Immune System – Part III
The delicate balance of the immune system between tolerance and autoimmunity
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ABSTRACT
The immune system consists of an intricate network of organs, cells, and molecules responsible for maintaining the body’s homeostasis and responding to aggression in general. Innate immunity operates in conjunction with adaptive immunity and is characterized by rapid response to aggression, regardless of previous stimulus, being the organism first line of defense. Its mechanisms include physical, chemical and biological barriers, cellular components, as well as soluble molecules. The organism first line of defense against tissue damage involves several steps closely integrated and constituted by different components of this system. The aim of this review is to restore the foundations of this response, which has high complexity and consists of several components that converge to articulate the development of adaptive immune response. We selected some of the following steps to review: perception and molecular recognition of aggressive agents; activation of intracellular pathways, which result in vascular and tissue changes; production of a myriad of mediators with local and systemic effects on cell activation and proliferation, synthesis of new products involved in the chemoattraction and migration of cells specialized in destruction and removal of offending agent; and finally, tissue recovery with restoration of functional tissue or organ.

Keywords: innate immunity, inflammation, autoimmunity, PAMPs, Toll-like receptors.

INTRODUCTION
A loss of the ability of the immune system of an individual to distinguish between self and non-self is observed in organ-specific and systemic autoimmune disorders. This ability, known as self-tolerance, is maintained in immunocompetent B and T cells both through central and peripheral mechanisms.

Loss of self-tolerance can have intrinsic or extrinsic causes. Intrinsic causes, i.e., those related to characteristics of the individual, are usually associated with polymorphisms in histocompatibility molecules, components of innate immunity, such as the Complement system and Toll-like receptors, components of acquired immunity, such as regulatory lymphocytes and cytokines, and hormonal factors, which are under genetic control. Environmental factors, such as bacterial and viral infections, exposure to physical and chemical agents, such as UV radiation, pesticides, and drugs, are examples of extrinsic causes.

Epidemiological studies have shown the importance of genetic factors in determining susceptibility to autoimmune
FAS apoptosis pathways are seen. Consequently, since patients, mutations in the genes encoding proteins of the homeostasia can be evaluated in autoimmune lymphoproliferative disorders and lymphomas. Apoptotic cells should be removed immediately by phagocytes, macrophages, and dendritic cells not directly involved in the response to pathogens, known as bystander activation; and molecular mimicry.

Regarding extrinsic causes, several recent reviews on the role of infections in the development of autoimmune diseases\textsuperscript{1-3} can be found in the literature, and associations between infections and exacerbation of autoimmune disorders are common. Infections can trigger loss of tolerance by several mechanisms. Among them are tissue damage and cellular necrosis, which expose cryptic epitopes in autoantigens or allow access of immunocompetent cells to normally isolated antigens; polyclonal T and B cell activation by microbial superantigens, such as toxins produced by \textit{S. aureus}; activation of immunocompetent cells not directly involved in the response to pathogens, known as bystander activation; and molecular mimicry.

Rheumatic fever after group A streptococcus β-hemolytic infection, in which antibodies against streptococcal M protein that cross-react with cardiac tissue can be found in the serum of those patients, is the classical example of molecular mimicry.

The process of programmed cell death, apoptosis, is important both for maintenance of central and peripheral tolerance and in controlling lymphocyte populations generated in the immune response. An increase in the rate of apoptosis can result in immunodeficiencies, and there is evidence that failure of apoptotic mechanisms or clearance of apoptotic cells can lead to the development of autoimmunity and lymphomas. Apoptotic cells should be removed immediately by phagocytes, macrophages, and dendritic cells, preventing exposure of autoantigens. The crucial role of apoptosis in maintenance of lymphocyte population homeostasis can be evaluated in autoimmune lymphoproliferative syndrome (ALPS), a rare human disorder. In those patients, mutations in the genes encoding proteins of the FAS apoptosis pathways are seen. Consequently, since lymphocytes do not undergo the usual process of apoptosis, there is progressive accumulation of these cells, resulting in lymphadenomegaly, hepatosplenomegaly, and autoreactive T lymphocytes. It is intriguing that autoimmune manifestations in this syndrome are related, predominantly, to the hematologic system, especially autoimmune hemolytic anemia and thrombocytopenia.

At least in some of the patients with SLE, the deficiency in apoptotic cell clearance seems to contribute for the pathophysiology of the disease. Genetic deficiencies of C1, C2, and C4 are associated with a greater prevalence of systemic lupus erythematosus, although the strength of this association is variable for each of those elements. Thus, 90% of individuals with C1q deficiency should develop SLE, usually with significant renal involvement. C4 deficiency would be associated with the development of SLE in 75% of the cases; and those with C2 deficiency can be asymptomatic, but a smaller proportion of those patients will develop SLE, although less severe.

Currently, evidence that innate immunity has an important role in the development of autoimmunity accumulates. The known relationship between Complement deficiencies and autoimmunity has been explained by the reduced clearance of immunocomplexes and apoptotic cells. Toll-like receptors that recognize pathogen-associated molecular patterns (PAMPs) represent another important link between innate and acquired immunity. Some of those receptors show specificity for nucleic acids, such as the autoantigens DNA and ribonucleoproteins. Inflammation triggered by innate immunity can stimulate the immune system, by a mechanism known as adjuvant effect. Inflammatory mediators induce expression of HLA class I and II molecules by cells in the damaged tissue, allowing those cells to work as antigen-presenting cells. In this situation, presentation of autoantigens in a context other than the usual, which might result in autoimmunity, is possible.

CENTRAL TOLERANCE OF T LYMPHOCYTES

Several aspects are relevant when considering the rupture of the mechanisms of tolerance, with consequent triggering and maintenance of autoimmune abnormalities. The multifactorial and polygenic nature of autoimmune disorders, with genes linked to disease susceptibility and others strictly linked to their severity is one of these aspects. Since the recombinant mechanisms of multiple gene segments responsible for coding immunoglobulins in B lymphocytes (BL) and TCR in T lymphocytes (TL) are random, receptors capable of recognizing self structures are most likely produced.
T cell precursors, originated in the bone marrow, migrate to the thymus where they undergo sequential modifications characterizing the different stages of TL differentiation. In the initial maturation stage, cellular proliferation of lymphocytes occurs in the outermost cortex region, with TCR gene rearrangement and expression of CD3, TCR, CD4, and CD8 molecules on the cell surface. As thymocytes mature, they migrate from the thymic cortex to the medulla. The thymic stroma consists of epithelial cells, macrophages, and bone marrow-derived dendritic cells, besides fibroblasts and extracellular matrix molecules. The interaction of thymocytes and cells of the thymic microenvironment is fundamental for cell proliferation, differentiation, and expression of surface molecules, such as CD4 and CD8, and creation of the repertoire of TL receptors.

Thymocytes that succeed in expressing the complete TCR molecule (αβ or γδ chains) undergo two distinct processes, positive selection followed by negative selection. Positive selection is based on usefulness criteria, mainly the affinity of the link between TCR and MHC complex (MHC restriction). Thymocytes undergo positive selection in the thymic cortex, where thymocytes with TCR capable of binding to the self-peptide-MHC complex are stimulated to mature and continue the maturation process. Thymocytes whose receptors do not recognize the self-MHC molecules die by apoptosis, ensuring that TLs are restricted to self-MHC. Positive selection also associates restriction of class I and II MHC molecules to TL subtypes, ensuring the CD8+ T cells are specific for peptides exposed on Class I MHC molecules, while CD4+ T cells are specific for peptides exposed on class II MHC molecules. In negative selection, thymocytes whose TCRs show strong affinity for the self-peptide-MHC complex are eliminated, therefore avoiding maturation of autoreactive TLs. In theory, immunologic self includes all epitopes (antigenic determinants) coded by the DNA of the individual, so all other epitopes are recognized as non-self.

During the development of thymocytes, the majority of those cells die by apoptosis (around 95%). This is due, mainly, to unsuccessful rearrangements of TCR chains and positive and negative selections, and only a small fraction (3% to 5%) will become mature TLs (Figure 1).

However, recent studies suggest that not all thymocytes with high affinity for autoantigens are destroyed by negative selection in the thymus. Some median- and high-affinity thymocytes survive and undergo a process known as non-deletional central tolerance that leads to the generation of immunosuppressor CD4+ T cells, known as naturally-occurring regulatory T cells or \textit{T}_{REGs}.

Figure 1
A. Schematic representation of positive and negative selections for T cells in the thymus. Double-positive thymocytes are in contact with self-peptides linked to MHC molecules on epithelial cells, in the thymic cortex, and on macrophages and dendritic cells, in the thymic medulla. Thymocytes whose TCRs are incapable of recognizing self-MHC and peptides die by apoptosis (negative selection). Thymocytes whose TCRs have high affinity for self-MHC and peptides are removed by apoptosis (positive selection). B. Schematic representation of central and peripheral tolerance mechanisms of B lymphocytes. Immature lymphocytes that show high affinity for self antigens in the bone marrow are eliminated by apoptosis (1) or undergo change of their antigenic specificity (2). Non-specific lymphocytes for self antigens and that underwent recombination (3) migrate into the periphery. Cells that encounter auto-antigens in the periphery and are activated are eliminated by apoptosis or become anergic. On the other hand, those that do not show specificity for self antigens are capable of generating an immune response when they encounter foreign antigens.
PERIPHERAL TOLERANCE OF T LYMPHOCYTES

Immunologic tolerance of TLs to self-antigens occurs, mainly, in the thymus. Cells that reach the periphery should be immunocompetent against non-self-antigens, but incapable of developing an immune response against self-antigens; however, this is not what is seen. Several studies have shown that small numbers of autoreactive cells are present in individuals without autoimmune disorders and they can be isolated from peripheral blood and lymphoid tissues. These observations have shown that autoreactive TLs that manage to evade the barriers of tolerance mechanisms and leave primary lymphoid organs can be found in the periphery. Therefore, thymic deletion of autoreactive TLs is not the only mechanism responsible for self-tolerance; its maintenance also involves the interaction of several peripheral immunologic mechanisms that operate continuously. Peripheral immunologic tolerance is organized in different and redundant mechanisms, such as immunological ignorance, deletion, inhibition, or suppression of autoreactive clones (Figure 2).

Immunological ignorance can be due to physical separation between antigens and TLs, such as that seen in the blood-brain barrier, or to insufficient levels of antigens to activate the respective TLs. Another mechanism, much more important, consists on antigen presentation in the absence of a co-stimulator or “second signal”. In this situation, failure of TL activation occurs, leading to anergy or cell death by apoptosis. Programmed death of TLs by apoptosis is triggered by the extrinsic apoptosis pathway involving the Fas molecule and its ligand (FasL). Increased expression of the Fas ligand in APCs presenting antigens can induce apoptosis of TLs through activation of the Fas molecule. Defects in TL signaling activation pathways associated with resistance to apoptosis apoptosis may lead to the persistence of autoreactive TL clones in the peripheral circulation.

As mentioned before, an important mechanism of peripheral tolerance consists of the absence of a co-stimulatory signal necessary for activation of TLs, besides the primary

Figure 2
Peripheral tolerance and induction mechanisms. T cells physically separated from their specific antigens, for example, by the blood brain barrier, cannot be activated, which is known as immunologic ignorance. T cells that express CD95 (FAS) on their surface can receive apoptosis induction signals from cells that express FAS-L, which is referred as deletion. One example of inhibition can be observed in the binding of CD152 to CD80 on APCs, inhibiting T cell activation. Regulatory T cells can inhibit or suppress other T cells, probably by producing inhibitory cytokines, such as IL-10 and TGF-β.
The delicate balance of the immune system between tolerance and autoimmunity

signal furnished by the binding between the TCR and the MHC-antigen complex. This secondary signal is obtained, mainly, by the interaction between the CD28 molecule on the surface of TLs and the B7 molecule family (CD80 and CD86), expressed on the surface of antigen-presenting cells (APCs). In the presence of pathogens, APCs are activated and increase the expression of B7 molecules to a high enough level to provide the second signal and production of IL-2 by TLs. In physiologic conditions and absence of inflammation, low levels of B7 molecules in APCs hinder complete activation of autoreactive TLs, favoring anergy or apoptosis when they encounter the antigens for which they are specific.

Inhibition of TLs can also be achieved by binding a B7 family co-stimulatory-competing molecule. In specific situations, APCs express CTL-4 (CD152) molecules, which have greater affinity for CD28 than B7 molecules (CD86 and CD80). However, unlike those molecules, CTLA-4 inhibits TLs, inducing apoptosis. In mice, blocking the CTLA-4 molecule accelerates the progression of autoimmune diabetes.

Mechanisms of immunosuppression also include several immunoregulatory cell populations whose basic characteristic is the capacity to produce immunosuppressor cytokines, such as IL-4, IL-10, and mTGF-β, besides being able to induce cell-cell contact-mediated suppression through surface molecules, such as CTLA-4. Immunoregulatory cells are involved in the modulation and control of pathogen elimination processes in places with tissue destruction, exposure of autoantigens, and production of proinflammatory cytokines, conditions that favor induction and maintenance of autoimmune events that need to be controlled. These cells work in a complex network of regulatory mechanisms aimed at ensuring modulation of immunologic responses in face of several antigens derived of infectious processes, tumors, alloantigens, autoantigens, and allergens.

Naturally-occurring regulatory T cells (T<sub>REG</sub>, CD4<sup>+</sup> CD25<sup>+</sup> CD127<sup>Low</sup>, Foxp3<sup>+</sup>), first described by Sakaguchi et al., T<sub>R</sub> cells, which regulate by producing IL-10 and suppress the development of some TL responses in vivo,<sup>11</sup> and TH3 cells, capable of suppressing target-cells by producing TGF-β,<sup>12</sup> are among the immunoregulatory TLs. Several other regulatory cells, such as CD8<sup>+</sup>CD28<sup>-</sup> TLs, NK-T cells, T<sub>øy</sub> cells, double-negative TLs, CD8<sup>+</sup>Qa1<sup>+</sup> TLs, and CD1<sup>+</sup> B cells, also exist, Figure 3 shows the main immunoregulatory cells.

The relevance of regulatory cell populations in autoimmune rheumatic diseases has been demonstrated in murine models, in which the absence or depletion of T<sub>REG</sub> cells triggers systemic autoimmune diseases with elevated titers of antinuclear antibodies, as well as organ-specific autoantibodies.<sup>14</sup> Important findings, such as functional, phenotypic, and quantitative defects of regulatory cells, have been reported in several human autoimmune rheumatic disorders, demonstrating their important role in the maintenance of immunologic tolerance and in the pathophysiological mechanisms of these disorders.

Studies of immunoregulatory T cells have been undertaken in disorders like rheumatoid arthritis, systemic lupus erythematosus, mixed connective tissue disease, primary Sjögren syndrome, Kawasaki disease, and Wegener’s granulomatosis. In these disorders, quantitative changes in these cells have

![Figure 3](https://example.com/figure3.png)

Schematic representation of the main immunoregulatory cells responsible for maintaining the mechanisms of peripheral tolerance.
been observed in affected tissues and/or peripheral circulation, functional defects, target-cell resistance to suppression, and even normal frequency and function. Conflicting results in the literature are most likely a consequence of the conceptual and technical evolution in this field, leading to heterogeneous methodological approaches among different studies, with the consequent discrepancy in results.

It is hoped that studies currently under way can elucidate more accurately the exact role of regulatory cells in different disorders and the real role of functional and quantitative changes in these cells in the rupture of tolerance seen in autoimmune diseases. Due to the multifactorial and multigenic characteristics, as well as the clinical heterogeneity of each autoimmune rheumatic disease, that are more like syndromes than nosological entities, the finding of numeric and/or functional changes in just a fraction of patients with a given disorder should not be surprising.

**EFFECTOR T LYMPHOCYTES AND AUTOIMMUNITY**

Approximately 20 years ago, based on the cytokines produced, effector CD4+ TLs were divided in two distinct subtypes, T helper 1 (Th1) and T helper 2 (Th2) cells. Some authors also favored the existence of a third cell population, Th0 cells, represented by undifferentiated lymphocytes capable of producing Th1 and Th2 cytokine profiles. Currently, it is clear that, after antigenic stimulation, naïve CD4+ TLs proliferate and differentiate, according to the local cytokine environment, in different effector subtypes with different characteristics (Th1, Th2, Th3, Th17), determined by their cytokine profile and functional properties.

As shown in Figure 4, Th1 cells are characterized, mainly, by the production of large quantities of IFN-γ, while Th2 cells produce IL-4, IL-5, and IL-13. T helper 1 response triggers late hypersensitivity mechanisms, activates macrophages, and are very efficient in eliminating intracellular pathogens. T helper 2 cells are more effective in helping the immune humoral response, triggering the production of immunoglobulins and eosinophilic inflammation responses, important in fighting extracellular pathogens. T helper 0 lymphocytes differentiate into Th1 or Th2 on an early stage of cellular activation. Characteristically, Th1 or Th2 cytokine profile direct the development of their own pathway, inhibiting expression of the opposite pattern. Thus, once the immune response is polarized into Th1 pattern, the Th2 pathway will be inhibited and vice-versa. This is due to regulation of the level of membrane receptors, differential expression of transcription factors, and epigenetic changes.

Unregulated, or exacerbated, immune effector responses can lead to the development of allergic and autoimmune disorders. T helper 1 cells are potentially proinflammatory and they have been associated with induction and progression of autoimmune diseases. However, studies with transgenic mice with deficiencies of IFN-γ or its receptor have shown that the loss of signaling associated with IFN-γ does not render one resistant to the development of autoimmunity. On the contrary, those animals are more susceptible. Since IFN-γ is one of the main Th1 cytokines, those observations lead to questioning of the exclusive role of Th1 cells in the pathophysiology of autoimmune diseases, leading to the search of other subtypes of TLs, distinct from Th1 cells, capable of inducing tissue inflammation and autoimmunity.

The need to understand the immunologic mechanisms responsible for tissue lesions on several chronic inflammatory disorders, and the development of studies on effector TL populations lead to the characterization of IL-17-producing TLs, called Th17. Recent studies have demonstrated that the specific subpopulation of IL-17-producing CD4+ T lymphocytes, more than Th1 cells, have a central role in the pathogenesis of experimental models of autoimmune diseases.

Studies on autoimmune disorders, such as rheumatoid arthritis, systemic lupus erythematosus, psoriasis, multiple...
sclerosis, systemic sclerosis, inflammatory bowel disease, ankylosing spondylitis, and juvenile idiopathic arthritis, demonstrated the presence of elevated levels of inflammatory products related to the Th17 effector pathway or even their direct participation on pathophysiological mechanisms.

Current concepts on the immunopathology of chronic inflammatory diseases indicate a central role of Th17 cells, which would be responsible for mediating early tissue inflammation, producing proinflammatory cytokines and chemokines responsible for recruiting Th1 cells to sites of inflammation. Even though T regulatory cells (T\(_{\text{REG}}\)) can also accumulate in those sites, the presence of inflammatory cytokines renders target cells less susceptible to immunomodulation and decreases the immunosuppressive power of T\(_{\text{REG}}\) cells. Recent studies have demonstrated a great flexibility in the differentiation process of CD4\(^+\) TLs, and the existence of a close relationship between the Th17 pathway and Th1 and T\(_{\text{REG}}\) pathways does exist.\(^6\) Depending on the stimulus and environmental conditions, Th17 cells can differentiate into either Th1 or T\(_{\text{REG}}\) cells, changing significantly the end-result of the immune response.\(^6\) In the current context, a new subpopulation of effector TLs, Th9 cells, has been proposed.\(^7\) Murine Th9 lymphocytes have recently been described as cells that produce large quantities of IL-9, an important cytokine in the responses against intestinal parasites.\(^8\) It has also been demonstrated that they result from the polarization of Th2 lymphocytes stimulated in the presence of TGF-β and IL-4, and that they do not present none of the transcription factors characteristic of Th1, Th2, Th17, or T\(_{\text{REG}}\) cells.\(^7\) The role of IL-9 in the pathophysiology of chronic allergic conditions has been well documented; however, little is known on the role of those cells in different human diseases.

Understanding those differentiation pathways and their imbalances in different autoimmune disorders will help in the development of therapeutic strategies aimed at broadening the action of regulatory cells, along with the control of effective inflammatory response.

**CENTRAL B LYMPHOCYTE TOLERANCE**

When membrane immunoglobulins, BL receptors, are expressed for the first time, when those cells are still in the bone marrow, autoreactive receptors can be produced as consequence of the random repertoire-generating process. To avoid the release of autoreactive BLs into the periphery, mechanisms of central tolerance exist and, in case they fail in eliminating those lymphocytes, peripheral tolerance mechanisms also exist. Deficiencies in those mechanisms can lead to the development of autoimmunity.

Immature BLs that show high affinity for self-antigens in the bone marrow are eliminated or undergo reactivation of RAG1 and RAG2 genes (which cause somatic hypermutation of hypervariable regions in the immunoglobulin genes) and express a new immunoglobulin chain, therefore showing new antigenic specificity. This process is known as receptor editing, and is an important mechanism through which occasional autoreactive BLs will lose their autoreactivity. If receptor editing fails in eliminating autoreactivity, cells are usually eliminated by apoptosis (Figure 1B). Occasionally, BLs that recognize self-antigens can leave for the periphery, but they show low expression of membrane immunoglobulins.

**PERIPHERAL TOLERANCE OF B LYMPHOCYTES**

When occasional autoreactive mature BLs encounter soluble autoantigens in the periphery, in the absence of helper TLs, i.e., of the second activation signal, they become anergic, being incapable of responding after new encounters with the antigen. If an anergic BL encounters an activated helper TL, it can be eliminated by the interaction between BL Fas and TL FasL. In the absence of normal co-stimulatory pathways, anergic B lymphocytes show greater sensitivity to apoptosis after binding of Fas to its ligand (FasL). In this context, chronic exposure to autoantigens in physiological environment (non-inflammatory conditions) can contribute to tolerance maintenance.

B lymphocytes that encounter autoantigens in the periphery cannot home into lymphoid follicles, probably due to failure in the expression of adequate chemokine receptors. There is evidence of the existence of peripheral tolerance mechanisms for BLs that develop autoreactive specificities, as result of somatic hypermutation, during the response to a non-self-antigen, in germ centers. In this case, it has been hypothesized that elevated local antigen concentrations would lead those clones to apoptosis.

All these mechanisms emphasize the fact that the mere existence of autoreactive BLs is not, in itself, damaging. Before an immune response is initiated, BLs need to encounter their antigens, receive effective help from TLs, and its intracellular signaling machinery should be able of creating a normal response.

**IDIOTYPIC NETWORK**

The idiotypic network theory, introduced in the decade of 1970, is based on the reciprocal interaction among variable regions of the antibodies produced by an individual. The idiotope is the portion of the variable region of the immunoglobulin
molecule that interacts with the antigen. Each idiotope can behave as an antibody, when it encounters its antigen, and as an antigen, when facing an anti-idiotype antibody. One can imagine this scenario as an interconnected network of antibodies with reciprocal reactivity for idiotopes, in such a way that a critical mass is formed, which contributes to the stability of the system. Extrinsic antigenic stimuli would represent a disturbance of this network, but they tend to be assimilated and return to their status quo.

In theory, the immune system would recognize, predominantly, itself, and the response to an antigen would be the result of the increase of a given antibody in the network, disturbing the homeostasis of the system. An increase in the first antibody, AB1, recognized by AB2, would lead to an increase of the latter that has a tendency to promote hyper-regulation of the former (AB1). The same would be seen regarding AB2, AB3, and so forth, always showing a tendency to restore the equilibrium of the system (Figure 5).

Recently, the interest on the study of idiotypic interactions, especially in the clinical context, such as in autoimmune diseases, has been renewed. In the last decade, idiotypic interactions of T cells have received increased attention. It has been proposed that anti-idiotypic T cells behave as regulatory T cells, controlling autoimmune diseases. Recently, experimental evidence has shown that idiotypic and anti-idiotypic T cells can coexist and form a dynamic idiotypic network in the same organism.

Expressive therapeutic effects of the use of natural antibodies (intravenous immunoglobulins) in autoimmune and inflammatory disorders represent the first example of clinical intervention based on those powerful regulatory interactions. It has been suggested that B cells and/or immunoglobulins can be used therapeutically to increase the diversity and restore the repertoire of TLs to improve its function in situations associated with a restricted repertoire. Thus, antibodies have a role not only in interaction and signaling among BLs, but they apparently modulate the development, maintenance, and function of TLs. It is believed that autoimmune diseases can be related to deficiencies in the control of autoreactive antibodies by the idiotypic network. Immunomodulation of autoimmune and inflammatory disorders using intravenous immunoglobulins can have relevant therapeutic effects. Idiotype interactions have been studied in myasthenia gravis and hemophilia A. Recently, an experimental therapy for type I diabetes, based on vaccines composed of idiotypic peptides, and vaccination with idiotypic and anti-idiotypic antibodies, for cancer, has been proposed.

DENDRITIC CELLS AND AUTOIMMUNITY

Dendritic cells (DCs) are immune system cells whose main function is processing and presenting antigens to other cells, being considered the most important antigen-presenting cells (AOCs). Dendritic cells have a fundamental role in the maintenance of both central and peripheral tolerance. Classically, DCs can be classified in two main subtypes, myeloid and plasmacytoid, that differ according to their origin, morphology, and products secreted. Myeloid DCs (mDCs) secrete large quantities of IL-2 while plasmacytoid DCs (pDCs) secrete IFN-α.

Dendritic cells can be found in the most variable anatomic sites and, once activated, they migrate to secondary lymphoid organs where they interact with TLs and BLs. Dendritic cells have a central role in immune responses, regulating their activation and progression. Since DCs should limit tissue damage while, at the same time, guaranteeing the ability to respond to pathogens, overall mechanisms of tolerance maintenance should work actively to maintain this fine equilibrium.

During viral infections, immunocomplexes containing DNA or RNA of apoptotic or necrotic cells are internalized by DCs and BLs, interacting with receptors TLR 7, 8, and 9, which, on their turn, will activate the production of Type I IFN. Plasmacytoid DCs, in particular, produce large quantities of type I IFN, which has a paracrine action on other DCs,
increasing even more immune activation (Figure 6). Several clinical observations confirmed the critical role of IFN-α in the etiopathogenesis of autoimmune disorders, suggesting that induction of autoimmunity does not require, necessarily, factors like molecular mimicry, but it can be related to tissue damage and activation of innate immune response in susceptible individuals. Several evidences have shown that activation of DCs via Toll-like receptors (TLRs), in genetically susceptible individuals, can induce autoimmunity through the production of proinflammatory cytokines, especially type I interferons (IFN-α and IFN-β).

**DENDRITIC CELLS AND AUTOIMMUNE DISEASES**

Dendritic cells are fundamental for the onset of immune responses and, in some autoimmune diseases, such as systemic lupus erythematosus (SLE), their immunostimulatory function is exacerbated, i.e., they are more effective in stimulating TL responses. Several studies have suggested that the blood of SLE patients behaves as an inductor of maturation and differentiation of DCs. Exposure of monocytes of healthy individuals to the serum of SLE patients results in fast generation of cells with DC morphology and function. In SLE, monocytes behave like DCs, inducing proliferation of allogeneic TLs, representing an important source of immunomodulatory mDCs. Since, in SLE, this function is inhibited by the use of anti-IFN-α antibodies, IFN-α seems to be the great inducer of maturation and differentiation of DCs. The main source of this cytokine are pDCs, and the most accepted mechanism to explain the elevated levels of IFN-α in the serum of SLE patients is the recognition of immunocomplexes, containing DNA or RNA of apoptotic or necrotic cells, that are internalized by pDCs, activating the IFN-α production pathway. Several clinical observations confirm the critical role of IFN-α on the etiopathogenesis of SLE. Treatment of tumors or infectious diseases with IFN-α is frequently associated with induction of autoantibodies (4-19%), and a variety of SLE symptoms have been observed in those patients. Mutations in genes involved in the production of IFN-α/β predispose the development of SLE by increasing the ability of pDCs to release those cytokines after activation. Other diseases, such as autoimmune thyroiditis and diabetes, are also marked by the action of INF-α.

In turn, rheumatoid arthritis and psoriasis are associated with the deleterious effects of TNF-α, a highly inflammatory cytokine that affects several cells and organ systems. It is believed that mDCs of those patients are important in the rupture of tolerance and exacerbation of TNF production stimulated by recognition of autoantigen-containing complexes.

Learning the proper manipulation of the behavior of those cells in different disorders is one of the challenges of modern immunology. If we succeed, those efforts will represent a valuable treatment alternative, either to induce immunity or to recover tolerance.
IMMUNOBIOLOGICALS

Immunobiological therapies used includes, mainly, cytokines, monoclonal antibodies, and soluble cytokine receptors. Several cytokines and their receptors, adhesion molecules, and cells that participate in the immune response, such as B and T lymphocytes, represent the targets of immunobiological therapy. Immunobiological therapy is used in different medical subspecialties including rheumatology, oncology, hematology, gastroenterology, neurology, and nephrology, among others.28,29

Monoclonal antibodies used usually belong to IgG class and receive the suffix “mab”, derived from the expression monoclonal antibody. This suffix varies according to the constitution of the non-variable portion of the monoclonal antibody. If it is a murine antibody, the suffix “omab” is used (for example, edrecolomab); if it is chimeric, i.e., with approximately 30% of murine molecule and 70% of human molecule, the suffix used is “ximab” (for example, infliximab); if it is humanized, and less than 10% of the monoclonal antibody is murine in origin, the suffix “zymab” is used (for example, epratuzumab); and if the antibody is completely human, the suffix “umab” is used (for example, adalimumab). Soluble cytokine receptors are fusion proteins (recombinant) composed by the receptor in question associated with the Fc portion of IgG1, and the suffix used is “cept” (for example, etanercept).30

Below, we will review immunobiological therapy applied to soluble mediators of the immune response and T and B lymphocytes used, especially in autoimmune disorders.

Biological therapy and cytokines

Biological agents can be used to inhibit the effects of cytokines, as seen with monoclonal anti-cytokine antibodies, or soluble receptors that bind cytokines and block their effects in target-cells. Cytokines can also be used in biological therapy through recombinant analogue proteins that mimic the effects of the original cytokine.

Proinflammatory cytokines, such as interleukin-1 (IL-1), TNF-α, and IL-6, are the main targets of anti-cytokine therapy, while the use of type I interferons is the main application of cytokine agonist therapy.

Anakinra is a non-glycosilated recombinant analogue of IL-1 receptor antagonist (IL-1Ra). Interleukin-1 receptor antagonist can be classified as a counter regulatory cytokine produced during inflammation, in response to the production of IL-1, which competes with IL-1β for type I IL-1 receptors, inhibiting its biologic effects.29

This agent was initially investigated, in several clinical assays, in rheumatoid arthritis (RA) and it was approved by the FDA (Food and Drug Administration), in 2001, for cases refractory to one or more DMARDs (disease-modifying anti-rheumatic drugs). However, the true role of anakinra in RA has yet to be determined, especially after the advent of other biological agents.31 Nonetheless, new studies have demonstrated good results when it is used in individuals with diabetes mellitus, acute gout, adult Still’s disease, systemic form of juvenile idiopathic arthritis, and in autoinflammatory syndromes, such as familial Mediterranean fever, TRAPS (tumor necrosis factor receptor-associated periodic syndrome), Muckle-Wells syndrome, and Schnitzler syndrome.32,33,34

Two new IL-1 antagonist agents, canakinumab and rilonacept, have been recently used in the treatment of rare diseases. Canakinumab is an anti-IL-1β human monoclonal antibody released in Europe and United States to treat the systemic form of juvenile idiopathic arthritis and cryopyrinopathies (autoinflammatory syndromes associated with defects of cryopyrin or pyrin genes). Rilonacept, known as IL-1Trap, is a fusion protein composed of the type I receptor of IL-1, the accessory protein of the IL-1 receptor, and the Fc fraction of human IgG1. Rilonacept binds and neutralizes circulating IL-1 before it binds to its receptors on the cell surface. It has been investigated in patients with autoinflammatory syndromes and in acute gout with good results.35

Anti-TNF-α agents represent the main biological agents currently used in rheumatology. Its main representatives include etanercept, infliximab, and adalimumab. Etanercept is a dimer that includes the p75 TNF receptor linked to the Fc fraction of IgG. It binds TNFα molecules, circulating or bound to effector cells, inhibiting the binding to its receptor and preventing the biologic effects of TNFα and TNFβ (lymphotoxin), but it does not cause cytotoxicity of cells that express TNFα on their surface.36

Infliximab is a chimeric anti-TNFα monoclonal antibody, i.e., it has a portion of human IgG1κ and a Fγ region from neutralizing murine antibodies with high affinity for human TNFα. Infliximab binds both free TNFα and TNFα bound to the cell surface, inducing lysis of those cells by antibody-mediated cytotoxicity. Unlike etanercept, infliximab does not bind TNF-β. Adalimumab is an anti-TNFα IgG1 recombinant human monoclonal antibody with a prolonged half-life, requiring subcutaneous administration every two weeks.36

Etanercept, infliximab, and adalimumab have been well investigated and approved for treatment of RA, juvenile idiopathic arthritis, ankylosing spondylitis (AS), and psoriatic arthritis. Infliximab and adalimumab have shown to be effective...
in controlling disease activity and maintenance of remission in Crohn’s disease (CD), while infliximab seems to be the only agent effective in controlling CD-associated fistulae. A second generation of anti-TNFα agents is currently being evaluated in different studies. Those agents include certolizumab pegol and golimumab.37,38

Certolizumab pegol (CDP870) is the pegylated fragment of the Fb portion of a humanized anti-TNFα monoclonal antibody whose efficacy in the treatment of RA and CD has been demonstrated. Due to its long half-life, certolizumab pegol can be administered subcutaneously once a month.39 Golimumab is an anti-TNFα human monoclonal antibody that has been evaluated in patients with RA, psoriatic arthritis, and ankylosing spondylitis (AS) with good results.40,41,42

Lenerecept and CDP571 are anti-TNFα agents that did not demonstrate clinical benefits in the treatment of disorders like as CD and RA, and its development has been discontinued. Pergusnercept was evaluated in patients with RA and showed to be superior to placebo only in one phase II study. Onerecept is the recombinant TNFα soluble receptor p55 that has been under investigation in the treatment of moderate to severe psoriasis. This agent did not show to be effective in CD induction therapy.29

Interleukin-6 (IL-6) is a cytokine whose actions affect different cells and high levels can be observed in several inflammatory diseases. It stimulates hepatocytes to produce acute phase proteins, such as C reactive protein, fibrinogen, and amyloid A protein, while at the same type it reduces the production of albumin. Recently, it has been described that IL-6 stimulates production of the peptide hepcidin that participates in the pathogenesis of inflammation-associated anemia. Tocilizumab, a humanized monoclonal antibody against the soluble IL-6 receptor, inhibits the proinflammatory effects of this cytokine. Clinical assays have demonstrated the efficacy of this biological agent in the treatment of RA, juvenile idiopathic arthritis, and Castleman’s disease.43

Interleukin-5 is a cytokine with Th2 profile that stimulates eosinophil production, activation, and maturation. Mepolizumab is an anti-IL-5 monoclonal antibody that has been investigated in patients with asthma, nasal polyps, and hypereosinophilic syndromes. Mepolizumab has been under investigation in Churg-Strauss syndrome on a phase I/II clinical trials.44 Interleukin-9 is a cytokine produced by Th2 and Th9 cells and it is associated with higher bronchial hyperreactivity, having a pathogenic role in asthma. A humanized monoclonal anti-IL-9 (MEDI-528) antibody has been evaluated in the treatment of asthma.45

Interleukin-17 is a proinflammatory cytokine produced by Th17 cells with an important role in the immune response to specific pathogens, such as extracellular bacteria and fungi, and the pathogenesis of autoimmune diseases, including RA. The safety of a monoclonal anti-IL-17 IgG4 antibody (LY2439821) has been demonstrated on a phase I study with RA patients.46

Interleukin-15 has an important role in the activation of TLs, including memory cells and NK cells. It has been shown that a monoclonal anti-IL-15 IgG1 antibody (HuMax-IL15) was well tolerated and effective in a phase I-II study in RA patients.47

The proinflammatory cytokines IL-12 and IL-23 share the same p40 subunit and they participate in the activation of NK cells and differentiation of CD4+ TLs into Th1 phenotype in response to IL-12, into Th17 in response to IL-23, along with the cytokines IL-1β, TGF-β, and IL-6. Two agents that affect the p40 subunit of IL-12 and IL-23 have been developed (ustekinumab and briakinumab), showing good results in the treatment of psoriasis.48 It has also been shown that ustekinumab is effective in the treatment of psoriatic arthritis.49

Tumor growth factor β is a cytokine with three isoforms (TGF-β1, TGF-β2, and TGF-β3), but several proteins belong to the TGFB superfamily. Aberrant expression of TGF-β1 and TGF-β2 is seen in patients with systemic sclerosis and it is implicated in the pathogenesis of cutaneous and pulmonary fibrosis. However, in a recent clinical assay, the use of an anti-TGF-β1 monoclonal antibody (CAT-192) did not show therapeutic efficacy in patients with systemic sclerosis.50 Lederlimumab (CAT-152) is an anti-TGF-β2 human monoclonal antibody that has been studied in the postoperative period of trabeculectomy for the treatment of glaucoma to avoid excessive cicatrization, presenting controversial clinical results.51

In the family of type I interferons (IFN), recombinant IFNα and IFNβ proteins are used in clinical practice, especially in the treatment of viral hepatitis (hepatitis virus B and C)52,53 and multiple sclerosis, respectively. In the latter, the antagonism of IFNβ against IFNγ, which is very important in the pathophysiology of multiple sclerosis,54 is the proposed mechanism of action. IFN-α has also been used in the treatment of mucocutaneous, ocular, and neurological manifestations of Behçet disease55 and Churg-Strauss syndrome.56 Fontolizumab is a humanized anti-IFNγ agent that has been evaluated in patients with CD, showing good results.

Receptor Activator for Nuclear Factor κ B Ligand (RANKL) is a cytokine of the IFNγ superfamily, produced by activated T lymphocytes, that affects osteoclast differentiation and maturation, stimulating bone resorption. RANKL is important in the pathophysiology of RA, psoriatic arthritis, and
osteoporosis in systemic inflammatory disorders. Denosumab is an anti-RANKL human monoclonal antibody that has been investigated in the treatment of RA, osteoporosis, bone tumors, multiple myeloma, and bone metastasis.

Biological therapy and the complement system

Eculizumab is a humanized anti-C5 monoclonal antibody that binds the 2b subunit of C5 convertase, blocking the cleavage of C5 into the proinflammatory component, C5a, as well as formation of the membrane attack complex (C5b-C9), therefore inhibiting cellular lysis. Eculizumab has been proven to be effective in the treatment of paroxysmal nocturnal hemoglobinuria.

Pexelizumab is a monoclonal antibody with high affinity for the C5 component and it also inhibits its cleavage by C5 convertase. This biological agent has been investigated in patients with acute myocardial infarction, but has not shown to be effective in reducing the infarct area in patients undergoing primary angioplasty and data on the reduction of mortality in acute myocardial infarction are controversial.

Anti-T lymphocyte biological therapy

Therapy with biological agents against therapeutic targets in TLs has been investigated extensively. Surface molecules [CD3, CD4, CD5, CD7, CD40 ligand (CD40L), and CD25] are the main targets; however, an indirect approach, i.e., against TL activation by inhibiting its interaction with antigen presenting cells and with B cells, can also be used.

Abatacept is the main anti-TL biological agent currently being used. It inhibits co-stimulation-dependent activation of TLs by antigen presenting cells. Abatacept is a soluble fusion protein consisting of the extracellular domain of CTLA-4 (cytotoxic T lymphocyte-associated antigen 4) linked to the Fc portion of human IgG1. It inhibits TL activation by binding to CD80 and CD86 molecules in antigen presenting cells, therefore blocking interaction with CD28 molecules in TLs. Since the affinity of CTLA-4 with CD80 and CD86 is much higher than that of CD28, abatacept hinders the binding of this molecule to CD80/86, preventing triggering of the second signal, which is fundamental for activation of TLs. Abatacept has been investigated extensively in RA with proven efficacy in several clinical trials. Some studies to evaluate the efficacy of abatacept in SLE, ANCA-associated vasculitis, giant cell arteritis, Takayasu arteritis, systemic sclerosis, and AS, are ongoing.

The molecule of belatacept is similar to abatacept, but its affinity for CD80 and CD86 molecules is ten times higher than that of abatacept. Some studies in patients undergoing renal transplantation have demonstrated efficacy similar to that of cyclosporin in avoiding acute rejection and higher than cyclosporin in preventing post-transplantation chronic nephropathy.

Ipilimumab and tremelimumab are anti-CTLA-4 monoclonal antibodies that increase activation of TLs and improve cancer immunity. Those agents have been evaluated in patients with melanoma and prostate, lung, and bladder neoplasias, besides hepatocellular carcinoma.

Alefacept is another biological agent that inhibits interaction between antigen presenting cells and T lymphocytes. It is a dimeric fusion protein composed by the extracellular portion of LFA-3 (leukocyte function antigen-3) and the Fc portion of IgG1. Alefacept binds the CD2 molecule in the surface of TLs, inhibiting its interaction with LFA-3, blocking TL activation. This agent has been successfully used in the treatment of psoriasis in patients with chronic lesions and indication of systemic therapy or phototherapy.

Efalizumab is a monoclonal antibody anti-LFA-1 (leukocyte functional antigen-1; an adhesion molecule present in T lymphocytes that binds to ICAM-1, allowing migration of activated T lymphocytes from the blood stream into tissues) monoclonal antibody. Efalizumab is currently indicated for treatment of psoriasis, but its use has been banned in the United States due to the increased risk of progressive multifocal leukoencephalopathy.

Natalizumab is an anti-integrin α4β1 monoclonal antibody, a molecule found on the surface of leukocytes, except neutrophils. Natalizumab blocks the integration between integrin α4β1 and the adhesion molecule VCAM-1. The use of this biological agent has been investigated in the treatment of multiple sclerosis and CD, however, the development of cases of progressive multifocal leukoencephalopathy indicates that it should be cautiously introduced in daily clinical practice.

Anti-CD40L antibodies were developed to inhibit TL stimulation by antigen presenting cells, and BL stimulation by T lymphocytes. Two anti-CD40L monoclonal antibodies have been investigated in patients with SLE. Hu5c9 was investigated in patients with lupus nephritis with good results, but the study was discontinued due to the development of thromboembolic phenomena. The other anti-CD40L antibody (IDEC 131) did not show benefits in the treatment of patients with lupus nephritis.

Anti-TL therapy can be divided in depletion and modulation of its function. Several biological agents for depletion of TLs have been developed. The first was the anti-CD7 monoclonal antibody, but studies have been discontinued due to the lack of clinical efficacy of murine and chimeric antibodies in RA patients. CD5 plus is an immune conjugate composed of an
Complement-dependent cytotoxicity: NK cells, macrophages, and monocytes bind to the complex rituximab-CD20 on the cell surface through their Fcγ receptors, producing BL cytolysis.

Complement-dependent cytotoxicity: the rituximab-CD20 complex binds to C1q and activates the complement cascade via the classical pathway, leading to lysis of BLs by the membrane attack complex.

Induction of BL CD20+ apoptosis.

In 1997, the use of rituximab in the treatment of non-Hodgkin's B cell lymphoma, follicular and low degree, refractory and relapsing, was approved. After approval for its use in lymphoma, rituximab was used in other types of lymphoma, in B lymphocyte disorders (Wäldestrom macroglobulinemia and multiple myeloma), and in Castleman's disease. The use of rituximab in the treatment of rheumatoid arthritis in patients who failed treatment with anti-TNFα has been approved. Rituximab has been investigated in the treatment of SLE in several case series and has presented varying results in controlling manifestations of the disease, especially refractory nephritis and neurologic involvement. It is curious that controlled clinical trials did not demonstrate efficacy in patients with nephritis or in patients with other SLE manifestations. Rituximab has been evaluated in two clinical trials in patients with Sjögren syndrome, demonstrating non-significant results regarding improvement of fatigue and xerostomia/xerophthalmia.

Rituximab has been evaluated in systemic vasculitis, especially in patients with Wegener’s granulomatosis and microscopic polyangiitis, in two clinical assays that demonstrated it was not inferior to cyclophosphamide on inducing remission. It has shown promising results in other autoimmune diseases, such as immunologic thrombocytopenic purpura, type II mixed cryoglobulinemia, and inflammatory myopathies, in series of cases, representing future perspective of benefits with the use of this drug.

Ocrelizumab is a second generation humanized anti-CD20 monoclonal antibody that has the same specificity of rituximab. Phase III clinical trials to evaluate its efficacy in patients with non-Hodgkin's lymphoma are ongoing, but studies that...
evaluated this agent in RA and SLE were discontinued due to lack of efficacy. A phase II study has been planned to evaluate the use of this agent in patients with reactivation and remission of multiple sclerosis.\textsuperscript{83}

Ofatumumab and veltuzumab are anti-CD20 monoclonal antibodies that inhibit activation of B lymphocytes\textsuperscript{77} that are currently in phase III studies for chronic lymphocytic leukemia and non-Hodgkin lymphoma. In RA, ofatumumab has been evaluated in phase II studies with patients with inadequate response to methotrexate and anti-TNFα.\textsuperscript{84}

Epratuzumab is a humanized anti-CD22 monoclonal antibody. CD22 is a surface glycoprotein restricted to B lymphocytes, but it migrates to the surface in mature B lymphocytes, disappearing when it differentiates into plasmacells.\textsuperscript{77} The use of epratuzumab in relapsing non-Hodgkin lymphoma and in that refractory to conventional treatment, including rituximab, has been under investigation; it has also been investigated in SLE, Sjögren syndrome, and RA.\textsuperscript{85} Preliminary results have demonstrated good tolerability and efficacy of this biological agent.\textsuperscript{86}

Belimumab (LymphoStat-B) is a human monoclonal antibody that specifically recognizes and inhibits the biological activity of BlyS (also known as BAFF), a molecule of the TNF superfamily that affects survival, maturation, and activation of B lymphocytes. Several cells (monocytes, macrophages, bone marrow stromal cells, synoviocytes, and astrocytes) produce BlyS, especially under interferon γ stimulation. Although belimumab is associated with a reduction in the number of circulating B lymphocytes, it only acts on soluble BlyS and it does not induce cytotoxicity.\textsuperscript{77,87} Phase II studies with belimumab are under way in patients with SLE and RA.\textsuperscript{88} In SLE, belimumab was well tolerated, but significant differences in disease activity and number of relapses were not observed when compared to placebo.\textsuperscript{89}

Atacicept (TACI-Ig) is a recombinant fusion protein that simulates the soluble receptor (TACI) and blocks the action of BlyS and APRIL. This drug has been evaluated in a phase I study in SLE and good safety was observed in different doses.\textsuperscript{90} Patients with SLE and RA are being recruited for phase II studies. Atacicept is also being evaluated in studies with patients with multiple sclerosis, chronic lymphocytic leukemia, multiple myeloma, and non-Hodgkin lymphoma.\textsuperscript{91}

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The delicate balance of the immune system between tolerance and autoimmunity


