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Influence of Innate Immunity on Immune Tolerance

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Abstract
This review mainly focuses on the mechanisms of peripheral immune tolerance within the perspectives of innate immunity. Healthy immune response requires balanced interaction of the highly specialized elements of immunity within a harmony. Innate immunity supported by microbial pattern recognition receptors, physical anatomical barriers and soluble effectors stands as the first line of defense against non-self-antigens. Innate receptors recognize major classes of pathogens and trigger immediate immune/inflammatory responses. The decisive action has been the key issue in skewing of immune reactivity to a pathogen or to tolerate self- and non-self-antigens. Non-responsiveness to self- or to harmless foreign antigens with means of multiple mechanisms is known as immune tolerance; a non-inflammatory, non-proliferative and suppressive response linked to suppressor molecules as CTLA-4 and cytokines like IL-10, TGF-β and IL-35, and also to non-inflammatory blocking antibody isotypes as IgG_4. Regulatory cells ascertain both induction and maintenance of peripheral tolerance. Allergic diseases, autoimmunity and transplant rejection are the best illustrations of immune tolerance loss. Adaptive immunity responsible for both establishment and maintenance of a long-lasting immune responsiveness is mainly fine-tuned by actions of innate immunity. Better understanding of the relationship between innate immunity and immune tolerance is a prerequisite both for better understanding of pathogenesis of tolerance-related diseases and also for development of novel therapeutic options. **Conclusion.** Recent evidences point the important roles of innate immunity for establishment of immune tolerance with decisive role in central mechanisms. In a peremptory way, a '*balanced tolerance*' is essential for the survival.

Key Words: Antigen-Presenting Cells ▪ Cytokines ▪ Central Immune Tolerance ▪ Innate Immunity ▪ Peripheral Immune Tolerance.

Introduction

Immunity is comprised of specific and non-specific responses generated by several tissues, cells and their products, which functions elegantly within the customized networks of interactions. This system has to distinguish pathogens from external innocuous antigens and self-antigens to determine the intensity and the class of immune responses to be generated. Non-responsiveness, either to self or to non-self antigens, is defined as immune tolerance that is formed and maintained by combined means of the central and peripheral immune tolerance mechanisms. Dysregulation or loss of immune tolerance may lead to the development of allergic disorders such as asthma, allergic rhinitis (AR), atopic dermatitis and more, besides autoimmunity, recurrent abortions and transplant rejections (1).

This review mainly focuses on the mechanisms of formation and maintenance of peripheral immune tolerance within the perspectives of innate immunity.

Immune System and Immune Tolerance

Both arms of innate and adaptive immune systems work in a harmony to generate healthy im-

mune responses. Epithelial barriers, dendritic cells (DCs) and phagocytes, natural killer (NK) cells, innate lymphoid cells (ILCs) and elements of the complement system constitute the major components of innate immune system, while T and B lymphocyte functions, generated antibodies, in addition to responses of effector cells such as mast cells, basophils and eosinophils form the adaptive arm of the immune system. It has to be noted that the adaptive immune responses require both recognition of specific antigens and innate immune signals for custom-tailoring of immunity to a particular antigen.

The immune system is capable of tolerating both self- and non-self-antigens by means of the central and peripheral immune tolerance mechanisms. This is crucial for immune homeostasis. A well-balanced tolerance facilitates the survival and stabilizes the organism, while excessive tolerance may lead to inadequate defense against the invading pathogens and inadequacy to limit the development of chronic infections and cancer. On the other hand, loss of tolerance to external antigens may induce hypersensitivity reactions as seen in allergic disorders, while loss of tolerance to self-antigens may lead to the development of autoimmune diseases (2).

Pregnancy stand as the best model to exemplify how optimum and perfect the immune tolerance has to be. Maternal immune system has to tolerate fetal antigens until delivery in order to avoid fetus rejection (3). Accordingly, it will be appropriate to interpret these functions of the immune system in a way that 'a healthy immune response' can be defined as generating optimum and sufficient responses against pathogens and cancer cells, while tolerating self-tissues, harmless environmental antigens and the commensal microorganisms.

Establishment of immune tolerance is determined by two major mechanisms: the central and the peripheral immune tolerance mechanisms. During development, in the case of recognition of self-antigens by progenitors of either B cells in bone marrow or T cells in thymus, the silencing or deletion of these cells form central tolerance. Although a great percentage of developing cells are deleted in central lymphoid organs, a small number of T cells can still escape from thymic deletion and reach to periphery (4, 5). This is regulated by means of peripheral tolerance through apoptosis, anergy and T regulatory (Treg) cell action in the secondary lymphoid organs (6). Peripheral tolerance mechanisms are also responsible for healthy immune responses to environmental antigens, including allergens (6). It has been depicted that the ratio of the effector T helper (Th)2 cells to the Treg cells for the same particular antigen form the basis in determining allergic or healthy response in an individual (7). Other immune cell subsets with regulatory capabilities also contribute to tolerance, including NK cells, ILCs and B regulatory (Breg) cells (7-9). The ultimate response to a certain antigen could be defined by interactions between the environment and the immune system, where the innate immunity indispensably contributes to the determination of the response type to a particular antigen (10). Better understanding of innate immunity is essential for establishment of the links between innate and adaptive immune systems and their interactions with the environmental stimuli.

Innate Immunity Defining the Fate: Immune Tolerance or Immune Response

The decisive factors on the crossroads of responsiveness or unresponsiveness are being deeply investigated nowadays. Studies have revealed indispensable roles of Treg cells in the establishment and the maintenance of peripheral tolerance. Treg cells belong to a specific sub-group of $CD4^+$ T cells of the adaptive immunity. They express the forkhead box P3 (FoxP3) transcription factor, and have suppressive capacities by production of cytokines such as interleukin (IL)-10, IL-35 and transforming growth factor (TGF)-β, and by surface expression of suppressor molecules as cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) (11-13). Treg cells contribute to allergen-specific tolerance and can be induced as a consequence of allergen-specific immunotherapy (AIT) (14, 15). In parallel with the mechanisms of allergen tolerance, a group of self-peptide specific $CD4^+$ T

cells can be converted to Treg cells under certain conditions and contributes to regulation of autoimmune responses in periphery (16). At first look, adaptive immune system which is responsible for the establishment and maintenance of a long-lasting antigen-specific tolerance is orientated with the early actions of innate immunity. In addition, early innate immunity is important to define the intensity and class of immune tolerance. For better understanding of the role of innate immunity in immune tolerance development, contribution of cellular players of innate immunity and their cytokines, as well as other factors will be discussed throughout this review.

Innate Cytokines Acting on Tolerance

Almost all immune cells like DCs, $CD4^+$ and $CD8^+$ T cells, B cells, NK cells, and ILCs have functional subsets, all of which are categorized according to their distinct surface receptors and cytokine secretion patterns. Cytokines such as IL-1β, IL-6 and tumor necrosis factor (TNF)-α exert proinflammatory properties, while IL-10, IL-27, IL-35 and TGF-β are known for their suppressive effects (17).

Innate Immune Functions of Interleukin-10 is crucial in establishment and maintenance of immune tolerance. IL-10 is the most widely investigated and the best-known suppressive cytokine to date. IL-10 is mainly produced by monocytes, Treg cells, Breg cells, a small fraction of NK cells, macrophages, as well as by DCs and mast cells (17). IL-10 has a direct innate effect, which suppresses antigen presentation and development of adaptive immunity. IL-10 limits production of proinflammatory cytokines such as TNF-α, IL-6 and IL-8, inhibits conversion of DCs from monocytes and restricts presentation capacity of antigen presenting cell (APC) by down-regulation of surface molecules as major histocompatibility complex (MHC)-II and CD86. Moreover, IL-10 controls T cell responses by restricting IL-2, IFN-γ and granulocyte-macrophage colony stimulating factor (GM-CSF) productions from T-cells and limits T cell proliferation (18, 19). IL-10 induces expression of a number of molecules with tolerogenic properties in human monocytes, macrophages and DCs, as well. Innate inflammatory cytokines like IL-1 contributes in tissue recruitment of leukocytes in response to an injury, by increasing the expression levels of adhesion molecules; intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 on endothelial cells. In contrast, IL-10 down-regulates the expression of both of these adhesion molecules, which results in decreased migration of proinflammatory cells at the site of tissue injury and in turn limits inflammation (20).

Studies highlighted the importance of IL-10 in maintenance of tolerance. DC-derived IL-10 production observed in respiratory tract of healthy individuals was found to be diminished in patients with AR and asthma (1). In the gastrointestinal tract, IL-10 is produced both by intestinal macrophages and by Treg cells and contributes to mucosal homeostasis. Loss of IL-10 receptor in APCs was related to epithelial damage in the intestines (21). Generation of peripheral tolerance in allergic individuals occurs by induction of Treg cells either by natural high dose allergen exposure, as seen in beekeepers (22) and in food allergic children (23), or induced by allergen-specific immunotherapy (24). Induced Treg cells produce high amounts of IL-10 and bring forth the above mentioned effects (25).

There are several instances, particularly in infections and tumors in which IL-10 detrimentally contributes to immunity. During leishmania infection, partial blockade of IL-10 in localized cutaneous leishmaniasis was found to be beneficial for patients (26). In addition, IL-10 contributes to impaired bacterial clearance in *Mycobacterium tuberculosis* infection (27). As revealed in a meta-analysis, increased serum levels of IL-10 in cancer patients with solid tumors or hematological malignancies was found to be predictive for a worse outcome (28). Taken together, this important immune regulatory cytokine has two opposite facets, while maintaining tolerance, the organism could become prone to both infections and cancer. Therefore, the general immune suppressor activity of IL-10 becomes detrimental in immune responses to infectious agents and tumors.

Transforming Growth Factor-Beta is known for its pleiotropic properties. It is produced by assortment of cells including lymphocytes, macrophages, eosinophils, epithelial cells and fibroblasts, and it influences almost all cell types due to widely expressed TGF-β receptors (17). TGF-β is among the most important cytokines produced by Treg cells, with well-known suppressive effects (29). TGF-β has anti-inflammatory effects on innate cells like NK cells, monocytes and macrophages (30). In addition, TGF-β influences adaptive immunity by suppression of Th1 and Th2 type $CD4^+$ T cells. It contributes to differentiation of Th17 and Th9 cell subsets from naïve T cells and plays a role in the induction of conventional Treg cells and also by particularly upregulating the expression of their signature transcription factor; FoxP3 (31). In airway diseases like asthma, chronic rhinosinusitis and allergic rhinitis, TGF-β contributes to both inhibition of T-cell responses and inflammation, and also to tissue repair and remodeling processes (32). In all of these diseases, a chronic mucosal wound that needs to be healed is a part of the pathogenesis and TGF-β is the key cytokine that comes to the scene for this activity. Immune suppression affects various cells around the wound and induces fibroblast proliferation at the same time. This effect cannot lead to full recovery, but causes a continuous inadequate healing process that is recognized as remodeling. TGF-β is well known with its contribution to tissue remodeling in allergic diseases, especially in allergic asthma, with hallmarks such as thickening of basal membrane, increased smooth muscle mass in bronchi, increased mucus secretion due to goblet cell hyperplasia and infiltration of immune cells to the inflamed area, all of which could contribute to a worse disease prognosis (33). TGF-β2 is overexpressed in fibroblasts of severe asthmatics, which leads to proliferation of epithelial cells (34). Though well-known as an immune regulatory cytokine, TGF-β has pleiotropic effects, therefore this cytokine could be responsible for irreversible changes in certain disease conditions. More studies are required to elucidate the contribution of TGF-β both in immune regulation and in remodeling and also in the balance between these key events.

Interleukin 27 belongs to IL-12 family of cytokines with both pro- and anti-inflammatory properties, which are attributed to plasticity and cross-talk of cytokine subunits in addition to shared utilization of receptors with other cytokines (35, 36). IL-27 is expressed by myeloid cells like monocytes, macrophages and DCs, while its receptors are expressed widely on leukocytes including NK cells and lymphocytes (37). IL-27 plays important roles in Th1 differentiation via controlling IL-12 responsiveness (38). Intracellular glutathione redox status in monocyte-derived DCs regulates production of IL-27, which contributes to Th-1-polarizing effects of DCs (39). Anti-inflammatory functions of IL-27 can be mediated by induction of IL-10 production in $FoxP3^-$ $CD4^+CD25^+$ T regulatory type-1 (Tr1) cells (40, 41), and IL-10 production of $CD4^+$ T cells under Th1 and Th2 polarizing settings can be augmented by IL-27 (41). On the other hand, IL-27 suppresses the development of $FoxP3^+$ inducible regulatory T cells (iTregs) and Th17 cells via differential effects on STAT1 transcription factor (42). Briefly, IL-27 regulates diverse Th cell subsets, limits excessive activation of T cells and may have potential applications both in AIT and controlling of autoimmune diseases (43).

Interleukin-35 is a recently discovered cytokine of IL-12 family with immune regulatory roles. It is produced by Breg, Treg, epithelial and vascular endothelial cells as well as by immature DCs (44-47). A minor Treg cell group, with capability of secreting IL-35, is dependent on the expression of FoxP3 (48). Treg-derived IL-35 limits Th1, Th2 and Th17 responses and controls inflammation (49, 50). IL-35 has been proposed to act on limitation of an already established inflammation and thus may exert regulatory effects in autoimmune diseases like rheumatoid arthritis and systemic lupus erythematosus. Increased levels of IL-35 in autoimmune disorders may indicate a compensatory attempt to down-regulate the inflammation (51). Contribution of IL-35 in the pathogenesis of allergic diseases is questioned. Murine models of asthma have pointed out suppressive roles of IL-

35 on airway hyper-reactivity and inflammation (52). A recent study in patients with grass pollen allergy asserted IL-35 as a novel immune regulator induced following grass pollen sub-lingual immunotherapy (53). On the other hand, this regulatory cytokines' potential detrimental effects within the frame of tumor immunology have been revealed. IL-10 and IL-35 produced by Treg cells in tumor micro environment cooperatively regulate anti-tumor immunity by promotion of T cell exhaustion, could underlie a tumor immune evasive strategy and form a potential immune resistance mechanism to tumor immunotherapy (54). Taken together, future studies are required for determination of properties of IL-35 to be utilized in therapeutic interventions.

Interleukin-37 is a member of IL-1 family of cytokines expressed in NK cells, monocytes, stimulated B cells and epithelial cells. Low physiological expression of IL-37 is upregulated by inflammation and anti-inflammatory roles for IL-37 have been proposed. Accordingly, IL-37 inhibits both innate and adaptive immune responses, decreases production of pro-inflammatory cytokines from DCs and macrophages, and contributes to a number of chronic autoimmune and inflammatory disorders, cardiac diseases, as well as cancer (55). Diminished production of IL-37 in human peripheral blood mononuclear cells of allergic asthmatics in comparison to their healthy counterparts have been reported. The same study also revealed remission of airway inflammation, cytokine production and mucus secretion in a mouse model of asthma (56). Recently, a study investigating the contribution of IL-37 to allergic inflammation has revealed diminished airway hyper-reactivity and pulmonary eosinophilia in a mouse model of house dust mite-induced asthma, in response to IL-37 (57).

Innate Immune Cells and Immune Tolerance

Antigen-presenting cells including DCs, macrophages and B cells contribute to the initiation of immune responses and are therefore important sentinels of immunity. DCs with superior antigen-presenting capacity engulf, phagocyte and process antigens into peptide fragments and then present on their surfaces to different subsets of T cells via MHC molecules. During antigen presentation, co-stimulatory signals to T cells are provided by molecules expressed as CD40, CD80 and CD86, and they also provide polarizing cytokines to T cells, all of which act together to define the fate of the T cell responses to be either on effector or regulatory side (58). Therefore, it may be hypothesized that the development of tolerance versus reactivity may be initially driven by DCs that are capable of forming links between innate and adaptive immunity with vital roles in the orchestration of immune responses (59). Maturation status of DCs together with antigen presentation levels define the outcome either to be tolerogenic or immunogenic (Figure 1). DCs at steady state conditions express low-levels of maturation markers such as MHC-II, CD40, CD80 and CD86, and inflammatory cytokines, which can be termed as immature and are known to promote induction of tolerance. Following activation by antigens, migration to the lymph nodes together with the initiation of DC maturation and up-regulation of these maturation markers are essential for T cell activation. On the other hand, some commensal microorganisms as well as tonic inflammatory signals are not sufficient to induce high-level expression of maturation markers in steady-state DCs. Therefore, these cells do not acquire immunogenic capacities (60). Diminished expression of MHC-II together with down-regulated levels of co-stimulatory molecules on DCs brings out their tolerogenic properties, which in turn leads to T cell suppression, a requisite for establishment of tolerance (60).

The fate of immature dendritic cells (DCs) following antigen uptake is determined by the innate immune response related factors present in the micromilieu. A DC uptaking the same antigen has a capacity to act in opposite ways according to the surrounding conditions. Under inflammatory conditions, the immature DC migrating from site of injury/inflammation to the lymph nodes will be initiated and during DC maturation, expression levels of MHC-II molecules together with the co-stimulatory molecules: CD80 and CD86 and in-

flammatory cytokines; IL-1, IL-12 and IFN-γ will be upregulated. The net results are increased antigen presenting cell (APC) capacity, which is sufficient for initiation of adaptive T-cell responses, and production of cytokines relevant with pathogen type that will drive induction of different Th subsets such as Th1, Th2 and Th17. The aberrant activation may have pathogenic consequences as seen in autoimmunity, allergic diseases and graft rejection. On the other hand, during antigen uptake, if there is no inflammation, if cytokines with known suppressive effects such as IL-10 and TGF-β are present, or if TLR2-, TLR7- or TLR9-triggering PAMPs are present in the micro milieu, then immature DCs will be converted to tolerogenic DCs characterized with low expression levels of CD80 and CD86 together with low MHC-II. These cells can produce TGF-β and IL-10, and they can express suppressive molecules such as immunoglobulin-like transcript (ILT)-2, ILT3 and ILT4. Vitamin D3 as well as retinoic acid and indoleamine 2,3-dioxygenase (IDO) contribute to the induction of tolerogenic DCs. These DCs have capacity to induce T regulatory cells, which have suppressive effects to prevent pathologies. (Blue arrows indicate tolerogenic conditions, while red arrows indicate inflammatory conditions.)

A great number of experimental studies have investigated the contribution of DCs to the establishment and maintenance of immune tolerance. A mouse model revealed that both production of IL-10 and down-regulation of MHC-II expression were important factors that contribute to the tolerance induced by IL-10-differentiated DCs (61). IL-10, produced by DCs or other cells, inhibits gene transcription of pro-inflammatory cytokines together with down-regulation of MHC-II and co-stimulatory molecule expressions in activated DCs. This results with restricted presentation of antigens to T cells and ends up with decreased T cell activation (62). Together with these well-known facts, a recent experimental study revealed upregulation of FoxP3 in mouse DCs under certain conditions, which could possibly have a potential application for future therapeutic approaches (63).

Inhibition of monocyte-conversion to inflammatory DCs by IL-35 was shown to ameliorate ovalbumin-induced allergic inflammation in mouse

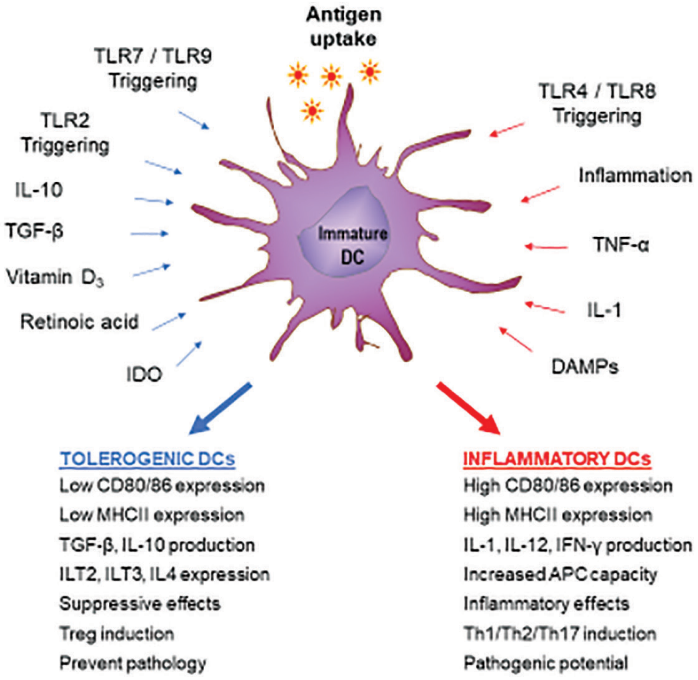

Figure 1. Influence of Innate Immune Factors on Dendritic Cell Responses.

models, leading to T-cell tolerance to ovalbumin antigen (64). A number of studies in humans has also tried to establish a link between DCs and immune tolerance. Studies investigating the oral cavity, the place where diverse environmental antigens are encountered such as commensal microbes, dietary antigens and allergens, have revealed the presence of several subsets of tolerogenic DCs with the capacity to induce immune regulatory responses in humans (65).

By oral administration of innocuous antigens such as food allergens, immune microenvironment is modulated, tolerogenic immune modulators are increased, all of which can inhibit inflammatory responses and lead to induction of tolerance (66, 67). Cross-talk between the host and microbiota has great importance in designation of immune responses. Important propensities of some bacteria and also their microbial particles for impelling DCs attitude towards a tolerogenic response have been reported. The tolerization of inflammatory epidermal DCs as well as skin Langerhans cells occur in response to toll-like receptor (TLR)2-mediated recognition of *Staphylococcus aureus* in atopic dermatitis skin (68). Lipopeptides of gram-negative bacteria are known to regulate immune responses. A potent analogue of synthetic lipopeptides; LP40 incorporates TLR2-dependent mechanisms in DCs that enhances production of IL-10 and IFN-γ, limits naïve T cell differentiation and indirectly limits production of IL-4 together with limitation of IgE- and promotion of IgG4-antibody isotypes. All these mentioned effects are potent limitation factors for Th2-type allergic inflammation (69).

Plasmacytoid DCs are known to produce anti-viral type-1 interferons upon TLR7 and TLR9 stimulation, and are revealed to contribute to the establishment of peripheral tolerance during AIT (70). Tonsils are strategically located lymphoid organs in the entrance of both respiratory and alimentary tracts, where first contact of the immune system with the food and respiratory antigens take place. Both pDCs and mDCs are present within the tonsils, with the dominance of pDCs that are co-localized with FoxP3$^+$ Treg cells. Diminished numbers of pDCs observed in tonsil samples of atopic individuals revealed significance of this APC subset in induction of immune tolerance (71). In contrast, mDCs and stimulations that can activate them are related with loss of allergen-specific T cell tolerance both in blood and tonsil samples of healthy, non-allergic individuals (72). Studies demonstrating the contribution of pDCs in prevention of atherosclerosis (73) and allograft rejection (74) also support the tolerogenic roles of pDCs by inducing Treg cells.

Current research has exposed functionally abnormal DCs in allergic patients, which may be modulated towards a tolerant state by interventions like AIT, corticosteroids, some DC-related cytokines as thymic stromal lymphopoietin (TSLP) (75). Studies have also intended to develop utilization of DCs in induction of tolerance. As an example, a mouse model utilizing allergoids coupled with mannan to be uptaken by oral mDCs via sublingual route has revealed the induction of FoxP3$^+$ Treg cells (76). Protocols for induction of DCs with tolerogenic capacities (tol-DCs) have been defined. Following differentiation of DC precursors with mediators like IL-10, TGF-β or dexamethasone, these cells acquire tolerogenic properties (77), which is promising for promotion of antigen-specific immune tolerance. DC-10, an inducible subset of tol-DCs, secretes sensible amounts of IL-10, expresses tolerogenic molecules including ILT2, ILT3, ILT4 and HLA-G and promotes anergy of T cells together with induction of Tr1 cells via IL-10 dependent ILT4/HLA-G pathway (62, 78). Studies have revealed important contribution of DC-10 in pregnancy and linked low frequencies of DC-10 in decidua of women with early miscarriage, which is of noteworthy (62). Understanding the DC biology is utmost important both for better illumination of their roles in precise-trimming of immune responses and possible utilization of DCs in future therapeutic interventions.

Contribution of Programmed Death Receptor in Immune Regulation Co-stimulatory signaling through B7 family members is a prerequisite both for activation and inhibition of T cell responses.

Programmed death-1 receptor (PD-1) was initially identified in T cells undergoing apoptosis (79) and is known to contribute to the establishment and maintenance of immunological tolerance. PD-1 has two ligands; PD-L1 (B7-H1) and PD-L2 (B7-DC), and triggering of PD-1 with these ligands contributes to negative regulation of T lymphocyte activation, proliferation, survival and cytokine production (80), and also induction of Treg cells (81). PD-1 is expressed in a number of cell types including T cells, B cells, monocytes, mesenchymal stem cells and Treg cells. Although having similar roles and binding to the same receptor, PD-L1 and PD-L2 have different expression patterns. PD-L1 expression is observed on macrophages, DCs and B cells, while expression of PD-L2 is limited to macrophages and DCs following activation (82-84). PD-1 has protective roles against autoimmune disorders through induction of T cell apoptosis and promotion of Treg cells while PD-1 blockade was reported to have promising outcomes in tumor immunotherapy (81). A number of studies have investigated the contribution of PD-L1 and PD-L2 in mechanisms of allergic disorders. PD-L2 and PD-L1 were claimed to have opposing effects in airway inflammation. PD-L2 was found to contribute to the regulation and suppression, while PD-L1 was claimed to be decisive for development of airway hyperreactivity in a mouse model of asthma (85). In addition, increased expression of PD-1 observed in nasal tissue samples of patients with chronic rhinosinusitis with nasal polyps was correlated both with disease severity and tissue expression of IL-5 (80). Further studies focused on contribution of these molecules in allergic inflammation may provide a potential for the discovery of new biomarkers potential and also novel therapeutic interventions.

Natural killer cells, a subset of lymphocytes, are best known for their cytotoxic properties against tumors and virus-infected cells. NK cells contribute to immune responses with their cytokine productions. Their activity is tightly regulated by the balance of cell surface inhibitory and activating receptors. Surface marker designation for phenotypic characterization of NK cells is as $CD3^-$ $CD16^+$ and $CD56^+$. Recent studies have revealed two major subsets of NK cells in peripheral blood with respect to their CD16 and CD56 expressions. NK cells with high expression of CD16 and low expression of CD56 ($CD16^+CD56^{dim}$) form the majority of the circulating NK cells, while the remaining NK group is characterized with no expression of CD16 and high expression of CD56 ($CD16^-$ $CD56^+$) (86, 87). These subsets have discrete functional properties; $CD16^+CD56^{dim}$ NK cell subset has cytotoxic properties and a limited potential for cytokine secretion (88). On the other hand, $CD16^-$ $CD56^+$ NK group has been characterized with high cytokine secretion competency, which may play roles both in inflammatory and regulatory properties, in response to various stimuli (9, 89). NK cell groups exist with respect to their cytokine secretion profiles analogous to Th1 and Th2 cells (90), while a subset of NK cells that produce IL-10 *in vitro* is termed as NK regulatory subset that can suppress antigen-specific T cell responses (9). A number of studies proposed roles for NK cell derived IL-10 in inflammatory disorders such as Behcet's disease and multiple sclerosis (91-93). NK cells respond to a variety of cytokines, such as IL-2, IL-15, IL-12 and IL-18 and the effectual microenvironment can modulate the functions of these innate cells. NK cells have the capacity to produce important cytokines such as IFN-γ, TNF, IL-5, IL-13, IL-10, GM-CSF and also chemokines such as CCL3, CCL4, CCL5 and CCL8, all of which propose important potentials for contribution to the regulation of immune responses (94, 95).

Better understanding of how NK cells contribute to the regulation of inflammation has an important value to find solutions for inflammatory diseases and tissue injury. In humans, NK cells were revealed to utilize cytotoxicity towards DCs, $CD4^+$ and $CD8^+$ T cells, which can limit excessive immune responses in viral infections. Suppression of $CD4^+$ T cells by NK cells in turn limits B cell-mediated humoral immunity against viruses, all of which are important for the establishment of a critical balance between efficient immunity versus excessive and tissue damaging inflammation (96-98). In a mouse model, where excessive

inflammation was triggered by lymphocytic choriomeningitis virus infection, complete depletion of NK cells or presence of NK cells, which lacks perforin-mediated cytotoxicity worsened the disease progression. $CD8^+$ T cell cytotoxicity was revealed to be controlled by NK cells in this model (99). For comparison, in asthma, a decrease in total NK cell counts, increased ratio of the cytotoxic NK cell subset with diminished cytotoxic capacity and an inverse correlation with lung function were observed (100). In another study, NK cells were found to contribute to eosinophil apoptosis in non-severe asthma and diminished NK activity due to the lack of a pro-resolving mediator; lipoxin A4 was observed in severe asthma (101). Taken together, understanding the roles of NK cells, the important sentinels of innate immunity, which contribute to both the aggravation and the regulation of inflammation beside their best-known action of cytotoxicity against the tumor cells and virus-infected cells, will enable us to utilize them as new therapeutic interventions.

Innate lymphoid cells are recently discovered subset of lymphocytes, which lack lineage markers and antigen-specific receptors of T or B cells. ILCs have been revealed to contribute to immune responses by their cytokine secretions, which are similar to their corresponding Th cell subsets as ILC1, ILC2 and ILC3 (similar to Th1, Th2 and Th17, respectively). They serve as a bridge in between innate and adaptive arms of immunity (102, 103). ILCs are oriented by lipid mediators, as well as cytokines produced by stromal, epithelial and myeloid cells and contribute to trimming of immune responses by their cytokine and mediator productions and via cell to cell contacts (104). ILCs contribute to both allergic and non-allergic diseases of inflammatory origin by producing cytokines and other mediators (104). ILC2s exert roles in allergic diseases by augmenting the Th2-type inflammation. Peripheral ILC2s which could contribute to induction of Th2 cells from naïve T cells were revealed to be diminished as a result of subcutaneous allergen immunotherapy in patients with severe seasonal allergic rhinitis (105). In line with this report, a recent study has revealed diminished numbers of peripheral blood ILC2s in response to AIT, in patients with AR (106). Likewise, a recent study has revealed regulation of ILC2 functions in allergic diseases by Tregs and their relevant cytokines, IL-10 and TGF-β (107).

Among ILCs, a specific subset have the capacity to enhance an immunologically tolerant state (108, 109). A recent study has reported a subset of IL-10 producing ILCs with regulatory functions, termed as ILCreg, which are present in the gut and were shown to have a unique gene identity that do not resemble classic ILCs or other Treg cells. TGF-β1 was determined to be produced by ILCregs during innate intestinal inflammation, and autocrine TGF-β1 has been revealed to sustain the expansion and maintenance of ILCregs, all of which propose a possible role for ILCs in maintenance of tolerance by innate immunity (8). Recently, retinoic acid, a vitamin A metabolite was shown to induce IL-10 producing ILCregs from ILC2s. This subset had a profile similar to Tregs by expression of CTLA-4 and CD25 together with diminished production of Th2-type cytokines (110). ILCs attract attention and are better characterized and understood day by day. Better understanding of their contribution to immune responses require extensive studies, however, as this rare subset of innate immune cells contributes to almost all diseases, it is expected that they may have roles beyond the current concepts.

Basophils and mast cells as their tissue counterparts; are members of innate immunity and contribute to Th2 immune responses. Mast cells and basophils are known to be loaded with granules and once triggered by cross linking of IgE bound to their Fcε receptors, they rapidly degranulate, synthesize and release intra-granular mediators including histamine, proteases, cytokines and lipid mediators (2). Reportedly, basophils that produce IL-3 promoted Th2-type airway inflammation in a mouse model (111). Th2 type immune responses are associated with immunity against parasites and when Th2 responses are directed to allergens, allergic reactions are manifested. In addition to their rapid effector responses to environmental stimuli as allergens, mast cells also exert modulatory effects

on immune responses (112). Functional interplay between mast cells and T cells has been proposed (113). Mast cells have regulatory and immune suppressive properties and are involved in the induction of immune tolerance (114). In experimental studies, mast cells were revealed to contribute to peripheral tolerance in response to Treg derived IL-9 (115, 116). On the other hand, bone marrow derived mast cells are known to induce an increase in the levels of $CD4^+CD25^+FoxP3^+$ Treg cells in a TGF-β1 dependent manner (117). Human mast cells produce both IL-10 and TGF-β which may contribute to immune regulation (118, 119). A recent study revealed basophil anergy in a house dust mite allergic population with an observed amelioration of AR. Basophil anergy was proposed to be a promising phenomenon both for being a biomarker and for a possible utilization in therapy, which deserves further investigations (120).

Other Players Acting on Immune Tolerance

Histamine*;* a biogenic amine, is an important mediator of allergic inflammation that induces vasodilatation, increases vascular permeability and contributes to type-1 hypersensitivity reactions. Histamine exerts its physiological functions like differentiation and proliferation of cells and hematopoiesis (121). Histamine has pleiotropic effects attributed to its 4 different receptors (H1R, H2R, H3R, H4R), all of which represent a complex immune regulatory role with discrete effects in relation with the receptor subtypes and their differential expressions (122). Among these receptors, H2R with relatively high expression on Th2 cells, contributes to the induction of Treg cells and induction of peripheral tolerance to allergens. In response to H2R up-regulation, IL-10 production increases and T cell stimulation is down-regulated. H2R has been shown to enhance the suppressive effects of TGF-β on T cells, which supports the role of histamine in H2R-mediated immune regulation (123-125). H2R has also been revealed to have effects on limitation of lung inflammation by regulation of lung invariant NKT (iNKT) cell responses (126). On the other hand, H4R is expressed in several hematopoietic cells and plays essential roles in the histamine-induced activation of DCs, monocytes, T cells, mast cells and eosinophils. H4R exerts its functions in both autocrine and paracrine manners. Triggering of H4R increases the expression of adhesion molecules, shapes and arranges the actin cytoskeleton of eosinophils that in turn lead to increased movement of these cells. H4R also plays roles in mast cells to mobilize calcium and induces chemotaxis without affecting degranulation, enabling the selective recruitment of these cells (127, 128).

Innate Mechanisms of Immune Tolerance-Related Disorders

As mentioned above, immune tolerance is a state of unresponsiveness to immune stimuli that otherwise have the capability to generate immune reactivity. Although suppression of this reactivity is relatively a desired condition when taken from the allergy and autoimmunity points of views, excessive suppression, in other words, superfluous tolerance, may promote emergence of chronic infections and cancer development (Figure 2). It is reasonable that, loss of tolerance may also be a key step in the development of allergic disorders, autoimmunity, host versus graft reactions following transplantations and miscarriages leading to infertility.

A fine balance between reactivity (effector functions) and non-reactivity (tolerance) should be established for a healthy state. Excessive tolerance to antigens could end up with tumor development and/or chronic infections, on the other hand, over reactivity could end up with allergic diseases, auto immunity, rejection of transplanted tissues or organs, and infertility. Cytokines acting on induction and maintenance of tolerance; IL-10, TGF-β, IL-27, IL-35 and IL-37 are secreted by cells that have regulatory capacities. Mast cells, basophils as well as APCs have capacity either to induce or break tolerance, which is determined by some specific stimulations. TLR7 and TLR9 support a tolerant state while triggering of TLR4 and TLR8 induce secretion of proinflammatory

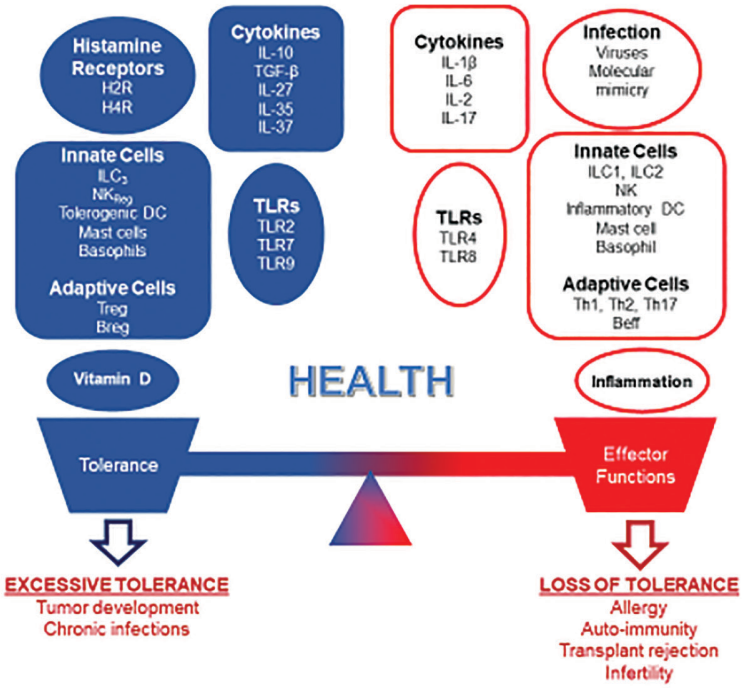

Figure 2. Contribution of Immunity to the Tolerant State.

cytokines as IL-1β, IL-6 and IL-17, and initiates inflammation. TLR2 triggering supports a tolerant state, binding of histamine to H2R and H4R also have tolerant properties. Viruses and molecular mimicry of bacteria could trigger inflammation and autoimmune diseases, respectively. Vitamin D has anti-inflammatory and tolerance promoting effects. (Blue groups indicate conditions acting to form a tolerant state while red groups indicate immune-response stimulating conditions. Beff: B effector cells.)

In allergic disorders, such as AR, asthma, atopic dermatitis, food allergy and more, both IgE mediated immediate hypersensitivity and/or delayed type T-cell mediated hypersensitivity reactions to ubiquitous antigens are observed (3, 24, 129). This may be accepted as a state of inability to tolerate these antigens, to which healthy individuals are normally non-responsive. As central tolerance mechanisms elicit discrimination of self and non-self, peripheral tolerance mechanisms generally shape the immune response with specificity to antigens and allergens. This is especially marked in allergic disorders, in which tolerance to allergens is not sufficient. In healthy individuals, regulatory T cells control and regulate both self-reactive and non-self-reactive T cell populations and prevent development of potential allergic disorders. Although allergen specific tolerance is a long-lasting state in healthy non-allergic individuals, sometimes, this stability may be disrupted by the activation of innate immune system such as by viral infections, which may lead to loss of peripheral tolerance (130). In the case of recognition of microbial particulates by TLR4 and TLR8, once triggered, these receptors induce inflammatory responses. In addition to this stimulation, in the presence of pro-inflammatory cytokines, IL-1β and IL-6 in the micro-milieu, proliferation of allergen specific $CD4^+$ T cells is triggered, which could lead to the loss of peripheral allergen specific tolerance (72).

Besides, loss of immunological tolerance to self-antigens is known as autoimmunity. Systemic lupus erythematosus, celiac disease, diabetes mellitus type 1 and multiple sclerosis are the leading examples of autoimmune disorders. The major pathologic mechanisms observed in these diseases

can be explained by T cell bypass due to superantigen production of polyclonal activation of B cells, especially by infections. Furthermore, in T cell-B cell discordance, aberrant B cell receptor mediated feedback results in perpetuating autoreactive B cells, which can also cause autoimmunity (131). Molecular mimicry is another presumed mechanism observed in autoimmunity, in which an exogenous antigen, which shares structural similarities with host antigens that may direct the immune response to self (132).

Immune tolerance also contributes to the progression of tumors. Briefly, altered antigen expressions and mutated protein structures, additional to elimination failure of the immune system, as seen in excessive tolerant states, may lead to tumor development. Several metabolic enzymes and various ligands may suppress T cell activation and proliferation. Myeloid-derived suppressor cells and Treg cells have been reported to be the major components of the immune suppressive tumor micro-environment (133). Thus, in cancer research controlling tolerance and enhancing immune responses to tumors by means of immune modulation are aimed (134). The recent studies include utilization of IgE-related anti-parasitic pathways for tumor clearance and more studies are required to reveal the success of this pathway (135).

Commentary

The strength and sustainability of immune tolerance is a defining factor in a number of diseases. Excessive tolerance may lead to insufficient defense against pathogens and may promote chronic infections as well as cancer. In contrast, from the clinical point of view, attenuated immune tolerance may manifest as allergic disorders, autoimmunity, organ transplant rejection and repeated miscarriages causing infertility.

The integrative mechanisms of central and peripheral tolerances work for achievement of a state of immune homeostasis for both the survival and the health of the organism. In the above-mentioned clinical conditions, which are presumed to be due to imbalances in immune tolerance mechanisms, novel treatment approaches that aim to warrant restoring this imbalance may provide a curative treatment option. Customization of treatment by means of precision medicine methods will enable better patient selection who will potentially benefit from personalized treatments (24, 136, 137). AIT, as an example of precision medicine approach in allergy, is the most acceptable way of induction of peripheral tolerance against the sensitizing allergens responsible for the signs and symptoms seen in allergic disorders (2, 138). In autoimmunity, although most of the patients are treated with immunosuppressive therapeutics in a non-specific manner, it is essential to induce the antigen-specific immunological tolerance to self or allogeneic antigens, while sustaining the entire immunity (139). Personalized strategies that target antigen presentation to T cells and implementing antigen-specific changes at the epitope and T cell receptor levels may provide novel insights into the management of autoimmune disorders and transplant rejections. Better understanding of the relationship between innate immunity and immune tolerance is a prerequisite for development of novel therapeutic options. The final response to a particular antigen/allergen can be defined by interactions between the environment and the immune system. As initial response to an environmental stimulus is driven by innate immunity, better understanding of immune tolerance mechanisms deeply relies on deeper knowledge on environmental stimuli and their innate recognition. Therefore, studies focused on exposome as well as microbiome that are gaining attendance nowadays will potentially contribute to this area. One should keep in mind that adaptive immunity that is responsible for both establishment and maintenance of a long-lasting immune responsiveness is mainly fine-tuned by actions of innate immunity. DCs form links between innate and adaptive arms and orchestrate immune responses differentially for each particular antigen. Understanding the DC biology is of great importance both for better illumination of their roles in precise-trimming of immune responses and for possible utilization of DCs in future therapeutic interventions.

Conclusion

Immune tolerance conveys unresponsiveness of immune system to relevant and substantial immune stimuli. A healthy immune status means generating sufficient responses against both pathogens and cancer cells, while tolerating self-tissues, commensal microorganisms as well as harmless environmental antigens. As both excessive and deficient immune tolerance lead to a number of disorders, evaluation of the tolerance intensity is important. Development of biomarkers that will reveal tolerance status is of great importance. In a peremptory way, a '*balanced tolerance*' is essential for the survival.

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