

T_H2 Cells in the Pathogenesis of Airway Remodeling

Regulatory T Cells a Plausible Panacea for Asthma

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Abstract

T-helper type 2 (T_H2) cells are one of the hallmarks of airway remodeling. The daunting task of regaining tolerance will be to regulate airway hyperresponsiveness (AHR) and remodeling in chronic asthma by balancing the ballet of T_H1 and T_H2 cells. The mechanism of tolerance appears to be modulated by a specialized subset of T cells called regulatory T cells (Tregs). Currently there are six subtypes of Tregs including CD4⁺CD25⁺ naturally occurring (N-Tregs), inducible naïve CD4⁺CD25⁻ T cells (T_R1), T_R1 memory phenotype, T-helper type 3 (T_H3), CD4⁺CD25⁺DX5⁺ natural killer T cells (T_RNKT), and CD4⁺CD25⁺CD8⁺ cytotoxic T cells (T_RCTC). The development of Tregs is controversial as to whether they occur in the thymus or peripheral lymphoid tissue. Studies have shown that N-Tregs are generated in the thymus and T_R1 cells occur in the periphery. Nevertheless, Tregs express an arsenal of molecular membrane markers: CD3, CD25, CD62L, CD69, BTLA, GITR, ICOS, Neuropilin-1 (Nrp-1), and PD-1. However, the most definitive marker is Forkhead Winged-Helix Transcriptional Factor Box p3 (Foxp3). The suppression of N-Tregs occurs by cell-to-cell contact, and low levels of IL-10 and moderate levels of TGF-β, but the primary mechanism involves the sequestration and activation of neighboring naïve CD4⁺CD25⁻ T cells to become T_R1 cells. In contrast, T_R1 cells exert their suppressive properties by copious secretion of IL-10 and TGF-β. These suppressive mechanisms occur by the inhibition of IL-2 production and the promotion of cell cycle arrest. The development of this specialized subset of T cells is an enigma, but their understanding will provide a plausible panacea for asthma.

Key Words

Airway remodeling
Asthma
FoxP3
Inflammatory cytokines
PD-1
Th-2 cells
T-regulatory cells
TR-1 cells

Introduction

Asthma is a chronic disease that is considered a major public health problem in industrialized societies, especially in the United States. The symptoms of asthma can occur from allergies, exercise, and genetic disposition. The hallmarks of asthma are pulmonary eosinophilia, inflammation of the bronchiolar tubes, and mucus hypersecretion. Asthma is a complex inflammatory disease that afflicts nearly 20.3 million Americans. Despite advances in research, the worldwide prevalence, morbidity, and mortality of asthma have dramatically increased over the last two decades (1). The development of asthma and other autoimmune diseases involves a breakdown in self-tolerance (2). Although autoreactive T cells are normally present in all individuals, clinical disease only affects a minority population, suggesting that mechanisms for peripheral self-tolerance must exist to completely control pathogenic T cells (3).

CD4⁺CD25⁺ regulatory T cells (Tregs) have re-emerged as a unique population of suppressor T cells that maintain peripheral immune tolerance (4). Over the past few years four types of Tregs have been identified. These include naturally occurring CD4⁺CD25⁺ (N-Tregs), inducible naïve CD4⁺CD25⁻ T cells (T_R1), T_R1 memory phenotype, and T helper type 3 (T_H3). To augment the canon of Tregs, several other phenotypes have recently been observed. These cells are CD4⁺CD25⁺DX5⁺ natural killer T cells (5) (T_RNKT) and CD4⁺CD25⁺CD8⁺ cytotoxic T cells (T_RCTC) (6). Tregs constitutively express the IL-2R α (CD25), inhibit IL-2 production, and promote cell cycle arrest in CD4⁺ and CD8⁺ T cells by a mechanism that requires cell-to-cell contact, are largely independent of IL-10, and partially dependent on TGF- β (7). In addition, cell contact utilizes membrane-bound molecules, although the nature of these molecules is still

elusive (8). However, the primary mechanism of suppression is the sequestration and activation of inducible naïve CD4⁺CD25⁻ T cells to become T_R1 cells.

The constitutive expression of CD25⁺ renders these cells anergic and suppressive and has the ability to control autoimmune diseases to include gastritis, colitis, thyroiditis, and autoimmune diabetes (7,9). In contrast, T_R1 cells exert their suppressive properties by a copious and lucrative secretion of IL-10 and TGF- β . Tregs are generated in the thymus and migrate to the periphery, whereas the T_R1 cells are induced in the periphery, in the presence of IL-10 and TGF- β . The T_H1 and T_H2 cell types without a doubt are the dubious cell types that insidiously wage war on the immune system and lay the foundation for the pathogenesis of asthma and autoimmune diseases. N-Tregs have re-emerged as the dominant peripheral mechanism of immune suppression of autoreactive T cells and a plausible panacea for resetting the thermostat of a disrupted immune system. In this review we have critically reviewed the current knowledge regarding the spawning of regulatory T cells and their functional properties for modulating immune response in the pathogenesis of asthma.

The Ballet of T_H1 and T_H2 Cells

Over the last decade it has been well established that the T_H1 and T_H2 cells play an intricate role in exasperating asthma. It has been shown that the role of T_H1 cell in airway inflammation is nominal, compared to the chemical actions that are elicited by the T_H2 cells (10,11). The development of these helper T cells is driven by naïve CD4⁺ T cells and antigen-presenting cells coupled with the release of two principal cytokines. The T_H1 cell is differentiated in response to IL-12, whereas T_H2 has a predilection for IL-4.

Dendritic cells producing IL-12 cause the upregulation of the transcriptional factor T-bet and the expression of interferon- γ . This cytokine causes the expression of IL-12R to induce the expression STAT4 (10,12,13). The production of interferon- γ blocks the development of IL-4, thereby driving a T_H1-polarized response. In contrast, after TCR stimulation, c-maf and GATA-3 (12) are activated, which induces the expression NFAT and AP-1 transcription factor (12,14), and translocation to the nucleus. Simultaneously, signaling transducer and activation for transcription-6 (STAT-6) is recruited and binds to the cytoplasmic domain of the IL-4R, where it is phosphorylated by the Tyk2, Jak1, and Jak3 leading to STAT6 dimerization and translocation to the nucleus. This activation triggers the expression of GATA3 (12,14), a T_H2-polarized response, and the production of the proallergic cytokines IL-4, IL-5, IL-9, and IL-13.

The T_H2 Cytokine Repertoire

The cytokine cocktail released from the T_H2 cell promotes the recruitment and mobilization of eosinophils and mast cells, which are the foot soldiers of the pathogenesis of acute and chronic asthma. The eosinophil is a potent modulator of airway remodeling (15). Eosinophils secrete a prodigious and lucrative source of TGF- γ (15). This multifaceted cytokine also regulates fibroblast and myofibroblast. These cells produce several extracellular matrix (ECM) proteins including collagens, proteoglycans, and tenascin (11,15,16). Because of the enriched cytokine environment, a crosstalk phenomenon creates and sustains this cell population. TGF- β released from the eosinophils is enhanced by mast cell-derived tumor necrosis factor- α (TNF- α) (15). In addition, tryptase released from mast cells also induces fibroblast proliferation, via proteinase-activated receptor 2

(PAR2) and peroxisome proliferator-activated receptor lambda through cyclooxygenase 2 (COX2) activity. Accordingly, fibroblast produces stem-cell factor (SCF) and granulocyte-macrophage-colony stimulating factor (GM-CSF), which promotes the survival of mast cells and eosinophils. The caustic cocktail of the mast cell consists of histamine and leukotrienes. Leukotriene is a potent chemo-attractant for eosinophil recruitment (17), and contributes to goblet cell hyperplasia, which causes airway occlusion by mucus, airway smooth muscle cell hyperplasia, and increased collagen deposits around the airways and in blood vessels within the lungs (11,15,16). (Fig. 1). Understanding what governs a T_H1 or T_H2 polarization is crucial for controlling the pathogenesis of asthma. At infancy, certain environments and genetic disposition promote the mechanism of tolerance, how to reset the thermostat of a disrupted immune system may be within a specialized subset of T cells called regulatory T cells.

Regulatory T Cells and Subsets

Naturally Occurring CD4⁺CD25⁺ Regulatory T Cells (N-Tregs)

Tregs are cells that constitutively express the CD25. They develop in the thymus, but are able to migrate to the peripheral lymphatic organs and have the capacity to suppress the proliferation of conventional T cells (T_H1 and T_H2 lymphocytes). These cells express the transcriptional factor Foxp3, the master regulatory gene that defines this T cell subset. N-Tregs suppress neighboring T cells by cell-to-cell contact by membrane-bound molecules (8), although the identification and characterization are not definitive. However, programmed death one (PD-1) has been implicated as a membrane molecule to regulate peripheral tolerance and prevention of

autoimmunity (18). In addition, GITR has been found to be expressed on N-Tregs and shows to be crucial in regulating immunity (19,20). However, the primary suppressive properties occur by sequestering and activating inducible Tregs.

CD4+CD25– Inducible Tregs (T_R1)

T_R1 cells were initially defined in studies of CD4⁺ T cells that had been activated in the presence of IL-10 and rendered anergic. Most studies define T_R1 cells by their development and the cytokine production profile. Several studies showed that IL-10–anergized CD4⁺ T cells contained the precursors of T_R1 cells, and that IL-10 was a critical factor for their differentiation (4,7,21–24). Importantly, T_R1 cells were shown to be involved in downregulation of immune responses in vitro and in vivo, via the lucrative production of the immunosuppressive cytokines IL-10 and TGF-β (7,21,24,25). In addition, several studies show that Tregs might function to induce the proliferation of the T_R1 cells and production of TGF-β. TGF-β inhibits the number of cells differentiated into T_H2 phenotype rather than the amount of T_H2 cytokines secreted by each differentiated T cell. The production of high levels of IL-10 coincides with the high levels of ICOS expression. ICOS is believed to be a switch mechanism for the production of IL-10. Other studies have also shown T_R1 cells producing high levels of IL-10 and TGF-β, moderate amounts of IFN-γ and IL-5, but little to no IL-2 or IL-4 (7,21,24–26). Other studies are not cohesive with the amount of TGF-β, but do agree on the amount of IL-10. A study conducted by Cohn and colleagues showed the development of T_R1 cells from transgenic CD4⁺ T cells from mice (27). This research group demonstrated that this population produced high levels of IL-10, but low levels of TGF-β, IFN-γ, IL-4, and IL-2. In addition, these cells proliferate poorly and, when activated, inhibit proliferation of bystander cells

(27). Furthermore, when these murine T_R1 cells were adoptively transferred into mice and activated with specific antigens, they protected from inflammatory bowel disease (28). Tregs and T_R1 cells are not immortal and within this subset several studies discovered memory Tregs.

T_R1 Memory Phenotypes

Other studies have shown that once the T_R1 cells are developed they differentiate into memory phenotypes via primary stimulation. Kemper and associates demonstrated that purified human CD4⁺ lymphocytes stimulated with CD3, CD46, and complement proteins C3b dimers developed into T_R1 cells (29). In addition, memory phenotypes developed after a primary and secondary stimulation. Upon the primary stimulation, CD45RA⁺CD45RO⁺ and naïve CD4⁺ T cells both synthesize IL-10 after activation with CD3/CD28/CD46 or CD3/CD46 plus IL-2. The CD45RA⁺CD45RO⁺ subpopulation (high responding cells) responded more robustly in that they produced IL-10 earlier and in greater quantities (about three- to eightfold more than naïve T cells) (28). Others studies agree that the CD45RO⁺ carries the suppressive activity. According to Levings and colleagues (30) upon isolation of pure populations of human CD25⁺CD4⁺ T cells contained both CD45RO⁺ and CD45RO[–] cells, but the suppressive activity is enriched in the CD45RO⁺ memory fraction. T_R1 cells proliferate slowly, and secrete no IL-2 or IL-4, but produce copious amounts of IL-10 and inhibit the proliferation of bystander cells both in vitro and in vivo (30). CD4⁺ cells can also differentiate into another cell type called the T_H3 cell.

T_H3 Cells

The regulatory T cell type 3 (T_H3) cells were described by Weiner and co-workers (31) as CD4⁺ T cells induced by oral antigen

in the mesenteric lymph nodes, which produce TGF- β and varying amounts of IL-4 and IL-10 (25). These cells suppress the development of experimental encephalomyelitis, suggesting that T cells producing TGF- β or IL-10 have regulatory or suppressive properties that inhibit the effects of pathogenic autoreactive T cells. Other studies agree that T_H3-generated cells only develop by oral and mucosal stimulation (25). The route of antigen administration does not propagate the development of T_H3 cell types. The conditions needed for the generation of T_H3 cells are poorly understood, and will require extensive research. Recent studies have re-categorized the T_H3 cells a helper cell to a regulatory T cell. Because T_H3 cells, however, function as suppressor rather than helper cells, we prefer to call these cells type 2, or T regulatory-2 (T_R2) cells, so as to group them with other regulatory T cells. Although regulatory T cells constitutively display CD25, both TR1 and TR2 cells can acquire this marker as well (25,32,33). The development of T_R2 cells in many studies has been through oral or mucosal stimulation. However, altered peptide (lower affinity) antigens can induce TGF- β -secreting T_R2 cells in the thymus (32,33).

CD4-CD25⁺CD8⁺ Cytotoxic T Cells (T_RCTL)

T_RCTL is dependent on transforming growth factor beta (TGF- β) for development. TGF- β has been reported to enhance considerably the proliferation of mouse CD8⁺ cells. This cytokine enhances human CD4⁺ cell expression of CD25, CTLA-4, CD40L, and TNFR2 (25,32–34), TGF- β increases TNF- α production by both CD4⁺ and CD8⁺ T cells (32,33). TGF- β accelerates activation-induced cell death of some T cells but protects others from apoptosis. In other studies, a brief exposure of CD8⁺ cells to IL-2 and TGF- β led to potent suppressive activity. Others have shown that CD8⁺ suppressor cells

can produce IL-10 and TGF- β (32,33). But the most recent study came from Lucas and colleagues, who compared CD8⁺CD25⁺ T cells from MHC Class II-deficient mice to Regulatory CD4⁺CD25⁺ T cells in several respects. (i) Both subsets were detected in the thymus and periphery, where they appear late in ontogeny. (ii) Peripheral CD4⁺ and CD8⁺ CD25⁺ T cells expressed CD44, GITR, and strongly expressed CTLA-4. (iii) Both subsets expressed Foxp3. (iv) Both subsets secreted large amounts of IL-10 in response to TCR stimulation (6). In addition, another study demonstrated CD8⁺ T cells primed with 4-1BB became suppressive against CD4⁺ T cells in a TGF- β dependent manner (6). A subsequent study confirmed this role for 4-1BB primed CD8 T cells, but demonstrated that suppression was IFN- γ dependent (35,36). Myers and associates (33) confirmed that these CD8 T cells required IFN- γ R. Indeed, CD8 appears to be a candidate member of the Treg canon; however, natural killer T cells have also debuted in the spotlight.

CD4-CD25⁺DX5⁺ Natural Killer T Cells (T_RNKT)

Natural killer T cells (T_RNKTs) are a subpopulation of T cells that express the surface marker DX-5⁺ and NK1.1 (Clone PK136) for mice and CD161 in humans. In addition, they are found in many tissues and they also reside in the lung tissue. Umetsu and colleagues have shown NKTs release two intoxicating cytokines, which are IL-4 and IL-13 at robust levels, that drive the cultivation a T_H2 response and thus the development of AHR (25,37). However, others do not concur with this evidence, suggesting that the mouse V α 14i NKT cells do not produce IL-4 and IL-13 (38). NKT suppression is cryptic, but it has been suggested that this subset is positioned to influence and interact with other N-Tregs and TR1 cells during early phases of the autoimmune response (35,39). It is an enigma if T_RNKTs drive or suppress AHR, a mystery

for suppressive activity (7,21,32,34). In the thymus and after birth CD4⁺CD25⁺ cells have the phenotype of naïve cells. In the periphery, they display the markers of previously activated cells as a consequence of their response to self and non-self antigens (7,21,32). In the thymus it has been shown the naïve Tregs have been induced by TGF- β and IL-2. Horowitz and colleagues (32,33) induced allo-stimulated total CD4⁺ CD25⁻ cells to develop Treg-like activity (21,24,32). The effect required the addition of both IL-2 and TGF- β to the cultures (21,24,30,32,34,40). Finally, the combination of IL-2 and TGF- β enhances the suppressive effects of purified mouse Tregs and the corresponding human subsets (32,34,40). It does appear that thymic epithelial cells play a role in the development and maintenance of regulatory T cells (32). Thymic CD4⁺ cells with high affinity TCRs for self-antigen become Tregs rather than being deleted. It is not unlikely that these CD4⁺ cells are recognizing self-antigens on thymic epithelial cells in the presence of TGF- β (32). Thymic epithelial cells can express tissue specific self-antigens, and some can produce TGF- β . Thus, if self-reactive CD4⁺ T cells respond to self-antigens in a milieu containing TGF- β , they may become CD25⁺ rather than being negatively selected (32).

In the Periphery

The thymus has been the epitome for the origin and conversion of regulatory T cells. However, there have been recent studies that have demonstrated that naïve CD4⁺CD25⁻ T cells can be induced in the periphery. Chen and colleagues (4) showed strong evidence that TGF- β converts naïve CD4⁺CD25⁻ T cells to CD25⁺ regulatory cells comes from our surprising findings that TGF- β and TCR co-stimulation induces transcription factor Foxp3 gene expression in CD4⁺CD25⁻ T cells. This peripheral induction of foxp3 is

dependent on the levels of TGF- β and TCR stimulation. They also showed that if TCR stimulation is absent, TGF- β fails to induce Foxp3 (4). TGF- β also appears to be a key modulator of Treg development, but its expression on Tregs is an enigma. It is suggested that TGF- β enhances its own production as evident by exogenous TGF- β upregulation of its own mRNA expression in CD4⁺ T cells (4,34). Indeed, this cytokine contributes to the development of T_R1 cells, but dendritic cells are definitely a factor in the conversion.

Immature dendritic cells have also been proposed as a means of Treg cell development in the periphery. In humans, injection of immature DCs pulsed with influenza peptide into volunteers rendered them unable to respond to a subsequent immunogenic stimulus (4,7,41). They concluded, based on this finding, that immature DCs constitutively expressing self-antigens would directly or indirectly induce a state of peripheral tolerance. Indirect mechanisms of tolerance may include the differentiation and activation of Tregs. In addition, although the types of Treg have not been identified in all studies, there is evidence that immature DCs produce IL-10 and induce a state of tolerance by triggering the differentiation and function of T_R1 cells in mice and humans (4,7). This group's findings were consistent with Morel and associates (41) that immature DCs do induce Treg cell development. The rediscovery of regulatory/suppressor cells has revealed that DC play an important role in the induction and maintenance of several regulatory T cell populations (41). Thus, an IL-10 secreting, nonproliferating human CD4⁺ T cell population with regulatory properties can be induced in vitro by repeated stimulation of naïve T cells with allogeneic immature DC (41). Furthermore, a study done by Akbari and colleagues (42) demonstrated within 24 h of respiratory expo-

sure to ovalbumin (OVA) pulmonary DCs found with the bronchial lymph nodes expressed IL-10 (42). These IL-10-producing DCs induced the development of CD4⁺ T cells to produce IL-4 and large amounts of IL-10, a cocktail associated with regulatory T cells. The progression of these IL-10-producing DCs after OVA exposure from an immature DC phenotype within the pulmonary tissue to the more mature phenotype in the bronchial lymph nodes was consistent with published data, which indicates that DCs mature as they migrate from peripheral tissue into the regional lymph nodes after acquiring antigen (42).

A most recent study done by Stock and associates (43) in the periphery was the use of splenic DCs (CD11c⁺ CD8 α ⁺) which were shown to produce IL-10 and IL-12 and drive the induction of regulatory T cells (42). The phenotype of these cells were like a T helper type 1-like regulatory cell which produce the cytokine milieu of IL-10 and interferon- γ (43). They also expressed T-bet the master gene for T_H1, Foxp3 the master gene of regulatory T cells, and ICOS, the inducible costimulatory molecule.

Function of the Regulatory T Cells

Regulatory T cells as the name implies regulate the functions of other neighboring T cells, which prevents the emergence of autoreactive T cells and curtails the immune response to antigens. These actions are facilitated by the release of cytokines such as IL-10 and TGF- β as well as cell-to-cell contact. The mode of suppression by Tregs elicited by IL-10 and TGF- β is possibly by the inhibition of IL-2 production (7). The action of cell-to-cell contact is drastically vague, but it appears to be orchestrated by cell cycle arrest in CD4⁺, CD8⁺ T cells (7), and NKTs. Other groups have found that the Tregs not only produce IL-10 and TGF- β , but also low amounts

of IFN- γ , but no IL-4 or IL-2 (4,26). The mechanism of cell-to-cell contact by N-Tregs is poorly understood; it has been suggested that several markers such as GITR or CD40 may be implicated but this remains to be elucidated. However, recently a group showed that the engagement of GITR triggered the activation of a molecule known as granzyme B (GZ-B) (44). GZ-B is a serine protease, secreted mainly by NK cells and CTLs, (44,45) and is largely responsible for the induction of apoptosis in the target cell (44). However, recent reports have shown that human CD4⁺ T cells are also able to synthesize GZ-B and perforin (46). Furthermore, studies by Grossman and co-workers (47) as well as others have shown that GZ-B is highly upregulated in activated human T cells bearing a Tr1 phenotype. In addition, Tregs in the human system mediate suppression with requirement for granzyme A (GZ-A). These results suggest a possible role for granzyme in mediating T cell suppression. The data presented in this study implicate that GZ-B/A plays a pivotal role in the suppressive capacity of murine Tregs (44,47).

Membrane Markers on Regulatory T Cells

Regulatory T cells have been difficult to isolate because of their identity. But over the last 5 yr many membrane markers have become unraveled. These markers are not only useful for identifying Tregs, but play a dominant role in their suppressive properties. In this review we will investigate several highly distinctive surface markers. Inducible costimulatory molecule (ICOS) is a marker of the B7 Family. ICOS is found on activated T_H1, T_H2, and Tregs. The ligand for ICOS is found on activated dendritic cells, macrophages, and monocytes. It has been reported to act as a switch for the production of IL-10. Intriguingly, ICOS^{high} T cells were

Table 1.

Molecular membrane markers	Function
CD25	IL-2 alpha chain receptor, found on regulatory T cells.
ICOS (CD275)	Inducible co-stimulatory molecule. May help facilitate the production of IL-10.
CTLA-4 (CD152)	Cytotoxic lymphocyte associated antigen. Downregulator or braking of T cell proliferation.
GITR	Glucocorticoid-induced tumor necrosis factor receptor. Triggers pro/antiapoptotic effects.
PD-1 (CD279)	Inhibits T cell proliferation by apoptosis.
BTLA (CD2 72)	Inhibition of IL-2 production on effector T cells.
CD40L	Cytokine production in naïve T cells.
CD62L	L-selectin is homing marker for naïve T-cells.
CD69	Early activation marker of naïve T cells.
CCR4 and CCR8	Chemokines, homing markers.
CD45RO	Memory regulatory T cells.
Neuropilin-1	Unknown.
Foxp3	Molecular transcription factor for regulatory T cell development and function.

linked to IL-10 production, ICOS^{medium} T cells secreted IL-4, IL-5, and IL-13, ICOS^{low} T cells were associated with IL-2, IL-3, IL-6, and IFN- γ production (48). The development of these IL-10-producing Treg cells was dependent on ICOSL-ICOS interactions. Airway hyperreactivity could be inhibited by adoptive transfer of these Treg cells into sensitized mice. These findings suggest a novel mechanism by which ICOS and IL-10 reduce airway inflammation associated with asthma and implicate ICOS in regulating respiratory tolerance (43,49).

Another member of the B7 family is programmed death one (PD-1). PD-1 is an immunoglobulin superfamily member and is related to CTLA-4 and CD-28 (48). It was originally identified in a T cell line undergoing programmed cell death (50,51); however, further studies have shown that its expression is associated with lymphocyte activation rather than cell death (50–53). PD-1 cytoplasmic region contains two tyrosines residues and has an immunoreceptor tyrosine-base inhibitor motif (ITIM) and immunoreceptor tyrosine-base switch motif (ITSM). The presence of the ITSM may be due to the fact that PD-1 is responsible for regulating a positive and negative signal for T cell activa-

tion. Another possibility for the ITSM is that there are two different ligands for PD-1, PD-L1 (B7-H1) and PD-L2 (B7-DC) (49–52). A third member of the B7 family is B cell and T cell lymphocyte attenuator (BTLA); it is similar to CTLA-4 because it behaves as a regulator for T cell development. BTLA is induced on activation of naïve T cells and is expressed by T_H1, T_H2 cells (49,54), and Tregs. Expression of BTLA is subsequently lost on highly polarized T_H2 cells, but is maintained on T_H1 cells (54). BTLA contains two cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIM) and undergoes inducible tyrosine phosphorylation and association with SHP-1 and SHP-2 (54). These three markers have been under the spotlight for Treg development and their suppressive properties, but there are many more to be investigated.

A marker that is not of the B7 family is Neuropilin-1. This membrane marker is a semaphorin III receptor originally described to be involved in axon guidance and more recently found in primary immune response (52,56). Being co-regulated with Foxp3, Nrp-1 represents a candidate marker for N-Tregs as it exhibits a high expression level in both polyclonal and monoclonal Tregs but not

in antigen-stimulated nonregulatory T cells (55) (CD4⁺CD25⁻ T cells) (Table 1).

Signaling Molecules and Transcriptional Factors for Treg Development

It has been demonstrated in many studies that IL-2 is necessary for regulatory T cell development, survival, and homeostasis. IL-2R is composed of an alpha (CD25) and beta (CD122) chain (57). However, two transcriptional factors have been identified to collaborate with IL-2R. These two molecular factors are JAK3 (Janus Kinase), a novel member of protein tyrosine kinase family, and STAT5 (signaling transducer and activator for transcription) (57,58). Burchill and colleagues (58) demonstrated that disruption of the STAT5 signaling pathway leads to the absence of CD4⁺CD25⁺ regulatory T cells, thereby allowing the unchecked development of activated CD4⁺ and CD8⁺ T cells. It has been proposed that STAT5 binds to the CD25 promoter and thereby promotes the increase in CD25 transcription. STAT5 binding sites have been found in the CD25 promoter and have been shown to promote CD25 upregulation following TCR stimulation. Mice that express STAT5 also express the key transcription factor Foxp3, and it was expressed at levels greater than or equal to wild-type mice CD4⁺CD25⁺ T cells (57,58). Taken together with their observation that STAT5 knockout mice CD4⁺ T cells show a dramatic decrease in Foxp3 expression, these results suggest that STAT5 activation is both necessary and sufficient for Treg development and survival (57,58).

Foxp3

Foxp3 is the most definitive means for the identification of regulatory T cells. In addition, mice and humans lacking this gene develop fatal immune wasting diseases. Mutations in the X-linked Foxp3 gene in scurfy mice lead to an autoimmune disease characterized by multiorgan lymphocytic infiltration

that results in enlarged spleen, lymph nodes, and liver, as well as dermatitis and severe runting (59–63). In humans, mutations in Foxp3 have been linked to autoimmune diseases including X-linked autoimmunity allergic dysregulation syndrome (64), X-linked autoimmunity immunodeficiency syndromes (64–66), X-linked syndrome (immune dysfunction), polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) (65–67). Scurfin, the protein product of Foxp3, is a member of the forkhead/winged-helix family of transcriptional regulators (63,65,66,68). Scurfin appears to act as a transcriptional repressor, suggesting that scurfin is a negative regulator of T cell activation and function (63). In mice lacking scurfin there is a severe deficiency in Tregs and overexpression of CD4⁺CD25⁻ T cells (69).

Concluding Remarks

In conclusion, treating patients with allergies, asthma, and autoimmune diseases will come from the understanding of how to control and prevent an autoreactive T cells. The renaissance of this regulatory T cell repertoire appears to be a plausible solution for these diseases. It will be interesting to see in future studies how T_RNKT and T_RCTC contribute to suppression or exacerbation of asthma and if these cells are Foxp3⁺ cells. Foxp3 appears to be crucial and possibly the turning point for the development and how this population of T cells orchestrates their suppressive properties. In addition, understanding the role of molecular markers such as PD-, ICOS, and Neuropilin-1 as they are present on Tregs will be pivotal. It is certainly obvious that a tremendous amount of research is required to understand the role of regulatory T cells and the prevention of asthma and autoimmune diseases.

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