Immune System – Part II Basis of the immunological response mediated by T and B lymphocytes

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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.

ORIGIN

Pluripotent stem cells from the bone marrow give origin to myeloid and lymphoid progenitor cells. The lymphoid progenitors, in turn, give origin to T and B lymphocytes and NK cells. The cells that will differentiate into T lymphocytes (TL) leave the bone marrow and migrate to the thymus, where the entire process of selection and maturation occurs. Only mature T lymphocytes leave the thymus and enter the circulation. The cells that will differentiate into B lymphocytes (BL) remain in the bone marrow and, at the end of the maturation stage, leave the bone marrow and enter the circulation, migrating to the secondary lymphoid organs (Figure 1).

B LYMPHOCYTES

The BL are initially produced in the vitelline sac and subsequently, during fetal life, in the liver and finally, in the bone marrow. The cells that will differentiate into BL remain in the bone marrow during the maturation process and the mature BL

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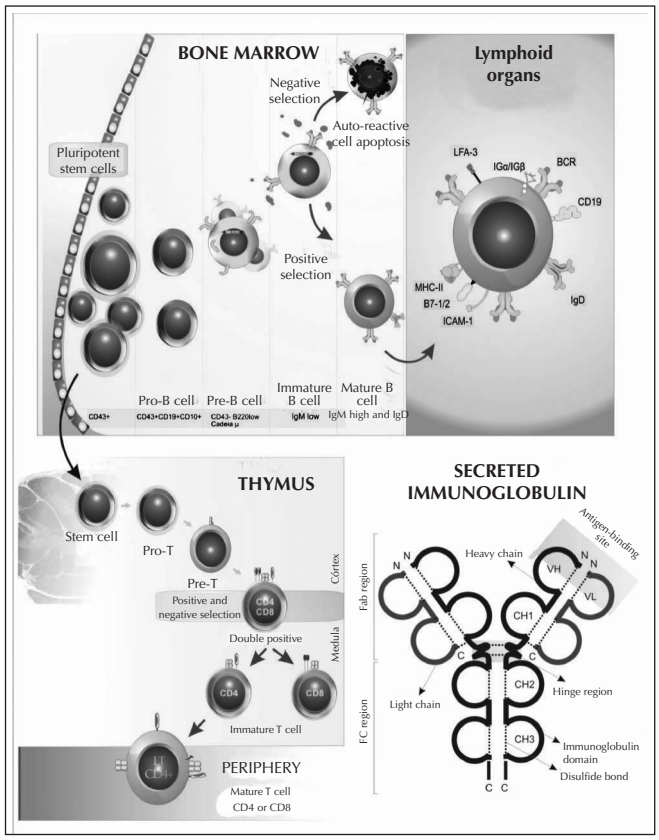

Figure 1. B-cell maturation stages in the bone marrow, from the pluripotent stem cell. Initially, the B lymphocytes go through proliferation cycles with concomitant expression of the B-cell receptor (BCR) chains. Cells that fail to express BCR are eliminated and cells that recognize self-proteins with high affinity are stimulated to undergo apoptosis (negative selection). The lower part of the figure shows T-cell development stages, from the migration of their precursors from the bone marrow to the thymus. The right lower portion of the figure shows the schematic view of a secreted IgG molecule.

leave the bone marrow and enter the circulation, migrating to the secondary lymphoid organs[1,2,3] (Figure 1).

The molecules responsible for the recognition of antigens in the BL are the membrane immunoglobulins, IgM and IgD. These are the counterpart of T-cell receptors (TCR) and, by analogy, are called B-cell receptors (BCR) in some contexts.

CHARACTERISTICS OF IMMUNOGLOBULINS

Each molecule of immunoglobulin (Ig) is constituted by two heavy chains and two light chains, linked by disulfide bonds. There are five types of heavy chains, called α, γ, δ, ε and μ, which define the classes of immunoglobulins, IgA, IgG, IgD, IgE and IgM. There are two types of light chains, kappa (κ) and lambda (λ). The specificity of the antigen binding is defined by the variable portion (Fab) of the molecule, constituted by the union of the variable regions of heavy and light chains of immunoglobulins. The characteristic properties of each class of Ig can be seen in Table 1.[1,3]

Table 1 – Basic characteristics of classes of immunoglobulins

Class	Structure	Properties
IgA	Dimeric and Monomeric	Found in gastrointestinal, respiratory and urogenital tract mucosa. Prevents the colonization by pathogens. Also present in saliva, tears and milk.
IgD	Monomeric	Membrane immunoglobulin. It is part of the membrane receptor of naïve B lymphocytes (BCR).
IgE	Monomeric	Involved in allergic and parasitic processes. Its interaction with basophils and mastocytes causes histamine release.
IgG	Monomeric	Main immunoglobulin of acquired immunity. It has the capacity to cross the placental barrier.
IgM	Monomeric Pentameric	It is part of the membrane receptor of naïve B lymphocytes (BCR). Form found in the serum, secreted early in acquired immune response.

ORGANIZATION OF IMMUNOGLOBULIN GENES

Each immunoglobulin chain is formed from gene segments that rearrange into a specific chain to constitute the complete chain (Figure 1). The variable portion of the heavy chain of immunoglobulins is codified by the segments VH, DH and JH. There are more than 50 VH, ~25 DH and 6 JH genes arranged sequentially in the chromosome, followed by the constant regions Cμ, Cδ, Cγ3, Cγ1, Cα1, Cγ4, Cγ2, Cε1 and Cα2. In the light chains, the segments are ~35 Vκ, 5 Jκ and only one Cκ segment; ~30 Vλ and 4 JλCλ sets.[2]

MATURATION OF B LYMPHOCYTES

The maturation of B lymphocytes start with pro-B cells that express three genes, TdT, RAG1 and RAG2, which command the gene recombination necessary for the production of immunoglobulins. The assembly of the heavy chain starts with the random combination of a D segment and a J segment, which subsequently bind to a V segment, defining the recognition specificity of the antibody that will be formed, regardless of

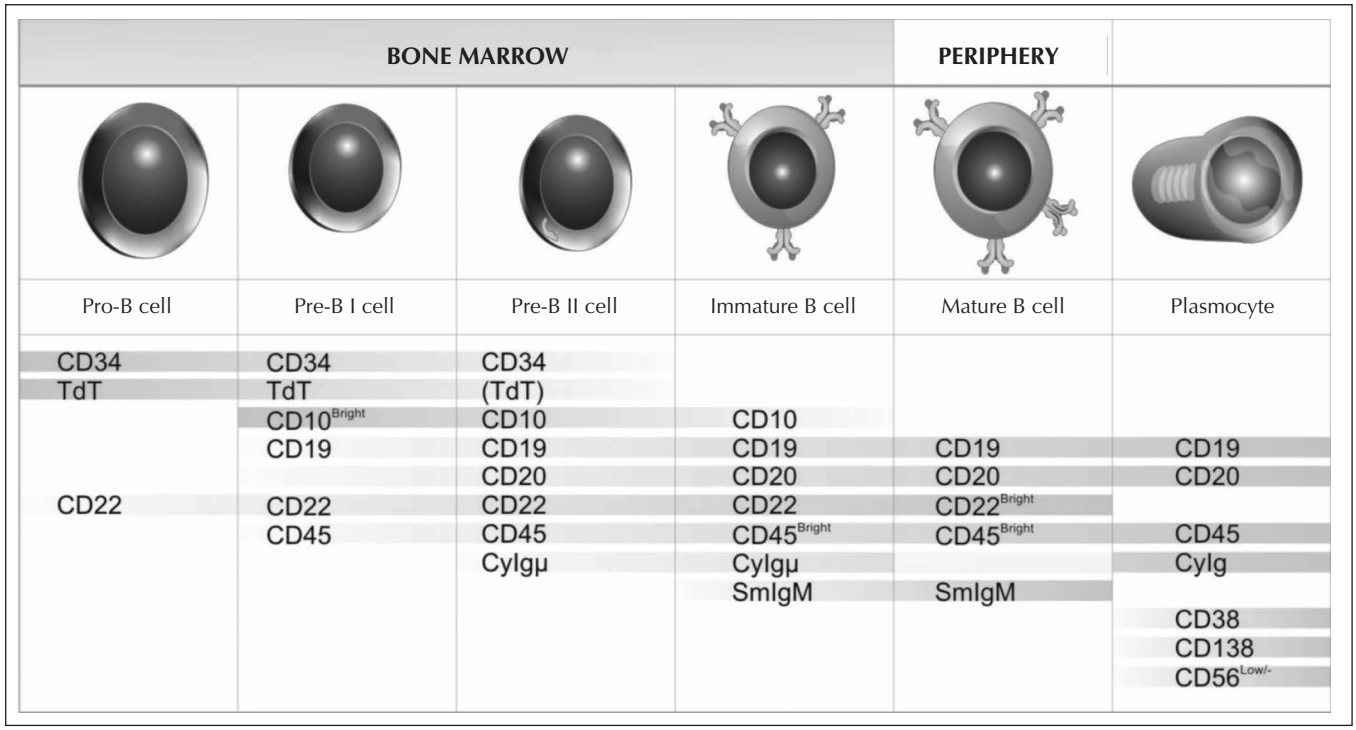

Figure 2. B-cell maturation stages with examples of immunophenotypic markers expressed in the normal development of these cells. CyIgμ - cytoplasmic μ chain; SmIgM - surface molecule IgM ; CyIg - cytoplasmic immunoglobulin.

the constant portion that will be part of the complete chain. This heavy chain is associated with an invariable chain and is expressed on the surface as a pre-BCR, together with the accessory molecules Igα and Igβ. Subsequently, the light chain k is rearranged and, if in the latter fails to do so, the λ chain is rearranged. Each BL presents, therefore, a single type of light chain associated with the heavy chain. The successful expression of a complete IgM on the surface of the BL leads to the maturation progression with subsequent production of membrane IgD.[3]

It is noteworthy the fact that every gene rearrangement occurs in the beginning of the maturation of the BL in the pre-B stage, still in the bone marrow and is completely independent from any contact with the antigen.[2]

The combinatorial process of the different segments that constitute the variable portions of the heavy and light chains and the different possibilities of association between them result in approximately 10^{11} different recognition specificities by the immunoglobulins.[1]

In order to restrict this repertoire, mechanisms of positive and negative selection act during the BL maturation. In the positive selection, the immature BL expressing functional membrane Ig molecules receive signs of survival to proceed with the maturation. In the process of negative selection, the immature BL, still in the bone marrow, which recognize self-antigens with high affinity, undergo apoptosis or initiate a process called receptor edition, in which the RAG genes are once again activated and another light chain V-J combination is generated to substitute the previous one, which is auto-reactive.[1,2,3] Some details of the dynamics of B cell maturation are shown in Figure 1.

IMMUNOPHENOTYPIC CHARACTERIZATION

The stages of BL maturation are characterized by a specific pattern of immunoglobulin gene expression and other membrane proteins expression that act as phenotypic markers of these developmental stages (Figure 2). These surface molecules have important and specific functions in the different phases of BL maturation. The existence of monoclonal antibodies specific for each one of these markers allows studies on the distribution and dynamics of BL lineage to be carried out. Moreover, monoclonal antibodies against some of these surface molecules can also be used with therapeutic purpose, reaching specific subpopulations of cells of the B lineage.

B LYMPHOCYTE ACTIVATION

The BL are responsible for the humoral immunity, characterized by the production and release of antibodies capable of neutralizing, or even destroying, the antigens (Ag) against which they were generated. In order to do so, the BL must be activated, which results in a process of proliferation and differentiation that culminates in the generation of plasma cells with the production of immunoglobulins with high-affinity for the antigen epitope that originated the response. For the activation to occur, it is necessary for the BCR to bind to an antigen epitope, which triggers a sequence of intracellular events. In addition to the antigen recognition, the activation of the BL also depends on a second activation signal.[1,2]

The BL receptor complex (BCR) includes, in addition to the membrane immunoglobulin, two peptide chains, Igα and Igβ, of which function is to initiate the intracellular signaling after the encounter with the antigen.[1,2]

The Igα and Igβ molecules contain activation motifs (ITAMs) that are phosphorylated after the binding of the antigen to the BCR complex and activate factors that promote the transcription of genes involved in the proliferation and differentiation of the BL (Figure 3-A).[2,7]

The BL also act as antigen-presenting cells, after the internalizing and processing the Ag bound to the surface receptor (BCR). The peptides generated by antigen processing are expressed in the membrane of the BL bound to the molecules of the major histocompatibility complex (MHC) class II, and presented to LTCD4+ (helper). The interaction between the peptide complex/MHC class II with the TL receptor (TCR) initiates a chain of events that lead the auxiliary TL to clonal expansion and production of cytokines that stimulate the proliferation and differentiation of BL (Figure 3-B).[1,2,7]

Proteins of the complement system also offer secondary activation signals through the receptor for the C3d fragment, called CR2 or CD21, expressed on the BL surface. The CD21 forms a complex with two other membrane proteins, CD19 and CD81, in BL, allowing the simultaneous recognition of C3d and of the antigen, by BCR. This binding promotes the start of the signaling cascade of both receptors, generating a much higher response when compared to the response of the antigen not bound to the C3d molecule.[1,2] The possibility of the C3d/CR2 complex acting as a second signal for the activation of BL guarantees the triggering of the response in the presence of microorganisms and antigens that activate the complement. This is also a mechanism of amplification of the humoral immune response, as the antibodies that are capable of activating the complement will result in a higher stimulus of the BL.[1,8]

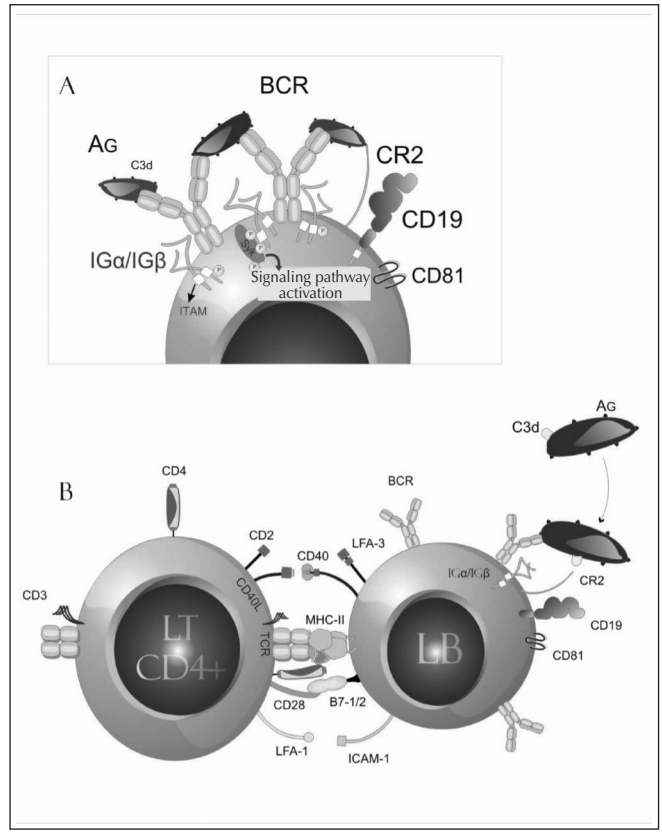

Figure 3. A. BCR and B cell activation complex. From the immunoglobulin cross-linking on the LB surface, biochemical events are triggered that initiate with the phosphorylation of ITAMs (immunoreceptor tyrosine-based activation motifs), with consequent activation of enzymes and biochemical intermediaries that will culminate in the activation of transcription factors that promote cell activation. B. Interaction of B lymphocyte with auxiliary T lymphocyte after antigen processing by B cells. Cytokine production by auxiliary T lymphocytes will result in the activation of the humoral immune response. Note the surface molecules involved in the activation process.

The BL potential to perform its functions after the activation of other receptors related to the innate immune response, such as the Toll-Like Receptors (TLRs), has been recently emphasized. It has been demonstrated that the BL express most of the TLRs, such as TLR-2, TLR-3, TLR-5 TLR-7 and TLR-9, responding to an enormous variety of ligands that can proteins, polysaccharides, lipids and others.[8]

The BL response to peptide antigens requires the assistance of helper TL and these antigens are, therefore, called "T-dependent antigens". Many non-protein antigens, with

repetitive epitopes, do not need the cooperation of the TL and are called "T-independent antigens".[1]

CHARACTERISTICS OF THE T-DEPENDENT RESPONSE

The humoral response to protein Ag requires antigen recognition by the helper TL, and cooperation with the antigen-specific BL, stimulating its clonal expansion, class switching, the affinity maturation and the differentiation in memory BL.[1]

TL expresses surface CD40L molecules that interact with its ligand, CD40, present on BL surface and also expresses CD28, that binds to the B7-1 (CD80) and B7-2 (CD86) molecules. The expression of the latter molecules is significantly increased in the activated BL membrane. These two pairs of molecules, CD40/CD40-L and CD28/B7, allow the transmission of stimulation signals and induce the production of several cytokines (Figure 3-B).[1,2]

IMMUNOGLOBULIN CLASS SWITCHING

The differentiation stage is characterized by significant alterations in the morphology of the BL and also by the change in the constant portion of the heavy chain of IgM or IgD to IgG, IgA or IgE, a process known as class switching. This stage involves complex molecular events, such as genomic DNA rearrangement and alternative splicing of messenger RNA. In this process, the variable portions of the heavy and light chains remain the same and consequently, the antigen specificity of the antibody is not altered, but the immune response becomes more diversified, as the different classes of Ig present different functional characteristics.[1,2]

The signal generated by the CD40/CD40L interaction seems to act globally at the beginning of the class switching process. Patients with a type of X-linked immunoglobulin deficiency present low expression of CD40L in activated TL and, consequently, the BL present a defect in the process of immunoglobulin class change, with an increase in serum levels of IgM, but not of IgG, IgA or IgE, making the patient more susceptible to pyogenic infections and the development of autoimmune diseases and lymphoma.[1,2]

AFFINITY MATURATION

At the start of the response, there is enough Ag to interact with both high and low-affinity BL and the produced antibodies are heterogeneous. Throughout the response, larger amounts of antibodies bind to Ag, decreasing its availability. In this phase, the BL with higher affinity, which interact better with the antigen determinant, are preferably stimulated. This process is called affinity maturation. The increase in the antibody affinity for a certain Ag, during the progression of the T-dependent humoral response, is the result of a somatic mutation in the Ig genes during the clonal expansion. Some of these mutations will generate cells capable of producing antibodies with high affinity; however, others can result in the decrease or even in the loss of the antigen-binding capacity. The positive selection guarantees the selective survival of antibody-producing BL with progressively higher affinity. During this process, the interaction CD40/CD40L is crucial, as well as the presence of T-lymphocyte derived soluble factors and, therefore, the affinity maturation occurs only in response to T-dependent antigens.[1,2,3]

As the memory BL persist after an exposure to T-dependent antigens, the antibodies produced in a secondary response have a higher mean affinity than those produced in a primary one. This process is important for the elimination of persistent or recurring antigens.[1,2]

CHARACTERISTICS OF THE PRIMARY AND SECONDARY ANTIBODY RESPONSES

The primary and secondary antibody responses to protein antigens differ both qualitatively and quantitatively.

Primary Response

The first contact with an antigen, due to natural exposure or vaccination, leads to the activation of naïve BL, which differentiate into antibody-producing plasmocytes and memory cells, resulting in the production of specific antibodies against the inducing antigen. After the start of the response, there is a phase with an exponential increase in antibody levels, followed by a phase called plateau, in which the levels do not change. That is followed by the last phase of the primary response, the decline phase, in which a progressive decrease in the number of circulating specific antibodies occurs.[1,2]

Secondary Response

When getting in contact with the antigen for the second time, there is an existing population of BL capable of recognizing this antigen due to the clonal expansion and memory cells generated at the primary response. The secondary response differs from the primary one in the following aspects: the

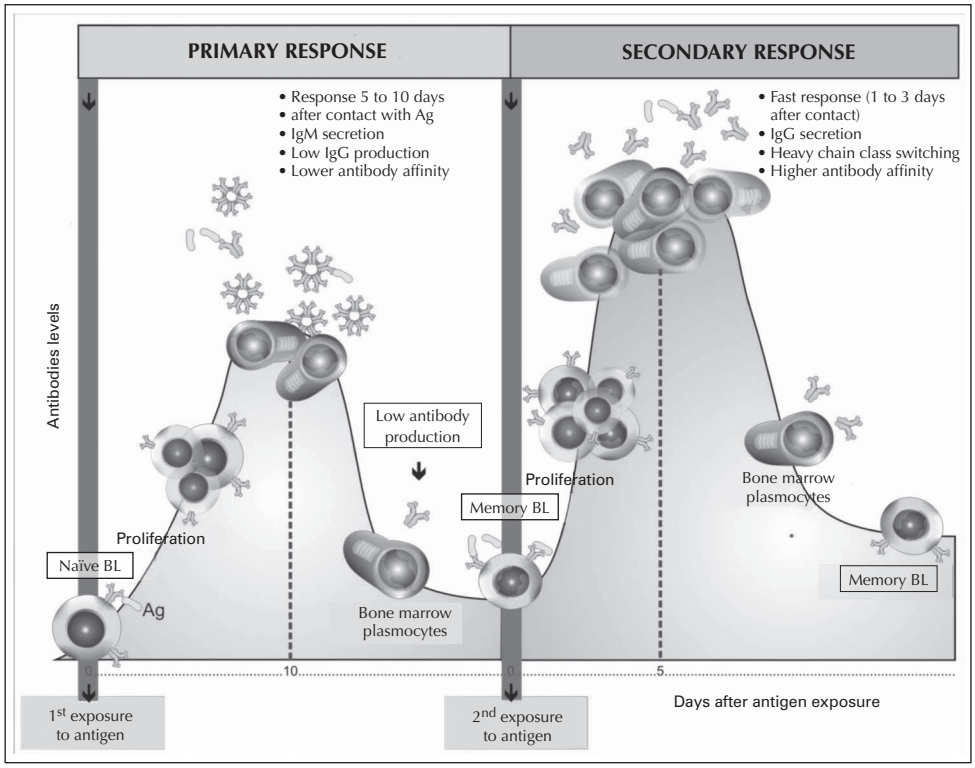

Figure 4. Schematic view of the primary and secondary phases of adaptive humoral immune response. The naïve B cells in the peripheral lymphoid tissues are activated after the contact with the antigen, proliferate and differentiate in antibody-secreting cells and memory B-cells. The secondary response is faster and occurs after the activation of memory B-cells, promoting the production of larger amounts of antibodies.

dose of antigen necessary to induce the response is smaller, the latency phase is shorter and the exponential phase is more marked; the production of antibodies is faster and higher levels are attained; the plateau phase is reached faster and last longer and the decline phase is slower and persistent (Figure 4).[1,2,3]

The extent of the secondary response also depends on the time interval since the initial contact with the antigen. The response will be lower if the interval is too short or too long. If it is too short, the antibodies still present form Ag/Ac complexes that are rapidly eliminated; if it is too long, it is possible that the memory cells will gradually decrease over time, although the capacity to trigger a secondary response can persist for months or years. The optimal period for the induction of the secondary response is just after the decrease of primary response antibodies levels below the limits of detection.[1,2]

IgM and IgG isotypes are produced in both types of response, primary and secondary; however, in the primary response, IgM is the main Ig and the production of IgG is lower and occurs later. In the secondary response, IgG is the predominant immunoglobulin. In both responses, the serum levels of IgM decrease rapidly, so that after one or two weeks, there is a marked decrease, whereas the production of IgG is persistent.[1,2] It is noteworthy the fact that the very sensitive immunoenzymatic tests can register low or residual levels of IgM for months, in some cases.

CHARACTERISTICS OF T-INDEPENDENT RESPONSE

T-independent antigens can stimulate antibody production in the complete or relative absence of TL. These antigens are usually non-protein, polymeric molecules that stimulate the production of low-affinity Ig that belong, mostly, to the IgM class. As usually there is no TL activation, the cytokines necessary for class switching, affinity maturation or memory BL formation will not be generated. The change to other Isotypes rarely occurs in the response to T-independent antigens.[1,2,3]

One example of the importance of T-independent antigens response is the humoral immunity in the presence of bacterial polysaccharides, a decisive host defense mechanism in the defense of the host against infections by encapsulated bacteria. Therefore, individuals with congenital or acquired deficiencies that affect the humoral response are especially susceptible to, and eventually die of infections by encapsulated bacteria. Another example of

response to T-independent antigens are the natural antibodies, present in the circulation of normal individuals and apparently produced without antigen exposure. Many of these natural antibodies recognize carbohydrates with low affinity and it is believed that they are produced by peritoneal BL, that is, B1 cells, stimulated by bacteria that colonize the gastrointestinal tract and by BL from the marginal zone of the lymphoid organs.

Antibodies against blood types A and B glycolipid antigens are another example of natural antibodies.[1,3]

REGULATORY B CELLS (B_{REGS})

The concept of regulatory BL (B_{REGS}) was introduced by Bhan and Mizoguchi[11] based on the study with BL-deficient mice (B^-) crossed with mice that did not express TCRα ($TCR\alpha^{-/-}$) and spontaneously developed chronic colitis. The mice that were doubly affected ($B^-/TCR\alpha^{-/-}$) presented earlier disease and more marked inflammation than the $TCR\alpha^{-/-}$ animals, but which had BL. It was surprisingly demonstrated that in this experimental model, it was possible to minimize the effect of colitis in the $B^-/TCR\alpha^{-/-}$ group, by the administration of purified immunoglobulins and colonic epithelial anti-cell autoantibodies. The observed improvement was accompanied by the increase in the clearance of apoptotic cells, favored by the autoantibodies produced by the LB_{REGS}.

Other studies in experimental models demonstrated the existence of cells with immunomodulatory capacity in the BL pool which, in a chronically inflamed environment, differentiate in a phenotype with high expression of CD1d, and capacity to produce IL-10 and suppress the inflammatory response.

The LB_{REGS} depend on antigen interaction and stimulation through CD40L and B7 molecule, but once activated, they produce IL-10 and TGF-β, suppressing the activation and differentiation of $LTCD4^+$, $LTCD8^+$ and NK/T cells, inhibit the activation of dendritic cells and stimulate the differentiation of TL regulators. When there is contact between the LB_{REGS} and naïve TL, there is inhibition of the TL activation and differentiation into TH1 (Figure 5).[12,13]

The study of LB_{REGS} in animal models clearly demonstrates the regulatory role of these cells in different diseases. The demonstration of the existence of these cells in humans will be very important for the understanding of autoimmune diseases. More than understanding the diversity of effects of anti-inflammatory cytokines that guarantee the functions of LB_{REGS}, their study reinforces the evidence of the physiological production of autoantibodies with a protective purpose.[11,12,13]

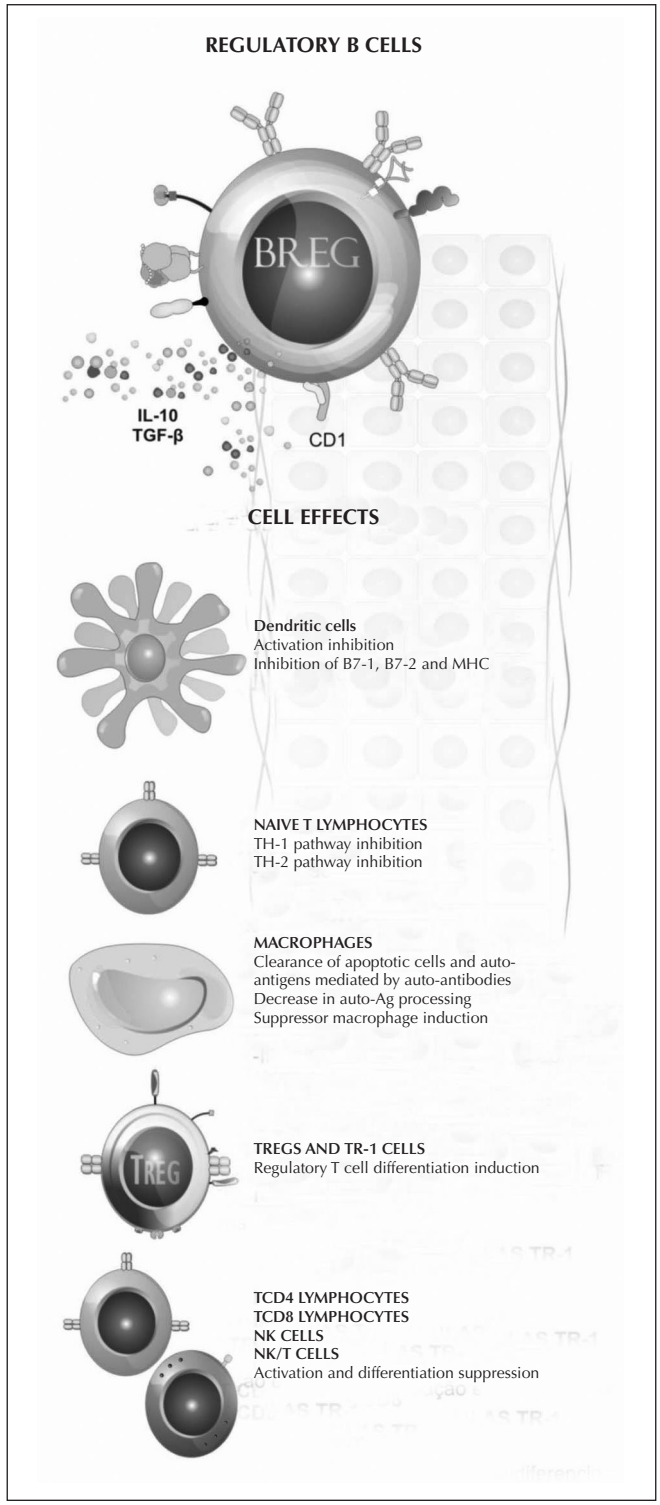

Figure 5. Regulatory mechanisms of the immune response induced by regulatory B cells (BREGs). The suppression mechanisms acting on different cell targets depend on the secretion of IL-10 and TGF-β.

SPECIAL SUBTYPES OF EFFECTOR B LYMPHOCYTES

In addition to conventional BL (B2), there are other BL subtypes, such as B1 and BL from the marginal zone (MZ-B) of the spleen, which act as true sentinels in specific anatomic sites, such as the peritoneal cavity, pleural cavity and peripheral zone of the spleen, respectively. These subtypes are very similar to the Tγδ lymphocytes and the NK/T lymphocytes, due to their location in specific zones and effector function, with the use of highly conserved receptors, but capable of recognizing a limited variety of pathogens. Both subtypes function as cells of the inate and adaptive immunity. Since they belong to both, innate and adaptive immune systems, theses cells probably represent a primitive and conserved form of immunity.[1,2,14]

B1 LYMPHOCYTES

The B1 lymphocytes constitute a distinct subpopulation of the conventional BL (B2). They have a strong self-renewal capacity and are found mainly in the pleural and peritoneal cavities and, at lower amounts, in the spleen. They are responsible for the production of most natural IgM and also of most IgM class antibodies, including antibodies against TL, dsDNA, erythrocytes and antibodies that recognize common bacterial constituents. It has been suggested that B1 lymphocytes constitute an evolutionary relic, originated from a primitive lineage of innate immunity that became the lymphoid cell of the adaptive system, but still maintains many characteristics of a cell from the innate system. The B1 lymphocytes seem to represent the first line of defense against systemic infections by viruses and bacteria and are of utmost importance for the body's homeostatic balance. They produce poly-reactive antibodies with low affinity, which are important for removal of old cells and/or cells that underwent cell stress and for protection against the development of autoimmune diseases and atherosclerosis.[15,16]

The B1 lymphocytes express low levels of the B220/CD45R, CD19, Mac-1 and IgD surface markers, high levels of surface IgM and are subdivided in two main groups: B1a and B1b. The B1a lymphocytes express the CD5 surface molecule at intermediate levels, whereas the B1b lymphocytes are $CD5^-$ and capable of differentiation into cells with myelomonocytic characteristics, with function and morphology similar to the macrophage. It was verified that the secretion of IL-10 by the B1 lymphocytes modulates the phagocytic activity of the macrophage *in vitro* and the presentation of antigens in the presence of IL-10 induces the tolerance or differentiation of TL into suppressor cells, indicating a possible immunomodulating role of this cell population.

B Lymphocytes from the marginal zone

The main populations of effector BL from the spleen are the BL from the marginal zone (MZ-B) and the follicular BL (FC-B). The MZ-B are specialized populations of BL located in the peripheral or marginal regions of the spleen, a region called splenic sinusoid. The macrophages from the marginal zone are also located in this site, which together with the MZ-B represent the first line of rapid defense against particulate antigens from the bloodstream. However, phenotypically, these lymphocytes are very similar to the conventional BL, also being classified in the B2 lymphocyte group.[14]

The FC-B, located in the lymphoid follicles, act at a more advanced stage of acquired response, generating memory BL and plasmocytes through the T-dependent humoral response, which has been addressed before in this review.[14]

The MZ-B, as well as the B1 lymphocytes, have a limited repertoire of antigen recognition, normally auto-reactive. However, although they are auto-reactive, they are also poly-reactive, with important specificity for both the removal of cell debris and the recognition of bacterial and viral antigens. Another important characteristic of these cells is their capacity to generate the so-called T-independent humoral responses, that is, they can respond avidly to antigen stimuli and differentiate into antibody-secreting cells without the help from a TL. Together, MZ-B and LB1 are part of the so-called natural-memory responses, as they rapidly generate effector cells at the initial stages of the immune response.[14]

T LYMPHOCYTES

The pre-T cells enter the thymic cortex through the arteries and during the process of selection and maturation, they migrate towards the bone marrow, from where they go into the circulation. The process of TL maturation involves the expression of a functional T cell receptor (TCR) and the co-receptors CD4 and/or CD8.[1]

The TL only recognize processed antigens, presented by MHC molecules on the surface of an antigen-presenting cell The TCR is expressed in the membrane of the TL in association with complex called CD3, which consists of five different proteins from the immunoglobulin family. The TCR is responsible for the recognition of the peptide-MHC molecule complex and the CD3, for the subsequent cell signaling.[1]

T CELL RECEPTOR (TCR)

The TCR is formed by two peptide chains of the immunoglobulin superfamily, with a variable region and a constant one, formed from gene segments which, during the maturation, under go a recombination process similar to the BCR. In around 95% of the circulating TL, the TCR is formed by the α and β chains. A small percentage of TL presents a TCR that consists of γ and δ chains. The α and δ chains are formed by the combination of three types of segments (V, J and C) and the β and γ chains, by four types of segments (V, D, J and C). There are approximately 70 deferent Vα segments, 60 Jα segments and only one Cα. For the β chain, there are approximately 50 Vβ segments, followed by two sets that consist of one Dβ, 6-7 Jβ and one Cβ. The γ and δ chains present lower variability.[17]

The great diversity of mature TL repertoire is generated by the process of somatic recombination, in which a given gene V, among the several possible ones, binds to a given gene J, or DJ combination. The diversity of the potential repertoire of the TL is approximately 10.[16] The recombination between the different segments is mediated by enzymes expressed only during the phase of lymphocyte maturation.[1,17]

T LYMPHOCYTE MATURATION

The process of TL maturation occurs in sequential phases that involve the somatic recombination and the expression of the TCR, cell proliferation, expression of the CD4 and CD8 co-receptors as well as positive and negative selection induced by self-antigen presentation by cells from the thymic stroma.[17]

Initially, there is the rearrangement of the genes in the β chain of the TCR and subsequently, of the α chain. The thymocytes, or immature lymphocytes, start to express low levels of CD4 and CD8 on the surface and therefore, are double-positive. At this phase, the thymocytes migrate towards the thymic medulla and enter in contact with the self Ag presented by the epithelial cells of the thymic stroma. Only those that bind to the MHC/Ag complex with adequate affinity receive stimulation to survive (positive selection). The thymocytes of which TCR do not present affinity for the self MHC undergo apoptosis due to the lack of stimulation (death by neglect).[17] The interaction with MHC molecules class I or II determines the differentiation of the thymocyte into $CD8^+$ or $CD4^+$ TL, respectively. Continuing the maturation process, the αβ thymocytes that survived the positive selection and express only CD4 or CD8 enter in contact in the medulla with dendritic cells and macrophages, extremely efficient antigen-presenting cells (APCs), that present self-Ag associated with MHC. The immature thymocytes that interact with high affinity with these complexes die due to apoptosis (negative selection). The cells that survive become mature TL, ready to leave the thymus and exercise their action in the periphery. Only around 5% of the cells that enter the thymus become mature TL[17] (Figure 1).

This process of thymic education aims at guaranteeing that the circulating TL are tolerant to the self-Ag, but capable of recognizing Ag that are foreign to the body, when presented by the self-MHC. However, the central mechanisms of tolerance are not absolute, as the auto-reactive TL can be found in the periphery. Among other mechanisms of peripheral regulation are different populations of regulatory TL, which act in the periphery preventing the development of autoimmunity.[17]

TL EFFECTORS

There are several subtypes of TL effectors. Classically, the two main subtypes are helper (Th) and cytotoxic TL, which present a αβ TCR receptor associated with and the co-receptor molecules, CD4 or CD8, respectively. The CD4 TL (Th) are responsible for the orchestration of other cells of the immune response in the eradication of pathogens and are also very important in the activation of BL, macrophages or even CD8 TL. The CD8 TL are involved mainly in antiviral responses and also have anti-tumor activity. Both subtypes present a very important role in the control of intracellular pathogens. Other subtypes of TL effectors, found especially in skin and mucosal barriers, are the TL γδ. These cells are important in the immune responses against antigens commonly found in these anatomic sites and represent a link between the innate and adaptive immune responses (Figure 6).[18]

Auxiliary CD4 T Lymphocytes (Th)

The T-helper (TH) lymphocytes are functionally subdivided by the pattern of the cytokines that they produce. During the stimulation supplied by an APC, a Th0 precursor lymphocyte can become a Th1, Th2 or Th17 lymphocyte, depending on the environment of present cytokines. Although morphologically indistinguishable, these cells present distinct patterns of secreted cytokines and, consequently, different effector responses[19] (Figure 6).

Th1 Lymphocytes

The Th1 lymphocytes produce large quantities of IL-2, which induce the proliferation of TL (including self-CD4-TL in autocrine form) and also induces the proliferation and increases the

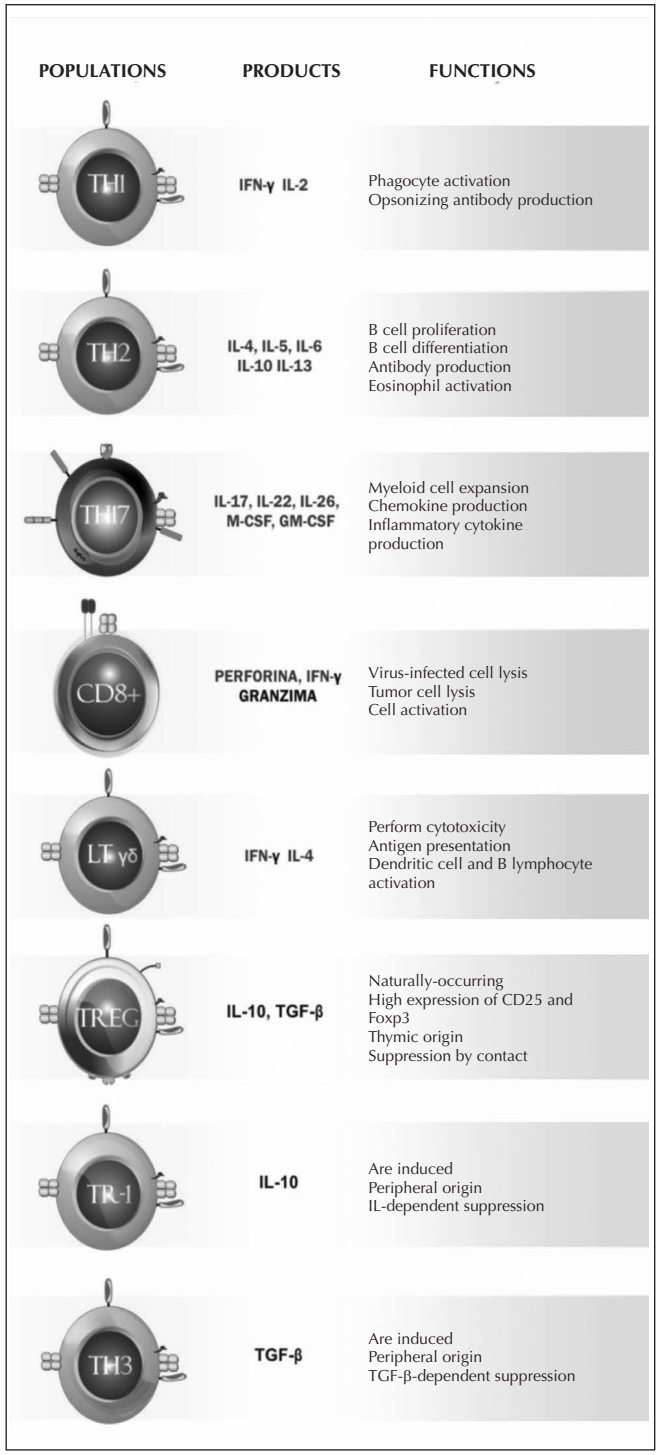

Figure 6. General characteristics of T cells, with emphasis on auxiliary T lymphocytes (subtypes TH1 and TH2), TH17 cells, cytolytic T lymphocytes, Tγδ lymphocytes and natural (TREGs) and induced regulatory T cells (TR-1 and Th3).

cytotoxic capacity of the CD8 TL. The other cytokine produced in large amounts by the Th1 lymphocyte is INF-γ, a cytokine that is very important in the activation of macrophages infected with intracellular pathogens, such as mycobacteria, protozoa and fungi, which also present a relevant role in the activation of CD8 TL.[18] Patients with immunodeficiency syndrome in whom the INF-γ receptor is absent, present severe infections by mycobacteria.[20] There is a positive feedback cycle in the action of the INF-γ on other Th0 lymphocytes, inducing their polarization towards the Th1 differentiation pathway and inhibiting the Th2 pathway.[19] The Th1 response is essential for the control of intracellular pathogens and possibly contributes to the pathogenesis of autoimmune rheumatic diseases, such as rheumatoid arthritis (RA) and multiple sclerosis (MS). However, in recent years, a new subpopulation of TL, the Th17 lymphocytes, has been considered responsible for the physiopathological process of these diseases.[21]

Th2 Lymphocytes

The second very important population of Th lymphocytes is the Th2 lymphocytes, which produces IL-4, IL-5, IL-6 and IL-10, favoring the production of antibodies.[18] The Th2 responses are associated with allergic diseases and infections by helminthes, as the IL-4 induces the change of immunoglobulin class in B lymphocytes into IgE and the IL-5 induces the production and activation of eosinophils. Similarly to INF-γ, IL-4 also promotes the positive feedback for the Th2 pathway and suppresses the Th1 pathway. In situations of immediate hypersensitivity, such as allergic diseases, the therapy aims at the Th2 immune desensitization and induction of allergen-specific Th1 responses.[18,19] In diseases known to be caused by Th1 lymphocytes, the Th2 cytokines have been considered to be protective, and therefore, the search for the alteration in the immune response pattern from Th1 to Th2 has been broadly studied, aiming at improving or re-establishing immunological tolerance. However, this bipolar paradigm has been recently reformulated, due to the recognition of new TL subtypes, mainly Th17 cells.[18,19].

Th17 Lymphocytes

The Th17 lymphocytes represent a new subtype of TL effectors important for the protection against infection by extracellular microorganisms. They were originally described in experimental models of autoimmune diseases, such as autoimmune encephalitis and collagen-induced arthritis, which were formerly believed to be mediated predominantly by Th1 cells. This new Th differentiation pathway started to be elucidated with the discovery of the IL-23 cytokine, which, together with IL-1

and IL-6, can lead to the development of autoimmune diseases in murine models due to its important pro-inflammatory role and as inductor of differentiation and activation of Th17 lymphocyte.[21] The Th17 lymphocytes produce IL-22 and IL-26 cytokines and cytokines from the IL-17 family. The latter are potent inducers of inflammation, inducing cell infiltration and the production of other pro-inflammatory cytokines.[21,22]

The deregulated production of IL-17 is associated with several autoimmune conditions, such as multiple sclerosis, inflammatory intestinal disease, psoriasis and lupus. In patients with RA, elevated levels of IL-17 were found in the synovia, where it functions as an important factor of osteoclast activation and bone resorption.[21,22]

The Th17 differentiation pathway is antagonized by Th1 and Th2 cytokines and, in some experimental models of autoimmunity caused by Th17 lymphocytes, the Th1 and Th2 cytokines have shown to be protective. The exact understanding of the mechanisms of Th polarization in humans is crucial for a better understanding of the physiopathological mechanisms of chronic inflammatory diseases and the possible development of more effective immunotherapy approaches.[21]

CYTOTOXIC TL (CD8)

The CD8-TL recognizes intracytoplasmic antigens presented by MHC molecules class I, which are expressed by practically all nucleated cells. Cells infected by viruses and tumor cells are normally recognized by the CD8-TL.[18] After adhering to the target-cells presenting an antigen associated with the MHC and adequate co-stimulation, the CD8-TL proliferate and, at a subsequent contact, can eliminate by cytotoxicity any cell that presents this specific antigen, regardless of the presence of co-stimulatory molecules. The CD8-TL induce the apoptosis in the target cell through the action of perforins and granzymes and can also lead to apoptosis through the expression of the Fas L receptor (CD95), which interact with the Fas molecule in the target cells.[18]

γδ TL

A small population of peripheral TL has TCR with limited diversity, consisting of γδ chains. These cells differ from the αβ TL, as their TCR can recognize antigens even in the absence of presentation by the MHC molecule, being then considered true sentinels of the body.[23,4]. The γδ TL also present immunological memory and respond more vigorously at a second antigen contact. The γδ TL exercise their effector functions in different ways, by cytotoxicity, for instance (a primary characteristic of CD8-TL). Therapeutic studies have explored this cytotoxic characteristic against tumor antigens. The γδ TL also have a auxiliary function, releasing cytokines such as INF-γ (Th1) or IL-4 (Th2) and can act as efficient APC, as they have a high capacity to present antigens to the αβ TL, mediating their activation and proliferation. The γδ TL are also capable of activating dendritic cells and BL, thus amplifying both cell and humoral immune response. These cells are commonly found in the first lines of defense of the body, such as mucosal and skin barriers, where they act as true sentinels that recognize molecular patterns, recognizing and presenting antigens, responding to them and also contributing to cell activation and proliferation in the immune system.[23]

The γδ TL opened the discussion on one of the contemporary dogmas of immunology, the division of the immune system in innate and adaptive. The innate immune system responds rapidly when it recognizes molecular patterns derived from pathogens and is capable of inducing adaptive immune responses through the release of cytokines and antigen presentation. In contrast, the adaptive response acts slowly during an antigen contact, but develops immunological memory and responds rapidly and vigorously at a subsequent contact (Figure 4). The γδ TL can be included in both arms of the immune response, as they exercise the functions of cells from both the innate immunity and the adaptive response. Its participation in the two types of response suggests that these cells represent a primitive and conserved form of immunity.[23]

REGULATORY TL

A great deal of evidence has demonstrated the importance of the different populations of regulatory TL in the maintenance of immunological auto-tolerance and in the control of autoimmune responses. Thus, there is great interest in the study of these cells and their potential use in the treatment of autoimmune diseases.[25]

The cells with immunoregulatory function present as basic characteristic the capacity to produce immunosuppressive cytokines, such as IL-4, IL-10 and TGF-β. They act in a complex network of regulatory mechanisms aimed at guaranteeing the modulation of immunological responses in the presence of several antigens from infectious agents, tumors, alloantigens, autoantigens and allergens. Among the TL with regulatory function are the naturally-occurring regulatory TL (T_{REGS} $CD4^+CD25^+$), described by Sakaguchi *et al* (1995),[26] the T_{R1} lymphocytes that produce IL-10 and suppress the development of some TL responses *in vivo* and the Th3 lymphocytes, capable

of preventing the development of autoimmune diseases in the presence of TGF-β production (Figure 6).[27] Other TL with regulatory function are the γδ TL, $CD8^+Qa\text{-}1^+$ cells, $CD8^+CD28^-$ ($CD8^+$ T_R) TL, the NK/T cells and the double-negative TL.[23,28,29]

NATURALLY-OCCURRING REGULATORY T LYMPHOCYTES - T_{REGS}

The T_{REGS} cells represent 5 to 10% of the total $CD4^+$ TL in peripheral blood and can also be isolated from the thymus. The T_{REGS} present in the thymus are naive cells, which, when go to the periphery, become activated and acquire a memory phenotype.[25]

The T_{REGS} present high levels of CD25 ($CD25^{HIGH}$ or $CD25^{BRIGHT}$) and express CD45RO, CD62L, CD122, HLA-DR, CD69, CD71 and the glucocorticoid-induced TNF α receptor (GITR), among others. They also express the transcription factor *Foxp3,* found predominantly in thymic and peripheral T_{REGS}, which seems to be very important for the development and function of T_{REGS} in both mice and humans.[25] Patients with a mutation in the *FOXP3* gene present the so-called IPEX (Immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome, an autoimmune disorder that affects multiple organs with the development of allergy and intestinal inflammatory disease. Apparently, these patients present impaired T_{REGS} development and consequent defect in the suppressor function, leading to a state of hyperactivation of TL, which become reactive against autoantigens, intestinal commensal bacteria or innocuous environmental antigens, developing autoimmune polyendocrinopathy, intestinal inflammatory disease or allergy, respectively.[30]

There is an interest in the study of regulatory TL due to their key role in the maintenance of mechanisms of auto-tolerance and immune response regulation.

In addition to the autoimmune disorders associated with the absence/deficiency of its function, there is interest regarding their study in situations of exacerbation of the immune response, such as in transplants, graft-versus-host disease (GVHD), in allergic processes and other conditions. In these situations, the approach would be to stimulate the number and the function of these cells from the administration of drugs, cytokines, co-stimulatory molecules or other elements that can potentially attain this finality.[25]

Double-Negative Regulatory T Lymphocytes

Most CD3+ TCRαβ TL also express CD4 or CD8 molecules. However, a new subpopulation of TL regulators with αβTCR receptor, but CD4-CD8- was described by Strober *et al*, in 1989.[29] The peripheral naturally-occurring double-negative TL with suppressor activity, called DN T_{REG} cells, are a subpopulation of cells present in lymphoid and non-lymphoid tissues. There is a small number of them, representing 1%-2% of the peripheral TL in humans, but that number can be increased in some autoimmune diseases. The DN T_{REG} cells produce predominantly INF-γ , TNF-α and a small quantity of TGF-β and are capable of suppressing the allogeneic and xenogeneic immune response mediated by CD4+ and CD8+ TL, as well as the response to self-antigens.[29,31]

The stimulus of the DN T_{REG} cells by specific antigens through the TCR is important for the development of the regulatory activity. The DN T_{REG} cells are capable of acquiring allopeptides from the peptide-MHC complexes of APC and presenting these peptides on their surface. This presentation allows the specific interaction of the DN T_{REG} cells with TL effectors reactive to the alloantigen, which, after this contact, progress to cell death.[32] Similarly to the CD4+CD25+ T_{REG} cells, the suppression by the DN T_{REG} cells also requires the cell-to-cell contact.

In the autoimmune lymphoproliferative syndrome (ALPS) type Ia, the patients present an accumulation of $CD3+TCR\alpha\beta^+$ DN T cells in the periphery, as a result of a defect in apoptosis. It is believed that the increase in the number of DN cells in these cases lead to the loss of CD4 and CD8 molecules by the senescent TL, which fail to die by apoptosis.[32] In this specific case, there is no evidence that these cells have a regulatory activity.

Recent data indicate that the DNT_{REG} cells can be increased in some autoimmune diseases, maybe as an attempt to control the effector cells. The complete characterization of the human DNT_{REG} cells might have important implications in the understanding and treatment of autoimmune diseases and the rejection of transplants[32].

$CD8^+CD28^-$ Regulatory T Lymphocytes

The cells immunophenotypically characterized as $CD8^+CD28^-$ recognize specifically antigens expressed mainly through APC in association with MHC class I, but are not activated as they lack the CD28 receptor. Instead, they suppress the proliferative response of alloreactive CD4+ TL in the surroundings. This suppressor effect is not mediated by cytokines, but requires cell-to-cell interaction between CD4+ Th lymphocytes, $CD8^+CD28^-$ TL suppressors and APC presenting allogenic antigens.[33]

The action mechanism of the $CD8^+CD28^-$ regulatory cells is not completely elucidated and few studies have characterized the function of these regulatory cells *in vitro*.

NK/T CELLS

Practically all cells that express αβTCR are MCH-restricted, that is, they recognize the antigen only in association with self-MHC molecules and express the co-receptors CD4 or CD8. A small population of TL express markers found in NK cells and are known as NK/T cells, which also seem to have an important role in the regulation of the immune response. The NK/T cells present expression of TCR with α chains with limited diversity. This TCR recognizes lipids bound to non-polymorphic molecules, called CD1, which are similar to the MHC class I.[34]

The NK/T cells seem to arise from the same precursor that originates conventional TL, but are positively selected after high-avidity interactions with glycolipids associated with CD1d molecules expressed by the epithelial or medullary cells of the thymic tissue.[35]

In spite of the limited repertoire, the NK/T cells present two different strategies to recognize pathogens. The first, observed in the recognition of Gram-negative bacteria, occurs through the signaling of Toll type receptors (TLR) by the LPS. The second occurs through the specific recognition of glycosyl ceramides present on the bacterial cell wall, presented by CD1d. The last signaling pathway guarantees the recognition of pathogens that do not present ligands for TLR on the cell wall.[36]

Due to the recognition of conserved glycolipids, both endogenous (iGbeta3) and exogenous, these cells are involved in allergic, inflammatory and tumor responses, as well as in autoimmunity, in addition to participating in the regulation of the immune response.[36]

PRESENTATION OF ANTIGENS AND TL ACTIVATION

The presentation of antigens to the TL starts with the antigen processing by the APC. The processing consists in the capture of the antigen, its proteolytic degradation to smaller fragments, transportation and accommodation of the antigenic peptides in the MHC molecule groove and finally its transposition from the MHC-peptide complex to the cell surface, for recognition by the TCR. The TL recognize the MHC-peptide complex via TCR, regardless of the cell compartment from where this antigen was obtained.[37]

Normally, the exogenous antigens, phagocyted or endocyted, are accommodated in MHC molecules class II, which interact with the TCR and the CD4 co-receptor on the TL surface.[1]

During the processing of intracellular antigens, protein molecules of cytosol, such as for instance the viral antigens, are integrated to the ubiquitin protein and directed to the proteasome, a catalytic unit capable of converting the cytosolic antigens in peptides. The peptides thus produced are conducted to the endoplasmic reticulum and associated with the MHC I molecules. The MHC I-peptide complexes are transported to the cell surface for posterior presentation to the $CD8^+$ T cells.[38]

For the activation of the TL to occur, after the recognition of the peptide by the TCR, a second signal is necessary, which is mediated by the interaction of several other co-stimulatory molecules present on the surface of the TL and the APC. Due to its importance in the regulation of the immune response, it is worth mentioning the co-stimulatory molecules that participate in the CD28-CD80 or CD28-CD86 interaction, which results in stimulation signals and the CD28-CTLA4 interaction, which promotes inhibitory signaling.[39,40]

FINAL CONSIDERATIONS

There are different populations of mature B cells that can be found in different anatomic sites, which very often present diversified functions. The B-1 and MZ-B cells seem to be pre-selected to react to antigens capable of generating T-independent responses, acting as innate-memory B cells. The follicular B cells act as precursors of the T-dependent immune responses and can undergo cell and molecular adaptations in response to antigen stimulation. As a result, there are responses mediated by long-lived mature plasmocytes, capable of synthesizing a substantial amount of antibodies that remain avid for several years. These T-dependent responses also create a B-cell memory compartment that does not secrete antibodies but responds vigorously to antigenic re-exposure.

The T cells have as one of their main characteristics, the auxiliary effector activity in the activation of other cell subtypes, mainly through the secretion of cytokines and the direct effector action on target cells, of which representative examples are the cytotoxic CD8 TL. Several subpopulations of TL, classified mainly by the pattern of secreted cytokines, have been described. The main effector subtypes are the Th1 lymphocytes (secretes INF-γ and IL-2), Th2 lymphocytes (secretes IL-4,IL-10 and IL-13) and Th17 lymphocytes (secretes IL-17, IL-21 and IL-22). Other subpopulations such as

NKT lymphocytes and γδ TL represent very heterogeneous populations regarding their functional capacity, sometimes acting as effectors and sometimes as regulatory lymphocytes. Regulatory lymphocytes are crucial for the control of practically all immune responses, acting on all cell subtypes of the innate and adaptive immunity.

The natural T_{REGS} originated in the thymus and the populations of regulatory TL induced in the periphery, including the $CD8^+Qa\text{-}1^+$, TL $CD8^+CD28^-$ and TL double-negative cells, are part of this pool of immunomodulating lymphocytes.

Not only the TL can exercise immunoregulation, but many times, during an effector response, the B cells can behave as both active effector cells and immunomodulating cells, called B_{REGS}. The latter are capable of controlling the magnitude of the humoral and cell response, bringing back the immune homeostasis and helping the maintenance of peripheral tolerance.

Hence, distinct compartments of antigen-specific T and B cells can be recruited in the effector response after a local or systemic stimulation. The molecular events necessary for their development, selection, migration and activation are still being investigated and the understanding of these pathways will allow, in the future, the specific manipulation of the cell and humoral effector pathways, facilitating the immunity against microorganisms and preventing diseases.

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