In The Beginning

Noel R. Rose BS AM MD PhD FCAP FAAAAI

Department of Pathology and Department of Molecular Microbiology and Immunology, The Johns Hopkins Schools of Medicine and Public Health, Baltimore, MD, USA

KEY WORDS: autoimmunity, tolerance, thyroiditis, myocarditis, infection

IMAJ 2015; 17: 74–79

In 1957, an article published in the *Journal of the American Medical Association* (*JAMA*) changed the immunologic world [1]. It summarized over 3 years of intensive research by a team of investigators at the State University of New York at Buffalo, proving for the first time that a common human disease, chronic lymphocytic (Hashimoto) thyroiditis, is due to autoimmunization. This review summarizes my own involvement in this body of work, initially at Buffalo and then at Johns Hopkins. I recount my personal background that brought me to it, and describe the subsequent changes in immunologic thinking and medical practice that stemmed from the original discovery.

THE DECADE OF THE 1950S AND BEFORE: GENESIS

My interest in biomedical research was ignited in the seventh grade. Our biology teacher brought into class his personal monocular microscope and gave us, as young students, the opportunity to look at our own bodies in a new way. I was amazed, as was Anton van Leewenhock two centuries before, at this unseen world of microorganisms thriving on our skins and between our teeth. Enthralled by the observation, I decided at an early age that studying the effects of our microbial inhabitants on human health and disease would be my future career.

In the subsequent years, I pursued all of the courses and research opportunities available in microbiology first at Yale University as an undergraduate and then at the University of Pennsylvania as a graduate student. Following receipt of a PhD degree from the University of Pennsylvania, I accepted a junior faculty appointment at the University at Buffalo with a commitment to complete my medical training and thus receive qualifications in both medicine and basic biologic science.

In Buffalo I joined the recently created Department of Bacteriology and Immunology under the leadership of Professor Ernest Witebsky who was already world famous for isolating the blood group A and B substances. He was long interested in the organ-specific antigens that distinguish the specialized cells of the body. Frequently called tissue-limited antigens, these substances reflect the unique function of each cell within the organ and provide valuable insights into the fundamentals of cellular differentiation and metabolism. Witebsky predicted that organ-specific antigens would be of great importance in understanding normal physiology as well as disease, and in developing immunologic therapy for cancer [2].

Most of Witebsky's personal research was conducted using alcoholic extracts of tissues which probably represented glycolipids of cell membranes. The rationale for this approach was based on the extensive studies of cardiolipin, the alcohol-soluble antigen used in the serologic test for syphilis. Witebsky was following the precepts of his teacher Hans Sachs, himself one of the two key students of the great immunologist, Paul Ehrlich.

When I joined his department, Witebsky suggested that I look at the organ-specific antigen from the thyroid gland, thyroglobulin. At that time it was considered one of the rare proteins with clear-cut organ specificity. However, the methods for studying proteins in their native state were still limited, and Witebsky suspected that the organ specificity of thyroglobulin might be an artifact due to denaturation of the protein during its preparation. Since I had good training in biochemistry, I set about developing a gentle method of preparing thyroglobulin by the step-wise addition of ammonium sulfate to simple saline extracts of thyroid. It was quite easy to prepare thyroglobulin with nearly 90% purity. By making thyroglobulin from a number of different species and then immunizing rabbits with the products I readily confirmed that this protein antigen is strongly organ specific. The rabbit antibodies reacted strongly with thyroglobulin and barely, if at all, with any other organ of the species. Moreover, rabbit antisera induced by thyroglobulins of one species cross-reacted with similarly prepared thyroglobulins from other mammalian species.

Despite my great care in preparing the thyroglobulin in a gentle fashion, Witebsky was still concerned that it may have been denatured. I decided, therefore, to perform the critical experiment. All of us as medical students had learned the dictum of Ehrlich, *horror autotoxicus*. It taught us that the body refuses to produce autoantibodies because they would inflict harm. As a disciple of Ehrlich and as a blood grouper, Witebsky was a strong, vocal advocate of the validity of the *horror autotoxicus* law and agreed that a native protein injected into the same animal would fail to induce antibody formation. Therefore, I

carefully prepared rabbit thyroglobulin and immunized rabbits with this material. Since the amount of thyroglobulin was very limited, I adopted a then new method of immunization employing complete Freund adjuvant. My first results were highly disturbing; injection of rabbit thyroglobulin into rabbits indeed did induce thyroglobulin-specific antibody. Fearing there might be allogeneic differences among rabbits, I went on to prepare thyroglobulin from a single rabbit and injected it into the same animal. Once more, autoantibodies appeared.

Despite these results, I felt strongly that I had prepared thyroglobulin in the most careful fashion. Suppose, I reasoned, I had actually immunized the rabbits with thyroglobulin in a natural (or nearly natural) state, what would happen to the rabbit's own thyroid gland? Therefore, I removed thyroids from the immunized animals and asked Kornel Terplan, professor of pathology, to examine them. He soon reported that the glands were grossly infiltrated with monocytic cells and granulocytes with evidence of fibrosis and even germinal center formation. It was, he suggested, a replica of the human disease, chronic lymphocytic (Hashimoto) thyroiditis.

In those days, the only autologous antigens known to induce an autoimmune response were the sequestered antigens in "privileged sites." They included antigens from the brain, the lens of the eye, and sperm. The idea that the thyroid gland, a well-vascularized tissue, could contain an autoantigen capable of inducing disease seemed highly unlikely. Before he would accept these startling results, Witebsky insisted that I reproduce the phenomenon in other species. I succeeded in doing so in guinea pigs and dogs. Some of the dogs, in fact, produced particularly severe thyroiditis. As a final step, I performed the ultimate experiment of preparing thyroglobulin from one lobe of a rabbit, used to immunize the very same animal. Its remaining thyroid lobe could then be shown to develop thyroid infiltration. For these surgical experiments, I turned to the chairman of surgery, Dr. John Paine, and his assistant, Dr. Richard Egan.

When all these results were assembled, Witebsky had to accept the reality that Ehrlich's biologic dictum of *horror autotoxicus* did not apply to thyroglobulin and that this human disease, chronic lymphocytic thyroiditis, was likely the result of autoimmunization. Through Dr. Paine's connections, we laboriously collected about a dozen sera samples from patients with various thyroid diseases and found that at least four of them contained potent antibodies to human thyroglobulin. These four patients had severe thyroiditis.

After 3 years of intensive work, we agreed that we had completed the circle of evidence to prove that human chronic lymphocytic thyroiditis is an example of a human disease caused by autoimmunization. In order to announce these results, Witebsky contacted Dr. John Talbot, former professor of medicine at Buffalo, who had just become Editor-in-Chief of *JAMA*. They agreed this journal was the appropriate one since it would be read not only by practicing physicians but also by the leading medical investigators. Witebsky and I spent a great deal of time preparing the article as thoroughly, but cautiously, as possible. As we concluded our labors and prepared to submit the paper, Witebsky exclaimed that it may well demolish an accepted medical dogma. Many enigmatic human diseases might now be attributed to autoimmunity. There should be some way, I decided, of critically evaluating the evidence before declaring a human disease to be autoimmune. Koch's Postulates had served infectious disease research very well for almost a century. A similar set of postulates might provide valuable guidelines for future studies of autoimmunity. From that came the four "Witebsky Postulates" which established a rational basis for declaring a human disease to be autoimmune in etiology.

The *JAMA* article created quite a stir. It was one of the 100 most cited papers in biology at that time. It generated, as Witebsky predicted, numerous claims that a particular human illness is due to autoimmunization. Years later, I revisited the original postulates and developed a three-tiered approach to decide that a human disease is due to autoimmunity based on direct, indirect and circumstantial levels of evidence [3].

As our work was being publicized, another line of investigation appeared that led to general acceptance of autoimmunity as an important cause of many human diseases. Dr. Peter Miescher, then in Basel, Switzerland, provided compelling evidence that the lupus erythematous (LE) cell described in the bone marrow of lupus patients by Hargraves represented phagocytosis of nuclear material [4]. This phagocytic phenomenon resulted from opsonization of the nucleus by antinuclear antibodies. As thyroiditis became the prototype of a large group of diseases that are mainly restricted to a single organ, lupus has served as the model for many other diseases in which broad autoimmune responses induce systemic effects.

The impact of the *JAMA* article not only influenced clinical medicine, it changed our fundamental understanding of the immune response. We soon had visits from two of the key figures who shaped modern immunology. Peter Medawar came from England and added self-tolerance in his formulation of immunologic tolerance acquired in embryonic life. F.M. Burnet from Australia incorporated in his theory of clonal selection the concept of clonal deletion to establish normal self-tolerance and the chance origin of forbidden clones to explain the exceptional occurrence of autoimmune disease. Thus, our paper had far-reaching and lasting effects on both basic and applied immunology.

THE DECADE OF THE 1960S: MECHANISMS

By 1960, the concept of autoimmune disease had become well entrenched. The number of human diseases believed to be of autoimmune origin increased almost daily. My own research program followed two pathways. The first was to apply the

new methods that I had learned to detecting autoantibodies and also to demonstrating cell-mediated responses to other immune related diseases, including immediate and delayed hypersensitivity responses [5,6]. I also tried to show that another organ-specific antigen, insulin, was capable of producing insulitis in rabbits [7]. The goal of an experimentally induced insulitis in animals has still not been achieved.

The second and more prominent direction of my research in the decade of the 1960s was to determine the actual mechanisms of pathogenic autoimmunity. While we could not consistently transfer thyroiditis by passive infusion of antiserum, thyroglobulin-specific antibodies were capable of inducing thyroiditis especially if the thyroid gland were genetically vulnerable, damaged or inflamed [8,9]. On the other hand, antigen-specific T cells could adoptably transfer thyroiditis to syngeneic but not allogeneic recipients [10].

As more information emerged on the differentiation of T cells in the thymus, we turned to quantitative studies of thymic depletion and restoration [11]. An unexpected finding of these experiments was the diversity of immune responses in thymus cell-restored mice, suggesting that the early thymus itself may actually contain cells that retard rather than promote the immune response. This led to a number of studies continuing for the next ten years showing that timed neonatal thymectomy hastened spontaneous thyroiditis in the OS chicken and the BUF rat [12,13]. These experiments suggested the presence in the thymus of natural thymic suppressors, which we later built into our conceptual models of the mechanism of self-tolerance and autoimmunity based on clonal balance [14-16].

A further question was the actual mechanism of tissue damage. To address that issue we developed cell cultures of thyroid cells, taking care that they retained their organ specificity by producing thyroglobulin. Using intricate cell culture techniques, we could show that direct contact between thyroglobulin-specific T cells and cultured thyroid cells induced injury and death of thyroid follicular cells [17,18].

A series of experiments began a detailed antigenic mapping of the thyroglobulin molecule. The first experiments involved separation of the peptide fragments following proteolytic digestion [19]. A number of years later **t**he results led to the discovery that autoantibodies to thyroglobulin present in normal euthyroid individuals differed from additional antibodies formed later in patients with clinical thyroiditis [20]. These findings clarified our earlier concepts of the difference between naturally occurring, benign autoimmunity and pathogenic autoimmune disease [21,22].

Another informative study related to a second organ-specific antigen of the thyroid gland, thyroperoxidase. Other investigators had shown that most thyroiditis patients produce antibody to thyroperoxidase in addition to antibody to thyroglobulin. In our hands, rabbits injected with thyroid cell suspension did not produce antibody to rabbit thyroperoxidase, in contrast to rhesus monkeys given rhesus thyroid cell suspensions [23]. The primates immunized with thyroid extracts produced antibody to thyroglobulin first. After lesions appeared in their thyroids they produced additional antibodies to thyroperoxidase. Based on these experiments we later suggested that *escalation* of the autoimmune response was an early signal of pathogenic autoimmunity [24].

As the 1960s ended, we were able to achieve another major goal, the induction of experimental thyroiditis in mice [25]. This task was accomplished largely through careful attention to the adjuvant as well as the strain of mouse used in active immunization. It opened the doors to studies of the genetics of autoimmune diseases.

THE DECADE OF THE 1970S: GENETICS

The 1970s opened with the first demonstration that the susceptibility of different strains of mice to experimentally induced thyroiditis was genetically determined. The most prominent genes regulating susceptibility were part of the major histocompatibility complex (MHC) [26]. This finding was soon confirmed in the spontaneous model of thyroiditis in the OS chicken, as well as both induced and spontaneous thyroiditis in the rat [27-29]. Today, virtually every autoimmune disease in experimental animals and humans is regulated by genes of the major histocompatibility complex. This MHC association represents one of the fundamental characteristics (the "common threads") shared by all autoimmune diseases. Within the MHC, we were able to resolve the active immunization process into the induction steps that depend primarily on MHC class II genetic determinants and severity of lesions in the thyroid gland, which resides mainly in MHC class I [30,31]. Subsequently, we took up studies of the genes outside of the MHC, which together account for more than half of the inherited susceptibility to thyroiditis. In this case, however, there is no single predominant family of genes, but rather a large number of disparate traits [32]. Each contributes a small amount to the final susceptibility. Most of the genes that have been studied were found to regulate the immune response.

These early investigations on thyroiditis in experimental animals are reflected in our current understanding of genetics of autoimmune disease generally. The induction of the immune response depends first upon recognition of the candidate antigen by genes of the MHC. Together they determine the specificity of the immune response. Non-MHC genes represent a consortium of non-antigen-specific traits that collectively regulate the normal homeostasis in the immune response on a daily basis. In the case of autoimmunity, there is a gradient from highly susceptible to relatively resistant mice in response to the candidate antigen [33]. Present evidence suggests that a similar situation occurs in human autoimmune disease.

By the end of the 1970s, we gained a good understanding of the inherited susceptibility to thyroiditis and witnessed its application to other autoimmune diseases. Collectively, however, all of these genetic traits seem to provide less than half of the actual risk. Some of the risk may be associated with hormonal influences or random post-genomic, epigenetic changes in the immune system itself. However, the largest contribution to risk seems to arise from environmental mediators. They represent the "triggers" of autoimmunity.

THE DECADE OF THE 1980S: ENVIRONMENT

In humans, many examples of environmental agents that precipitate autoimmune diseases in genetically susceptible individuals have been described, yet in only a few instances has a cause-and-effect relationship been established [34,35]. In experimental animals, the most common environmental mediators appear to be infections.

In 1981, I moved with a number of colleagues to the Johns Hopkins University [Figure 1]. Johns Hopkins provides a particularly favorable setting for combined fundamental and clinical research. As the first university in the United States founded specifically to support advanced research and education, the medical school has, from the beginning, accepted only students who have completed a broad undergraduate curriculum. In fact, modern American medical education is modeled after the Hopkins plan. Two years of intensive study of the basic biologic sciences underlying health and disease are followed by 2 years of applied clinical clerkships in a university academic hospital. Although modified many times in many ways, this basic plan transformed medicine from a trade to a scholarly discipline. The Hopkins model now marks medical education throughout the world.

Figure 1. Dr. Rose examining a gel in his laboratory at Johns Hopkins, 1982

At Johns Hopkins, I had the opportunity of establishing a department devoted to infection and immunity. To emphasize the role of microbial infection in the induction of autoimmune disease we expanded our earlier investigations of inflammatory diseases of the heart instigated by viral infection. From detailed study has come the first clear evidence of how infection could initiate autoimmune disease. Briefly, we found that coxsackievirus B3, one of the common viral causes of myocarditis in humans, induces acute infectious myocarditis in all strains of mice [36]. Although most mice recover from the viral disease spontaneously, a few inbred strains develop a chronic disease that we established as the result of an organ-specific immune response to cardiac myosin [37,38].

This model, the first example of virus-induced autoimmune disease where the antigen was defined, has proved to be of incredible value in discovering the underlying immunopathic mechanisms [39]. Virus-induced damage inflicted on the cardiac cells induces expression of the intracellular cardiac myosin. In the presence of cardiac inflammation, also induced by the virus, the antigen is presented to CD4 T helper cells, which are able to initiate pathogenic autoimmunity. Recent investigations have shown that the Th17 pathway is essential for the induction of inflammatory myocarditis [40]. However, the signature cytokine, interleukin (IL)-17, does not determine the severity of inflammation. Instead, IL-17 is the critical component in determining whether a mouse with autoimmune myocarditis will proceed to further fibrotic remodeling of the heart in the form of dilated cardiomyopathy [41,42].

These experiments have provided the opportunity to dissect the step-wise progression from viral infection to autoimmune inflammatory myocarditis to subsequent fibrotic cardiac remodeling and dilated cardiomyopathy. Although somewhat chaotic on first observation, the steps can be predictable. It provides hope that human immune mediated disorders can be assessed in terms of the inflammatory pathways activated and the future outcome determined [43]. Equally important, identification of the key inflammatory mediators provides opportunities to prognose, predict and intervene therapeutically. By the end of the 1980s, we could see our goal of moving from reductionistic, mechanistic experiments to translational investigations involving earlier diagnosis, more accurate prognosis and more targeted interventions [44].

THE DECADE OF THE 1990S: EPIDEMIOLOGY

In 1997, our group published another highly cited article that changed the immunologic landscape [45]. Starting with 24

well-defined autoimmune diseases, we assembled the existing publications on incidence and prevalence based on the most reliable data available. Three autoimmune diseases are relatively common in North America and Western Europe: Graves' disease, chronic lymphocytic thyroiditis and rheumatoid arthritis. All of the other autoimmune diseases are relatively rare. As a group, the autoimmune diseases are among the largest clinical and public health problems in the industrialized countries. Many of these diseases are increasing in incidence. Interestingly, all the autoimmune diseases co-occur with some other autoimmune conditions, signifying some measure of shared genes. The autoimmune diseases are truly a family [46].

This knowledge of the relatedness of the autoimmune diseases also points to the prospect that genetic data combined with biochemical and immunologic early biomarkers may soon be able to predict, on an individual basis, the onset of some autoimmune diseases. By defining the precise causative antigen, early interventions to prevent or abort the condition may be feasible [47].

THE DECADE OF THE 2000S: POLICY ISSUES

Part of the responsibility of an investigator is to share the essence of his research findings with professional colleagues and with the general public. Much of my recent activity relates to promoting the concept that autoimmune diseases are a family of related human diseases. Basic science has shown that the autoimmune diseases share common threads [48]. They represent a category of disease based on their common etiology, loss of self-tolerance. The autoimmune diseases, collectively, are among the major causes of illness and death in the industrialized countries. They are comparable in prevalence to other main categories of disease, cancer and heart disease, although they are still relatively unrecognized. Health professionals must realize that autoimmune diseases as a group are relatively common and that they can be life-long and life-threatening. Although cures for any autoimmune disease are not presently at hand, most patients can be maintained by supportive treatment. However, many of the most effective treatments are risky and expensive. Our vision turns to prevention [47].

The general public has begun to read about autoimmune diseases in widely circulating journals and newspapers. Patients and their families need to understand more about their causation and course. That the incidence is increasing suggests that the industrialized environment may be an important factor in their etiology. There is some suggestion that autoimmune diseases are now rising in other lesser developed countries as industrialization proceeds. Autoimmune diseases are an international health priority.

A proportion of my own efforts has been to develop a Research Center for Autoimmune Disease at Johns Hopkins. It is designed to bring together the many faculty members in the university community who work on any aspect of tolerance and autoimmunity. Especially important is its mission of connecting investigators and clinicians interested in different autoimmune diseases and discovering the parallels and synergies. I was pleased to have the opportunity of chairing the Autoimmune Disease Coordinating Committee of the National Institutes of Health, which is charged with coordinating research policy at the national level. On the public side, I work with the American Autoimmune Related Disease Association to increase public awareness and understanding of all the autoimmune diseases. Finally, the International Congresses of Autoimmunity have promoted research on autoimmunity as a major cause of disease throughout the world.

For the past 60 years, I have been privileged to see autoimmunity emerge as an important component of human medicine, to witness the importance of studying autoimmunity as a part of the normal physiology, to participate in expanding the scope of autoimmune disease research, both inward to the molecular and genetic levels and outward to the clinic. It has been a wonderful journey of discovery but we still have many miles to go.

Acknowledgments

I am pleased to thank Starlene Murray and Deborah H. Rose for editorial assistance

Correspondence

Dr. N.R. Rose

Dept. of Pathology, The Johns Hopkins Schools of Medicine and Public Health, Baltimore, MD 21205, USA

email: nrrose@jhmi.edu

References

1. Witebsky E, Rose NR, Terplan K, Paine JR, Egan RW. Chronic thyroiditis and autoimmunization. *JAMA* 1957; 164: 1439-47.
2. Witebsky E, Rose NR, Shulman S. Studies of normal and malignant tissue antigens. *Cancer Res* 1956; 16: 831-41.
3. Rose NR, Bona C. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol Today* 1993; 14: 426-30.
4. Miescher P. The antigenic constituents of the neutrophilic leukocyte with special reference to the L.E. phenomenon. *Vox Sang* 1957; 2 (3): 145-58.
5. Arbesman CE, Rose NR, Kantor SZ, Beede RB. Immunologic studies of ragweed-sensitive patients. I. Specificity and sensitivity of hemagglutination reactions. *J Allergy* 1960; 31: 317-32.
6. Girard JP, Rose NR, Kunz ML, Kobayashi S, Arbesman CE. In vitro lymphocyte transformation in atopic patients: induced by allergens. *J Allergy* 1967; 39: 65-81.
7. Fellenberg von R, Rose NR. Antigenicity of insulin. *Int Arch Allergy* 1968; 33: 454-72.
8. Vladutiu AO, Rose NR. Transfer of experimental autoimmune thyroiditis of the mouse by serum. *J Immunol* 1971; 106: 1139-42.
9. Jaroszewski J, Sundick RS, Rose NR. Effects of antiserum containing thyroglobulin antibody on the chicken thyroid gland. *Clin Immunol Immunopathol* 1978; 10: 95-103.
10. Twarog FJ, Rose NR. Transfer of autoimmune thyroiditis of the rat with lymph node cells. *J Immunol* 1970; 104: 1467-75.
11. Mayhew B, MacGillivray MH, Rose NR. Immunologic restoration of thymectomized mice by thymus cell suspensions and the duration of their effect. *Proc Soc Exp Biol Med* 1968; 128: 1217-21.

12. Welch P, Rose NR, Kite JH Jr. Neonatal thymectomy increases spontaneous autoimmune thyroiditis. *J Immunol* 1973; 110: 575-7.
13. Silverman DA, Rose NR. Neonatal thymectomy increases the incidence of spontaneous and methylcholanthrene-enhanced thyroiditis in rats. *Science* 1974; 184: 162-3.
14. Rose NR. The role of the thymus in spontaneous autoimmune thyroiditis. *Ann N Y Acad Sci* 1975; 249: 16-24.
15. Rose NR. Current concepts of autoimmune disease. *Transplant Proc* 1988; 20: 3-10.
16. Rose NR, Talor E. Antigen-specific immunoregulation and autoimmune thyroiditis. *Ann N Y Acad Sci* 1991; 636: 306-20.
17. Rose NR, Kite JH, Jr, Doebbler TK, Brown RC. In vitro reactions of lymphoid cells with thyroid tissue. In: Amos B, Koprowski H, eds. Cell-Bound Antibodies. Philadelphia: Wistar Institute Press, 1963: 19-33.
18. Creemers P, Rose NR, Kong YM. Experimental autoimmune thyroiditis: in vitro cytotoxic effects of T lymphocytes on thyroid monolayers. *J Exp Med* 1983; 157: 559-71.
19. Stylos WA, Rose NR. Splitting of human thyroglobulin. II. Enzymatic digestion. *Clin Exp Immunol* 1969; 5: 285-97.
20. Bresler HS, Burek CL, Hoffman WH, Rose NR. Autoantigenic determinants on human thyroglobulin. II. Determinants recognized by autoantibodies from patients with chronic autoimmune thyroiditis compared to autoantibodies from healthy subjects. *Clin Immunol Immunopathol* 1990; 54: 76-86.
21. Esquivel PS, Rose NR, Kong YM. Induction of autoimmunity in good and poor responder mice with mouse thyroglobulin and lipopolysaccharide. *J Exp Med* 1977; 145: 1250-63.
22. Rose NR, Mackay IR. Introduction – autoimmunity versus autoimmune diseases. In: Rose NR, Mackay IR, eds. The Autoimmune Diseases, Chapter 1. Orlando, FA: Academic Press, Inc, 1985: xxv-xxvi.
23. Andrada JA, Rose NR, Kite JH, Jr. Experimental thyroiditis in the rhesus monkey. IV. The role of thyroglobulin and cellular antigens. *Clin Exp Immunol* 1968; 3: 133-51.
24. Rose NR. Autoimmune regulation: through the crystal ball. *Clin Exp Immunol* 2007; 147: 9.
25. Rose NR, Twarog FJ, Crowle AJ. Murine thyroiditis: importance of adjuvant and mouse strain for the induction of thyroid lesions. *J Immunol* 1971; 106: 698-704.
26. Vladutiu AO, Rose NR. Autoimmune murine thyroiditis. Relation to histocompatibility (H-2) type. *Science* 1971; 174: 1137-9.
27. Bacon LD, Kite JH Jr, Rose NR. Relation between the major histocompatibility (B) locus and autoimmune thyroiditis in obese chickens. *Science* 1974; 186: 274-5.
28. Rose NR. Differing responses of inbred rat strains in experimental autoimmune thyroiditis. *Cell Immunol* 1975; 18: 360-4.
29. Lillehoj HS, Beisel K, Rose NR. Genetic factors controlling the susceptibility to experimental autoimmune thyroiditis in inbred rat strains. *J Immunol* 1981; 127: 654-9.
30. Tomazic V, Rose NR, Shreffler DC. Autoimmune murine thyroiditis. IV. Localization of genetic control of the immune response. *J Immunol* 1974; 112: 965-9.
31. Beisel KW, David CS, Giraldo AA, Kong YM, Rose NR. Regulation of experimental autoimmune thyroiditis: mapping of susceptibility to the I-A subregion of the mouse H-2. *Immunogenetics* 1982; 15: 427-30.
32. Beisel KW, Kong YM, Babu KSJ, David CS, Rose NR. Regulation of experimental autoimmune thyroiditis: influence of non-H-2 genes. *J Immunogenetics* 1982; 9: 257-65.
33. Rose NR, Kong YM, Okayasu I, Giraldo AA, Beisel K, Sundick RS. T-cell regulation in autoimmune thyroiditis. *Immunol Rev* 1981; 55: 299-314.
34. Nyland JF, Caturegli P, Rose NR. Environment/drug-induced human autoimmune disease. Immune System Toxicology. In: Lawrence D, ed. Comprehensive Toxicology. Philadelphia: Elsevier Academic Press, Ltd, 2010; 5: 440-51.
35. Miller FW, Pollard KM, Parks CG, et al. Criteria for environmentally associated autoimmune diseases. *J Autoimmunity* 2012; 39(4): 253-8.
36. Wolfgram LJ, Beisel KW, Herskowitz A, Rose NR. Variations in the susceptibility to coxsackievirus B_3-induced myocarditis among different strains of mice. *J Immunol* 1986; 136: 1846-52.
37. Wolfgram LJ, Beisel KW, Rose NR. Heart-specific autoantibodies following murine coxsackievirus B_3 myocarditis. *J Exp Med* 1985; 161: 1112-21.
38. Neu N, Rose NR, Beisel KW, Herskowitz A, Gurri-Glass G, Craig SW. Cardiac myosin induces myocarditis in genetically predisposed mice. *J Immunol* 1987; 139: 3630-6.
39. Rose NR, Beisel KW, Herskowitz A, et al. Cardiac myosin and autoimmune myocarditis. In: Evered D, Whelan J, eds. Ciba Foundation Symposium 129: Autoimmunity and Autoimmune Disease. Chichester, UK: John Wiley & Sons, Ltd, 1987: 3-24.
40. Cihakova D, Rose NR. Pathogenesis of myocarditis and dilated cardiomyopathy. *Adv Immunol* 2008; 99: 95-114.
41. Baldeviano GC, Barin JG, Talor MV, et al. Interleukin-17A is dispensable for myocarditis but essential for the progression to dilated cardiomyopathy. *Circ Res* 2010; 106: 1646-55.
42. Wu Lei, Ong SuFey, Talor MV, et al. Cardiac fibroblasts mediate IL-17A-driven inflammatory dilated cardiomyopathy. *J Exp Med* 2014; 211(7): 1449-64.
43. Rose NR. Critical cytokine pathways to cardiac inflammation. *J Interferon Cytokine Res* 2011; 31: 1-6.
44. Rose NR. Prediction and prevention of autoimmune disease: a personal perspective. *Ann NY Acad Sci* 1007; 1109: 117-28.
45. Jacobson DL, Gange SJ, Rose NR, Graham NMH. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin Immunol Immunopathol* 1997; 84: 223-43.
46. Eaton WW, Rose NR, Kalaydjian A, Pedersen MG, Mortensen PB. Epidemiology of autoimmune diseases in Denmark. *J Autoimmunity* 2007; 29 (1): 1-9.
47. Rose NR. Moving from prediction to prevention. *The Scientist* 2007 May (Suppl): 76-78.
48. Rose NR. Autoimmune diseases: tracing the shared threads. *Hosp Practice* 1997; 32: 147-54.

Capsule

A comparative encyclopedia of DNA elements in the mouse genome

The laboratory mouse shares the majority of its protein-coding genes with humans, making it the premier model organism in biomedical research, yet the two mammals differ in significant ways. To gain greater insights into both shared and species-specific transcriptional and cellular regulatory programs in the mouse, the Mouse ENCODE Consortium has mapped transcription, DNase I hypersensitivity, transcription factor binding, chromatin modifications and replication domains throughout the mouse genome in diverse cell and tissue types. By comparing with the human genome, Yue et al. not only confirm substantial conservation in the newly annotated potential functional sequences, but also find a large degree of divergence of sequences involved in transcriptional regulation, chromatin state and higher order chromatin organization. Their results illuminate the wide range of evolutionary forces acting on genes and their regulatory regions, and provide a general resource for research into mammalian biology and mechanisms of human diseases.

Nature 2014; 515: 355

Eitan Israeli

Vitamin D Antibodies in Systemic Sclerosis Patients: Findings and Clinical Correlations

Narin N. Carmel MD[1,2*], Pnina Rotman-Pikielny MD[2*], Alexey Lavrov MD[3] and Yair Levy MD[2]

[1]Golda-Hasharon Hospital, Rabin Medical Center Campus, Petah Tikva, Israel
Departments of [2]Internal Medicine E and [3]Anesthesiology, Meir Medical Center, Kfar Saba, affiliated with Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

ABSTRACT: **Background:** Vitamin D is a pivotal factor in calcium homeostasis and exerts immunomodulatory effects. Hypovitamin D has been demonstrated in systemic sclerosis (SSc) patients and may be related to more severe disease of longer duration and with extensive skin involvement.

Objectives: To seek anti-vitamin D antibodies in SSc patients, as found by previous research in patients with systemic lupus erythematosus (SLE).

Methods: The study included 54 SSc patients and 41 volunteers. Immunoglobulin (Ig) G and IgM autoantibody levels against 25(OH)D and 1,25(OH)D were obtained from patients and controls and were compared. SSc patients were assessed for autoantibody profile and disease severity.

Results: Vitamin D antibodies were present in 87% of SSc patients and 42% of controls. Higher levels of anti-25(OH)D IgM antibodies were detected in SSc patients compared to controls (0.48 ± 0.22 vs. 0.29 ± 0.29, respectively, $P = 0.002$); however, IgG levels were lower in the SSc patients. No such discriminative effect was found regarding anti-1,25(OH)D antibodies between SSc and controls. No correlation was found between vitamin D antibodies and other autoantibodies, disease severity, or target organ damage.

Conclusions: To the best of our knowledge, this is the first study of these novel anti-vitamin D antibodies in SSc patients and the first time a correlation between IgM 25(OH) vitamin D antibodies and scleroderma has been identified. Further research on the pathophysiological significance and therapeutic potential of vitamin D is required.

IMAJ 2015; 17: 80–84

KEY WORDS: scleroderma, systemic sclerosis (SSc), vitamin D, vitamin D antibodies, vitamin D deficiency, vitamin D insufficiency, 25-hydroxyvitamin D (25(OH)D), dihydroxyvitamin D (1,25(OH)D)

Vitamin D is a pivotal factor in disorders that involve calcium metabolism, such as osteoporosis and osteomalacia. In vitro studies have demonstrated that vitamin D also exerts immunomodulatory effects. Vitamin D receptors were identified in several types of immune cells and this substance plays a key role both in reaction enhancement of the naïve immune system and in restraint of the type 1 response of the adaptive immune system [1]. The number of vitamin D receptors on CD4 T cells correlates with their immune activity. Adding 1,25(OH) D to CD4 T lymphocytes inhibits proliferation of T helper (Th)-1 cells, but increases the activity of Th-2 cells and related cytokines interleukin (IL)-5 and IL-10 [2]. Vitamin D also affects the naïve immune system via Th-17, which plays a central role in neutrophil activity and inflammatory response. These activities are suppressed by 1,25(OH)D via inhibition of IL-23 and IL-6 production [3].

Systemic sclerosis (SSc) is a chronic connective tissue disease of unknown etiology that causes widespread microvascular damage and excessive deposition of collagen in the skin and internal organs [4]. Clinical associations exist between the pattern of organ involvement (limited vs. systemic disease) and scleroderma-specific autoantibodies, such as antinuclear antibody, anti-centromere antibody and anti-topoisomerase-1 antibody (anti-Scl-70) [5]. However, the etiopathogenesis of the disease and the role of various antibodies in organ involvement and disease manifestations remain unclear.

A high prevalence of vitamin D deficiency was noted among patients with autoimmune diseases [6–8], especially systemic lupus erythematosus (SLE) [9]. Moreover, patients with SLE and severe vitamin D deficiency demonstrated a more severe disease course [10]. These observations led to the hypothesis that vitamin D deficiency may exacerbate autoimmune conditions [6,10]. Vitamin D treatment in animal models of autoimmune diseases, primarily SLE, led to significant improvements [11]. However, similar results have not been fully achieved in humans, and the role of vitamin D treatment in the course of autoimmune diseases has yet to be studied [12].

SSc patients tend to have very low vitamin D levels. This may be attributed to several characteristics of the disease, including disseminated skin involvement and renal injury that may interfere with vitamin D synthesis, as well as vitamin D malabsorption in cases of advanced intestinal disease. Similar to SLE patients, SSc patients with vitamin D deficiency demonstrate a lengthier and more severe disease [13], particularly regarding lung involvement [14,15]. An inverse cor-

*The first two authors contributed equally to this study

relation was found between the severity of skin involvement, including fibrosis and calcinosis, and vitamin D blood levels [16,17]. However, other studies did not confirm the association between the severity of scleroderma and vitamin D deficiency [18,19]. Another possible explanation for vitamin D deficiency in patients with autoimmune diseases is the presence of neutralizing autoantibodies to vitamin D. Few studies on vitamin D antibodies in these diseases have been conducted. A preliminary study in patients with SLE demonstrated higher levels of anti-vitamin D antibodies compared to those with anti-phospholipid syndrome or pemphigus vulgaris [20].

The above studies, combined with conflicting information regarding the role of vitamin D status in SSc patients, motivated us to examine anti-vitamin D antibodies in SSc patients. We investigated the presence and levels of IgG and IgM antibodies to 25-hydroxyvitamin D (25(OH)D) and dihydroxyvitamin D (1,25(OH)D) in a cohort of SSc patients followed regularly in our outpatient clinic. A complete autoimmune antibody history was obtained and the pattern and severity of organ damage characterized. The main objective was to investigate vitamin D antibodies in a cohort of scleroderma patients. We also tried to detect a correlation between vitamin D antibodies and specific organ involvement and disease severity.

PATIENTS AND METHODS

The study population comprised 54 consecutive patients diagnosed with SSc (both limited and systemic manifestations) classified according to the American College of Rheumatology (ACR) 1980 criteria [21]. All patients were followed at the Meir Medical Center outpatient clinic. Fifty-five patients with scleroderma enrolled in the study (44 with systemic disease, 11 with limited manifestation). Clinical data included age, disease duration, organ involvement, the presence of autoantibodies, and vitamin D levels prior to treatment initiation. Forty-one volunteers from the hospital staff served as the control group. Controls were questioned about their current health status including smoking, medical history, pregnancy status, regular consumption of medications including oral contraceptives, hormone replacement therapy, and use of nutritional supplements. Blood samples were also tested for vitamin D levels.

SSC SEVERITY SCORE (SCSS)

A numeric evaluation of disease severity in five organ systems (lung, kidney, skin, gastrointestinal, joints) on a scale ranging from 0 (no injury) to 3 (severe injury) was obtained. Lung injury regarding interstitial lung disease and pulmonary hypertension was estimated. This ranking system correlates with other severity scales, such as that of Medsger et al. [22]. The scores were calculated based on medical record data for the five organ systems, with a range of 0 (healthy) to 15 (severe pan-systemic illness).

DETECTION OF AUTOANTIBODY LEVELS

- **Anti-vitamin D antibody determination**

For antibody determination, 95-well enzyme-linked immunosorbent assay (ELISA) plates (Maxisorp, Nunc, Denmark) were coated overnight at 4°C with 1,25(OH) vitamin D (Sigma, St. Louis, MO, USA) 5 g/ml and absolute ethanol. The plates were blocked with 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 1 hour at 37°C. Following washing with PBS, the sera from the subjects were added at a dilution of 1:100 in 1% BSA and PBS for 4 hours at room temperature. Immunoglobulin binding was probed by goat anti-human IgG antibodies conjugated to alkaline phosphatase (Jackson ImmunoResearch, West Grove, PA, USA) for 1 hr at 37°C. The plates were developed using phosphatase substrate (Sigma) and read at optical densities (OD) of 405 nm using an ELISA reader (Anthos HT2, Anthos Labtech Instruments, Salzburg, Austria).

- **Vitamin D measurement**

The commercial kit, LIAISON 25(OH) vitamin D (DiaSorin Inc., Saluggia, Italy) was used to measure serum concentration of 25(OH) vitamin D. A low 25(OH) vitamin D level was set as < 30 ng/ml, while < 10 ng/ml was considered insufficient.

STATISTICAL ANALYSIS

Descriptive statistics included counts, mean, median, standard deviation, minimum and maximum values for continuous measurements, and contingency tables with counts and percents for categorical measurements. Comparison of anti-vitamin D levels between the treatment and control group were assessed using the Mann-Whitney test. Receiver operating characteristic (ROC) curve was applied to identify the discriminative ability of anti-vitamin D to identify the best discriminative threshold, which was determined to be 0.3 U.

A logistic regression model including vitamin D metabolite antibodies, age and gender subclasses was applied to identify independent factors that could predict SSc. Statistical analyses were carried out using IBM SPSS software, version 22.0. Significance level was defined as $P = 0.05$.

RESULTS

DEMOGRAPHICS AND GENERAL PATIENT PROFILE

The study included 95 subjects (54 SSc patients and 41 healthy controls). The scleroderma patients were significantly older than the control group (52.6 ± 14.7 vs. 35.7 ± 8.5 years, $P < 0.001$) and there were more females in both groups (87.3% and 85.4%, respectively) [Table 1]. Although measurement of vitamin D levels was not part of the current research, we obtained vitamin D levels that were taken prior to beginning vitamin D therapy: 82.1% of the patients had documented vitamin D deficiency (< 30 ng/dl) compared to only 9.8% of controls.

Table 1. Demographic characteristics of scleroderma patients versus controls

	Scleroderma (n=54)	Controls (n=41)	*P* value
Age (years)	52.6 ± 14.7	35.7±8.5	‹ 0.001
Female gender	48 (88.9%)	35 (85.4%)	0.757
Comorbidities*	31 (57.4%)	6 (14.6%)	‹ 0.001
Smoker	5 (9.1%)	11 (26.8%)	0.029
Documented vitamin D insufficiency	82.1% (n=39)	9.8% (n=41)	
Systemic/limited scleroderma	44/10 (79.6%/20.4%)	Irrelevant	
Length of disease (years, range)	10 ± 6.6 (2–43)	Irrelevant	
Scleroderma severity score (range)	5.56 ± 2.5 (2–11)	Irrelevant	
Antibody profile		Irrelevant	
Antinuclear antibody (ANA)	52 (98.1%)		
Anti-topoisomerase I (Scl-70)	28 (51.9%)		
Anti-centromere	10 (18.9%)		

Data are presented as n (%) or mean ± SD
*Comorbidities included hypertension, hyperlipidemia, diabetes mellitus, asthma and obesity

Table 2. Vitamin D antibody levels in scleroderma patients and controls

	Scleroderma patients	Controls	*P* value*
Anti 25(OH)D IgG	0.23 (0.11–1.94)	0.26 (0.13–1.85)	0.049
Anti 25(OH)D IgM	0.48 (0.11–1.10)	0.29 (0.04–1.61)	0.002
Anti 1,25(OH)D IgG	0.31 (0.13–1.34)	0.25 (0.14–0.63)	0.887
Anti 1,25(OH)D IgM	0.21 (0.07–1.46)	0.27 (0.05–1.79)	0.367

**P* values by Mann-Whitney test

Table 3. Risk factor for scleroderma determined by vitamin D antibodies, age and gender calculated by regression model analysis

			95% CI	
Variable	OR	*P* value	Lower limit	Upper limit
Anti 25 (OH) vitamin D IgG	4.8	0.034	1.1	20.3
Anti 25 (OH) vitamin D IgM	7.5	0.003	2.0	27.5
Anti 1,25(OH) vitamin D IgG	.90	0.868	.30	3.0
Anti 1,25(OH) vitamin D IgM	.90	0.862	.30	3.0
Age	1.1	0.000	1.0	1.2
Gender	1.6	.5820	.30	9.6

Scleroderma Severity Score (ScSS) ranged from 2 to 11 with an average of 5.6 ± 2.5, implying severe injury in at least two organ systems and moderate damage in three organ systems. Half the patients had a score of 6 or less and 75% had a score of less than 8. Severe degrees of involvement were noted in the following systems: muscular (n=5), skin (n=6), gastrointestinal (n=3), and renal (n=2).

PRESENCE AND SIGNIFICANCE OF VITAMIN D ANTIBODIES

Vitamin D antibodies were present in 87% of SSc patients and 42% of controls. There were significant differences in the levels of anti-25(OH)D antibodies between the scleroderma and control groups. IgM levels were higher in SSc patients compared to controls (0.48 vs. 0.29, $P = 0.02$, Mann-Whitney test). In contrast, IgG antibodies were lower in scleroderma patients (0.23 vs. 0.26, $P = 0.005$, Mann-Whitney test). It should be noted that anti-1,25(OH)D subtype IgG antibodies also demonstrated a higher trend but the difference was not statistically significant ($P = 0.878$) [Table 2].

ROC curve analysis was applied to identify the anti-vitamin D antibody 25(OH) D and 1,25(OH)D thresholds that best discriminate between SSc patients and controls. A cutoff level/threshold of 0.30 U/L had 87.0% sensitivity and 58% specificity, whereas a threshold of 0.20 U/L demonstrated higher sensitivity of 92.6% but lower specificity of only 42.5%. At a cutoff of 0.3, 47 patients (87%) and 17 controls (42%) were positive to the antibodies. A logistic regression model demonstrated that age and anti-25(OH)D IgM and IgG levels were significantly related to scleroderma disease, whereas higher levels of anti-25(OH)D IgM (> 0.3) increased the risk of SSc, with odds ratio (OR) of 7.5 and 95% confidence interval (CI) 2.0–27.5, $P <$ 0.003. Lower levels of anti-1,25(OH)D IgG (< 0.3) increased the risk of SSc (OR 4.79, 95%CI 1.1–20.3, $P < 0.035$). For age, the OR was 1.0 (95%CI 1.0–1.2) [Table 3].

Borderline differences in ScSS were noted between patients with anti-25(OH)D and those without antibodies (66 vs. 5 respectively, $P = 0.114$). For the correlation between ScSS and antibodies, only anti-1,25(OH)D IgM demonstrated a weak, negative correlation with borderline significance ($r = -0.272$, $P = 0.056$). Anti-25(OH)D IgM had a weakly positive correlation with Scleroderma Severity Subscale related to muscle involvement ($r = 0.288$, $P = 0.035$). There was no significant difference in anti-25(OH)D or anti-1,25(OH)D levels in 15 patients with severe involvement of one or more systems compared to subjects with less severe disease. No further association was identified between vitamin D antibodies and targeted system injury or other autoimmune antibody profile subtypes.

More female subjects had anti-25(OH)D values ≥ 0.2 (89.6% vs. 57.1%, $P = 0.055$) compared to males. A higher incidence of anti-25(OH)D (threshold > 0.3) was noted among all female participants in the study (74.7% vs. 46.2%, $P = 0.049$).

DISCUSSION

In addition to its well-known functions regarding bone metabolism, vitamin D, a fat-soluble vitamin, has significant effects on the immune system. Several in vitro studies have demonstrated its efficacy in preventing and treating autoimmune diseases [11,23,24].

SSc patients have a high prevalence of vitamin D deficiency [17]. Hypovitamin D in SSc patients can be attributed to skin

involvement and renal injury that might interfere with vitamin D synthesis and malabsorption due to advanced intestinal disease. Vitamin D antibodies may also cause vitamin D deficiency in SLE patients, as demonstrated by Carvalho et al. [20] in a preliminary study. However, there is no information regarding vitamin D antibodies in patients with SSc.

The present study presents novel data regarding vitamin D antibodies in SSc patients. A total of 95 subjects, 54 of whom had SSc, were tested for both 25(OH) D and 1,25(OH)D antivitamin D antibodies and two subtypes of antibodies, IgM and IgG. Varying levels of anti-25(OH)D, especially the IgM subclass, differentiated SSc patients from controls. Furthermore, anti-25(OH)D IgM was higher in the SSc group than the controls (0.48 ± 0.22 vs. 0.39 ± 0.33, $P = 0.013$), while IgG was lower (0.27 ± 0.26 vs. 0.34 ± 0.29, $P = 0.026$). There were no statistically significant differences regarding anti-1,25(OH)D antibodies between groups, although a slight trend was noticed.

Few studies on vitamin D antibodies in autoimmune diseases have been conducted, mostly without antibody subtypes or vitamin D metabolite-related analysis [20,25]. Carvalho and team [20] found vitamin D antibodies in a subset of patients with SLE. The results in SSc patients presented here are in concert with those of Carvalho's study, demonstrating the presence of these unique antibodies. Different autoantibodies have been described in various frequencies, but it has not been determined whether they are all pathogenic in SLE. However, the association found by Carvalho et al. between anti-vitamin D antibodies and anti-dsDNA in SLE patients suggests that despite their low frequency in the cohort presented here (4%, n=7), anti-vitamin D antibodies may play a role in SLE pathogenesis. However, this breakthrough study did not perform subclass analyses; therefore, we lack information regarding the role of IgM vitamin D antibodies in SLE.

An Indonesian study by Handono [25] showed a similar trend: 70.63% of SLE patients had low (< 30 ng/ml) vitamin D serum levels. Autoimmune antibodies were frequently present in these patients: anti-dsDNA in 70.30% and anti-vitamin D in 64.81%. Serum levels of 25(OH) vitamin D correlated negatively with anti-dsDNA and anti-vitamin D antibodies ($r = -0.416$, $P = 0.032$, and $r = -0.537$, $P = 0.041$, respectively). The authors postulated that low vitamin D levels in patients with SLE may be caused by anti-vitamin D antibodies.

The present study did not find a statistically significant difference between patients and controls in antibody titers against the active metabolite 1,25(OH) vitamin D. It is important to remember that the major circulating form of vitamin D is 25(OH)D and its level is currently considered the best indicator of vitamin D stores. Since 25(OH)D is the dominant form in circulation, with a longer half-life than that of 1,25(OH)D, it may well be a more immunogenic form.

In this study, anti-vitamin D was detected among both SSc patients and healthy controls. These results are consistent with previous research. Carvalho et al. detected a high frequency of vitamin D antibodies among healthy controls, raising the hypothesis that antibodies develop in individuals who consume high doses of vitamin D. Unfortunately, we do not have data regarding vitamin D intake/exposure among the controls, but since they are relatively young (mean age 35.7 years) and healthy, we assume that most did not consume vitamin D regularly.

The literature lacks information regarding subtype analysis and metabolite dominance of vitamin D antibodies. Based on our literature review, this analysis has not been published for any autoimmune disease.

Unlike previous reports that demonstrated a correlation between anti-vitamin D antibodies and anti-dsDNA antibodies among SLE patients, no such correlation was observed in the current study. Moreover, no other autoantibody levels correlated with anti-vitamin D levels.

This study was limited by a relatively small cohort for an antibody study. The healthy controls were not perfectly matched with the study group. In addition, the lack of a validated, standardized, commercial assay for vitamin D antibodies was a major limitation. Therefore, we determined normal values based on differences between SSc patients and controls. Moreover, a single measurement from one blood sample is another limitation, which is common in antibody studies due to the cost of the assay. In addition, vitamin D measurements were not part of the study. Reliable data on oral intake of milk products, vitamin D supplementation and sun exposure among the healthy controls were lacking.

CONCLUSIONS

To our knowledge this is the first report of these novel anti-vitamin D antibodies in SSc patients. Our study is also innovative because it includes measurement of antibodies for both vitamin D metabolites and subtype antibodies analysis. We showed that SSc patients have higher levels of IgM anti-25(OH) D antibodies. Our results raise questions about a possible additional immunogenic role of both vitamin D metabolites. The observation that vitamin D antibodies are more frequent in SSc patients can also aid in differentiating scleroderma patients from healthy controls. Further studies with a larger cohort are required to confirm these findings and to examine the utility of these novel anti-vitamin D antibodies as a diagnostic and prognostic marker in SSc patients.

Correspondence
Dr. P. Rotman-Pikielny
Dept. of Medicine E, Meir Medical Center, Kfar Saba 44281, Israel
Phone: (972-9) 747-2671
Fax: (972-9) 744-0085
email: pnina.rotman@clalit.org.il

References

1. Linker-Israeli M, Elstner E, Klinenberg JR, Wallace DJ, Koeffler HP. Vitamin D(3) and its synthetic analogs inhibit the spontaneous in vitro immunoglobulin production by SLE-derived PBMC. *Clin Immunol* 2001; 99 (1): 82-93.

2. Borgogni E, Sarchielli E, Sottili M, et al. Elocalcitol inhibits inflammatory responses in human thyroid cells and T cells. *Endocrinology* 2008; 149 (7): 3626-34.
3. Daniel C, Sartory NA, Zahn N, Radeke HH, Stein JM. Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th17 to a Th2 and regulatory T cell profile. *J Pharmacol Exp Ther* 2008; 324 (1): 23-33.
4. Viswanath V, Phiske MM, Gopalani VV. Systemic sclerosis: current concepts in pathogenesis and therapeutic aspects of dermatological manifestations. *Indian J Dermatol* 2013; 58 (4): 255-68.
5. Hinchcliff M, Varga J. Systemic sclerosis/scleroderma: a treatable multisystem disease. *Am Fam Physician* 2008; 78 (8): 961-8.
6. Arnson Y, Amital H, Shoenfeld Y. Vitamin D and autoimmunity: new aetiological and therapeutic considerations. *Ann Rheum Dis* 2007; 66 (9): 1137-42.
7. Cantorna MT, Mahon BD. Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. *Exp Biol Med (Maywood)* 2004; 229 (11): 1136-42.
8. Zold E, Szodoray P, Gaal J, et al. Vitamin D deficiency in undifferentiated connective tissue disease. *Arthritis Res Ther* 2008; 10 (5): R123.
9. Huisman AM, White KP, Algra A, et al. Vitamin D levels in women with systemic lupus erythematosus and fibromyalgia. *J Rheumatol* 2001; 28 (11): 2535-9.
10. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; 357 (3): 266-81.
11. Abe J, Nakamura K, Takita Y, Nakano T, Irie H, Nishii Y. Prevention of immunological disorders in MRL/l mice by a new synthetic analogue of vitamin D3: 22-oxa-1 alpha,25-dihydroxyvitamin D3. *J Nutr Sci Vitaminol (Tokyo)* 1990; 36 (1): 21-31.
12. Antico A, Tampoia M, Tozzoli R, Bizzaro N. Can supplementation with vitamin D reduce the risk or modify the course of autoimmune diseases? A systematic review of the literature. *Autoimmun Rev* 2012; 12 (2): 127-36.
13. Calzolari G, Data V, Carignola R, Angeli A. Hypovitaminosis D in systemic sclerosis [Letter]. *J Rheumatol* 2009; 36 (12): 2844, author reply 2845.
14. Vacca A, Cormier C, Piras M, Mathieu A, Kahan A, Allanore Y. Vitamin D deficiency and insufficiency in 2 independent cohorts of patients with systemic sclerosis. *J Rheumatol* 2009; 36 (9): 1924-9.
15. Caramaschi P, Dalla Gassa A, Ruzzenente O, et al. Very low levels of vitamin D in systemic sclerosis patients. *Clin Rheumatol* 2010; 29 (12): 1419-25.
16. Serup J, Hagdrup H. Vitamin D metabolites in generalized scleroderma. Evidence of a normal cutaneous and intestinal supply with vitamin D. *Acta Derm Venereol* 1985; 65 (4): 343-5.
17. Arnson Y, Amital H, Agmon-Levin N, et al. Serum 25-OH vitamin D concen–trations are linked with various clinical aspects in patients with systemic sclerosis: a retrospective cohort study and review of the literature. *Autoimmun Rev* 2011; 10 (8): 490-4.
18. Belloli L, Ughi N, Marasini B. Vitamin D in systemic sclerosis. *Clin Rheumatol* 2011; 30 (1): 145-6.
19. Gambichler T, Chrobok I, Höxtermann S, Kreuter A. Significantly decreased serum 25-hydroxyvitamin d levels in a large German systemic sclerosis cohort [Letter]. *J Rheumatol* 2011; 38 (11): 2492-3, author reply 2494.
20. Carvalho JF, Blank M, Kiss E, Tarr T, Amital H, Shoenfeld Y. Anti-vitamin D, vitamin D in SLE: preliminary results. *Ann N Y Acad Sci* 2007; 1109: 550-7.
21. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. *Arthritis Rheum* 1980; 23 (5): 581-90.
22. Medsger TA Jr, Silman AJ, Steen VD, et al. A disease severity scale for systemic sclerosis: development and testing. *J Rheumatol* 1999; 26 (10): 2159-67.
23. Lemire JM, Ince A, Takashima M. 1,25-Dihydroxyvitamin D3 attenuates the expression of experimental murine lupus of MRL/l mice. *Autoimmunity* 1992; 12 (2): 143-8.
24. Larsson P, Mattsson L, Klareskog L, Johnsson C. A vitamin D analogue (MC 1288) has immunomodulatory properties and suppresses collagen-induced arthritis (CIA) without causing hypercalcaemia. *Clin Exp Immunol* 1998; 114 (2): 277-83.
25. Handono K. Vitamin D Serum level and disease activity in patients with systemic lupus erythematosus. *Int J Pharm Sci Inven* 2013; 2 (2): 35-40.

Capsule

A conserved response for tissue repair

Upon injury or infection, the body releases chemicals that trigger tissue repair and pathogen clearance. Because the medical community needs new therapeutic leads in this era of growing antibiotic resistance, identifying these molecules is a high priority. Dalli and team looked for these factors in mice infected with self-resolving *Escherichia coli*, in human breast milk, and in regenerating planaria. They identified two related molecules, conserved across these organisms, which promoted pathogen clearance, reduced inflammation, and accelerated tissue regeneration. Scientists will need to carry out further studies to determine whether these chemicals have similar properties in humans.

Proc Natl Acad Sci USA 2014;10.1073/pnas.1415006111

Eitan Israeli

Capsule

Detection of self-reactive CD8+ T cells with an anergic phenotype in healthy individuals

Immunological tolerance to self requires naturally occurring regulatory T (T_{reg}) cells. Yet how they stably control autoimmune T cells remains obscure. Maeda et al. show that Treg cells can render self-reactive human CD8+ T cells anergic (i.e., hypoproliferative and cytokine hypoproducing upon antigen restimulation) in vitro, likely by controlling the co-stimulatory function of antigen-presenting cells. Anergic T cells were naïve in phenotype, lower than activated T cells in T cell receptor affinity for cognate antigen, and expressed several co-inhibitory molecules, including cytotoxic T lymphocyte-associated antigen-4 (CTLA-4). Using these criteria, they detected in healthy individuals anergic T cells reactive with a skin antigen targeted in the autoimmune disease vitiligo. Collectively, their results suggest that T_{reg} cell-mediated induction of anergy in autoimmune T cells is important for maintaining self-tolerance.

Science 2014; 346: 1536

Eitan Israeli

Small-Medium Vessel Vasculitides: is the Complement System a Potential Forgotten Target?

Eleonora Ballanti MD, Maria S. Chimenti MD PhD and Roberto Perricone MD

Unit of Rheumatology, Allergology and Clinical Immunology, Department of Internal Medicine, University of Rome Tor Vergata, Rome, Italy

ABSTRACT: Systemic vasculitides are a group of uncommon diseases characterized by blood vessel inflammation. The complement system is involved in the pathogenesis and clinical manifestations of several autoimmune diseases, including systemic vasculitides. This enzymatic system is a component of the innate immune system. Its main function was initially believed to be limited to the recognition and elimination of pathogens, but research in recent years has demonstrated the important role that complement proteins play in modulating adaptive immunity and in bridging innate and adaptive responses. Its activation is also critical for the development of T cell immunity and natural antibodies as well as for the regulation of autoreactive B cells. In systemic vasculitides, particularly small-medium vessel vasculitides, the complement system has been shown to contribute to the development of inflammatory damage. In view of these crucial functions, the complement system represents an attractive therapeutic target for a wide range of diseases, including vasculitic disorders.

IMAJ 2015; 17: 85–92

KEY WORDS: complement system, vasculitides, anti-complement therapy

For Editorial see page 110

The systemic vasculitides are a group of uncommon diseases characterized by blood vessel inflammation. The reported annual incidence of these relatively uncommon disorders is 40–54 cases per 1 million persons [1]. The incidence appears to be affected by geography, age, and seasonal factors. Vasculitis may be limited to skin or other organs, or it may be a systemic disorder with multiple manifestations [2].

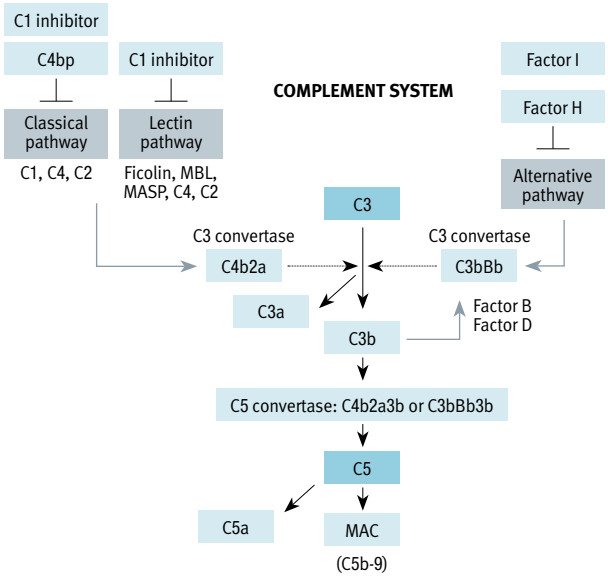
Complement is a part of the innate immune response, involved in adaptive response and crucial functions of the immune system

Since there are no diagnostic criteria for the primary systemic vasculitides, physicians must rely on experience and disease definitions. Recently, the Chapel Hill Consensus Conference (CHCC 2012) nomenclature defined 10 primary vasculitides based on vessel size (large, medium, small). In particular, the CHCC 2012 adopted the recommendations of the American College of Rheumatology, the American Society of Nephrology, and the European League Against Rheumatism (EULAR) to replace 'Wegener's granulomatosis' with 'granulomatosis with polyangiitis' (GPA) [3]. Churg-Strauss syndrome was replaced with the term 'eosinophilic granulomatosis with polyangiitis' (EGPA) to provide continuity within the anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis group. Henoch-Schönlein purpura was now named 'IgA vasculitis' (IgAV) based on the established evidence that the defining pathological feature is abnormal immunoglobulin A deposition in vessel walls [3].

The pathogenesis of these diseases is characterized by the presence of leukocytes in the vessel associated with the production of wall inflammatory damage. Depending on the specific vasculitic disorder, affected vessels vary in size, type and location. Many vasculitic disorders are caused by immune complexes (IC), which implies that the activation of the complement system is a pathogenic mechanism. The complement system is a component of the innate immune system, which includes physical, cellular and chemical elements [4]. Its immunoregulatory functions were recently demonstrated: i.e., the complement proteins play an important role in modulating both adaptive and innate immunity responses. This enzymatic system comprises more than 30 plasma and membrane-bound proteins [4]. The activation of these proteins occurs through three pathways: classical, alternative, and lectin. All three pathways are activated according to a cascade system, with activation of one factor leading to the activation of the next [Figure 1]. The contribution of complement to the development of inflammatory damage was confirmed through a series of elegant studies, with a body of accumulated data demonstrating that the activation of the complement system is also critical for the development of T cell immunity, the development of natural antibodies, and the regulation of autoreactive B cells [4,5].

The diagnosis of vasculitides relies on the recognition of a compatible clinical presentation supported by specific laboratory or imaging tests and confirmatory histology. ANCA testing has been especially valuable in defining a subgroup of small vessel vasculitides. Treatment is based on clinical manifestations and level of organ involvement. Evidence on traditional immunosuppressive therapies, such as methotrexate and cyclo-

Figure 1. Activation of the complement system. Activation of the complement system occurs through three pathways: classical, alternative, and lectin. All the pathways lead to the cleavage of C3 and finally converge at the activation of C5, with possible formation of the membrane attack complex (MAC). To avoid excess complement activation the complement system is tightly controlled by several regulatory proteins (including C1 inhibitor, C4 binding protein, Factor I, Factor H shown in the figure) that act at different points of complement cascade



phosphamide, has been collected. Newer approaches, e.g., the use of anti-tumor necrosis factor-alpha (anti-TNFa) or anti-B cell therapies, are being tried in refractory cases [6].

ANCA-ASSOCIATED VASCULITIDES

The ANCA-associated vasculitides (AAV) are a group of diseases with predominant inflammation of small vessels and the presence of detectable ANCA in serum. Due to common features, it is accepted that AAV share common pathogenic mechanisms. A new nomenclature has recently been introduced, with AAV including GPA, microscopic polyangiitis (MPA), EGPA and renal limited vasculitis [2]. Research on AAV has shown significant advances in the last two decades. Environmental toxins have been implicated, such as silica, a potential activator of the inflammasome complex that, among other functions, generates the active cytokine interleukin (IL)-1 [7]. Infections due to several microbes including *Staphylococcus aureus* have been linked repeatedly to the pathogenesis of vasculitis. Toxin from *Staph. aureus* is a potent activator of the NLRP3 inflammasome, suggesting potential links between different environmental agents and their pro-inflammatory effects in vasculitis. Infection has also been implicated in the formation of ANCA, specifically lysosomal-associated membrane protein 2 (LAMP-2) [8]. Homology between the middle portion of the complementary proteinase 3 (cPR3) sequence and *Staph. aureus* proteins may induce anti-complementary PR3 antibodies that, in turn, induce anti-PR3 antibodies via an anti-idiotypic response and ANCA vasculitis. These small vessel vasculitides are characterized by necrotizing inflammation of the vessel wall, particularly of small arteries, arterioles, capillaries and venules, in conjunction with the presence of ANCAs [9]. ANCAs are autoantibodies produced in response to neutrophil cytoplasmic enzymes and represent a useful marker for the diagnosis of systemic vasculitis such as GPA, MPA and EGPA. These autoantibodies can be detected using immunofluorescence assay (IFA) and are classified according to dyeing patterns into cytoplasmic-ANCA (c-ANCA) or perinuclear-ANCA (p-ANCA). Enzyme-linked immunosorbent assay (ELISA), currently the most popular assay, can detect myeloperoxidase (MPO)- and proteinase 3 (PR3)-ANCAs [10].

The majority of p-ANCAs and c-ANCAs have been proven to be equivalent to MPO- and PR3-ANCAs, respectively. Patients with MPA (50–80%) and EGPA (2–50%) are positive for p-ANCA, and those with GPA (75–90%) are positive for c-ANCA. However, a small percentage (5–20%) of GPA patients may have p-ANCA, while positivity for c-ANCA may be observed in 10–50% of MPA and 3–35% of EGPA patients [11]. In patients with active systemic GPA, MPA or EGPA, ANCAs are reported to be positive in over 80%, 70% or 50% of cases, respectively [10]. In particular, however, some patients are negative for both MPO- and PR3-ANCAs while other clinical findings strongly suggest the presence of systemic vasculitis, which makes diagnosing systemic vasculitis insidious [10]. In addition to the role of autoantibodies, T cells also participate in disease mechanisms. T cells are localized in inflammatory lesions related to AAV, and granuloma formation is considered T cell dependent [12]. In this respect, alterations of T cell immunity such an abnormal T cell activation and dysfunction of T regulatory cells have been described [13].

Complement participates in the pathogenesis of several small-medium vessel vasculitides including ANCA-associated vasculitides, cryoglobulinemia, urticarial vasculitides and IgA vasculitis

In AAVs, the adaptive immune response, embodied by the ANCAs, interacts with innate immunity, especially with neutrophils and the complement system. Together, these elements target the endothelium, causing necrotizing vasculitis [14]. Among the different soluble mediators involved in ANCA vasculitis, components of the alternative complement pathway are emerging as forerunners since the elegant demonstration of protection from disease in C5 and factor-B knockout mice. In vitro data demonstrate that in AAVs the complement system

constitutes an amplification loop for ANCA-induced neutrophil activation. Schreiber et al. [15] showed that supernatants from ANCA-activated neutrophils activate the complement system via the alternative pathway, resulting in the production of C5a, among others [15]. C5a was able to prime neutrophils for ANCA-induced activation, and blocking the C5a receptor on neutrophils abrogated this process.

Murine models have shown that complement depletion prevented MPO-ANCA glomerulonephritis, and mice deficient in C5 or complement factor B did not develop pauci-immune necrotizing crescentic glomerulonephritis, characterized by the relative lack of immunoglobulin and complement deposition on kidney biopsy immunofluorescence [15]. In agreement with these experimental data, the complement components MAC (membrane attack complex), C3d and factor B could be detected in diseased glomeruli of patients with AAVs. The alternative pathway component factor B co-localized with MAC, but the classical pathway component C4d could not be detected [16]. Interestingly, AAV in the kidney is not quite so pauci-immune as once thought. Although immune complexes containing IgG or IgA are generally lacking in the classic lesions of ANCA-associated glomerulonephritis, non-specific IgM deposits and certainly complement deposition are often present. The anaphylatoxin C5a not only primes neutrophils for an ANCA-induced respiratory burst, but C5a receptor-deficient animals are protected against development of glomerulonephritis [15]. AAV are not associated with hypocomplementemia. Moreover, serum C3 and C4 levels are not a sensitive indicator of complement involvement because certain forms of glomerulonephritis and vasculitis that have substantial vascular deposits of complement are not associated with hypcomplementemia. According to these findings, compounds interfering with the complement cascade should be explored as therapeutic options for AAV. The pathogenesis of AAVs is summarized in Figure 2.

In vasculitic disorders, the complement system could be a possible target for therapeutic purposes

CRYOGLOBULINEMIC VASCULITIS

Cryoglobulins (CG) are an abnormal group of serum proteins that share the common property of reversible precipitation at low temperatures. The majority of CG are either intact monoclonal immunoglobulins or immune complexes in which one component, usually IgM, exhibits antibody activity to IgG. The latter are known as mixed CG [17]. Cold-precipitate, monoclonal or polyclonal immunoglobulins can occur in a variety of diseases, including plasma cell or lymphoid neoplasms, chronic infection, and inflammatory diseases. With the discovery of the hepatitis C virus (HCV), it became established that the majority of cases of cryoglobulinemia are related to HCV infection [Figure 3]. Essential mixed cryoglobulinemia demonstrates a prominent association with HCV infection (> 90%). It is a systemic vasculitis (leukocytoclastic vasculitis) affecting cutaneous vessels and multiple visceral organs [18].

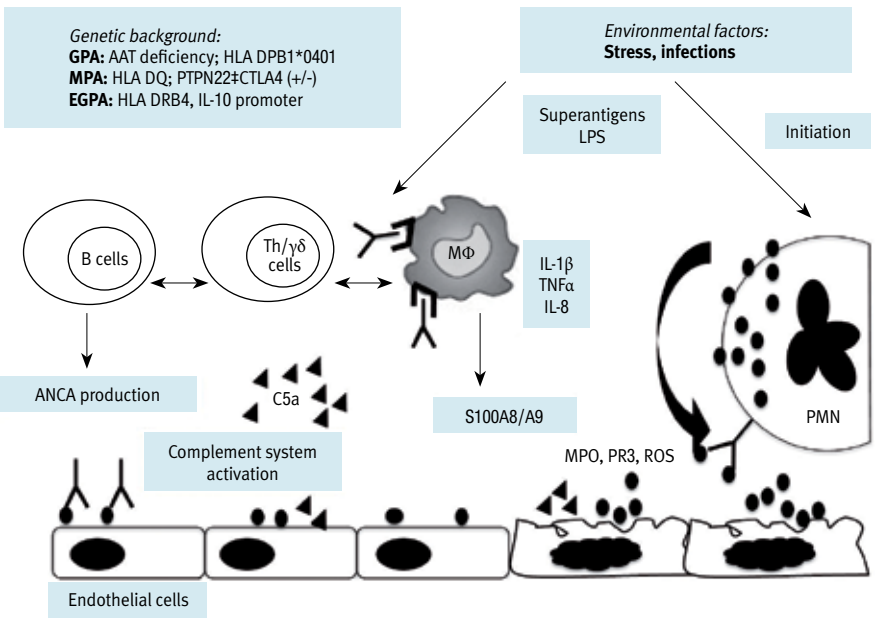
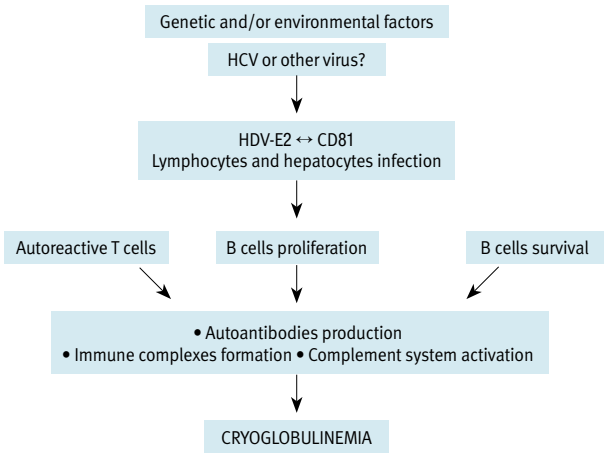


Figure 2. Pathogenic mechanisms of ANCA-associated vasculitis. Proposed model for anti-neutrophil cytoplasmic autoantibody (ANCA)-mediated vascular inflammation through alternative pathway complement activation. Neutrophils are primed by cytokines to express ANCA antigens (myeloperoxidase and proteinase-3) at the cell surface and then adhere to susceptible endothelium, and ANCA antibodies interact with the ANCA antigens, resulting in neutrophil activation. The ANCA-activated neutrophils release factors (properdin, factor B, proteases, ROS and MPO) that can directly damage the endothelium but also activate the alternative complement pathway with the generation of the powerful neutrophil chemoattractant C5a. This complement activation amplifies neutrophil influx and activation eventually culminating in the severe necrotizing inflammation of the vessel wall

ANCA = anti-neutrophil cytoplasmic autoantibody, AAT = alpha-1 anti-trypsin, PTPN22 = protein tyrosine phosphatase non-receptor type 22, CTLA4 = cytotoxic T lymphocyte antigen 4, LPS = lipopolysaccharide, B = B cells, Th = T helper, MΦ = monocytes/macrophages, IL-1β = interleukine 1-beta, TNFα = tumor necrosis factor-alpha, PMN = polymorphonuclear cells, MPO= myeloperoxidase, PR3 = proteinase 3, ROS = oxygen radicals

Figure 3. Pathogenesis of essential mixed cryoglobulinemia (ECM). In a genetically predisposed patient, a known antigenic stimulus (e.g., HCV) or unknown stimuli act on immune system cells. HCV is the major pathogenetic factor involved in EMC. It can stimulate the immune system as lymphotropic virus or as B cell activator. The result is a polyclonal activation of B cells and the production of autoantibodies and immune complexes. Those classically precipitate at low temperatures and are called cryoprecipitates. The cause of the precipitation could be structural modification of immunoglobulin variable light and heavy chains and a reduction in sialic acid or galactose in the constant fragment (Fc). HCV-E2 envelope protein can bind the CD81 expressed on B cells and hepatocytes. Interaction between CD81 and HCV-E2 increases the frequency of VDJ rearrangement and bcl-2 activation, with inhibition of B cell apoptosis, increased survival and autoantibody production. IC deposition as well as complement system activation leads to wall vessel damage. The histological outcome is a leukocytoclastic vasculitis [16,17]



Monoclonal CG are usually associated with hematological disorders, whereas mixed CG are found in many infectious and systemic disorders. The classical pathway of the complement system is usually activated in both essential and secondary cryoglobulinemia. Decreased C4 and C2 levels are observed together with slightly altered C3 levels in the disease course. Late complement components are insignificantly affected, although modest elevations have been reported. Diminished serum levels of complement components may reflect ongoing consumption by CG-containing IC [4]. Cryoglobulinemic vasculitis can be characterized by palpable purpura, arthritis, weakness, neuropathy and glomerulonephritis [18,19]. Although the presence of glomerulonephritis is associated with an overall poor prognosis, progression to end-stage renal failure is uncommon. Besides the detection of serum cryoglobulin itself, low C4 is often proposed as a serologic criterion for the diagnosis and classification of mixed cryoglobulinemia and may provide surrogate evidence of the presence of cryoglobulinemia. Moreover, HCV-mixed cryoglobulinemic patients who respond to treatment show a decrease in serum cryoglobulin levels and an increase in C4 serum levels [20].

URTICARIAL VASCULITIS

Urticarial vasculitis (UV) is a pathologic entity characterized by recurrent episodes of urticaria, with the histopathologic features of leukocytoclastic vasculitis, mainly involving capillaries and venules. UV may be idiopathic or associated with chronic diseases such as infections, hematologic diseases, connective tissue diseases, particularly systemic lupus erythematosus (SLE) and Sjögren's syndrome, and malignancy. Differing from urticaria, UV lesions last longer than 24 hours and may leave residual hyperpigmentation [21]. UV is an immune complex-mediated disease. Circulating antigen-antibody complexes initially form in the blood and then deposit in the vessel walls. Complement is activated through the classical pathway and C3a and C5a are generated. Based on the level of complement activation and consumption, several syndromes are described that differ only in severity and prognosis [22]. Normocomplementemic urticarial vasculitis (NUV) is a mild disorder with prevalent cutaneous involvement and normal serum complement levels. Conversely, hypocomplementemic urticarial vasculitis (HUV) is a multi-organ disease with possible gastrointestinal, musculoskeletal, renal, pulmonary and ophthalmologic involvement, and low level of serum complement proteins. The antigen involved in immune complex formation may be autologous (self-antigen) or exogenous (infective agent, drug). IgG autoantibodies to the collagen-like region of C1q in serum from HUV patients have been described frequently. These autoantibodies may account for the lowering of serum C1q in this syndrome and can be found in patients with other diseases, especially autoimmune conditions. In particular, anti-C1q has been detected in SLE (61%), rheumatoid arthritis (20%), scleroderma (15%), Sjögren's syndrome (15%), mixed connective tissue disease (15%), and even in chronic HCV infection (38%) [22]. Because of the presence of these autoantibodies in a large percentage of patients with both UV and SLE, some authors have hypothesized that these diseases could represent different expressions of the same autoimmune disorder. In SLE patients, anti-C1q levels correlate with the severity of skin and renal involvement. In UV, their pathogenic significance is not clearly elucidated but seems to be associated even with lung disease [23]. The diagnosis of UV is suggested by a typical clinical presentation and supporting laboratory tests (low levels of C3, C4 and C1q, presence of anti-C1q antibodies, high erythrocyte sedimentation rate), but is always confirmed by skin biopsy.

OTHER SMALL VESSEL VASCULITIDES

IgA vasculitis (IgAV) and IgA nephropathy (IgAN) are currently considered related diseases. Both diseases show similar

histological patterns and IgA abnormalities. The common clinical feature of IgAN is an indolent progressive disease with slowly increasing proteinuria and loss of renal function associated with episodes of macroscopic hematuria in half the patients. In the majority of patients, Henoch-Schönlein purpura nephritis (HSPN) is characterized by acute onset followed by full recovery [24]. The activation of the complement pathway is likely to be involved in the pathophysiology of glomerular lesions. Glomerular deposition of MBL, L-ficolin, MASP and C4d are observed in the vast majority of patients with HSPN and IgAN. These findings, together with the absence of C1q, are supportive of the predominant activation of the complement system by the lectin pathways as a pathophysiologic mechanism. The deposition of complement fragments derived from the activation of the lectin pathway has been shown to be associated with a higher degree of proteinuria and hematuria as well as with more severe histological lesions in both HSPN and IgAN patients [24,25]. These findings emphasize the need for further studies to assess the potential significance of measuring blood and urinary complement activation products and MAC to evaluate disease activity and potential therapeutic targets.

TREATMENT OF SMALL AND MEDIUM VESSEL VASCULITIDES

The treatment of vasculitides includes three phases: induction of remission, maintenance, and treatment of relapse. Treatment strategies for main small and medium vessel vasculitides are summarized in Table 1. Remission should be induced rapidly, balancing potential target organ damage against drug toxicity. Maintenance with immunosuppression should limit the amount of corticosteroid use and prevent relapse. Concomitant medication is used to treat or prevent adverse events from immunosuppressive treatment.

A combination of intravenous or oral cyclophosphamide (CYC) and glucocorticoids is recommended for inducing remission of generalized primary small and medium vessel vasculitis [6]. EULAR recommends that patients with ANCA-associated vasculitis be categorized according to different levels of severity to assist in treatment decisions [6]. In particular, patients with different levels of disease severity respond to different treatment protocols. The severity and extent of the disease classify patients into five groups: localized, early systemic, generalized, severe, and refractory. A combination of oral or parenteral methotrexate (MTX) and glucocorticoid can be used as a less toxic alternative to CYC for the induction of remission in non-organ-threatening or non-life-threatening ANCA-associated vasculitis [26]. Remission maintenance therapy consists of a combination of low dose glucocorticoid therapy with one of the following: azathioprine (AZA), leflunomide (LEF), or MTX, which were selected on the basis of randomized controlled trials [27]. In fact, the toxicity of long-term CYC makes it an unattractive option for this purpose. Remission maintenance therapy should be continued for at least 18–24 months as early cessation of therapy is associated with an increased risk of relapse [27].

Table 1. Management of ANCA-associated vasculitides, cryoglobulinemic vasculitis and urticarial vasculitis

Disease	Disease stage	Treatment
ANCA-associated vasculitides	Induction of remission	
	Localized	Co-trimoxazole for GPA
	Early systemic	MTX+GC
	Generalized	CYC+GC or RTX+GC
	Severe	As generalized + PE
	Refractory	IVIG, RTX, IFX, ATG, MMF, 15-deoxyspergualin
	Maintenance of remission	AZA or MTX or LEF or MMF + GC
Urticarial vasculitis	Cutaneous	Antihistamines, colchicine, dapsone, hydroxychloroquine, indomethacin, GC
	Extracutaneous or chronic cutaneous	GC
	Severe systemic or chronic GC resistant	AZA, CYC, CYA, MMF
Cryoglobulinemic vasculitis	Asymptomatic	Monitoring
	Mild-moderate	Low-medium dose GC ± LAC ± other symptomatic drugs
	Moderate-severe	Peg-IFN+Riba (in HCV patients) Low-medium dose GC
	Severe-rapidly progressive	CYC (or RTX) + GC + PE (± antiviral agents)

Therapeutic strategy should be established on the basis of the activity/severity of diseases and adjusted for the single patient

GPA = granulomatosis with polyangiitis, GC = glucocorticoids, CYC = cyclophosphamide, CYA = cyclosporine A, MTX = methotrexate, RTX = rituximab, PE = plasma exchange, IVIG = intravenous immunoglobulin, IFX = infliximab, ATG = anti-thymocyte globulin, MMF = mycophenolate mofetil, AZA = azathioprine, LEF = leflunomide, LAC = low antigen content diet, Peg-IFN = pegylated interferon, Riba = ribavirin

Alternative immunomodulatory therapy choices should be considered for patients who do not achieve remission, or relapse, on maximal doses of standard therapy. Treatment options in refractory disease include rituximab (RTX) and TNFa antagonists as well as intravenous immunoglobulins (IVIG), deoxyspergualin and anti-thymocyte globulin (ATG) [28,29]. In particular, RTX has emerged as an alternative for CYC in the remission induction of patients with generalized and severe disease [30].

Plasma exchange is reserved for selected patients with rapidly progressive severe renal disease in order to improve renal survival; it proved superior to methylprednisolone pulses in a controlled trial [31].

Treatment of cryoglobulinemic vasculitis should be established on the basis of the activity/severity of disease and adjusted for the individual patient. In the presence of active chronic hepatitis, eradicating the HCV infection should be attempted; severe, rapidly progressive disease must be treated with steroids and immunosuppressive agents, as in other systemic vasculitides. In HCV patients with acute and severe manifestations, some authors suggest combined or sequential therapy with RTX and antiviral agents [32].

Patients with urticarial vasculitis usually require antihistamines for the symptomatic relief of the pruritus. Systemic glucocorticoids are the mainstay treatment at the initial dose of 0.5–1.0 mg/kg per day. Indomethacin, colchicine, dapsone and hydroxychloroquine have been shown to be effective and to minimize side effects due to steroids. For severe or refractory HUVS, high doses of glucocorticoids may be needed, associated or not with other agents such as AZA, CYC, mycophenolate mofetil (MMF), cyclosporine A (CYA), and MTX. Plasma exchange has also been used [33]. The principles of treatment for UV and cryoglobulinemia are presented in Table 1.

Treatment of IgAV needs to balance the risk of chronic kidney disease (CKD) versus the risk and cost of the treatment. In the case of minimal renal symptoms such as microhematuria, short duration macroscopic hematuria or mild proteinuria, patients may not need treatment because of the low CKD risk. In the case of nephritic syndrome or nephrotic proteinuria, even without clinical nephrotic syndrome, treatment with glucocorticoids might be recommended [24]. The addition of immunosuppressive drugs might be considered when improvement is delayed or in situations of higher risk, even if results from randomized clinical trials with CYC were not encouraging. Plasma exchange should be considered promptly in patients in whom steroids and immunosuppressive drugs are not effective, or even initially when nephritic and nephrotic syndromes are associated with a high percentage of crescents [24].

THERAPIES TARGETING COMPLEMENT IN SMALL–MEDIUM VESSEL VASCULITIDES

A link between the complement system and tissue damage during ischemic, inflammatory and autoimmune diseases is increasingly recognized. This makes the complement system an attractive target for the treatment of a wide range of diseases, such as connective tissue diseases, glomerulonephritis, myocarditis, multiple sclerosis, type I diabetes mellitus, asthma, myocardial infarction, paroxysmal nocturnal hemoglobinuria, and vasculitides [34]. However, side effects potentially associated with the modulation of complement system in the long term must be considered. Prolonged systemic suppression may, for instance, increase the susceptibility to bacterial infections [35].

Several compounds interfering with the complement system cascade have been studied in experimental models for autoimmune diseases. The main therapeutic strategies are inhibition of complement activation components, inhibition of complement receptors, and inhibition of MAC [34].

Few studies on the use of anti-complement agents in vasculitic disorders have been conducted. Inhibitors of the C5a receptor (C5aR), a receptor distributed to a variety of immune cells including neutrophils, monocytes and dendritic cells, are in the early phases of investigation.

In 2012, Bekker et al. [36] presented a phase II clinical trial of CCX168 (a C5aR Inhibitor) in patients with a clinical diagnosis of granulomatosis with polyangiitis, microscopic polyangiitis or renal-limited vasculitis (CLEAR: C5aR inhibitor on Leukocytes Exploratory ANCA-associated Renal vasculitis). This multinational, randomized, double-blind, placebo-controlled clinical trial is being performed in 60 subjects in Europe. The primary objective of this clinical trial is to assess the safety and tolerability of CCX168. Secondary objectives include assessment of the feasibility of reducing or eliminating corticosteroids in the treatment of ANCA-associated renal vasculitis without the need for rescue corticosteroids, and evaluation of the effect of CCX168 treatment on renal function and ANCA disease. At the same meeting Dairaghi and colleagues [37] presented both animal and human phase I data on CCX168. This compound blocked C5aR activation following oral dosing in both humans and C5aR-humanized mice (hC5aR knockin mice). In the anti-MPO mouse model, CCX168 at a dose of 30 mg/kg achieved near-maximal inhibition of glomerulonephritis, with significant reduction of glomerular crescent formation and glomerular necrosis. The therapeutic benefit was associated with C5aR blockade on blood neutrophils ranging from 87 to 93%. In the phase I human clinical study, CCX168 was well tolerated, with excellent oral bioavailability and dose proportional increases in systemic exposure. Analysis of the human data revealed that 30 mg CCX168 twice daily provided excellent coverage of C5aR on human blood leukocytes. Further multicenter studies are needed to better define the long-term outcomes and safety profile of these new therapeutic agents. Only two complement modulators have been approved for use in humans to date: one is eculizumab, which binds to the complement protein C5, inhibiting its cleavage, and is indicated for the treatment of paroxysmal nocturnal hemoglobinuria [38]; the other is plasma-derived C1 esterase inhibitor, indicated for the treatment of hereditary angioedema [39].

Modulation of the complement system is one of the benefits associated with the use of high dose intravenous immunoglobulins (IVIg) in autoimmune conditions. The complement system-modulating effect exhibited by IVIg can be explained by several mechanisms. First, activated C3 and C4 may bind to immunoglobulin molecules, which then serve as scavengers, hence avoiding in situ deposition of these fragments [28]. Second, C1q may bind to immunoglobulin, leading to a deviation of C1 binding from its target to the IVIg. Third, IVIg

may enhance the inactivation of C3 in complex with immunoglobulins and thus down-regulate C3 convertase activity [39]. Finally, IVIg are able to evoke a mild and controlled activation of the complement system. This is not harmful and may reduce the pathological activation observed in the pathogenesis of autoimmune disease [28]. A broadly applicable anti-C therapeutic agent to treat acute and chronic conditions should be inexpensive, highly specific, have a long plasma half-life or be active orally, and able to block the pathological activation of the complement system while causing minimal disruption of the systemic complement function [40].

None of the currently available agents meet these requirements, but data derived from preclinical studies and initial clinical trials suggest that complement modulation could become an important therapeutic strategy in autoimmune conditions, including vasculitides, in the next decades. Based on the fact that AAV are infrequent conditions, further multicenter trials are needed to explore these potential new therapeutic targets.

CONCLUSIONS

Systemic vasculitides, in particular small-medium vessel vasculitides, are characterized by inflammation of blood vessel walls, IC deposition and activation of complement system. We have reviewed the contribution of complement system in the pathogenesis of these diseases. In particular, a close interaction has been demonstrated between complement system fragments, ANCA and systemic inflammation. The activation of complement system is crucial for the initiation, maintenance and perpetuation of the inflammatory process. Specific therapeutic regimens, including immunosuppressive drugs used to treat small vessel vasculitides significantly improves the prognosis and quality of life of affected patients but are known to be hampered by serious side effects and toxicity. According to the pathogenesis of systemic vasculitides, compounds interfering with the complement cascade are being explored as new therapeutic options in autoimmune diseases including ANCA-associated vasculitides and could represent a promising strategy in the near future.

Correspondence
Dr. M.S. Chimenti
Unit of Rheumatology, Allergology and Clinical Immunology, Dept. of Internal Medicine, University of Rome Tor Vergata, Via Montpellier 1, 00133, Rome, Italy
Phone: (39-6) 725-96287
Fax: (39-6) 209-00358
email: maria.sole.chimenti@uniroma2.it

References

1. Reinhold-Keller E, Herlyn K, Wagner-Bastmeyer R, Gross WL. Stable incidence of primary systemic vasculitides over five years: results from the German vasculitis register. *Arthritis Rheum* 2005; 53: 93-9.
2. Waller R, Ahmed A, Patel I, Luqmani R. Update on the classification of vasculitis. *Best Pract Res Clin Rheumatol* 2013; 27: 3-17.
3. Jennette JC. Overview of the 2012 revised International Chapel Hill Consensus Conference nomenclature of vasculitides. *Clin Exp Nephrol* 2013; 17: 603-6.
4. Ballanti E, Perricone C, Greco E, et al. Complement and autoimmunity. *Immunol Res* 2013; 56: 477-91.
5. Carroll MC. The role of complement in B cell activation and tolerance. *Adv Immunol* 2000; 74: 61-88.
6. Mukhtyar C, Guillevin L, Cid MC, et al. EULAR recommendations for the management of primary small and medium vessel vasculitis. *Ann Rheum Dis* 2009; 68: 310-17.
7. Franchi L, Eigenbrod T, Nunez G. Cutting edge: TNF-alpha mediates sensitization to ATP and silica via the NLRP3 inflammasome in the absence of microbial stimulation. *J Immunol* 2009; 183: 792-6.
8. Mariathasan S, Weiss DS, Newton K, et al. Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* 2006; 440: 228-32.
9. Tervaert JW, van der Woude FJ, Fauci AS, et al. Association between active Wegener's granulomatosis and anticytoplasmic antibodies. *Arch Intern Med* 1989; 149: 2461-5.
10. Brouwer E, Tervaert JW, Horst G, et al. Predominance of IgG1 and IgG4 subclasses of anti-neutrophil cytoplasmic autoantibodies (ANCA) in patients with Wegener's granulomatosis and clinically related disorders. *Clin Exp Immunol* 1991; 83: 379-86.
11. Langford CA. 15. Vasculitis. *J Allergy Clin Immunol* 2003; 111 (2 Suppl): S602-12.
12. Lamprecht P, Csernok E, Gross WL. Effector memory T cells as driving force of granuloma formation and autoimmunity in Wegener's granulomatosis. *J Intern Med* 2006; 260: 187-91.
13. Wilde B, Thewissen M, Damoiseaux J, et al. T cells in ANCA-associated vasculitis: what can we learn from lesional versus circulating T cells? *Arthritis Res Ther* 2010; 12: 204.
14. Kallenberg CG. Pathophysiology of ANCA-associated small vessel vasculitis. *Curr Rheumatol Rep* 2010; 12: 399-405.
15. Schreiber A, Xiao H, Jennette JC, Schneider W, Luft FC, Kettritz R. C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. *J Am Soc Nephrol* 2009; 20: 289-98.
16. Xiao H, Schreiber A, Heeringa P, Falk RJ, Jennette JC. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am J Pathol* 2007; 170: 52-64.
17. Lamprecht P, Gause A, Gross WL. Cryoglobulinemic vasculitis. *Arthritis Rheum* 1999; 42: 2507-16.
18. Cacoub P, Saadoun D. Hepatitis C virus infection induced vasculitis. *Clin Rev Allergy Immunol* 2008; 35: 30-9.
19. Tarantino A, Anelli A, Costantino A, De Vecchi A, Monti G, Massaro L. Serum complement pattern in essential mixed cryoglobulinaemia. *Clin Exp Immunol* 1978; 32: 77-85.
20. Sansonno D, De Re V, Lauletta G, Tucci FA, Boiocchi M, Dammacco F. Monoclonal antibody treatment of mixed cryoglobulinemia resistant to interferon alpha with an anti-CD20. *Blood* 2003; 101: 3818-26.
21. Grotz W, Baba HA, Becker JU, Baumgärtel MW. Hypocomplementemic urticarial vasculitis syndrome: an interdisciplinary challenge. *Dtsch Arztebl Int* 2009; 106: 756-63.
22. Chang S, Carr W. Urticarial vasculitis. *Allergy Asthma Proc* 2007; 28: 97-100.
23. Lienesch DW, Sherman KE, Metzger A, Shen GQ. Anti-Clq antibodies in patients with chronic hepatitis C infection. *Clin Exp Rheumatol* 2006; 24: 183-5.
24. Davin JC. Henoch-Schonlein purpura nephritis: pathophysiology, treatment, and future strategy. *Clin J Am Soc Nephrol* 2011; 6: 679-89.
25. Espinosa M, Ortega R, Gómez-Carrasco JM, et al. Mesangial C4d deposition: a new prognostic factor in IgA nephropathy. *Nephrol Dial Transplant* 2009; 24: 886-91.
26. de Groot K, Rasmussen N, Bacon PA, et al. Randomized trial of cyclophosphamide versus methotrexate for induction of remission in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum* 2005; 52: 2461-9.
27. Pagnoux C, Mahr A, Hamidou MA, et al. Azathioprine of methotrexate maintenance for ANCA-associated vasculitis. *N Engl J Med* 2008; 359: 2790-803.
28. Ballanti E, Di Muzio G, Novelli L, Perricone C, Perricone R. Churg-Strauss syndrome with neurologic manifestations: successful treatment with intravenous

immunoglobulins. *IMAJ* 2012; 14: 583-5.

29. Svetlicky N, Blank M, Zandman-Goddard G. The beneficial effects of intravenous immunoglobulin for antineutrophil cytoplasmic antibody-positive vasculitis. *IMAJ* 2012; 14: 568-9.
30. Polachek A, Caspi D, Elkayam O. Granulomatosis and polyangiitis: the rituximab option. *IMAJ* 2013; 15: 193-4.
31. Casian A. Plasma exchange for severe renal vasculitis: long-term follow-up of the MEPEX trial. *Clin Exp Immunol* 2011; 164 (Suppl 1): 52.
32. Saadoun D, Resche Rigon M, Sene D, et al. Rituximab plus Peg-interferon-alpha/ribavirin compared with Peg-interferon-alpha/ribavirin in hepatitis C-related mixed cryoglobulinemia sequential therapy with antiviral agents. *Blood* 2010; 116: 326-34.
33. Jara LJ, Navarro C, Medina G, Vera-Lastra O, Saavedra MA. Hypocomplementemic urticarial vasculitis syndrome. *Curr Rheumatol Rep* 2009; 11: 410-15.
34. Holers VM. The complement system as a therapeutic target in autoimmunity. *Clin Immunol* 2003; 107: 140-51.
35. Mizuno M. A review of current knowledge of the complement system and the therapeutic opportunities in inflammatory arthritis. *Curr Med Chem* 2006; 13: 1707-17.
36. Bekker P, Dairaghi D, Miao S, Powers JP, Jaen JC, Schall TJ. Oral C5a receptor antagonist CCX168 in a phase 2 Renal Association–European Dialysis and Transplant Association (ERA–EDTA) Congress, 2012, Paris.
37. Dairaghi D, Leleti M, Miao S, et al. Clinical dose selection of the C5a receptor antagonist CCX168 for the phase 2 ANCA-associated renal vasculitis clinical trial (the CLEAR trial), 49th European Renal Association–European Dialysis and Transplant Association (ERA–EDTA) Congress, 2012, Paris.
38. Parker CJ. Management of paroxysmal nocturnal hemoglobinuria in the era of complement inhibitory therapy. *Hematol Am Soc Hematol Educ Program* 2011; 2011: 21-9.
39. Cicardi M, Zanichelli A. Replacement therapy with C1 esterase inhibitors for hereditary angioedema. *Drugs Today* 2010; 46: 867-74.
40. Morgan BP, Harris CL. Complement therapeutics; history and current progress. *Mol Immunol* 2003; 40: 159-70.

Capsule

Autophagy is essential for effector CD8+ T cell survival and memory formation

The importance of autophagy in the generation of memory CD8+ T cells in vivo is not well defined. Xu et al. report that autophagy was dynamically regulated in virus-specific CD8+ T cells during acute infection of mice with lymphocytic choriomeningitis virus. In contrast to the current paradigm, autophagy decreased in activated proliferating effector CD8+ T cells and was then upregulated when the cells stopped dividing just before the contraction phase. Consistent with those findings, deletion of the gene encoding either of the autophagy-related molecules Atg5 or Atg7 had little to no effect on the proliferation and function of effector cells, but these autophagy-deficient effector cells had survival defects that resulted in compromised formation of memory T cells. These studies define when autophagy is needed during effector and memory differentiation and warrant reexamination of the relationship between T cell activation and autophagy.

Nature Immunol 2014; 15: 1152

Eitan Israeli

Capsule

Identifying a rare population of dendritic cells capable of enhancing the function of cytotoxic T cells

Immune evasion is a hallmark of cancer, and tumors progress despite the accumulation of immune cells within a tumor and at the margin. By performing a systematic analysis of immune cell components in mouse tumor models and human specimens, Broz and colleagues identified a rare population of dendritic cells capable of enhancing the function of cytotoxic T cells. Although much is known on how the immune system functions to prevent disease, the mechanisms by which cytotoxic immune cells are suppressed in tumors are poorly understood. The authors used mouse models of breast cancer and melanoma, as well as human melanoma specimens, to dissect the myeloid lineage, which consists of tumor-associated macrophages (TAMs), monocytes, and dendritic cells that have the capacity to present antigens to T cells and induce cytotoxic T cell function. The authors identified a rare subset of dendritic cells (CD11b+/CD103+) that had a distinct transcription factor signature (IRF8, BATF3, and ZBTB46) as demonstrated by the reduction of this cell population in knockout mice lacking one of these transcription factors. The homing of this dendritic population was independent of colony-stimulating factor 1, which is essential for TAM infiltration, and dependent on granulocyte macrophage colony-stimulating factor (GM-CSF) and the Fms-related tyrosine kinase 3 (Flt3) ligand. These cells had elevated phagocytic capacity compared with TAMs and could stimulate cytotoxic T cells, whereas ablation of this dendritic cell population decreased the efficacy of adoptive cytotoxic T cell therapy in vivo. Bioinformatics analysis revealed that patients with a reduction in these dendritic cells had a much poorer prognosis in multiple cancers, including breast, lung, and head and neck tumors. Cancer immunotherapy is under intense investigation and offers the potential for therapeutic intervention in a variety of cancer types. The identification of stimulatory dendritic cells may provide an additional approach for immune checkpoint inhibition strategies to generate a favorable environment for the function of cytotoxic T cells.

Cancer Cell 2014: 10.1016/j.ccell.2014.09.007

Eitan Israeli

Interleukin-1: Ariadne's Thread in Autoinflammatory and Autoimmune Disorders

Luca Cantarini MD PhD[1]*, Giuseppe Lopalco MD[2]*, Marco Cattalini MD[3], Antonio Vitale MD[1], Mauro Galeazzi MD[1] and Donato Rigante MD[4]

[1]Research Center of Systemic Autoimmune and Autoinflammatory Diseases, Rheumatology Unit, Policlinico Le Scotte, University of Siena, Siena, Italy
[2]Interdisciplinary Department of Medicine, Rheumatology Unit, Policlinico of Bari, Bari, Italy
[3]Immunology and Rheumatology Department, Pediatric Clinic, University of Brescia and Spedali Civili di Brescia, Brescia, Italy
[4]Institute of Pediatrics, Università Cattolica Sacro Cuore, Rome, Italy

ABSTRACT: Autoinflammatory and autoimmune disorders are characterized by chronic activation of the immune system, which leads to systemic self-directed inflammation in genetically predisposed individuals. Mutations in inflammasome-related proteins have been associated with autoinflammatory disorders, and the link between inflammasome and autoimmune disorders is becoming increasingly clear. As researchers learn more about these two areas, other disorders that were once thought to be autoimmune are now being considered autoinflammatory, or as having at least an autoinflammatory component. This review depicts the role of interleukin-1 as "Ariadne's thread" on the path through the labyrinth of autoinflammatory and autoimmune disorders and emphasizes the blurred boundary between innate and adaptive immune systems.

IMAJ 2015; 17: 93–97

KEY WORDS: interleukin-1 (IL-1), autoinflammation, autoimmunity, anakinra, canakinumab

Interleukin-1 (IL-1), the first cytokine discovered (in the 1980s), is a potent mediator of inflammation. It coordinates systemic host defense responses to pathogens or various injuries, and the whole IL-1 system represents an attractive target for different therapeutic interventions. The identification of a new cytoplasmic complex of proteins, called inflammasomes which sense intracellular danger- or pathogen-associated molecular patterns and regulate IL-1β activation and secretion, has enhanced our understanding of the role of IL-1 in biology and many disease processes. Dysregulation of the inflammasome is the cause of a family of monogenic autoinflammatory disorders. This dysregulation has also been involved in the pathogenesis of different multifactorial polygenic diseases with an autoinflammatory component, and even in different autoimmune diseases.

Dysregulation of the inflammasome is the pivotal cause in the pathogenesis of autoinflammatory disorders and seems also to participate in different systemic autoimmune conditions

Classical autoimmune disorders are associated with the presence of autoantibodies and autoantigen-specific T cells; however, in addition to the classical major histocompatibility complex (MHC) class II-associated diseases, such as systemic lupus erythematosus and rheumatoid arthritis, the autoimmunity paradigm has also been the dominant conceptual framework when considering the pathogenesis of a range of other chronic inflammatory conditions, such as inflammatory bowel disease and systemic onset juvenile idiopathic arthritis.

Several difficulties arise when the autoimmunity concept is evoked to describe the self-directed tissue inflammation encountered in many diseases, such as hereditary periodic fevers, now framed in the context of autoinflammatory disorders, for which autoantibody, autoreactive T cells, and MHC allelic associations are lacking [1].

FROM MONOGENIC TO MULTIFACTORIAL AUTOINFLAMMATORY DISORDERS

Advances in cellular and molecular biology have revealed that impaired control of innate immune system generates the so-called autoinflammatory disorders, a group of heritable diseases characterized by unprovoked attacks of systemic inflammation in the absence of autoantibodies and autoreactive T cells [2]. Following the discovery of the familial Mediterranean fever (FMF) gene in 1997 and the tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS) gene in 1999, there has been an extraordinary revolution in the understanding of monogenic autoinflammatory disorders, with identification of multiple genes and new clinical entities, all characterized by subverted mechanisms of inflammation. Their unifying pathogenetic mechanism is a dysregulation of the inflammasome, which leads to overproduction of IL-1β [3]. At a clinical level, autoinflammatory disorders

*The first two authors contributed equally to this study

Table 1. Cinical trials in progress related to interleukin-1 blockade in the monogenic autoinflammatory disorders

	Phase	Status	Study	Disease	Clinical Trial.gov Identifier
Anakinra	3	Recruiting	Anakinra in adult patients with colchicine-resistant FMF	FMF	NCT01705756
	1	Completed	The use of anakinra in the treatment of familial cold autoinflammatory syndrome	FCAS	NCT00214851
Canakinumab	3	Recruiting	Efficacy, safety and tolerability of ACZ885 in pediatric patients with the following cryopyrin-associated periodic syndromes: familial cold autoinflammatory syndrome, Muckle-Wells syndrome and CINCA syndrome	CAPS	NCT01576367
	3	Completed	The safety and efficacy of canakinumab in patients aged ≥ 4 years diagnosed with cryopyrin-associated periodic syndromes in Canada	CAPS	NCT01105507
	3	Completed	Efficacy and safety study of canakinumab administered for 6 months (24 weeks) in Japanese patients with cryopyrin-associated periodic syndromes followed by an extension phase	CAPS	NCT00991146
	2	Completed	Evaluation of the safety and efficacy of canakinumab in pediatric patients with colchicine-intolerant or colchicine-resistant FMF	FMF	NCT01148797
	2	Completed	Efficacy and safety of canakinumab in patients with colchicine-resistant FMF	FMF	NCT01088880
	3	Active	Efficacy, safety and tolerability of ACZ885 in pediatric patients with the following cryopyrin-associated periodic syndromes: familial cold autoinflammatory syndrome, Muckle-Wells syndrome and CINCA syndrome	CAPS	NCT01302860
	–	Recruiting	Clinical outcomes and safety: a registry study of canakinumab patients (B-confident)	CAPS	NCT01213641
	2	Active	Canakinumab in patients with active hyper-IgD syndrome	MKD	NCT01303380
	2	Completed	Efficacy and safety study of ACZ885 in patients with tumor necrosis factor receptor-associated periodic syndrome	TRAPS	NCT01242813
	3	Recruiting	Efficacy and safety of canakinumab in patients with hereditary periodic fevers	HPFs	NCT02059291

CAPS = cryopyrin-associated periodic syndromes, FMF = familial Mediterranean fever, HPFs = hereditary periodic fever syndromes, MKD = mevalonate kinase deficiency syndrome, TRAPS = tumor necrosis factor receptor-associated periodic syndrome

are defined by recurrent fever attacks with constantly increased acute-phase reactants and several inflammatory phenomena involving skin, joints, serosal membranes, etc., that usually start in childhood and are mostly induced by IL-1β-directed pro-inflammatory cascade. Therefore, monotherapy with blocking IL-1 agents often leads to a reduction in disease severity in most autoinflammatory disorders [4]. Table 1 presents a list of the most recent clinical trials on this group of diseases.

Recent advances in our knowledge on the pathogenesis of many multifactorial polygenic disorders with a presumed auto-inflammatory component have paved the way for the introduction of novel therapeutic modalities. Type 2 diabetes mellitus (T2DM) is profoundly influenced by inflammasome activation-dependent IL-1 release: high serum concentrations of glucose lead to increased IL-1β production in human β cells, which is followed by NF-kB activation, Fas signaling up-regulation, and β cell apoptosis. Different scientists have found that oligomers of islet amyloid polypeptide, a protein deposited in the pancreas of patients with T2DM, might trigger NLRP3 inflammasome, enhancing mature IL-1β production and resulting in a progressive decrease in the number of β cells [5]. Several studies have also been performed to prove IL-1 blockade as an effective strategy for β cell function and glycemic control [6]. Other evidence derives from the positive effects of a single dose of canakinumab on HbA1c levels [7]. Clinical trials are in progress to determine whether gevokizumab might improve glycemic control in subjects with T2DM in combination with metformin (ClinicalTrials.gov NCT01144975, NCT01066715, NCT00513214) [8]. However, further results are needed to give anti-IL1 agents a role in the management of T2DM.

Interleukin-1 plays a critical role in the complex pathways linking innate and adaptive immune systems

Patients with idiopathic recurrent acute pericarditis (IRAP) are usually characterized by absence of autoantibodies or self-reactive T cells, and are brilliant responders to IL-1 inhibition [9]. In addition, IRAP may occur in patients with FMF and TRAPS, disclosing the diagnosis of these two autoinflammatory disorders [10], and may also be the only clinical symptom in patients carrying low-penetrance TRAPS-related mutations [11]. A multicenter study evaluating the incidence of TRAPS mutations in patients with IRAP has demonstrated that positive family history of pericarditis, failure of treatment with colchicine, and need for immunosuppressive agents are crucial diagnostic clues [12]. Fifteen young patients with IRAP were evaluated after treatment with anakinra, revealing a 95% reduction in recurrences [13]. A phase IV study to prove anakinra efficacy in patients with IRAP is ongoing (ClinicalTrials.gov NCT02219828).

Many pro-inflammatory cytokines have a critical role in orchestrating the body's reaction to monosodium urate (MSU) and calcium pyrophosphate dihydrate crystals: both these crystals activate the NLRP3 inflammasome, resulting in intense production of bioactive IL-1β [14]. Further evidence for the role of IL-1β in the pathogenesis of gout is shown by the demonstration that IL-1β receptor-deficient mice are not susceptible to MSU-induced inflammation. Further proof of the concept that IL-1 is basically involved in patients with gout

and pseudogout derives from the favorable results obtained with anakinra in an open-label study [15]. In line with these findings, Vitale et al. [16] recently reported three patients with chronic tophaceous gout unresponsive to standard therapy, in whom anakinra led to remarkable amelioration of joint symptoms within 24 hours. Interestingly, the patients were also affected by T2DM and, along with reduced joint symptoms, also achieved a marked improvement in glycemic control [16]. Moreover, a large study on crystal-induced arthritis demonstrated the superior therapeutic effect of canakinumab and rilonacept as compared to corticosteroids [17,18]. A phase III study testing canakinumab efficacy in the prevention of gout attack recurrence is now ongoing for patients intolerant or unresponsive to colchicine (ClinicalTrials.gov NCT01362608).

Adult-onset Still's disease (AoSD) is a rare inflammatory disorder of undisclosed etiology, characterized by increased pro-inflammatory cytokines in the serum [19] and substantial risk of macrophage activation syndrome which occurs as a severe complication with a higher mortality rate than in children with systemic onset juvenile idiopathic arthritis, the pediatric counterpart of AoSD. Robust evidence on IL-1 involvement in the pathogenesis of AoSD derives from the beneficial effects observed when treating these patients with IL-1 antagonists. Notably, in an open randomized multicenter study in 22 patients with AoSD, monotherapy with anakinra has been effective in those refractory to conventional treatments such as corticosteroids and methotrexate [20]. Also, canakinumab and rilonacept have proven effective in inhibiting IL-1 in AoSD [21,22].

Anti-interleukin-1 agents may represent new weapons for treating autoimmune disorders, displaying, like autoinflammatory conditions, self-directed systemic inflammation in genetically predisposed individuals

THE MELTING POT OF AUTOIMMUNE DISORDERS

The pathogenesis of rheumatoid arthritis (RA) has actually been related to multiple pro-inflammatory cytokines, such as TNFα and IL-1β [23], and understanding RA pathophysiological mechanisms has clarified the role of cytokines as new potential targets for biological therapy. In this regard anakinra, alone or in combination with methotrexate, has been evaluated in several controlled studies of patients with RA, improving the disease course and preventing radiological progression of joint damage [24]. A phase II dose-finding study also evaluated the favorable response of canakinumab in patients with active RA despite ongoing methotrexate therapy [25].

The etiology of chronic uveitis remains uncertain, though this condition is the most frequent extra-articular sign of different systemic autoimmune rheumatologic disorders, such as the oligoarticular variant of juvenile idiopathic arthritis, seronegative spondyloarthritis, and Behçet's disease (BD). The inflammatory process leading to uveitis is mainly driven by Th-17 cells and directed by many pro-inflammatory cytokines, chiefly TNFα and IL-1β [26]. In fact, anakinra, canakinumab and gevokizumab might suppress immune mediated ocular inflammation not only in animal models but also in different diseases in which severe uveitis is part of the clinical spectrum, such as Blau syndrome, early-onset sarcoidosis, and BD [27-29]. Three multicenter phase III clinical trials are underway to test the safety and efficacy of gevokizumab in treating active non-infectious uveitis (ClinicalTrials.gov NCT01684345), quiescent non-infectious uveitis (ClinicalTrials.gov NCT01747538), and BD-associated uveitis (ClinicalTrials.gov NCT01965145), whereas a phase II clinical trial with gevokizumab is presently being conducted for patients with scleritis (ClinicalTrials.gov NCT01835132).

The pathogenesis of BD is still unknown, and continuous efforts are in progress to characterize its biologic background where genetic and environmental factors cooperate, suggesting that the disease lies probably at the crossroad between autoimmune and autoinflammatory syndromes. The role of innate immunity in BD has been suggested not only by increased levels of IL-1 in both serum and synovial fluid but also by the relevant effect obtained with IL-1 inhibition [30]. A pilot study is currently testing the clinical efficacy of anakinra (ClinicalTrials. gov NCT01441076). Canakinumab has also shown positive results [31]. Additional evidence for the role of IL-1β in BD derives from a trial with gevokizumab that has led to complete resolution of intraocular inflammation in both uveitis and retinal vasculitis [32].

Sjogren's syndrome (SS) is characterized by infiltration of mononuclear cells in the salivary and lacrimal glands, leading to dryness of both mouth and eyes. Although its pathogenesis remains unknown, several studies have hypothesized that subverted cytokine pathways might contribute to the pathological setting of the syndrome. Highly increased concentrations of IL-1 have been found in the salivary fluid and peripheral blood of patients with SS, suggesting this cytokine as the master regulator in the development of SS local and systemic manifestations [33]. In addition, an imbalance between salivary IL-1 and IL-1 receptor antagonist (IL-1Ra) most likely promotes the typical inflammatory lesions in the mouth. Some authors have also hypothesized that IL-1β might display a proteolytic activity, disrupting acinar and ductal gland structure [34]. Anti-IL-1 agents are now regarded as potential treatment tools, and a randomized double-blind placebo-controlled trial has revealed that IL-1 inhibition with anakinra is able to reduce fatigue in patients with SS [35]. Recent data from a prospective double-blind randomized trial also demonstrate that targeting IL-1 by topical application of anakinra reduces dry eye disease-related symptoms [36].

Chronic diffuse interstitial wall inflammation in the lung is the precondition for progressive pulmonary fibrosis, which characterizes all interstitial lung diseases. Alveolar macrophages are involved in different pulmonary inflammatory processes through IL-1 hypersecretion after a host of exogenous and endogenous stimuli [37]. Several studies have revealed the presence of IL-1β in the pulmonary fibrous tissue, suggesting a causative link between IL-1 and fibrosis [38]. A genetic variability in the IL1RN gene, encoding the physiological IL-1Ra, may also contribute to the pathogenesis of idiopathic pulmonary fibrosis [39]. NLRP3 inflammasome is probably involved in pneumoconiosis [40], and a phase I/II study is underway to test skin gene expression after administration of the IL-1 inhibitor rilonacept in patients with systemic sclerosis (ClinicalTrials.gov NCT01538719), though further studies are required to explore the exact role of IL-1 in the pathogenesis of pulmonary inflammatory processes in their entirety.

CONCLUSIVE REMARKS

This review has shown how the inflammasome works as an activating platform for the release of bioactive pro-inflammatory IL-1β. Dysegulation in this cascade may be caused by mutations in the genes coding for inflammasomal components and/or their interaction partners, leading to autoinflammatory scenarios. However, the contribution of deregulated inflammasomes to the field of autoimmune disorders, such as RA and SS, was recently suggested and corroborated by the efficacy of IL-1 blockade in these conditions. New avenues for the therapy of such diseases will be paved in the near future and our way of managing these patients will likely be revolutionized.

Correspondence
Dr. L. Cantarini
Rheumatology Unit, Policlinico "Le Scotte", University of Siena, Viale Bracci 1, 53100 Siena, Italy
Phone: (39-577) 233-393
Fax: (39-577) 40450
email: cantariniluca@hotmail.com

References

1. Joosten LA, Netea MG, Dinarello CA. Interleukin-1β in innate inflammation, autophagy and immunity. *Semin Immunol* 2013; 25: 416-24.
2. Rigante D, Vitale A, Lucherini OM, Cantarini L. The hereditary autoinflammatory disorders uncovered. *Autoimmun Rev* 2014; 13: 892-900.
3. Cantarini L, Rigante D, Brizi MG, et al. Clinical and biochemical landmarks in systemic autoinflammatory diseases. *Ann Med* 2012; 44: 664-73.
4. Rigante D, Frediani B, Galeazzi M, Cantarini L. From the Mediterranean to the sea of Japan: the transcontinental odyssey of autoinflammatory diseases. *Biomed Res Int* 2013; 2013: 485103.
5. Masters SL, Dunne A, Subramanian SL, et al. Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1β in type 2 diabetes. *Nat Immunol* 2010; 11: 897-904.
6. Larsen CM, Faulenbach M, Vaag A, Ehses JA, Donath MY, Mandrup-Poulsen T. Sustained effects of interleukin-1 receptor antagonist treatment in type 2 diabetes. *Diabetes Care* 2009; 32: 1663-8.
7. Hensen J, Howard CP, Walter V, Thuren T. Impact of interleukin-1β antibody (canakinumab) on glycaemic indicators in patients with type 2 diabetes mellitus: results of secondary endpoints from a randomized, placebo-controlled trial. *Diabetes Metab* 2013; 39: 524-31.
8. Cavelti-Weder C, Babians-Brunner A, Keller C, et al. Effects of gevokizumab on glycemia and inflammatory markers in type 2 diabetes. *Diabetes Care* 2012; 35: 1654-62.
9. Cantarini L, Lopalco G, Selmi C, et al. Autoimmunity and autoinflammation as the yin and yang of idiopathic recurrent acute pericarditis. *Autoimmun Rev* 2014; pii: S1568-9972(14)00217-1.
10. Rigante D, Cantarini L, Imazio M, et al. Autoinflammatory diseases and cardiovascular manifestations. *Ann Med* 2011; 43: 341-6.
11. Cantarini L, Lucherini OM, Vitale A, et al. Expanding spectrum of TNFRSF1A gene mutations among patients with idiopathic recurrent acute pericarditis. *Intern Med J* 2013; 43: 725-7.
12. Cantarini L, Rigante D, Merlini G, et al. The expanding spectrum of low-penetrance TNFRSF1A gene variants in adults presenting with recurrent inflammatory attacks: clinical manifestations and long-term follow-up. *Semin Arthritis Rheum* 2014; 43: 818-23.
13. Finetti M, Insalaco A, Cantarini L, et al. Long-term efficacy of interleukin-1 receptor antagonist (anakinra) in corticosteroid-dependent and colchicine-resistant recurrent pericarditis. *J Pediatr* 2014; 164: 1425-31.e1.
14. Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 2006; 440: 237-41.
15. So A, De Smedt T, Revaz S, Tschopp J. A pilot study of IL-1 inhibition by anakinra in acute gout. *Arthritis Res Ther* 2007; 9: R28.
16. Vitale A, Cantarini L, Rigante D, Bardelli M, Galeazzi M. Anakinra treatment in patients with gout and type 2 diabetes. *Clin Rheumatol* 2014 [Epub ahead of print].
17. Schlesinger N, De Meulemeester M, Pikhlak A, et al. Canakinumab relieves symptoms of acute flares and improves health-related quality of life in patients with difficult-to-treat gouty arthritis by suppressing inflammation: results of a randomized, dose-ranging study. *Arthritis Res Ther* 2012; 13: R53.
18. Schumacher Jr HR, Evans RR, Saag KG, et al. Rilonacept (interleukin-1 trap) for prevention of gout flares during initiation of uric acid-lowering therapy: results from a phase III randomized, double-blind, placebo-controlled, confirmatory efficacy study. *Arthritis Care Res* 2012; 64: 1462-70.
19. Agarwal SK. Biologic agents in rheumatoid arthritis: an update for managed care professionals. *J Manag Care Pharm* 2011; 17 (9 Suppl B): S14-18.
20. Alten R, Gomez-Reino J, Durez P, et al. Efficacy and safety of the human anti-IL-1beta monoclonal antibody canakinumab in rheumatoid arthritis: results of a 12-week, phase II, dose-finding study. BMC Musculoskelet Disord 2011; 12: 153.
21. Selmi C. Diagnosis and classification of autoimmune uveitis. *Autoimmun* Rev 2014; 13: 591-4.
22. Aróstegui JI, Arnal C, Merino R, et al. NOD2 gene-associated pediatric granulomatous arthritis: clinical diversity, novel and recurrent mutations, and evidence of clinical improvement with interleukin-1 blockade in a Spanish cohort. *Arthritis Rheum* 2007; 56: 3805-13.
23. Simonini G, Xu Z, Caputo R, et al. Clinical and transcriptional response to the long-acting interleukin-1 blocker canakinumab in Blau syndrome-related uveitis. *Arthritis Rheum* 2013; 65: 513-18.
24. Gül A, Tugal-Tutkun I, Dinarello CA, et al. Interleukin-1β-regulating antibody XOMA 052 (gevokizumab) in the treatment of acute exacerbations of resistant uveitis of Behçet's disease: an open-label pilot study. *Ann Rheum Dis* 2012; 71: 563-6.
25. Hamzaoui K, Hamza M, Ayed K. Production of TNF-alpha and IL-1 in active Behçet's disease. *J Rheumatol* 1990; 17: 1428-9.
26. Cantarini L, Vitale A, Scalini P, et al. Anakinra treatment in drug-resistant Behçet's disease: a case series. *Clin Rheumatol* 2013 - http://dx.doi.org/10.1007/s10067-013-2443-8.
27. Vitale A, Rigante D, Caso F, et al. Inhibition of interleukin-1 by canakinumab as a successful mono-drug strategy for the treatment of refractory Behçet's disease: a case series. *Dermatology* 2014; 228: 211-14.
28. Solomon A, Dursun D, Liu Z, Xie Y, Macri A, Pflugfelder SC. Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. *Invest Ophthalmol Vis Sci* 2001; 42: 2283-92.

29. Azuma M, Motegi K, Aota K, Hayashi Y, Sato M. Role of cytokines in the destruction of acinar structure in Sjögren's syndrome salivary glands. *Lab Invest* 1997; 77: 269-80.

30. Willeke P, Schotte H, Schlüter B, et al. Interleukin-1beta and tumour necrosis factor-alpha secreting cells are increased in the peripheral blood of patients with primary Sjögren's syndrome. *Ann Rheum Dis* 2003; 62: 359-62.

31. Dubost JJ, Perrier S, Afane M, et al. IL-1 receptor antagonist in saliva; characterization in normal saliva and reduced concentration in Sjögren's syndrome (SS). *Clin Exp Immunol* 1996; 106: 237-42.

32. Norheim KB, Harboe E, Gøransson LG, Omdal R. Interleukin-1 inhibition and fatigue in primary Sjögren's syndrome – a double blind, randomised clinical trial. *PLoS One* 2012; 7: e30123.

33. Koretzky GA, Elias JA, Kay SL, Rossman MD, Nowell PC, Daniele RP. Spontaneous production of interleukin-1 by human alveolar macrophages. *Clin Immunol Immunopathol* 1983; 29: 443-50.

34. Barlo NP, van Moorsel CH, Korthagen NM, et al. Genetic variability in the IL1RN gene and the balance between interleukin (IL)-1 receptor agonist and IL-1β in idiopathic pulmonary fibrosis. *Clin Exp Immunol* 2011; 166: 346-51.

35. Cassel SL, Eisenbarth SC, Iyer SS, et al. The NALP3 inflammasome is essential for the development of silicosis. *Proc Natl Acad Sci USA* 2008; 105: 9035-40.

36. Artlett CM, Sassi-Gaha S, Rieger JL, Boesteanu AC, Feghali-Bostwick CA, Katsikis PD. The inflammasome activating caspase 1 mediates fibrosis and myofibroblast differentiation in systemic sclerosis. *Arthritis Rheum* 2011; 63: 3563-74.

37. Choi JH, Suh CH, Lee YM, et al. Serum cytokine profiles in 868 patients with adult onset Still's disease. *J Rheumatol* 2003; 30: 2422-7.

38. Stabile A, Bertoni B, Ansuini V, La Torraca I, Sallì A, Rigante D. The clinical spectrum and treatment options of macrophage activation syndrome in the pediatric age. *Eur Rev Med Pharmacol Sci* 2006; 10: 53-9.

39. Lequerre T, Quartier P, Rosellini D, et al. Interleukin-1 receptor antagonist (anakinra) treatment in patients with systemic-onset juvenile idiopathic arthritis or adult onset Still disease: preliminary experience in France. *Ann Rheum Dis* 2008; 67: 302-8.

40. Kontzias A, Efthimiou P. The use of canakinumab, a novel IL-1β long-acting inhibitor, in refractory adult-onset Still's disease. *Semin Arthritis Rheum* 2012; 42: 201-5.

Capsule

Rational design of small molecules as vaccine adjuvants

Adjuvants increase vaccine potency largely by activating innate immunity and promoting inflammation. Limiting the side effects of this inflammation is a major hurdle for adjuvant use in vaccines for humans. It has been difficult to improve on adjuvant safety because of a poor understanding of adjuvant mechanism and the empirical nature of adjuvant discovery and development historically. Wu et al. describe new principles for the rational optimization of small-molecule immune potentiators (SMIPs) targeting Toll-like receptor 7 as adjuvants with a predicted increase in their therapeutic indices. Unlike traditional drugs, SMIP-based adjuvants need to have limited bioavailability and remain localized for optimal efficacy. These features also lead to temporally and spatially restricted inflammation that should decrease side effects. Through medicinal and formulation chemistry and extensive immunopharmacology, the authors show that in vivo potency can be increased with little to no systemic exposure, localized innate immune activation, and short in vivo residence times of SMIP-based adjuvants. This work provides a systematic and generalizable approach to engineering small molecules for use as vaccine adjuvants.

Sci Transl Med 2014; 6: 263ra160

Eitan Israeli

Capsule

Intravenous immunoglobulin may be an effective therapy for refractory, active diffuse cutaneous systemic sclerosis

Poelman et al. sought to retrospectively review a single-center experience using intravenous immunoglobulin (IVIG) for the treatment of refractory, active diffuse cutaneous systemic sclerosis (dcSSc). The mean modified Rodnan Skin score (mRSS) at baseline was compared to the mRSS at 6, 12, 18, and 24 months post-IVIG initiation by the paired Student *t*-test. Changes in mRSS at 6 and 12 months were also compared to data from historical controls of three large, negative, multicenter, randomized clinical trials of other medications [D-penicillamine (D-pen), recombinant human relaxin (relaxin), and oral bovine type I collagen (collagen)], and to patients treated with mycophenolate mofetil (MMF) alone using the Student *t*-test. Thirty patients were treated with adjunctive IVIG (2 g/kg/month) for refractory active dcSSc. The mean baseline mRSS of our cohort was 29.6 ± 7.2, and this significantly decreased to 24.1 ± 9.6 (n = 29, $P = 0.0011$) at 6 months, 22.5 ± 10.0 (n = 25, $P = 0.0001$) at 12 months, 20.6 ± 11.8 (n = 23, $P = 0.0001$) at 18 months, and 15.3 ± 6.4 (n = 15, $P < 0.0001$) at 24 months. The mean change in mRSS at 6 months was not significantly different in the IVIG group (-5.3 ± 7.9) compared to the relaxin trial (-4.8 ± 6.99, $P = 0.74$) or MMF group (-3.4 ± 7.4, $P = 0.26$); however, at 12 months, the mean change in mRSS was significantly better in the IVIG group (-8 ± 8.3) than in the D-pen (-2.47 ± 8.6, $P = 0.005$) and collagen (-3.4 ± 7.12, $P = 0.005$) groups, and was comparable to the group of primary MMF responders (-7.1 ± 9, $P = 0.67$). The authors suggest that IVIG may be an effective adjunctive therapy for active dcSSc in patients in whom other therapies failed.

J Rheumatol 2014;doi:10.3899/jrheum.140833

Eitan Israeli

Is B Cell-Targeted Therapy Effective in Systemic Lupus Erythematosus?

Daphna Paran MD[1] and Yaakov Naparstek MD[2]

[1]Department of Rheumatology, Tel Aviv Sourasky Medical Center, affiliated with Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[2]Department of Medicine, Hadassah Medical Center and Hebrew University-Hadassah Medical School, Jerusalem, Israel

ABSTRACT: In the past decade we have witnessed a dramatic change in the management of autoimmune diseases, such as rheumatoid arthritis, due to the development of new biologic drugs designed to target key mediators in the autoimmune process. However, the development of similar target-specific drugs for the management of SLE has not been as successful. The B cell has long been considered central to the pathogenesis of SLE and has been regarded as an important target for biologic drugs. Several B cell-targeted drugs have been developed and although the mechanisms seem promising, most of the studies published to date have failed to achieve their primary endpoints, leading to an ongoing debate regarding the role of B cell therapy in SLE. The present report discusses the pros and cons of B cell-targeted therapy in SLE, reviews the clinical studies, and offers possible explanations for the discrepancies between randomized control studies and real-life experience.

IMAJ 2015; 17: 98–103

KEY WORDS: systemic lupus erythematosus (SLE), B cell-targeted therapy, B cell depletion, belimumab, randomized control trials (RCTs)

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease affecting predominantly young women of childbearing age. Disease manifestations range from a mild disease involving one or two systems to a multisystem, severe and sometimes life-threatening disease. In managing this condition the chief goals are to control active disease, achieve remission, and prevent irreversible organ damage while avoiding drug-induced side effects.

B cells seem to play an important role in the pathogenesis of SLE, but no B cell-depleting therapy in RCTs has achieved a satisfactory therapeutic effect in this disease

The only drugs approved by the Food and Drug Administration (FDA) for SLE since 1955 are hydroxychloroquine and corticosteroids, although multiple immunosuppressant drugs – methotrexate, azathioprine, mycophenolate mofetil (MMF), cyclosporine and cyclophosphamide (CYC) – have been used over the years to control the severe manifestations. These drugs lead to generalized immunosuppression, with multiple adverse effects including predisposition to infection, malignancy and infertility. Moreover, they are not universally effective, with partial or no response in many cases. In view of this poor benefit-risk profile, new drugs have recently been developed to target specific immune cells or cytokines thought to be central to the disease pathogenesis with the aim of achieving better control of the disease with fewer side effects.

B cells have a central role in the pathogenesis of SLE and exert multiple effects. B cells not only produce pathogenic autoantibodies but have additional pivotal roles in the autoimmune process: they act as antigen-presenting cells, provide co-stimulatory signals for T cell activation and differentiation, secrete and respond to cytokines, link innate and acquired immunity by Toll-like receptors, affect follicular dendritic cell differentiation, and help shape the architecture of peripheral lymphoid organs. Alteration of these B cell functions may lead to breach of tolerance and autoimmune disease. Loss of B cell tolerance occurs very early in SLE, as shown by Arbuckle et al. [1] who looked at serum samples from the U.S. Defense Serum Repository and found that autoantibodies are typically present many years before the onset of SLE while patients are still asymptomatic. Subsets of B cells are altered in SLE with an increase in transitional B cells, memory cells and plasma cells and an increase in a subset of autoreactive B cells (9G4+) in blood and peripheral lymphoid organs. Autoreactive B cells lead to development of autoreactive memory B cells and plasma cells.

Indeed belimumab, the first FDA-approved drug for the treatment of SLE in more than 50 years, is a B cell-targeted therapy. There is currently an ongoing debate regarding the role of B cell-targeted therapy in SLE due to the modest effect of belimumab in two large phase III randomized control trials (RCT) in contrast to the seemingly dramatic effect of B cell depletion therapy with rituximab in open-label studies, while rituximab failed to show a significant effect in two randomized control studies.

This review will discuss the pros and cons of B cell-targeted therapy in SLE, review the clinical studies and reports,

and offer possible explanations for the discrepancies between studies and real-life experience.

MECHANISMS OF B CELL TARGETING

Several mechanisms of B cell targeting have been studied to date, including inactivation of autoreactive B cells via tolerance induction, B cell depletion using monoclonal antibodies that bind B cell surface antigens, blockade of B cell survival factors, and blockade of co-stimulatory signals.

Although the mechanisms of action of these drugs are promising, all these drugs except for belimumab have failed to show clinical benefit [2]. This might be due to the complex effects and counter-effects of the multiple arms of the immune system and/or to flaws in study design, which will be discussed.

• INDUCTION OF TOLERANCE

To date, induction of tolerance has been attempted with two drugs, abetimus and edratide, but they have not achieved their primary endpoints in their respective clinical studies. Abetimus is composed of four identical strands of dsDNA, covalently linked to a small molecule platform that cross-links B cell receptors (BCRs) leading to anergy or deletion of autoreactive B cells. The drug rapidly reduces anti-double-stranded DNA (anti-dsDNA) antibody levels by formation and clearance of drug-antibody complexes. In view of the important role of anti-dsDNA antibodies in the pathogenesis of lupus nephritis, abetimus was studied as a possible agent to prevent renal flares. However, two pivotal trials (phase II and III, n=317) with a large number of lupus nephritis patients did not demonstrate a significant prolongation in time until renal flare. There were no significant side effects [3]. Edratide is a designed peptide (hCDR1) based on the sequence of the complementarity-determining region (CDR)1 of a human anti-DNA monoclonal antibody that bears the major idiotype 16/6Id. hCDR1 was shown to ameliorate the serological and clinical manifestations of induced or spontaneously developed lupus in mice. The beneficial effects of hCDR1 were associated with the down-regulation of pathogenic cytokines: interleukin-1beta (IL-1β), interferon-gamma (IFNγ) and IL-10, and the up-regulation of the immunosuppressive cytokine transforming growth factor-beta (TGFβ). Treatment with hCDR1 significantly reduced production of the B cell stimulator (BLyS) in lupus-prone mice, reduced T cell apoptosis and induced CD4+CD25+Foxp3+ regulatory cells [4]. However, a phase II randomized, double-blind, placebo-controlled, multiple-dose study to assess the efficacy and safety of edratide in SLE did not meet its primary endpoint.

• B CELL DEPLETION

► Rituximab

B cell depletion with rituximab (RTX) is currently approved for the treatment of rheumatoid arthritis and antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis [5-7]. Given the central role of B cells in SLE, B cell depletion seems an attractive mechanism for the management of SLE as well. Indeed, open-label uncontrolled studies have reported a beneficial effect of RTX in more than 400 patients worldwide. The majority of patients were refractory to multiple immunosuppressants including CYC. The major manifestations treated successfully with RTX include nephritis, severe thrombocytopenia and hemolytic anemia, severe central nervous system (CNS) manifestations, and severe skin disease. A retrospective 7 year analysis of the first 50 patients with refractory SLE who were treated at University College London reported the 6 month clinical outcome as well as the long-term safety profile [8]. Of the 45 patients available for follow-up at 6 months, 19 (42%) achieved remission, and 21 (47%) reached partial remission after one cycle of RTX. B cell depletion resulted in a decrease in median global BILAG scores from 12 to 5 ($P < 0.0001$) and median anti-dsDNA antibody titers from 106 to 42 IU/ml ($P < 0.0001$), and an increase in the median C3 level from 0.81 to 0.95 mg/L ($P < 0.02$) at 6 months. Five serious adverse events were observed. The authors concluded that RTX is an effective treatment for patients with active SLE whose disease has failed to respond to standard immunosuppressive therapy. Two European registries reported similar results. The French AutoImmunity and Rituximab registry described 136 patients with SLE who were treated with RTX. Overall response, according to the SELENA-SLEDAI score was observed in 71% of the patients.. Efficacy did not differ significantly between patients receiving RTX monotherapy and those receiving concomitant immunosuppressive agents who had higher baseline disease activity. Among responders, 41% experienced a relapse of disease, with a response in 91% after retreatment with RTX [9]. A pooled analysis of the efficacy of RTX from European cohorts diagnosed with biopsy-proven lupus nephropathy (LN) who were treated with RTX yielded 164 patients. RTX was administered in combination with corticosteroids (99%) and immunosuppressive agents in 124 patients (76%) (cyclophosphamide in 58 and mycophenolate in 55). At 6 and 12 months, respectively, response rates were 27% and 30% for complete response (CR), 40% and 37% for partial response (PR) and 33% for no response. Significant improvement in 24 hour proteinuria ($P = 0.006$), serum albumin ($P < 0.001$) and protein/creatinine ratio ($P < 0.001$) at 12 months was observed. The authors concluded that RTX may be an effective option for

Although RCTs designed to assess the efficacy of rituximab in SLE have failed to achieve their primary endpoints, rituximab use has been included in the ACR and EULAR guidelines for the management of patients with refractory lupus nephritis (class III/IV) who have not responded to mycophenolate mofetil and cyclophosphamide

patients with LN, especially those refractory to standard treatment or who experience a new flare after intensive immunosuppressive treatment [10].

When looking at the immunological effects of RTX, clinical response correlates with B cell depletion, is not dependent on serologic response, and may precede decline in autoantibody levels. This observation suggests a beneficial effect on autoantibody-independent functions of B cells. Patients who are serologic responders enjoy a more prolonged clinical response (> 3 years vs. 6 months). RTX reduces anti-dsDNA and antinucleosome antibodies by 30–40% but does not reduce anti-Ro or protective antibodies. Response to treatment is better in extractable nuclear antigen antibody (ENA)-negative patients. These observations suggest that short-lived plasma cells (rapidly proliferating B cell clones) produce anti-dsDNA, anticardiolipin and antinucleosome antibodies which are affected by RTX, as compared to long-lived plasma cells which produce anti-Ro, anti-RNP and protective antibodies and are not affected by B cell depletion with RTX. B cell depletion has the potential to partially restore tolerance. In patients who respond well to RTX and maintain prolonged clinical remission there is a progressive disappearance of lupus-specific antibodies (anti-dsDNA antibodies, 9G4 antibodies), repopulation with an increased number of naïve B cells, and a dramatic and prolonged expansion of transitional B cells – "resetting" the immune system and possibly reinstituting tolerance [11].

Belimumab is the only B cell-targeted therapy found in RCTs to be effective for SLE, leading to an ongoing debate on the role of this treatment as well as on the importance of study design of RCTs in lupus

Despite these promising reports, the two large randomized controlled studies designed to assess the efficacy of RTX in non-renal lupus (EXPLORER) [12] and lupus nephritis (LUNAR) [13] did not achieve their respective primary endpoints. The EXPLORER was a phase II/III RCT and included 257 patients with moderately to severely active extrarenal SLE (≥ 1 BILAG A in > 50% of patients or ≥ 2 BILAG B) despite ongoing immunosuppression. All patients received high dose prednisone on entry (more than 60% received an average dose of 45.9 ± 16.4 mg/day). In addition, the primary endpoints were stringent. A major clinical response required achieving BILAG C in all systems at week 24 without a moderate or severe flare by week 52, and a partial response was defined as a BILAG C in all systems at week 24 and maintaining this for 16 weeks, BILAG B in only one system by week 24 without a new BILAG A or B by week 52.

No differences were observed between placebo and RTX in the primary and secondary efficacy endpoints. However, a beneficial effect of RTX on the primary endpoint was observed in the preplanned subgroup analysis of African Americans and Hispanics. This trial enrolled patients with active SLE and used aggressive background treatment, which could have masked the beneficial effect of the addition of RTX to standard of care therapy. In addition, the definitions of a response were stringent and differed from what might be expected in a real-life setting. A quantifiable immunological effect was seen with the reduction in anti-dsDNA and anticardiolipin antibodies and CD8 memory T cells. An enhanced effect on anti-dsDNA was seen in anti-dsDNA-positive and ENA-negative patients, as well as an increase in complement (in all with anti-dsDNA at baseline) and an increase in platelets in patients with initial low levels [14-16].

The LUNAR trial enrolled 144 patients with active lupus nephritis (class III or IV). All patients were treated with high dose steroids and mycophenolate mofetil and were randomized 1:1 to receive RTX or placebo. The overall (complete and partial) renal response rates were 45.8% among the 72 patients receiving placebo and 56.9% among the 72 patients receiving RTX ($P = 0.18$). The primary endpoint (superior response rate with RTX) was not achieved; however, there were more responders in the RTX group at 12 months (56.9% vs. 45.8%), increased improvement in proteinuria (32% vs .9%), and fewer patients required rescue treatment with CYC (0% vs. 8%). A possible true benefit may have been missed due to the small sample size. In addition, a longer follow-up may have demonstrated a more beneficial effect since the time to true proteinuria remission is 2 years [17]. Similar to the EXPLORER trial, effective background therapy with steroids and MMF was used in both arms. MMF and steroids are effective in a large percentage of patients with LN and their use may have masked the beneficial effect of RTX treatment.

Several explanations for the discrepancies between real-life experience with RTX in SLE and the results of RCTs have been suggested [16]. The majority of patients in uncontrolled studies had severe and sometimes life-threatening disease, refractory to immunosuppressants which were usually not continued, while in the RCTs life-threatening cases were excluded and all cases received background immunosuppression. The endpoints in RCTs are stringent and any changes in corticosteroid or immunosuppressive doses would be considered non-responders, while in real life dose adjustments are common. In addition, RCTs used high dose steroids which may mask a beneficial effect, whereas in the real-life setting concomitant use of high dose steroids is uncommon. The possible synergistic effect of a combination of CYC and RTX as used in real-life refractory cases has not been assessed in RCTs. RCTs set predefined endpoints and do not assess long-term benefits, which may be especially relevant when assessing the effects on lupus nephritis.

Indeed, despite the fact that RTX was not shown to be efficacious in lupus nephritis in RCTs, the recent guidelines of both the EULAR and the ACR for the management of LN have recommended the use of RTX in patients with LN refractory to MMF and to CYC based on the real-life experience reports in

refractory cases [18,19]. One could argue that RTX indeed may have a role in refractory cases but not in early cases, such as those enrolled in the RCTs. This argument was recently challenged as well by the promising reports of the beneficial effect of RTX treatment early on in LN, employing a regimen with minimal steroids and MMF [20]. Lightstone and team [20] report the results of their study on the first 50 patients in the rituxilup cohort where patients with LN received two doses of RTX 1000 mg (given 2 weeks apart) accompanied by two doses of intravenous methylprednisolone 500 mg and MMF with no additional steroids. Renal remission was defined as serum creatinine not higher than 15% above baseline; complete remission (CR) was defined as urine protein:creatinine ratio (PCR) < 50 mg/mmol, partial remission (PR) if PCR > 50 mg/mmol but non-nephrotic and > 50% reduction. Ninety percent of patients (45/50) achieved complete or partial remission (CR 72%, persistent PR 18%) [21]. Moreover, Moroni et al. [22] recently reported the first attempt to compare RTX to MMF or CYC as a regimen for remission induction in LN. Although this was a small study of 54 patients, complete remission was achieved in 70.6% of patients on RTX, in 52.9% on MMF, and in 65% on CYC. Partial remission was reached in 29.4% on RTX, 41.2% on MMF, and 25% on CYC. The authors conclude that RTX seemed to be at least as effective as MMF and CYC pulses in inducing remission, especially when considering that patients treated with RTX had more negative renal prognostic factors in this study.

► Ocrelizumab

Evaluation of another anti-CD20 antibody, ocrelizumab, a humanized rather than a chimeric anti-CD20 antibody, in two phase III studies – the BEGIN and the BELONG that were similar to the EXPLORER and LUNAR – were stopped prematurely due to an increase in infections [16,23].

► Epratuzumab

Epratuzumab is a monoclonal antibody that targets CD22, a B cell-specific surface antigen involved in B cell signaling. The mechanism of action is still not fully defined but data indicate that it selectively modifies B cell activation and function [24]. The first two international randomized controlled trials (ALLEVIATE 1 and 2) evaluating epratuzumab in patients with moderately to severely active SLE were discontinued prematurely because of interruption in drug supply [25]. The results of the EMBLEM study, a phase IIb trial to assess the efficacy and safety of epratuzumab in patients with moderate to severe SLE, were published recently. Epratuzumab led to a modest decrease of about 30% in B cells, without a change in immunoglobulin levels [24]. In this study the proportion of responders was higher in all epratuzumab groups compared with placebo, but the overall treatment effect was not statistically significant [24]. Multicenter phase III studies with epratuzumab in patients with SLE are currently ongoing.

• BLOCKADE OF B CELL SURVIVAL FACTORS

► Belimumab

While the use of RTX has not been approved for the treatment of SLE, another B cell-targeted therapy, belimumab, is the first drug approved for the treatment of SLE in 50 years. Belimumab is a human immunoglobulin (Ig)-G1λ monoclonal antibody that binds soluble B lymphocyte stimulator (BLyS) and inhibits its biologic activities. Elevated BLyS levels correlate with increased SLE disease activity. Belimumab was approved by the FDA in 2011 for the treatment of active SLE (not including severe lupus nephritis or CNS disease) refractory to standard therapy. The efficacy of belimumab was demonstrated in two large RCTs (BLISS 52, BLISS 76) with more than 800 patients in each study. Pooled data showed a beneficial effect in 50.6% of belimumab-treated patients versus 46.2% ($P < 0.0001$) in the placebo arm [26,27]. The large number of participants allowed sufficient power to demonstrate a modest beneficial effect. In addition, the use of a novel composite endpoint, the SLE responder Index (SRI), has allowed the detection of reduction in disease activity without worsening in other organ systems.

These study design issues might explain why RTX, a relatively potent drug with a marked biologic effect and dramatic clinical effects in uncontrolled studies, has not achieved the primary endpoint in RCTs, while belimumab has overcome the hurdles of FDA approval with statistically significant effects in well-designed trials. There are, however, several questions that remain to be clarified regarding the use of belimumab in real-life patients with SLE [28], including the clinical relevance of the modest differences as compared to the placebo arm as well as the possible role of belimumab in the treatment of refractory severe disease and renal or central nervous system lupus. The cost-benefit ratio should be addressed as well.

► Atacicept

Atacicept (TACI-IgG) is a humanized fusion protein that binds BLys and APRIL (a proliferation-inducing ligand) and might be more effective than belimumab in the management of lupus. A phase II/III trial of atacicept in LN was terminated after the enrollment of six patients due to an unexpected decline in serum IgG and the occurrence of serious infections; however, in retrospect these complications may have been due to concomitant treatment with MMF [29]. Results of an RCT of subcutaneous atacicept 75 mg or 150 mg, or placebo twice weekly for 4 weeks, then weekly for 48 weeks in patients with moderate to severe SLE, were recently published. There was no difference between atacicept 75 mg and placebo for flare rate or time to first flare (as defined by BILAG A or B). Analysis of atacicept 150 mg suggested benefit [30].

THE FUTURE OF B CELL-TARGETED THERAPIES IN SLE

Following the failure of RCTs to demonstrate a beneficial effect of B cell depletion with RTX in non-renal lupus and LN,

many questions have been raised regarding this mechanism of intervention:

- Are we depleting both pathogenic and protective B cells, hence not showing benefit?
- RTX depletes short-lived plasma cells but does not affect pathogenic long-lived plasma cells. Could interventions directed against the plasma cell be more effective?
- Could RTX be an effective approach in early SLE and not only in refractory cases?
- Is combination treatment of RTX with CYC synergistic and necessary for a significant beneficial effect?
- Have RCTs failed to show a beneficial effect with RTX due to flaws in study design?

Several points should be addressed in future study designs:

- A large number of patients should be recruited to achieve sufficient statistical power
- A sufficiently long follow-up is needed to detect a beneficial effect: in LN trials a follow-up of at least 2 years is required to detect proteinuria remission and at least 5 years are required to discern differences in maintenance therapies
- Background therapies including corticosteroids and concomitant immunosuppressants need to be at the lowest dose possible to avoid the masking of a beneficial effect. A good example would be the two well-designed RCTs of RTX in the treatment of ANCA-associated vasculitis, where RTX was compared to CYC without additional background immunosuppression and a well-outlined protocol for corticosteroid dose and tapering was used in both arms [6,7]
- Clinically meaningful endpoints are needed to better define response in this heterogenous disease.

Although the use of biologics for the management of patients with SLE has lagged behind the dramatic change in the management of rheumatoid arthritis, significant advances have been made. Better designed future studies, better understanding of immunological disturbances in individual SLE patients at different times in the course of disease, and better definition of protective and pathogenic mechanisms of different B cell populations with possible targeting of selective populations may aid in the development of effective B cell-targeted therapies for the treatment of SLE.

Correspondence
Dr. D. Paran
Dept. of Rheumatology, Tel Aviv Sourasky Medical Center, Tel Aviv 64239, Israel
Phone: (972-3) 697-3668
Fax: (972-3) 697-4577
email: parand@netvision.net.il; daphnap@tlvmc.gov.il

References

1. Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med* 2003; 349: 1526-33.
2. Gatto M, Kiss E, Naparstek Y, Doria A. In-/off-label use of biologic therapy in systemic lupus erythematosus. *BMC Med* 2014; 12: 30.
3. Horowitz DM, Furie RA. Abetimus sodium: a medication for the prevention of lupus nephritis flares. *Expert Opin Pharmacother* 2009; 10: 1501-7.
4. Sthoeger ZM, Sharabi A, Molad Y, et al. Treatment of lupus patients with a tolerogenic peptide, hCDR1 (Edratide): immunomodulation of gene expression. *J Autoimmun* 2009; 33: 77-82.
5. Emery P, Deodhar A, Rigby WF, et al. Efficacy and safety of different doses and retreatment of rituximab: a randomised, placebo-controlled trial in patients who are biological naive with active rheumatoid arthritis and an inadequate response to methotrexate (Study Evaluating Rituximab's Efficacy in MTX inadequate responders (SERENE)). *Ann Rheum Dis* 2010; 69: 1629-35.
6. Jones RB, Tervaert JWC, Hauser T, et al. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med* 2010; 363: 211-20.
7. Stone H, Merkel PA, Spiera R, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med* 2010; 363: 221-32.
8. Lu TY, Ng KP, Cambridge G, et al. A retrospective seven-year analysis of the use of B cell depletion therapy in systemic lupus erythematosus at University College London Hospital: the first fifty patients. *Arthritis Rheum* 2009; 61: 482-7.
9. Terrier B, Amoura Z, Ravaud P, et al. Club Rhumatismes et Inflammation. Safety and efficacy of rituximab in systemic lupus erythematosus: results from 136 patients from the French AutoImmunity and Rituximab registry. *Arthritis Rheum* 2010; 62: 2458-66.
10. Díaz-Lagares C, Croca S, Sangle S, et al. UK-BIOGEAS Registry. Efficacy of rituximab in 164 patients with biopsy-proven lupus nephritis: pooled data from European cohorts. *Autoimmun Rev* 2012; 11: 357-64.
11. Anolik JH. B cell biology: implications for treatment of systemic lupus erythematosus. *Lupus* 2013; 22: 342-9.
12. Merrill JT, Neuwelt CM, Wallace DJ, et al. Efficacy and safety of rituximab in moderately-to-severely active systemic lupus erythematosus: the randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. *Arthritis Rheum* 2010; 62: 222-33.
13. Rovin BH, Furie R, Latinis K, et al. Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab study. *Arthritis Rheum* 2012; 64: 1215-26.
14. Cambridge G, Leandro MJ, Teodorescu M, et al. B cell depletion therapy in systemic lupus erythematosus: effect on autoantibody and antimicrobial antibody profiles. *Arthritis Rheum* 2006; 54: 3612-22.
15. Tew GW, Rabbee N, Wolslegel K, et al. Baseline autoantibody profiles predict normalization of complement and anti-dsDNA autoantibody levels following rituximab treatment in systemic lupus erythematosus. *Lupus* 2010; 19: 146-57.
16. Reddy V, Jayne D, Close D, Isenberg D. B-cell depletion in SLE: clinical and trial experience with rituximab and ocrelizumab and implications for study design. *Arthritis Res Ther* 2013; 15 (Suppl 1): S2.
17. Jónsdóttir T, Zickert A, Sundelin B, Henriksson EW, van Vollenhoven RF, Gunnarsson I. Long-term follow-up in lupus nephritis patients treated with rituximab – clinical and histopathological response. *Rheumatology* 2013; 52: 847-55.
18. Bertsias GK, Tektonidou M, Amoura Z, et al. Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. *Ann Rheum Dis* 2012; 71: 1771-82.
19. Hahn BH, McMahon MA, Wilkinson A, et al. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res* 2012; 64: 797-808.
20. Lightstone L. Minimising steroids in lupus nephritis – will B cell depletion pave the way? *Lupus* 2013; 22: 390-9.
21. Condon MB, Ashby D, Pepper RJ, et al. Prospective observational single-centre cohort study to evaluate the effectiveness of treating lupus nephritis with rituximab and mycophenolate mofetil but no oral steroids. *Ann Rheum Dis* 2013; 72 (8): 1280-6.
22. Moroni G, Raffiotta F, Trezzi B, et al. Rituximab vs mycophenolate and vs cyclophosphamide pulses for induction therapy of active lupus nephritis: a clinical observational study. *Rheumatology* 2014; 53: 1570-7.
23. Mysler EF, Spindler AJ, Guzman R, et al. Efficacy and safety of ocrelizumab in active proliferative lupus nephritis: results from a randomized, double-blind,

phase III study. *Arthritis Rheum* 2013; 65: 2368-79.

24. Wallace DJ, Kalunian K, Petri M, et al. Efficacy and safety of epratuzumab in patients with moderate/severe active systemic lupus erythematosus: results from EMBLEM, a phase IIb, randomised, double-blind, placebo-controlled, multicentre study. *Ann Rheum Dis* 2014; 73: 183-90.
25. Wallace DJ, Gordon C, Strand V, et al. Efficacy and safety of epratuzumab in patients with moderate/severe flaring systemic lupus erythematosus: results from two randomized, double-blind, placebo-controlled, multicentre studies (ALLEVIATE) and follow-up. *Rheumatology* 2013; 52: 1313-22.
26. Furie R, Petri M, Zamani O, et al. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. *Arthritis Rheum* 2011; 63: 3918-30.
27. Navarra SV, Guzmán RM, Gallacher AE, et al. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. *Lancet* 2011; 377: 721-31.
28. Ramos-Casals M, Sanz I, Bosch X, Stone JH, Khamashta MA. B-cell-depleting therapy in systemic lupus erythematosus. *Am J Med* 2012; 125: 327-36.
29. Ginzler EM, Wax S, Rajeswaran A, et al. Atacicept in combination with MMF and corticosteroids in lupus nephritis: results of a prematurely terminated trial. *Arthritis Res Ther* 2012; 14 (1): R33.
30. Isenberg D, Gordon C, Licu D, Copt S, Rossi CP, Wofsy D. Efficacy and safety of atacicept for prevention of flares in patients with moderate-to-severe systemic lupus erythematosus (SLE): 52-week data (APRIL-SLE randomised trial). *Ann Rheum Dis* 2014 Jun 20 [Epub ahead of print].

Capsule

Metformin as adjunct antituberculosis therapy

The global burden of tuberculosis (TB) morbidity and mortality remains immense. A potential new approach to TB therapy is to augment protective host immune responses. Singhal et al. report that the antidiabetic drug metformin (MET) reduces the intracellular growth of *Mycobacterium tuberculosis* (*Mtb*) in an AMPK (adenosine monophosphate-activated protein kinase)-dependent manner. MET controls the growth of drug-resistant *Mtb* strains, increases production of mitochondrial reactive oxygen species, and facilitates phagosome-lysosome fusion. In Mtb-infected mice, use of MET ameliorated lung pathology, reduced chronic inflammation, and enhanced the specific immune response and the efficacy of conventional TB drugs. Moreover, in two separate human cohorts, MET treatment was associated with improved control of *Mtb* infection and decreased disease severity. Collectively, these data indicate that MET is a promising candidate host-adjunctive therapy for improving the effective treatment of TB.

Sci Transl Med 2014;6:263ra159

Eitan Israeli

Capsule

The genetics of febrile seizures

Febrile seizures – seizures caused by a high fever – occur in as many as 5% of young children. Often the fever is a result of a viral illness, but vaccine-induced fevers can also cause seizures and are considered an adverse effect of immunization. Family and twin studies confirm a strong genetic component underlying risk for febrile seizures. Genes have been identified for some epilepsy syndromes, but genetic risk factors for "simple" or self-limited febrile seizures have been elusive. Two recent studies advance our understanding of genetic susceptibility to fever-related seizures. Schubert et al. (*Nature Genet* 2014; 10.1038/ng.3130) used exome sequencing in two large families with multiple affected individuals to identify rare variants that segregate with fever-related seizures. They identified mutations in *STX1B* in both families as well as in four unrelated affected individuals. Rare variants in STX1B were associated with a broad range of seizures, ranging from simple febrile seizures to more severe epilepsy conditions, so further study is required to understand what role this gene plays in run-of-the-mill simple febrile seizures. The second study is a genome-wide association study (GWAS) by Feenstra et al. (*Nature Genet* 2014; 10.1038/ng.3129) in which they identify common genetic variants associated with risk for simple febrile seizures. They compared three groups of individuals: 929 children who had a febrile seizure after receiving the measles-mumps-rubella (MMR) vaccine, 1070 who had febrile seizures unrelated to the MMR vaccine, and 1999 controls without febrile seizures. They identified four loci associated with risk of febrile seizures overall and confirmed the associations in an independent case-control cohort. Two risk variants lie within well-known epilepsy genes that encode sodium channel subunits, *SCN1A* and *SCN2A*, so these results are not entirely surprising. Notably, variants in a different gene, *ANO3*, were associated with the highest risk of febrile seizures. Little is known about how this gene, which encodes a transmembrane protein that belongs to a family of chloride channels, might be related to seizure susceptibility. The discovery highlights new areas for research and, potentially, therapy. The fourth locus was not linked to a gene, but to a genomic region previously associated with magnesium levels – another area worthy of investigation. Perhaps most interesting, the authors identified two loci that are specific for risk of febrile seizures after the MMR vaccine, both of which are in genes that are involved in the immune response to infection: *IFI44L* and *CD46*. These results suggest that MMR vaccine-related seizures may be a subtype of simple febrile seizures.

Eitan Israeli

IMAJ • VOL 17 • FEBRUARY 2015

Livedo Reticularis: An Enigma

Shirish R. Sangle MBBS MD and David P. D'Cruz MD FRCP

Lupus Research Unit, Guy's and St Thomas' Hospital NHS Foundation Trust and Kings College Medical School, London, UK

KEY WORDS: livedo reticularis, thrombosis, pregnancy morbidity, accelerated atherosclerosis, livedoid vasculopathy

IMAJ 2015; 17: 104–107

Livedo reticularis, as the name suggests, is a livedoid discoloration of the skin in a reticular pattern. In 1907 Ehrmann described two types of livedo: livedo racemosa which has an incomplete (broken) reticular lattice work pattern, and livedo reticularis where the reticular pattern comprises complete circles (unbroken) [1]. The concept was that pathological livedo was racemosa and the reticularis pattern was non-pathological. However, in clinical practice there is no clear-cut differentiation between these forms of livedo, and often "livedo reticularis" is the term used to describe all types of livedo.

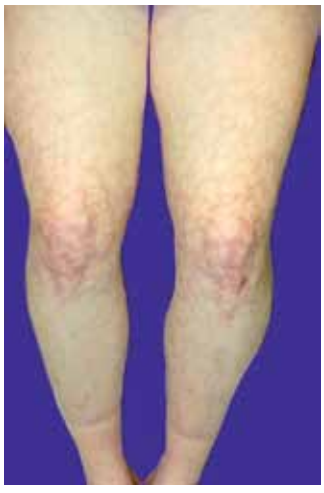
Broadly speaking, livedo is divided into physiological and pathological livedo. Physiological livedo (cutis marmorata) is commonly seen on the legs of young healthy women in cold weather and varies according to temperature (rewarming). This lattice-like pattern is a result of livedoid discolouration due to anastomoses between vascular cones in the skin where desaturated blood stagnates [2] [Figure 1].

The classification of pathological livedo was not clear until recently. However, in the revised classification criteria for antiphospholipid syndrome (APS), pathological livedo is defined as "the persistent, non-reversible with rewarming, violaceous, red and blue, reticular or mottled pattern of the skin of trunk, arms or legs, consisting of regular unbroken circles (livedo reticularis) or irregular broken pattern (racemosa)." Here the concept was based on the extent and fixed pattern of livedo rather than the type of livedo. This was further classified according to the width of livedo (< or > 10 mm) – large and fine livedo racemosa and large and fine livedo reticularis. It is not clear whether the width of the livedo has any implications for the observed pathology in the skin [3]. The term livedoid vasculitis is used for cutaneous ulcers with livedo and is generally of the racemosa type [4].

Certain drugs such as amantadine used in the treatment of Parkinson's disease and multiple sclerosis can induce livedo, which may occur in up to 28% of patients receiving amantadine. Skin biopsies of livedo in these patients did not show any vasculitis and it was thought to be secondary to depleted catecholamines [5].

Livedo reticularis is an independent marker of arterial and venous thrombosis, pregnancy morbidity, and possibly accelerated atherosclerosis

Figure 1. Livedo reticularis



PREVALENCE

The first description of an association between livedo reticularis and cerebrovascular accidents came from Sneddon in 1965 [6]. He described six patients, one man and five women, with cerebrovascular accidents who had livedo reticularis. All patients were negative for LE cells and had no clinical features of systemic lupus erythematosus (SLE) or polyarteritis nodosa, although none appear to have been tested for the lupus anticoagulant. Interestingly, most of the patients were hypertensive and one patient underwent a renal biopsy which showed hypertensive changes [6]. Livedo is also observed in various autoimmune diseases such as livedoid vasculopathy, SLE with or without antiphospholipid (Hughes) syndrome (APS), thromboangiitis obliterans, primary thrombocytopenia, polyarteritis nodosa, and polycythemia vera [7]. Although livedo may present in isolation as seen in Sneddon's syndrome, there is a strong correlation between APS and livedo. In his first description of APS, Hughes included livedo as part of a syndrome associated with arterial and venous thrombosis, spontaneous abortions, neurological manifestations, thrombocytopenia and livedo [8]. Livedo is the most commonly observed cutaneous lesion in APS [9]. In their study of 200 APS patients, Frances et al. [10] found livedo in 25.5%. Livedo can be an initial manifestation in primary APS in up to 40% of patients and more frequently in up to 70% of patients with SLE and APS [11]. Similar findings were described in a cohort of 1000 patients with APS. In this cohort, livedo was observed in

20.4% of patients and was significantly more prevalent in women (26% vs. 16%) than in men [12].

There is a strong association between livedo and anticardiolipin antibodies (aCL). Weinstein and co-authors [13] found livedo in almost 50% of patients with SLE. In this cohort of 78 patients there was significant correlation between immunoglobulin G (IgG) aCL and severe to moderate livedo. On the other hand, the prevalence of the lupus anticoagulant was not significantly different between the livedo and non-livedo groups [13].

CLINICAL ASPECTS

• ARTERIAL AND VENOUS THROMBOSIS

Arterial and venous thrombosis was observed in patients with livedo irrespective of the presence of antiphospholipid antibodies (aPL). The relationship between livedo and stroke was first described by Sneddon [6]. Frances et al. [14] described 46 patients with livedo of whom 27 were aPL negative. They found both arterial and venous thrombosis in both groups (positive and negative aPL), and skin biopsies showed arterial occlusions in both groups [14]. Furthermore, Frances et al. reported a significant correlation between livedo and seizures, cardiac valvular defects, systemic hypertension, and Raynaud's phenomenon. Our own studies confirmed that renal artery stenosis in APS was associated with livedo [15]. In a large series of 308 patients, Toubi and colleagues [16] noted an increased prevalence of migraine, stroke and seizure disorder in patients with livedo and APS.

• PREGNANCY MORBIDITY

Pregnancy morbidity was also higher in our patients with SLE and livedo who were negative for antiphospholipid antibodies [17]. These findings further confirmed the observations of Frances et al. [14] that pregnancy loss is more common in livedo patients with and without aPL. Livedo may be independently associated with pregnancy loss in patients who are negative for aPL.

The underlying pathology involves prothrombotic as well as immunologic processes

• ACCELERATED ATHEROSCLEROSIS

A preliminary controlled study in our unit found that ankle brachial pressure index (ABPI) was abnormal in patients with livedo as compared to those without livedo [18]. Taking a clue from this finding, we further investigated ABPI, pulse wave velocity (PWV) and pulse contour analysis in four groups consisting of 74 patients with APS, SLE and APS, and patients with livedo only. In this study, 41 patients had livedo and 33 did not. The PWV was significantly abnormal in the livedo group compared to the non-livedo group. In both groups there were almost equal numbers of patients with APS and SLE [19]. These results suggest that vascular dysfunction may be associated with livedo.

• LIVEDOID VASCULITIS (LV)

Livedo may present as a cutaneous ulcer (livedoid vasculopathy, LV), also known as livedo reticularis ulcerations, and atrophie blanche may be a consequence. This usually affects the legs bilaterally and is more common in summer [20-22]. It may affect all age groups but is more common in young females. Initially, it starts with a painful papular rash that develops into an ulcer. It subsequently heals, leaving a porcelain white scar that is often star-shaped: atrophie blanche [23]. LV was originally described as a clinical manifestation of vasculitis; however, the present concept is that it is a vaso-occlusive phenomenon with thrombosis of intradermal venules [24,25]. LV has been described as secondary LV when associated with a coagulation defect otherwise known as primary or idiopathic.

PATHOPHYSIOLOGY

The pathophysiology of livedo is not clearly defined. The results of skin biopsy depend on the site of the biopsy. Wohlrab and team [26] noted that biopsies from the center of the livedo lesion (white area) had better yield than other areas. Histological findings of livedo were similar in primary APS and APS associated with SLE.

Zelger et al. [27] described 15 patients with Sneddon's syndrome of whom 12 had skin biopsies. Only small to medium-sized arteries of the dermis-subcutis boundary were found to be involved. Lesions follow a distinct course. Zelger et al. described four stages. An initial phase (stage I), characterized by the attachment of lymphohistiocytic cells and detachment of endothelial cells (endothelitis), is followed by an early phase (stage II), which displays partial or complete occlusion of the lumen by a plug of lymphohistiocytic cells and fibrin. In an intermediate phase (stage III), the occluding plug is replaced by proliferating subendothelial cells accompanied by dilated capillaries in the adventitia of the occluded vessel. The late phase (stage IV) shows fibrosis and shrinkage of the affected vessels [27]. Sepp and colleagues [28] studied skin specimens of 18 patients with Sneddon's syndrome, and reported that CD3+, UCHL-1+, and HLA-DR+ cells constituted a significant proportion of the inflammatory infiltrate in the early stages, whereas in later stages, endothelial cells and leukocytes were scarce. These findings indicate the possibility of an inflammatory and/or immunological process involved in the pathogenesis of livedo. There were no significantly different histological findings of livedo on skin biopsies in primary APS and APS associated with SLE [10].

In contrast to these findings, histopathological changes were different in patients with livedoid vasculitis (LV) and are characterized as follows:

- *Initial stage:* Occlusion of dermal vessels, intravascular fibrin deposition and thrombosis with no evidence of sig-

nificant inflammation [24]. In this study there was a high incidence of positive aPL and low levels of tissue plasminogen activators. Indeed treatment with recombinant tissue plasminogen activator helped in resistant cutaneous ulcers that had failed to respond to conventional therapies [29]

- *Intermediate stage:* This stage is characterized by hyalinization in the dermis and sometimes endothelial proliferation. It should be noted that fibrinoid deposition is present in all stages [24]
- *Late stage:* Direct immunofluorescence usually demonstrates deposition of immunoglobulin, fibrin and complement components. In the initial stages here is deposition of fibrin on the vessel walls, and deposition of immunoglobulins and complement is detected in later stages. Schroeder et al. [30] showed that immunoglobulins and complement components (C1q, C3, and properdin) were localized in diseased vessel walls, suggesting an immune pathogenesis.

Thus, LV is a thrombo-occlusive condition with coagulation defects and/or fibrinolysis. Similarly, the description "atrophie blanche" may be attributed to conditions such as LV and other ulcerative conditions on the lower limbs [22].

In summary, there is a wide variation in the histopathological findings in livedo reticularis and LV. The spectrum includes inflammation, thrombosis, hyalinization, and immunological pathology. This probably demonstrates more than one pathogenic mechanism. In addition, as mentioned earlier, abnormal ABPI and PWV suggests accelerated atherosclerosis [18,19].

DIFFERENTIAL DIAGNOSIS

There are a number of other pathological conditions that may present as livedo. Polyarteritis nodosa and antineutrophil cytoplasmic antibody (ANCA)-positive vasculitis, involving small and middle-size arteries may present as cutaneous leg ulcers associated with livedo. A skin biopsy may be confirmatory, showing true vasculitis. Other autoimmune inflammatory diseases such as cryoglobulinemic vasculitis and pyoderma gangrenosum may also manifest in a similar way [4,31,32].

TREATMENT

A variety of treatment approaches have been used and no single treatment has been found ideal. In view of the increased risks of cerebrovascular accident and arterial and venous thrombosis, patients are advised to modify traditional risk factors with measures such as weight loss, lipid-lowering therapies, blood pressure control, diabetes management, smoking cessation, and avoidance of estrogens including contraceptive pills and hormone replacement therapies. Antiplatelet agents such as low dose aspirin, clopidogrel, dipyridamol, and vasodilators such as nifedipine and pentoxyphylline, have been tried prophylactically but the benefits of these therapies are inconsistent [33]. Low molecular weight heparin and oral anticoagulants have shown promising results [34,35].

Despite these treatment options, livedoid ulcers are often resistant to therapy. Several approaches have been tried with variable success. Systemic and intradermal corticosteroids have shown little benefit [33]. Considering the low levels of tissue plasminogen activator, recombinant tissue plasminogen has been tried with some success in difficult-to-treat LV [30,36]. Therapeutic modalities such as intravenous immunoglobulins and hyperbaric oxygen have also been tried with some success [37,38].

SUMMARY

Livedo reticularis is a common cutaneous manifestation of APS and may be a prognostic marker of more severe disease. It is associated with arterial and venous thrombosis and pregnancy morbidity irrespective of the presence of antiphospholipid antibodies. Recent results suggest the possibility of an association with accelerated atherosclerosis in patients with livedo. Given the similarities between APS and livedo (aPL negative), experts in this field believe that livedo may represent the so-called seronegative antiphospholipid syndrome [39], although the exact relationship of livedo with seronegative APS remains to be elucidated.

Livedo reticularis may be a marker of "seronegative" antiphospholipid (Hughes) syndrome

LV may present as painful cutaneous ulcers that are often difficult to treat. The underlying pathology involves prothrombotic as well as immunological processes with some overlap with APS. Treatment remains challenging and results are often variable.

Correspondence
Dr. D.P. D'Cruz
Louise Coote Lupus Unit, 4th Floor, Tower Wing, Guy's Hospital, London SE1 9RT, UK
Phone: (44-207) 188-9756
Fax: (44-207) 188-3574
email: david.d'cruz@kcl.ac.uk

References

1. Ehrmann S. Ein neues Gefaβsymptom bei Lues. *Wiener Med Wochenchrift* 1907; 57: 777-82.
2. Uthman IW, Khamashta MA. Livedo racemosa: a striking dermatological sign for the antiphospholipid syndrome. *J Rheumatol* 2006; 33: 2379-82.
3. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; 4: 295-306.
4. Criado PR, Rivitti EA, Sotto MN, de Carvalho JF. Livedoid vasculopathy as a coagulation disorder. *Autoimmun Rev* 2011; 10: 353-60.
5. Shealy CN, Weeth JB, Mercier D. Livedo reticularis in patients with parkinsonism receiving amantadine. *JAMA* 1970; 212: 1522-3.
6. Sneddon IB. Cerebrovascular lesions and livedo reticularis. *Br J Dermatol* 1965; 77: 180-5.

7. Kraemer M, Linden D, Berlit P. The spectrum of differential diagnosis in neurological patients with livedo reticularis and livedo racemosa. A literature review. *J Neurol* 2005; 252: 1155-66.
8. Hughes G. Connective tissue diseases and the skin. The 1983 Prosser-White Oration. *Clin Exp Dermatol* 1984; 9: 535-44.
9. Asherson RA, Khamashta MA, Ordi-Ros J, et al. The "primary" antiphospholipid syndrome: major clinical and serological features. *Medicine (Baltimore)* 1989; 68: 366-74.
10. Francès C, Niang S, Laffitte E, Pelletier Fl, Costedoat N, Piette JC. Dermatologic manifestations of the antiphospholipid syndrome: two hundred consecutive cases. *Arthritis Rheum* 2005; 52: 1785-93.
11. Asherson RA, Mayou SC, Merry P, Black MM, Hughes GR. The spectrum of livedo reticularis and anticardiolipin antibodies. *Br J Dermatol* 1989; 12: 215-21.
12. Cervera R, Piette JC, Font J, et al. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis Rheum* 2002; 46: 1019-27.
13. Weinstein C, Miller MH, Axtens R, Buchanan R, Littlejohn GO. Livedo reticularis associated with increased titers of anticardiolipin antibodies in systemic lupus erythematosus. *Arch Dermatol* 1987; 123: 596-600.
14. Francès C, Papo T, Wechsler B, Laporte JL, Biousse V, Piette JC. Sneddon syndrome with or without antiphospholipid antibodies. A comparative study in 46 patients. *Medicine (Baltimore)* 1999; 78: 209-19.
15. Sangle S, D'Cruz D, Wajanat J, et al. Renal artery stenosis in antiphospholipid syndrome and hypertension. *Ann Rheum Dis* 2003; 62: 999-1002.
16. Toubi E, Krause I, Fraser A, et al. Livedo reticularis is a marker for predicting multi-system thrombosis in antiphospholipid syndrome. *Clin Exp Rheumatol* 2005; 23: 499-504.
17. Sangle S, D'Cruz DP, Hughes GR. Livedo reticularis and pregnancy morbidity in patients negative for antiphospholipid antibodies. *Ann Rheum Dis* 2005; 64: 147-8.
18. Sangle S, Christodoulou C, Paul S, Hughes GR, D'Cruz DP. The point prevalence of an abnormal ankle-brachial index in antiphospholipid antibody negative patients with livedo reticularis: a controlled study. *Ann Rheum Dis* 2008; 67: 276-7.
19. Sangle SR, Tanikawa A, Schreiber K, Zakalka M, D'Cruz DP. The prevalence of abnormal pulse wave velocity, pulse contour analysis and ankle-brachial index in patients with livedo reticularis: a controlled study. *Rheumatology (Oxford)* 2013; 52: 1992-8.
20. Feldaker M, Hines EA Jr, Kierland RR. Livedo reticularis with summer ulcerations. *AMA Arch Derm* 1955; 72: 31-42.
21. Feldaker M, Hines EA Jr, Kierland RR. Livedo reticularis with ulcerations. *Circulation* 1956; 13: 196-216.
22. Alavi A, Hafner J, Dutz JP, Mayer D, Sibbald RG, Criado PR, Livedoid vasculopathy: an in-depth analysis using a modified Delphi approach. *J Am Acad Dermatol* 2013; 69: 1033-42.
23. Stevanović DV Atrophie blanche. A sign of dermal blood occlusion. *Arch Dermatol* 1974; 109: 858-62.
24. Criado PR, Rivitti EA, Sotto MN, Valente NY, Aoki V, Carvalho JF, Livedoid vasculopathy: an intriguing cutaneous disease. *An Bras Dermatol* 2011; 86: 961-77.
25. Papi M, Didona B, De Pità O, et al. Livedo vasculopathy vs small vessel cutaneous vasculitis: cytokine and platelet P-selectin studies. *Arch Dermatol* 1998; 134: 447-52.
26. Wohlrab J, Fischer M, Wolter M, Marsch WC. Diagnostic impact and sensitivity of skin biopsies in Sneddon's syndrome. A report of 15 cases. *Br J Dermatol* 2001; 145: 285-8.
27. Zelger B, Sepp N, Schmid KW, Hintner H, Klein G, Fritsch PO. Life history of cutaneous vascular lesions in Sneddon's syndrome. *Hum Pathol* 1992; 23: 668-75.
28. Sepp N, Zelger B, Schuler G, Romani N, Fritsch P. Sneddon's syndrome – an inflammatory disorder of small arteries followed by smooth muscle proliferation. Immunohistochemical and ultrastructural evidence. *Am J Surg Pathol* 1995; 19: 448-53.
29. Klein KL, Pittelkow MR. Tissue plasminogen activator for treatment of livedoid vasculitis. *Mayo Clin Proc* 1992; 67: 923-33.
30. Schroeter AL, Diaz-Perez JL, Winkelmann RK, Jordan RE. Livedo vasculitis (the vasculitis of atrophie blanche). Immunohistopathologic study. *Arch Dermatol* 1975; 111: 188-93.
31. George T. Livedoid vasculopathy. Pathogenesis, diagnosis and treatment of cutaneous infarction. *Hautarzt* 2011; 62: 627-34.
32. Hairston BR, Davis MD, Pittelkow MR, Ahmed I. Livedoid vasculopathy: further evidence for procoagulant pathogenesis. *Arch Dermatol* 2006; 142: 1413-18.
33. Maessen-Visch MB, Koedam MI, Hamulyák K, Neumann HA. Atrophie blanche. *Int J Dermatol* 1999; 38: 161-72.
34. Goerge T, Weishaupt C, Metze D, et al. Livedoid vasculopathy in a pediatric patient with elevated lipoprotein(a) levels: prompt response to continuous low-molecular-weight heparin. *Arch Dermatol* 2010; 146: 927-8.
35. Di Giacomo TB, Hussein TP, Souza DG, Criado PR. Frequency of thrombophilia determinant factors in patients with livedoid vasculopathy and treatment with anticoagulant drugs – a prospective study. *J Eur Acad Dermatol Venereol* 2010; 24: 1340-6.
36. Deng A, Gocke CD, Hess J, Heyman M, Paltiel M, Gaspari A. Livedoid vasculopathy associated with plasminogen activator inhibitor-1 promoter homozygosity (4G/4G) treated successfully with tissue plasminogen activator. *Arch Dermatol* 2006; 142: 1466-9.
37. Kreuter A, Gambichler T, Breuckmann F, et al. Pulsed intravenous immunoglobulin therapy in livedoid vasculitis: an open trial evaluating 9 consecutive patients. *J Am Acad Dermatol* 2004; 51: 574-9.
38. Yang CH, Ho HC, Chan YS, Liou LB, Hong HS, Yang LC. Intractable livedoid vasculopathy successfully treated with hyperbaric oxygen. *Br J Dermatol* 2003; 149: 647-52.
39. Rodriguez-Garcia JL, Bertolaccini ML, Cuadrado MJ, et al. Clinical manifestations of antiphospholipid syndrome (APS) with and without antiphospholipid antibodies (the so-called 'seronegative APS'). *Ann Rheum Dis* 2012; 71: 242-4.

Capsule

Synaptic, transcriptional and chromatin genes disrupted in autism

The genetic architecture of autism spectrum disorder involves the interplay of common and rare variants and their impact on hundreds of genes. Using exome sequencing, Rubeis and collaborators show that analysis of rare coding variation in 3871 autism cases and 9937 ancestry-matched or parental controls implicates 22 autosomal genes at a false discovery rate (FDR) < 0.05, plus a set of 107 autosomal genes strongly enriched for those likely to affect risk (FDR < 0.30). These 107 genes, which show unusual evolutionary constraint against mutations, incur de novo loss-of-function mutations in over 5% of autistic subjects. Many of the genes implicated encode proteins for synaptic formation, transcriptional regulation and chromatin-remodeling pathways. These include voltage-gated ion channels regulating the propagation of action potentials, pacemaking and excitability-transcription coupling, as well as histone-modifying enzymes and chromatin remodelers – most prominently those that mediate post-translational lysine methylation/demethylation modifications of histones.

Nature 2014; 515: 209

Eitan Israeli

New Autoantibodies in Inflammatory Myopathies: Diagnostic Value and Relationship with Clinical Phenotypes

Albert Selva-O'Callaghan MD PhD[1] and Ricard Cervera MD PhD FRCP[2]

[1]Department of Internal Medicine, General Hospital Vall D'Hebron, Universitat Autònoma de Barcelona, Barcelona, Catalonia, Spain
[2]Department of Autoimmune Diseases, Hospital Clínic, Barcelona, Catalonia, Spain

KEY WORDS: inflammatory myopathies, polymyositis, dermatomyositis, inclusion body myositis, autoantibodies

IMAJ 2015; 17: 108–109

Classification of idiopathic inflammatory myopathies (i.e., myositis) has always been difficult. In their seminal work on polymyositis and dermatomyositis in 1975, Bohan and Peter [1] were the first to establish a rational classification. Although still useful today, it has some limitations. Certain inflammatory myopathies, such as the recently described inclusion body myositis, are not included, and dystrophies such as dysferlinopathy might be misdiagnosed as polymyositis using these criteria. In 1991, Dalakas [2] proposed a new classification that focuses on pathological criteria and enables classification of patients with dermatomyositis, polymyositis, or inclusion body myositis. The main limitation of this system is that polymyositis, which is now considered a diagnosis of exclusion [3], is likely to be over-diagnosed. In fact, some authors suggest it may be as rare as unicorns, dragons and other mythological beasts [4,5].

Love et al. [6], also in the 1990s, reported that the presence of specific autoantibodies (anti-Mi2, anti-Jo1, anti-SRP) or associated autoantibodies (e.g., anti-Ro60/52, anti-La, anti-RNP, anti-PM/Scl) could be of help for subclassifying myositis, with each antibody being ascribed to a characteristic clinical phenotype. Such is the case of antisynthetase syndrome, characterized by the presence of myositis, arthritis, fever, Raynaud's phenomenon, and interstitial lung disease, associated with anti-Jo1 antibodies.

Since Love's publication and particularly in the last 5 years, several new autoantibodies have been described and related to specific clinical phenotypes, and this has often implied the use of specific diagnostic or therapeutic approaches. For example, anti-TIF1γ (formerly known as *anti-p155* based on its molecular weight on protein immunoprecipitation analysis), has proved to be a good marker of cancer-associated myositis [7,8]. Anti-MDA5 antibodies, which are strongly associated with rapidly progressive interstitial lung disease in patients with clinical amyopathic dermatomyositis [9], and the recently described autoantibodies against 3-hydroxy-3-methylglutaryl-coenzyme reductase (anti-HMGCR), which identify a subset of patients with statin-related myopathy and histological findings of immune mediated necrotizing myopathy [10], are other examples of the utility of these myositis-specific or -associated antibodies in diagnosing and classifying the various myositis groups.

The last member of the network is the recently described anticortactin antibody [11-13]. At present, anticortactin seems to be only a myositis-associated autoantibody, but a specific related phenotype may emerge as additional myositis cohorts are analyzed. According to recently published data [11, 12], anticortactin antibody can be used as a marker only of autoimmune myositis, which is important because other myopathies such as dystrophies or metabolic muscle diseases can mimic true autoimmune polymyositis. Nonetheless, cortactin is a ubiquitous protein that plays several roles in our organism. It acts in the assembly of actin filament in muscle as well as in adhesion and migration of non-muscle cells, particularly neoplastic cells. Furthermore, cortactin seems to be implicated in tumor cell motility and metastasis, and it has been recognized for its association with cancer progression [14]. Therefore, anticortactin antibodies could also be relevant in patients with cancer-associated myositis.

As often occurs in science, the discovery of this autoantibody was a matter of chance, a serendipitous phenomenon. Researchers found an unexpected band on blot studies performed to confirm the results of enzyme-linked immunosorbent assay (ELISA)-positive anti-MDA5 and anti-HMGCR antibodies. On further analysis by mass spectrometry, the band was found to be cortactin. In parallel, other researchers found the same autoantibody in seronegative myasthenia gravis patients. The next logical step would be to investigate whether these antibodies are directed against the same or different epitopes of the molecule in myositis and myasthenia gravis patients, and to identify them.

Considering the emerging data, it seems that the diagnosis and classification of myositis will rely not only on clinical grounds and muscle pathology but also on the presence of myositis-specific and associated autoantibodies [15]. As occurred with anticortactin antibodies, identification of new autoantibodies will further help clinicians in the task of diagnosing and treating patients with inflammatory myopathy.

Acknowledgment
This study was funded in part by a grant from the Spanish Ministry of Health and Consumer Affairs (PI12-01320)

Correspondence
Dr. A. Selva-O'Callaghan
Siracusa,12 bis "A", Barcelona, Catalonia, Spain
email: aselva@vhebron.net

References

1. Bohan A, Peter JB. Polymyositis and dermatomyositis. *N Engl J Med* 1975; 292: 344-7, 403-7.
2. Dalakas MC. Polymyositis, dermatomyositis and inclusion-body myositis. *N Engl J Med* 1991; 325: 1487-98.
3. Milisenda JC, Selva-O'Callaghan A, Grau JM. The diagnosis and classification of polymyositis. *J Autoimmun* 2014; 48-49: 118-21.
4. van der Meulen MF, Bronner IM, Hoogendijk JE, et al. Polymyositis: an overdiagnosed entity. *Neurology* 2003; 61: 316-21.
5. Amato AA, Griggs RC. Unicorns, dragons, polymyositis, and other mythological beasts. *Neurology* 2003; 6: 288-9.
6. Love LA, Leff RL, Fraser DD, et al. A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. *Medicine (Baltimore)* 1991; 70: 360-74.
7. Labrador-Horrillo M, Martínez MA, Selva-O'Callaghan A, et al. Anti-TIF1γ antibodies (anti-p155) in adult patients with dermatomyositis: comparison of different diagnostic assays. *Ann Rheum Dis* 2012; 71: 993-6.
8. Trallero-Araguás E, Rodrigo-Pendás JÁ, Selva-O'Callaghan A, et al. Usefulness of anti-p155 autoantibody for diagnosing cancer-associated dermatomyositis: a systematic review and meta-analysis. *Arthritis Rheum* 2012; 64: 523-32.
9. Chen Z, Cao M, Plana MN, et al. Utility of anti-melanoma differentiation-associated gene 5 antibody measurement in identifying patients with dermatomyositis and a high risk for developing rapidly progressive interstitial lung disease: a review of the literature and a meta-analysis. *Arthritis Care Res (Hoboken)* 2013; 65: 1316-24.
10. Mammen AL, Chung T, Christopher-Stine L, et al. Autoantibodies against 3-hydroxy-3-methylglutaryl-coenzyme a reductase (HMGCR) in patients with statin-associated autoimmune myopathy. *Arthritis Rheum* 2011; 63: 713-21.
11. Labrador-Horrillo M, Martínez MA, Selva-O'Callaghan A, et al. Identification of a novel myositis-associated antibody directed against cortactin. *Autoimmun Rev* 2014; 13: 1008-12.
12. Gallardo E, Martínez-Hernández E, Titulaer MJ, et al. Cortactin autoantibodies in myasthenia gravis. *Autoimmun Rev* 2014; 13: 1003-7.
13. Berrih-Aknin S. Cortactin: A new target in autoimmune myositis and myasthenia gravis. *Autoimmun Rev* 2014; 13: 1001-2.
14. Martinez-Quiles N. Emerging roles of hematopoietic lineage cell-specific protein 1 in the immune system. *OA Immunology* 2013; 1: 2.
15. Hilton-Jones D. Myositis mimics: how to recognize them. *Curr Opin Rheumatol* 2014; 26: 663-70.

Capsule

Human genetics shape the gut microbiome

Host genetics and the gut microbiome can both influence metabolic phenotypes. However, whether host genetic variation shapes the gut microbiome and interacts with it to affect host phenotype is unclear. Goodrich et al. compared microbiotas across › 1000 fecal samples obtained from the Twins UK population, including 416 twin pairs. The authors identified many microbial taxa whose abundances were influenced by host genetics. The most heritable taxon, the family Christensenellaceae, formed a co-occurrence network with other heritable bacteria and with methanogenic Archaea. Furthermore, Christensenellaceae and its partners were enriched in individuals with low body mass index (BMI). An obese-associated microbiome was amended with *Christensenella minuta*, a cultured member of the Christensenellaceae, and transplanted to germ-free mice. *C. minuta* amendment reduced weight gain and altered the microbiome of recipient mice. These findings indicate that host genetics influence the composition of the human gut microbiome and can do so in ways that impact host metabolism.

Cell 2014; 159: 789

Eitan Israeli

Capsule

A microRNA up-regulated in asthma airway T cells promotes T_H2 cytokine production

MicroRNAs (miRNAs) exert powerful effects on immunological function by tuning networks of target genes that orchestrate cell activity. Simpson et al. sought to identify miRNAs and miRNA-regulated pathways that control the type 2 helper T cell (T_H2 cell) responses that drive pathogenic inflammation in asthma. Profiling miRNA expression in human airway-infiltrating T cells revealed elevated expression of the miRNA miR-19a in asthma. Modulating miR-19 activity altered T_H2 cytokine production in both human and mouse T cells, and T_H2 cell responses were markedly impaired in cells lacking the entire miR-17~92 cluster. miR-19 promoted T_H2 cytokine production and amplified inflammatory signaling by direct targeting of the inositol phosphatase PTEN, the signaling inhibitor SOCS1 and the deubiquitinase A20. Thus, up-regulation of miR-19a in asthma may be an indicator and a cause of increased T_H2 cytokine production in the airways.

Nature Immunol 2014; 15: 1162

Eitan Israeli

"Intellectual property has the shelf life of a banana"

Bill Gates (born 1955), American business magnate, philanthropist, investor, computer programmer, inventor, and co-founder of Microsoft, the world's largest PC software company. He has pursued a number of philanthropic endeavors, donating large amounts of money to various charitable organizations and scientific research programs through the Bill & Melinda Gates Foundation

New Therapeutic Approaches to Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitides: Looking at Tomorrow

Alessandra Soriano MD

Department of Clinical Medicine and Rheumatology, Campus Bio-Medico University, Rome, Italy

KEY WORDS: antineutrophil cytoplasmic antibodies (ANCA), ANCA-associated vasculitis, small vessel vasculitis, alternative pathway, complement

IMAJ 2015; 17: 110–111

Almost 50 years have passed since the addition of cyclophosphamide to steroids, which became established as the main therapeutic regimen for patients with small vessels vasculitides. This derived mainly from the contribution of Fauci et al. who had amassed considerable clinical and therapeutic experience in the early 1970s [1,2]. This approach has represented the standard therapy for small vessels vasculitides for more than two decades, at least until the first randomized trial performed by the European Vasculitis Study Group in 2003 [3], when reducing the cumulative dosage of cyclophosphamide, via a switching therapy schedule with azathioprine or methotrexate, was proposed as maintenance therapy after the induction of disease remission. This option was then supported by further evidence over the years [4].

More recently, the introduction of B cell-targeted therapy has undoubtedly been one of the major breakthroughs in the management of these disorders, defined more precisely today as antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitides (AAV). A substantial equivalence between the anti-CD20 monoclonal antibody rituximab and the standard immunosuppressive therapy for the induction of complete remission was shown by the RAVE-ITN trial in 2010 [5]. Nonetheless, the rates of relapse have not been markedly improved by the use of rituximab induction [6], and the maintenance of remission is still one of the most intriguing therapeutic challenges in the field of AAV.

The latest evidence dates back to last November, when Guillevin and team [7] reported the superiority of repeated doses of rituximab for AAV relapse prevention as compared with azathioprine during a total follow-up period of 28 months. However, as observed by Jayne in his editorial in the same issue of the *New England Journal of Medicine* [8], "the duration of the follow-up after the last dose of rituximab was short […] thus, the problem of longer term relapse risk and the need for further observation and therapy remain," as does the problem of the high incidence of rituximab-related severe adverse events [8].

Despite the expansion of the therapeutic armamentarium, the issue of severe side effects related to the above therapies indeed remains a pivotal point, together with the difficulty in restoring, or preserving in the long term, the renal function of affected patients [9]. In addition, the relapse rate within 5 years of the diagnosis of AAV is still very high, especially in patients with proteinase 3 (PR3)-AAV [9].

Therefore, the challenges in the approach to AAV are focused on identifying new therapeutic targets, which eventually will be better tolerated and more specific. Furthermore, new insight into AAV pathogenesis might be useful to identify additional disease markers in order to improve monitoring of the disease and prediction of relapse [9].

What about future research directions for AAV therapies? Although the role of B cells in AAV has not been completely elucidated, and given the evidence of the impressive efficacy of rituximab in AAV, it has been postulated that other B cell targets could be potential therapeutic alternatives in AAV management. Ofatunumab, a novel anti-CD20 monoclonal antibody, has shown a potent ability in binding CD20, leading to greater complement-dependent cytotoxicity [10]. Epratuzumab, the anti-CD22 monoclonal antibody that acts as an immunomodulatory agent by inducing B cell anergy, has been tested in systemic lupus erythematosus (SLE) and might be a potential option in some AAV cases [11]. Belimumab, the anti-BAFF monoclonal antibody approved for SLE, is currently under investigation as another option for preventing AAV relapse (ClinicalTrials.gov No NCT01663623).

Furthermore, the contribution of the alternative complement pathway in the pathogenesis of AAV has been investigated intensely in recent years, despite the fact that AAV are usually considered pauci-immune diseases in which immune complex and complement deposition are typically absent or scarce [12]. Alternative pathway activation has been related to different neutrophil-mediated diseases, including AAV, but the exact mechanisms through which this pathway and the neutrophils interact remain largely unstudied.

Xiao et al. [13] were the first to suggest a critical role for complement in the induction of necrotizing crescentic glomerulonephritis through their mouse model of ANCA disease. The authors transferred anti-MPO antibodies, induced in MPO-deficient mice, into wild-type mice, thus determining crescentic glomerulonephritis. When the recipient mice were deficient in complement C5 or factor B, they did not develop the disease; on the contrary, glomerulonephritis was detected in the same animal models when they were deficient in

C4, a factor of the classical and mannose-binding lectin pathways of complement. The same group also showed that pretreating mice with C5-inhibiting monoclonal antibody prevented the development of renal damage.

In 2009 Xing et al. [14] detected several components of the alternative pathway, such as factor B, factor P (properdin), C3d and the membrane attack complex in glomeruli and in small blood vessels of kidney biopsy specimens from AAV patients. The levels of C5a, a cleavage product of complement C5 with anaphylatoxic and chemotactic features, which is responsible for the amplification of the inflammatory response and represents the final event in the activation of that pathway, were then found by Yuan et al. [15] to be elevated in active AAV in patients' plasma and urine. The authors later demonstrated the alternative pathway activation in the circulation of a larger population of patients with active AAV and confirmed the strong increase in plasma levels of C5a, as compared with inactive AAV patients [16].

The rationale for inhibiting C5a underlies the ongoing phase 2 trial (CLEAR; ClinicalTrials.gov No NCT01363388), which is investigating the role of CCX168, a novel orally administered small molecule inhibitor of C5a receptor (C5aR), in patients with AAV [17].

In the present issue of this journal, Ballanti and colleagues [18] pointed their attention to the complement system as a potential target for novel therapeutic approaches to small-medium vessel vasculitides. The authors reviewed the contribution of complement system in the pathogenesis of such diseases and discussed the rationale of blocking the alternative complement pathway, which participates in several innate and adaptive immune functions. Intriguingly, it has been observed that ANCA-activated neutrophils themselves release factors that might contribute to the activation of complement system [13]. Myeloperoxidase (MPO) has been shown to mediate the alternative pathway-induced C3 deposition in some models [19]. There is evidence that properdin, present in secondary neutrophil granules, is instrumental in determining this event because it can directly interact with neutrophil components, namely MPO, but also with proteinase 3 (PR3), elastase and cathepsine G. Properdin is the only known 'positive regulator' in the complement system that acts as initiator of the alternative complement pathway after neutrophil degranulation. Given its 'pattern recognition' capability, properdin might represent another potential target to be investigated in the near future [19]. However, given the role of the alternative pathway and particularly of properdin pivotal in maintaining health, there is concern about possible side effects related to this approach. Clearly, discovering further details of the pathophysiological mechanisms of AAV will set the basis for a wider spectrum of potential therapeutic targets.

The growing knowledge of innate immunity mechanisms coming from basic research – not least the release of neutrophil extracellular traps (NETs), a process referred to as 'NETosis' in the course of AAV – will certainly open new perspectives on these devastating diseases whose biological mechanisms still represent a fascinating and stimulating conundrum. Finally, due to the rarity of AAV conditions, they represent a prime example of a research area that might take advantage of international research cooperation programs, given the limited number of patients, the fragmentation of research itself, and the lack of widespread expertise in such a field. For all these reasons, multicenter studies and clinical trials are crucial to confirm the preliminary evidence and to orientate both basic and clinical research [20].

Correspondence
Dr. A. Soriano
Dept. of Clinical Medicine and Rheumatology, Campus Bio-Medico University, Rome, Italy
Phone: (39-622) 541-1340
Fax: (39-622) 541-456
email: soriano.alessandra@gmail.com

References

1. Fauci AS, Haynes BF, Katz P, Wolff SM. Wegener's granulomatosis: prospective clinical and therapeutic experience with 85 patients for 21 years. *Ann Intern Med* 1992; 98: 76-85.
2. Hoffman GS, Kerr GS, Leavitt RY, et al. Wegener granulomatosis: an analysis of 158 patients. *Ann Intern Med* 1992; 116: 488-98.
3. Jayne D, Rasmussen N, Andrassy K, et al.; European Vasculitis Study Group. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N Engl J Med* 2003; 349: 36-44.
4. Pagnoux C, Mahr A, Hamidou MA, et al.; French Vasculitis Study Group. Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. *N Engl J Med* 2008; 359: 2790-803.
5. Stone JH, Merkel PA, Spiera R, et al.; RAVE-ITN Research Group. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med* 2010; 363; 221-32.
6. Specks U, Merkel PA, Seo P, et al.; RAVE-ITN Research Group. Efficacy of remission-induction regimens for ANCA-associated vasculitides. *N Engl J Med* 2013; 369: 417-27.
7. Guillevin L, Pagnoux C, Karras A, et al.; French Vasculitis Study Group. Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. *N Engl J Med* 2014; 371: 1771-80.
8. Jayne D. Extending the indications for rituximab in ANCA-associated vasculitis. *N Engl J Med* 2014; 371: 1839-40.
9. Sanders JS, Abdulahad WH, Stegeman CA, Kallenberg CG. Pathogenesis of antineutrophil cytoplasmic autoantibody-associated vasculitis and potential targets for biologic treatment. *Nephron Clin Pract* 2014; 128: 216-223.
10. Cheson BD. Ofatunumab, a novel anti-CD20 monoclonal antibody for the treatment of B-cell malignancies. *J Clin Oncol* 2010; 28: 3525-30.
11. Wallace DJ, Kalunian K, Petri MA, et al. Efficacy and safety of epratuzumab in patients with moderate/severe active systemic lupus erythematosus: results from EMBLEM, a phase IIb, randomised, double-blind, placebo controlled, multicentre study. *Ann Rheum Dis* 2014; 73: 183-90.
12. Wilde B, van Paassen P, Witzke O, Tervaert JW. New pathophysiological insights and treatment of ANCA associated vasculitis. *Kidney Int* 2011; 79: 599-612.
13. Xiao H, Schreiber A, Heeringa P, Falk RJ, Jennette JC. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am J Pathol* 2007; 170: 52-64.
14. Xing GQ, Chen M, Liu G, et al. Complement activation is involved in renal damage in human antineutrophil cytoplasmic autoantibodies associated pauci-immune vasculitis. *J Clin Immunol* 2009; 29: 282-91.
15. Yuan J, Gou SJ, Huang J, Hao J, Chen M, Zhao MH. C5a and its receptors in human anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis. *Arthritis Res Ther* 2012; 14: R140.
16. Gou SJ, Yuan J, Chen M, Yu F, Zhao MH. Circulating complement activation in patients with anti-neutrophil cytoplasmic antibody-associated vasculitis. *Kidney Int* 2013; 83: 129-37.
17. Furuta S, Jayne D. Emerging therapies in anti-neutrophil cytoplasm antibody-associated vasculitis. *Curr Opin Rheumatol* 2014; 26: 1-6.
18. Ballanti E, Chimenti MS, Perricone R. Small-medium vessel vasculitides: is the complement system a potential forgotten target? *IMAJ* 2015; 17: 85-92.
19. O'Flynn J, Dixon KO, Faber Krol MC, Daha MR, van Kooten C. Myeloperoxidase directs properdin-mediated complement activation. *J Innate Immun* 2014; 6: 417-25.
20. Holle JU, Gross WL. Treatment of ANCA-associated vasculitides (AAV). *Autoimmun Rev* 2013; 12: 483-6.

Tumor Necrosis Factor-Alpha at the Crossroad between Rheumatoid Arthritis and Autoimmune Cholangitis

Carlo Selmi MD PhD[1,2], Elena Generali MD[1,2] and Luca Cantarini MD PhD[3]

[1]Division of Rheumatology and Clinical Immunology, Humanitas Research Hospital, Rozzano, Milan, Italy
[2]BIOMETRA Department, University of Milan, Milan, Italy
[3]Research Center of Systemic Autoinflammatory Diseases and Behçet's Disease Clinic, Department of Medical Sciences, Surgery and Neurosciences, University of Siena, Siena, Italy

KEY WORDS: autoimmune cholangitis, inflammatory arhritis, anti-tumor necrosis factor-alpha (anti-TNFα), biologic agents

IMAJ 2015; 17: 112–113

We have been largely convinced by data presented in recent years that common factors outnumber differences between autoimmune diseases, particularly when pathogenetic mechanisms are evaluated [1,2], as in the case of B or CD8+ T cells [3], with obvious therapeutic implications [4,5]. Nonetheless, in some cases this may be a double-edged sword, as represented by liver autoimmunity induced by biologic drugs [6], while in others the frequent coexistence of multiple autoimmune conditions allows evaluation of new treatments in unsuspected diseases [7]. In this issue of *IMAJ* Kovacs and colleagues [8] describe a case in which two paradigmatic organ-specific and systemic autoimmune diseases, i.e., primary biliary cirrhosis (PBC) and rheumatoid arthritis (RA), were successfully treated by targeting tumor necrosis factor-alpha (TNFα) with etanercept.

RA and PBC are both autoimmune diseases of largely unknown etiology sharing some similarities, such as the presence of specific serum autoantibodies (namely rheumatoid factor, anti-citrullinated peptides for RA, and anti-mitochondrial antibodies for PBC), the possibility of seronegative patients, and the striking predominance of female patients [9]. RA is a chronic systemic inflammatory disease affecting approximately 0.5–1% of the general population, where genetics, environmental risk factors (such as smoking) and autoimmunity contribute to disease onset. RA is characterized by symmetric articular involvement, chronic synovitis and systemic inflammation with significant cardiovascular morbidity [10,11]. The pathogenesis of RA is based on chronic inflammation that initiates when a T cell is activated by an endogenous trigger, which in turn activates macrophages and fibroblasts to produce TNFα, interleukin (IL)-6, IL-17, IL-1 and IL-23. Also B cells are involved in RA pathogenesis, as the presence of specific autoantibodies demonstrates, and once started, inflammation is chronically maintained by the continued afflux of new inflammatory cells in the area. In contrast, PBC is a rare chronic autoimmune cholestatic liver disease, affecting about 20/1,000,000 men and 20/100,000 women, mostly perimenopausal, in the general population [12]. Serum anti-mitochondrial antibody (AMA) is found in 95% of patient sera, usually at high titers, and the disease is largely asymptomatic [2].

Similar to RA, PBC etiology remains enigmatic, and apparently both genetics and environmental agents have a role in the development of the disease [13-15], as is well demonstrated by monozygotic twins [16]. Both innate immunity and adaptive immunity are involved in the development of PBC [17], but the role of TNFα in PBC has not been clarified. Its expression appears to be enhanced in biliary epithelial cells, possibly leading to PBC cholestasis and inflammation, while some studies have shown opposite findings. Its expression, nonetheless, decreases after ursodeoxycholic acid (UDCA) therapy, which is currently the only approved therapy for PBC. TNFα genetic polymorphisms have been evaluated to assess whether they are associated with a higher risk of PBC developing compared to the general population, but different results have been reported, particularly in genome-wide studies.

Despite the similarities between these two diseases, the therapeutic approaches are very different. In fact, in RA, there is a broad armamentarium of drugs, such as disease modifying anti-rheumatic drugs (DMARDs) and biologic agents, especially directed at blocking TNFα as well as other cytokines such as IL-6 and, most recently, intracellular signaling inhibitors. In contrast, the PBC therapeutic cornerstone remains UDCA, and the only ultimate cure is liver transplantation [2]. In recent years however, different therapeutic approaches are being evaluated in this rare disease, mainly because of unsatisfactory responses to the standard therapy. Methotrexate (MTX) and other immunosuppressant drugs, as well as biologic agents such as rituximab, have been evaluated, while the newest promising data have been provided for obeticholic acid. In the case of PBC, one should be aware that liver tests in general and alkaline phosphatase levels in particular are the only outcome measures to be used in clinical trials because changes in liver histology occur over several years.

Etanercept is a soluble recombinant TNFα receptor used in the treatment of different rheumatic and dermatologic diseases

(i.e., RA, polyarticular juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, plaque psorisias). It is administered subcutaneously at a dosage of 50 mg/week, but its use is hampered by adverse events including latent infection reactivation, recurrent infections, and by injection site reactions; apart from that the treatment is generally well tolerated. It has been used off-label for vasculitis, especially Wegener's granulomatosis, but it did not show efficacy.

When two different autoimmune conditions are approached, the treatment options are sometimes limited, especially when putative drugs for one disease, e.g., MTX, may potentially cause a worsening of the other condition. Cases of PBC associated with rheumatic conditions treated with anti-TNFα agents have been reported previously, with conflicting outcomes. The first, by Spadaro et al. in 2008 [18], was a report on anti-TNFα treatment for a patient with both PBC and RA. However, since infliximab treatment did not yield a satisfactory clinical response and there was no amelioration of liver test abnormalities, etanercept was started and led to a prompt clinical response and liver test normalization. Second, Ogata et al. [19] also reported the case of a woman with PBC and RA treated with etanercept that resulted in improvement in both clinical symptoms and liver test abnormalities. A third case of PBC associated with RA treated with etanercept was reported by Kubo et al. [20], and in this case, as well, etanercept improved the results of liver tests.

The response to etanercept in the previous cases and in the case reported in this issue of *IMAJ* suggests that TNFα is important in PBC pathogenesis. Thanks to our better understanding of disease pathogenesis, novel biological treatments targeting not only TNFα will likely develop in the next few years and data from comorbidities will be crucial. As examples, tocilizumab may ameliorate RA, and limit systemic sclerosis overlap, PBC, and generalized lymphadenopathy. Beyond the therapeutic implications, the present case report [8] illustrates the possibility that liver test abnormalities observed in rheumatic diseases may be unrelated to DMARDs but may represent the coexistence of PBC with its common manifestations. Furthermore, novel treatment targets are being developed as the pathogenesis of these diseases becomes more clear; this will lead hopefully to more specific treatments with less serious adverse events and to tackling more than one pebble in the mosaic of autoimmunity.

Correspondence
Dr. C. Selmi
Division of Rheumatology and Clinical Immunology, Humanitas Clinical and Research Center, via A. Manzoni 56, 20089 Rozzano, Milan, Italy
Tel: (39-02) 8224-5129
Fax: (39-02) 8224-2298
email: carlo.selmi@unimi.it

References

1. Selmi C, Lu Q, Humble MC. Heritability versus the role of the environment in autoimmunity. *J Autoimmun* 2012; 39: 249-52.
2. Selmi C, Bowlus CL, Gershwin ME, Coppel RL. Primary biliary cirrhosis. *Lancet* 2011; 377: 1600-9.
3. Carvalheiro H, da Silva JA, Souto-Carneiro MM. Potential roles for CD8(+) T cells in rheumatoid arthritis. *Autoimmun Rev* 2013; 12: 401-9.
4. Chighizola CB, Favalli EG, Meroni PL. Novel mechanisms of action of the biologicals in rheumatic diseases. *Clin Rev Allergy Immunol* 2014; 47: 6-16.
5. Epis OM, Giacomelli L, Deidda S, Bruschi E. Tight control applied to the biological therapy of rheumatoid arthritis. *Autoimmun Rev* 2013; 12: 839-41.
6. Efe C. Drug induced autoimmune hepatitis and TNF-alpha blocking agents: is there a real relationship? *Autoimmun Rev* 2013; 12: 337-9.
7. Tani C, D'Aniello D, Delle Sedie A, et al. Rhupus syndrome: assessment of its prevalence and its clinical and instrumental characteristics in a prospective cohort of 103 SLE patients. *Autoimmun Rev* 2013; 12: 537-41.
8. Kovacs A, Siminischi GA, Baksay A, Gall A, Takacs M, Szekanecz Z. Successful etanercept treatment of primary biliary cirrhosis associated with rheumatoid arthritis. *IMAJ* 2015; 15: 114-16.
9. Floreani A, Franceschet I, Cazzagon N, et al. Extrahepatic autoimmune conditions associated with primary biliary cirrhosis. *Clin Rev Allergy Immunol* 2014; May 9. [Epub ahead of print]
10. Bartoloni E, Alunno A, Bistoni O, Gerli R. Cardiovascular risk in rheumatoid arthritis and systemic autoimmune rheumatic disorders: a suggested model of preventive strategy. *Clin Rev Allergy Immunol* 2013; 44: 14-22.
11. Hollan I, Meroni PL, Ahearn JM, et al. Cardiovascular disease in autoimmune rheumatic diseases. *Autoimmun Rev* 2013; 12: 1004-15.
12. Podda M, Selmi C, Lleo A, Moroni L, Invernizzi P. The limitations and hidden gems of the epidemiology of primary biliary cirrhosis. *J Autoimmun* 2013; 46: 81-7.
13. Miller FW, Pollard KM, Parks CG, et al. Criteria for environmentally associated autoimmune diseases. *J Autoimmun* 2012; 39: 253-8.
14. Selmi C, Leung PS, Sherr DH, et al. Mechanisms of environmental influence on human autoimmunity: a national institute of environmental health sciences expert panel workshop. *J Autoimmun* 2012; 39: 272-84.
15. Selmi C, Lu Q, Humble MC. Heritability versus the role of the environment in autoimmunity. *J Autoimmun* 2012; 39: 249-52.
16. Bogdanos DP, Smyk DS, Rigopoulou EI, et al. Twin studies in autoimmune disease: genetics, gender and environment. *J Autoimmun* 2012; 38: J156-69.
17. Selmi C, Meroni PL, Gershwin ME. Primary biliary cirrhosis and Sjogren's syndrome: autoimmune epithelitis. *J Autoimmun* 2012; 39: 34-42.
18. Spadaro A, Scrivo R, Riccieri V, Valesini G. Effect of tumor necrosis factor alpha antagonists in a patient with rheumatoid arthritis and primary biliary cirrhosis. *Revue Rhum* 2008; 75: 87-9.
19. Ogata A, Terabe F, Nakanishi K, et al. Etanercept improved primary biliary cirrhosis associated with rheumatoid arthritis. *Revue Rhum* 2009; 76: 105-7.
20. Kubo S, Iwata S, Saito K, Tanaka Y. Successful treatment of primary biliary cirrhosis with etanercept in a patient with rheumatoid arthritis. *Revue Rhum* 2011; 78: 535-6.

"Don't tell me the moon is shining; show me the glint of light on broken glass"

Anton Chekhov (1860-1904), Russian dramatist, author and physician. Apart from his must-esteemed short stories, he is most known for his plays: *The Seagull*, *Uncle Vanya*, *Three Sisters* and *The Cherry Orchard*. "Medicine is my lawful wife," he once said, "and literature is my mistress"

"Fear prophets and those prepared to die for the truth, for as a rule they make many others die with them, often before them, at times instead of them"

Umberto Eco (b. 1932), Italian philosopher, literary critic and novelist. He is best known for his groundbreaking 1980 historical mystery novel *Il nome della rosa* (*The Name of the Rose*)

Successful Etanercept Treatment for Primary Biliary Cirrhosis Associated with Rheumatoid Arthritis

Attila Kovács MD PhD[1], Adelina G. Siminischi MD[1,2], Beáta Baksay MD[1], András Gáll MD[2], Mária Takács MD[1] and Zoltán Szekanecz MD PhD[3]

[1]Hospital and Outpatient Clinic of Hungarian State Railways, Szolnok, Hungary
[2]Kátai Gábor Hospital and Outpatient Clinic, Karcag, Hungary
[3]Division of Rheumatology, Department of Internal Medicine, University of Debrecen Faculty of Medicine, Debrecen, Hungary

KEY WORDS: rheumatoid arthritis (RA), primary biliary cirrhosis (PBC), biological therapy, etanercept

IMAJ 2015; 17: 114–116

For Editorial see page 112

Primary biliary cirrhosis (PBC) is a progressive chronic hepatic disease of autoimmune pathogenesis. It is characterized by intrahepatic cholestasis due to intrahepatic bile duct destruction, leading to fibrosis and nodular hepatocellular regeneration. Its exact etiopathogenesis remains unknown [1,2]. The development of the disease is associated with the production of characteristic autoantibodies. The main clinical manifestations of PBC include jaundice, pruritus and xanthelasmata, as well as secondary consequences of malabsorption such as osteoporosis. PBC may be associated with autoimmune manifestations in other organs, such as autoimmune thyroid disease, rheumatoid arthritis (RA), vasculitis, systemic sclerosis, Sjögren's syndrome, and diabetes mellitus [1,2]. The goals of treatment are symptom relief and prevention of further bile duct obstruction and destruction. Pruritus is relieved by cholestyramine, a bile acid sequestrant that prevents the reabsorption of bile acids in the gastrointestinal tract, and by the enzyme inducer phenobarbital. Routine care includes immunosuppressive agents such as corticosteroids, azathioprine, cyclosporine and methotrexate; drugs that may dampen tissue fibrosis including D-penicillamine and colchicine; as well as ursodeoxycholic acid that reduces the concentration of bile acids. As discussed below, there have been attempts to administer biologics to PBC patients. Finally, if cholestasis persists and pruritus becomes unbearable, liver transplant is the only option [1,2].

Rheumatoid arthritis (RA) is also a chronic inflammatory disease involving multiple joints. The development of bone and cartilage erosions may lead to destruction of joint structure and consequent disability [3]. Due to the progressive nature of the disease, early aggressive therapy is of utmost importance [3]. Until the last decade, treatment included the administration of corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), and synthetic disease-modifying drugs (DMARDs). In the new millennium, the introduction of biological agents was a real breakthrough. Indeed, considerable clinical improvement and the reduction of radiological progression could be obtained by the administration of anti-tumor necrosis factor (TNF)agents and biologics attacking other targets [3]. We present the case of a woman with coexisting RA and PBC who received anti-TNF therapy.

PATIENT DESCRIPTION

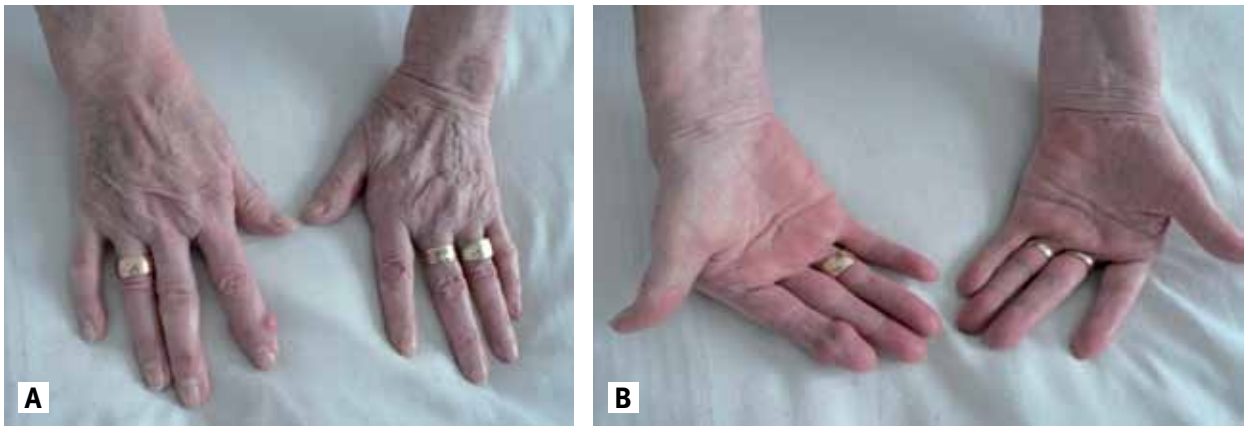
A 67 year old woman had a negative medical history prior to 2005. In that year, elevated hepatic enzymes (alkaline phosphatase, ALP and gamma-glutamyltransferase, GGT), high total bilirubin levels, jaundice and hepatomegaly were observed. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were within the normal range. She underwent cholecystectomy and reoperation was performed twice due to postoperative abscess formation. Despite the surgery, ALP and GGT remained continuously elevated.

In 2006, polyarthritis mostly affecting the small joints of the hand, morning articular stiffness, as well as immunoglobulin (Ig) M rheumatoid factor (RF) positivity lead to the diagnosis of seropositive RA established by a local rheumatologist. DMARD treatment (leflunomide 20 mg/day) was initiated but had to be discontinued due to the sustained elevation of hepatic enzymes. Until 2008 she received methylprednisolone 4 mg/day.

The patient was first admitted to our hospital in Szolnok in August 2012 due to high RA activity. Her main signs and symptoms included sclera jaundice, palpable liver, as well as swollen and tender metacarpophalangeal joints, reduced grip strength, gross interosseus atrophy, and swelling of the right ankle joint.

Laboratory results showed elevated acute-phase reactants: erythrocyte sedimentation rate (ESR) 50 mm/hr and C-reactive protein (CRP) 24.4 mg/L; markedly elevated GGT (702 U/L) and ALP (743 U/L) but only slightly elevated AST (35 U/L) and ALT (41 U/L), and anemia (hemoglobin 117 g/L). Abdominal ultrasonography showed status after cholecystectomy with thickening of intrahepatic bile duct walls but no obstruction of the extrahepatic flow. Since the active RA had to be treated, despite the elevated hepatic enzymes it was decided to admin-

Synovitis of small joints **[A]** is reduced following etanercept treatment **[B]**



ister very low dose methotrexate (MTX) (5 mg/week). Yet, even this very low dose resulted in further increase of GGT (791 U/L) and ALP (762 U/L), and MTX treatment had to be discontinued.

In September 2010, a more detailed hepatological examination was performed. Hepatitis B and C virus serology tests were negative, and immunoserologic tests revealed antinuclear antibody (ANA) positivity (titer > 160). Other autoantibodies (anti-smooth muscle, antimitochondrial, antiparietal cell, antimyeloperoxidase, antiproteinase 3, anticentomere and anticytoplasmic antibodies) were all negative. Based on these observations, PBC was diagnosed and ursodeoxycholic acid therapy was immediately given. Because of increasing RA activity, sulfasalazine was initiated and later combined with chloroquine. However, this combined DMARD therapy had to be stopped due to intense stomach pain, nausea and vomiting. The patient still received continuous low dose methylprednisolone therapy. The concomitant secondary osteoporosis was treated with calcium, vitamin D, and annual infusions of zoledronic acid.

The patient maintained high disease activity (DAS28 5.74) despite corticosteroid treatment, and we therefore considered biological therapy. There was no hepatologic or other contraindications. In December 2010, laboratory tests still showed seropositivity (RF 48 IU/ml, anti-CCP > 500 U/ml), high inflammatory activity (ESR 46 mm/hr, CRP 9.9 mg/L), ANA and anti-dsDNA negativity.

In February 2011, etanercept 50 mg once a week was initiated subcutaneously. As early as within the first 2 weeks the patient noted a considerable amelioration of her arthritic symptoms [Figure 1]. Much to our surprise, after 2 weeks of therapy, hepatic function tests also showed significant improvement (GGT 189 U/L, ALP 341 U/L). This favorable tendency was observed throughout the course of biological therapy (August 2011: ESR 33 mm/hr, CRP 10.3 mg/L, GGT 141 U/L, ALP 356 U/L), but transient ANA positivity (67.1 U/ml) was observed.

During biological therapy the patient's general state was stable. Her arthritic symptoms were relieved to such an extent that in May 2011 the administration of corticosteroids could be terminated. However, in January 2012, at the semiannual pulmonary checkup the chest X-ray indicated an undefined cavernous bean-sized malformation in the right apex of the lung. It was suggested that the treatment be discontinued until a more precise diagnosis could be established. Sputum culture was Koch-negative. In March 2012 a chest computed tomography (CT) depicted pulmonary emphysema and fibrotic scarring, but there was no sign of malignancy or cavity formation. The pulmonologist agreed to the reintroduction of etanercept therapy. Interestingly, increased RA activity and increasing GGT (198 U/L) and ALP (409 U/L) were again observed during the biologic-free period. Thus, in April 2012 etanercept was reintroduced and a few weeks later the patient again reported considerable relief of her arthritic symptoms, and by June 2012 hepatic tests again showed improvement (GGT 164 U/L, ALP 380 U/L). These changes during etanercept treatment and biologic-free periods suggest that anti-TNF therapy exerted significantly beneficial effects on both RA and PBC.

COMMENT

PBC is a chronic autoimmune disease characterized by injury of intrahepatic bile duct epithelia. PBC may be associated with other autoimmune conditions including RA [1-3]. Although 95% of PBC patients are seropositive for antimitochondrial antibodies (AMAs) directed against the inner mitochondrial membrane, pyruvate dehydrogenase and 2-oxoacid enzymes, AMA-negative cases do not differ clinically from those positive to AMA. Furthermore, TNFα and associated genes are involved in the pathogenesis of PBC [2]. There have been case reports on the biological treatment of PBC, but anti-TNF agents have not yet become a part of routine care [1]. Biologics may be primarily used in cases of PBC associated with arthritis, when the underlying rheumatic condition is the main indication for this treatment [1,3].

Our Hungarian colleagues, Laduver et al. [4], presented a similar case in 2009. Spadaro et al. [5] also reported a case of RA-PBC where, due to high disease activity, infliximab was administered initially but had little effect on RA activity or hepatic function test results. Etanercept treatment later resulted in a considerable reduction of activity in both diseases.

In conclusion, anti-TNF biologics appear to be hugely successful in the treatment of arthritis and other chronic inflammatory diseases. As suggested by our case and other case reports, in duly justified cases, the administration of TNFα inhibitors should be extended to the treatment of other immunopathologic diseases, such as PBC.

Correspondence
Dr. Z. Szekanecz
University of Debrecen Faculty of Medicine, Institute of Medicine, Dept. of Rheumatology, Nagyerdei str 98, Debrecen, 4032, Hungary
Phone/Fax: (36-52) 255-091
email: szekanecz.zoltan@med.unideb.hu

References

1. Neuberger J. Primary biliary cirrhosis. *Lancet* 1997; 350 (9081): 875-9.
2. Gershwin ME, Mackay IR. The causes of primary biliary cirrhosis: convenient and inconvenient truths. *Hepatology* 2008; 47 (2): 737-45.
3. Myasoedova E, Davis JM 3rd, Crowson CS, Gabriel SE. Epidemiology of rheumatoid arthritis: rheumatoid arthritis and mortality. *Curr Rheumatol Rep* 2010; 12 (5): 379-85.
4. Laduver A, Keszthelyi P. Effect of etanercept treatment in a patient with primary biliary cirrhosis and rheumatoid arthritis. *Hun Rheumatol* 2009; 50: 158.
5. Spadaro A, Scrivo R, Riccieri V, Valesini G. Effect of tumor necrosis factor alpha antagonists in a patient with rheumatoid arthritis and primary biliary cirrhosis. *Joint Bone Spine* 2008; 75 (1): 87-9.

Capsule

A step toward better vaccine adjuvants

The receptor TLR4 stimulates immune signaling pathways. It can do so through two adaptor proteins: MyD88, which can trigger undesirable inflammatory responses, and TRIF, which stimulates immune responses. Currently, adjuvants to boost immune responses to vaccines are developed with the idea that their structure determines the adaptor protein that TLR4 will use. However, Kolb and fellow-researchers suggest that TLR4 signaling is inherently biased toward the TRIF-dependent pathway, particularly in the context of type I interferon signaling. The findings may help in the development of more effective vaccine adjuvants that enhance immune responses without triggering potentially harmful inflammatory reactions.

Sci Signal 2014; 7: ra108
Eitan Israeli

Capsule

One clock for you and your microbes

Disrupting our circadian rhythms increases the risk of developing diabetes, obesity, cancer, and cardiovascular disease, but scientists do not fully understand why. Thaiss et al. report that conditions that cause jet lag change the composition and activities of gut microbes in mice, which can lead to metabolic disease. Gut microbe composition no longer fluctuated diurnally in mice with disrupted circadian rhythms, but normal rhythmic feeding or the transplantation of gut microbes from normal mice restored this oscillation. Normal mice that received gut microbial transplants from jet-lagged humans or mice that experienced a change in their day-night schedule gained weight and developed symptoms of metabolic disease.

Cell 2014; 159: 514
Eitan Israeli

Capsule

The gut microbiota influences blood-brain barrier permeability in mice

Pivotal to brain development and function is an intact blood-brain barrier (BBB), which acts as a gatekeeper to control the passage and exchange of molecules and nutrients between the circulatory system and the brain parenchyma. The BBB also ensures homeostasis of the central nervous system (CNS). Branistre et al. report that germ-free mice, beginning with intrauterine life, displayed increased BBB permeability compared to pathogen-free mice with a normal gut flora. The increased BBB permeability was maintained in germ-free mice after birth and during adulthood and was associated with reduced expression of the tight junction proteins occludin and claudin-5, which are known to regulate barrier function in endothelial tissues. Exposure of germ-free adult mice to a pathogen-free gut microbiota decreased BBB permeability and up-regulated the expression of tight junction proteins. These results suggest that gut microbiota-BBB communication is initiated during gestation and propagated throughout life.

Sci Transl Med 2014; 6: 263ra158
Eitan Israeli

Autoimmune Pitfalls of Anti-Tumor Necrosis Factor-Alpha Therapy

Siniša Roginić MD[1], Alan Jelić MD[2], Asja Stipić-Marković MD PhD[3], Artukovic Marinko MD[3], Irena N. Artuković MD[4] and Martinović K. Dušanka MD PhD[5]

[1]Department of Internal Medicine, General Hospital Zabok, Zabok, Croatia
[2]Department of Internal Medicine, General Hospital Sisak, Sisak, Croatia
[3]Department of Clinical Immunology, Pulmonology and Rheumatology, Sveti Duh Clinical Hospital, Zagreb, Croatia
[4]Institute for Cardiovascular Diseases Prevention and Rehabilitation, Zagreb, Croatia
[5]Department of Rheumatology and Clinical Immunology, University Hospital Split, Split, Croatia

KEY WORDS: anti-tumor necrosis factor (anti-TNF), drug-induced lupus, etanercept, autoimmunity, antibody

IMAJ 2015; 17: 117–119

Biological therapy refers to the use of medication tailored to specifically target an immune or genetic mediator of disease. It has revolutionized the treatment of chronic inflammatory diseases, such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriatic arthritis (PsA) and inflammatory bowel diseases (IBD) [1,2]. Most medications are directed against tumor necrosis factor-alpha (TNFα) (infliximab, adalimumab, etanercept, golimumab, certolizumab pegol) and others against interleukin (IL)-1 (anakinra), IL-6 (tocilizumab), CD20 (rituximab), and CD28-CD80/86-mediated T cell co-stimulation (abatacept). The most extensive clinical experience was gathered from treating RA patients with anti-TNF agents. Since their introduction in 1998, over a million patients have been treated.

However, increasing clinical data warn us of the potential dangers of disabling TNF-mediated immunological signaling – namely, increased incidence of infections, solid and hematological tumors, demyelinizing disease, cardiovascular incidents, and induction of autoimmunity [1,2]. Induction of autoantibodies (antinuclear antibody, anti-dsDNA) under biological therapy is well recognized: elevated titers were found in 11–13% of patients treated with etanercept and 3–98% treated with infliximab, depending on the indication and type of antibodies determined [1,3]. Most of these patients had no clinical manifestations, but some of them developed autoimmune syndromes: vasculitis, systemic lupus erythematosus (SLE), interstitial lung disease, inflammatory myopathies, antiphospholipid syndrome, etc. [4].

We present the case of a patient who developed drug-induced lupus erythematosus (DIL) after etanercept was introduced into his treatment for severe RA. Etanercept is a soluble p75 TNF-receptor fusion protein conjugated to the Fc region of human immunoglobulin (Ig) G used since 1998 to treat RA, AS, PsA and juvenile idiopathic arthritis. It is given by self-injection under the skin once or twice a week (50 mg/week). DIL is generally a milder version of the idiopathic disorder that is traditionally associated with production of antihistone antibodies. This pattern is now changing, in part due to the many new drugs – both conventional and biological – that have been introduced into clinical practice for treating autoimmune diseases [4]. DIL has been recognized as a side effect of more than 80 drugs since its first description in association with sulfadiazine. Despite frequent induction of autoantibodies in treated patients, development of DIL under anti-TNF therapy is uncommon; De Bandt et al. [5] reported a 0.19% prevalence for infliximab and 0.18% for etanercept.

PATIENT DESCRIPTION

A 45 year old man presented at the emergency room complaining of fever (41.6° CF), dry cough and left-sided chest pain of 2 weeks duration, with no improvement following a prescribed course of antibiotics. He had been diagnosed with rheumatoid arthritis 2 years previously and was initially treated with leflunomide and methotrexate, which were switched to etanercept and methotrexate 2 months before the present admission. Pre-treatment PPD and Quantiferon test were negative. After 2 weeks of treatment, he developed erythema on the trunk and extremities, but the treatment was continued since the joint symptoms abated significantly. Other medications included low dose prednisone (5 mg), folic acid, pantoprazol and occasional non-steroidal anti-inflammatory drugs (NSAID). The patient is allergic to sulfasalasine and is a former smoker.

Upon arrival he appeared ill, diaphoretic, with resting rate 130/80 mmHg, pulse 130/min and respiratory rate 22/min. His skin was covered with a maculopapular scaling rash in centripetal distribution. Head and neck examination revealed "moon face" aspect, without palpable lymph nodes. Lung auscultation on the lower left field showed no breathing sounds. Metacarpophalangeal and proximal interphalangeal joints were symmetrically swollen and tender on palpation. The rest of the physical examination was unremarkable. Chest X-ray showed left-sided

pleural effusion without infiltrate. Based on the patient's general appearance, iatrogenic immunosuppression and laboratory findings of neutrophilia with left shift, elevated C-reactive protein (CRP, 243.3 mg/L) and procalcitonin (1.97 µg/L), a regimen of broad-spectrum antibiotic and antifungal treatment was started. Other findings included mild normocytic normochromic anemia (lowest hemoglobin level 109 g/L) and positive direct Coomb's test without hemolysis. Despite antibiotics and antifungal medication the patient remained febrile without clinical improvement. Extensive microbiological and imaging workup (repeated blood, sputum, pleural effusion and swab cultures, computed tomography scan of brain and chest, transesophageal echocardiogram, abdominal ultrasound) failed to show any source of infection. CT scan showed bilateral pleural effusion and small pericardial effusion. Pleural fluid biochemistry and cytology showed exudates with abundant lymphocytes, repeated cultures for *Mycobacterium tuberculosis* returned negative. PPD and Quantiferon test were repeated and were also negative.

A diagnosis of drug-induced lupus (DIL) was assumed, considering the preexisting rash correlating with etanercept therapy, pericardial and pleural effusions, fever of unknown origin and other general symptoms. Further testing included ultrasound of hands and wrist joints (minimal joint effusion, improvement compared to the pre-etanercept period), skin biopsy with direct immunofluorescence assay (granular C3 and IgM deposits on epidermodermal junction) and battery of autoantibodies (ANA, antinucleasome and antihistone antibodies were positive while the other antibodies were negative).

After 3 weeks, antimicrobial therapy was stopped and prednisone 20 mg/day was introduced leading to gradual improvement. Fever and general symptoms abated completely; the rash and joint stiffness remained but subsided significantly. The patient was discharged with gradual tapering of corticosteroids; no other biological agent was prescribed.

COMMENT

Clinical experience with anti-TNF biological therapy is rapidly increasing, and we are becoming aware of potential risks from altering the complex and incompletely understood cytokine network. Side effects can be cumbersome leading to treatment cessation and serious morbidity, which prevents their wide use as first-line medications. Most articles on anti-TNF (particularly etanercept)-induced lupus are reports on individual cases, with only two larger retrospective studies of 32 and 7 patients respectively [4,5]. Since the first description of etanercept-induced lupus erythematosus in 2002, fewer than 50 cases have been published and, to our knowledge, this is the first described case in Croatia. In the previous reports, timing of the onset and cessation of the SLE features also strongly support a drug-related effect of etanercept. SLE developed after a mean of 7.7 months (range 3–24 months) of commencing etanercept therapy [4,5]. Our patient's symptoms began only 2 weeks after initiating therapy. The most common clinical feature of DIL is skin rash, and skin biopsy was not performed in most of the published cases while in our patient it showed granular IgG and complement deposits. There was no significant life-threatening organ involvement such as kidney, lung (interstitial lung disease), heart, or central nervous system. Our patient's clinical presentation is consistent with the typical clinical features previously described in DIL, including severe general and joint symptoms and rash; however, he also had serositis which has been described in only 3% of cases (in the largest series of 37 patients) [4].

Serologic and clinical manifestations of DIL differ from those in idiopathic SLE, but there is also a difference between "classic" DIL (e.g., procainamide) and anti-TNF DIL. Patients with anti-TNF DIL generally have a milder clinical picture, with higher prevalence of rash than those with classic DIL, more inconsistent elevation of positive ANA, anti-dsDNA and antihistone antibodies [4]. The pathophysiology of DIL is controversial. Induction of antibodies is the first step and occurs in all described cases. In RA patients treated with infliximab, ANA developed in 29–76.7% and anti-dsDNA in 10–29%. Also, in etanercept-treated RA patients, 11–36.3% had developed ANA and 5–15% developed anti-dsDNA [3,4].

The question remains: why does anti-TNF therapy decrease titers of rheumatoid factor and anti-CCP antibodies, concurrently inducing ANA and anti-dsDNA. TNF might up-regulate the cellular expression of the adhesion molecule CD44, which has a role in the clearance of apoptotic material by phagocyte. Reduced CD44 expression by anti-TNF can potentially induce SLE by abnormal clearance of apoptotic materials. Experimental models also show that agents or events that modify T cell DNA methylation may induce autoimmunity by causing over-expression of T cell LFA-1 (lymphocyte function-associated antigen 1, CD11a/CD18).

Cytokine imbalance is another attractive hypothesis: TNF, overexpressed in RA, exerts inhibitory effects on plasmatocytoid dendritic cell expansion and systemic release of interferon-alpha (IFNα), a culprit of SLE. Therefore, blocking TNF function may cause DIL by inducing IFNα secretion.

There are no consensual diagnostic criteria for DIL, but most authors use the following: i) a temporal relationship between clinical manifestations and anti-TNF therapy, ii) at least one serologic American Congress of Rheumatology (ACR) criterion of SLE (e.g., ANA, anti-dsDNA), and iii) at least one non-serologic ACR criterion (e.g., arthritis, serositis, hematologic disorder, malar rash) [5]. Our patient fulfilled 5 of 11 ACR criteria.

The mainstay of DIL treatment is cessation of the incriminating drug, which is sufficient in most cases [4,5]. Some patients require glucocorticoid therapy and only in rare instances another immunosuppressant. There are reports of successful treatment with rituximab. The prognosis is good, with the vast majority of patients suffering no permanent damage and recovering completely, although recovery can be prolonged (median 8 weeks, range 3–16 weeks, in one case 6 months) [5].

DIL is a relatively novel side effect of anti-TNF biological therapy. In fact, it might be a logical consequence of altering immune system signaling, considering the high frequency of autoantibody induction during anti-TNF therapy.

Recognizing patients who are likely to develop clinical manifestations is a future challenge, and early recognition of this potentially harmful condition is mandatory. It is also prudent to check baseline autoantibody levels before initiating biological therapy and not to prescribe it to patients with SLE.

Correspondence
Dr. S. Roginić
Dept. of Internal Medicine, General Hospital Zabok, Bracak 8, Zabok 49210, Croatia
Phone: (385-4) 920-4480
Fax: (385-4) 922-7727
email: sinisa.roginic@gmail.com

References

1. Shoenfeld Y, Blank M, Abu-Shakra M, et al. The mosaic of autoimmunity: prediction, autoantibodies, and therapy in autoimmune diseases [Review]. *IMAJ* 2008; 10: 13-19.
2. Ling E, Ofer-Shiber S, Goren O, Molad Y. Outcome of patients with rheumatoid arthritis: cross-sectional study of a single-center real-world inception cohort. *IMAJ* 2013; 15 (12): 758-62.
3. Atzeni F, Turiel M, Capsoni F, Doria A, Meroni P, Sarzi-Puttini P. Autoimmunity and anti-TNF-alpha agents [Review]. *Ann N Y Acad Sci* 2005; 1051: 559-69.
4. Ramos-Casals M, Brito-Zerón P, Muñoz S, et al. Autoimmune diseases induced by TNF-targeted therapies: analysis of 233 cases. *Medicine (Baltimore)* 2007; 86 (4): 242-51.
5. De Bandt M, Sibilia J, Le Loët X, et al. Systemic lupus erythematosus induced by anti-tumour necrosis factor alpha therapy: a French national survey. *Arthritis Res Ther* 2005; 7 (3): 545-51.

Capsule

Limbal stem cells to treat scarring and prevent blindness

Our corneas – the transparent structures that allow us to see – are easily damaged by trauma and infection, which can cause scarring or blindness. Although corneas can be transplanted, transplants are limited by immune responses and by a shortage of cornea donors. Basu et al. devised a cell-based approach to prevent corneal scarring. They obtained stem cells from the human limbus (the region between cornea and sclera), which could be differentiated into keratocytes (corneal cells). The stem cells actively regenerated new corneal tissue when encased in a fibrin gel and applied to the wounded surface of the eye in mice. Such cells could potentially be obtained directly from a patient to treat scarring and prevent blindness.

Sci Transl Med 2014; 6: 266ra172

Eitan Israeli

Capsule

An ancient defense system eliminates unfit cells from developing tissues during cell competition

The recognition and elimination of unfit or mutant cells in cell competition is reminiscent of the detection of pathogens by the innate immune system. In Drosophila, the Toll and immune deficiency (IMD) signaling pathways govern the innate immune response to a broad range of pathogens and activate the NFκB transcription factor homologs Relish (Rel), Dorsal (dl), or Dorsal-related immunity factor (Dif). The conceptual similarities between innate immunity and cell competition led us to investigate whether the Toll and IMD pathways were required for cell competition in Drosophila wing disks. In their analysis of both *Myc*-induced and *Minute*-induced cell competition Meyer et al. revealed requirements for two related but distinct cohorts of components from the IMD and Toll pathways. Both signaling cohorts required the extracellular ligand Spätzle and non-canonical Toll-related receptors (TRRs) and led to elimination of the less-fit loser cells by inducing NFκB-dependent activation of pro-apoptotic genes. However, their analysis uncovered interesting differences between the signaling module deployed in each competitive context. In *Myc*-induced competition, elimination of wild-type loser cells required four of the nine TRRs encoded in the Drosophilagenome (*Toll-2*, *Toll*-3, *Toll-8*, and *Toll-9*) in non-redundant roles. By contrast, elimination of *RpL14*–/+ cells in Minute-induced competition required only *Toll-3* and *Toll-9*. Furthermore, the NFκB factor activated downstream of the TRRs was also context-dependent. Signal transduction within wild-type loser cells led to selective activation of Relish, whereas the death of *RpL14*–/+ loser cells in Minute-induced competition required Dorsal and Dif. These results suggest that signaling from the different TRR subsets influenced which NFκB factor was activated. Finally, although in each competitive context apoptosis of the relatively less fit cells was induced, the specific death-inducing gene expressed was determined by specifically activated NFκB factor. The authors conclude that in two genetically distinct contexts of cell competition, the ancient innate immune defense response system is activated and drives the elimination of the cells perceived as relatively less fit. In each competition paradigm, different signaling modules are employed, suggesting that the genetic identity of the competing cell populations influences the pathway that is activated. These results thus provide evidence for evolutionary adaptation of TRR-NFκB signaling modules in an organismal surveillance system that measures internal tissue fitness rather than external pathogenic stimuli.

Science 2014; 346: DOI: 10.1126/science.1258236

Eitan Israeli

Transverse Myelitis Activation Post-H1N1 Immunization: A Case of Adjuvant Induction?

Adam Austin MD[1], Angela Tincani MD[2], Shaye Kivity MD[1,3,4], María-Teresa Arango MSc[3,5] and Yehuda Shoenfeld MD FRCP MaACR[1,3]

[1]Sackler Faculty of Medicine, University of Tel Aviv, Tel Aviv, Israel
[2]Department of Rheumatology and Clinical Immunology, SpedaliCivili and University of Brescia, Brescia, Italy
[3]Zabludovicz Center for Autoimmune Diseases and [4]Borenstein Talpiot Medical Leadership Program 2013, Sheba Medical Center, Tel Hashomer, Israel
[5]Doctoral Program in Biomedical Sciences Universidad del Rosario, Bogota, Colombia

KEY WORDS: transverse myelitis, adjuvants, autoimmunity, immunization, autoimmune syndrome induced by adjuvants (ASIA)

IMAJ 2015; 17: 120–122

Innovations in vaccination technology have helped eradicate many diseases throughout the Western and developed worlds. During the last 50 years vaccines have diminished the incidence of once commonplace pediatric and adult diseases. Although vaccine technology is safe, non-specific reactions following the administration of immunizations sometimes occur, which typically include site-reaction transient flu-like symptoms. However, recent evidence has spotlighted the development of autoimmune phenomena post-vaccination in sporadic cases [1]. These adverse reactions are thought to be due to associated adjuvants and foreign antigens. Recently, the autoimmune syndrome induced by adjuvants (ASIA) was defined, which includes related diseases such as the Gulf War syndrome, macrophage myofasciitis syndrome, siliconosis, and post-vaccination phenomena [2].

The current understanding is that vaccine adjuvants play an essential role in the pathogenesis of autoimmunity in these patients. They typically consist of aluminum salts, which are added to help enhance the response of the host innate and adaptive immune system [2]. In a minority of patients, the adjuvants stimulate the creation of autoantibodies as well as the appearance of clinical symptoms such as myalgia, myositis, arthralgia, chronic fatigue, sleep disturbances, neurological manifestations and cognitive impairment. The increasing number of cases in the literature linking vaccine adjuvants and autoimmunity has helped substantiate this relationship [3]. For instance, there is evidence demonstrating a connection between various defined rheumatologic illnesses with immunizations, such as systemic lupus erythematosus developing after the hepatitis B virus (HBV) and human papillomavirus vaccinations. Furthermore, a possible association between various autoimmune syndromes and the varicella and measles-mumps-rubella vaccinations has also been documented [4].

In the general population, autoimmune manifestations of a neurological nature following immunization have also been reported. These post-vaccination neurological complaints consist predominately of neuropathy, encephalitis, vasculitis and demyelination. A literature review showed that neuromuscular conditions, such as Guillain-Barre syndrome (GBS), myasthenia gravis, optic neuritis and inflammatory myopathies, have a temporal relationship with the HBV and hepatitis A vaccines as well as numerous other vaccines. It has also been reported that cases of multiple sclerosis have been triggered by the administration of the HBV vaccine. Additionally, there is a correlation between the cellular *Bordetella pertussis* portion of the diphtheria-tetanus-pertussis vaccine and increased risk of seizure. However, since many of these neuromuscular autoimmune illnesses are rare in themselves, a direct relationship cannot be confirmed.

During the spring of 2009, an eruption of new influenza cases was quickly denoted "the swine flu." The H1N1 vaccination was created for four influenza A viruses and its efficacy led to a reduction in H1N1 infectivity rates and hospital admissions. While some H1N1 vaccines were associated with flu-like symptoms typically 4 to 7 days post-administration, a very small population of patients developed more severe side effects, including autoimmune and neuroimmune phenomena.

Although the etiology of transverse myelitis, a rare inflammatory spinal-cord condition, is largely unknown, there is growing evidence in the literature that an autoimmune syndrome induced by the adjuvant phenomenon is responsible in some cases for the disease pathogenesis [4]. According to a recent multi-analysis conducted by our research team, 37 cases of transverse myelitis developed within one month post-vaccination that used various common immunizations [5]. Similar to the immune reaction of infectious diseases, vaccine adjuvants cause autoimmunity in a similar manner, including molecular mimicry, epitope spreading, up-regulation of cytokines, and polyclonal activation of B and T lymphocytes [5].

In this report, we will attempt to further the discussion by illustrating a case of transverse myelitis 2 months post-vaccination with the influenza A (H1N1) immunization.

PATIENT DESCRIPTION

A 41 year old man presented to our department with headache, leg paresthesia and

sensory loss of 2 months duration. The patient had been diagnosed with psoriasis 9 years earlier and was initially prescribed only a topical medication to treat the skin lesions. His disease history is significant for one exacerbation 5 years after his original diagnosis, which manifested as an abrupt eruption of psoriatic lesions in the scalp and nails. The patient also developed arthritis of the hands and left foot dactylitis shortly thereafter. He was then treated with prednisone 5 mg/day and methotrexate 20 mg/week, and has been in complete remission without any medication for the past 2 years.

The patient's vaccination history was significant for both the influenza A (H1N1) and yellow fever immunizations. The yellow fever vaccine was administered 16 months before onset of the initial neurological symptoms. He reported myalgia, arthralgia, fatigue, xerostomia and non-bilious non-blooding emesis for a period of 8 days post-vaccination. Two months before the appearance of neurological symptoms, he had received the influenza A (H1N1) vaccination and no acute adverse reaction occurred. Two months later, the patient consulted with a family physician due to the gradual appearance of an occipital headache and generalized fatigue. He was sent for magnetic resonance imaging (MRI), which demonstrated a mild disk protrusion at the level of C5-C6 without spinal cord compromise. Cervicobrachialgia was suspected for which analgesics and anti-inflammatory medications were prescribed, resulting in slight amelioration of his symptoms.

Owing to the persistence of his neurological complaints, the patient was admitted to our department for further workup. Upon admission, he complained of continued occipital-located headache, which persisted throughout the day and night. He also complained of xerostomia without signs of xerophthalmia. He reported paresthesia of both legs with any movement of his neck. He denied recent trauma, visual disturbances, nausea, vomiting or recent fever. On physical examination, he appeared well and in no acute distress. His vital signs and cardiovascular and respiratory systems were unremarkable. His abdomen was soft and non-tender. Neurological examination was significant only for a loss of transient vibratory sensation in his thighs bilaterally. He denied neck stiffness and displayed no meningeal signs.

His blood cell count and liver and muscle enzymes were within normal limits. Protein electrophoresis, electrophoresis of immunoglobulins and complement were within the normal reference range. Furthermore, serological testing was negative for rheumatoid factor, antinuclear antibodies (including specific testing for anti-RNP, Sm, Ro, La), anti-DNA, anticardiolipin antibodies (immunoglobulins G and M), lupus anticoagulant, and perinuclear and cytoplasmic antineutrophil cytoplasmic antibodies (p-ANCA and c-ANCA). Tests for *Mycobacterium tuberculosis*, including the purified protein derivative test and polymerase chain reaction, were normal. Enzyme-linked immunosorbent assay (ELISA) for anti-aquaporin 4 antibodies was also negative. A lumbar puncture was performed and cerebral spinal fluid (CSF) demonstrated normal appearance, cytology and biochemistry. CSF testing for VDRL, parasitic and viral serologies were negative, and electrophoresis gamma was 18% (7–14%) with absent monoclonal bands.

A spinal cord MRI highlighted three medullar hyperintense lesions on T2 that enhanced after gadolinium use: two appeared at D1 and D5, which were at the left paramedian region, and did not exceed 0.5 cm in diameter, and one was at the level of D7, reaching 1 cm in diameter, also left paramedian. A brain MRI demonstrated no abnormalities. Further neurological evaluation was normal, including electroneuromyography of all four limbs and visual evoked potential.

The patient was diagnosed with transverse myelitis based on clinical symptoms and the MRI findings. He was treated initially with three pulses of intravenous methylprednisolone 1000 mg. Additionally, oral corticosteroids and azathioprine were administered as maintenance therapy. Approximately 3 months after admission, he experienced a total remission of all neurological symptoms. At follow-up 7 months after admission to our department, the patient's brain and spinal cord MRIs demonstrated no signs of demyelinating lesions.

COMMENT

We describe a 41 year old man who experienced leg paresthesia and sensory loss 2 months after immunization with the influenza A H1N1 vaccination. The clinical laboratory and imaging results were compatible with the diagnosis of transverse myelitis, while a direct etiology was not defined. The patient was successfully treated with corticosteroids and azathioprine. The fact that the symptoms appeared 2 months after the vaccination suggests an immune mediated reaction to the immunization, or ASIA syndrome [5]. The patient's background of psoriatic arthritis, together with a history of inflammatory reaction following yellow fever immunization, may suggest an autoimmune tendency.

The seasonal influenza virus may cause neurological symptoms; the incidence of encephalopathy and delirium during infection is as high as 1:100,000 especially among children. Nevertheless, a small minority of patients may suffer from neurologic-autoimmune phenomenon following the H1N1 vaccination. The most notable example of an influenza vaccine causing an autoimmune process is that of Guillain-Barre syndrome after the induction of the Influenza A/New Jersey vaccination in 1976. The incidence of GBS was significantly higher in the population receiving the vaccination. A recent meta-analysis of the relation between GBS and the influenza A (H1N1) 2009 monovalent inactivated vaccine showed a slightly increased risk of the condition developing.

In a large Swedish cohort of one million patients receiving the Pandremrix Influenza A (H1N1) monovalent-adjuvanted vaccine, there was an increase in the risk of Bell's palsy and various paresthesias. Since the 2009 H1N1 pandemic, there has also been a reported spike in the incidence of narcolepsy in Europe that may be associ-

ated with the swine-influenza vaccination. This finding reinforces the view of some researchers in the field of sleep disorders that the impairment of orexin-producing neurons in the brains is associated with an autoimmune process.

Additionally, there are at least three case reports in the literature that describe episodes of transverse myelitis that occurred after H1N1 immunization. In one report, a 44 year old man suffered from fever and various neurological symptoms, including right leg paresthesia, one month after receiving the influenza A (H1N1) vaccine. The patient demonstrated hyperdense MRI lesions at the level of C6 and C7 and was diagnosed with TM. In South America, a second report chronicled a 52 year old woman who developed a thoracic intramedullary lesion one week after immunization with a H1N1-trivalent vaccine without evidence of other causes of the episode. A third case of transverse myelitis was described after the administration of the monovalent A (H1N1) nasal influenza vaccine in a 27 year old woman.

In conclusion, the induction of transverse myelitis post-immunization is plausible in view of the increasing frequency of case reports in the medical literature demonstrating this phenomenon as well as the growing biological evidence of a post-vaccination autoimmune pathogenesis.

Correspondence
Dr. S. Kivity
Zabludovicz Center for Autoimmune Diseases, Sheba Medical Center, Tel Hashomer 52621 Israel
Phone: (972-3) 530-8070
Fax: (972-3) 530-3673
email: kivitys@gmail.com

References

1. Blank M, Israeli E, Shoenfeld Y. When APS (Hughes syndrome) met the autoimmune/inflammatory syndrome induced by adjuvants (ASIA). *Lupus* 2012; 21: 711-14.
2. Shoenfeld Y, Agmon-levin N. ASIA-autoimmune/inflammatory syndrome induced by adjuvants. *J Autoimmun* 2011; 36: 4-8.
3. Zafrir Y, Agmon-Levin N, Paz Z, Shilton T, Shoenfeld Y. Autoimmunity following Hepatitis B vaccine as part of the spectrum of 'Autoimmune (Autoinflammatory) Syndrome induced by Adjuvants' (ASIA): analysis of 93 cases. *Lupus* 2012; 21: 146-52.
4. Schattner A. Consequence or coincidence? The occurrence, pathogenesis and significance of autoimmune manifestations after viral vaccines. *Vaccine* 2005; 23: 3876-86.
5. Agmon-Levin N, Kivity S, Szyper-Kravitz M, Shoenfeld Y. Transverse myelitis and vaccines: a multi-analysis. *Lupus* 2009; 18: 1198.

Capsule

A receptor tyrosine kinase signals to YAP

The Hippo pathway limits cell proliferation by inhibiting the activity of the transcriptional coactivator YAP. In contrast, cell proliferation is stimulated by the binding of growth factors to tyrosine receptor kinases, such as the binding of neuregulin to ERBB4. Neuregulin binding also triggers the cleavage of ERBB4. Haskins et al. found that a fragment containing the intracellular domain of ERBB4 interacted with and activated YAP. Breast cancer cell migration induced by neuregulin was blocked by knocking down YAP. Thus, ERBB4 could promote tumor aggressiveness both through receptor tyrosine kinase signaling and by stimulating YAP.

Sci Signal 2014; 7: ra116 and pe29

Eitan Israeli

Capsule

Chimpanzee adenovirus vector Ebola vaccine — preliminary report

The unprecedented 2014 epidemic of Ebola virus disease (EVD) has prompted an international response to accelerate the availability of a preventive vaccine. A replication-defective recombinant chimpanzee adenovirus type 3-vectored ebola virus vaccine (cAd3-EBO), encoding the glycoprotein from Zaire and Sudan species that offers protection in the non-human primate model, was rapidly advanced into phase 1 clinical evaluation. Ledgewood et al. conducted a phase 1, dose-escalation, open-label trial of cAd3-EBO. Twenty healthy adults, in sequentially enrolled groups of 10 each, received vaccination intramuscularly in doses of $2x10^{10}$ particle units or $2x10^{11}$ particle units. Primary and secondary end-points related to safety and immunogenicity were assessed throughout the first 4 weeks after vaccination. In this small study, no safety concerns were identified; however, transient fever developed within 1 day after vaccination in two participants who had received the $2x10^{11}$ particle-unit dose. Glycoprotein-specific antibodies were induced in all 20 participants; the titers were of greater magnitude in the group that received the 2x1011 particle-unit dose than in the group that received the $2x10^{10}$particle-unit dose (geometric mean titer against the Zaire antigen, 2037 vs. 331; $P = 0.001$). Glycoprotein-specific T cell responses were more frequent among those who received the $2x10^{11}$ particle-unit dose than among those who received the $2x10^{10}$ particle-unit dose, with a CD4 response in 10 of 10 participants versus 3 of 10 participants ($P = 0.004$) and a CD8 response in 7 of 10 participants versus 2 of 10 participants ($P = 0.07$). Reactogenicity and immune responses to cAd3-EBO vaccine were dose dependent. At the $2x10^{11}$ particle-unit dose, glycoprotein Zaire-specific antibody responses were in the range reported to be associated with vaccine-induced protective immunity in challenge studies involving non-human primates. Clinical trials assessing cAd3-EBO are ongoing.

N Engl J Med 2014; DOI: 10.1056/NEJMoa1410863

Eitan Israeli

What to do when the Diagnosis of Giant Cell Arteritis and Takayasu's Arteritis Overlap

Nurit Katz-Agranov MD[1], Amir Tanay MD[2], Daniel J. Bachar MD[3] and Gisele Zandman-Goddard MD[1,4]

[1]Department of Medicine C, [2]Allergy, Immunology & Rheumatology Unit, and [3]Angiography Unit, Department of Radiology, Wolfson Medical Center, Holon, Israel
[4]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

KEY WORDS: giant cell arteritis (GCA), Takayasu's arteritis (TA), upper limb claudication, vasculitis, brachio-axillary arteries

IMAJ 2015; 17: 123–125

Giant cell arteritis (GCA) is the most common form of systemic necrotizing vasculitis in people aged 50 years and older and preferentially involves the extracranial branches of the carotid artery and temporal artery. Biopsy is considered the gold standard for establishing the diagnosis. However, GCA may involve the aorta and its major branches, most commonly the brachiocephalic and subclavian arteries, with symptoms including upper/lower limb claudication and other signs of limb ischemia in more than 10% of the cases. The prevalence of involvement on radiography is even higher. Involvement of these large vessels makes it difficult to distinguish GCA from Takayasu's arteritis (TA), the second most common necrotizing vasculitis, which also involves large and medium branches of the aorta but usually affects a younger population [1].

We report an unusual case of vasculitis, classified as both GCA and TA, based on American College of Rheumatology (ACR) 1990 classification criteria, and illustrate the complexity of diagnosis and treatment in such overlapping cases.

PATIENT DESCRIPTION

A 78 year old woman was referred to our hospital due to severe pain in her left hand which began 5 months before her presentation. The pain, distributed from her left wrist up to her left shoulder, was described as continuous, accompanied by tingling and pallor not related to exertion. There was a further history of 2 year unintentional weight loss, jaw claudication, paresthesia and myalgia, the latter attributed to statin therapy. The patient denied any history of headaches or visual impairment.

Physical examination on admission revealed a blood pressure difference between arms, 125/80 mmHg on the right and undetectable on the left, weakened brachial and radial pulse on the left, and a murmur heard over the axilla and brachial artery on the right; no murmurs were heard over the subclavian arteries. No signs of acral ischemia were noted. The neurological examination was non-contributory. The rest of the examination was unremarkable.

Laboratory investigations during hospitalization revealed an elevated sedimentation rate (ESR) of 54 mm/hour (0–29 mm/h); the complete blood count and other routine laboratory tests including chemistry, liver, renal and thyroid functions as well as lipid profile were all within normal limits. Serology including rheumatoid factor, antinuclear antibody (ANA), antineutrophil cytoplasmic antibodies (ANCA), anti-DNA antibodies, cryoglobulins, C3 and C4 levels, anti-Ro/La antibodies, anticardiolipin antibodies, and hepatitis serologies were all negative. Lupus anticoagulant, anticardiolipin immunoglobulins (Ig) M and G, and anti-β2-GPI-IgM values were within normal limits.

Computed tomography angiography (CTA) demonstrated arterial thickening, occlusion of the left axillary artery, stenosis of the right axillary artery and a narrowing of both brachial arteries, left more than right. An additional chest and abdominal CTA performed for further assessment demonstrated similar findings, without evidence of micro-aneurysms or occlusion of visceral or pulmonary arteries or other involvement of the abdominal aorta. Ophthalmologic examination revealed no signs of retinal vasculitis. Due to the patient's advanced age, high ESR and slightly reduced left temporal artery pulse on follow-up examination along with the typical angiographic findings, GCA was suspected; prednisone 60 mg/day was started promptly followed by a unilateral left temporal artery biopsy which showed no signs of inflammation. After 3 weeks of prednisone therapy the patient reported reduction in the severity of the pain in her left arm, which had by then disappeared completely during rest but was exacerbated only by exertion. Therapy with steroids and later with methotrexate and azothiaprine led to several side effects. With steroid therapy alone, which was tapered down, the patient showed clinical improvement as well as normalization of ESR. However, throughout the treatment the patient was non-compliant with her medications.

Follow-up CT angiograms demonstrated neither progression nor amelioration of the vasculitis. Considered an atypical case of GCA, the patient was conservatively treated with steroids and was followed in the outpatient clinic for 2 years. Medical therapy was challenging due to partial remission, drug-induced complications, and non-compliance throughout treatment. Irreversible damage was revealed in the left brachial and axillary arteries on the angiogram, and residual

Table 1. Reported cases of GCA/LVV presenting as upper limb claudication

Case	Diagnosis	Age/ gender	Symptoms	Involved arteries	Treatment	Outcome	Reference
1	GCA	78/ F	Persistent left arm pain	Bilateral, axillobrachial	High dose CS, then tapered off	Partial clinical improvement post-CS therapy	Katz-Agranov *IMAJ* (present case)
2	GCA	76/ M	Vertigo, left arm claudication	Bilateral external carotid, bilateral axillobrachial	High dose CS followed by methotrexate due to relapse and CS intolerance	Clinical improvement post-CS therapy, several relapses, later remission	Kolossváry . *Int Angiol* 2005; 24: 202
3	LVV	71/ F	Intermittent left arm claudication	Internal L. carotid, L. subclavian	Multiple surgical bypass surgeries and CS therapy	Clinical improvement, later a severe relapse involving subclavian-axillary necessitating additional surgical bypass. Then clinically stable	Lambert *Clin Rheumatol* 1996; 15: 174
4	LVV	65/ F	Weakness of both arms, worse on exertion	Bilateral axillary, L. vertebral	CS therapy along with pharmacological bilateral cervical sympathectomy	Significant clinical improvement following surgery. Relapsed and treated with high dose CS therapy, then clinically stable	Lambert
5	LVV	63/ F	Bilateral upper extremity claudication	L. subclavian, R. axillary	High dose CS therapy and R. carotid artery endarterectomy	Clinical improvement, low dose CS maintenance therapy	Lambert
6	GCA	62/ F	Vascular symptoms of upper limb and deterioration in general condition	Bilateral post-vertebral	High dose CS therapy	Asymptomatic on long-term follow-up, no angiographic improvement on follow-up	Ninet *Am J Med* 1990; 88: 13
7	GCA	65/ F	Vascular symptoms of upper limb	Bilateral axillary	High dose corticosteroid therapy	Partial clinical improvement, no angiographic improvement on follow-up. Remained dependent on low dose CS maintenance therapy	Ninet
8	GCA	67/ F	Vascular symptoms of upper limb and deterioration in general condition	L. brachial-axillary, bilateral humoral	High dose corticosteroid therapy and transitory anticoagulation therapy	Clinical improvement; asymptomatic with maintenance therapy; angiographic normalization of affected arteries	Ninet
9	GCA	73/ F	Vascular symptoms of upper limb and deterioration in general condition	Bilateral subclavian axillary (NS)	High dose CS therapy and transitory anticoagulation therapy	Clinical improvement, no angiographic improvement	Ninet
10	GCA	70/ F	Vascular symptoms of upper limb	L. subclavian, R. axillary	High dose CS therapy	Persistence of intermittent claudication requiring maintenance therapy then lost to follow-up	Ninet
11	GCA	71/ F	Weakness and pain in upper extremities, chronic headaches	R. subclavian, R. axillary	High dose CS therapy	Clinical improvement, restored peripheral pulses	Van Damme *Angiology* 1989; 40: 593
12	GCA	78/ F	Gangrene of L. thumb, weakness in both upper extremities, painful mastication	L. subclavian, L. axillary, R. subclavian, R. axillary	Bypass surgery, high dose CS therapy	Clinical improvement, restored peripheral pulses	Van Damme
13	GCA / LAI	67/ F	Occipital headaches, bilateral arm claudication	Bilateral axillary	High dose CS therapy	NS	Stanson *Am J Roentgenol* 1976; 127: 957
14	GCA / LAI	67/ F	Claudication of left arm in patient with polymyalgia rheumatica	Bilateral axillary	High dose CS therapy	Clinical improvement, restoration of R. arm pulses	Stanson
15	GCA / LAI	59/ F	Arm claudication	Bilateral subclavian, bilateral axillary, R. internal mammary	High dose CS therapy	NS	Stanson
16	GCA / LAI	71/ F	Isolated arm claudication	L. axillary, R. brachial	High dose CS therapy	NS	Stanson

GCA = giant cell arteritis, LVV = large vessel vasculitis, TA = temporal artery, LAI = large artery involvement, CS = corticosteroids, NS = not specified

pain and functional impairment in both arms were reported by the patient.

COMMENT

Due to the clinical presentation and findings, our patient qualified for both GCA and TA, according to the 1990 ACR clinical criteria, as she met three of the five criteria for GCA and three of six for TA.

Involvement of the aorta and its large vessels has long been considered an unusual manifestation of GCA and more characteristic of TA. Hence, the involvement of large vessels in GCA can cause overlap in the diagnostic criteria of GCA and TA, both of which have similar histological abnormalities and share pathogenic pathways. Nevertheless, distinguishing between these diseases is thought to be important for defining the appropriate treatment and surveillance because, unlike GCA, TA has a chronic course with relapses and remissions and is associated

with substantial morbidity and mortality ranging from 3% to 15% [2]. The diagnosis is often delayed and is usually established only when arterial stenoses or occlusions are present. Its course usually extends for many years with varying degrees of activity, and there may be ongoing inflammation even in the absence of symptoms. Only 20% of patients have a monophasic and self-limited disease. Therefore, long-term immunosuppressive therapy is considered necessary to avoid complications. According to various studies, vascular interventions are performed in more than 50% of cases [2]. Although the course of illness in large vessel GCA is usually benign and comparable to that of classic GCA, in some studies vascular intervention is undertaken [3,4].

In a recent study comparing angiographic findings of GCA and TA, lesions in both vasculitides were generally symmetrical in paired arteries and contiguous in the aorta with only a few differences in patterns of arterial involvement. Carotid and mesenteric arterial disease was seen more frequently in TA, axillary disease was more frequent in GCA, and a tendency towards asymmetric involvement of the subclavian artery was seen in TA with a high frequency of left subclavian artery involvement in contrast to symmetric subclavian with concomitant axillary involvement in GCA. Most arterial lesions were stenotic or occlusive and no significant differences were observed between TA and GCA regarding the type of arterial lesions [5].

We searched the literature for GCA/TA overlap cases and found only one case, in English, which raised the question of large vessel GCA versus TA. There are, however, anecdotal reports and case series of GCA variants/subtypes, such as atypical or "pulseless large vessel (LV-GCA), involving upper extremities" or "isolated form of GCA" [Table 1]. In many of these cases the presenting symptom was similar to that in our patient, namely upper extremity pain and/or claudication, while others presented with severe signs of limb ischemia, Raynaud's phenomenon or constitutional symptoms. Characteristic symptoms of GCA, such as headache or visual disturbances, were absent in many cases, as in ours. The arteries most commonly involved were the subclavian, axillary and brachial arteries, and there was a female predominance similar to that in TA. Similar to our patient, biopsy-negative cases were common. Most cases, however, had clinical and radiographic findings sufficient for diagnosis, and responded to steroid therapy.

Therefore, subtle differences in the distribution of the arterial disease between GCA and TA suggest that they may even exist on a continuum within the same disease in which disease expression may be influenced by age-related factors such as hormonal, immunologic and vascular factors.

Treatment is challenging in both GCA and TA. Currently, the best evidence-based treatments include high dose steroids. However, 40.8–48% of GCA patients and 46–84% of TA patients require additional immunosuppressive agents to achieve remission and as steroid-sparing agents. Many patients received biologic agents (mainly infliximab), followed by methotrexate, azathioprine, cyclophosphamide or cyclosporine, which are particularly effective in cases of GCA resistant to adjunctive therapy with methotrexate/azathioprine. Various medical regimens are sufficient for partial or complete remission [3] in GCA and TA. However, in resistant TA patients interventional methods such as balloon angioplasty (percutaneous transluminal coronary angioplasty) and/or surgery (bypass, embolectomy, etc.) may be beneficial. These same interventional methods may be considered in resistant cases of GCA involving large vessels [4]. The disadvantage of invasive therapy is re-occlusion or restenosis and peri-procedural complications [4].

Our case presents the difficulties in managing a case of GCA/TA overlap. We conclude that there may be need for a revision of the diagnostic criteria for these large vessel vasculitides in an era of advanced radiological technologies, and emphasize the need to screen for involvement of large vessels in cases of GCA. Acknowledging that GCA may overlap with TA could result in a more prompt diagnosis and accurate management where the use of interventional treatment should be further investigated. These issues should be taken into account when devising new classification criteria for large vessel vasculitides. Our unusual case of GCA emphasizes the difficulty in diagnosis, treatment and surveillance of large vessel vasculitis based on current diagnostic criteria.

Correspondence
Dr. G. Zandman-Goddard
Head, Dept. of Internal Medicine C, Wolfson Medical Center, Holon 58100, Israel
Phone: (972-3) 503-8674
Fax: (972-3) 502-8810
email: goddard@wolfson.health.gov.il

References

1. Nesher G. The diagnosis and classification of giant cell arteritis. *J Autoimmunity* 2014; 48-49: 73-5.
2. Alibaz-Oner F, Zehra Aydin S, Direskeneli H. Advances in the diagnosis, assessment and outcome of Takayasu's arteritis. *Clin Rheumatol* 2013; 32: 541-6.
3. Czihal M, Piller A, Schroettle A, Kuhlencordt PJ, Schulze-Koops H, Hoffmann U. Outcome of giant cell arteritis of the arm arteries managed with medical treatment alone: cross-sectional follow-up study. *Rheumatology* (Oxford) 2013; 52: 282-6.
4. Assie C, Janvresse A, Plissonnier D, Levesque H, Marie I. Long-term follow-up of upper and lower extremity vasculitis related to giant cell arteritis: a series of 36 patients. *Medicine* (Baltimore) 2011; 90: 40-51.
5. Grayson PC, Maksimowicz-Mckinnon K, Clark TM, et al. Distribution of arterial lesions in Takayasu's arteritis and giant cell arteritis. *Ann Rheum Dis* 2012; 71: 1329-34.

"A government big enough to give you everything you want is strong enough to take everything you have"

Thomas Jefferson (1743-1826), American Founding Father, the principal author of the Declaration of Independence, and the third President of the United States. He was a spokesman for democracy

Digital Ulcers, Systemic Sclerosis Sine Scleroderma and Paraneoplastic Phenomena Responding to Bosentan Therapy

Itzhak Sharabi MD[1], Amir Tanay MD[2,3] and Gisele Zandman-Goddard MD[1,3]

[1]Department of Medicine C, and [2]Allergy, Immunology & Rheumatology Clinic, Wolfson Medical Center, Holon, Israel
[3]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

KEY WORDS: digital ulcers, systemic sclerosis sine scleroderma, paraneoplastic disease, systemic sclerosis, bosentan

IMAJ 2015; 17: 126–127

Paraneoplastic syndrome is defined as a constellation of signs and symptoms that are secondary to the presence of a malignancy and can present as an atypical autoimmune disease. Regression of the rheumatologic disorder occurs following treatment of the underlying malignancy [1]. Systemic sclerosis (SSc) is a severe multiorgan disease characterized by widespread fibrosis and extensive vascular damage, and activation of the immune system with production of autoantibodies. The vascular involvement of SSc manifests as Raynaud's phenomenon (RP) and digital ulcers (DU) [2]. Most patients with SSc will develop limited or diffuse scleroderma while systemic sclerosis sine scleroderma is a rare variant of SSc [3]. We present the case of a patient with systemic sclerosis sine scleroderma who later developed lung cancer.

PATIENT DESCRIPTION

A 69 year old woman, a current smoker with a history of 80 pack-years, complained of pain and blue discoloration of the third digit of her right hand that began a few days earlier. Her past history included moderate chronic obstructive lung disease with 55% FEV1 on pulmonary function tests, malignant neoplasm of breast post-left lumpectomy and radiotherapy in 2008, gastroesophageal reflux disease, hypertension and hyperlipidemia. She denied trauma to this hand, had not taken a new medication in the past 6 months, had not experienced thrombotic events in the past, and neither she nor her family had a history of Raynaud's phenomenon. There was no history of decreased appetite, weight loss, change in cough, or dyspnea. Her relevant medical therapy included aspirin, omepradex, cilazapril, simvastatin and tamoxifen.

The rheumatology examination revealed tenderness in the third digit of her right hand with transient blue discoloration, without evidence of calcinosis, thickening of the skin on the face, trunk or limbs, sclerodactyly, telangiectasis, or arthritis. Routine laboratory tests, including complete blood count, liver, renal and thyroid function, hypercoagulability tests, cardiolipin immnunoglobulins (Ig) G and M antibodies and antinuclear antibodies (ANA), were all within normal limits. Ultrasound/Doppler of the right hand was normal. A computed tomography (CT) and CT angiography of the chest and abdomen and an echocardiogram were normal.

The patient was initially diagnosed with ischemia of the hand due to diffuse atherosclerosis. Despite treatment with enoxaparin and aspirin, the findings persisted. Over the next 2 months, her situation worsened further with cyanosis of all five digits of the right hand and in the fourth and fifth digits of the left hand accompanied by severe pain requiring therapy with opiates.

Treatment with iloprost 2 ng/kg/min IV over 6 hours was initiated for 3 weeks together with high doses of oxycontin. Nevertheless, the patient eventually developed autoamputation of the distal phalanxes of the third and fourth digits of her right hand.

Repeated serology for hypercoagulability, vasculitis and cancer markers were normal. However, a repeated serology in the same laboratory revealed elevated titers of ANA 1:320 and anticentromere antibodies that were initially negative during the first hospital admission. The patient was diagnosed with systemic sclerosis sine scleroderma where digital ulcers and the appropriate serological tests were the only manifestations of disease.

She was treated with bosentan 125 mg twice a day as a monotherapy and the response was immediate and dramatic. No new digital ulcers developed. Within 8 weeks, the necrotic areas disappeared and she could discontinue opiate therapy. The patient developed calcinosis of the finger beds.

One year after the initial presentation, she underwent a repeated CT chest scan for persistent cough which showed a 1 cm nodule in the left lower lobe and a 8 mm nodule in the left upper lobe. Fine-needle aspiration and pulmonary nodule biopsy from the two nodules revealed an adenocarcinoma on histology. Left video-assisted thoracoscopic surgery (VATS) wedge resection was performed to the nodule of the left lower lobe. Histology depicted adenocarcinoma, without vascular invasion (stage pT2A). In addition, stereotactic body radiotherapy (SBRT) to the nodule of the left upper lobe was performed. Follow-up

FDG-PET-CT (fluorodeoxyglucose-positron emission tomography-CT) revealed no pathological activity in either nodule, indicating complete remission. During the entire period the patient continued therapy with bosentan. No recurrence of digital ulcer occurred over 2 years of follow-up.

COMMENT

This is an unusual presentation of SSc sine scleroderma where the hallmark single feature was severe digital necrotic ulcers. The patient did not develop thickened skin, Raynaud's phenomenon or visceral disease, and the diagnosis was based on the single manifestation and appropriate autoantibody status which included elevated titers of ANA and anticentromere antibodies. Hence, we could not classify the patient as limited or diffuse scleroderma [4]. The unusual manifestation of SSc sine scleroderma was actually the expression of an occult cancer that became clinically evident within a year. In one study, SSc sine scleroderma was found in 8.3% of 947 patients in a SSc cohort. The diagnosis was determined in patients who suffered from Raynaud's phenomenon, positive ANA, and one visceral involvement. These patients were more often females, had anticentromere antibodies, and 12.5% were referred from vascular surgery due to digital ulcers. Digital ulcers were present in 24.1% of these patients. These findings correlate with our case [3]. The unusual findings prompted us to search for paraneoplastic disease. The literature lacks information regarding the systemic sclerosis sine scleroderma subset and the association with paraneoplastic syndrome.

We reviewed the literature and identified another 14 reported cases of digital ulcer as a paraneoplastic syndrome of lung cancer [Table 1]. The male to female ratio was 1.8:1, and the age range was 40–78 years in males and 52–69 years in females. Eighty percent of the patients developed digital ischemia/ulcer or Raynaud's syndrome within a year prior to diagnosis of lung carcinoma; 33%, including our patient, had elevated titers of ANA, and only 13% had elevated anticentromere antibody titers. Of the 15 reported cases, 5 had adenocarcinoma.

Table 1. Paraneoplastic digital ulcers and/or paraneoplastic Raynaud's syndrome in lung carcinoma

No.	Author, year	Age/ Gender	Serologic investigation	Histological type of lung cancer
1	Sharabi et al. (present)	69/ F	ANA positive 1:320, anticentromere positive, anticardiolipin negative	Adenocarcinoma
2	Adedayo, 2012	40/ M	ANA, antipospholipid antibody, anticardiolipin, lupus anticoagulant – all negative	Non-small cell
3	Moulakakis, 2010	70/ M	N/A	Lung Ca diagnosed by CT only
4	Schildmann, 2010	78/ M	ANA negative	Non-small cell
5	Schmid, 2008	62/ F	ANA, RF, cryoglobulin, ANCA, HIV – all negative	Non-small cell
6	Kopterides, 2004	65/ F	ANA, antiphospholipid anticardiolipin, RF and cryoglobulin – all negative	Adenocarcinoma
7	Wong, 2003	62/ M	ANA, RF, extractable nuclear negative	Non-small cell
8	Imandi, 2002	73/ M	ANA positive 1:1280	Small cell
9	Vaidya, 2001	52/ F	ANA positive 1:320 elevated IgG anticardiolipin	Non-small cell
10	Wang, 1996		Elevated cryoglobulinemia	Non-small cell
11	Arrowsmith, 1991	63/ M	Positive Hep2 cell substrate for centromere-specific ANA 1:2560	Small cell
12	Wilmalaranta, 1987	69/ M	ANA, RF, cryoglobulin – all normal	Adenocarcinoma
13	Field, 1986	70/ M	ANA, RF cryoglobulin – all negative	Small cell anaplastic carcinoma
14	Petri, 1985	62/ M	ANA positive 1:320	Adenocarcinoma
15	Domz, 1961	50/ F	Elevated cryoglobulinemia	Adenocarcinoma

ANA = antinuclear antibody, N/A = not available, RF = rheumatoid factor, ANCA = antineutrophil cytoplasmic antibodies, HIV = human immunodeficiency virus

Bosentan, an endothel-1 antagonist, is beneficial for digital ulcers in SSc [5]. Compared to the 14 other cases of paraneoplastic digital ischemia/ulcer that we reviewed, our case was the only one, to our knowledge, where bosentan was initiated and proved effective as treatment for paraneoplastic digital ulcer, showed an excellent response on healing, and prevented new ulcers despite ongoing cancer.

In conclusion, this case emphasizes that patients who develop unusual manifestations of systemic sclerosis, like SSc sine scleroderma with digital ischemia and digital ulcer in whom the accepted causes have been excluded, deserve the fullest investigation in an attempt to discover an occult malignancy. We emphasize that the digital ulcers responded immediately to therapy with bosentan, before the malignancy was removed.

Correspondence

Dr. G. Zandman-Goddard

Dept. of Medicine C, Wolfson Medical Center, Holon 58100, Israel

Phone: (972-3) 502-8674

Fax: (972-3) 502-8810

email: goddard@wolfson.health.gov.il

References

1. Racanelli V, Prete M, Minoia C, Favino E, Perosa F. Rheumatic disorders as paraneoplastic syndromes. *Autoimmunity Rev* 2008; 7: 352-8.
2. Gabrielli A, Avvedimento EV, Krieg T. Scleroderma. *N Engl J Med* 2009; 360: 1989-2003.
3. Van den Hoogen F, Khanna D, Fransen J, et al. 2013 Classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum* 2013; 65: 2737-47.
4. Marangoni RG, Rocha LF, Del Rio APT, et al. Systemic sclerosis sine scleroderma: distinct features in a large Brazilian cohort. *Rheumatology* 2013; 52: 1520-4.
5. Matucci-Cerinic M, Denton CP, Furst DE, et al. Bosentan treatment of digital ulcers related to systemic sclerosis: results from the RAPIDS-2 randomised, double-blind, placebo-controlled trial. *Ann Rheum Dis* 2011; 70: 32-8.

Immune Mediated Myopathy following Long-Term Statin Therapy

Abdulla Watad MD[1,2], Alessandra Soriano MD[1,5], Hananya Vaknine MD[4], Yehuda Shoenfeld MD FRCP MaACR[1,3] and Howard Amital MD MHA[1,2,3]

[1]Zabludowicz Center for Autoimmune Diseases and [2]Department of Medicine B, Sheba Medical Center, Tel Hashomer, Israel
[3]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[4]Institute of Pathology, Wolfson Medical Center, Holon, Israel
[5]Department of Clinical Medicine and Rheumatology, Campus Bio-Medico University of Rome, Italy

KEY WORDS: polymyositis, statins, immune-mediated myopathy, atorvastatin

IMAJ 2015; 17: 128–129

Immune-mediated myopathies (IMMs) are a heterogeneous group of acute, subacute and chronic acquired diseases involving the skeletal muscle, all characterized by the presence of moderate to severe muscle weakness and inflammation. The most common IMMs are dermatomyositis (DM), polymyositis (PM), necrotizing autoimmune myositis (NAM), and inclusion body myositis. Among the IMMs, PM is rare and constitutes a single entity that often remains a diagnosis of exclusion. It occurs in subjects above the age of 18, manifesting as proximal muscle weakness, elevated creatine kinase (CK) levels, and a characteristic electromyographic pattern. Indeed, it is often misdiagnosed due to the lack of a unique clinical phenotype. In PM the fundamental immune process is mediated by CD8+ cytotoxic T cells that invade non-necrotic muscle fibers expressing major histocompatibility complex (MHC)-I antigen, whereas in dermatomyositis an abundance of B cells penetrate the fascicles between the myofibers [1].

Toxic myopathy is an uncommon side effect of statin therapy, and in most cases it presents as a self-limited condition that subsides with discontinuation of the drug. It is important to differentiate statin-associated IMMs from toxic myopathy, because patients with IMMs do not recover after discontinuing the drugs and often require immunosuppressive therapy. Among all the IMMs, polymyositis induced by statins has been described rarely in the literature [3]. We describe the case of a patient who developed biopsy-proven polymyositis following prolonged treatment with atorvastatin.

PATIENT DESCRIPTION

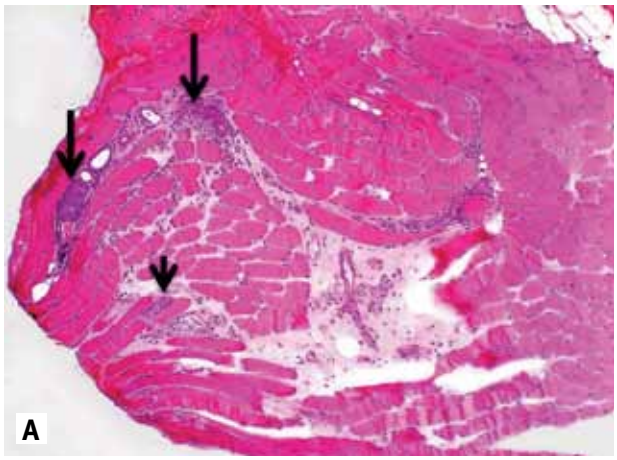
A 71 year old woman was admitted to our hospital due to weakness, myalgia, dry mouth and weight loss over the previous 3 months. Her general practitioner noticed proximal weakness, widespread tenderness and swelling of her left leg, with no other significant findings on physical examination. Initially non-steroidal anti-inflammatory drugs (NSAIDs) were prescribed but there was no significant improvement. The patient had no respiratory, abdominal or urinary symptoms, and no arthralgia, skin rash, fever, or nocturnal sweats. Her medications included atenolol 25 mg/day and atorvastatin 20 mg/day because of essential hypertension and hypercholesterolemia over the previous 5 years. There was no family history of autoimmune diseases. She was engaged in aerobic exercises three times a week, but a few weeks before admission she was unable to perform basic activities.

Blood tests revealed a significant increase in CK (> 4000 IU/L) and slightly elevated transaminases and C-reactive protein (CRP, 6.54 mg/dl). On admission, her temperature was 36.6°C, blood pressure 157/77 mmHg, pulse 70 beats/min, and weight 62 kg. On physical examination the lungs were clear, no heart murmurs were heard, lymph nodes were not palpable, and there was no hepatosplenomegaly, skin pigmentation or lesions. The only notable finding was proximal symmetric weakness of 3/5 of the four limbs with minimal swelling of the left leg.

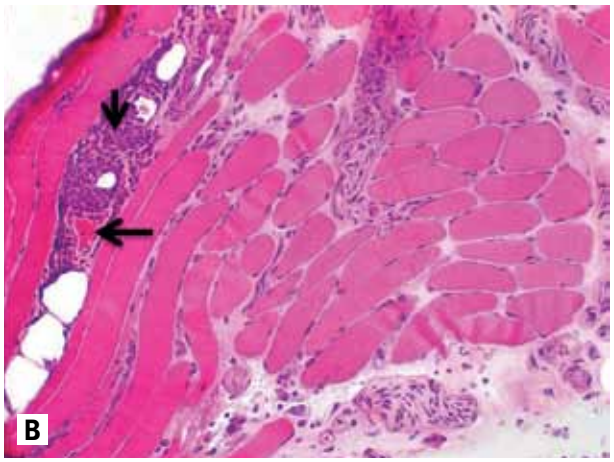
Autoantibody screening (including anti-Jo-1, anti-PL-7, anti-PL-12, anti-SRP, anti-Mi-2, anti-Ku, anti-Scl-70, anti PM-Scl) was negative except for antinuclear antibodies with a fine speckled pattern at 1:160 titer. Anti-HMG CoA reductase was also negative. The electrocardiogram was normal, a whole-body computed tomography scan did not demonstrate relevant findings and neither did a gynecological examination.

An electromyogram showed signs of myopathy with denervation features in proximal muscles of the upper limbs, while the nerve conduction study revealed superficial peroneal nerve dysfunction on the right; no further signs of neuropathy were detected. A muscle biopsy was performed, revealing several foci of endomysial perivascular infiltrate, with concomitant evidence of muscle fiber necrosis, suggestive of polymyositis [Figure 1].

Prednisone was initiated at a dosage of 60 mg/day, leading to clinical improvement and rapid decrease in levels of CK, liver function enzymes, and acute-phase reactants. Steroid tapering was subsequently performed by adding methotrexate (MTX) at the maximum dosage of 15 mg/week. After one year of surveillance the patient continues to enjoy a complete clinical remission.



[A] Low power view depicting variation in muscle fiber size along with endomysial mononuclear inflammatory cell infiltrates (long arrow) and necrotic muscle fiber (short arrow). Hematoxylin & eosin x40



[B] A higher power view demonstrates that the infiltrate is both perivascular (long arrow) and insinuating between skeletal muscle fibers within the endomysium (short arrow). Hematoxylin & eosin x100

COMMENT

Statins are commonly prescribed medications for the treatment of hyperlipidemia and have a good safety profile. However, in 5%–20% of cases the therapy has to be discontinued because of side effects [2]. One of the most common side effects involves the musculoskeletal system. Patients often complain of myalgias, but myositis and rhabdomyolysis have also been recorded. Myopathic symptoms produced by statins generally resolve within several months following cessation of the medication. Nevertheless, in rare cases statins may trigger IMMs that do not resolve despite discontinuing therapy and immunosuppressive treatment is required [2].

A recent literature review by Padala and Thomson [3] to identify statin-associated inflammatory and necrotizing myopathies yielded 10 cases of PM, 14 of DM, and 63 of statin-related NAM. Among the PM cases reported, only one was histologically well defined, six were defined as "probable" (by the presence of three of four criteria for the diagnosis of PM) and three as "possible" (by the presence of two of the four criteria), according to the Bohan and Peter diagnostic criteria [3]. In all the cases, underlying malignancy and connective tissue disorders had previously been excluded. Anti-Jo-1 was found positive only in 3 cases, while antinuclear antibodies (ANA) were positive in 6 of 10 cases. The mean duration of statin exposure symptoms was 47.8 months (range 2 weeks to 42 months) [3].

Bohan and Peter's diagnostic criteria for PM include symmetric proximal muscle weakness progressing over weeks to months; muscle biopsy showing myofiber necrosis, phagocytosis, regeneration, variation in fiber diameter, and an inflammatory exudate; elevation of serum skeletal muscle enzymes; and electromyography showing low-amplitude, small, polyphasic motor units. In our patient, all four criteria were fulfilled, confirming this as a definitive case of PM. This case report supports the fact that statin therapy could cause PM even after long-term therapy.

To the best of our knowledge, only two other similar cases have been previously described in the literature. In 2001 Folzenlogen [4] reported the case of a 76 year old man with evidence of serologically and biopsy-proven PM, responsive to both the interruption of atorvastatin and immunosuppressive treatment; Fauchais et al. [5] reported another case of probable PM in a 54 year old woman who developed proximal muscle weakness, Raynaud's phenomenon and non-specific sclerodermic changes of the hands 4 months after initiation of atorvastatin.

In conclusion, muscular symptoms in a patient on long-term statin treatment could be the first indication of polymyositis due to this treatment and should encourage physicians to perform ANA screening, especially in cases of proximal muscular weakness and increased muscle enzyme levels.

Correspondence
Dr. A. Watad
Dept. of Medicine B, Sheba Medical Center, Tel Hashomer 52621, Israel
Phone: (972-3) 530 2435
Fax: (972-3) 535-4796
email: watad.abdulla@gmail.com

References

1. Dalakas MC. Pathogenesis and therapies of immune-mediated myopathies. *Autoimm Rev* 2012; 11: 203-6.
2. Mohassen P, Mammen LA. Statin-associated autoimmune myopathy and HMGCR autoantibodies. *Muscle Nerve* 2013; 48: 477-83.
3. Padala S, Thompson PD. Statins as a possible cause of inflammatory and necrotizing myopathies. *Atherosclerosis* 2012; 222: 15-21.
4. Folzenlogen D. A case of atorvastatin combined toxic myopathy and inflammatory myositis. *J Clin Rheumatol* 2001; 7: 340-5.
5. Fauchais AL, Iba Ba J, Maurage P, et al. Polymyositis induced or associated with lipid-lowering drugs: five cases. *Rev Med Interne* 2004; 25: 294-8.

Etanercept-Induced Pneumonitis: Severe Complication of Tumor Necrosis Factor-Alpha Blocker Treatment

Abdulla Watad MD[1,2], Marina Perelman MD[3], Ribhi Mansour MD[2], Yehuda Shoenfeld MD FRCP MaACR[1,4] and Howard Amital MD MHA[1,2,4]

[1]Zabludowicz Center for Autoimmune Diseases, [2]Department of Medicine B, and [3]Pulmonary and Mediastinal Pathology Unit, Department of Pathology, Sheba Medical Center, Tel Hashomer, Israel

[4]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

KEY WORDS: etanercept, tumor necrosis factor (TNF) inhibitors, pneumonitis, interstitial lung disease (ILD), rheumatoid arthritis (RA)

IMAJ 2015; 17: 000–000

Tumor necrosis factor-alpha (TNFα) is a pleomorphic cytokine with both immune and non-immune biologic effects. The past decade has witnessed a vast increase in our understanding of TNFα biology, resulting in the widespread use of several TNF inhibitors (TNFi) for the treatment of rheumatoid arthritis (RA) and other rheumatic diseases. However, we acknowledge that TNFi may have a variety of adverse effects. Aside from infectious pulmonary complications, which have been extensively reviewed, non-infectious complications of TNFi have only recently been recognized. We report the case of a patient with RA who developed pneumonitis following treatment with etanercept.

PATIENT DESCRIPTION

A 63 year old, non-smoking, woman presented with dry cough and intermittent dyspnea that had persisted for 4 months. She had a 10 year history of RA, hypertension and diabetes mellitus type 2 and had been treated with methotrexate (MTX) at a dose of 7.5 mg weekly, prednisone 5 mg/day, atenolol 50 mg/day, aspirin 100 mg/day, insulin glargine 73 IU/day, and metphormin 850 mg twice daily. Her disease activity was not adequately controlled with this treatment and entanercept 50 mg weekly was initiated 16 weeks before presentation. Following treatment with etanercept, her arthritic symptoms abated and laboratory parameters such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) normalized. About 3 months prior to her presentation, she developed dyspnea and a dry cough.

On admission, her physical examination revealed swelling and tenderness of the metacarpophalangeal joints in her hands and knees, fine wheezes on the right lower lobe of the lung, absence of heart murmurs, bilateral moderate edema in her legs, fever of 39°C, 85% hemoglobin saturation (SPO2) in room air and 94% with oxygen supplement, respiratory rate 30, heart rate 71, and speech dyspnea associated with recruitment of the accessory respiratory muscles. Laboratory studies showed a white cell count of 8600/μl (81% of neutrophils), lactate dehydrogenase 376 IU/L and CRP 173 mg/L. Other common blood chemistries were within normal limits. Chest X-ray [Figure 1A] revealed bilateral interstitial infiltrates, enhanced on the right side. A chest computed tomography demonstrated ground-glass opacities with bilateral peripheral consolidations [Figure 1B].

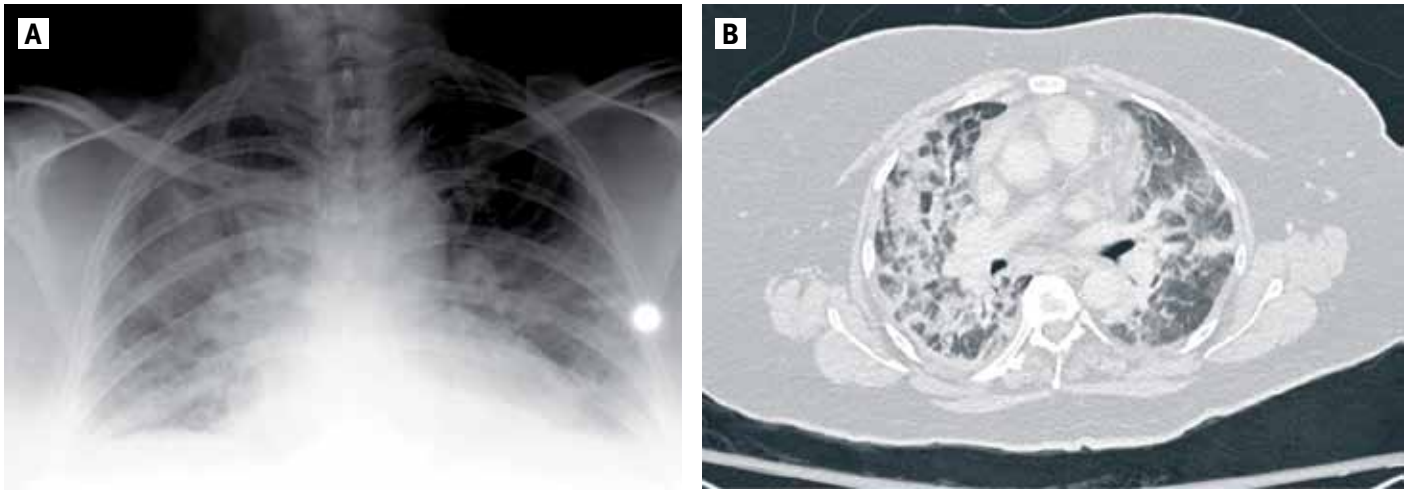
Serology tests for human immunodeficiency virus and cytomegalovirus were also negative. Bronchoscopy with bronchoalveolar lavage was performed and was negative for *Pneumocystis jiroveci*. Echocardiogram did not reveal significant pathological findings. Video-assisted thoracoscopic surgery biopsy of lung [Figure 2] demonstrated diffuse homogenous thickening of the alveolar septae with chronic inflammatory infiltrate, associated with reactive hyperplasia of type II pneumocytes. Some of the alveolar spaces contained fibrin, partially with organizing fibrin. Occasional hyaline membranes were seen. No microorganisms were identified on periodic acid-Schiff, Grocott methenamine-silver, Gram or Ziehl-Neelsen stains. Immunohistochemical stains for Pneumocystis and cytomegalovirus were negative. The above morphological findings were compatible with a cellular-type non-specific interstitial pneumonia pattern, associated with features of acute and organizing diffuse alveolar damage. These morphological patterns of lung injury can be seen in autoimmune diseases, lung drug toxicities, and infections.

Treatment with ceftriaxone and azithromycin was initiated, and cotrimoxazole was later added for *Pneumocystis jiroveci* pneumonia. Since there was no improvement with the antibiotic regimen, the dosage of steroid treatment was increased. Despite the high dose of steroids, the patient continued to deteriorate and become hypoxic despite oxygen supplement, without any improvement using an oxygen reservoir mask or BIPAP. The patient was intubated and transferred to the intensive care unit.

COMMENT

Drug-induced interstitial lung disease (ILD) has been reported in the past as a rare but severe adverse effect of disease-modifying antirheumatic drugs (DMARDs) such as gold and MTX. A meta-analysis of 21 studies with 8276 RA patients conducted by

Figure 1. [A] Enlarged cardiac silhouette with bilateral pulmonary interstitial infiltrates, enhanced primarily on the right side. **[B]** Chest computed tomography demonstrating ground-glass opacities with multiple bilateral peripheral consolidations



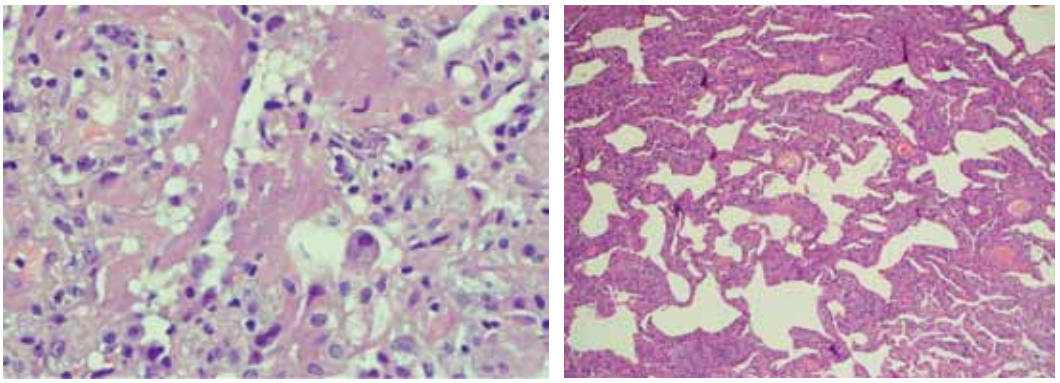


Figure 2. [A] Diffuse thickening of interstitium by chronic inflammatory infiltrate (Hematoxylin & eosin x 100). **[B]** Higher magnification showing hyaline membranes (H&E x 400)

Conway et al. [1] for the period 1990–2011 showed that MTX was not associated with an increased risk of total adverse respiratory events and there was no difference in the risk of pulmonary death among MTX-treated RA patients. However, they found an increased risk of pneumonitis among MTX-treated patients (relative risk 7.0, 95% confidence interval 1.57–31.05). Treatment of such events consisted of drug cessation and sometimes the addition of corticosteroids especially in patients who remain symptomatic following MTX withdrawal.

The general prognosis of MTX-induced acute and subacute lung toxicity is usually favorable. Some patients present with subsequent respiratory failure. A review of 123 published cases of MTX-induced pneumonitis, including 62 RA cases, demonstrated a mortality rate of 13% secondary to respiratory disease [1]. A total of 122 cases of new onset (62%) or exacerbation (38%) of ILD secondary to treatment of systemic autoimmune diseases with biological drugs were collected and analyzed by the BIOGEAS registry project [2]. The underlying disease was RA in 89% of the patients, but cases also occurred in patients with inflammatory bowel disease, ankylosing spondylitis, psoriatic arthritis and other rheumatic diseases [2]. Except for five cases that emerged during rituximab treatment, all the patients were treated with anti-TNF, with etanercept and infliximab each accounting for almost 50% of cases while only 3 cases were associated with adalimumab. Interestingly, two-thirds of the patients had used MTX in the past but only 16% were current users. Only a few ILD cases among patients undergoing therapy with adalimumab have been described [3]. In addition, a patient with RA who had received tocilizumab for 8 months experienced a fatal acute exacerbation of ILD [4].

Post-marketing surveillance studies in Japan indicated that the frequencies of drug-related ILD in Japanese RA patients treated with infliximab or etanercept were 0.5% and 0.6%, respectively [5]. In accordance with findings in TNF antagonist-naïve RA patients, men were affected by ILD twice as frequently as women. ILD is also a frequent extraarticular complication

of RA, and it is unclear whether treatment with TNF antagonists truly raises the risk of such occurrences developing. Very few cases of ILD have been reported in anti-TNF-treated patients with ankylosing spondylitis, which is less often associated with ILD. Several reports underlined the occurrence of ILD in the first months following initiation of anti-TNF therapy, which strongly suggests a causal link [2,3,5].

In this report we describe a patient with RA without known extra-articular involvement who presented with an acute pulmonary disease following initiation of therapy with etanercept. Given the increasing use of biological therapy, we believe that clinicians should be alert to this association and learn to recognize it early in the disease. The literature stresses that early intervention results in better chances of survival.

Correspondence
Dr. A. Watad
Dept. of Medicine B, Sheba Medical Center, Tel Hashomer 52621, Israel
Phone: (972-3) 530-2435
Fax: (972-3) 535-4796
email: watad.abdulla@gmail.com

References

1. Conway R, Low C, Coughlan RJ, O'Donnell MJ, Carey JJ. Methotrexate and lung disease in rheumatoid arthritis – a meta-analysis of randomized controlled trials. *Arthritis Rheum* 2014; 66: 803-12.
2. Perez-Alvarez R, Perez-de-Lis M, Diaz-Lagares C, et al. Interstitial lung disease induced or exacerbated by TNF-targeted therapies: analysis of 122 cases. *Semin Arthritis Rheum* 2011; 41: 256-64.
3. Komiya K, Ishii H, Fujita N, et al. Adalimumab-induced interstitial pneumonia with an improvement of pre-existing rheumatoid arthritis-associated lung involvement. *Intern Med* 2011; 50: 749-51.
4. Kawashiri SY, Kawakami A, Sakamoto N, Ishimatsu Y, Eguchi K. A fatal case of acute exacerbation of interstitial lung disease in a patient with rheumatoid arthritis during treatment with tocilizumab. *Rheumatol Int* 2012; 32: 4023-6.
5. Takeuchi T, Tatsuki Y, Nogami Y, et al. Post-marketing surveillance of the safety profile of infliximab in 5000 Japanese patients with rheumatoid arthritis. *Ann Rheum Dis* 2008; 67: 189-94.

Capsule

RNA viruses promote activation of the NLRP3 inflammasome through a RIP1-RIP3-DRP1 signaling pathway

The NLRP3 inflammasome functions as a crucial component of the innate immune system in recognizing viral infection, but the mechanism by which viruses activate this inflammasome remains unclear. Wang et al. found that inhibition of the serine-threonine kinases RIP1 (RIPK1) or RIP3 (RIPK3) suppressed RNA virus-induced activation of the NLRP3 inflammasome. Infection with an RNA virus initiated assembly of the RIP1-RIP3 complex, which promoted activation of the GTPase DRP1 and its translocation to mitochondria to drive mitochondrial damage and activation of the NLRP3 inflammasome. Notably, the RIP1-RIP3 complex drove the NLRP3 inflammasome independently of MLKL, an essential downstream effector of RIP1-RIP3-dependent necrosis. Together our results reveal a specific role for the RIP1-RIP3-DRP1 pathway in RNA virus-induced activation of the NLRP3 inflammasome and establish a direct link between inflammation and cell-death signaling pathways.

Nature Immunol 2014; 15: 1126

Eitan Israeli

Capsule

Variation at HLA-DRB1 is associated with resistance to enteric fever

Enteric fever affects more than 25 million people annually and results from systemic infection with *Salmonella enteric serovar typhi* or paratyphi pathovars A, B or C1. Dunstan et al. conducted a genome-wide association study of 432 individuals with blood culture-confirmed enteric fever and 2011 controls from Vietnam. We observed strong association at rs7765379, odds ratio (OR) for the minor allele = 0.18, $P = 4.5 \times 10–10$, a marker mapping to the HLA class II region, in proximity to HLA-DQB1 and HLA-DRB1. The authors replicated this association in 595 enteric fever cases and 386 controls from Nepal and also in a second independent collection of 151 cases and 668 controls from Vietnam. Imputation-based fine-mapping across the extended MHC region showed that the classical HLADRB1*04:05 allele (OR = 0.14, $P = 2.60 \times 10–11$) could entirely explain the association at rs7765379, thus implicating HLADRB1 as a major contributor to resistance against enteric fever, presumably through antigen presentation.

Nature Genet 2014; doi:10/1038/ng.3143

Eitan Israeli

"Suppose you were an idiot. And suppose you were a member of government. But then I repeat myself"

Mark Twain (1835-1910), American author and humorist. He wrote *The Adventures of Tom Sawyer* and its sequel, *Adventures of Huckleberry Finn*, the latter often called "the Great American Novel"