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Molecular and Functional Heterogeneity of T regulatory cells

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Abstract

Naturally occurring CD4+ T regulatory (Treg) cells are produced during maturation in the thymus and have a mandatory role in maintaining homeostasis and immune quiescence. Development and function of Treg cells depends on the transcription factor forkhead box P3 (Foxp3), which is necessary and sufficient for Treg cell function. Currently emerging evidence indicates Treg cells display molecular and functional heterogeneity and can be categorized into naïve and effector- or memory-like cells, which can produce effector cytokines supporting the idea that Treg cells retain plasticity. The role of Treg cells that acquire these properties remains unclear and is currently under intense investigation. In this review, we summarize recent advances on the differentiation of effector- or memory-like Treg cells, the impact of the cytokine milieu on the molecular and functional heterogeneity of Treg cells, and the clinical implications of the heterogeneity and specialization of Treg cells.

Keywords

T regulatory cells; Th1; Th2; Th17; autoimmunity

Introduction

Naturally occurring $CD4^+$ T regulatory (T_{reg}) cells produced during the normal process of maturation in the thymus are specialized for immune suppression. Disruption of the development and function of T_{reg} cells is a primary cause of autoimmune and inflammatory diseases [1]. T_{reg} cells express transcription factor forkhead box P3 (Foxp3), which is considered the most specific marker to identity the T_{reg} lineage [2–4]. Extensive studies have demonstrated that mutation of Foxp3 or down-regulation of Foxp3 expression in T_{reg} cells leads to reduction of T_{reg} cell numbers and loss of T_{reg} suppressive activity and induces immune dysregulation [5–7], strongly suggesting that Foxp3 plays a dominant role in the development and function of T_{reg} cells. T_{reg} cells also express ‘signature’ surface markers such as CD25, CTLA-4 and GITR but most of these markers lack specificity and can be expressed by activated conventional $CD4^+$ T cells [8–10]. Therefore, a definitive or highly specific surface marker identifying naturally occurring T_{reg} cells is lacking thus far.

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T_{reg} cells display heterogeneity in cell surface phenotype and regulatory functions. T_{reg} cells can be categorized into naïve and effector- or memory-like cells distinguished by the developmental stage, phenotype and migration properties [11]. Interestingly, recent studies have revealed that T_{reg} cells particularly effector- or memory-like T_{reg} cells were able to produce effector cytokines such as IFN-γ and IL-17 under various conditions [12, 13], supporting the idea that T_{reg} cells are not at the final stage of their differentiation but retain plasticity. These observations led to the conclusion that natural T_{reg} cells are a heterogeneous population consisting of a committed T_{reg} lineage and an uncommitted subpopulation with developmental plasticity [14]. Overall, the fate of T_{reg} cells appears to be associated with the anatomical location, microenvironment cytokine milieu and the status of other immune cells present in the same microenvironment. Although the role of committed T_{reg} cells in the maintenance of self-tolerance and immune homeostasis is well documented, the role of T_{reg} cells, which acquire the ability to produce effector cytokines remains unclear and is currently under intense investigation. In this review, we summarize recent advances in our understanding of the maturation of effector-or memory-like T_{reg} cells, the impact of the cytokine milieu on the phenotype and functional plasticity of T_{reg} cells and the clinical implications of the functional specialization of T_{reg} cells.

1. Phenotypic and functional properties of naïve and effector- or memory-like T_{reg} cells

Naturally occurring T_{reg} cells develop within the thymus through a $CD25^{hi}CD4^{+}CD8^{-}$ intermediate in a process that depends on multiple factors including T-cell receptors (TCRs) engaging thymic MHC/self-peptide ligands [15–17] and the presence of γc cytokines IL-2, IL-7 and IL-15 [18, 19]. $Foxp3^{+}$ T_{reg} cells generated in thymus, like their naïve conventional T cells, express L-selectin (CD62L) and the chemokine receptor CCR7 and preferentially migrate into peripheral secondary lymphoid organs [20, 21]. Within lymph nodes, T_{reg} cells appear to function by interacting with dendritic cells and limiting their ability to effectively prime naïve T cells thereby blocking the differentiation of autoreactive T cells and maintaining the homeostasis [22–24].

T_{reg} cells also play an essential role in balancing effector cell-mediated immunity in response to infection. To function properly *in vivo*, T_{reg} cells are required to come into physical proximity to their targets regardless of whether they function through cell contact- or cytokine-based mechanisms [22, 25]. The ability of T_{reg} cells to traffic from secondary lymphoid organs to peripheral tissues is controlled by expression of various of homing receptors and such event is associated with the functional maturation of T_{reg} cells [26]. Although the mechanisms underlying the maturation of T_{reg} cells and the differential expression of homing receptors in T_{reg} cells *in vivo* have not been fully elucidated, extensive research has demonstrated that T_{reg} cells are heterogeneous in their differentiation stage, tissue localization and expression of effector molecules of suppression [1]. These findings have provided a framework for further understanding of how specialized subsets of T_{reg} cells might carry out divergent tasks in regulating ongoing immune reactions.

1.1 Developmental stage and migratory properties of T_{reg} cells

$CD4^{+}CD25^{+}$ T_{reg} cells in infants express high levels of CD62L and CCR7, which allow T_{reg} cells to migrate into lymphoid tissues. However, a rapid down-regulation of CCR7 expression occurs in T_{reg} cells between birth and 18 months of age. Moreover, the majority of the T_{reg} cells in cord blood and in infants up to 18 month of age express the gut-homing integrin α4β7, while only a minor subset of T_{reg} cells express CCR4, a chemokine receptor associated with extra-intestinal homing [26–28]. In contrast, the majority of T_{reg} cells isolated from adult blood express CCR4 while only a minority of cells express α4β7 [29]. At

3 years of age the expression of α4β7 is significantly reduced and the expression of CCR4 is significantly increased on T_{reg} cells, suggesting that the switch of homing receptors on T_{reg} cells occurs at 3 years of age concomitantly with other signs of maturation [26]. Interestingly, the switch of homing receptors on T_{reg} cells reflects not only their migratory properties but also corresponds to their memory phenotype. Conventional T cells and T_{reg} cells up to 18 month of age express higher levels of CD45RA, the cell surface marker associated with a naïve phenotype of T cells, compared with the corresponding T cells from adults. However, at 3 years of age, the expression of CD45RA is significantly reduced on the $CD25^{hi}$ T cells compared with those at 18 month of age, while the expression of CD45RO, the cell surface marker for memory phenotype of T cells, is increased [26].

The dynamic development of homing receptor expression is also associated with the functional maturation of T_{reg} cells. The fetal intestine is sterile at birth, but colonization by a variety of microorganisms begins directly after delivery [30]. Studies using mouse models show that exposure to bacterial antigens favor the generation and expansion of functional T_{reg} cells [31–33]. In fact, T_{reg} cells from germ-free mice are less suppressive than those from conventional or colonized but pathogen-free mice [31, 32]. It was reported that the majority of the T_{reg} cells in umbilical cord and infant blood expressed α4β7 but not CCR4. This expression profile resulted in migration of T_{reg} cells into intestinal secondary lymphoid tissues, suggesting that the gut might be the primary site of antigen exposure for T_{reg} cells in early life. Upon encounter with antigen, T_{reg} cells gain distinct homing characteristics mediated by de novo expression of organ-specific adhesion molecules and chemokine receptors and that leads to a potentially widespread distribution of T_{reg} cells later in life. Indeed, T_{reg} cells can be found in most non-lymphoid tissues even in the absence of any overt inflammation [22].

1.2 Phenotypic characterization and immune function of effector- or memory-like T_{reg} cells

Studies from Hamann group first demonstrated that T_{reg} cells could be subdivided into populations resembling naïve T cells and effector- or memory-like T cells based on expression of homing receptors. The integrin $\alpha_E\beta7$ (CD103) discriminates the distinct subsets of murine $CD4^+$ T_{reg} cells. $\alpha_E\beta7^+$ cells represent effector- or memory-like T_{reg} cells that predominantly enter peripheral tissues and inflamed effector sites. These cells displayed potent suppressive activity compared to $CD25^+$ $\alpha_E\beta7^-$ T_{reg} cells *in vitro*. Moreover, *in vivo* these cells were capable of suppressing acute inflammatory reactions in antigen induced arthritis [11]. Interestingly, Rap1 activation has a critical role in regulating the generation of $CD103^+$ T_{reg} cells, as mice transgenic for the constitutively active form of Rap1 display a significant increase in the proportion of $CD103^+$ T_{reg} and prominent lymphopenia [34]. CCR6 and other surface markers are also used to distinguish naïve and effector- or memory-like T_{reg} cells.

T_{reg} cells with an effector- or memory-like phenotype might arise as a result of T_{reg} cell activation, presumably owing to recognition of self- or non-self-antigen [25, 26]. In spite of the fact that T_{reg} cell specificity is still poorly characterized, there is evidence that TCR recognition and TCR signaling have a key role in influencing the phenotype, function and localization of T_{reg} cells *in vivo* [17, 25]. Conditioning loss of TCR signaling due to inactivation of $p56^{Lck}$ function in T_{reg} cells did not significantly affect Foxp3 expression. However, $p56^{Lck}$-deficient T_{reg} cells fail to suppress immune responses of other cells. Moreover, $p56^{Lck}$-deficient T_{reg} cells highly express CD62L while remain incapable of upregulating $\alpha_E\beta7$ and CCR6 upon encountering antigen [17]. Thus, sequential molecular and signaling events may occur during the development and functional specialization of T_{reg} cells. Naïve T_{reg} cells and T_{reg} cells in early life are activated *in vivo* presumably through the recognition of self-antigen or commensal microbes resulting in expression of homing

receptors and functional maturation (Figure 1). Instability is further enhanced during activation and expansion of T_{reg} cells particularly effector- or memory-like T_{reg} cells.

2. Stability and functional specialization of T_{reg} cells

The therapeutic potential of T_{reg} cells was envisioned decades ago because of their unique suppressive function. These cells have been widely used in various conditions from transplantation to autoimmune diseases [35–37]. However, clinical implementation of their potent immune regulatory activity has been challenging [38]. Recent findings suggest that T_{reg} cells are heterogeneous in functions and phenotypes and not all T_{reg} cells are at terminal stage of differentiation but rather display a significant degree of plasticity. Specifically, T_{reg} cells can lose their suppressive activity and become conventional effector cells [39] or acquire the ability to secrete effector cytokines such as IFN-γ and IL-17 while retaining their suppression function [12, 40]. Current research is focused on understanding the driving forces and mechanisms underlying the plasticity of T_{reg} cells and on identifying factors that can either promote or reverse the stability of T_{reg} cells in the context of autoimmune and inflammatory diseases.

2.1 T_{reg} cells control distinct immune responses

$CD4^+$ T helper (Th) cells play critical roles in orchestrating the adaptive immune responses mainly through secreting various cytokines and chemokines that activate and/or recruit targets cells. More than two decades ago, Mosmman and Coffman recognized that effector $CD4^+$ T cells can be divided into two distinct populations with unique functions: IFN-γ-producing Th1 cells and IL-4-producing Th2 cells [41]. It was found that Th1 cells help to combat intracellular pathogens and that Th2 cells mediate host defense against extracellular parasites [42–45]. Recently, a new effector Th lineage, the IL-17- producing Th17 cells was discovered [46, 47] and these cells are involved in immune responses against extracellular bacteria and fungi [48]. Production of polarizing cytokines that drive the lineage differentiation is dictated by the cytokine milieu and the type of pathogen encountered [25]. IFN-γ and IL-12 direct the differentiation of Th1 cells upon encountering intracellular pathogens, while IL-4 induces Th2 cells during infection with large mucosal parasites; TGF-β and IL-6 promote the development of Th17 cells during infection with extracellular bacterial or fungi [42, 45, 49].

Functional specialization of $CD4^+$ effectors cells is controlled by the differential expression of lineage specific transcription factors, such as T-bet for Th1 cells, GATA, IRF4 for Th2 cells, RORγt, IRF4 and Stat3 for Th17 cells. These lineage-specific transcription factors regulate distinct gene expression programs that are involved in determining cytokine production and migration properties [50–54]. Abnormal activation of any subset of $CD4^+$ effector cells is harmful to host tissues. Aberrant activation of Th1 cells is considered as the critical event in most organ-specific autoimmunity while aberrant activation of Th2 cells is considered responsible for allergic inflammatory diseases and asthma. Thus far, some of the autoimmune responses have been shown to be mediated by Th17 cells [55]. Therefore, regulation and control of immune responses mediated by $CD4^+$ effector cells is of critical importance. Active immune suppression by T_{reg} cells is a central mechanism for control of pathogenic immune responses. Defects in function of T_{reg} cells can result in Th1, Th2 and Th17 -mediated inflammatory diseases [56–58].

Several groups have reported that T_{reg} cells can use canonical $CD4^+$ effector cell-associated transcription factors to restore or maintain immune homeostasis during polarized Th1, Th2 and Th17 cell-mediated immune responses [25]. In response to IFN-γ, $Foxp3^+$ T_{reg} cells upregulate the Th1-specifying transcription factor T-bet. Subsequently, T-bet promotes expression of the chemokine receptor CXCR3 on T_{reg} cells and results in accumulation of T-

bet$^+$ T_{reg} cells in sites of Th1 cell-mediated inflammation. T-bet-deficient T_{reg} cells display impaired proliferation during Th1-mediated immune response and subsequently, these T_{reg} fail to suppress the expansion of Th1 cells [57]. Similarly, IRF4, transcription factor involved in the control IL-4 production and the differentiation of Th2 cells is required for T_{reg} cell-mediated control of Th2-type inflammatory responses. Mice in which *Irf4* is specifically deleted within Foxp3$^+$ T_{reg} cells develop a lymphoproliferative disease that is associated with a selective increase in the number and frequency of IL-4 and IL-5-producing CD4$^+$ T cells [58]. Moreover, deletion of Stat3 in Foxp3$^+$ T_{reg} cells results in development of spontaneous fatal intestinal inflammation that is characterized by excessive IL-17 production but with normal levels of Th1 and Th2-associated inflammatory cytokines, indicating that in the absence of Stat3, T_{reg} cells selectively lose their immune suppressive function on Th17-type immune responses [56]. Taken together, the aforementioned studies suggest that T_{reg} cells utilize distinct molecular programs to control Th1, Th2 and Th17 – type responses. Although the mechanism by which T-bet, IRF4 and Stat3 control T_{reg} cell activity during Th1-, Th2- and Th17-mediated responses is still unclear, these transcription factors are likely to be involved in mechanisms that control the migration and functional properties of T_{reg} cells.

Based on these findings, a model emerges in which selective expression or activation of transcription factors associated with Th1, Th2 and Th17 lineage differentiation drives the development of molecularly diverse and functionally specialized T_{reg} subsets armoring them with the machinery required to restrain responses mediated by distinct types of CD4$^+$ effector T cells [25] as illustrated in Figure 2. These new findings may have significant clinical implications because isolation or generation of functionally specialized T_{reg} cells may improve T_{reg} cell-mediated treatments targeted specifically to control Th1, Th2, and Th17-mediated inflammatory diseases. Therefore, identification of mechanisms underlying the differentiation and development of such functionally specialized T_{reg} cells and developing methods to selectively isolate and expand the different subsets of T_{reg} cells will be highly beneficial.

2.2 Plasticity of T_{reg} cells and the underlying mechanisms

Although the importance of T_{reg} to control responses of other cell types is well established, T_{reg} cells can become unstable in certain experimental conditions. Specifically, Foxp3$^+$ T_{reg} cells can lose their inhibitory function and even become pathogenic effector cells in autoimmune settings [39]. The propensity of T_{reg} cells to convert to other cell types or to acquire the ability to produce inflammatory cytokines is a real concern, as they might have the potential to exacerbate conditions that they are intended to treat. However, the ability of T_{reg} cells to appropriately adapt to a defined setting that promotes the generation of a specific subset of effector cells while sustaining a regulatory program, might be critical for their ability to regulate immune responses mediated by this specific effector cell population.

(a). Unstable expression of Foxp3 in T_{reg} cells—The development and function of T_{reg} cells is critically dependent on expression of Foxp3 [59]. The suppressive activity of T_{reg} cells with attenuated Foxp3 expression is nearly abolished and interestingly, these cells preferentially become Th2-type effectors [7]. It has also been observed that decreased Foxp3 expression is associated with human immune disorders [60, 61]. These findings suggest that decreased Foxp3 expression may be responsible for the instability and conversion of T_{reg} cells into effector cells. The molecular and functional features conferred to T_{reg} cells by Foxp3 have been extensively investigated [62]. These studies revealed that Foxp3 protein amplified and stabilized genes encoding cell surface or secreted molecules, including Fgl2, CD73, CD39, TRAIL or CTLA-4, which are normally upregulated in conventional T cells upon TCR stimulation and are capable of mediating negative feedback regulation of T-cell

activation. In addition, Foxp3 enforces repression of TCR-mediated immune responses such as secretion of effector cytokines including IL-4, IFN-γ, TNF-α, IL-17 and IL-21. These observations suggest that Foxp3 might solidify T_{reg} lineage stability through modification of cell surface and signaling molecules resulting in adaptation of signals required to induce and maintain T_{reg} cells.

It was thought that Foxp3$^+$ T_{reg} cells are stable *in vivo* and Foxp3 expression is controlled by Foxp3 itself through a positive feedback loop [63]. However, a series of reports have challenged this view. T_{reg} cells isolated from inflammatory sites have lower expression of Foxp3, which might account for increased susceptibility to autoimmunity [62]. Adoptive transfer experiments suggested that these unstable T_{reg} cells can become autoreactive effector T cells as a consequence of Foxp3 instability [39]. Moreover, in a lymphopenic setting, a fraction of adoptively transferred Foxp3$^+$ T_{reg} cells displays unstable expression of Foxp3 and can convert to follicular helper T cells and promote the formation of germinal centers in mouse Peyer's patches [64]. Because the levels of Foxp3 expression are crucial for the stabilization of T_{reg}, many studies have investigated the forces that regulate expression of Foxp3 in post-tymic T_{reg} cells. It has been determined that IL-6 acts in synergy with IL-1 to downregulate Foxp3 expression via a pathway dependent on the transcription factor Stat3 [65]. Evidence of active regulation of Foxp3 stability is provided by the observation that loss of Dicer, which regulates expression of microRNAs, resulted in normal development of thymic T_{reg} cells, but downregulation of Foxp3 and dysfunction of T_{reg} cells in the periphery. Moreover, Dicer deficient T_{reg} cells lost their suppressive function *in vitro* and Dicer deficient mice developed fatal systemic autoimmune disease [66].

It is currently accepted that autoimmunity is caused by imbalance of pathogenic T cells and Foxp3$^+$ T_{reg} cells [67, 68]. This could be due to a defect in T_{reg} cells that allows pathogenic cells to escape regulation and mediate disease [69]. With the emerging findings, the alternative possibility could be entertained that instability of Foxp3 results in generation of pathogenic effector- or memory-like T cells that themselves promote autoimmunity [39].

(b). IL-17-secreting Foxp3$^+$ T_{reg} cells—Several groups have now described the conversion of T_{reg} cells into Th17 phenotype induced by appropriate inflammatory stimuli [13, 40, 70–72]. These observations are particularly relevant in the context of cell-based therapy. The reciprocal relationship between T_{reg} cells and Th17 cells has been established since the discovery of Th17 cells [65]. Expression of RORγt is observed in Foxp3$^+$ T_{reg} cells; however, Foxp3 is able to physically bind to RORγt and to inhibit the transcriptional activity of RORγt thereby blocking IL-17 production [73]. Therefore, under steady conditions T_{reg} cells are unable to produce IL-17. In the presence of IL-6 or other appropriate inflammatory stimuli, a fraction of Foxp3$^+$ T_{reg} cells acquire a phenotype resembling Th17 cells and this event may be associated with instability of Foxp3 expression.

Recently, we determined that T_{reg} cells can be converted into IL-17$^+$ T_{reg} cells under physiologic conditions in the absence of exogenous polarizing proinflammatory cytokines [74]. This process requires the presence of antigen presenting cells and conventional CD4$^+$ T cells. Under these conditions, stimulation of conventional and T_{reg} cells in the presence of antigen presenting cells selectively promotes the differentiation of T_{reg} but not conventional CD4$^+$ T cells into IL-17$^+$ cells. IL-1β was identified to play an essential role in the differentiation of T_{reg} cells into IL-17$^+$ cells and activation of MAPK pathways may have an active role in this differentiation process. IL-17-producing T_{reg} cells express enhanced levels of RORγt and retain Foxp3 expression, albeit at levels slightly lower compared with IL-17$^-$ T_{reg} cells isolated from the same culture conditions. Our studies strongly suggest that Foxp3$^+$ T_{reg} cells are subjected to differentiation that is imposed by other immune cells

during activation. This subset of differentiated T_{reg} cells may acquire the ability to regulate immune responses induced by specific populations of effector T cells.

In human, IL-17^+ T_{reg} cells have been identified under various conditions [13, 40, 75, 76]. Human T_{reg} cells expressing CD4, CD25 and Foxp3 but not CD45RA can differentiate into IL-17-producing cells in the presence of allogeneic antigen presenting cells and the cytokine IL-2 or IL-15. This differentiation process is enhanced by exogenous IL-1, IL-23 and IL-21, whereas IL-6 and TGF-β do not affect the emergence of IL-17^+ T_{reg} cells [13]. Although IL-1 and IL-6, were previously described as important factors in the development of human Th17 cells from conventional $CD4^+$ T cells [77], IL-1 plays the dominant role in promoting IL-17 production from T_{reg} cells [13, 75]. Several studies in human showed that these T_{reg} cells retain their suppressive function *in vitro* [40, 78]. It has also been determined that $CD45RO^+$ memory-like T_{reg} cells but not naïve T_{reg} cells are capable of producing IL-17 upon TCR-mediated stimulation in the presence of the combination of IL-1β, IL-2, IL-23 and IL-21 [40, 76]. A recent study reported that naïve $CD45RA^+$ T_{reg} cells retain stable CpG methylation across the *RORC* locus even upon prolonged ex vivo expansion and as a consequence, they display only a marginal tendency to express RORγ and develop into IL-17-producing cells. In contrast, stimulation-induced DNA demethylation of *RORC* occurs selectively in $CD45RA^-$ memory-like T_{reg} cells, irrespective of their Foxp3 expression levels [79]. This finding may explain why $CD45RO^+$ memory-like T_{reg} cells are more susceptible to conversion into IL-17^+ cells compared to naive T_{reg} cells.

Recent studies identified IL-17^+ T_{reg} cells in inflamed intestinal mucosa of patients with Crohn's disease [80]. These cells were also found to selectively accumulate in the colititc microenvironment and to associate with cancer [78]. Although the biological relevance of this conversion *in vivo* is unknown, these findings indicate the existence of IL-17^+Foxp$3^+$$T_{reg}$ cells *in vivo* and strongly suggest that these cells might be associated with progression of inflammation and development of cancer.

Conclusions

The existence and importance of T_{reg} cells have been well documented over the past decade. T_{reg} cells have the potential to be used as cell-based therapy in various conditions from bone marrow and organ transplantation to autoimmune and infectious diseases. Because of the unique function and clinical potential of T_{reg} cells, the study of T_{reg} cells represents one of the major areas of research in immunology during the last 10 years. Such effort has led to significant advances in understanding the development of T_{reg} cells and the molecular mechanisms underlying their suppressive function. However, in the past 3 years a new twist has unexpectedly developed regarding the function and fate of T_{reg} cells. It is no known that like conventional T cells, T_{reg} cells are a heterogeneous population that is not at a terminal stage of the differentiation but displays a significant degree of plasticity, which allows them to convert into cells with different effector properties. To date, only the rudimentary mechanisms of this phenomenon have been identified. Greater understanding of the mechanisms underlying this conversion process as well as the function and fate of converted T_{reg} cells might shed light to the immunopathology of immune diseases and may lead to identification of targets for pharmacological intervention for T_{reg} cell differentiation thereby improving the efficacy of T_{reg} cell-based therapies.

Highlights

T regulatory cells (Treg cells)

Treg plasticity

Treg cell specialization

Treg cell homing markers

Effector, memory Treg cells

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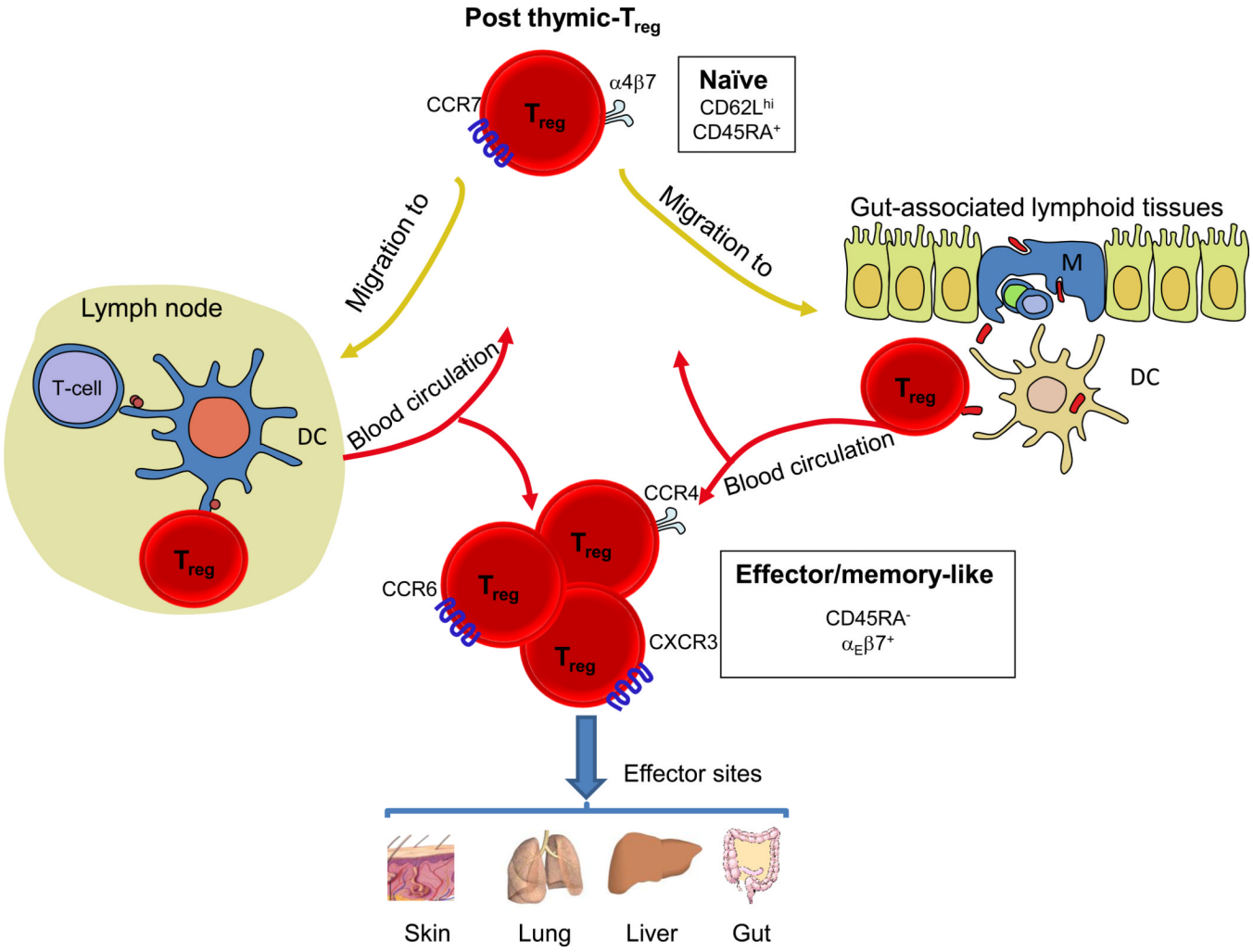

Figure 1. Post-thymic differentiation of T_{reg} cells

The majority of T_{reg} cells at birth are naïve cells and express high levels of CD62L, CCR7 and the gut homing receptor $\alpha_4\beta_7$ allowing them to migrate into secondary lymphoid organs and gut-associated lymphoid tissues where naive T_{reg} cells encounter self-antigens and commensal microbes. Subsequently, T_{reg} cells express diverse homing receptors, which render them capable of migrating to specific tissues and organs. For instance, expression of CCR4 is required for migration to skin, lung and other inflamed tissues; T-bet-dependent expression of CXCR3 is important for localization of T_{reg} cells to inflamed liver; T_{reg} cells expressing CCR6 are able to migrate to Th17 cell-mediated inflammatory sites. Upon recognition of antigen T_{reg} cells, like conventional naïve T cells, lose their naïve phenotype and become effector- or memory-like cells expressing distinct surface markers. Thus, post-thymic differentiation of T_{reg} cells occurs through a sequence of events that involve antigen recognition, migration and functional maturation, which eventually determine the specific function and fate of T_{reg} cells.

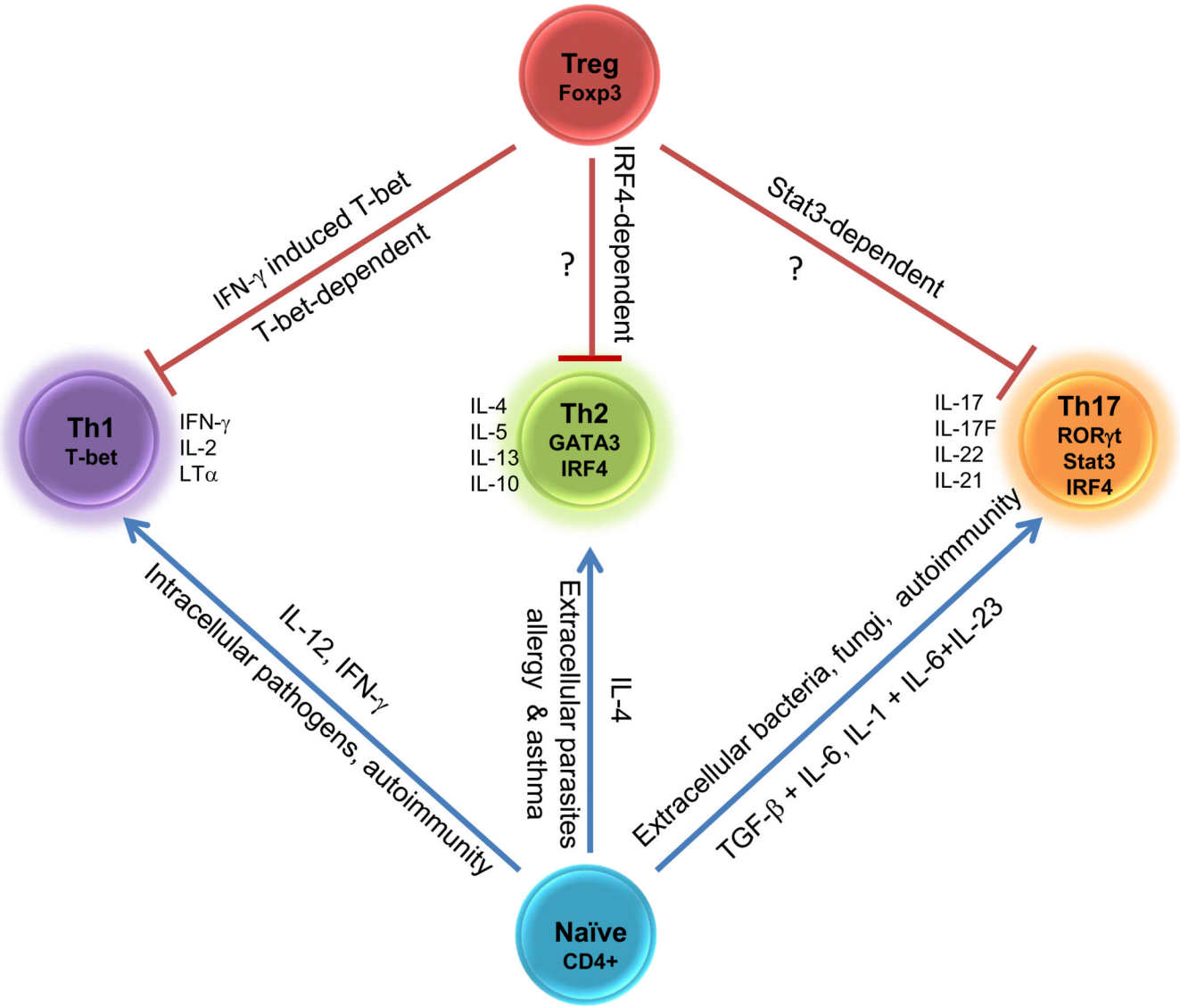

Figure 2. A model of functional specialization of T_{reg} cells

The differentiation of naïve $CD4^+$ T cells into distinct effector subsets, such as Th1-, Th2- and Th17- cells is controlled by key transcription factors that are induced by different environmental factors including the type of pathogen and cytokine milieu. It seems that T_{reg} cells can also differentiate into distinct subsets that are capable of regulating responses of individual T effector cell populations. Thymus-derived $Foxp3^+$ T_{reg} cells differentiated in the presence of IFN-γ express T-bet and specifically suppress the function and expansion of Th1 cells. IRF4 deficient T_{reg} cells are incapable of suppressing Th2 cells but retain their suppressive activity on Th1 and Th17 cells. T_{reg} cells lacking Stat3 are unable to control Th17 cell-mediated inflammation. Although the driving forces regulating expression of IRF-4 and Stat3 in T_{reg} cells have not yet been identified, it seems that T_{reg} cells might use specific molecular programs controlled by transcription factors to restrain particular types of immune responses mediated by distinct effector T cell subsets.