The Mosaic of Autoimmunity: Prediction, Autoantibodies, and Therapy in Autoimmune Diseases – 2008

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The most significant development in our understanding of autoimmunity in the last 5 years relates to our ability to predict autoimmune diseases.

Predictivity of autoimmunity

For more than two decades, the detection of serum autoantibodies has been used for the diagnosis and classification of autoimmune diseases. In addition, some autoantibodies have a prognostic significance or are used as markers for disease activity. In recent years a new piece in the mosaic of autoimmunity has clearly emerged – namely, the predictive value of autoantibodies. Indeed, many autoantibodies can be detected in the preclinical phase of autoimmune diseases many years before the disease becomes apparent; furthermore, they have a high diagnostic positive predictive value [1].

Among autoimmune rheumatic diseases, antibodies to RNP, Sm, dsDNA, cardiolipin, Ro and La have a PPV for systemic lupus erythematosus of 94–100%. According to the type of antibody, the appearance can precede clinical diagnosis by 7–10 years with a frequency that varies from 32% to 78% at the moment of diagnosis [2]. In subjects with scleroderma, anti-centromere and anti-topoisomerase I antibodies are detectable, with a PPV of 100%, up to 11 years before clinical manifestations. In rheumatoid arthritis, the rheumatoid factor has a predictivity of 52–88%, depending on the study, while for anti-cyclic citrullinated peptide antibodies the predictivity is much higher, reaching 97%. If the rheumatoid factor and anti-CCP antibodies are both present, PPV rises to 100%. These two antibodies have even been detected in the serum up to 14 years before patients manifested the first symptoms of the disease.

Anti-Ro and anti-La antibodies were detected on average 5 years before the appearance of overt clinical signs and symptoms of Sjögren's disease in 73% of asymptomatic mothers who had given birth to a child with autoantibody-associated congenital heart block and who later developed Sjögren's syndrome.

Anti-nucleosome antibodies were found in 67% of patients with primary antiphospholipid syndrome up to 11 years before the development of SLE. Their PPV was 100%. Additionally, anti-cardiolipin antibodies may help to predict cases of SLE shifting to secondary APS [3].

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PPV = positive predictive value

CCP = cyclic citrullinated peptide
SLE = systemic lupus erythematosus
APS = antiphospholipid syndrome

In organ-specific autoimmune diseases – such as primary biliary cirrhosis, Addison's disease, Hashimoto's thyroiditis, type 1 diabetes, celiac disease and Crohn's disease – the predictive value of each antibody characteristic for a specific disease is similar to that for the autoantibodies in autoimmune rheumatic diseases [4].

Anti-thyroid peroxidase antibodies have been shown to be a good predictor for postpartum thyroid dysfunction. A high anti-TPO antibody level immediately postpartum can predict thyroiditis with 97% sensitivity, 91% specificity, and a PPV of 92%.

In anti-mitochondrial antibody-positive subjects without clinical or biochemical signs of hepatic damage, anti-mitochondrial antibodies can be detected up to 25 years before the clinical manifestation of primary biliary cirrhosis. Their PPV is higher than 95%.

Antibodies specific for pancreatic islet cells, insulin, 65 kD glutamic acid decarboxylase and tyrosine phosphatase-like (IA-2) protein are predictive markers for type 1 diabetes. Their PPV is 43%, 55%, 42% and 29%, respectively

The risk of first-degree relatives of patients with diabetes developing the disease grows progressively with the duration of follow-up and with the number of positive autoantibodies, being 2%, 25%, and 70%, with one, two and three or four positive antibodies. Adrenal cortex autoantibodies may precede disease onset by up to 10 years and their PPV is about 70%.

Anti-*Saccharomyces cerevisiae* antibodies as markers for Crohn's disease were detected in the sera of apparently healthy subjects on average 3 years before the disease became overt [5]. Their diagnostic sensitivity was 31% but the specificity and the predictive value were both 100%.

The predictive value of anti-tissue transglutaminase and anti-endomysial antibodies for celiac disease onset is 50–60%. If the patient carries the HLA-DQ2 or DQ8 antigens, known to be genetic markers for susceptibility to celiac disease, the PPV of the autoantibodies approaches 100%.

In summary, it is now clear that many autoantibodies have the ability to predict the development of an autoimmune disease in asymptomatic persons. It is also clear that the progression towards a given autoimmune disease, and its severity, can be predicted from the type of antibody, the antibody level, and the number of antibodies present.

The early diagnosis of autoimmune diseases made possible by the predictive capability of these antibodies is especially important when the disease progression can be prevented by avoiding environmental factors that may trigger or worsen the disease, or when a specific therapy is available and effective [6].

Finally, the predictive value of autoantibodies relies on the diagnostic accuracy of the laboratory methods used. Although sensitivity is an important requisite of diagnostic tests, the weight for prediction is based mainly on their diagnostic specificity. Hence, a correct evaluation of the predictive significance of autoantibodies can only be based on assays performed with methods ensuring high analytical and diagnostic specificity.

TPO = thyroid peroxidase antibodies

The role of autoantibodies

The healthy immune system is tolerant to the molecules of which the body is composed. A circulating natural antibody repertoire was detected in healthy individuals. Wide-ranging natural antibodies were found to react with self-molecules and are therefore defined as autoantibodies. In a recent study that employed an antigen microarray informatics technology, autoantibodies were detected even before birth [7]. When self-tolerance is disturbed as a result of inflammatory processes, exposure to chemicals, molecular mimicry with pathogens, vaccination, receptor editing, radiation and genetic background, what follows is dysregulation of the immune system, resulting in the emergence of an autoimmune disease. Elevated autoantibody titers will be detected, followed by autoantibody spread [8]. Not only do autoantibodies serve as biomarkers for different autoimmune disease activity and predict an autoimmune state, but different autoantibody patterns can mark resistance to the disease [9]. As biomarkers, the autoantibodies may represent a status of disease activity or predict a future pathogenic condition such as anti-PDH which may appear 30 years before primary biliary cirrhosis. Organ-specific diseases are associated with autoantibodies specific to the main affected organ, like anti-myelin basic protein and anti-myelin oligodendrocyte glycoprotein in multiple sclerosis, anti-thyroglobulin and TPO in thyroiditis, and insulin and glutamic acid decarboxilase autoantibodies in diabetes mellitus type 1. In systemic autoimmune diseases such as systemic lupus erythematosus, more than 100 autoantiboides were detected [10]. All these autoantibodies represent clusters of autoantibodies with organ specificities. For example, the use of glomerular proteome arrays of 30 antigens known to be expressed in the glomerular milieu, as well as sera from lupus patients, revealed five distinct clusters of immunoglobulin G autoreactivity in the sera of lupus patients. Whereas two of these IgG reactivity clusters (DNA/chromatin/glomeruli and laminin/myosin/matrigel/vimentin/heparan sulphate) showed association with disease activity, the others such as: histones, vitronectin/collagen/chondroitin sulphate, and entactin/fibrinogen/hyaluronic acid, did not. Other studies showed the importance of the anti-α-actinin association with glomerulonephritis flares in lupus.

Some of the nephrophilic autoantibodies react also with DNA, such as anti-laminin or anti-actinin. This resembles the general cross-reactivity of anti-DNA antibodies to diverse nuclear compartments, glycoproteins, glycolipids, oligossacharides or phospholipids [11].

Apart from nephritis, a cluster of autoantibodies associated with central nervous system involvement in lupus patients has emerged in recent years. This cluster includes the anti-ribosomal phosphoprotein and anti-N-methyl-d-aspartate in patients with neuropsychiatric SLE [12]. Other neuropsychiatric SLE-associated autoantibodies include antibodies targeting neuronal structures, brain-reactive autoantibodies, NMDA receptors, MAP-2, ganglioside, neurofilament, and glial fibrillary acidic protein [13].

Anti-endothelial autoantibodies are an example of a tissue-

IgG = immunoglobulin isotype G
NMDA = N-methyl-D-aspartate

specific population of autoantibodies, which may target different molecules on endothelial cells in a direct way or via intermediate molecules, and are associated with various autoimmune diseases. The AECA may target microvascular or macrovascular endothelial cells addressing molecular weight molecules that range between 25 and 200 kD. AECA target endothelial cells by recognizing heparane sulfate for example, or via endothelial cell-binding molecules such as platelet factor-4, beta-2 glycogen protein-I, DNA, histones, phosphatidylserine, and proteoglycans such as proteinase-3. The autoimmune diseases associated with AECA include SLE, APS, rheumatoid arthritis, vasculitis, heparin-induced thrombocytopenia, thrombotic thrombocytopenic purpura, Kawasaki disease, micropolyarteritis, Behçet's disease, and systemic sclerosis. AECA may affect endothelial cells in different pathways, leading to a procoagulant state or taking part in inflammatory processes, cell destruction, or protection.

Therapy of autoimmune diseases
Biological therapies and autoimmunity

During the last two decades major progress has been achieved in understanding the pathogenic mechanisms involved in chronic inflammatory autoimmune diseases. The identification of the specific and major roles that cytokines, peptides and cells play in the pathophysiology of various rheumatic and other chronic diseases has allowed the pharmaceutical industry to generate a wide range of new biological therapeutic interventions directed against those cytokines and/or cells.

Biological therapies directed against tumor necrosis factor were the first to show significant clinical efficacy with an accepted safety profile. Other biologics include anti-interleukin-1, anti-IL-6, anti-B lymphocyte stimulator (Blys), anti-CD20 and many others.

Three anti-TNFα agents are available: infliximab (monoclonal murine-human chimeric anti-TNF), etanercept (a fusion protein of two p75 chains of the TNFα receptor and the Fc portion of IgG1), and adalimumab (a human monoclonal recombinant IgG1 anti-TNFα). These agents are used for the treatment of severe rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis, vasculitis, and/or inflammatory bowel disease. Among patients with RA, therapy with anti-TNFα agents is associated with the reduction of titers of rheumatoid factor and the highly RA-associated anti-CCP antibody. Post-marketing studies have found that prolonged treatment with anti-TNFα agents may be associated with increased risk for the development of various infections, the occurrence of clinical and laboratory autoimmune manifestations and, possibly, increased risk for malignancy.

Autoimmunity associated with anti-TNFα therapy could be manifested by the presence of autoantibodies, mainly antinuclear antibody, and less frequently by the development of an overt autoimmune disease [14]. Several autoantibodies – including antinuclear antibody, anti-dsDNA, anti-SS/A, anti-SS/B, antineutrophil

cytoplasmatic antibodies and antiphospholipid antibodies – have been detected in the sera of patients treated with anti-TNFα agents. However, antinuclear antibody and anti-DNA were the most common autoantibodies induced by anti-TNFα and mainly by infliximab treatment.

Infliximab therapy is associated with the generation of antinuclear antibody and anti-dsDNA in the sera of 63–95% and 3–57% of patients with RA, 72% and 68% of 28 patients with severe psoriasis, 42–53% and 17–35% of patients with Crohn's disease, and in 85% and 31% respectively of patients with ankylosing spondylitis.

Etanercept and adalimumab treatment induced antinuclear antibodies and anti-DNA only in 11% and 15% of chronic RA patients and in 13% and 5% of patients with spondyloarthropathy [14,15]. In one study etanercept therapy was not shown to trigger the generation of autoantibodies.

Cases of SLE and vasculitis were reported following anti-TNF therapy. French national surveys revealed 12 cases of SLE, 10 cases of limited lupus, and 39 cases of vasculitis following anti-TNFα treatment. The patients had RA, ankylosing spondylitis, and spondyloarthropathies. Most of these patients were treated with infliximab, while only a few cases developed following etanercept and adalimumab therapy. The vasculitis was limited to the skin in the majority of cases, but systemic vasculitis affecting kidneys, lungs, pericardium and nervous system was also reported.

A significant proportion of the autoantibodies generated following anti-TNFα therapy belong to the IgM isotype, suggesting that those autoantibodies belong to the natural autoantibodies class and are probably produced as a result of polyclonal activation of B cells following the injection of chimeric antibody. In addition, infliximab may increase the number of apoptotic T cells. Because of a possible defect in the clearance of apoptotic cells in lupus, it may induce generation of autoantibodies to diverse cell compartments.

Peptide immunotherapy

Traditionally, autoimmune diseases have been treated with simple palliation of symptoms, with hormonal replacement when possible, and with potent non-specific immunosuppressive drugs. Many of the alternative therapies currently under investigation are attempting to spare most of the immune system, while interfering only with those components that are responsible for the specific autoimmune disease. One of these approaches is based on immunomanipulation employing antigenic peptides, which may bind to major histocompatibility complex molecules to form complexes recognized by T cells. Peptide-mediated immunotherapy has been studied in a number of experimental models of autoimmune diseases, among them experimental autoimmune encephalomyelitis, SLE and APS.

EAE is an autoimmune inflammatory disease of the CNS mediated by CD4^{+} Th1 cells that recognize the immunizing antigen. EAE can be induced by several myelin proteins, such as MBP,

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AECA = anti-endothelial autoantibodies
IL = interleukin
TNF = tumor necrosis factor
RA = rheumatoid arthritis

EAE = experimental autoimmune encephalomyelitis
MBP = myelin basic protein

PLP and MOG. Cop-1 is a synthetic amino acid copolymer that stimulates MBP immunologically and was demonstrated to suppress EAE when administered parenterally [16]. Cop-1 leads to activation of Th2 suppressor cells, which are activated by shared suppressive determinants between MBP and cop-1 to secrete Th2 and Th3 cytokines. Oral administration of cop-1 resulted in inhibition of EAE induction. Furthermore, oral cop-1 was more effective than oral MBP in suppressing EAE in rats. It appears that the *in vivo* effect of cop-1 relates to its acting as an altered peptide ligand that preferentially induces regulatory cells (Th2 and Th3) which cross-react with MBP and potentially with other myelin antigens.

Experimental SLE can be induced in naive mice by active immunization with a pathogenic anti-DNA mAb, bearing the 16/6 idiotype, manifested by high levels of autoantibodies that include anti-DNA and antinuclear protein antibodies as well as 16/6Id antibodies. The 16/6Id-immunized mice also develop lupus-associated clinical symptoms (e.g., leukopenia, proteinuria, kidney damage). The pathogenic and therapeutic potential of two synthetic peptides was studied; they were designed based on the complementarity-determining regions of a pathogenic anti-DNA mAb that bears the 16/6 Id. Immunization of high responder mouse strains with the CDR-based peptides led to production of autoantibodies and clinical manifestations characteristic of experimental SLE. The CDR-based peptides, however, could prevent autoantibody production in neonatal mice that were immunized later either with the peptide or with the pathogenic autoantibody. Furthermore, the peptides inhibited specific proliferation of lymph node cells of mice immunized with the same peptide or with the pathogenic anti-DNA mAb. It was later shown that the administration of the peptides starting at the time of disease induction prevented the development of clinical manifestations of the disease [17]. Prevention of SLE induction was shown to be associated mainly with a decrease in the levels of IL-2, interferon-gamma and TNFα, while the secretion transforming growth factor-beta was elevated. Amelioration of the clinical manifestations of an already established experimental SLE correlated with a dramatic decrease in TNFα secretion, elevated levels of TGFβ, and immunomodulation of the Th1 and Th2 type cytokines to levels close to those observed in healthy mice [17].

An experimental model for APS can be induced in naive mice by active immunization with pathogenic anti-β2GPI autoantibodies [18]. The mice exhibit anti-β2GPI antibodies as well as thrombocytopenia, prolonged activated partial thromboplastin time, low fecundity and increased rate of fetal resorptions. Synthetic peptides that share structural similarity with the putative phospholipid binding region of the β2GPI molecule, and high homology to cytomegalovirus, were able to induce antiphospholipid Abs and anti-β2GPI Abs in NIH/Swiss mice [19]. Previously, using a hexapeptide phage display library, Blank et al. [20] identified three

hexapeptides that react specifically with pathogenic anti-β2GPI mAbs which cause endothelial cell activation and induce experimental APS. These peptides specifically inhibited, both *in vitro* and *in vivo*, the biological functions of the anti-β2GPI monoclonal Abs. High homology was found between one of these hexapeptides (TLRVYK) and peptidic domain of various bacteria and viruses. Mice immunized with these microbial pathogens developed anti-β2GPI autoantibodies that reacted with the TLRVYK peptide. Furthermore, these antibodies were able to induce experimental APS in naïve mice [21]. These results established a mechanism of molecular mimicry in experimental APS, demonstrating that bacterial peptides homologous with the β2GPI molecule induce pathogenic anti-β2GPI antibodies along with APS manifestations.

IVIg therapy in autoimmunity and related disorders

Intravenous immunoglobulin is used to treat a number of immune deficiencies and autoimmune diseases [22]. Several mechanisms have been proposed to explain the effects of IVIg on immune modulation, such as the anti-idiotype modulation of pathogenic autoantibodies [22]. We recently reported on the presence of antibodies that bind to BAFF (B cell-activating factor of the TNF family). In *vitro* IVIg binding prevented BAFF from exerting its anti-apoptotic effect on B cells. These anti-BAFF immunoglobulins might amend deleterious effects of BAFF in B cell-mediated autoimmune diseases [23]. Animal models of SLE, APS and scleroderma have been successfully treated with IVIg [22]. Additionally, disease-specific fractions of IVIg were purified and used to treat animal models of SLE (against anti-dsDNA) [22] and APS (against anti-β2GPI) [24]. This disease-specific IVIg demonstrated even better therapeutic effects in both disease models (SLE and APS) than whole IVIg. Disease-specific fractions of IVIg may allow for the production of more effective and multiple therapeutic units from every batch of IVIg, with the potential of lowering costs and enhancing the availability of IVIg, an expensive and scarcely available resource.

In SLE patients we have used IVIg successfully to treat extreme cases of bone marrow suppression, as well as neuropsychiatric, serositis, cardiopulmonary and bone marrow SLE manifestations [23,25]. Similarly, in women with recurrent fetal loss due to APS, IVIg was able to reduce the abortion rate [22]. Vasculitides, such as Churg-Strauss syndrome and Wegener's granulomatosis, and skin fibrosis in patients affected by scleroderma improved after IVIg treatment [22]. In addition, a reduction in joint pain with a significant recovery of joint function has been reported in systemic sclerosis patients with severe and refractory joint involvement treated with IVIg [26].

We have further reviewed and investigated the safety of IVIg therapy [27]. In a large cohort of patients treated with high dose IVIg during a mean of 3.8 ± 3.5 years, no severe adverse events (thromboembolism, renal failure or anaphylaxis) were recorded when safety measures as well as slow-infusion rates were enforced.

MOG = anti-myelin oligodendrocyte glycoprotein
CDR = complementary determining region
TGF = transforming growth factor
β2GPI = beta-2-glycoprotein-I
Abs = antibodies

IVIg = intravenous immunoglobulin
BAFF = B cell-activating factor

T regulatory cells [28,29]

Among the several mechanisms that play a role in maintaining peripheral self-tolerance is a unique CD4$^+$CD25$^+$ population of naturally occurring regulatory T cells, which actively prevent both the activation and the effector function of autoreactive T cells that have escaped different mechanisms of tolerance. Experimental *in vivo* studies have demonstrated that the absence of regulatory T cells allows the induction of organ and non-organ-specific autoimmune diseases, while the addition of this T cell population can prevent or delay these diseases. Many studies have shown the benefit of targeting this cell population by restoring self-tolerance. Therapies that could possibly increase the suppressive ability of Treg cells were shown to improve that course of autoimmune diseases.

Treg cells and autoimmune diseases

Alterations in the apoptotic properties of Treg cells may favor the ratio of responder CD4$^+$CD25$^-$ T cells to suppressor CD4$^+$CD25$^+$ T cells, thus leading to the breakdown of self-tolerance and permitting excessive inflammation and autoimmunity. In murine models, impaired secretion of TGFβ and IL-10 seem to be essential for the development of arthritis, however the role of these cytokines in human RA is not yet well established.

Higher frequencies of CD4$^+$CD25$^+$ Treg cells were found in synovial fluid compared to that in the peripheral blood of RA patients. In this respect, a recent study was undertaken to investigate the regulatory capacity of autologous peripheral blood Treg cells in contact with synovial tissue cell cultures, and to evaluate their presence in peripheral blood, synovial tissue and synovial fluid of patients with RA [30]. RA synovial tissue cell cultures exhibited spontaneous expression of IFNγ which was abrogated by the depletion of CD3$^+$ T cells and specifically reduced by co-culture with autologous peripheral blood Treg cells. The amount of Foxp3 transcripts, however, was lower in the synovial membrane than in peripheral blood or synovial fluid. The T-beta/Foxp3 ratio correlated with both the higher grade of synovial tissue lymphocyte infiltration and higher disease activity. This study shows the efficacy of autologous Treg cells in reducing the inflammatory activity of synovial tissue cell cultures *ex vivo* in human RA.

In addition, many studies investigated the status of Treg cells in patients with SLE. In one of the first studies, the level of CD4$^+$CD25$^+$ T cells was evaluated in the peripheral blood of patients with SLE. In that study, 94 SLE patients, 52 RA patients and 50 age and gender-matched healthy individuals were enrolled. In terms of CD4$^+$CD25$^+$ T cells, defined as having a fluorescence intensity of CD25 expression exceeding 100, SLE patients still had significantly lower levels than the normal controls, expressed as percentages of peripheral blood mononuclear cells (1.7 ± 1.3 vs. 3.7 ± 1.3%, $P < 0.05$). In that study, although decreased CD4$^+$CD25$^+$ T cells were found in SLE patients, no correlation was found between the levels of Treg cells and disease activity in SLE. In contrast to these results, a later study was able to demonstrate that the frequency of CD4$^+$CD25$^+$ Treg cells was significantly decreased in patients with active SLE compared with inactive patients and with controls, and was also inversely correlated with disease activity, as assessed by SLEDAI scores (r = -0.59, P = 0.001) and serum anti-dsDNA levels (r = -0.65, $P <$ 0.001) [31].

In another recent study, the transcription factor Foxp3 was measured in Treg cells of 43 patients with SLE. One group comprised 20 newly admitted patients with the first manifestations of the disease, and the second group included patients who were treated with cytostatics and steroids. The results revealed a significant decrease in CD4$^+$CD25$^+$ and CD4$^+$CD25high T cell numbers in patients from group I compared with the control and group II patients. Co-expression of Foxp3 on Treg cells was significantly reduced in both groups regardless of the therapy. The ability of Tregs to suppress the proliferation of autologous CD8$^+$ and CD4$^+$ T cells was significantly reduced in both groups of patients compared to the healthy donors.

Targeting T regulatory cells

In vitro activation of CD4$^+$CD25 high Treg cells from patients with active SLE increased the expression of FoxP3 and restored their suppressive function, suggesting that strategies to enhance the function of these cells might benefit patients with autoimmune diseases. This strengthened the idea of using various therapeutic regimens to restore self-tolerance by improving the function of Treg cells.

In one of our previous studies we demonstrated a higher sensitivity of Treg cells to undergo spontaneous apoptosis in patients with active RA [28]. Alterations in CD4$^+$CD25$^+$ cell apoptosis and cell count were found to correlate with RA disease activity. Here again, the reversal of these deviations from normal was documented in association with the beneficial outcome of infliximab therapy. In another recent study, infliximab therapy was shown to give rise to a CD4$^+$CD25high Foxp3 Treg cell population, which mediated suppression via TGFβ and IL-10, and lacked CD62L expression, thereby distinguishing this Treg cell subset from natural Treg cells present in healthy individuals and patients with active RA [32]. These results suggest that anti-TNFα therapy in RA patients generates a newly differentiated population of Treg cells, which compensates for the defective natural Treg cells.

Glucocorticoids were also reported to affect the activity of Treg cells on the basis of FoxP3 and cytokine expression. FoxP3 mRNA expression was significantly increased in asthmatic patients receiving inhaled glucocorticoid treatment, systemic glucocorticoid treatment, or both. The frequency of CD25$^+$ memory CD4$^+$ T cells and transient FoxP3 mRNA expression by CD4$^+$ T cells significantly increased after systemic glucocorticoid treatment. In addition, glucocorticoids induced IL-10 and FoxP3 expression in short-term and long-term cultures *in vitro*. Thus, up-regulation of Treg cells could become one of the tools by which self-tolerance is restored.

In agreement with all the above, we have shown for the first time that IVIg was proven by a unique mechanism to enhance

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Treg = T regulatory cells
IFNγ = interferon-gamma

SLEDAI = SLE Disease Activity Index

the suppressive activity of CD4$^+$CD25$^+$ Treg cells [29]. In that study we demonstrated that the *in vitro* addition of IVIg to CD4$^+$ cells increased intracellular expression of IL-10, TGFβ and FoxP3 when we gated on CD4$^+$CD25high T cells, suggesting that IVIg has the capability of directly affecting Treg cells. The up-regulation of Treg cell suppressive activity by IVIg was accompanied by decreased TNFα secretion by CD4$^+$ effector cells [Figure 1].

The mechanisms by which IVIg could possibly affect the function of Treg cells are still not sufficiently clear. Increased expression of intracellular IL-10 in Treg cells could inhibit the production of pro-inflammatory cytokines by Th1 such as TNFα. In this regard, IVIg treatment resulted in the down-regulation of the Th1-type cytokine TNFα, and the up-regulation of the Th2-type cytokine IL-10. As supported by several experimental studies, IVIg regulates crucial steps of T cell-mediated immune responses. These effects involve the modulation of activation, proliferation, differentiation, apoptosis, and effector mechanisms of T cells. The pattern of IVIg-T cell interactions is complex, as IVIg may directly bind to regulatory structures on T cells, or modulate T cell functions indirectly via soluble or cellular components of the immune system.

Primary biliary cirrhosis as a paradigm of the mosaic of autoimmunity [33-39]

Primary biliary cirrhosis is considered a model autoimmune disease because of its signature anti-mitochondrial autoantibody, the homogeneity of clinical characteristics, and the specificity of biliary ductular cell pathology. Twenty years ago Gershwin et al. [40] reported the cloning and identification of the E2 component of pyruvate dehydrogenase as the immunodominant autoantigen of PBC, allowing for vigorous dissection of T and B lymphocyte responses against PDC-E2 and development of several valid experimental models. There has also been considerable research into the biology of biliary ductular cells, which includes the unique properties of apoptosis in which there is exposure of PDC-E2 to the effector processes of the immune system. The proximal cause of PBC is autoimmunity directed against well-identified mitochondrial located autoantigens in individuals with inherited deficits of immune tolerance. The potential initiator of PBC includes *inter alia* particular environmental xenobiotics; pathogenesis is aided and abetted by genetic weaknesses in mechanisms of immune regulation; and subsequent multilineage immunopathology impacts upon uniquely susceptible biliary ductular cells to culminate clinically in the chronic autoimmune cholangiolitis of PBC. Gershwin and co-workers emphasize the very high degree of specificity of antibodies to PDC-E2 and the subsequent development of clinical PBC, and the belief that immunoreactivity with recombinant PDC-E2, after a long latency, is strongly indicative of development of clinical disease.

PBC, like most polygenic autoimmune diseases, clearly belongs to the "complex disease" category that is attributable to combined effects of multiple environmental and behavioral influences,

PBC = primary biliary cirrhosis
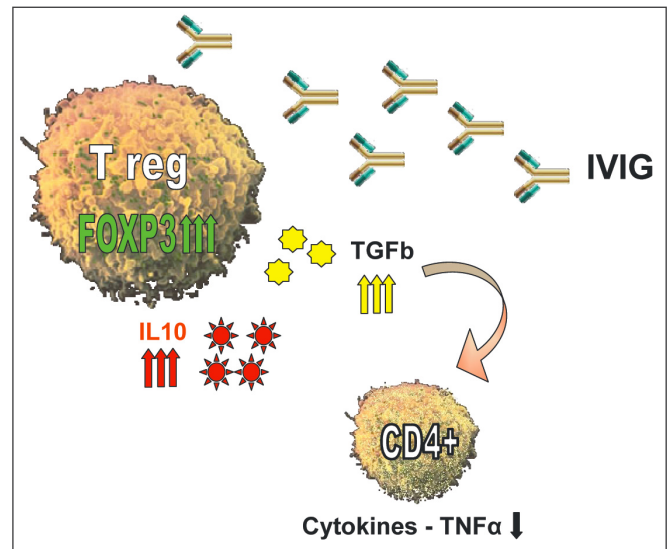PDC-E2 = E2 component of pyruvate dehtdrogenase

Figure 1. Targeting T regulatory cells in autoimmune diseases. The incubation of IVIg with CD4$^+$CD25$^+$ Treg cells increased the expression of Foxp3 and the production of TGFβ and IL-10. The co-culture of IVIg with Treg cells increased also their suppressive ability, and decreased TNFα production by CD4$^+$CD25$^-$ T cells.

genetic elements, and perhaps chance. We deconstruct these components into: a) chemical xenobiotic-dependent initiation, b) an initial deficit in natural immune tolerance that includes most of the genetic contribution and is permissive for maintenance of the disease, and c) a vulnerability of the primarily affected biliary epithelial cell by reason of its particular biology.

Tolerance is essentially the absence in the periphery of T and B cells with receptors of sufficient affinity to recognize/react with self, including the presence and effects of Treg cells [39]. If one or the other of these is deficient, the individual – whether mouse or human – is on the knife's edge of anti-self reactivity; which a) might not ever happen; b) happens regularly in "highly" predisposed individuals whenever a native (unaltered) cell fragment encounters an undeleted anti-self lymphocyte under immunogenic conditions, as in autoimmune NOD or NZB mice; or c) happens occasionally when the immune system of a "moderately" predisposed individual is confronted with a near-self-antigen under immunogenic conditions. Indeed, we submit that whether it is a chemical synthetic xenobiotic or a bacterial mimic, immunization under appropriate circumstances (genetically tolerance-deficient host, use of complete Freund adjuvant), can lead to autoimmune pathology including disease of a similar nature to that seen in human PBC. Early predictions of PBC may lead to a better therapeutic outcome [37-39].

In this article we have summarized two of the main advances in our understanding of the mosaic of autoimmunity, as expressed in PBC – the prediction and treatment of autoimmunity.

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