

Beneficial autoimmunity participates in the regulation of rheumatoid arthritis

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1. ABSTRACT

Antigen specific T cells and B cells recognize their target determinants by antigen specific receptors that are being rearranged in a random manner. These cells then undergo negative and positive selection processes that limit, albeit not eliminate, the escape of self-reactive T and B cells capable of eliciting autoimmune responses. The above processes are referred to as “central selection”, and their outcome is the “central tolerance”. Auto-reactive T and B cells escaping central tolerance are then subjected to peripheral mechanisms that restrain their auto-aggressive behavior. Different types of regulatory T cells are key players in maintaining actively induced peripheral tolerance. In patients suffering from various autoimmune disorders autoreactive T and/or B cells that escaped central tolerance also circumvented regulatory T cells that could, potentially, eradicate their pathogenicity in the periphery. We have found an additional regulatory mechanism that restrains the harmful activity of these cells at that time. It includes autoimmune B cells that produce neutralizing autoantibodies against numerous inflammatory mediators,

mostly cytokines and chemokines, which participate in destructive autoimmunity. These autoantibodies restrain the harmful consequences of inflammatory autoimmune conditions such as in Rheumatoid Arthritis. Interestingly, this antibody production is elicited during autoimmune diseases, and to a much lesser extent during local inflammation. The specificity of this response is highly restricted to determinants with minimal cross reactivity to other known gene products. Thus, the immune system allows selective breakdown of tolerance in autoimmune conditions. The findings that this beneficial response is turned on by the autoimmune condition, and then regulated by its progression further imply for the existence of a programmed regulatory response of “Beneficial autoimmunity”. In the current review we describe how this mechanism was discovered in experimental models of Rheumatoid arthritis and Multiple sclerosis, demonstrate its importance in the natural regulation of these diseases, and finally explore its relevance to human diseases.

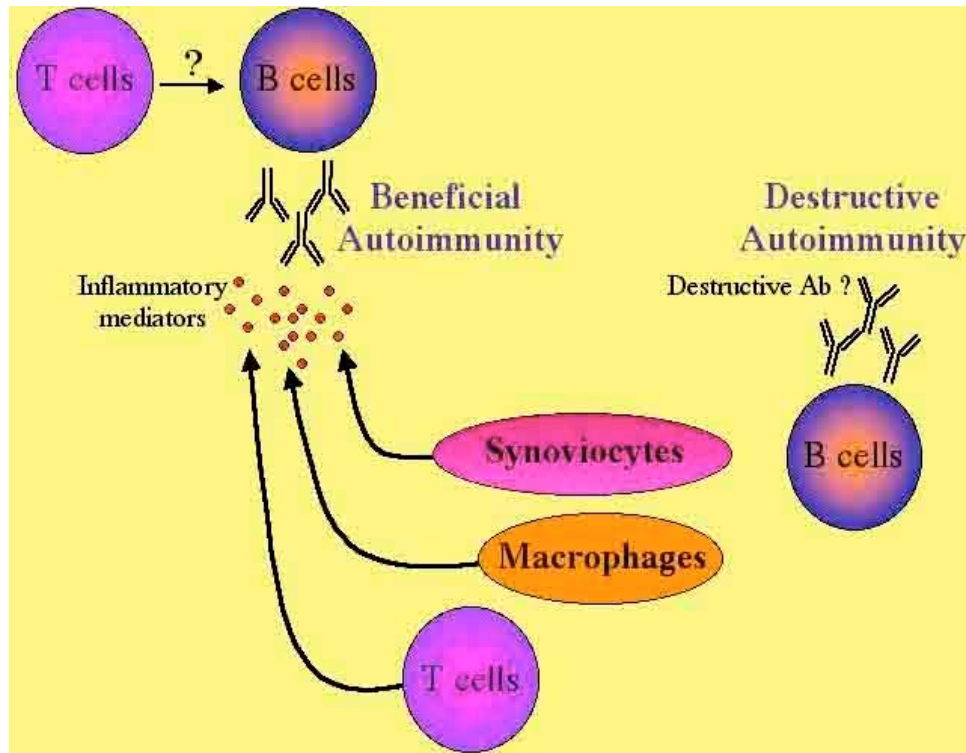


Figure 1. A schematic view of the dynamics of the inflammatory process in the arthritic joint, in which the function of inflammatory mediators produced by various cells in the joint is partially neutralized by beneficial autoimmunity.

2. INTRODUCTION

In inflammatory autoimmune diseases, auto-reactive T cells recognize self-components as foe and induce a destructive response. It is believed that at the initiation of different autoimmune diseases, these cells are being activated by infectious pathogens in patients carrying the appropriate genetic background. This may explain, in part, the low concordance in the prevalence of these diseases (less than 30%) between identical twins (1, 2).

Infectious invaders can initiate an inflammatory autoimmune disease by two different mechanisms of action: elicitation of a direct cross-reactive response between self and foe, or initiation of a bystander auto-reactive response. According the first mechanism of action, the auto-reactive cells are activated by microbial determinants that possess high similarity to self-antigens. These cells then home to different organs, including the autoimmune site, where they propagate and induce inflammation (3). The alternative mechanism includes bystander activation of autoimmune cells (4). In this case, an infectious invader, with no cross-reactivity to self, induces a local inflammatory process. As a result, dendritic cells are being activated via Toll Like Receptors (TLR's)/CD14 molecules (5, 6) and present both microbial antigens, as well as self antigens obtained from apoptotic/necrotic cells in the inflamed tissue to autoimmune T cells which commence the inflammatory process. For example, subjecting SJL mice to Theiler's virus, that has no cross-reactivity to Central Nervous Systems (CNS) determinants, eventually leads to the development of

Experimental Autoimmune Encephalomyelitis (EAE), with an apparent response to CNS determinants (4, 7).

The continuing inflammatory autoimmune process can be viewed as a multi-sequential event in which antigen specific T cells interact with their target determinants to initiate a secondary influx of effector T cells, B cells, monocytes and neutrophils, all of which participate in the initiation, propagation and maintenance of the inflammatory condition. Major tools with which these cells communicate and directly damage tissues are the inflammatory cytokines and chemokines (8). These soluble mediators, particularly chemokines, also activate adhesion molecules and attract leukocytes to the site of inflammation (9-16). Among the key cytokines that direct inflammatory autoimmune disorders are: $\text{TNF-}\alpha$, IL-1, IL-12, IL-15, IL-17, IL-18, IL-23 (17-30), and the recently discovered IL-27 (31-33). These cytokines direct tissue destruction (8), polarization of non-polarized CD4^+ T cells into inflammatory Th1 cells over non-inflammatory Th2 (25-31), or support the proliferation and function of polarized Th1 cells and other leukocytes, including B cells (31, 34). As for chemokines, the role of MCP-1, MIP-1 α , IP-10, RANTES, and MIF has well been documented in experimental models (35-47) now being extended to clinical trials. IL-8 is also an important potential target for therapy of Rheumatoid Arthritis (RA) and related diseases (48).

What regulates the dynamics of inflammatory autoimmune diseases? So far two major regulatory cytokines were identified: IL-10 and TGF- β . During the immune response, antigen specific regulatory T cells

named Tr1, characterized by high IL-10 production, and Th3 cells, producing TGF- β , were found capable of restraining the destructive function of autoimmune Th1 cells (49-52). It is believed that during the inflammatory process, in the presence of IL-10, initially produced by activated macrophages, and of IL-2, mostly produced by CD4⁺ Th1 cells, some antigen specific none-polarized T cells (Thnp) are being polarized into IL-10 producing Tr1 cells (50). These cells then restrain the progression of the autoimmune condition. Similarly, in the presence of TGF- β , some of these Thnp cells are driven into Th3 cells that also suppress inflammation (52). Two other cytokines that are thought to encompass anti-inflammatory properties are IL-4 and IL-13, mainly produced by another subtype of antigen specific T cells named Th2 (53-57). The anti-inflammatory role of these cytokines, particularly IL-4, in the regulation of RA has well been studied in different experimental models of the diseases (58-61). It has been shown that of the regulatory cytokines IL-10 mRNA expression is the most dominant in the synovial tissues from patients in early stages of rheumatoid, reactive, and undifferentiated arthritis (61), though it is not clear whether it is transcribed by Tr1 cells there.

In addition to antigen specific regulatory T cells, the immune system generated another type of regulatory T cells that continuously express the alpha chain of the IL-2 receptor (CD25). These CD4⁺CD25⁺ T cells actively restrain the function of inflammatory CD4⁺ T cells, by mechanisms yet to be fully identified (62-64). Recent studies suggest that not only do these cells exist in human, but they may also play a role in regulating the pathogenesis of inflammatory autoimmune diseases (65), including RA (66-68), and its experimental models (69, 70). In a very recent manuscript, published in the August 2004 issue of the *Journal of Experimental Medicine*, Ehrenstein *et al* show that in RA patients most CD4⁺CD25⁺ T cells are in an anergic state, and therefore are incapable of suppressing inflammatory functions (71). However, treatment with a monoclonal antibody against TNF- α (Infliximab) restored the capacity of CD4⁺CD25⁺ regulatory T cells isolated from these patients to inhibit cytokine production and to convey a suppressive phenotype (71). This may explain, in part, the beneficial effect of anti-TNF- α therapy in RA (72).

All antigen specific regulatory T cells, and possibly CD25⁺ regulatory T cells restrain the function of auto-reactive Th1 cells by blocking their ability to produce inflammatory mediators. We have recently identified a novel mechanism by which the immune system also inhibits the destructive reactivity of these mediators. We found that some of the auto-reactive B cells that escaped negative selection are specific to proinflammatory cytokines and chemokines, and that during autoimmune conditions, and to a much lesser extent during local inflammatory reactions, these cells become activated and elicit an autoantibody response against few of these mediators (73). By doing so, they suppress the pathological consequences of self-destructive immunity (73). Using a modification of DNA vaccination, we could selectively either amplify, or totally eliminate the "Beneficial

autoimmune response" to single mediators, and therefore evaluate their contribution to the natural regulation of disease (73). For example, while amplification of beneficial autoimmunity to the single key mediator TNF- α markedly suppressed experimentally induced RA, its exclusion profoundly aggravated disease severity (73). This enabled us to evaluate the contribution of this type of regulatory response to the regulation of the disease (73). Subsequently, we have noticed that patients suffering from RA, but not Osteoarthritis (OA), display a similar type of antibody production to this inflammatory cytokine. This extends our findings and their relevance in human autoimmunity (73). The current review explores this interesting issue.

3. SELF-SPECIFIC AUTOIMMUNE CELLS ESCAPE CENTRAL SELECTION AND CAN POTENTIALLY INDUCE INFLAMMATORY AUTOIMMUNE DISEASES

Antigen specific T cells and B cells recognize their target determinants by their antigen specific T cell receptor (TCR) and antigen specific B cell receptor (BCR), respectively. These receptors are generated through a random rearrangement process. Since this is a random process, lymphocytes whose receptors can recognize one of the body's own components are also generated, and can potentially induce autoimmunity (74, 75). These lymphocytes then undergo a process that is commonly referred to as "Central selection" that results in limitation, but not total elimination, of these auto-reactive cells. This process of successful selective deletion of auto-reactive cells leads to the development of a state of resistance against autoimmunity, which is referred to as "Central tolerance" (74, 75). B cells and T cells undergo a different path of differentiation. B cells differentiate in the bone marrow. The vast majority of those recognizing self-antigens undergo a rapid stage of negative selection by apoptosis. Alternatively, some self-specific B cells can rearrange their receptor in a process that is referred to as receptor editing (76). Even though negative selection is highly restrictive, and the vast majority of B cells entering the process undergo apoptosis and never enter the peripheral immune system, many self-specific B cells escape central tolerance and can potentially be involved in destructive autoimmunity (76). In fact, the repertoire of antigen specific auto-reactive B cells is probably much larger than the repertoire of antigen specific autoimmune T cells that undergo a different process of negative and positive selection, as described below.

T cells differentiate in the thymus. In contrast to B cells that recognize target determinants by their BCR and its secreted form (antibody) directly, T cells recognize target antigens only when presented by major histocompatibility proteins (MHC). Thus, T cell selection in the thymus includes positive selection for TCR that recognizes self-MHC and negative selection against those recognizing self antigens presented by the MHC. Altogether more than 90% of T cells that differentiate in the thymus undergo apoptosis, some of which do not get positive signals by self MHC, and about two thirds of those that are positively

selected subsequently undergo negative selection (77). Despite this highly selective process, and similarly to B cell selection, this system is also leaky, and antigen specific autoimmune cells escape central tolerance (74). In fact, the leaking in T cell selection could be more dangerous than in the B cell compartment. After all, it is the T cells that regulate the harmful function of auto-reactive B cells (i.e. T dependent responses).

How important is central selection? Recent studies identified a functional mutation in a transcription factor that is highly expressed in thymic medullary epithelial cells, named autoimmune regulator gene (AIRE). This mutation resulted in the development of severe autoimmunity in human and mice (78, 79), which could be explained by an aberrant deletion of auto-reactive T cells in the thymus (78, 79). This further emphasizes the important role of central selection in the prevention of autoimmunity.

After deletion of auto-reactive T cells and B cells, those escaping central selection are subjected to peripheral mechanisms that restrain their potential harmful effect (80). Sections 4 and 5 discuss the role of inflammatory T cells and the cytokines and chemokines they produce in the initiation and progression of autoimmunity (section 4), and how regulatory T cells and the cytokines they produce restrain this destructive activity (section 5).

4. INFLAMMATORY CYTOKINE AND CHEMOKINES AND ADHESION MOLECULES DIRECT THE FUNCTION AND MIGRATORY PROPERTIES OF AUTOIMMUNE T CELLS AND ARE THEREFORE IMPORTANT POTENTIAL TARGETS FOR THERAPY

The development of a T cell mediated autoimmune condition can be viewed as a multi-sequential process in which antigen specific effector T cells enter the autoimmune site and interact with their target antigen presented by activated antigen presenting cells (APCs), including residual APCs, invading macrophages and dendritic cells. As a result, inflammatory cytokines and chemokines that are being produced at the autoimmune site attract a secondary influx of other T cells, including those that are not specific to the target antigen, monocytes and neutrophils, to commence the inflammatory process (3, 81, 82). At all times invading T cells and macrophages undergo Fas-FasL mediated apoptosis (83). Thus, administration of anti FasL antibodies during ongoing EAE, a T cell mediated autoimmune disease of the central nervous system, inhibits apoptosis of auto-reactive T cells at the autoimmune site and subsequently aggravated the disease (84). Apoptosis of invading cells at the autoimmune site requires continuing influx of invading cells to replace those dying there. Therefore, therapies that interfere in this event could potentially ameliorate, or inhibit the progression of these diseases. Indeed twelve years ago, together with L. Steinman from Stanford University, and T. Yednock, at the time at Athena Neurosciences, we have demonstrated, for the first time that a monoclonal antibody to the alpha-4 chain of alpha-4 beta-1 integrin (VLA-4) could prevent the accumulation of T cells and monocytes at the autoimmune

site and reverse EAE (85). This particular antibody has successfully finished phase III clinical trial in the USA and is about to enter the market as a potential drug for MS (86).

The function of VLA-4, and several other adhesion molecules, is dependent on their prior activation, a process that is directed by chemokines (13, 14, 87-89), and shear forces (16). Chemokines are small (~8-14 kDa), structurally related proteins that regulate cell trafficking through interactions with a subset of seven-transmembrane, G protein-coupled receptors (90). Based on the positions of the first two cysteines, the chemokines can be divided into four highly conserved, but distinct supergene families C, C-C, C-X-C and C-X3-C. The C-C family is primarily involved in the activation of endothelium and chemoattraction of T cells and monocytes to the site of inflammation (11, 90, 91). Of these families, more is known about the role of different C-C chemokines, and of CXCR3 and one of its three ligands, named CXCL10 (also known as Interferon-gamma inducible protein 10, IP-10) in the regulation of T cell mediated autoimmunity. The first study on the subject was published nine years ago. In this study, Karpus et al. blocked EAE in mice by immunizing them with rabbit anti-mouse polyclonal antibodies against Macrophage Inflammatory Protein-1 α (MIP-1 α , CCL3) (39). Gong et al. used an antagonist of another C-C chemokine named monocyte chemoattractant protein 1 (MCP-1, CCL2) to inhibit arthritis in the MRL-lpr mouse model (37). Subsequently, two complementary studies showed that mice lacking MCP-1 or its CCR2 receptor are EAE resistant (35, 92). Barnes et al. used anti-human RANTES (CCL5) antibodies to ameliorate adjuvant-induced arthritis (AA) in the Lewis rat (93). Six years ago C. Mackay and his colleagues identified that the chemokine receptor CXCR3 is highly expressed on inflammatory Th1 cells, but not Th2 cells (94, 95). This may suggest that blockade of the interaction between CXCR3 and its ligands could suppress inflammatory autoimmune diseases. Indeed, later on two groups, the one led by W. Karpus and mine, independently identified one of the three CXCR3 ligands (IP-10) as a major target for therapy of EAE (45, 96). We have also extended our study to AA (44).

Neutralizing antibodies, peptide antagonists and small molecule based drugs the antagonize the interaction of different CC chemokines and their receptors, particularly the interaction between MCP-1 and its CCR2 receptor, as well as monoclonal antibodies to CXCR3 and IL-8, are currently at different stages of clinical trials in RA and other inflammatory diseases, so far with a limited success (97). Another potential way by which the interaction of chemokines and their target receptors could be inhibited is by using small molecule receptor antagonists. On this subject an CCR1 antagonist is now being tested in clinical trials aiming at suppressing RA (98).

Thus far the most successful therapy for RA, and related diseases, has been achieved by neutralizing the function of TNF- α either by mAb or by a recombinant soluble receptor for TNF- α (8, 99-102). The underlying mechanism includes direct neutralization of the proinflammatory, destructive, properties of this cytokine,

and, as very recently shown, restoration of the suppressed activity of CD4⁺CD25⁺ regulatory T cells (71). TNF- α is produced at the site of inflammation by invading Th1 cells, macrophages and residual cells at the inflamed joint. The successes of anti TNF- α therapy in RA encouraged the extension of this way of therapy to inflammatory autoimmune diseases that are related to RA, and to other inflammatory autoimmune diseases, like Intestinal Bowel Diseases (IBD). It appears that anti TNF- α based therapy has been found more successful for vast majority of patients suffering from RA and related diseases, than those experiencing other inflammatory autoimmune diseases, including IBD. Additionally, within the RA patients it appears that a notable portion of the patients display resistance to anti TNF- α based therapy. This has motivated investigators to develop complementary/alternative therapeutic approaches. Similarly to TNF- α , IL-1 is a proinflammatory cytokine that is thought to manifest a direct inflammatory role in the regulation of disease (17). IL-1 receptor antagonist (IL-1ra) is a natural inhibitor of IL-1. Its overexpression could successfully suppress experimentally induced RA, (24). Clinical trials using recombinant IL-1ra, administered alone, or in combination with other drugs, are now being conducted (97). Another related cytokine that exerts similar/complementary biological activities is Interleukin-6 (IL-6). It is a pleiotropic cytokine that regulates the immune response, inflammation, and hematopoiesis. Overproduction of IL-6 plays pathologic roles in rheumatoid arthritis (RA), and the blockade of IL-6 may be therapeutically effective for the disease. A clinical trial has recently been undertaken to evaluate the safety and efficacy of a humanized anti-IL-6 receptor antibody, MRA, in patients with RA (103).

TNF- α is largely produced by antigen specific Th1 cells, which are believed to be the driving force of the inflammatory process in T cell mediated autoimmune diseases (81). During the initiation, and progression of inflammatory autoimmunity, antigen specific non-polarized CD4⁺ cells (Thnp) are being polarized either to pathogenic Th1 cells or non-pathogenic Th2 cells (55). Nine years ago Leonard et al demonstrated that neutralizing the function of IL-12 could suppress EAE (30). Three years later we were the first to demonstrate that neutralizing IL-18 also suppressed the disease in a similar mechanism (29). These studies were later extended to different inflammatory autoimmune diseases (27, 28, 104-106). It appears that during their early antigen specific activation, antigen specific Thnp cells express receptors for IL-12, IL-18 and IL-4. Under a cytokine milieu that is enriched with IL-18 and IL-12, ligation of these cytokines to their target receptors would initiate a signaling cascade via STAT4 and T-BET, resulting in a significant polarization into Th1, whereas if the cytokine milieu is enriched with IL-4, these Thnp cells will be preferentially polarized into Th2 via STAT6 or STAT5 and GATA3 signaling pathways (107-110). Therefore, neutralizing antibodies that block IL-12 or IL-18 suppress inflammatory autoimmune diseases. IL-23 and IL-27 are newly discovered cytokine that also participate the promoting Th1 responses (26, 32, 33, 111-118). A recent study in IL23^{-/-} mice explored its critical role in the pathogenesis of EAE (25). The role of IL-27 in the

regulation of autoimmunity has yet to be defined. In a very recent study we have shown that neutralizing IL-27 suppresses AA (31). In this particular study we also showed that IL-27 not only polarizes Thnp into Th1, but also potentiates the function of long-term CD4⁺ memory Th1 cells (31). Other cytokines that have shown to play a significant role in the regulation of T cell mediated autoimmunity, particularly experimentally induced arthritis are IL-17 and IL-15 (18-23, 119, 120). These cytokine and their receptors also serve as targets for antibody/soluble receptor based therapy in RA (97).

5. PERIPHERAL REGULATORY MECHANISMS THAT RESTRAIN THE HARMFUL FUNCTION OF INFLAMMATORY AUTOIMMUNITY

What regulates the function of autoimmune T cells and restrains their potential pathogenic consequences? T cells that escape central tolerance are subjected to regulatory mechanisms that restrain their pathogenicity. Based on their specificity, they can be divided into natural and adaptive regulatory T cells (121). The first type includes regulatory T cells that continuously express CD25. These CD4⁺CD25⁺ T cells actively restrain the function of inflammatory, CD4⁺ T cells, by mechanisms yet to be fully identified (62-64). They express a unique transcription factor named FoxP3 (122-124). Individuals that display genetic modifications in this gene suffer from a severe X-linked immunodeficiency syndrome named Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked syndrome (IPEX) (125). These recent observations further substantiate the key role of “natural” regulatory cells in maintaining active tolerance.

Antigen specific effector T cell reactivity is dependent on their basic polarization. During the initiation, and progression of an inflammatory autoimmunity antigen specific non-polarized CD4⁺ cells (Thnp) are being polarized either to pathogenic Th1 cells or non-pathogenic Th2 cells or (55). Interestingly, even though altering the Th1/Th2 balance into Th2 suppresses autoimmunity (27-30, 104-106), the direct administration of these cells has been found ineffective in suppressing ongoing autoimmunity (45, 126). In contrast, the administration of antigen specific TGF- β producing Th3 cells (49, 52), or IL-10 producing Tr1 cells effectively suppressed these diseases (49-52). It is believed that during the inflammatory process, in the presence of IL-10, initially produced by activated macrophages, and of IL-2, mostly produced by CD4⁺ Th1 cells, some antigen specific non-polarized T cells (Thnp) are being polarized into IL-10 producing Tr1 cells (50). These cells then restrain the progression of the autoimmune condition. Similarly, in the presence of TGF- β some of these Thnp cells are driven into Th3 cells that also suppress inflammation (52). Previous studies of H. Wiener and his group showed that oral administration of self-autoimmune antigens might effectively select antigen specific Th3 cells capable of suppressing autoimmunity (49, 127-130). My group has recently shown that Tr1 cells participate in the natural regulation of T cell mediated autoimmunity and that their function could be amplified by soluble peptide therapy (51). Selective amplification of

antigen specific regulatory T cells is indeed a major challenge in clinical trials.

In patients suffering from various autoimmune disorders autoreactive T and/or B cells escaped central tolerance and circumvented active tolerance induced by various regulatory T cells that could, potentially, eradicate their pathogenicity. We have found an additional regulatory mechanism that at that time restrains the harmful activity of these cells at that time.

6. TARGETED DNA VACCINATION TECHNOLOGY ENABLED THE DISCOVERY OF A NOVEL ANTIBODY BASED REGULATORY MECHANISM THAT RESTRAINS THE DESTRUCTIVE ARM OF THE IMMUNE SYSTEM

Twelve years ago, in a pioneer study D.C. Tang, M. DeVit and S.A. Johnston showed that administration of a plasmid DNA encoding human growth hormone could lead to the production of neutralizing antibodies to its gene product (131). The authors assumed that tolerance against growth hormone was broken due to the minor differences between human and mouse growth hormone. Six years later we showed that this technology, with modifications (Tang et al used gene gun delivery, whereas we are using naked plasmid DNA vaccines) could be used to breakdown tolerance to self-chemokines (41). Our approach included immunization with a mammalian expression vector, encoding a selected proinflammatory cytokine or chemokine, under the control of a strong viral promoter (CMV). The same vector also drives the expression of bacterial CpG as a recurring immunostimulatory sequence (ISS) (132, 133), which signals via TLR9 (134, 135), triggering a breakdown of tolerance and establishing an immune response against gene products encoded by the vaccines. At first, we demonstrated that “vaccination” via administration of plasmid DNA constructs encoding various C-C chemokines, followed by later induction of EAE, elicited antibody production against the chemokines encoded by the plasmid DNA and at the same time suppressed the disease (41). Chemokine specific purified antibodies isolated from these protected animals could then be used to transfer the beneficial effects of each vaccine (41). Later we showed that this strategy could be used to rapidly treat ongoing autoimmunity and that neutralizing antibodies generated in response to the gene product of each vaccine can adoptively transfer the high state of resistance (41, 43-45, 136, 137).

Throughout these studies, we have repeatedly observed some unexpected phenomena. The elicitation of beneficial autoantibody production was dependent on induction of an autoimmune disease, and was regulated by the immune system in accordance with disease progression. Thus, autoantibody production against the vaccine-encoded product regressed to baseline levels shortly after remission in acute EAE, whereas in chronic AA these antibodies were continuously produced, at a very high titer. (41, 43-45, 136, 137). Moreover, therapy of ongoing diseases led to a very rapid (within less than 48h)

production of highly specific beneficial autoantibodies (IgG2a) that could effectively transfer diseases resistance (41, 43-45, 136, 137). The most reasonable explanation for these unexpected findings is that targeted DNA vaccines amplify a pre-existing anti-self regulatory response, which, by itself, is capable of limiting, albeit not preventing, the emerging autoimmune condition.

In a very recent study, G. Wildbaum, M. Nahir and myself explored the validity of this interesting concept (73). The study included experimental work in an animal model of RA that has been extended to human patients. We shall first discuss the experimental work, and later on its relevance for human diseases. Because of its significance in the pathogenesis of RA, TNF- α was selected to be at the focus of our study. To address the question whether target DNA vaccines encoding TNF- α do amplify an existing response, we mapped the determinants that naturally produced antibodies to TNF- α recognize on TNF- α . We found that these antibodies interacted with 3 determinants on TNF- α that displayed no cross reactivity to any known gene product, and that targeted DNA vaccines encoding TNF- α augment only these responses. Thus, natural breakdown of tolerance is highly specific and restrictive. We have purified TNF- α specific natural antibodies from AA rats and used them to treat other sick rats in adoptive transfer experiments. These antibodies could effectively suppress ongoing diseases (73). We have therefore decided to name them Natural Protective Antibodies (NPA). We then looked for a novel way to specifically eliminate the production of NPA to TNF- α during AA, which would enable us to measure their real involvement in the natural regulation of the disease.

How important are these NPA to the natural regulation of disease? Neonatal administration of a self-antigen may result in an inability to mount a significant immune response against it in adult life (i.e. neonatal tolerance) (138). This approach has recently been extended to plasmid DNA vaccines (139). We used our TNF- α encoding DNA plasmid to induce neonatal tolerance to its gene product and studied the consequences of this abolition on the development and progression of AA. The results of these experiments clearly showed that NPA to TNF- α manifest a pivotal role in the natural regulation of disease, and while amplification of this response is beneficial for the host, its selective elimination leads to its aggravation (73).

7. ON MEN AND MICE: EXPLORING THE BASIC FINDINGS AND RELEVANCE IN RHEUMATOID ARTHRITIS

Therapies that neutralize the function of TNF- α suppress RA but not Osteoarthritis (OA) (97). We decided to learn, in double-blind set of experiments, if patients “know” what could be beneficial for them. In collaboration with M. Nahir, chief of the Rheumatology Department at the RAMBAM Medical Center in Israel, we have tested the potential production of NPA to TNF- α . We showed that the vast majority (>70%) of patients suffering from RA, but

not OA, had significant levels of autoantibodies directed against TNF- α . Thus, similarly to the immune system in experimental models, the human immune system also selectively generates a beneficial autoimmune response to restrain self-destructive immunity.

An interesting question is referred to the link between the severity of the disease and the antibody titer. It is possible that patients that develop severe RA do so because they fail to generate high antibody titer against TNF- α that would restrain their disease. Alternatively, the severity of the disease promotes the amplification of anti TNF- α antibody production. None of these possibilities may explain why about 25% of the RA patients do not produce autoantibodies against TNF- α . We (N. Karin G. Wildbaum & M. Nahir) are now trying to define whether patients that fail to respond to anti TNF- α therapy are those naturally producing low anti TNF- α antibody titer.

It should be noted that autoantibodies to the inflammatory chemokine IL-8 have been previously found in RA patients (140). Moreover, in this study levels of circulating free anti-IL-8 IgG autoantibodies were found to display a significant correlation with inflammatory parameters and disease severity (140). This may suggest that IL-8 could be a highly relevant candidate target for therapy.

From a clinically oriented perspective, the finding that NPABs to TNF- α exist in RA patients, and that DNA vaccines could amplify such a response, may pave the way for novel therapies that would be based on active immunization with plasmid DNA vaccine, viral vectors (141), or even modified TNF- α , at the protein level (142). The major limitations of antibody/soluble-receptor therapy are the high cost and our limited ability to produce large amounts of engineered receptor or human/humanized mAb for therapy. Active immunization would probably be a much more affordable way of therapy. However, the major limitation of the active immunization approach is the incapability to control its outcome. That is, a patient suffering from RA could potentially have to live, probably for lifetime, with an elevated titer to TNF- α . So far this limitation restricts the potential use of active vaccines, including DNA vaccines, as a way to effectively augment beneficial autoimmunity.

8. ACKNOWLEDGMENT

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