Treg Cell Differentiation: From Thymus to Peripheral Tissue

David M. Richards*,1, Michael Delacher*,1, Yael Goldfarb‡,1, Danny Kägebein*, Ann-Cathrin Hofer*, Jakub Abramson‡,2, Markus Feuerer*,2,3

*Immune Tolerance, Tumor Immunology Program, German Cancer Research Center (DKFZ), Heidelberg, Germany
†Faculty of Biology, Department of Immunology, Weizmann Institute of Science, Rehovot, Israel
‡Corresponding author: e-mail address: m.feuerer@dkfz.de

Contents
1. Introduction 176
2. Thymic Treg Cells 176
   2.1 Which Signals Are Important for tTreg Cell Generation in the Thymus? 176
   2.2 Which Cells Are Important for tTreg Cell Generation? 180
   2.3 Is Aire Important for tTreg Cell Generation? 183
3. Further Differentiation of Treg Cells in the Periphery 185
   3.1 Do Treg Cells Undergo Further Differentiation in the Secondary Lymphoid Tissues? 185
   3.2 Do Treg Cells Undergo Further Differentiation in Peripheral Nonlymphoid Tissues? 187
4. Future Perspectives 196
Acknowledgments 197
References 197

Abstract
Regulatory T cells (Tregs) are crucial mediators of self-tolerance in the periphery. They differentiate in the thymus, where interactions with thymus-resident antigen-presenting cells, an instructive cytokine milieu, and stimulation of the T cell receptor lead to the selection into the Treg lineage and the induction of Foxp3 gene expression. Once mature, Treg cells leave the thymus and migrate into either the secondary lymphoid tissues, e.g., lymph nodes and spleen, or peripheral nonlymphoid tissues. There is growing evidence that Treg cells go beyond the classical modulation of immune responses and also play important functional roles in nonlymphoid peripheral tissues. In this review, we summarize recent findings about the thymic Treg lineage differentiation as well as the further specialization of Treg cells in the secondary lymphoid and in the peripheral nonlymphoid organs.

1 Equally contributed first authors.
2 Equally contributed last authors.
1. INTRODUCTION

Foxp3+ regulatory T (Treg) cells are a subpopulation of CD4+ T cells, critical for the maintenance and regulation of immunological homeostasis and self-tolerance. Although Treg cells comprise only 3–5% of the peripheral blood T cell pool, they are important for regulating the activation of the immune system and preventing pathological reactivity to self (autoimmunity) and/or harmless antigens (allergy). The critical role of Treg cells within the immune system is best demonstrated by the lethal phenotypes resulting from their functional deficiency.1,2 This is well illustrated by the scurfy mice carrying a mutated Foxp3 gene, which consequently exhibit lymphoproliferation, develop fatal multiorgan autoimmunity, and die by the age of 24 days.3,4 Similarly, humans with an X-linked FOXP3 mutation suffer from the fatal immune dysregulation polyendocrinopathy enteropathy syndrome.5 Moreover, there is growing evidence that Treg cells go beyond the classical modulation of immune responses and also play important functional roles in various peripheral nonlymphoid tissues.6 In fact, it is becoming clear that tissue-specific subsets of Treg cells exist and seem to develop in response to organ-specific environmental signals. In this review, we summarize recent findings about the origin, phenotype and function of thymic, secondary lymphoid, and peripheral nonlymphoid tissue-resident Treg cells.

2. THYMIC TREG CELLS

It is now well established that there are two main pathways for the generation of Treg cells in vivo. The majority of functionally mature Treg cells are produced in the thymus, where recognition of self-antigen by certain clones leads to their deviation into the thymus-derived Foxp3+ Treg (tTreg) cell lineage. The second pathway of Treg cell generation is in the periphery, where naïve CD4+Foxp3− T cells encounter their cognate antigens and, under certain conditions, differentiate into periphery-derived CD4+Foxp3+ Treg (pTreg) cells.7

2.1 Which Signals Are Important for tTreg Cell Generation in the Thymus?

The past two decades have seen a true explosion of research into the development of tTreg cells. The early studies highlighted a central role for high-affinity T cell receptor (TCR) interactions with self-peptides in tTreg cell
development.\textsuperscript{8,9} These and several other subsequent reports set the stage for the still prominent hypothesis that developing thymocytes recognize self-antigen with high affinity, leading to clonal deviation from the conventional T cell (Tconv) fate, and differentiate into tTreg cells.\textsuperscript{10} Although interactions between the TCR and self-peptides presented by the major histocompatibility complex (MHC) are required for tTreg cell development, as demonstrated by the lack of tTreg cells in mice lacking conventional MHC molecules, the exact mechanisms that determine the choice between tTreg cell generation and negative selection of self-reactive T cells are still not defined.\textsuperscript{11} Moreover, the requirement of a high-affinity interaction has recently been put into question. Specifically, tTreg cells can be generated from thymocytes with varying TCR affinities for their cognate antigen, suggesting that the spectrum of affinities required for tTreg cell generation is much broader than previously thought.\textsuperscript{12} Nevertheless, the idea that productive tTreg cell differentiation ensues from interactions that lie between the signaling strength required for positive selection on the one side and clonal deletion on the other side still remains the prevalent view underlying the generation of Treg cells in the thymus.

Given that signaling strength plays a pivotal role in tTreg cell induction, it seems reasonable that costimulatory signals would also be required in this process. Indeed, in parallel to TCR activation, signaling via the CD28 coreceptor has also been shown to play an important part in the initiation of the tTreg cell differentiation program. Specifically, deficiency in CD28 or its corresponding ligands, CD80/CD86 (B7-1/B7-2), was shown to result in an about 80% decrease in the frequency of Treg cells.\textsuperscript{13–15} Interestingly, these mice did not develop autoimmunity, presumably because of the corresponding impact of diminished costimulation on Tconv cell activation, including self-reactive T cells.\textsuperscript{16} Moreover, CD28 was shown to have a cell-intrinsic role in the induction of \textit{Foxp3}, as well as \textit{Gitr} and \textit{Ctla-4} in double-positive (DP) thymocytes, indicating that it regulates tTreg cell development and function at multiple levels.\textsuperscript{14} Other costimulatory molecules have also been implicated in tTreg cell differentiation. Specifically, deletion of either CD154 (CD40L) or CD40 was shown to result in a threefold decrease in tTreg cell frequency.\textsuperscript{17} However, this decrease is not attributed to the direct role of CD154/CD40 in tTreg cell generation, but rather to the maintenance of tTreg cell homeostasis and survival. Another recent report suggested that tumor-necrosis factor receptor (TNFR) family members, such as GITR and OX40, could couple TCR signal strength to tTreg cell differentiation and thus support tTreg cell generation as well.\textsuperscript{18}
Cytokine signaling is another important element that regulates development of hematopoietic cells in general and T cells in particular. Although many of the initial studies focused on pTreg cell differentiation, it has now become evident that cytokines also play an important role in tTreg cell differentiation. Since both tTreg and pTreg cells are characterized by high expression of IL-2Rα (CD25), it has been hypothesized that IL-2 plays a pivotal role in all Treg cell development. Initial studies, however, demonstrated that IL-2 is not absolutely required for tTreg development, as the frequency of tTreg cells was reduced by only 50% in IL-2- or CD25-deficient mice. Nevertheless, more recent studies have begun to shed light on the role that IL-2 signaling plays in tTreg cell development. First, in mixed bone marrow (BM) chimera experiments, the frequency of tTreg cells is decreased by about fivefold in CD25-deficient, compared to CD25-sufficient donor cells, suggesting that IL-2 is particularly important in a competitive environment with normal thymocytes. Second, it is likely that the absence of IL-2 can be compensated for by other common γ-chain cytokines in the thymus, such as IL-7 or IL-15. Indeed, analysis of common γ-chain receptor-, IL-2-, IL-7-, IL-15-, STAT5-, or IL-2Rβ (CD122)-deficient mice revealed further declines in the frequency of tTreg cells. Because neither IL-7 nor IL-15 deficiency by itself affects tTreg cell production, it is the prevailing view that IL-2 is the principal common γ-chain cytokine required for tTreg cell development. However, it seems that IL-7 and IL-15 can, at least in part, compensate for its loss. This notion is supported by the in vitro finding that both IL-2 and IL-15 can support Treg differentiation from tTreg precursors. Another cytokine that seems to play an essential role in Treg cell development is transforming growth factor-β (TGF-β). Although TGF-β is known to be important for the conversion of CD4⁺Foxp3⁻ cells into pTreg cells in the periphery, it was for a long time believed to be dispensable for tTreg cell generation. This notion was mostly based on the observation that adult mice lacking TGF-βRII demonstrate fairly normal tTreg cell frequencies, while the pTreg cell pool was severely diminished. Thus, these data set the prevailing view that although TGF-β is crucial for pTreg cell homeostasis and maintenance of Foxp3 expression, it is not absolutely necessary for tTreg cell differentiation. However, subsequent studies demonstrated that T cell-specific ablation of the TGF-β-receptor results in a significant diminution of the first wave of neonatal tTreg cell production, around day 4 after birth, suggesting that Treg cell differentiation in the neonatal and adult thymus might differ in their requirement for TGF-β. Moreover, mice deficient for both TGF-β and IL-2 are completely deprived
of tTreg cells, suggesting that TGF-β might compensate for IL-2 deficiency and induce Foxp3 expression. How and when IL-2 and TGF-β signaling pathways intersect in the thymus to generate Foxp3+ cells, however, remains to be further elucidated.

The downstream mechanisms critical for tTreg cell development involve an orchestrated action of various transcription factors, which activate expression of the master regulator transcription factor Foxp3. Specifically, TCR-CD28 costimulation induces various signaling pathways that culminate in the activation of transcription factors including NFκB, AP-1, and NFAT. Indeed, inactivation of genes involved in NFκB activation such as protein kinase C-θ (PKC-θ), CARD-containing MAGUK protein 1 (CARMA1), TAK1, and IkappaB kinase (IKKb) leads to defective tTreg cell generation. Of the five NFκB family members (i.e., NFκB1, NFκB2, RelA, RelB, and c-Rel), c-Rel has been pinpointed as having the most central role in tTreg cell development. First, c-Rel is highly expressed in tTreg cells. Second, c-Rel-deficient mice show severe deficiencies in tTreg cell frequencies compared with normal frequencies in NFκB1-null mice and an intermediate reduction in RelA-deficient mice. Paradoxically, activation of the PI3K–Akt pathway, which is also downstream of the TCR-CD28 signaling, was shown to repress tTreg cell differentiation. Specifically, the inhibition of the Akt pathway was recently found to be critical for activation of the Foxo1 and Foxo3a transcription factors, which in turn translocate to the nucleus and collaborate with NFκB and other factors to induce Foxp3 transcription. Therefore, tTreg cell development seems to strongly depend on the balance between the NFκB and the PI3K–Akt signals, which are likely determined by the quality (i.e., strength and duration) of antigen stimulation. Parallel to the transcription factors directly activated by TCR-CD28 costimulation, additional transcription factors were found to operate as secondary modulators of Foxp3 expression. As already mentioned above, STAT5, a key transcription factor activated by γ-chain cytokine signaling, is essential for tTreg cell differentiation. Moreover, members of the nuclear receptor 4a (Nr4a) family of orphan nuclear receptors (Nr4a1, Nr4a2, and Nr4a3) were also found to be critical for Foxp3 expression, as triple-deficient mice were found to display a diminished tTreg cell repertoire and lethal autoimmune phenotypes. The role of TGF-β-induced transcription factors, like the Smad family, in tTreg cell development still remains controversial. The current prevalent view suggests that TGF-β/Smad signaling promotes tTreg cell generation by restraining negative selection rather than direct transcriptional control of Foxp3.
In conclusion, several models have been proposed to explain the development of tTreg cells. Based on the current knowledge, the following model seems to reflect the known aspects of Treg cell development in the thymus. In the first step, TCR stimulation of CD4 single-positive (SP) thymocytes results in the generation of CD25+Foxp3− CD4SP Treg cell precursors. As such, the TCR affinity can be high (but below the threshold that would induce negative selection), medium, or low (but not too low as to insufficiently activate the TCR). The critical element determining the tTreg cell fate decision, however, seems to be the balance between NFκB and PI3K/Akt signaling, which are both triggered by the TCR−CD28 costimulation. In the second step, the CD25+Foxp3− CD4SP Treg cell precursors need to respond to common γ-chain cytokines, predominantly IL-2, as well as to other stimuli (e.g., TGF-β), in order to initiate the transcriptional machinery controlling Foxp3 gene expression and the subsequent tTreg cell generation (Fig. 1). Interestingly, while IL-2, TGF-β, and costimulation via CD28 are all required for the development of tTreg cells, they are dispensable for Tconv cell development in the thymus, thus highlighting the unique role of these factors in controlling the tTreg cell developmental program.

2.2 Which Cells Are Important for tTreg Cell Generation?

Developing tTreg cells require interactions with other thymic populations to complete their differentiation process. As tTreg cells are mostly located within the thymic medulla, it has been proposed that the unique microenvironment provided by medulla-resident stromal cells is required for tTreg cell generation. Specifically, both medullary thymic epithelial cells (mTECs) and thymic dendritic cells (tDCs) have been identified as the key players in this process by providing both antigens for TCR stimulation and the necessary costimulatory signals required for tTreg cell development. The importance of the thymic medulla, and specifically mTECs, for controlling the tTreg cell developmental program is well exemplified by studies in which an enlarged mTEC compartment correlated with higher tTreg cell frequencies. In contrast, a severely diminished mTEC compartment, due to impaired noncanonical NFκB signaling, correlated with significantly reduced tTreg cell frequencies. Furthermore, it has been demonstrated that the CD25+Foxp3− Treg cell precursors, at the CD69+CCR7+CCR9− stage, require physical interaction with RelB+ mTECs in order to develop into mature Foxp3+ tTreg cells. Moreover, reduced MHC-II expression
on mTECs resulted in a smaller proportion of CD4 SP thymocytes being deleted and a higher differentiation rate into the tTreg cell fate. Nevertheless, the optimal environment for tTreg cell development seems to be provided by the cooperative action of both mTECs and tDCs. Specifically, it has been demonstrated that mTECs not only supply antigen to tDCs for

Figure 1 Development of tTreg cells is a multistep process. In the first step, TCR stimulation (with low, mid, or high affinities) on CD4+CD8− single-positive thymocytes results in the generation of CD25+Foxp3− CD4SP Treg cell precursors. In the second step, the CD25+Foxp3− CD4SP Treg cell precursors respond to common γ-chain cytokines, predominantly IL-2, as well as to other stimuli (e.g., TGF-β), which are provided in part by macrophages phagocytosing apoptotic T cell clones. This, together with TCR-CD28 signals, initiates transcriptional machinery involving multiple transcription factors (e.g., NFkB, Nr4a, Foxo1, STAT5) controlling Foxp3 gene expression and the subsequent tTreg cell generation. A critical element determining the tTreg cell fate decision is the balance between the NFkB and the PI3K/Akt pathways triggered by the TCR-CD28 costimulation. The development of tTreg cells does not require a dedicated APC, but rather entails a high degree of flexibility in the stromal cell types involved. The cooperative action of mTECs and tDCs, however, plays a pivotal role in this process. Aire-mediated expression of tissue-restricted antigens in mTECs is critical for the induction of a unique subpopulation of tTreg cells during the perinatal period.
subsequent cross-presentation to developing tTreg cells, but themselves act as professional antigen-presenting cells (APCs), responsible for tTreg cell generation. More recently, it was shown that mTECs and BM-derived thymic APCs contribute nonredundantly to tTreg cell generation, implying that each population presents a different array of self-antigens to developing thymocytes. In particular, Batf3-dependent CD8α+CD11c+ DCs were found to be responsible for the cross-presentation of about 50% of the Aire-dependent antigens, demonstrating an intimate and nonredundant crosstalk between thymic populations.

Other thymic populations have also been implicated in controlling tTreg cell development, including cortical thymic epithelial cells (cTECs), thymic B cells, and apoptotic thymocytes. Specifically, DP Foxp3+ cells, which express high levels of CCR7, localize in the cortex, whereas CD4 SP Foxp3+ Treg cells localize in the medulla. Specifically, experiments utilizing K14-Ab1-/- mice, with expression of MHC-II restricted to cTECs, were able to demonstrate that the thymic cortex is sufficient for supporting the generation of Foxp3+ tTreg cells and that these “cortical” tTreg cells rapidly migrate to the medulla via a CCR7-dependent mechanism. The role of thymic B cells in tTreg cell development was shown using BAFF-transgenic mice, which have a twofold increase in the number and frequency of thymic tTreg cells and an increase in thymic output as measured by Helios+Foxp3+ Treg cells in the periphery. Moreover, tTreg cell frequencies are decreased in B cell-deficient mice and BM chimeras from B cell-deficient donors into wild-type or BAFF-transgenic hosts also demonstrate a reduction in the tTreg cell population. Finally, a unique role for apoptotic thymocytes in tTreg cell development was delineated in an effort to explain the perplexing observation of delayed tTreg cell emergence on day 3 after birth. Although some previous studies suggested that the late tTreg cell onset is due to the incomplete structural organization of the thymic medulla immediately after birth, some noticed that tTreg cell emergence coincides with the previously reported massive rise in thymocyte apoptosis beginning 2 days postpartum. Hence, it was hypothesized that thymic apoptosis may play a critical role in tTreg cell development. Indeed, it has been found that TGF-β, which has been shown to be secreted by phagocytes following the engulfment of apoptotic cells, has an indispensable role at the early tTreg cell generation (discussed above). This is well illustrated by experiments where augmented apoptosis resulted in higher tTreg cell numbers and frequencies, while inhibition of apoptosis had the opposite effect, both in adult and in neonatal thymi.
In summary, tTreg cell differentiation does not seem to require a dedicated APC, but rather entails a high degree of flexibility of the stromal cell types involved. However, the cooperative action of mTECs and tDCs in the thymic medulla seems to play a pivotal role in this process.

2.3 Is Aire Important for tTreg Cell Generation?

The central role of the Autoimmune regulator (Aire) gene in the negative selection of self-reactive thymocytes is well established; however, its contribution to the selection and generation of tTreg cells has remained controversial for over a decade. Several early studies demonstrated that Aire does not impinge on Foxp3+ Treg cell generation as Aire-deficient mice exhibit unremarkable changes in Treg cell number and frequency or their proliferative and suppressive capabilities. Moreover, crossing Foxp3-mutant scurfy mice with Aire-deficient mice did not exacerbate lymphoproliferation nor did it change the profile of target organs afflicted by autoimmune attack in the scurfy mice. Therefore, the contention that Aire’s primary function is to mediate negative selection of autoreactive T cells rather than positive selection or clonal diversion of tTreg cells became the prevalent view in the field.

Nevertheless, these original studies were somewhat contrasted by the observed defects in the Treg cell compartment of human patients with autoimmune polyendocrine syndrome type 1 (APS1) who carry a mutated AIRE gene. Although these patients were found to have normal frequencies of circulating CD25hiCD4+ Treg cells, they had reduced expression of FOXP3, resulting in compromised Treg cell suppressive capacity. Moreover, several subsequent reports challenged the original studies in mice by showing mild, though significant, reduction in tTreg cell frequencies and numbers in Aire-deficient mice. The first evidence supporting the view that Aire-dependent expression of tissue-restricted antigens (TRAs) in mice regulates tTreg cell development was demonstrated using an Aire-hemagglutinin (HA) transgenic mouse, which expresses an HA peptide under the control of the Aire promoter in mTECs. Specifically, in experiments using double transgenic mice, where Aire-driven HA peptides presented on MHC-II molecules of mTECs are recognized by transgenic HA-specific T cell clones, it was demonstrated that Aire-driven expression of the HA antigen in mTECs is sufficient to generate antigen-specific tTreg cells. Moreover, HA-expressing mTECs were shown to be more effective inducers of Treg cells ex vivo than HA-expressing tDCs, implicating that
Aire-expressing cells and Aire-dependent expression of target genes in mTEC are critical for the generation of tTreg cells in mouse models. In addition, the direct involvement of Aire-dependent expression of a specific TRA in the selection of tTreg cells was recently described by the identification of a specific recurring tumor-infiltrating tTreg cell clone termed MJ23. These MJ23 Treg cells were found to develop in the thymus and to be specific for an Aire-dependent prostate antigen, rather than a tumor-specific antigen, implying that a subpopulation of Aire-dependent Treg cells may be co-opted by tumors developing within the corresponding organ. Moreover, most recent studies based on deep sequencing of the Treg cell TCRα chain repertoire in a fixed TCRβ chain model, in both Aire-sufficient and Aire-deficient mice, demonstrated that Aire is involved in both the selection process and deletion of Treg cells. Aire shaped the Treg cell TCR repertoire at the polyclonal stage and specifically affected lower frequency TCR clones, an effect that could be easily overlooked when the entire polyclonal Treg cell population, albeit restricted, is examined.

These data challenged an earlier report that suggested the Aire-dependent TRA expression in mTECs is dispensable for TCR diversity and selection of dominant TCRs on Treg cells.

Most recently, the role of Aire in shaping the tTreg cell repertoire was further elucidated in a very elegant study by the Benoist–Mathis group who identified a unique subpopulation of Aire-dependent perinatal tTreg cells that are critical for the induction of immune self-tolerance. Although the expression of Aire has previously been shown to be critical early in life for the establishment of central tolerance, the mechanism underlying this phenomenon remained unclear. Recently, it was demonstrated that Treg cell ablation using the NOD.Foxp3-DTR system during the first 10 days of life resulted in multiorgan autoimmunity typical of Aire-deficient mice on the NOD background, accompanied by severe weight loss and ultimately mortality by 24 days of age. Interestingly, such a dramatic outcome was not evident in mice depleted of Treg cells at a later time-point. To demonstrate Aire dependence of this autoimmune phenotype, Treg cells from Aire-sufficient and Aire-deficient mice were transferred into Treg cell-depleted perinates. Indeed, only Treg cells from Aire-sufficient mice were capable of protecting against the development of Aire-like autoimmunity, indicating Aire-dependent generation of Treg cells during a short perinatal window. Lineage tracing of the Treg cells using the NOD.Foxp3eGFP-CreERT2xR26Y reporter mouse, in which tamoxifen injection labels existent GFP+ Treg cells with YFP, allowed further investigation of the perinatal
Treg cell population. These double-labeled cells were shown to persist for over 2 months, and when peripheral Treg cells from perinates were transferred into Aire-deficient recipients, they were capable of significantly reversing the autoimmune phenotype. In contrast, this therapeutic effect was not achieved following transfer of GFP^+YFP^+ Treg cells from older mice or GFP^+YFP^- Treg cells of either age, demonstrating that the perinatal Treg cell population has long-term functional significance. 69

In conclusion, Aire seems to be critical for the induction of a unique subpopulation of tTreg cells during the perinatal period. 69 This, together with its well-defined role in clonal deletion of self-reactive thymocytes, extends the notion of a “layered” immune system and further blurs the virtual border between central and peripheral modes of immunological self-tolerance.

3. FURTHER DIFFERENTIATION OF TREG CELLS IN THE PERIPHERY

Following development in the thymus, Treg cells enter the periphery where they circulate through the secondary lymphoid tissues as well as the peripheral nonlymphoid tissues. Interestingly, circulating peripheral Treg cells can also reenter the thymus and control thymic tTreg output (Fig. 1).72 Treg cells in the periphery have heterogeneous expression patterns of activation/memory markers, adhesion molecules, and homing receptors. Originally, Treg cells were subdivided into subsets based on these expression patterns; however, the current Treg cell subset concept arose from evidence describing the diversity of Treg cell suppressor mechanisms. These mechanisms are dynamic and depend on the target cells, the location, and the inflammatory context. Collectively, it is becoming clear that the environmental signals present during Treg cell activation contribute to a further differentiation into unique Treg cell subsets that express genetic signatures mimicking that of their target cells or tissues (Table 1).6,73,74

3.1 Do Treg Cells Undergo Further Differentiation in the Secondary Lymphoid Tissues?

The first Treg cell subsets were identified in the secondary lymphoid tissues, i.e., spleen and lymph nodes, where they make up about 10–15% of the CD4^+ T cell pool. Treg cells were initially divided into naïve and memory subsets based on their function and differential expression of the surface molecules CD62L and CD103.75,76 It has long been known that Tconv cells develop into distinct subsets depending on the cytokine signals that are
present during their activation. For example, T helper 1 (TH1) cells develop in the presence of IFN-\(\gamma\) and IL-12, TH2 cells in the presence of IL-4, and TH17 in the presence of TGF-\(\beta\) and IL-6. These Tconv cell subsets are stabilized (and can be identified) by the differential expression of master transcription factors, such T-bet and GATA3. The potential for matched symmetry between Tconv cell subsets and Treg cell subsets has led many investigators to examine the importance of these transcription factors in Treg cell function. In fact, Treg cell-specific deletion of certain transcription factors resulted in the impaired suppression of only certain Tconv cell subsets. For example, Treg cell-specific deletion of IRF4 or GATA3 resulted in inability to suppress TH2 responses.\(^{77–79}\) In addition, Treg cell-specific deletion of STAT3 or T-bet resulted in the inability to suppress TH17 and TH1

<table>
<thead>
<tr>
<th>Location</th>
<th>Frequency (%)</th>
<th>Function(s)</th>
<th>Subset(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen and LN</td>
<td>10–15</td>
<td>Systemic autoimmune disease</td>
<td>Follicular Treg cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TH(_H)-specific subsets:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T-bet(^+) Treg cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GATA3(^+) IFN-(\gamma) Treg cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>STAT3(^+) Treg cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bcl6(^+) Treg cells</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>10–50</td>
<td>Metabolic control</td>
<td>PPAR-(\gamma)^+ Treg cells</td>
</tr>
<tr>
<td>Skin</td>
<td>30–90</td>
<td>Psoriasis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contact dermatitis</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>&lt;10</td>
<td>Asthma</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pulmonary fibrosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transplantation</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>5–40</td>
<td>Autoimmune hepatitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HCV infection</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>5–15</td>
<td>Type 1 diabetes</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>10–50</td>
<td>Postinjury muscle regeneration</td>
<td>Areg(^+) Treg cells</td>
</tr>
<tr>
<td>Gut</td>
<td>10–50</td>
<td>Intestinal barrier homeostasis</td>
<td>FFAR2(^+) Treg cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tolerance to commensal bacteria</td>
<td></td>
</tr>
</tbody>
</table>

Frequency: relative fraction of Foxp3\(^+\)CD25\(^+\) Treg cells of the CD4\(^+\) T cell pool. Functions: physiological or pathological situations in which these cells have been implicated.
responses, respectively. Finally, in germinal centers located in the secondary lymphoid tissues, the follicular Treg cell (TFR) and follicular Tconv cell (TFH) subsets develop and survive due to their expression of the transcription factors Blimp–1 and Bcl–6. In fact, Treg cell-specific deletion of Bcl–6 resulted in inability to suppress TFH responses. In summary, it is clear that specific subsets of Treg cells in the secondary lymphoid tissues are able to regulate immune responses and maintain homeostasis by specifically controlling T cell and B cell responses.

3.2 Do Treg Cells Undergo Further Differentiation in Peripheral Nonlymphoid Tissues?

Recent evidence suggests that environmental signals found in peripheral nonlymphoid tissues are also responsible for the development of tissue-specific Treg cell subsets. The proportion of tissue-specific Treg cell subsets within tissues is difficult to determine due to differences between inflammatory and steady-state conditions as well as the presence of both long-term (resident) and short-term (migratory) Treg cells.

The identification of the first tissue-specific Treg cell subset in visceral adipose tissue (VAT) opened the possibility that each tissue might harbor a distinct Treg cell subset that is responsible for controlling inflammation and maintaining tissue homeostasis. These VAT-specific Treg cells exhibited a distinct gene signature compared to Treg cells isolated from secondary lymphoid tissues. More recent reports have shown that peripheral nonlymphoid tissue-specific Treg cells can be distinguished from secondary lymphoid tissue Treg cells based on their phenotype and function. In the following sections, we will address the identification, differentiation, and function of these peripheral tissue-specific Treg cell subsets in a number of nonlymphoid tissues.

3.2.1 Treg Cells in the Adipose Tissue

Most studies about “fat” Treg cells focus on VAT-resident Treg cells. They are a dynamic population of Treg cells that increase in frequency between 5 and 25 weeks of age in mice. After reaching a plateau of about 50% of all CD4+ T cells, their frequency declines to about 10% in 40-week-old animals. In contrast, this population dynamic has not been observed in the splenic Treg cell compartment. The origin of these VAT-specific Treg cells was investigated through the analysis of the TCR repertoire and little overlap was identified between secondary lymphoid tissue- and VAT-specific Treg cells, which excluded a random influx of short-term
The VAT Treg cell TCR signatures were also unique compared to VAT-resident Tconv cells, ruling out peripheral conversion as a significant source of VAT Treg cells. Currently, it is hypothesized that an antigen-specific signal based on TCR specificity leads to retention of specific Treg cells in the VAT, followed by the expansion of these clones in situ. It has recently been reported that VAT Treg colonization happens within the first weeks of age and is later independent of thymic input. Additionally, the accumulation of VAT Treg cells depends on antigen presentation by MHC-II and soluble mediators, specifically IL-33. It is still not entirely clear how and where the specialized phenotype is generated, but it is tempting to speculate that once Treg cells reside in the VAT, they initiate a specific gene expression program leading to a discrete VAT-specific Treg cell signature, with more than 2000 genes being differently expressed between secondary lymphoid tissue- and VAT-specific Treg cells, whereby still 60% of the “typical” Treg cell signature remains intact (most notably CD25, GITR, CTLA-4, OX40, Foxp3, and KLRG1). This translates into the expression of specific chemokine receptors, namely CCR1, CCR2, CCR3, CCR5, CCR9, and CXCR6, while CCR6, CCR7, and CXCR3 are downregulated. Furthermore, VAT Treg cells acquire new functional abilities, such as taking up fat droplets via the expression of CD36, and producing more IL-10 than their splenic counterparts. Upon close examination of the VAT Treg cell gene expression signature, peroxisome proliferator-activated receptor-γ (PPAR-γ) was identified as the key driver of VAT Treg cell accumulation, phenotype, and function. This definitive study demonstrated that selective deletion of PPAR-γ in Treg cells caused only a VAT-specific decrease in Treg cell frequency and severely impinged on the specialized phenotype of VAT-specific Treg cells. Similarly, treatment of wild-type animals with a PPAR-γ inhibitor caused a decline only in VAT-resident GATA3+ Treg cells, not in splenic Treg cell frequency.

While all these changes occur under physiological (i.e., homeostatic) conditions, several studies focused on the importance of VAT-resident Treg cells in dysregulated metabolic conditions. Feeding animals a high-fat diet (HFD) leads to diet-induced obesity (DIO) with body weight increase and insulin resistance. During disease progression, an increase in the frequency and activation status of proinflammatory macrophages as well as CD4+ and CD8+ T cells can be observed. In contrast, the frequency of VAT Treg cells declined. In two sensitive loss-of-function experiments, Treg cell-depleted Foxp3-DTR mice and Treg-specific PPAR-γ-deficient mice showed an increase in proinflammatory cytokine
expression, as well as glucose and insulin levels, which indicated an emerging insulin resistance. Similar conclusions can be drawn from studies with both DIO- and leptin-deficient mice. Mitogenic anti-CD3 monoclonal antibody (mAb) was injected into HFD-fed mice and a selective increase in CD4+Foxp3+ Treg cell number was observed in VAT, almost to a level comparable to lean animal controls. After this treatment, improved glucose tolerance and insulin sensitivity, transient weight loss, and an increase in both antiinflammatory T cell and macrophage populations in VAT were observed. In leptin-deficient mice (ob/ob), the complete absence of functional leptin leads to increased body weight followed by severe insulin resistance and elevated liver enzymes. Additionally, oral and nasal administration of anti-CD3 mAb plus β-glucosylceramide showed fat-specific induction of CD4+Foxp3+ Treg cells, without systemic Treg cell expansion. Decreased amounts of proinflammatory cytokines (e.g., TNFα) were observed in fat tissue, along with decreased cellular infiltrates. On a broader scale, long-term decreases in blood glucose levels, liver enzyme levels, and blood cholesterol were also observed. Recently, another approach was used to study the impact of VAT Treg cell depletion in obesity models. It took advantage of the db/db mouse which lacks leptin receptor signaling, leading to obesity, hyperphagia, hyperglycemia, and diabetic nephropathy. Treg cells were systemically depleted with an anti-CD25 mAb, and increased fasting blood glucose levels, insulin resistance, increased proinflammatory cytokine expression, and renal cell infiltration were observed. When adoptively transferring Treg cells, insulin sensitivity was improved, CD8+CD69+ T cell infiltrates decreased, and damage to renal organs was reduced.

In summary, these studies have shown a profound ability of VAT-resident Treg cells to modulate obesity-associated insulin resistance, proinflammatory cytokine secretion, and cell infiltration. The definitive study identifying PPAR-γ also provided insight into the mechanisms of action. When treating obese animals with pioglitazone, a PPAR-γ agonist and insulin-sensitizing agent, Treg cells in VAT, but not in spleen or liver, were selectively enriched. Their gene expression profile shifted more toward the VAT-specific signature and they showed enhanced expression of CD36, enabling them to take up lipids. From these data, it is possible to infer that one of the pioglitazone-specific targets is VAT-resident Treg cells, probably enhancing their protective phenotype by increasing their frequency and potency and thereby contributing to reduced insulin resistance. This study showed, as a proof of concept, that organ-specific targeting of Treg cells is possible. Taken together, these findings strengthen the
hypothesis that VAT-resident Treg cells are pivotal agents controlling physiology and pathology of VAT. A recent study analyzed Treg cells in the brown adipose tissue (BAT). In contrast to the energy-storing white adipose tissue, the BAT and the inducible brown-in-white (brite) adipocytes are specialized in the dissipation of energy in the form of heat in a process called uncoupling thermogenesis. This study defined a BAT-specific Treg subset with implications for the regulation of energy homeostasis in response to environmental stress.

### 3.2.2 Treg Cells in the Skin

Skin-resident Treg cells, also called cutaneous Treg cells, represent a high percentage of CD4+ T cells in the skin. In humans, they can account for 40–90% of all CD4+ T cells and they are similarly high in mice. This population is maintained through migration of Treg cells into the dermis via E- and P-selectin binding in a CCR4-dependent manner as well as local induction of pTreg cells. For example, UV irradiation causes RANK-L induction in keratinocytes, which in turn drives the activation of Langerhans cells (LC). These activated LC then induce pTreg cell differentiation in an IL-10- and OX-40-dependent manner. Interestingly, there are few overlapping TCR sequences between Treg cells and Tconv cells in the skin. This is consistent with data from the VAT but opens questions as to the origin and antigen specificity of the converted pTreg cells. Skin Treg cells have a unique surface expression profile. They express CD44 and CD103, along with high levels of the chemokine receptors CCR4, CCR5, CCR6, and CCR7. In addition, they express high levels of activation markers (e.g., CTLA-4, CD25, and ICOS).

The influence of cutaneous Treg cells on the development of skin disease has been studied in both humans and mice. Several studies have investigated the importance of Treg cells during the development of contact hypersensitivity (CHS) in mice, a model for contact dermatitis (reviewed in Ref. 101). CHS begins with the “sensitization phase,” where hapten-linked epidermal peptides are presented to naïve T cells in skin-draining lymph nodes. This is followed by the “elicitation phase,” where reexposure of the skin to haptens causes antigen-specific inflammation. Treg cell depletion results in increased skin inflammation and it has been demonstrated that Treg cells can influence both phases of CHS development. For example, Treg cells can control the magnitude of T cell activation during sensitization. Furthermore, several studies showed that intravenous administration of Treg cells 1 day before elicitation can suppress inflammatory cell infiltration in an
IL-10-dependent manner. Finally, the selective depletion of Treg cells during elicitation resulted in enhanced and prolonged pathology, again indicating the importance of Treg cells in CHS.

Another very prevalent immune-mediated skin disease in humans is psoriasis and it is characterized by thickening of the epidermis and massive infiltration of both myeloid and lymphoid cells. There are conflicting reports about the role of Treg cells in this disease, but a general correlation has been established from patient studies showing increasing numbers of infiltrating Treg cells and disease severity. It is hypothesized that, even though enriched in psoriatic lesions, these Treg cells have impaired regulatory function and/or effector cells can resist their suppressive effects. Finally, it has also been observed that patient-derived psoriatic Treg cells differentiate easily into Th17 cells in vitro, in contrast control Treg cells, which raises questions about the origin and stability of this population. In the study of another human autoimmune skin disease, Bullous pemphigoid (BP), a significant reduction of Treg cells in patients has been observed in the peripheral blood. This is similar to findings in patients suffering from systemic sclerosis and morphea, a connective tissue disease with autoimmune involvement. These patients have fewer Treg cells in both skin lesions and peripheral blood, fewer IL-10- and TGF-β-producing cells in skin, and lower serum IL-10 and TGF-β levels. The same observation has been made in dermatomyositis, a rare disease affecting muscles and skin. Here, Treg cell frequency and overall TGF-β-producing cell numbers are reduced in peripheral blood and skin lesions.

Taken together, these findings in both mouse models (CHS) and human patients (psoriasis, BP, systemic sclerosis, and dermatomyositis) show an important role of Treg cells for skin-related physiology and pathology. Whereas, in most cases, the sheer reduction in Treg cell frequency in skin might promote a more severe autoimmune phenotype, psoriasis disputes this dogma by displaying an even increased frequency of Treg cells in skin lesions. It will be important to determine a specific phenotype of cutaneous Treg cells to understand their molecular makeup and identify key transcription factors responsible for their adaption to the cutaneous environment. This might enable a deeper understanding of their pathophysiological role in healthy and autoimmune-inflamed skin disease.

### 3.2.3 Treg Cells in the Lung

Respiration causes a constant influx of many immunogenic antigens such as plant and animal proteins. Therefore, the lung depends on complex systems
to ensure tolerance to harmless antigens while guaranteeing protection against pathogens. Anatomically, the lung can be separated into conducting airways and lung parenchyma. Ciliated and secretory epithelial cells, DCs, macrophages, and CD8+ T cells are abundant in these tissues. In addition, the presence of bronchial–associated lymphoid tissue (BALT) as local means to support B cell and T cell proliferation has been described.\textsuperscript{109} In this secondary lymphoid tissue, homing of Treg cells in a model of pulmonary fibrosis (PF) in CCR7-deficient animals has been observed.\textsuperscript{110} But the steady-state presence, frequency, and function of Treg cells in BALT remain elusive. The alveolar space is mostly populated by macrophages. The underlying parenchyma also contains macrophages, along with DCs, B cells, T cells, and mast cells.\textsuperscript{109} When measuring Treg cell frequency in the lung, many studies do not discriminate between airway and parenchyma. Hence, it has been observed that Treg cells account for only a small percentage of CD4 T cells in the murine (<10% of CD4 T cells\textsuperscript{109}) and human lung (<3%\textsuperscript{111}) under physiologic conditions. They have been reported to highly express CCR4, with less than 15% being CD103+ as well.\textsuperscript{109}

Treg cells in lung tissue have mostly been studied in the context of PF, asthma, and transplantation. In PF, repeated inflammatory and regenerative phases cause excessive collagen deposition and lung scarring, which decreases oxygen-uptake capacity. CCR7-deficient mice challenged with bleomycin to induce PF showed increased numbers of Treg cells, indicating that loss of CCR7 might retain Treg cells in the inflamed lung and thereby decrease inflammation and pathology.\textsuperscript{110} Lung-resident Treg cells have also been implicated in asthma. Here, genetic and environmental factors produce chronic inflammation of the lung. If there is a failure of Treg cell recruitment, excessive tissue repair and wound healing indicative of chronic asthma prevail.\textsuperscript{112} In asthmatic children, a decrease in Treg cell frequency in the bronchoalveolar lavage (BAL) fluid has been described, along with an impaired ability of Treg cells to suppress TH2 responder cells.\textsuperscript{113} Controversially, other studies show increased Treg cell numbers in patients with severe allergic pneumonitis, but they show impaired suppressive capacity.\textsuperscript{114} Interestingly, treatment with steroids has been shown to promote Treg cell activity and might partially explain the therapeutic benefit of this treatment.\textsuperscript{113} Next to chronic pulmonary disease, Treg cells have also been studied in the context of lung transplantation. It has been reported that increased numbers of Treg cells in the transplanted tissue correlate with protection from acute rejection. Once a rejection episode is resolved, Treg cell numbers decrease in frequency. Furthermore, it has been noted that Treg cells
infiltrate lung tissue before or at the same time as effector T cells, enabling
Treg cell frequency in BAL fluid to be used as a prognostic marker of rejec-
tion and bronchitis obliterans syndrome. In summary, lung-resident Treg
cells are an attractive target to modulate disease severity in common airway
diseases such as PF and asthma. In a recent study, the protein kinase CK2 has
been implicated to be specifically required for lung-associated Treg cells to
prevent excessive TH2 responses in the lung. This example highlights the
need for a more detailed profiling of the lung-associated Treg cell
populations to evaluate their detailed molecular phenotype and to develop
novel treatment strategies.

3.2.4 Treg Cells in the Liver

Immune tolerance is an essential requirement for the homeostasis of the liver
for two main reasons. First, the liver is permanently exposed to food and
microbial products from the intestine. Second, as a highly metabolic
organ, the liver produces a multitude of self-antigens. Therefore, a well-
performing self-tolerance system mediated by, among others, Treg cells is
important.

The observation that the liver is a unique organ in regard to immune
tolerance was first shown by the “liver tolerance effect” in 1967 when it
was demonstrated that liver allografts were accepted even with MHC mis-
matches without immunosuppression. Furthermore, nonliver allografts
were accepted if cotransplanted with a liver from the same donor. It
has also been shown that expression of extra-hepatic antigens in the liver
results in conversion of Tconv cells to highly suppressive pTreg cells that
inhibit immune reactions to the antigen in affected organs.

The frequencies of Treg cells among CD4 T cells range from 5% to 10%
in mice and can reach up to 30–40% in chronic hepatitis C virus
(HCV)-infected patients. Liver Treg cells were shown to express higher
levels of CTLA-4, GITR, and CD103 than splenic Treg cells, thus resem-
bling an effector/memory Treg cell phenotype. Additionally, they were able
to suppress hepatitis development in a TGF-β-dependent manner. It was
also observed that frequencies of liver-specific Treg cells increased up to 20%
in C57BL/6 mice after concanavalin A (ConA) treatment in a ConA-
induced hepatitis model.

Studies have shown an important role of Treg cells in the prevention
of autoimmune hepatitis (AIH). For example, a reduction of Treg
cell frequencies was identified in patients with AIH, in addition to an
inverse correlation between Treg cell numbers and clinic markers such as titers of liver-related autoantibodies. The functional properties of Treg cell-mediated suppression have also been analyzed and showed that direct contact of Treg cells to target cells leads to secretion of IL-10 and TGF-β, both of which are important for the prevention of AIH.

Besides AIH, Treg cells are also crucial during the progression of hepatic cancer and chronic infections of HCV. In hepatic cancer, several studies showed that higher numbers of Treg cells in the lesion are associated with cancer progression and poor prognosis for patients. In fact, the balance between CD8+ T cells and Treg cells seems to be important for overall and disease-free survival. Comparable tendencies have been observed during chronic HCV infections. Here, high numbers of Treg cells are associated with virus persistence. It was shown that Treg cells directly suppress HCV-specific CD8+ T cells in an IL-10- and TGF-β-independent, but cell contact-dependent, manner. In summary, Treg cells are essential players in the immune tolerance of the liver. Although their roles under pathological conditions have been well studied, it will be important to further investigate their function under physiological conditions.

3.2.5 Treg Cells in the Pancreas

The prominent role of Treg cells in the pancreas is highlighted by the observation that patients and mice lacking Treg cells (e.g., due to mutations in the Foxp3 gene) develop Type 1 diabetes (T1D). Self-reactive T cells destroy the insulin-producing β-cells of the pancreas and thereby initiate T1D. Most studies about pancreatic Treg cells focus on this situation.

It was shown that the frequency of Treg cells in the pancreas of NOD mice, a mouse strain that spontaneously develops T1D, was at about 10% of CD4+ T cells. Transcriptional analysis of pancreatic Treg cells revealed a higher expression of IL-10, GITR, and ICOS compared to lymph node Treg cells. Furthermore, an upregulation of CD103, IL1R2, S100a6, and the chemokine receptors CCR5, CXCR3, and CCR2 were found. Chemokine receptors, like the aforementioned, play a pivotal role in the homing and circulation of immune cells. For CXCR3, it was observed that it is important for homing of T cells into inflamed islets in NOD mice. Similarly, it was shown that double-stranded adeno-associated virus serotype 8 (dsAAV8) vector-mediated expression of CCL22 (ligand of CCR4) in islets leads to Treg cell recruitment and prevention of diabetes onset.
Besides the transfer of *ex vivo* expanded Treg cells, strategies targeting the IL-2 signaling pathway are promising for the treatment of T1D. In several studies, it was shown that low-dose IL-2 treatment or IL-2 produced by activated effector T cells can boost Treg cell activation/expansion and thus reverse established diabetes in NOD mice.\textsuperscript{139,140}

Another disease setting in which Treg cells are involved is the development of pancreatic ductal carcinoma. A significantly higher number of Treg cells were found in ductal carcinoma lesions compared to normal pancreas tissue, and poor prognosis was associated with high Treg frequency in the lesion.\textsuperscript{141} Moreover, these tumor-infiltrating Treg cells secrete TGF-\(\beta\) and IL-10.\textsuperscript{142}

In summary, pancreatic Treg cells play a decisive role in the immune homeostasis of the pancreas and imbalances in their frequencies or function can lead to severe pathologies such as T1D. Currently, most observations are derived from disease models, where migratory Treg cells are also being recruited. The specialization and function of long-term tissue-resident Treg cells in the pancreas are still unclear.

### 3.2.6 Treg Cells in the Muscle Tissue

Treg cells are only a small cell population in healthy muscle tissue, about 10\% of all muscle-resident CD4\(^+\) T cells. Since they are very limited, no molecular characterization is yet available for muscle-resident Treg cells under physiological conditions. Therefore, this specific Treg cell population is mostly studied under pathological conditions, such as muscular dystrophies or induced muscle injuries, where accumulation of proinflammatory CD4\(^+\) and CD8\(^+\) T cells seems to promote disease progression.\textsuperscript{143,144} In a recent study, acute muscle injury was induced via the administration of cardiotoxin, which causes myofiber necrosis. Interestingly, Treg cells rapidly accumulated in skeletal muscle and increased in frequency to about 50\% of all CD4 T cells within 7 weeks and showed a distinct gene expression profile.\textsuperscript{145} Similarly, Treg cells are also elevated in dystrophic muscles of mice harboring a muscular dystrophy (mdx) mutation, leaving them deficient of dystrophin, and human Duchenne muscular dystrophy patients. Under these conditions, muscle Treg cells display an activated phenotype in both mice and humans, and their selective depletion in mdx mutant mice leads to enhancement of immune cell infiltration and type 1 inflammatory response, resulting in even more impaired myofiber repair.\textsuperscript{145} In contrast to this, treatment with IL-2/anti-IL-2 mAb complexes increased Treg cell frequency and IL-10 concentrations in muscle tissue, decreasing myofiber injury.\textsuperscript{146}
Moreover, osteopontin seems to regulate Treg cell number and/or stability since muscles from osteopontin–deficient and mdx mutant mice display increased Treg cell numbers along with decreased fibrosis. Manipulation of mdx–dystrophic mice to increase or decrease muscle Treg cell numbers resulted in improved or impaired muscle repair, respectively. The muscle–repair factor amphiregulin was identified to be expressed specifically by muscle-specific Treg cells, enhancing the differentiation of muscle cell precursors and lowering the expression of proteins associated with fibrosis. In conclusion, muscle-specific Treg cells play an important role once muscle damage has occurred. They can limit damage by suppressing the development of type 1 inflammatory responses and express factors to actively enhance muscle repair and thereby promote faster recovery.

3.2.7 Treg Cells in the Gastrointestinal Tract

The gut contains a large reservoir of both secondary lymphoid tissue-resident Treg cells as well as nonlymphoid tissue-resident Treg cells. In addition, it is the main location of pTreg cell generation. Both tTreg and pTreg cells are necessary for maintaining intestinal barrier homeostasis, especially in the colon. Lamina propria-resident Treg cells can be distinguished from secondary lymphoid tissue Treg cells by the expression of free fatty acid receptor 2 (FFAR2, also known as GPR43). The development of these gut tissue-specific Treg cells is dependent on unique environmental signals, which require products produced by commensal bacteria. For example, short-chain fatty acids (SCFA), products of bacterial metabolism, are required for the maintenance of intestinal Treg cell homeostasis, including homing and proliferation potential. Importantly, it was shown that these effects were dependent on Treg cell expression of FFAR2, the receptor for SCFA. In summary, these reports show that specialized Treg cells in the gut and commensal bacteria have developed unique communication ways to limit immune activation and establish gut homeostasis.

4. FUTURE PERSPECTIVES

The differentiation of Treg cells in the thymus depends on the antigen repertoire that is available at a given time-point. This concept is highlighted by a recent study showing that a unique subpopulation of Aire-dependent perinatal tTreg cells, critical for the induction of immune self-tolerance, can only be generated during the perinatal phase. It is not entirely clear if this first wave of Treg cells is indeed critically to seed all peripheral tissues. It is
tempting to speculate that such a perinatal wave would once colonize the peripheral tissues in an antigen-specific fashion and could be self-sustained. This would be in analogy to the tissue-resident macrophages, which are believed to be of yolk sac and fetal liver origin and fairly independent of additional input of monocyte-derived macrophages under steady-state homeostasis. The difference, of course, is the antigen specificity of the TCR. On the other hand, tissue-resident memory CD8+ T cells can be established after, e.g., an infection in the skin during adulthood. Therefore, tissue-resident Treg cells might have a dual origin, a perinatal one and some tissue Treg cells may be established as a result of a specific tissue need in the adulthood.

The proportion of tissue-specific Treg cells within tissues is difficult to determine and characterize, especially in pathological conditions due to the presence of both long-term (resident) and short-term (migratory) Treg cells. In the future, the field needs to establish more precisely the molecular mechanisms that are required to differentiate into different subsets of tissue-resident Treg cells. PPAR-γ and VAT Treg cells are a good example for such a tissue-specific modifier. This information will yield specific markers and novel targets to modify Treg cells in a tissue-specific manner.

ACKNOWLEDGMENTS

This review was supported by grants from the Helmholtz Association of German Research Centers (HGF; HZ-NG-505) to M.F.; the German-Israeli Helmholtz Research School in Cancer Biology to M.D.; the DKFZ Postdoctoral Fellowship Program to D.K.; the Cooperation Program in Cancer Research of the DFKZ and Israel’s Ministry of Science, Technology and Space (MOST) to M.F., A.-C.H., and J.A.; Israel Science Foundation and Sy Syms foundations to J.A.

REFERENCES


