Frontier Issues in Autoimmunity: Publications in 2009–2010

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When searching PubMed for an article among the thousands of published medical studies, reviews and case reports of the last two years, one cannot help but notice the dominance of autoimmunity and autoimmune diseases. The main topics of these publications are genetics and epigenetics in autoimmune diseases, the role of B cells and the benefit of targeting them, biological drugs, and infections and autoimmune diseases.

Epigenetics and geoepidemiology in autoimmunity were discussed in more than 300 studies, whereas the role of targeting B cells, particularly their depletion, was discussed in more than 1000 studies in 2010 alone. In this review we summarize the impact of epigenetics and geoepidemiology in autoimmunity and at the same time convey the enthusiasm of immunologists/rheumatologists regarding the targeting of B cells to better treat autoimmune diseases.

EPIGENETICS AND AUTOIMMUNITY

Interactions between environmental and genetic factors are proposed to explain why autoimmunity afflicts certain individuals but not others [1]. Epigenetics is the study of mitotically heritable changes in phenotype (alterations in gene expression) that occur without direct alterations of the DNA sequence [2]. These epigenetic changes include: a) methylation of DNA by the covalent addition of a methyl group to a cytosine residue in a CpG site; b) post-translational modification of the amino acid tails of histones by means of acetylation, phosphorylation, methylation, sumoylation, or ubiquitylation; and c) the aberrant expression of microRNAs, each of which is capable of post-transcriptionally regulating the expression of a cohort of cognate target genes [3,4]. Collectively, these three major epigenetic mechanisms affect interactions of DNA with transcriptional factors, transcript stability, DNA folding, nucleosome positioning, chromatin compaction, and higher-order nuclear organization in a manner that determines whether a gene or a set of genes is silenced or activated and when and where a gene will be expressed. They therefore play crucial roles in determining the transcriptional programs of differentiating or differentiated tissues. This is why epigenetics is recognized today as a key mechanism underlying the development of autoimmunity and immune disorders [5].

Environmentally induced epigenetic changes play a key role in autoimmune disease pathogenesis, especially in those genetically predisposed

Recent advances have been made in our understanding of these changes. In asthma, for example, it is believed that environmental exposures during time periods when there is greater susceptibility to epigenetic regulation may be responsible for developmental plasticity, and these periods coincide with time periods when the asthma phenotype is known to be variable and changing [6]. Considerable work was recently conducted on the induction of these epigenetic changes following exposure to air pollution and folate ingestion during pregnancy and the correlation with asthma and atopy. It was found that the differentiation of Th1 and Th2 is regulated by epigenetic changes in various ways. By repressing epigenetic modifications, such as histone methylation, interferon-gamma transcription is down-regulated in developing Th2 cells. DNA methylation also plays an important role in the control of Th2 cytokine expression and stabilization during T cell development, for instance, in the production of interleukin-4 [7]. Epigenetic mechanisms controlling the development of regulatory T cells are just beginning to be explored. Studies have focused mainly on FoxP3, a pivotal element essential for Treg development and function. Epigenetic changes are a prerequisite for FoxP3 expression, stabilization and Treg suppressive function [8].

Systemic lupus erythematosus is an autoimmune disease where environmentally induced epigenetic changes contribute to disease pathogenesis in those genetically predisposed [9]. Similar interactions were found also in rheumatoid arthritis and scleroderma. The skin is exposed to a wide variety of environmental stimuli, such as ultraviolet radiation, and is thus prone to develop autoimmune conditions like atopic dermatitis, psoriasis and vitiligo. Such changes generate changes in the control of cellular processes [10], thereby promoting the development of autoreactive lymphocytes in SLE, synoviocyte proliferation in rheumatoid arthritis, or neural demyelination in multiple

Treg = regulatory T cells
SLE = systemic lupus erythematosus

sclerosis. It is well known that some SLE-inducing drugs, such as procainamide and hydralazine, affect T cell DNA methylation and thereby cellular function, and that identical changes in T cell DNA methylation and cellular function are found in patients with SLE, again implicating epigenetic mechanisms in the pathogenesis of human SLE and perhaps other autoimmune diseases [11].

Advances in understanding the genetic and epigenetic machinery and in biotechnology have laid the groundwork for potent and specific molecular targeting therapies by gene therapy and have just begun to be investigated for treating autoimmune disorders. Preclinical animal model studies have provided the proof-of-concept for multiple potential applications, but human clinical trials on gene therapy in autoimmunity are still in their infancy. The recent success of Phase I/II clinical trials of gene therapy in rheumatoid arthritis and multiple sclerosis and the development of biotechnology in target identification, as well as gene delivery systems, have moved these concepts from the realm of science fiction to reality [12].

Advances in understanding the genetic and epigenetic machinery together with progress in biotechnology have led to the groundwork for potent and specific molecular gene-targeting therapies

GEOEPIDEMIOLOGY AND AUTOIMMUNITY

Geoepidemiology of autoimmune diseases describes the burden of these illnesses across geographic regions and ethnic populations. Furthermore, geoepidemiology may bear important clues to the genetic and environmental triggering mechanisms of autoimmunity [13]. The geoepidemiology of autoimmune diseases could be described as the method used to compare epidemiological data of these diseases across different geographic regions and populations, and in this process identifies causative genetic, environmental and socioeconomic factors. A growing body of geoepidemiology data of autoimmune diseases has emerged in recent years. Rheumatoid arthritis is one of these disease states that was extensively investigated. A destructive bone and joint disease, RA has a differential pattern of incidence and prevalence among populations [14,15]. It is a multifactorial disease that results from interactions between genetic and environmental factors. The most associated environmental factor was smoking and is linked to worse outcome. The main genetic predisposition to RA was found to be HLA-DRB1 and the tyrosine-phosphatase gene PTPN22. The clinical manifestations of SLE show geographic, genetic and ethnic variation, being less severe in European ancestry than in African, Asian and Hispanic populations [16]. The frequency of familial SLE overall appears to be in the range of 8–10%, the rates being similar in Europeans, Latin Americans, African Americans, Afro-Caribbeans, and some Asian populations. However, Israeli Arab and Omani patients were reported to be exceptions, showing rates of 24% and 48%, respectively. Kidney involvement is a more common phenomenon in non-European patients. Renal damage has been reported to progress faster in African and Hispanic American and African and Chinese Canadian SLE patients compared to their European counterparts. The progression to chronic renal failure was also more common in these ethnic groups. Central nervous system involvement in European, African and Chinese Canadian SLE patients was not significantly different, although this was more frequent in African compared to European Canadian patients [17]. Antiphospholipid antibodies are detectable in 1–5% of asymptomatic healthy subjects and display a higher prevalence in SLE and RA patients than in other autoimmune disorders. However, not all aPL-positive SLE patients display this clinical manifestation. In this regard, it was found that ethnicity influences the prevalence of aPL. Immunoglobulin-G aCL prevalence ranged from 2% in an Afro-Caribbean population to 51% in a report from India. Chinese patients are generally considered to have a lower risk of thrombosis than Caucasians [17].

Environmental factors have also been implicated in the pathogenesis of anti-neutrophil cytoplasmic antibody-related vasculitides. These include silica, *Staphylococcus aureus* infection, and drugs. Inhaled silica may cause accelerated apoptosis of neutrophils, which trigger the development of ANCA-related vasculitides. It also activates mediastinal macrophages to express cytokines that attract those neutrophils and stimulate T cells. Homology between *S. aureus* peptides and PR3 could underlie the development of PR3-ANCA. Drugs such as propylthiouracil were reported to be a cause of ANCA-related vasculitides, but with better prognosis. Thus, the spectrum of these diseases is broad and is related to geographic region and genetic background, suggesting that there is a wide range of initiating factors. Other conditions, like autoimmune liver diseases, thyroiditis and joint inflammation, were also thoroughly investigated with regard to their geoepidemiological pattern. As a result we have a better understanding of the pathophysiology and can provide better treatment. The incidence rates of autoimmune thyroid diseases were found to be slightly higher among Asians when compared with Caucasians. The lowest rates were consistently found among Black Africans, both indigenous and outside Africa. The racial factor was the only one that could explain this ethnographic difference. On the other hand, the incidence of Graves' disease is rising rapidly in urban South African blacks, possibly reflecting the increasing dietary iodine intake that is tied with the process of urbanization [18].

RA = rheumatoid arthritis

aPL = antiphospholipid antibodies
ANCA = anti-neutrophil cytoplasmic antibodies

THE ERA OF TARGETING B CELLS

B cells are recognized as a main player in the field of autoimmune diseases, such as SLE, rheumatoid arthritis and many others. Self-reactive B cells have a seminal role in the development of these diseases much beyond their ability to produce autoantibodies, being antigen-presenting cells and the source for many important cytokines both immunostimulatory and immunomodulatory. Self-reactive B cells were shown to be the source of autocrine cytokines such as IL-10 and IL-6. The increased level of both these cytokines in SLE was reported to occur in correlation with disease activity, the production of other proinflammatory cytokines, and with increased titers of anti-dsDNA antibodies. In this respect, the impact of FcγIIB on the regulation of BCR (B cell receptor) was evaluated and shown to preferentially limit the activation of high-affinity autoreactive B cells. Therefore, FcγIIB can influence the activation of dendritic cells through immune complex-mediated mechanisms [19-21].

The expansion of Toll-like receptor 9-expressing B cells was reported to be a crucial step in the development of active SLE. The proportion of B cells expressing TLR-9 was higher among patients with inactive disease. This was the case among plasma cells and memory B cells in patients with active SLE. Enhanced induction of class II HLA and other antigen-presenting properties following TLR-9 stimulation was also documented in B cells from patients with active disease. Autoreactive B cells persist in SLE patients even during remission in the circulating B cell memory pool. TLR-9-dependent activation of these could be one of the various mechanisms involved in SLE relapses. In this respect, TLR-9 signaling increases IL-10 and IL-6 production by autoreactive B cells, and the opposite is also true, where in an autocrine fashion IL-10 and IL-6 also enhance B cell differentiation and the development of inflammatory immune responses [22].

B cells, namely autoreactive B cells, are main players in autoimmune diseases and should therefore be therapeutically targeted

The role of B cell-activating factor should also be considered when discussing autoimmunity. The over-expression of BAFF receptor and increased B cell sensitivity to BAFF were shown to be additional factors inducing autoimmune diseases, increasing the proinflammatory properties of B cells such as the secretion of tumor necrosis factor and interferons. Increased serum levels of BAFF were found in many autoimmune diseases to be in correlation with disease activity and titers of pathogenic autoantibodies [23].

Taking all the above into account, B cell-targeting strategies are considered a frontier therapy in the battle against many autoimmune diseases. Rituximab, a mouse-human chimeric monoclonal antibody against the B cell-specific antigen CD20, was the first B cell-targeted therapy proved in double-blind placebo-controlled trials in RA and approved in 2006. On the basis of data from many studies, the addition of RTX to methotrexate significantly reduced the symptoms of rheumatoid factor-seropositive RA (as assessed by the American College of Rheumatology 20, 50, and 70 response criteria), inhibited radiological progression and, importantly, is considered to be safe [24]. In a recent study by Gusman Moreno [25] the administration of RTX to 131 patients with different autoimmune conditions other than RA, mainly in which humoral responses were aberrant, appeared to be beneficial and safe. The current status of B cell depletion therapy in autoimmune diseases other than RA was evaluated by an international group of experts. Preliminary data support this regimen in otherwise refractory patients. However, due to the lack of evidence-based data from large controlled trials, at this stage RTX should be considered on an individual basis only [26].

The alteration of polyclonal B cell immunity following B cell depletion, mainly memory B cells, was shown to be beneficial in SLE patients whose disease is resistant to other immunosuppressive therapies. In agreement with this, RTX was reported in additional studies to improve the outcome of patients with Sjogren's syndrome [27-29]. In a multicenter randomized double-blind double-dummy trial, RTX was compared with cyclophosphamide for remission induction of severe ANCA-associated vasculitis. Rituximab therapy was not inferior to daily cyclophosphamide treatment for induction of remission, and was even superior in relapsing disease, thus becoming an option for refractory disease [30,31].

The benefit of RTX was further proven in other immune-mediated conditions. In one study, it allowed a reduction in the doses of steroids and immunosuppressive drugs in 22 patients defined as having steroid-dependent nephritic syndrome [32]. The effectiveness of RTX was also demonstrated in reducing hepatitis B virus-related B cell over-activation and rheumatoid factor-secreting B cell clones as well as mixed cryoglobulinemia. Current data are supportive of the therapeutic potential of depleting B cells, which alters antioxidant low density lipoprotein antibodies in patients with atherosclerosis [33,34].

A survey of Pubmed and EMBASE yielded 21 articles dealing with the effect of RTX on immunoglobulin and autoantibodies in SLE. Rituximab tended to diminish total immunoglobulin, but still within normal ranges, more from IgM than IgG. Rheumatoid factor titer decreased by 30–60% six months after RTX, whereas anticyclic citrullinated peptide antibody titers declined much less. Most studies reported a

IL = interleukin
TLR = toll-like receptor
BAFF = B cell-activating factor

RTX = rituximab
Ig = immunoglobulin

marked decrease of anti-dsDNA and C1q antibody levels, but not of anti-extractable nuclear antigen antibodies [35].

At this stage we are still unable to define which subgroup(s) of patients will respond to RTX and therefore must try to identify any possible predictive factors that could better predict response. In this respect, it is suggested that clinical response to RTX therapy is associated with lower interferon (IFNγ) and BAFF levels, the Fcγ receptor III genotype, and the C/G-174 polymorphism of IL-6. Moreover, a good clinical response correlates with rheumatoid factor positivity, but not anti-CCP antibody positivity. The initial non-response to RTX depends on circulating pre-plasma cell numbers at baseline and incomplete depletion; also, synovial B cells are not sufficiently eliminated by RTX therapy. So far, anti-CD20 therapy has only been approved for RA; however, continuous data suggest that RTX is valid and safe for other autoimmune diseases, such as SLE, Sjogren's syndrome, vasculitides, and some cases of multiple sclerosis [36].

A further step in targeting B cells was the introduction of anti-BAFF therapy (belimumab), a fully human monoclonal antibody that inhibits the biologic activity of the soluble form BAFF and was developed for the treatment of SLE, RA and potentially other autoimmune disorders. In a Phase I trial aiming to evaluate the safety, biologic activity and pharmacokinetics of belimumab, 70 patients with mild-to-moderate SLE were enrolled in a double-blind randomized study and followed for 84 to 105 days. Significant reductions in median percentages of CD20+ B cells were observed in patients treated with a single dose of belimumab vs. placebo (day 42 $P = 0.004$, and day 84 $P = 0.003$) and in patients treated with two doses of belimumab vs. placebo (day 105 $P = 0.03$). In this preliminary study it was concluded that belimumab was well tolerated and reduced peripheral B cell levels in SLE patients [37]. In 2008, a Phase II trial (double-blind placebo-controlled) in 449 patients with SLE demonstrated that belimumab stabilized SLE for more than 2.5 years. Later, the clinical benefit of belimumab in SLE was further proven in two large Phase 3 trials (unpublished). Response rates were 57.6% and 43.2% for 10 mg/kg belimumab compared with 43.6 and 33.8% for placebo in BLISS-52 and BLISS-76 respectively. When articles found in a PubMed search and data presented at international conferences are summarized, belimumab was found to be well tolerated in the treatment of RA during 24 weeks and SLE during 3 years. It significantly decreased rheumatoid factor levels and reduced symptoms of RA. It also significantly reduced symptoms of SLE and decreased anti-dsDNA antibody titers, mainly among patients with positive baseline anti-dsDNA or antinuclear antibodies [38,39].

The better understanding of pathogenetic mechanisms of RA and other autoimmune diseases led to the development of new anticytokine inhibitors such as anti-IL-6 (tocilizumab) and other new B cell-depleting therapies such as epratuzumab, a monoclonal antibody against the B cell surface antigen CD22. Some pharmaceutical companies are focusing on developing small molecule inhibitors (e.g., p38, JAK or Syk) with possible oral administration that hold promise in various autoimmune diseases [40]. Thus, future data will hopefully show that B cell-targeting therapies are indeed beneficial in autoimmune diseases, and further progress in this direction is encouraged.

IfNγ = interferon-gamma
CCP = cyclic citrullinated peptide

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