Molecular Targets of FoxP3$^+$ Regulatory T Cells

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Abstract: The important roles of FoxP3$^+$ T cells in many immunological or cancerous diseases are now well established. The research field is now moving in the direction to fine-control the generation, migration, expansion, and function of FoxP3$^+$ cells in an effort to prevent and cure specific types of diseases. Potential molecular targets to regulate FoxP3$^+$ T cells are reviewed in this article.

Key Words: FoxP3, regulatory T cells, tolerance, diseases, migration, chemokine receptors.

INTRODUCTION

FoxP3$^+$ regulatory T cells play essential roles in suppression of autoimmunity and hyperimmune responses. FoxP3$^+$ cells are generated in the thymus, and these thymus-generated naïve FoxP3$^+$ cells mainly migrate to secondary lymphoid tissues for their own antigen priming and suppression of the activation of other immune cells. Antigen priming induces a dramatic change in trafficking receptors for FoxP3$^+$ regulatory T cells which is required for their migration out of secondary lymphoid tissues and into various non-lymphoid tissues. Antigen priming also induces conversion of some FoxP3$^-$ T cells into FoxP3$^+$ cells. Insufficient or excessive activities and numbers of FoxP3$^+$ T cells have been reported in various pathological conditions. Insufficient numbers or activities of FoxP3$^+$ T cells are implicated with autoimmunity and overactive immune responses leading to tissue damage, while excessive numbers of FoxP3$^+$ T cells are implicated with cancers and chronic infection by pathogens. Therefore, control of FoxP3$^+$ regulatory T cells has clear therapeutic potentials for a number of diseases. FoxP3$^+$ regulatory T cells preferentially express many surface and intracellular molecules that can serve as molecular targets to either increase or decrease their numbers or activities. The basic biology of the FoxP3$^+$ cells and functions of the FoxP3$^+$ cell-associated molecules are reviewed in this article. FoxP3$^+$ cells are often called CD4$^+$CD25$^+$ regulatory cells. "CD4$^+$CD25$^+$ cells" or "regulatory T cells (Tregs)," however, is not the best term for FoxP3$^+$ cells, because many CD4$^+$CD25$^+$ cells are FoxP3$^-$ conventional cells, and regulatory T cells include not only FoxP3$^+$ cells but also some FoxP3$^-$ cells with suppressive functions. For simplicity, we will use the term "FoxP3$^+$ T cells" to refer to the largely overlapping FoxP3$^+$ and CD4$^+$ CD25$^+$ regulatory T cell populations in this review.

FoxP3, A CELL-LINEAGE DETERMINING TRANSCRIPTION FACTOR

FoxP3$^+$ T cells are defined by their expression of the transcription factor FoxP3. FoxP3 is a member of the forkhead/winged-helix family of transcriptional regulators [1]. The mouse protein is called FoxP3, while the human protein is called FOXP3. The FoxP3/FOXP3 proteins have 4 functional domains: repressor, zinc-finger, leucine zipper, and forkhead (FKH) domains [2] (Fig. **1**). The Fox family proteins bind DNA sequences defined by a core DNA sequence (5'-A(A/T)TRTT(G/T)R-3" ;R=pyrimidine) [3, 4]. The repressor domain is composed of two sub-domains: the first half of the repressor domain is involved in general transcriptional repression by FoxP3, and the second half of the domain is required for repression of NF-AT and NF-kB-mediated transcription [5, 6]. The role of the zinc-finger domain is unknown. The leucine zipper domain is thought to mediate dimerization of transcription factors [6]. The FKH domain mediates FoxP3 binding to the core DNA sequence [7] and is required also for nuclear localization of FoxP3. Enforced expression of FoxP3 in mouse CD4 T cells turns conventional naïve T cells into regulatory T cells [8, 9]. Therefore, FoxP3 would be an important target to control the development of FoxP3$^+$ regulatory T cells.

FoxP3$^+$ CELLS REGULATE IMMUNE RESPONSES IN A WIDE VARIETY OF DISEASES

Abnormal numbers or activities of FoxP3$^+$ regulatory T cells are implicated with autoimmune diseases, transplantation rejection, infectious diseases, and cancers. FoxP3$^+$ regulatory T cells can suppress the onset and/or progression of many diseases including diabetes [10], autoimmune encephalomyelitis [11, 12], thyroiditis [13], inflammatory bowel disease [14], systemic lupus erythematosus [15], rheumatoid arthritis[16], and gastritis [13, 17]. FoxP3$^+$ cells can suppress also immune responses leading to graft rejection and graft-versus-host disease [18-20]. While FoxP3$^+$ cells primarily function to limit tissue damage in the hosts, they can also help pathogens to evade the immune system, leading to chronic infection [21]. FoxP3$^+$ cells are highly enriched in many tumor types. For example, melanoma [22], cervical carcinoma [23], gastrointestinal tract cancer [24], gastric cancer [25], lung cancer [26], ovarian cancer [26], colorectal cancer [27], breast cancer, pancreas adeno caricinoma [28], head and neck cancer [29], hepatocellular carcinoma [30], leukemia and lymphoma (adult T cell leukemia/lymphoma [31], Hodgkin's lymphoma [32], and chronic lymphocytic

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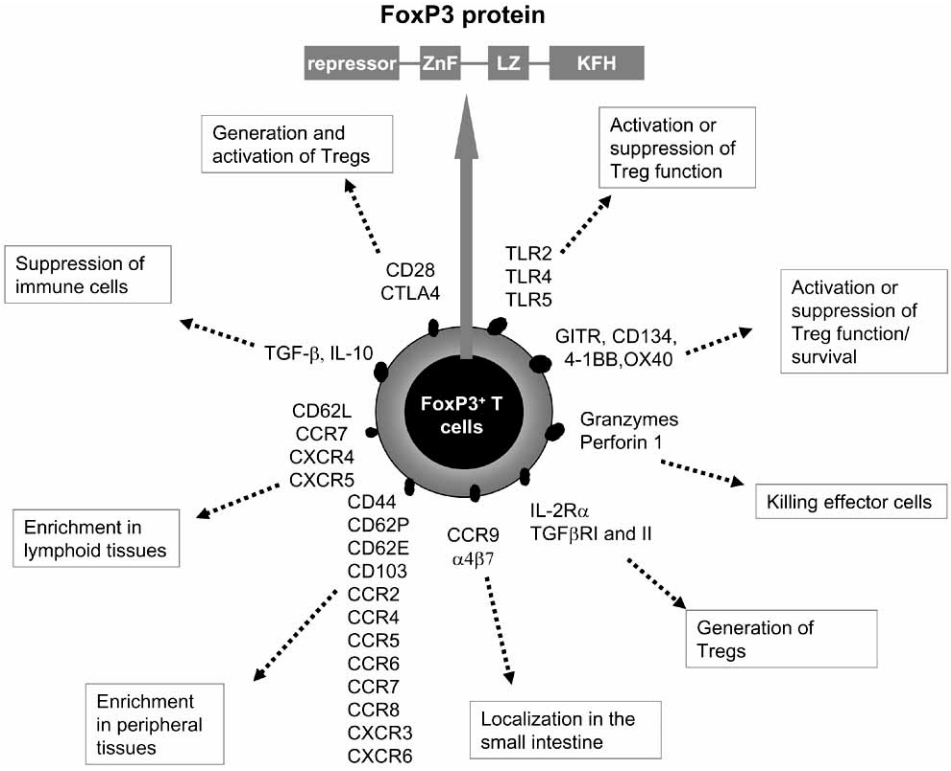

Fig. (1). Molecular control points of FoxP3+ T cells. FoxP3+ regulatory T cells express the transcription regulator FoxP3, which is believed to confer them the FoxP3+ T cell-specific gene expression pattern and suppressive functions. It is advantageous to reduce the numbers of FoxP3+ regulatory T cells to boost immune responses to vaccines and tumor cells. On the other hand, the numbers of functional FoxP3+ T cells in inflamed organs or tissues should be increased to suppress chronic inflammation and autoimmune diseases. FoxP3+ regulatory T cells express many molecules that regulate their generation, expansion, survival, migration, and function. Therefore, these molecules would serve as important control points for FoxP3+ regulatory T cells. Enhancement of the generation and survival of FoxP3+ regulatory T cells would be possible by regulating TGF-β receptors, IL-2Rα, CD28, CTLA-4, and 4-1BB. FoxP3+ T cells use a number of trafficking receptors such as chemokine receptors and adhesion molecules to migrate to various organs and tissue sites. Control of these trafficking receptors would be an effective strategy to regulate numbers of FoxP3+ T cells specifically in an organ of interest without depleting all of the FoxP3+ T cells in the body. FoxP3+ regulatory T cells express a number of other receptors such as TLRs and co-stimulation receptors to turn on and off their suppressive functions. For example, activation of GITR and OX40 can turn off the suppressive function of FoxP3+ T cells. FoxP3+ T cells express other effector molecules such as suppressive cytokines (TGF-β1 and IL10) and granzymes to suppress and kill inflammatory cells respectively. Activation or blocking of these molecules would be useful in control of the activity of FoxP3+ T cells.

leukemia [33] all have greatly increased numbers of FoxP3+ cells. Depletion of FoxP3+ cells resulted in regression of some tumors in mice [34]. The depletion also enhanced anti-tumor immunity against a murine colorectal tumor, CT26. Infusion of antigen-specific FoxP3+ cells before implantation of tumors was effective in suppression of anti-tumor cytotoxicity of CD8 cells, and this function of FoxP3+ cells was dependent on TGF-β1 [35]. Thus, FoxP3+ regulatory T cells represent another barrier to effective tumor immunity in animals and humans [36].

GENERATION OF FoxP3+ T CELLS

CD25 has been used as a surrogate marker for FoxP3+ T cells because the majority of FoxP3+ T cells express CD25 [13]. This method, usually, works well because ~90% of FoxP3+ T cells are CD25+. Upon immunization or activation *in vitro*, many FoxP3- T cells acquire CD25 expression, limiting the use of this method. Recently, antibodies to mouse FoxP3 or human FOXP3 have been available, allowing more definitive identification of FoxP3+ cells [37]. Additionally, FoxP3 GFP or RFP-knock-in mice have been generated [38, 39]: In these mice, GFP or RFP are expressed after an internal ribosome entry site (IRES) inserted at the end of the FoxP3 open reading frame to co-express GFP/RFP and FoxP3 in the same cells. This method allowed detection of FoxP3+ T cells based on GFP/RFP expression and tracking the generation of FoxP3+ cells in the thymus. FoxP3+ T cells are made in the thymus along with conventional naïve T cells. The majority of the thymus-generated FoxP3+ T cells are CD4+ single positive (SP) but small numbers of CD4+CD8+ double positive (DP) cells also exist [38, 40, 41]. Therefore, it is thought that FoxP3+ T cells can be generated at the DP stage, and the DP FoxP3+ T cells would differentiate into CD4+ SP FoxP3+ T cells for emigration. Also, some T cells may gain the expression of FoxP3 at the SP stage. In addition, FoxP3+ T cells can be made from conventional naïve T cells in the periphery upon antigen priming [42]. The majority of the thymus-generated FoxP3+ T cells constitutively express activated T cell-associated antigens such as CD25, cytotoxic T-lymphocyte-associated 4 (CTLA4), and gluco-

corticoid-induced tumor necrosis factor receptor family-related gene (GITR) along with a number of other molecules, described later in this review. Many of these molecules are important for induction, proliferation and functions of $FoxP3^+$ T cells, and, thus, can serve as molecular targets to regulate the numbers or activities of $FoxP3^+$ T cells.

MIGRATION OF $FoxP3^+$ CELLS

Similar to conventional T cells, the migration capacity of $FoxP3^+$ T cells is thought to be important for their effector functions *in vivo*. This is particularly true for $FoxP3^+$ T cells because they require cell-cell contact to suppress target cells. From an immunologist's point of view, the body is composed of lymphoid and non-lymphoid tissues. Lymphoid tissues can be further divided into primary and secondary lymphoid tissues. Thymus is the primary lymphoid tissue where naïve T cells are generated. Thymus-generated naïve T cells emigrate to the blood circulation. Lymph nodes, spleen, Peyer's patches and other organ-associated lymphoid tissues function as secondary lymphoid tissues where antigens and antigen presenting cells are collected to activate naïve T cells. In a manner similar to conventional naïve T cells, thymus-generated $FoxP3^+$ T cells express appropriate trafficking receptors and migrate mainly into secondary lymphoid tissues [43-45]. As homing receptors for secondary lymphoid tissues, L-selectin and CCR7 play important roles [46-49]. Peripheral node addressin (PNAd), the counter receptor for L-selectin, is expressed on high endothelial cells in lymph nodes. L-selectin mediates weak cell-cell interaction between naïve T cells and endothelial cells in secondary lymphoid tissues to initiate entry of lymphocytes into the tissues. $\alpha4\beta7$ can mediate rolling and adhesion of lymphocytes on high endothelial venules in Peyer's patches and other mucosal tissue sites [50, 51]. Direct migration of thymus-generated naïve $FoxP3^+$ T cells to non-lymphoid tissues such as intestine, bone marrow, and peritoneal cavity is a rare event [52]. It is the secondary lymphoid tissues where $FoxP3^+$ T cells acquire tissue-specific trafficking capacities [52]. $FoxP3^+$ T cells, antigen-primed in MLN and Peyer's patches, up-regulate the mucosal tissue homing receptors CCR9 and $\alpha4\beta7$. $FoxP3^+$ T cells, antigen-primed in skin-draining peripheral lymph nodes, acquire the skin-homing related receptor CCR8 [52]. Thus, $FoxP3^+$ T cells undergo trafficking receptor switches in thymus and secondary lymphoid tissues to acquire necessary receptors to migrate from organ to organ [53]. The migration of $FoxP3^+$ T cells to the secondary lymphoid tissues is thought to be important for suppression of the antigen priming of conventional T cells, and it can limit the generation of inflammatory memory/effector T cells. The migration of $FoxP3^+$ T cells from secondary lymphoid tissues to non-lymphoid tissues would be important for suppression of inflammatory T cells at effector sites.

POTENTIAL MECHANISMS OF TARGET CELL SUPPRESSION BY $FoxP3^+$ CELLS

The list of target cells that $FoxP3^+$ T cells can suppress, at least *in vitro*, is becoming lengthy. The list, now, includes $CD4^+$ T cells [54], $CD8^+$ T cells [55], CD1d-restricted NKT cells [56], monocytes/macrophages [57], naïve/memory B cells [58], dendritic cells [59], and NK cells [60]. It is incompletely understood how $FoxP3^+$ T cells inhibit the target cells [12, 14, 19, 61-66]. A number of potential mechanisms have been proposed. Two suppressive cytokines TGF-β and IL-10 are implicated in target cell suppression by $FoxP3^+$ T cells. The roles of these cytokines are discussed later in this review in more detail. The CTLA4, expressed by $FoxP3^+$ T cells, can suppress antigen presenting cells through B7 molecules [67, 68]. B7-CTLA4 interaction induces expression of indoleamine 2,3-dioxygenase (IDO), an enzyme that depletes the amino acid tryptophan [69]. Heme oxygenase (HO)-1 is induced by forced expression of FoxP3 in T cells [70]. HO-1 generates carbon monoxide [71], which has anti-inflammatory effects and suppresses the IL-2 production by T cells [72, 73]. Regulatory T cells highly express granzyme A and granzyme B, and kill target cells in perforin-dependent and independent mechanisms [74-76].

MOLECULAR TARGETS OF $FoxP3^+$ CELLS

$FoxP3^+$ T cells highly express a number of proteins that are preferentially expressed in effector T cells, some of which can be used to control the induction, migration, expansion, and, function of $FoxP3^+$ T cells. These molecules can be grouped into costimulation receptors, cytokines and cytokine receptors, Toll-like receptors (TLR), chemokine and adhesion receptors, and others (Table **1**). To be balanced, information on both the positive and negative roles of these molecules for $FoxP3^+$ regulatory T cells is discussed below.

Costimulation receptors: Glucocorticoid-induced TNF receptor (GITR/TNFRSF18) is a type I transmembrane protein, composed of three cysteine pseudorepeats in the extracellular domain and the intracellular domain [77, 78]. $FoxP3^+$ T cells highly express GITR [77, 78]. Agonistic anti-GITR antibodies can abrogate the suppressive function of $FoxP3^+$ T cells [79, 80]. The abolishment of suppression could be mediated also by the GITR expressed by target cells [81]. In this case, GITR activation would make the target cells resistant to the suppressive function of $FoxP3^+$ T cells. Therefore, GITR is a potentially useful target to control the function of $FoxP3^+$ T cells.

OX40 is another member of the TNF receptor family with a costimulatory function for $CD4^+$ and $CD8^+$ T cells. It is constitutively expressed on most $FoxP3^+$ cells, whereas it is expressed only by activated cells among conventional $CD4^+$ and $CD8^+$ T cells [82]. OX40 ligand (OX40L or CD134L) is expressed by antigen-presenting cells (B cells, dendritic cells, and macrophages) and activated endothelial cells [83, 84]. Activation of OX40 with an agonistic antibody abrogated the suppressive function of $FoxP3^+$ cells *in vitro* and *in vivo* in a graft-versus-host-disease model [85].

4-1BB is also constitutively expressed by $CD4^+CD25^+$ T cells [86]. Stimulation of 4-1BB can induce proliferation of $CD4^+CD25^+$ T cells, and the 4-1BB-stimulated T cells have suppressive functions *in vitro*. Although this study did not rigorously demonstrate that most of the 4-1BB-stimulated T cells were $FoxP3^+$ T cells, the results suggested that 4-1BB is a potential costimulator for $FoxP3^+$ T cells. In a hapten (trinitrobenzene sulfonate, TNBS)-induced colitis model in mice, treatment with agonistic anti-4-1BB antibodies ameliorated the colitis [87]. Contradictorily, it has also been reported that $FoxP3^+$ T cells of 4-1BB-deficient mice had no problem in suppression of T cell-induced colitis [88].

Table 1. Important Regulatory Molecules of FoxP3$^+$ Cells

Name(s)	General Protein Structure/Category	Ligands	Function for FoxP3$^+$ Cells
CD28	Type I transmembrane glycoprotein	CD80/B7-1, CD86/B7-2	Co-stimulation
CTLA4 (CD152)	Type I transmembrane glycoprotein	CD80/B7-1, CD86/B7-2	Co-stimulation
OX40 (CD134; TNFRSF4)	Type I transmembrane glycoprotein	OX40-Ligand/ TNFSF4	Survival/proliferation
4-1BB (CD137; TNFRSF9)	Type I transmembrane glycoprotein	4-1BB-Ligand/ TNFSF 9	Co-stimulation and expansion
GITR/ TNFRSF18	Type I transmembrane glycoprotein	GITR Ligand/ TNFSF18	Control of effector function
IL-2Rα (CD25)	Type I transmembrane glycoprotein Forms heterotrimers	IL-2	Expansion Differentiation Peripheral induction
TGFβRI and II	Form a heteromeric receptor complex	TGF-β1	Induction, differentiation, and expansion
IL-10	Secretory glycoprotein	IL10R /CD210	Effector function
TLR2	Type I transmembrane glycoprotein	Bacterial lipoproteins and other cell wall components	Regulation of function and expansion
TLR4	Type I transmembrane glycoprotein	Bacterial lipopolysaccharide	Regulation of function and expansion
TLR5	Type I transmembrane glycoprotein	Certain bacterial flagellins	Regulation of function and expansion
CCR4	7-Transmembrane-G-protein-coupled receptor	CCL17/CCL21	Chemokine receptor
CCR5	7-Transmembrane-G-protein-coupled receptor	CCL2/CCL3/CCL4/CCL5	Chemokine receptor
CCR6	7-Transmembrane-G-protein-coupled receptor	CCL20	Chemokine receptor
CCR7	7-Transmembrane-G-protein-coupled receptor	CCL19/CCL21	Chemokine receptor
CCR8	7-Transmembrane-G-protein-coupled receptor	CCL1	Chemokine receptor
CXCR4	7-Transmembrane-G-protein-coupled receptor	CXCL12	Chemokine receptor
CD103	Type I transmembrane glycoprotein	E-cadherin	Integrin/adhesion
LAG3 (CD223)	Type I transmembrane glycoprotein	MHC class II	Induction and effector function
Nrp-1	Type I transmembrane glycoprotein	Semaphorin 3A, PLGF-2, VEGF-165	Unclear
Granzyme A (CTLA-3)	Secreted cytoplasmic granule serine protease	NA*	Target cell lysis
Granzyme B (CTLA-1)	Secreted cytoplasmic granule serine protease	NA*	Target cell lysis
Perforin 1	Cytoplasmic granule integral membrane protein	NA*	Polymerizes into transmembrane tubules for target cell lysis

*NA, not applicable.

CD28 and its ligands B7-1 and B7-2 play critical roles in generation of FoxP3$^+$ T cells in the thymus. FoxP3$^+$ T cells are absent in CD28-deficient mice and B7-1/2 deficient mice [10]. The signals from CD28 activation also regulate the proliferation and survival of FoxP3$^+$ cells in the periphery [89]. FoxP3$^+$ T cells constitutively express CTLA-4. There have been contradictory reports on the role of CTLA4 in the FoxP3$^+$ T cell biology. Blocking anti-CTLA4 antibodies abrogated the suppressive activity of FoxP3$^+$ T cells *in vitro* and *in vivo* in a colitis model [90]. However, FoxP3$^+$ T cells from CTLA4-deficient mice develop and function normally [91]. While activation of the CLTA4 on conventional T cells is suppressive, activation of CTLA4 on FoxP3$^+$ T cells can be co-stimulatory for these cells [92].

Cytokines and Cytokine Receptors

Most FoxP3$^+$ T cells in humans and mice express the IL-2Rα chain CD25. Ironically, FoxP3$^+$ T cells do not or poorly produce its ligand IL-2. IL-2 is required to expand and maintain FoxP3$^+$ T cells, but high levels of IL-2 are inhibitory for the suppressive function of FoxP3$^+$ T cells *in vitro* [93]. The important role of IL-2 signaling in generation of FoxP3$^+$ cells is supported by decreased numbers and regulatory function of FoxP3$^+$ T cells in humans deficient with STAT5b, a transcription factor that mediates the IL-2 signaling [94]. Induction of FoxP3 by IL-2 is mediated by binding of STAT3 and STAT5 proteins to a highly conserved STAT-binding site located in the first intron of the FOXP3 gene [95]. The fact that FoxP3$^+$ cells are reduced in numbers in the thymus and periphery of Il2rγ deficient mice suggests that IL-2 signaling is required for generation and maintenance of FoxP3$^+$ cells *in vivo* [96]. IL-2 or IL2Rα deficiency does not affect the generation of FoxP3$^+$ cells in the thymus but decreases their numbers in the periphery [96].

The Role of TGF-β Proteins in the Effector Function of FoxP3$^+$ Cells is Debatable

It is well established that TGF-β1 can induce the generation of FoxP3$^+$ cells from naïve CD4$^+$ T cells. The frequency of FoxP3$^+$ cells was increased in secondary lymphoid organs and the thymus of a transgenic mouse strain over-expressing TGF-β1 [97]. In TGF-ß1–deficient mice, FoxP3$^+$ cells are reduced in numbers in the periphery but not in the thymus, suggesting that TGF-ß1 is involved in the expansion of FoxP3$^+$ cells in the periphery but is not required for their generation in the thymus [98]. A blocking anti-TGF-β1 antibody was able to completely suppress the function of mouse FoxP3$^+$ cells *in vitro* [92], but the result was not reproduced in other studies [99, 100]. It has also been reported that the FoxP3$^+$ cells from TGF-ß1-/- mice were still able to suppress target T cells, and the target T cells from TGF-ß1–insensitive Smad3$^{-/-}$ mice were suppressed by FoxP3$^+$ cells *in vitro* [99], downplaying the role of this cytokine in the effector function of FoxP3$^+$ cells. *In vivo*, however, the FoxP3$^+$ cells from TGF-ß1-/- mice did not protect recipient SCID mice from colitis after naïve T cell transfer, supporting the essential function of TGF-β1 *in vivo* [101]. In another study, it was shown that the effector T cells that cannot respond to TGF-β1 can escape the control by FoxP3$^+$ cells [102]. Taken together, the role of TGF-ß1 in peripheral induction or expansion of FoxP3$^+$ cells is firmly established, while the role of TGF-ß1 in effector functions of FoxP3$^+$ cells needs to be clarified in the future.

FoxP3$^+$ cells produce IL-10 upon TCR stimulation [103, 104]. However, blockade of IL-10 did not affect the suppressive function of human FoxP3$^+$ cells *in vitro* [105, 106]. In contrast, the FoxP3$^+$ cells from IL-10-deficient mice were unable to suppress myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), suggesting that IL-10 is involved in FoxP3$^+$ cell-mediated suppression of certain inflammatory diseases [107]. Also, a blocking anti-IL-10 receptor antibody was able to suppress the function of regulatory T cells in experimental autoimmune thyroiditis [108].

Toll-Like Receptors (TLR)

Mouse FoxP3$^+$ cells selectively express Toll-like receptors (TLR)-4, -5, -7, and -8. The TLR-4 ligand lipopolysaccharide (LPS) can enhance the survival, proliferation, and effector function of FoxP3$^+$ cells *in vitro* [108]. Human FoxP3$^+$ cells express TLR5, and activation of human FoxP3$^+$ cells in the presence of flagellin (the ligand of TLR5) increased their suppressive capacity and the expression levels of FOXP3 [109]. FoxP3$^+$ cells are significantly reduced in numbers in TLR2–/– mice, and TLR2 triggering by the TLR2 ligand Pam3Cys augmented the proliferation of FoxP3$^+$ cells *in vitro* and *in vivo* [110]. TLR2 activation by bacterial lipoprotein can expand FoxP3$^+$ cells [111]. It was found that the expanded FoxP3$^+$ cells were temporarily inactive but recovered their suppressive activity when the concentrations of TLR2 ligands (and infection) subsided. TLR ligands can activate dendritic cells for production of IL-6, and IL-6 can make pathogen-specific T cells resistant to the suppressive effect of FoxP3$^+$ cells [112]. Thus, TLRs and their ligands are important regulators of expansion and function of FoxP3$^+$ cells.

Chemokine and Adhesion Receptors

FoxP3$^+$ T cells express various chemokine receptors and adhesion molecules to migrate from a tissue site to the blood circulation and vice versa. Therefore, these trafficking receptors would serve as useful targets to control the tissue distribution of FoxP3$^+$ T cells. Among the ~20 chemokine receptors identified so far, memory and effector-associated chemokine receptors such as CCR4, CCR5, CCR6, CCR8, CXCR3 and CXCR6 are preferentially expressed by FoxP3$^+$ T cells compared to FoxP3$^-$ T cells [113]. CCR4 and CCR8 are implicated with T cell migration to the tissue sites of Th2 cell responses and to some tumors [113, 114]. On the other hand, CCR5 and CXCR3 are implicated with T cell homing to sites of Th1 immune responses. The ligand of CCR6 is highly expressed in Peyer's patches [115]. FoxP3$^+$ T cells express adhesion molecules such as αEβ7, α4β7, and cutaneous lymphocyte antigen (CLA) [116-119]. αEβ7 (a receptor for E-cadherin) mediates T cell interaction with epithelial cells. α4β7 (a receptor for MAdCAM-1) is a homing receptor for mucosal tissues [120]. Cutaneous lymphocyte antigen (CLA) is frequently expressed by human skin-homing lymphocytes. Small molecules or peptides that specifically block certain chemokine receptors including CCR3, CCR5 and CXCR4 have been developed [121, 122], and these antagonists would be useful in control of the migration of FoxP3$^+$ T cells.

Other Potential Targets

There are other molecules highly expressed by FoxP3$^+$ T cells and potentially important for regulation of their function. Lymphocyte activation gene-3 (LAG-3; CD223) is constitutively expressed by FoxP3$^+$ T cells [123]. LAG3 is a CD4-related molecule that binds MHC class II, and LAG3-MHC interaction decreases antigen priming of CD4$^+$ T cells [124]. Blockade of LAG3 suppressed the FoxP3$^+$ T cell function *in vitro* and *in vivo*, while over-expression of LAG3 in conventional CD4$^+$ T cells was able to convert these cells into suppressor cells, suggesting a potentially important role of LAG3 in generation and effector function of FoxP3$^+$ T

cells [123]. Neuropilin-1 (Nrp1) is a semaphorin III receptor and is involved in axon guidance and formation of the immunological synapse [125, 126]. Nrp-1 is constitutively expressed by FoxP3$^+$ T cells [127], but the role of Nrp-1 in FoxP3$^+$ T cell function is unclear. Expression of granzyme A and B by FoxP3$^+$ regulatory T cells has been reported. Granzyme A and granzyme B can mediate target cell killing by FoxP3$^+$ T cells *in vitro* [74-76]. The roles of granzyme A and B in FoxP3$^+$ T cell function *in vivo* remain to be documented. Along with suppressive cytokines, granzymes are potentially important effector molecules of FoxP3$^+$ T cells.

CONCLUDING REMARKS

In the last decade, we have witnessed a great progress in understanding of the biology of FoxP3$^+$ regulatory cells. The therapeutic functions of FoxP3$^+$ cells in many immunological or cancerous diseases are now well established. It is now important to fine-control the generation, migration, expansion, and function of FoxP3$^+$ cells in an effort to prevent and cure diseases in a selective manner. As reviewed in this article, FoxP3$^+$ cells express many surface and intracellular molecules that are important for their biology (Fig. **1**). These molecules would serve as potential targets to regulate the numbers and/or activities of FoxP3$^+$ T cells. As the products of high throughput genomics techniques such as microarray gene expression analysis, it is expected that the list of potential molecular targets of FoxP3$^+$ cells will be even more extended in the near future. One important caveat is that most of these molecules are also expressed by activated conventional (FoxP3$^-$) CD4$^+$ or CD8$^+$ T cells. This poses potential challenges in specifically targeting FoxP3$^+$ cells versus activated conventional T cells. More investigation on selective expression and involvement of the FoxP3$^+$ T cell-associated molecules in the biology of FoxP3$^+$ versus FoxP3$^-$ T cells is required to sort out this issue.

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REFERENCES

[1] Coffer, P. J.; Burgering, B. M. *Nat. Rev. Immunol.,* **2004,** *4*, 889.
[2] Ziegler, S. F. *Annu. Rev. Immunol.,* **2006,** *24*, 209.
[3] Kaufmann, E.; Muller, D.; Knochel, W. *J. Mol. Biol.,* **1995,** *248*, 239.
[4] Schubert, L. A.; Jeffery, E.; Zhang, Y.; Ramsdell, F.; Ziegler, S. F. *J. Biol. Chem.,* **2001,** *276*, 37672.
[5] Bettelli, E.; Dastrange, M.; Oukka, M. *Proc. Natl. Acad. Sci. USA,* **2005,** *102*, 5138.
[6] Lopes, J. E.; Torgerson, T. R.; Schubert, L. A.; Anover, S. D.; Ocheltree, E. L.; Ochs, H. D.; Ziegler, S. F. *J. Immunol.,* **2006,** *177*, 3133.
[7] Kaestner, K. H.; Knochel, W.; Martinez, D. E. *Genes Dev.,* **2000,** *14*, 142.
[8] Hori, S.; Nomura, T.; Sakaguchi, S. *Science,* **2003,** *299*, 1057.
[9] Khattri, R.; Cox, T.; Yasayko, S. A.; Ramsdell, F. *Nat. Immunol.,* **2003,** *4*, 337.
[10] Salomon, B.; Lenschow, D. J.; Rhee, L.; Ashourian, N.; Singh, B.; Sharpe, A.; Bluestone, J. A. *Immunity,* **2000,** *12*, 431.
[11] Kohm, A. P.; Carpentier, P. A.; Anger, H. A.; Miller, S. D. *J. Immunol.,* **2002,** *169*, 4712.
[12] Furtado, G. C.; Olivares-Villagomez, D.; Curotto de Lafaille, M. A.; Wensky, A. K.; Latkowski, J. A.; Lafaille, J. J. *Immunol. Rev.,* **2001,** *182*, 122.
[13] Sakaguchi, S.; Sakaguchi, N.; Asano, M.; Itoh, M.; Toda, M. *J. Immunol.,* **1995,** *155*, 1151.
[14] Singh, B.; Read, S.; Asseman, C.; Malmstrom, V.; Mottet, C.; Stephens, L. A.; Stepankova, R.; Tlaskalova, H.; Powrie, F. *Immunol. Rev.,* **2001,** *182*, 190.
[15] Lee, J. H.; Wang, L. C.; Lin, Y. T.; Yang, Y. H.; Lin, D. T.; Chiang, B. L. *Immunology,* **2006,** *117*, 280.
[16] Morgan, M. E.; Sutmuller, R. P.; Witteveen, H. J.; van Duivenvoorde, L. M.; Zanelli, E.; Melief, C. J.; Snijders, A.; Offringa, R.; de Vries, R. R.; Toes, R. E. *Arthritis Rheum.,* **2003,** *48*, 1452.
[17] Suri-Payer, E.; Amar, A. Z.; Thornton, A. M.; Shevach, E. M. *J. Immunol.,* **1998,** *160*, 1212.
[18] Chen, J.; Huoam, C.; Plain, K.; He, X. Y.; Hodgkinson, S. J.; Hall, B. M. *Transplant Proc.,* **2001,** *33*, 163.
[19] Wood, K. J.; Sakaguchi, S. *Nat. Rev. Immunol.,* **2003,** *3*, 199.
[20] Oluwole, S. F.; Oluwole, O. O.; DePaz, H. A.; Adeyeri, A. O.; Witkowski, P.; Hardy, M. A. *Transpl. Immunol.,* **2003,** *11*, 287.
[21] Rouse, B. T.; Sarangi, P. P.; Suvas, S. *Immunol. Rev.,* **2006,** *212*, 272.
[22] Ghiringhelli, F.; Larmonier, N.; Schmitt, E.; Parcellier, A.; Cathelin, D.; Garrido, C.; Chauffert, B.; Solary, E.; Bonnotte, B.; Martin, F. *Eur. J. Immunol.,* **2004,** *34*, 336.
[23] Fattorossi, A.; Battaglia, A.; Ferrandina, G.; Buzzonetti, A.; Legge, F.; Salutari, V.; Scambia, G. *Gynecol. Oncol.,* **2004,** *92*, 106.
[24] Sasada, T.; Kimura, M.; Yoshida, Y.; Kanai, M.; Takabayashi, A. *Cancer,* **2003,** *98*, 1089.
[25] Kawaida, H.; Kono, K.; Takahashi, A.; Sugai, H.; Mimura, K.; Miyagawa, N.; Omata, H.; Ooi, A.; Fujii, H. *J. Surg. Res.,* **2005,** *124*, 151.
[26] Woo, E. Y.; Chu, C. S.; Goletz, T. J.; Schlienger, K.; Yeh, H.; Coukos, G.; Rubin, S. C.; Kaiser, L. R.; June, C. H. *Cancer Res.,* **2001,** *61*, 4766.
[27] Somasundaram, R.; Jacob, L.; Swoboda, R.; Caputo, L.; Song, H.; Basak, S.; Monos, D.; Peritt, D.; Marincola, F.; Cai, D.; Birebent, B.; Bloome, E.; Kim, J.; Berencsi, K.; Mastrangelo, M.; Herlyn, D. *Cancer Res.,* **2002,** *62*, 5267.
[28] Liyanage, U. K.; Moore, T. T.; Joo, H. G.; Tanaka, Y.; Herrmann, V.; Doherty, G.; Drebin, J. A.; Strasberg, S. M.; Eberlein, T. J.; Goedegebuure, P. S.; Linehan, D. C. *J. Immunol.,* **2002,** *169*, 2756.
[29] Schaefer, C.; Kim, G. G.; Albers, A.; Hoermann, K.; Myers, E. N.; Whiteside, T. L. *Br. J. Cancer,* **2005,** *92*, 913.
[30] Unitt, E.; Rushbrook, S. M.; Marshall, A.; Davies, S.; Gibbs, P.; Morris, L. S.; Coleman, N.; Alexander, G. J. *Hepatology,* **2005,** *41*, 722.
[31] Berger, C. L.; Tigelaar, R.; Cohen, J.; Mariwalla, K.; Trinh, J.; Wang, N.; Edelson, R. L. *Blood,* **2005,** *105*, 1640.
[32] Marshall, N. A.; Christie, L. E.; Munro, L. R.; Culligan, D. J.; Johnston, P. W.; Barker, R. N.; Vickers, M. A. *Blood,* **2004,** *103*, 1755.
[33] Beyer, M.; Kochanek, M.; Darabi, K.; Popov, A.; Jensen, M.; Endl, E.; Knolle, P. A.; Thomas, R. K.; von Bergwelt-Baildon, M.; Debey, S.; Hallek, M.; Schultze, J. L. *Blood,* **2005,** *106*, 2018.
[34] Onizuka, S.; Tawara, I.; Shimizu, J.; Sakaguchi, S.; Fujita, T.; Nakayama, E. *Cancer Res.,* **1999,** *59*, 3128.
[35] Chen, M. L.; Pittet, M. J.; Gorelik, L.; Flavell, R. A.; Weissleder, R.; von Boehmer, H.; Khazaie, K. *Proc. Natl. Acad. Sci. USA,* **2005,** *102*, 419.
[36] Pure, E.; Allison, J. P.; Schreiber, R. D. *Nat. Immunol.,* **2005,** *6*, 1207.
[37] Roncador, G.; Brown, P. J.; Maestre, L.; Hue, S.; Martinez-Torrecuadrada, J. L.; Ling, K. L.; Pratap, S.; Toms, C.; Fox, B. C.; Cerundolo, V.; Powrie, F.; Banham, A. H. *Eur. J. Immunol.,* **2005,** *35*, 1681.
[38] Wan, Y. Y.; Flavell, R. A. *Proc. Natl. Acad. Sci. USA,* **2005,** *102*, 5126.
[39] Fontenot, J. D.; Rasmussen, J. P.; Williams, L. M.; Dooley, J. L.; Farr, A. G.; Rudensky, A. Y. *Immunity,* **2005,** *22*, 329.
[40] Sakaguchi, S. *J. Clin. Invest.,* **2003,** *112*, 1310.
[41] Fontenot, J. D.; Dooley, J. L.; Farr, A. G.; Rudensky, A. Y. *J. Exp. Med.,* **2005,** *202*, 901.
[42] Kretschmer, K.; Apostolou, I.; Hawiger, D.; Khazaie, K.; Nussenzweig, M. C.; von Boehmer, H. *Nat. Immunol.,* **2005**, *6*, 1219.

[43] Kim, C. H. *Arch. Immunol. Ther. Exp. (Warsz)*, **2007**, *55*, 151.
[44] Lim, H. W.; Broxmeyer, H. E.; Kim, C. H. *J. Immunol.,* **2006,** *177*, 840.
[45] Lim, H. W.; Hillsamer, P.; Kim, C. H. *J. Clin. Invest.,* **2004,** *114*, 1640.
[46] Forster, R.; Schubel, A.; Breitfeld, D.; Kremmer, E.; Renner-Muller, I.; Wolf, E.; Lipp, M. *Cell,* **1999,** *99*, 23.
[47] Warnock, R. A.; Campbell, J. J.; Dorf, M. E.; Matsuzawa, A.; McEvoy, L. M.; Butcher, E. C. *J. Exp. Med.,* **2000,** *191*, 77.
[48] Stein, J. V.; Rot, A.; Luo, Y.; Narasimhaswamy, M.; Nakano, H.; Gunn, M. D.; Matsuzawa, A.; Quackenbush, E. J.; Dorf, M. E.; von Andrian, U. H. *J. Exp. Med.,* **2000,** *191*, 61.
[49] Warnock, R. A.; Askari, S.; Butcher, E. C.; von Andrian, U. H. *J. Exp. Med.,* **1998,** *187*, 205.
[50] Berlin, C.; Bargatze, R. F.; Campbell, J. J.; von Andrian, U. H.; Szabo, M. C.; Hasslen, S. R.; Nelson, R. D.; Berg, E. L.; Erlandsen, S. L.; Butcher, E. C. *Cell,* **1995,** *80*, 413.
[51] Hamann, A.; Andrew, D. P.; Jablonski-Westrich, D.; Holzmann, B.; Butcher, E. C. *J. Immunol.,* **1994,** *152*, 3282.
[52] Lee, J. H.; Kang, S. G.; Kim, C. H. *J. Immunol*., **2007**, *178*, 301.
[53] Lim, H. W.; Broxmeyer, H. E.; Kim, C. H. *J. Immunol.,* **2006,** *177*, 840.
[54] Thornton, A. M.; Shevach, E. M. *J. Exp. Med.,* **1998,** *188*, 287.
[55] Piccirillo, C. A.; Shevach, E. M. *J. Immunol.,* **2001,** *167*, 1137.
[56] Azuma, T.; Takahashi, T.; Kunisato, A.; Kitamura, T.; Hirai, H. *Cancer Res.,* **2003,** *63,* 4516.
[57] Taams, L. S.; van Amelsfort, J. M.; Tiemessen, M. M.; Jacobs, K. M.; de Jong, E. C.; Akbar, A. N.; Bijlsma, J. W.; Lafeber, F. P. *Hum. Immunol.,* **2005,** *66*, 222.
[58] Lim, H. W.; Hillsamer, P.; Banham, A. H.; Kim, C. H. *J. Immunol.,* **2005,** *175*, 4180.
[59] Cederbom, L.; Hall, H.; Ivars, F. *Eur. J. Immunol.,* **2000,** *30,* 1538.
[60] Smyth, M. J.; Teng, M. W.; Swann, J.; Kyparissoudis, K.; Godfrey, D. I.; Hayakawa, Y. *J. Immunol.,* **2006,** *176*, 1582.
[61] Francois Bach, J. *Nat. Rev. Immunol.,* **2003,** *3*, 189.
[62] Shevach, E. M. *Nat. Rev. Immunol.,* **2002,** *2*, 389.
[63] McHugh, R. S.; Shevach, E. M. *J. Immunol.,* **2002,** *168*, 5979.
[64] Levings, M. K.; Bacchetta, R.; Schulz, U.; Roncarolo, M. G. *Int. Arch. Allergy Immunol.,* **2002,** *129*, 263.
[65] Karim, M.; Bushell, A. R.; Wood, K. J. *Curr. Opin. Immunol.,* **2002,** 14, 584.
[66] Shevach, E. M. *J. Exp. Med.,* **2001,** *193*, F41.
[67] Annunziato, F.; Cosmi, L.; Liotta, F.; Lazzeri, E.; Manetti, R.; Vanini, V.; Romagnani, P.; Maggi, E.; Romagnani, S. *J. Exp. Med.,* **2002,** *196*, 379.
[68] Manzotti, C. N.; Tipping, H.; Perry, L. C.; Mead, K. I.; Blair, P. J.; Zheng, Y.; Sansom, D. M. *Eur. J. Immunol.,* **2002,** *32*, 2888.
[69] Grohmann, U.; Orabona, C.; Fallarino, F.; Vacca, C.; Calcinaro, F.; Falorni, A.; Candeloro, P.; Belladonna, M. L.; Bianchi, R.; Fioretti, M. C.; Puccetti, P. *Nat. Immunol.,* **2002,** *3*, 1097.
[70] Choi, B. M.; Pae, H. O.; Jeong, Y. R.; Kim, Y. M.; Chung, H. T. *Biochem. Biophys. Res. Commun.,* **2005,** *327*, 1066.
[71] Otterbein, L. E.; Soares, M. P.; Yamashita, K.; Bach, F. H. *Trends Immunol.,* **2003,** *24*, 449.
[72] Sato, K.; Balla, J.; Otterbein, L.; Smith, R. N.; Brouard, S.; Lin, Y.; Csizmadia, E.; Sevigny, J.; Robson, S. C.; Vercellotti, G.; Choi, A. M.; Bach, F. H.; Soares, M. P. *J. Immunol.,* **2001,** *166*, 4185.
[73] Pae, H. O.; Oh, G. S.; Choi, B. M.; Chae, S. C.; Kim, Y. M.; Chung, K. R.; Chung, H. T. *J. Immunol.,* **2004,** *172*, 4744.
[74] Grossman, W. J.; Verbsky, J. W.; Barchet, W.; Colonna, M.; Atkinson, J. P.; Ley, T. J. *Immunity,* **2004,** *21*, 589.
[75] Gondek, D. C.; Lu, L. F.; Quezada, S. A.; Sakaguchi, S.; Noelle, R. J. *J. Immunol.,* **2005,** *174*, 1783.
[76] Zhao, D. M.; Thornton, A. M.; DiPaolo, R. J.; Shevach, E. M. *Blood,* **2006,** *107*, 3925.
[77] Nocentini, G.; Giunchi, L.; Ronchetti, S.; Krausz, L. T.; Bartoli, A.; Moraca, R.; Migliorati, G.; Riccardi, C. *Proc. Natl. Acad. Sci. USA,* **1997,** *94*, 6216.
[78] Kwon, B.; Yu, K. Y.; Ni, J.; Yu, G. L.; Jang, I. K.; Kim, Y. J.; Xing, L.; Liu, D.; Wang, S. X.; Kwon, B. S. *J. Biol. Chem.,* **1999,** *274*, 6056.
[79] McHugh, R. S.; Whitters, M. J.; Piccirillo, C. A.; Young, D. A.; Shevach, E. M.; Collins, M.; Byrne, M. C. *Immunity,* **2002,** *16*, 311.
[80] Shimizu, J.; Yamazaki, S.; Takahashi, T.; Ishida, Y.; Sakaguchi, S. *Nat. Immunol.,* **2002,** *3*, 135.
[81] Stephens, G. L.; McHugh, R. S.; Whitters, M. J.; Young, D. A.; Luxenberg, D.; Carreno, B. M.; Collins, M.; Shevach, E. M. *J. Immunol.,* **2004,** *173*, 5008.
[82] Gramaglia, I.; Weinberg, A. D.; Lemon, M.; Croft, M. *J. Immunol.,* **1998,** *161*, 6510.
[83] Stuber, E.; Neurath, M.; Calderhead, D.; Fell, H. P.; Strober, W. *Immunity,* **1995,** *2*, 507.
[84] Brocker, T.; Gulbranson-Judge, A.; Flynn, S.; Riedinger, M.; Raykundalia, C.; Lane, P. *Eur. J. Immunol.,* **1999,** *29*, 1610.
[85] Valzasina, B.; Guiducci, C.; Dislich, H.; Killeen, N.; Weinberg, A. D.; Colombo, M. P. *Blood,* **2005,** *105,* 2845.
[86] Zheng, G.; Wang, B.; Chen, A. *J. Immunol.,* **2004,** *173*, 2428.
[87] Lee, J.; Lee, E. N.; Kim, E. Y.; Park, H. J.; Chang, C. Y.; Jung, D. Y.; Choi, S. Y.; Lee, S. K.; Lee, K. W.; Kwon, G. Y.; Joh, J. W.; Kim, S. J. *Immunol. Lett.,* **2005,** *101*, 210.
[88] Maerten, P.; Kwon, B. S.; Shen, C.; De Hertogh, G.; Cadot, P.; Bullens, D. M.; Overbergh, L.; Mathieu, C.; Van Assche, G.; Geboes, K.; Rutgeerts, P.; Ceuppens, J. L. *Clin. Exp. Immunol.,* **2006,** *143*, 228.
[89] Tang, Q.; Henriksen, K. J.; Boden, E. K.; Tooley, A. J.; Ye, J.; Subudhi, S. K.; Zheng, X. X.; Strom, T. B.; Bluestone, J. A. *J. Immunol.,* **2003,** *171*, 3348.
[90] Liu, H.; Hu, B.; Xu, D.; Liew, F. Y. *J. Immunol.,* **2003,** *171*, 5012.
[91] Boden, E.; Tang, Q.; Bour-Jordan, H.; Bluestone, J. A. *Novartis Found. Symp.,* **2003,** *252*, 55.
[92] Nakamura, K.; Kitani, A.; Strober, W. *J. Exp. Med.,* **2001,** *194*, 629.
[93] Thornton, A. M.; Shevach, E. M. *J. Exp. Med.,* **1998,** *188*, 287.
[94] Cohen, A. C.; Nadeau, K. C.; Tu, W.; Hwa, V.; Dionis, K.; Bezrodnik, L.; Teper, A.; Gaillard, M.; Heinrich, J.; Krensky, A. M.; Rosenfeld, R. G.; Lewis, D. B. *J. Immunol.,* **2006,** *177*, 2770.
[95] Zorn, E.; Nelson, E. A.; Mohseni, M.; Porcheray, F.; Kim, H.; Litsa, D.; Bellucci, R.; Raderschall, E.; Canning, C.; Soiffer, R. J.; Frank, D. A.; Ritz, J. *Blood,* **2006,** *108*, 1571.
[96] Fontenot, J. D.; Rasmussen, J. P.; Gavin, M. A.; Rudensky, A. Y. *Nat. Immunol.,* **2005,** *6*, 1142.
[97] Schramm, C.; Huber, S.; Protschka, M.; Czochra, P.; Burg, J.; Schmitt, E.; Lohse, A. W.; Galle, P. R.; Blessing, M. *Int. Immunol.,* **2004,** *16,* 1241.
[98] Marie, J. C.; Letterio, J. J.; Gavin, M.; Rudensky, A. Y. *J. Exp. Med.,* **2005,** *201*, 1061.
[99] Piccirillo, C. A.; Letterio, J. J.; Thornton, A. M.; McHugh, R. S.; Mamura, M.; Mizuhara, H.; Shevach, E. M. *J. Exp. Med.,* **2002,** *196*, 237.
[100] Yamagiwa, S.; Gray, J. D.; Hashimoto, S.; Horwitz, D. A. *J. Immunol.,* **2001,** *166*, 7282.
[101] Nakamura, K.; Kitani, A.; Fuss, I.; Pedersen, A.; Harada, N.; Nawata, H.; Strober, W. *J. Immunol.,* **2004,** *172*, 834.
[102] Fahlen, L.; Read, S.; Gorelik, L.; Hurst, S. D.; Coffman, R. L.; Flavell, R. A.; Powrie, F. *J. Exp. Med.,* **2005,** *201*, 737.
[103] Dieckmann, D.; Plottner, H.; Berchtold, S.; Berger, T.; Schuler, G. *J. Exp. Med.,* **2001,** *193*, 1303.
[104] Stephens, L. A.; Mottet, C.; Mason, D.; Powrie, F. *Eur. J. Immunol.,* **2001,** *31*, 1247.
[105] Taams, L. S.; Smith, J.; Rustin, M. H.; Salmon, M.; Poulter, L. W.; Akbar, A. N. *Eur. J. Immunol.,* **2001,** *31*, 1122.
[106] Walker, M. R.; Kasprowicz, D. J.; Gersuk, V. H.; Benard, A.; Van Landeghen, M.; Buckner, J. H.; Ziegler, S. F. *J. Clin. Invest.,* **2003,** *112*, 1437.
[107] Zhang, X.; Koldzic, D. N.; Izikson, L.; Reddy, J.; Nazareno, R. F.; Sakaguchi, S.; Kuchroo, V. K.; Weiner, H. L. *Int. Immunol.,* **2004,** *16*, 249.
[108] Gangi, E.; Vasu, C.; Cheatem, D.; Prabhakar, B. S. *J. Immunol.,* **2005,** *174*, 7006.
[109] Crellin, N. K.; Garcia, R. V.; Hadisfar, O.; Allan, S. E.; Steiner, T. S.; Levings, M. K. *J. Immunol.,* **2005,** *175*, 8051.
[110] Sutmuller, R. P.; den Brok, M. H.; Kramer, M.; Bennink, E. J.; Toonen, L. W.; Kullberg, B. J.; Joosten, L. A.; Akira, S.; Netea, M. G.; Adema, G. J. *J. Clin. Invest.,* **2006,** *116,* 485.
[111] Liu, H.; Komai-Koma, M.; Xu, D.; Liew, F. Y. *Proc. Natl. Acad. Sci. USA,* **2006,** *103*, 7048.
[112] Pasare, C.; Medzhitov, R. *Science,* **2003,** *299*, 1033.
[113] Kim, C. H. *Curr. Opin. Hematol.,* **2005,** *12*, 298.

[114] Kim, C. H.; Rott, L.; Kunkel, E. J.; Genovese, M. C.; Andrew, D. P.; Wu, L.; Butcher, E. C. *J. Clin. Invest.,* **2001,** *108*, 1331.
[115] Williams, I. R. *Ann. N Y Acad. Sci.,* **2006,** *1072*, 52.
[116] Stassen, M.; Fondel, S.; Bopp, T.; Richter, C.; Muller, C.; Kubach, J.; Becker, C.; Knop, J.; Enk, A. H.; Schmitt, S.; Schmitt, E.; Jonuleit, H. *Eur. J. Immunol.,* **2004,** *34*, 1303.
[117] Cavani, A.; Nasorri, F.; Ottaviani, C.; Sebastiani, S.; De Pita, O.; Girolomoni, G. *J. Immunol.,* **2003,** *171*, 5760.
[118] Huehn, J.; Siegmund, K.; Lehmann, J. C.; Siewert, C.; Haubold, U.; Feuerer, M.; Debes, G. F.; Lauber, J.; Frey, O.; Przybylski, G. K.; Niesner, U.; De La Rosa, M.; Schmidt, C. A.; Brauer, R.; Buer, J.; Scheffold, A.; Hamann, A. *J. Exp. Med.,* **2004,** *199*, 303.
[119] Iellem, A.; Colantonio, L.; D'Ambrosio, D. *Eur. J. Immunol.,* **2003,** *33*, 1488.
[120] Butcher, E. C.; Williams, M.; Youngman, K.; Rott, L.; Briskin, M. *Adv. Immunol.,* **1999,** *72*, 209.
[121] Allen, S. J.; Crown, S. E.; Handel, T. M. *Annu. Rev. Immunol.,* **2007,** *25*, 787.
[122] Tamamura, H.; Tsutsumi, H.; Masuno, H.; Fujii, N. *Curr. Med. Chem.,* **2007,** *14*, 93.
[123] Huang, C. T.; Workman, C. J.; Flies, D.; Pan, X.; Marson, A. L.; Zhou, G.; Hipkiss, E. L.; Ravi, S.; Kowalski, J.; Levitsky, H. I.; Powell, J. D.; Pardoll, D. M.; Drake, C. G.; Vignali, D. A. *Immunity,* **2004,** *21*, 503.
[124] Huard, B.; Tournier, M.; Hercend, T.; Triebel, F.; Faure, F. *Eur. J. Immunol.,* **1994,** *24*, 3216.
[125] Neufeld, G.; Cohen, T.; Shraga, N.; Lange, T.; Kessler, O.; Herzog, Y. *Trends Cardiovasc. Med.,* **2002,** *12*, 13.
[126] Khan, A. A.; Bose, C.; Yam, L. S.; Soloski, M. J.; Rupp, F. *Science,* **2001,** *292*, 1681.
[127] Bruder, D.; Probst-Kepper, M.; Westendorf, A. M.; Geffers, R.; Beissert, S.; Loser, K.; von Boehmer, H.; Buer, J.; Hansen, W. *Eur. J. Immunol.,* **2004,** *34*, 623.

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