

Regulatory T Cells: Banned Cells for Decades

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Regulatory T cells (Tregs), either thymic derived or peripherally induced, suppress a variety of physiological and pathological immune responses, and the absence of this cell subset has been shown to result in severe systemic autoimmunity. Since their acceptance almost two decades ago, intensive research aiming to characterize the phenotype, to elucidate the suppressive activity, and to decipher the migratory behavior of Tregs has been performed. A substantial number of studies, however, focused on understanding whether defects in Treg numbers and function contribute to the development and progression of inflammatory, autoimmune, and malignant disorders, and how Treg numbers/function might be modulated to treat patients with autoimmune diseases or cancer. In the skin, an organ that is constantly exposed to the environment, Tregs are known to be critically involved not only in the maintenance of skin homeostasis but also in the regulation of cutaneous immune responses. In this review, we present an overview on recent data concerning Treg development and expansion, the molecular mechanisms underlying their immunosuppressive activity, and the modulation of Treg function. Furthermore, we discuss the role of Tregs in cutaneous inflammatory and autoimmune disorders.

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INTRODUCTION

Immune responses need to be controlled and be turned down after completion, as without downregulation immune reactions are at high risk to become pathologic. In the past, CD4⁺CD25⁺ regulatory T cells (Tregs) have been shown to have a crucial role in dampening immune responses against pathogens and in maintaining immunological self-tolerance

and immune homeostasis. In addition, Tregs represent an important cellular mechanism by which tumors evade immunosurveillance (Sakaguchi, 2005; Shevach, 2009). Hence, as key regulators of immune responses, Tregs have to be considered as therapeutic targets. Manipulation of numbers and/or suppressive activity of Tregs was shown to be useful in the treatment of various diseases, including autoimmune disorders, allograft rejection, graft-versus-host disease, and cancer (Shevach, 2009; Chen and Oppenheim, 2011). Accordingly, depletion of Tregs led to severe systemic autoimmunity and provoked immune responses to commensal bacteria in the intestine, causing inflammatory bowel disease (Singh *et al.*, 2001; Ohkura *et al.*, 2011), but on the other hand resulted in efficient antitumoral or antimicrobial immunity (Belkaid and Rouse, 2005). Moreover, antigen-specific expansion of Tregs was used to treat allergic diseases and establish immunological tolerance to organ grafts, as well as prevent graft-versus-host disease after bone marrow transplantation (Ohkura *et al.*, 2011).

Naturally occurring Tregs constitute 5–10% of total CD4⁺ T cells in mice and healthy humans, are produced in the thymus as a distinct mature subpopulation of T cells, and are characterized by the expression of the forkhead family transcription factor, Foxp3, as well as characteristic cell-surface markers, including a variety of co-stimulatory and co-inhibitory molecules (CD28, cytotoxic T lymphocyte antigen 4 (CTLA-4), inducible co-stimulator, etc.), tumor necrosis factor (TNF)/TNF receptor family members (RANKL, GITR, OX40, 4-1BB, etc.), chemokine and Toll-like receptors (CCR2, CCR4, CCR5, CCR6, CCR7, CCR8, CXCR3, CXCR4, TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, TLR8, and TLR9), and a variety of immunosuppressive cytokines (IL-10, transforming growth factor (TGF)- β , IL-35, etc.; reviewed in Lu and Rudensky, 2009; Shevach, 2009; Campbell and Koch, 2011; Chen and Oppenheim, 2011; Ohkura *et al.*, 2011). In addition to naturally occurring thymic-derived CD4⁺CD25⁺Foxp3⁺ Tregs, Foxp3⁺ T cells with suppressive activity, including IL-10-secreting Tr1 cells or TGF- β -induced Th3 cells, have been described (Mills and McGuirk, 2004; Wu *et al.*, 2007), which are thought to have a significant role in the maintenance of peripheral tolerance. However, at present, little is known about the contribution of Foxp3⁺ Tregs to ordinary immune responses and to sustaining self-tolerance. Thus, this review will focus on Foxp3⁺ Tregs and discuss the characteristics and migratory behavior of this cell subset.

COMPLEX CONTROL OF FOXP3 EXPRESSION

Initially, the Foxp3 gene was identified in *Scurfy* mice, and mutations in the Foxp3 locus were associated with X-linked autoimmune disease, dysregulated T-cell function,

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Abbreviations: AD, atopic dermatitis; CTLA-4, cytotoxic T lymphocyte antigen 4; LC, Langerhans cell; SLE, systemic lupus erythematosus; TGF- β , transforming growth factor; TNF, tumor necrosis factor; Treg, regulatory T cells

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hyperactivation of CD4⁺ T cells, and overproduction of pro-inflammatory cytokines (Brunkow *et al.*, 2001). In humans, mutations in the *FOXP3* gene have been linked to Treg deficiency, causing a severe systemic autoimmune disease called immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (Bennett *et al.*, 2001; Gambineri *et al.*, 2008). In mice and humans, Foxp3 has a crucial role for the function of Treg, as ectopic retroviral transduction of the *Foxp3* gene into conventional CD4⁺CD25⁻ T cells converts them to CD4⁺CD25⁺ Tregs able to suppress the proliferation of effector T cells *in vitro* and to inhibit the development and progression of systemic T-cell-mediated autoimmune disorders *in vivo* (Fontenot *et al.*, 2003; Hori *et al.*, 2003; Loser *et al.*, 2005a). Moreover, after retroviral Foxp3 transduction, CD4⁺ T cells upregulated the expression of Treg-associated markers such as CTLA-4, CD25, and neuropilin-1 (Loser *et al.*, 2005a). The functional and phenotypic hallmarks of CD4⁺ T cells ectopically expressing Foxp3 are similar to those observed in naturally occurring thymic-derived Tregs, indicating that Foxp3 is indeed a key regulator of Treg development and Treg function. However, in humans, FOXP3 can be induced upon activation in conventional CD4⁺ T cells, which do not show any suppressive activity, indicating differences in the regulation of Foxp3 expression between mice and humans, and, furthermore, suggesting that FOXP3 is less reliable in defining human Tregs than mouse Tregs (Allan *et al.*, 2007; Tran *et al.*, 2007; Miyara *et al.*, 2009).

During Treg development in the thymus, Foxp3 expression is controlled by signaling pathways, including TCR signaling, co-stimulation, cytokine production, and binding of various transcriptional activators to the Foxp3 promoter (Kim and Leonard, 2007; Samon *et al.*, 2008; Tone *et al.*, 2008; Kim *et al.*, 2009). After TCR engagement with self-peptide/major histocompatibility complex ligands, Foxp3 gene expression is initiated by the binding of the transcription factors, activator protein 1, nuclear factor of activated T cells, cyclic AMP-responsive element-binding protein, and activating transcription factor, to the promoter region or to an intronic Foxp3 sequence proposed to serve as an enhancer (Mantel *et al.*, 2006; Kim and Leonard, 2007; Lu and Rudensky, 2009). Besides TCR signaling and transcription factor binding, CD28/B7-1- or CD28/B7-2-mediated co-stimulation is required to stabilize Foxp3 expression, as the absence of either CD28 or its ligands B7-1 and B7-2 results in reduced numbers of Foxp3⁺ thymocytes and peripheral Tregs (Loser *et al.*, 2005b; Tai *et al.*, 2005). Moreover, *in vitro* studies have shown that increased activation of the Akt (acutely transforming retrovirus AKT8 in rodent T-cell lymphoma)-mammalian target of rapamycin pathway (a main target of CD28 signaling) caused impaired Foxp3 expression and thymic Treg generation (Haxhinasto *et al.*, 2008). In contrast, inhibition of phosphatidylinositol-3-kinase, and thereby of Akt activation, induced Foxp3, and mice with an inactive phosphatidylinositol-3-kinase isoform showed significantly increased numbers of thymic Treg (Patton *et al.*, 2006; Sauer *et al.*, 2008). TCR and co-stimulatory signals seemed insufficient to induce Foxp3 expression and functional Treg

in the thymus, as mice lacking IL-2 or the IL-2 receptor developed a severe lymphoproliferative disease and exhibited significantly reduced numbers of Foxp3⁺ Tregs (Furtado *et al.*, 2002; Malek *et al.*, 2002, 2008; Fontenot *et al.*, 2005; Burchill *et al.*, 2007; Vang *et al.*, 2008), suggesting a two-step model for Treg generation in the thymus. First, a TCR signal in combination with the interaction of co-stimulatory molecules results in the upregulation of CD25 and the development of a Treg precursor, which in a second step receives an IL-2 signal leading to the induction of Foxp3 expression. Of note, IL-2-mediated induction of Foxp3 in Tregs requires the activity of Jak 1 and 3 (JAK1/JAK3) and the signal transducer and activator of transcription 5 (STAT5; Burchill *et al.*, 2008; Lio and Hsieh, 2008; Lu and Rudensky, 2009), as *Jak3*-deficient mouse mutants lack Foxp3 expression completely and conditional knockout mice lacking STAT5 in thymocytes show strongly reduced numbers of Foxp3⁺ cells, whereas overexpression of a constitutively active form of STAT5 results in the expansion of Treg (Burchill *et al.*, 2007, 2008; Yao *et al.*, 2007).

Besides IL-2, TGF- β was demonstrated to be important for Treg generation. Although the contribution of TGF- β to thymic development of Tregs is still discussed intensely, TGF- β is central for the homeostasis of naturally occurring Tregs, as well as for Foxp3 upregulation and induction of suppressive activity in conventional CD4⁺ T cells (Chen *et al.*, 2003; Zheng *et al.*, 2004; Marie *et al.*, 2005; Liu *et al.*, 2008). However, this acquired Treg phenotype is unstable, as most of the TGF- β -induced Tregs lose Foxp3 expression and suppressor function in the absence of the cytokine, indicating that stable Foxp3 expression is a prerequisite for the maintenance of suppressive activity in Tregs (Floess *et al.*, 2007; Huehn *et al.*, 2009). More recently, epigenetic regulation was identified as a crucial factor for controlling stable Foxp3 expression by modulating the accessibility of the *Foxp3* promoter for binding of transcription factors (Huehn *et al.*, 2009). In particular, the methylation of three distinct and highly conserved CpG motifs in the human and mouse Foxp3 promoter results in the condensation of chromatin, whereas demethylation of these CpG motifs leads to the relaxation of chromatin and thereby to an increased transcriptional accessibility of the *Foxp3* gene (Huehn *et al.*, 2009). In naturally occurring thymic-derived Tregs, the CpG motifs of the Foxp3 promoter are demethylated almost completely, whereas they are methylated in resting conventional CD4⁺ T cells (Kim and Leonard, 2007; Janson *et al.*, 2008), indicating an amplified Foxp3 transcription in Tregs. Interestingly, demethylation of the Foxp3 promoter was found to be increased in conventional CD4⁺ T cells after TGF- β treatment, whereas neutralizing TGF- β upregulated the methylation of CpG motifs in the Foxp3 promoter, and thus completely abrogated the transient expression of Foxp3 (Tran *et al.*, 2007). Together, these data suggest a role for DNA demethylation in the regulation of stable Foxp3 expression in thymic-derived natural Tregs. In contrast, T cells that express Foxp3 only transiently, such as TGF- β -induced Tregs, are characterized by a methylated Foxp3 promoter (Baron *et al.*, 2007; Floess *et al.*, 2007; Polansky *et al.*, 2008).

MECHANISMS OF TREG-MEDIATED SUPPRESSION

Tregs inhibit the differentiation, activation, proliferation, cytokine secretion, or migration of conventional T cells. However, despite the increasing evidence of Tregs' involvement in immune regulation, relatively little is known about the molecular mechanisms underlying Treg-mediated suppression. By performing *in vitro* suppression assays or by using various mouse models and intravital microscopy, many mechanisms for Treg-mediated suppressor function have been suggested (Sakaguchi *et al.*, 2009; Shevach, 2009). In particular, *in vitro* assays revealed a cell contact-dependent mechanism, as Tregs separated from the responder cells by a semipermeable membrane were unable to inhibit effector cell proliferation, and, moreover, culture supernatants from antigen-stimulated Tregs failed to exhibit suppressive activity (Takahashi *et al.*, 1998; Thornton and Shevach, 1998). When analyzing the cell contact-dependent suppressor mechanism in more detail, Grohmann *et al.* (2002) could show that Tregs induce the expression of indoleamine 2,3-dioxygenase in dendritic cells, a potent regulatory molecule mediating the catabolism of the amino acid tryptophan into the proapoptotic kynurenine, which is particularly toxic to T cells. Notably, this process was dependent on CTLA-4, a surface marker constitutively expressed on Tregs, as blocking CTLA-4 signaling by anti-CTLA-4 reversed the Treg-mediated suppression of effector T-cell proliferation (Thornton *et al.*, 2004). To strengthen the relevance of CTLA-4 for the suppressive activity of Treg, Wing *et al.* (2008) selectively deleted CTLA-4 and demonstrated that mutant mice with a deletion of CTLA-4 expression in Tregs developed a systemic autoimmune disease similar to that observed in *Scurfy* mice. Cellular analysis revealed that the CTLA-4 deficiency altered neither the thymic development nor the peripheral homeostasis of Tregs but downregulated their suppressive activity. Thus, CTLA-4 deficiency in Tregs alone was sufficient to abrogate its suppressor function (Wing *et al.*, 2008; Shevach, 2009).

Another suppressive mechanism used by Tregs to inhibit effector T-cell differentiation and proliferation is the catalytic inactivation of adenosine triphosphate (ATP) by CD39, which represents the dominant ectoenzyme in the immune system, hydrolyzing tissue-destructive ATP to AMP (Borsellino *et al.*, 2007). Notably, as retroviral transduction of CD4⁺CD25⁻ conventional T cells with Foxp3 induced the expression of CD39, and CD39-deficient Tregs were dysfunctional, proliferated in response to anti-CD3/anti-CD28 stimulation, and failed to suppress the proliferation of responder T cells, the investigators concluded that CD39, and thus the degradation of ATP to AMP, might be crucial for the Treg function (Shevach, 2009). These observations were proven in a cutaneous contact allergy model, showing that Tregs expressing CD39 efficiently suppressed the ear swelling response in mice upon challenge with an obligate contact allergen, whereas CD39-deficient Tregs were not suppressive (Ring *et al.*, 2009). Interestingly, in allergen-challenged ears CD39-expressing Tregs inhibited the adhesion of activated effector T cells to inflamed endothelium by downregulating the expression of E- and P-selectin on vascular endothelial cells (Ring *et al.*, 2009, 2010), blocking ear swelling and erythema.

In another approach, several groups used DNA microarray analyses to elucidate the molecular mechanisms of Treg suppressive activity. Comparing the gene expression profiles of Tregs with those of naive or recently activated T cells revealed a substantial number of genes. Among these are TNF family members such as the glucocorticoid-induced TNF receptor-related protein (GITR, CD357), OX40 (CD134), and 4-1BB (CD137; McHugh *et al.*, 2002; Shimizu *et al.*, 2002; Valzasina *et al.*, 2005; So *et al.*, 2008), which were controversially presented as negative regulators of Treg-mediated suppression. All three markers are constitutively expressed on Tregs but are also upregulated on Foxp3⁻ effector T cells upon antigen stimulation, and it has been claimed that engagement of GITR, OX40, or 4-1BB reverses the suppressor function of Tregs (Stephens *et al.*, 2004; Valzasina *et al.*, 2005; So *et al.*, 2008). Accordingly, preincubation of Tregs with an agonistic anti-OX40 antibody, e.g., resulted in the loss of suppressor function in an *in vivo* mouse model of graft-versus-host disease and, moreover, led to increased antitumoral immunity, as evidenced by complete rejection of established tumors (Piconese *et al.*, 2008). In addition, gene expression studies of Tregs compared with naive or effector CD4⁺ T cells enabled the identification of suppressive humoral factors such as the galactoside-binding proteins, galectin-1 and galectin-10, or the immunosuppressive cytokine IL-35, which is a member of the IL-12 family and a heterodimer comprising Ebi3 and IL-12 α /p35 (Collison *et al.*, 2007; Garin *et al.*, 2007; Kubach *et al.*, 2007; Tang and Bluestone, 2008; Vignali *et al.*, 2008). Interestingly, antibody-mediated blockade of galectin-1 or galectin-10 reduced significantly the inhibitory effects of human and mouse Tregs; moreover, Tregs from galectin-1-deficient mouse mutants showed a reduced suppressive activity compared with Tregs from wild-type controls, suggesting that galectins are essential for Treg function (Garin *et al.*, 2007; Kubach *et al.*, 2007). IL-35 is constitutively expressed in Tregs, and it has been demonstrated that ectopic expression of IL-35 confers suppressive activity to naive CD4⁺ T cells. Furthermore, treatment of naive human or mouse T cells with IL-35 induced a regulatory phenotype, whereas recombinant IL-35 suppressed T-cell proliferation *in vitro*, as well as *in vivo*, suggesting IL-35 as a cytokine required for maximal suppressive activity in Tregs (Collison *et al.*, 2007, 2010). Together, these findings indicate that several mechanisms, including cell contact-dependent mechanisms and those mediated by soluble factors, are involved in Treg suppressor function. However, most of the suppressive mechanisms have been elucidated *in vitro*. Further *in vivo* studies might be required to decipher whether these mechanisms also apply *in vivo*.

MODULATION OF TREG FUNCTION

As Tregs have a crucial role in maintaining the balance of autoimmunity and immunosuppression, the numbers and the activity or tissue-specific migration of this cell population must be controlled tightly. In this context, signaling between RANK (CD265)-expressing epidermal antigen-presenting Langerhans cells (LCs) and RANKL (CD254)-overexpressing

keratinocytes in K14-RANKL transgenic mice has been shown to result in the increased capacity of LC to induce the proliferation of Tregs. This phenomenon is possibly mediated by the elevated expression of DEC205 (CD205) and CD86, as well as increased IL-10 secretion, factors that have previously been associated with the expansion of Tregs via antigen-presenting cells (Mahnke *et al.*, 2003; Loser *et al.*, 2006; Loser and Beissert, 2009). In support of this suggestion, depleting LC from K14-RANKL transgenic epidermis caused a substantial reduction of Treg numbers in regional lymph nodes, indicating that antigen-presenting cells stimulated via RANK-RANKL interactions can maintain peripheral numbers of Tregs (Loser *et al.*, 2006). At least in skin, one of the regulators of RANKL expression is active vitamin D3, which is enzymatically produced from an inactive precursor by LC upon inflammation or exposure to environmental factors such as UV light (Kim *et al.*, 2007; Sigmundsdottir *et al.*, 2007; Sigmundsdottir and Butcher, 2008). Notably, active vitamin D3 has been demonstrated to enhance Treg function (Gorman *et al.*, 2007), suggesting that active vitamin D3 upregulates RANKL and, subsequently, LC stimulated via RANK-RANKL signaling migrate to regional lymph where they induce the Treg proliferation (Loser and Beissert, 2009).

After expansion in secondary lymphoid organs, the activity of Tregs and their homeostatic and migratory properties are regulated precisely. In particular, the expression of specific adhesion molecules and tissue-specific chemokine receptors are critical for targeting Tregs to both lymphoid and non-lymphoid tertiary organs. Accordingly, in addition to their well-known presence in secondary lymphoid tissues, Tregs are detectable in non-lymphoid tissues even in the absence of inflammation (Sather *et al.*, 2007; Campbell and Koch, 2011). Recently, gene expression analyses revealed the relevance of tissue homing receptors for the appropriate localization and function of Tregs. Expression of the α E integrin (CD103), as well as the chemokine receptor CC-chemokine receptor 4 (CCR4), and the ability to produce E- and P-selectin ligands by the activity of α -(1,3)-fucosyltransferase VII are important for Treg migration toward the skin (Campbell and Koch, 2011). Consequently, deletion of CD103, CCR4, or α -(1,3)-fucosyltransferase VII resulted in the development of cutaneous autoimmune diseases (Sather *et al.*, 2007; Dudda *et al.*, 2008; Freyschmidt *et al.*, 2010). Besides their constitutive circulation through tertiary tissues such as skin, Treg migration to non-lymphoid organs is increased significantly during inflammation. However, the expression of inflammation-induced homing receptors on Tregs is largely dependent on the type of tissue and the kind of inflammation (Campbell and Koch, 2011). For example, Th17-mediated inflammation, such as that observed in experimental autoimmune encephalomyelitis or psoriasis, is characterized by high levels of the CCR6 ligand CCL20 in inflammatory lesions; thus, CCR6 expression on Tregs is essential for optimal recruitment to sites of Th17-mediated inflammation (Yamazaki *et al.*, 2008; Loser, unpublished observation). In contrast, Th1-mediated inflammation characterized by elevated IFN- γ concentrations promotes the expression of the CXCR3-chemokine receptor 3

(CXCR3) ligands, CXCL9, CXCL10, and CXCL11, in inflammatory lesions. Hence, in a mouse model of Th1-driven, concanavalin A-induced hepatitis, CXCR3-expressing Tregs migrate to inflamed liver tissue (Santodomingo-Garzon *et al.*, 2009). Apart from these examples, a variety of other tissue homing receptors have been identified on Tregs, including CCR2, CCR4, CCR9, CCR10, CXCR4, CXCR6, α E β 7 integrin, α 4 β 7 integrin, and P- and E-selectin ligands (Campbell and Koch, 2011; Loser, unpublished observations), suggesting that Tregs might use a combination of homing receptors to control their migration to tertiary tissues during inflammation.

Having migrated to inflammatory tissues, Treg suppressor function and Treg survival are controlled by cytokines and a variety of small molecules. Influencing Treg development, viability or activity is of therapeutic interest, and therefore the role of various cytokines and small molecules on Treg suppressive activity has been investigated in detail. As mentioned previously, binding of IL-2 to the IL-2 receptor controls Foxp3 expression via STAT5. Thus, IL-2 was shown to upregulate the proliferation of Tregs in cancer patients, and blocking IL-2 signaling led to reduced numbers of Tregs in clinical responders (Wei *et al.*, 2007). Moreover, administration of IL-2 stimulated the CXCR4 expression on Tregs and, thus, enabled Treg migration toward the CXCR4 ligand CXCL12 in a tumor microenvironment; this may therefore result in Treg accumulation in tumors (Ohkura *et al.*, 2011). Rapamycin, an immunosuppressive drug inhibiting IL-2 signaling and thereby blocking the activation of T and B cells, has been demonstrated to promote the expansion of Tregs by inhibition of effector T-cell proliferation (Battaglia *et al.*, 2006). Accordingly, treatment of non-obese diabetic mice with rapamycin prevents the induction of Type 1 diabetes, and furthermore expands naturally occurring Foxp3⁺ Tregs. In addition, *in vitro* treatment of Tregs from Type 1 diabetes patients with rapamycin upregulated their suppressive activity (Monti *et al.*, 2008), suggesting that rapamycin might promote the generation/expansion of Tregs *in vitro* and *in vivo*. More recently, the sphingosine 1-phosphate receptor agonist FTY720, which is normally used to prevent graft rejection during organ transplantation, was shown to increase the suppressive activity of Tregs *in vitro*, and furthermore to upregulate the expression of Foxp3 and the secretion of anti-inflammatory cytokines such as IL-10 and TGF- β (Zhou *et al.*, 2009). Hence, FTY720 might be suitable as a therapeutic alternative for the treatment of autoimmune disorders. Besides these rather indirect mechanisms to modulate Treg function using immunosuppressive drugs, such as retinoic acid, rapamycin, or FTY720, Treg activity can be controlled directly by targeting Treg-associated molecules with monoclonal antibodies. Accordingly, in mouse experiments, the *in vivo* administration of anti-GITR-, anti-OX40-, or anti-Folate Receptor 4 antibodies reduced significantly the numbers of peripheral Tregs, resulting in increased antitumoral immunity (Ko *et al.*, 2005; Yamaguchi *et al.*, 2007; Piconese *et al.*, 2008). Similar observations were made in human patients with metastatic melanoma: the administration of the CD25-directed immunotoxin RFT5-SMPT-dgA induced a transient reduction in

peripheral Treg numbers *in vivo* (Powell *et al.*, 2008). Together, different mechanisms are known to control the homeostasis, suppressor function, and migratory properties of Tregs, including antibodies or small molecules interfering with the suppressive activity of Tregs; active vitamin D3 and RANKL, involved in regulating Treg expansion; and chemokine receptors or particular integrins functioning as homing receptors to modulate Treg migration toward inflammatory tertiary tissues.

TREGS IN INFLAMMATORY SKIN DISORDERS

Tregs exist in almost all tertiary tissues, and a particularly high number of Tregs has been detected in skin (Dudda *et al.*, 2008; Tomura *et al.*, 2010). To analyze the function of cutaneous Tregs, Dudda *et al.*, 2008) reconstituted Foxp3-deficient *Scurfy* mice with Tregs lacking the ability to migrate to the skin, which resulted in the rapid development of cutaneous inflammation and suggested that skin-resident Tregs have a key role in maintaining immune homeostasis at this site. Hence, it is conceivable that sterile cutaneous inflammatory and autoimmune diseases might be associated with impaired numbers or function of Tregs in lesional skin. Atopic dermatitis (AD) is one of the most common inflammatory skin diseases. Although the majority of studies deciphering the pathophysiology of AD have focused on the role of Th2 cells, more recent reports indicate the importance of Tregs. When comparing the numbers of Tregs in peripheral blood from AD patients and healthy donors, several groups reported increased numbers of Tregs; moreover, Treg expansion was positively associated with disease severity in AD (Ou *et al.*, 2004; Hijnen *et al.*, 2009; Ito *et al.*, 2009). However, other studies revealed equal levels of Tregs in peripheral blood from AD patients and healthy donors (Brandt *et al.*, 2009). Similar to the observations made in peripheral blood, Treg numbers in lesional skin from AD patients were found to be rather inconsistent. Whereas Verhagen *et al.* (2006) did not detect Foxp3⁺ Tregs in skin, Schnopp *et al.* (2007) have shown substantial numbers of Tregs to be present in cutaneous lesions (Verhagen *et al.*, 2006; Schnopp *et al.*, 2007). These contradictory results might be explained by the different treatments of AD patients receive, as some common AD treatments, such as cyclosporine, glucocorticoids, and UV irradiation, are known to affect Treg numbers (Brandt *et al.*, 2009; Hijnen *et al.*, 2009; Loser and Beissert, 2009; Baumgrass *et al.*, 2010; Stary *et al.*, 2011).

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the loss of immune tolerance to self, leading to the activation and expansion of autoreactive lymphocytes, as well as the production of autoantibodies and immune complexes targeting multiple organs, including the skin. It is generally accepted that the malfunction of the immune system in SLE is based on a dysregulation of T-cell tolerance. As at least in murine models of SLE central tolerance mechanisms were unaffected, the breakdown of tolerance seems to emerge in the periphery, and thus abnormalities in Treg numbers and/or function have been suggested to contribute to T and B cell hyperreactivity. Accordingly, most—but not all—studies revealed decreased

numbers of CD4⁺CD25^{hi} Tregs in peripheral blood from SLE patients; furthermore, the reduced Treg levels were found to be inversely correlated with disease activity (Lee *et al.*, 2006; Mellor-Pita *et al.*, 2006; Gerli *et al.*, 2009; Buckner, 2010). However, these results could not be confirmed when Foxp3 was used as a marker for Tregs, suggesting that in SLE patients the expression of Foxp3 might be dissociated from the CD4⁺CD25^{hi} Treg phenotype (Lin *et al.*, 2007; Bonelli *et al.*, 2008, 2009). Besides Treg numbers, the immunosuppressive activity of Tregs has been studied intensively in SLE patients, and several groups observed a defect in suppressor function, which correlated with disease progression (Alvarado-Sanchez *et al.*, 2006; Venigalla *et al.*, 2008; Miyara *et al.*, 2009; Suen *et al.*, 2009), whereas others could not confirm such functional deficiencies (Vargas-Rojas *et al.*, 2008; Zhang *et al.*, 2008). These contradictory results might be explained partially by the different methods used for Treg isolation and quantification of suppressive activity. Moreover, the majority of studies to assess Treg numbers and Treg function was carried out in peripheral blood, whereas there is little or almost no information on the aberration of Treg numbers and/or function in lymphoid tissues or lesional skin. In SLE patients, disease activity varies between phases of relative remission and disease flares. Hence, it is conceivable that Treg expansion might be similarly inconsistent. Recently, Bonelli *et al.* (2009, 2010) identified a CD4⁺Foxp3⁺CD25[−] Treg subset with impaired suppressor function that was specifically induced in phases of active disease in SLE patients. Despite the large numbers of studies that have been performed, the role of Tregs during SLE development and progression remains elusive.

In psoriasis (an autoimmune-related chronic inflammatory skin disease strongly associated with the activity of Th17 cells and the Th17-driving cytokine IL-23), by contrast, Treg function has been shown to be impaired (Sugiyama *et al.*, 2005; Nair *et al.*, 2009). Recently, Bovenschen *et al.* (2011) convincingly explained the underlying molecular mechanism by demonstrating that in patients with severe psoriasis Tregs can differentiate toward IL-17-expressing Tregs that contribute to the perpetuation of autoimmunity. This conversion was associated with the loss of Foxp3 expression, whereas the levels of the Th17-specific transcription factor retinoic acid-related orphan receptor γ t (ROR γ t) were unchanged. Notably, the cytokine IL-23, together with TCR stimulation, induced the IL-17 secretion in Tregs and contributed to the downregulation of Foxp3 (Bovenschen *et al.*, 2011). As those converted IL-17⁺ Tregs were enriched in lesional versus non-lesional skin of psoriasis patients, a crucial role of Treg conversion for psoriasis development and progression is highly likely. Thus, the observations by Bovenschen *et al.* add a new aspect to psoriasis research: the investigators suggest a scenario in which, driven by the psoriatic cytokine milieu, *bona fide* Foxp3⁺ Treg coexist in lesional skin with IL-17⁺Foxp3[−]ROR γ t⁺ Tregs that potentially become pathogenic Th17 cells.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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