

For reprint orders, please contact:
reprints@future-drugs.com



Regulation of humoral immunity by FoxP3⁺ regulatory T cells

Chang H Kim

FoxP3⁺ T cells play critical roles in the regulation of B-cell function and humoral immune response. FoxP3⁺ T cells migrate to the T and B zones of lymphoid organs and the border area between the two areas. Not only can FoxP3⁺ T cells suppress B cells directly, but they also suppress B-cell response indirectly through suppressing T-helper cells. Suppression of T-helper or B cells by FoxP3⁺ T cells results in limited activation and proliferation of B cells. Consequentially, class-switch recombination and antibody production in B cells are suppressed by FoxP3⁺ T cells. Furthermore, FoxP3⁺ T cells can kill activated B cells directly. FoxP3⁺ T cells can suppress both autoreactive B cells and antibody responses to foreign antigens. The suppressive activity of FoxP3⁺ T cells is regulated by cytokines, costimulators and products of pathogens and normal microflora (i.e., Toll-like receptor ligands), which may allow selective regulation of immune responses to self-antigens and commensals versus pathogens by FoxP3⁺ cells. Insufficient function or numbers of FoxP3⁺ T cells in the body could lead to aberrant B-cell response and autoimmune diseases in multiple organs. Therefore, FoxP3⁺ T cells are an important immune component that reins in potentially over-active or self-reactive humoral immunity.

Expert Rev. Clin. Immunol. 2(6), 859–868 (2006)

FoxP3⁺ cells as regulatory T cells

Regulatory T cells (T_{regs}) were initially identified for their suppressive activity on immune response to self-antigens [1–7], but it was later found that they can also suppress immune responses to alloantigens [8–10], tumors [11,12] and pathogens [13,14]. The existence of T_{regs} that induce immune tolerance to self-antigens was reported many decades ago [15–20]; however, the research on T_{regs} in the 1970s was based on the incorrect hypotheses that the function of T_{regs} would be mediated by soluble factors that do not exist [21]. In the mid-1990s, it was demonstrated that T_{regs} express CD25 (the interleukin [IL]-2 receptor α -chain) [22]. This finding energized the research field and we have witnessed a remarkable progress in characterization of T_{regs} in the last decade.

Although T_{regs} can be induced from non-T_{regs} in the periphery in certain conditions, the presence of thymus-derived regulatory cells is firmly established [5,23,24]. A key feature of thymus-derived T_{regs} in mice and humans is that they

express FoxP3 (the product of the mouse *Foxp3* gene) or FOXP3 (the human version). FoxP3/FOXP3 is a member of the fork-head/winged-helix family of transcriptional regulators. FoxP3 induces regulatory function in T cells and increases the expression of cytotoxic T lymphocyte antigen (CTLA)-4 and CD25, but decreases IL-2 production [25–28]. FoxP3 binds and suppresses nuclear factor (NF) of activated T cells (NFAT) and NF- κ B [29], key transcription factors required for activation of immune cells. Therefore, FoxP3 acts as the T_{reg}-lineage-specific transcription-regulation factor, while other factors play similar roles in T-cell differentiation into different T-cell lineages, such as T-helper (Th)1 cells (signal transducers and activators of transcription [Stat]4, Stat1, T-box expressed in T cells and interferon [IFN] regulatory factor-1) and Th2 cells (c-Maf, NFATc, Stat6, and GATA-3) [30].

In addition to the FoxP3⁺ cells of thymic origin, T_{regs} can be generated in response to antigens in the periphery [31], pre-existing FoxP3⁺

CONTENTS

FoxP3⁺ cells as regulatory T cells

Regulation of B-cell response

Migration of FoxP3⁺ T_{regs} to B-cell sites of secondary lymphoid & nonlymphoid tissues

FoxP3⁺ cells regulate B-cell response

Impacts of FoxP3⁺ cells on diseases of the humoral immune system

Expert commentary & five-year view

Key issues

Acknowledgements

References

Affiliation

Laboratory of Immunology and Hematopoiesis, Department of Pathobiology, Purdue Cancer Center, Purdue University, West Lafayette, IN 47907, USA
Tel.: +1 765 494 0976
Fax: +1 765 494 9830
chkim@purdue.edu

KEYWORDS:
antibodies, B cells, chemokine, FoxP3, migration, regulatory cells

T_{regs} [32], antigen-presenting cells [33–36] and cytokines (tumor growth factor [TGF]- β , IL-2, -10, -4 and -13) [37–40]. Furthermore, there have been reports on the roles of estrogen [41], vitamin D3 [42] and prostaglandin E2 [43] in the generation of T_{regs} . However, not all T-cell subsets with suppressive functions express FoxP3 [44], and not all FoxP3⁺ T cells express CD25 [45,46]. Although most FoxP3⁺ cells are CD4⁺ T cells [23,24,47], some are CD8⁺ cells [45,48]. Generally, T-cell function is mediated by cytokines or cytokine-like molecules on cell surface. For example, Th1 cells produce IFN- γ , which activates macrophages and other cells for cell-mediated immunity. Th2 cells produce IL-4, -5 and -13, which act on B cells, eosinophils and other cell types for antihelminth function and immunoglobulin (Ig)E response [49]. By contrast, FoxP3⁺ T cells express different effector molecules (e.g., TGF- β , CTLA4 and/or IL-10) and do not or poorly produce naïve and Th1/2 effector cytokines (e.g., IL-2, -4 and IFN- γ) [50,51]. It should also be appreciated that there are different types of CD4⁺ T_{regs} in addition to the FoxP3⁺ T cells. There include Tr1 and Th3 cells. Tr1 cells are generated from naïve T cells in response to IL-10 and produce IL-10 as their major effector cytokine [52], whereas Th3 cells are induced in response to TGF- β [38,53]. Some of Tr1 cells or Th3 cells could be FoxP3⁺ T cells, but they are independent of FoxP3 expression [54].

Regulation of B-cell response

Antibody-mediated immunity begins with naïve B cells in secondary lymphoid tissues. Naïve B cells are generated in bone marrow, but migrate to secondary lymphoid tissues for functional maturation. Naïve B cells are collected within the primary follicles of lymph nodes, Peyer's patches, spleen and other secondary lymphoid tissues [55–57]. Primary follicles are formed adjacent to T-cell zones where many T cells are localized. In the absence of antigens that activate naïve B cells and Th cells, naïve B cells migrate in and out of the primary follicles and circulate in the blood. Upon antigenic stimulation, B cells undergo activation and differentiation. B cells undergo initial activation at the boundary of B-cell follicles and T-cell zones. In the spleen, they become plasmablasts in marginal-zone-bridging zones (area between the T zone and the red pulp). Protein antigens that are able to activate both Th and B cells are called T-dependent antigens [56]. T-dependent antigens induce class-switch recombination and somatic hypermutation in antibody genes. During this process, B cells receive important stimulatory signals, such as CD40L and cytokines from Th cells [58,59]. Alternatively, T-independent antigens can directly activate B cells and induce IgM and limited IgG responses without forming memory [60].

T-dependent antigens induce formation of germinal centers (GCs). GCs are sites of B-cell activation and differentiation to generate B cells that make class-switched and high affinity antibodies to T-dependent antigens [61,62]. Although GC B cells are of the majority, T cells, follicular dendritic cells (FDCs) and dendritic cells (DCs) are also present in GCs and regulate activation, selection and differentiation of GC B cells [61–63]. T cells in GCs (GC T cells) produce IL-10 and express CD40L

and inducible costimulator (ICOS). GC T cells activate GC B cells and induce somatic hypermutation and class-switch recombination [63]. GC T cells are highly efficient in helping GC B cells, and induce expression of activation-induced cytosine deaminase (AID) and class-switch recombination in developing B cells for production of class-switched Igs, such as IgG_{1–4}, IgA_{1&2} and IgE in humans.

Migration of FoxP3⁺ T_{regs} to B-cell sites of secondary lymphoid & nonlymphoid tissues

An important feature of the suppression mediated by FoxP3⁺ T_{regs} is the requirement for physical cell–cell contact with target cells, which implies that FoxP3⁺ T cells must migrate next to target cells to suppress them. T cells must express appropriate trafficking receptors to migrate from blood to tissue sites [64]. Expression of CCR7 and CD62L are required for T cells to enter secondary lymphoid tissues, $\alpha 4\beta 7$ and CCR9 to migrate to the small intestine. Chemokine receptors (e.g., CCR4, 8 and 10) are implicated with skin-homing T cells. Chemoattractant receptors (e.g., CCR1, 2, 4, 5, CXCR3, 6 and CRTH2) and adhesion molecules (e.g., E- and P-selectin ligands) are implicated with T-cell migration to sites of inflammation. Adhesion molecules mediate attachment of T cells on endothelial cells (called rolling and firm adhesion), and chemoattractants regulate the activity of integrins and induce chemotaxis [65]. T_{regs} and conventional T cells share expression of many trafficking receptors, and it appears that they co-migrate with conventional T cells to secondary lymphoid and nonlymphoid tissues [66]. It was reported that human CD4⁺CD25⁺ T_{regs} are located in perivascular areas of fibrous septa of thymus [67]. These T_{regs} express CCR8, and its chemokine ligand CCL1, expressed by either thymic medullary macrophages or fibrous septa epithelial cells, could mediate such a localization in the thymic medulla. In addition to CCR8, CD4⁺CD25⁺ T_{regs} in human peripheral blood express the Th2-type chemokine receptor CCR4 [68]. These circulating CD4⁺CD25⁺ T_{regs} preferentially express cutaneous lymphocyte antigen (CLA) over the gut-homing receptor $\alpha 4\beta 7$ [69], which indicates that many of the circulating T_{regs} can migrate to the skin. However, many T_{regs} are found in intestinal mucosa in humans [70] and it was later shown that approximately 45% of memory FoxP3⁺ T cells, present in human peripheral and neonatal blood, actually express $\alpha 4\beta 7$ [66]. Importantly, T_{regs} are also found in T- and B-cell areas (also called follicles) of secondary lymphoid tissues, where initiation of humoral immunity occurs.

In secondary lymphoid tissues, many CD4⁺CD25⁺ T_{regs} are found in T-cell zones [48,71]. These T_{regs} mainly express CCR7 and are chemotactically attracted to its ligand CCL19, a chemokine expressed in the T-cell zones. However, this migration behavior rapidly changes upon antigenic stimulation of T_{regs} . Upon activation, T_{regs} upregulate CXCR5 and are attracted chemotactically to a different chemokine CXCL13, which is expressed in the B-cell zones and GCs of secondary lymphoid tissues. Therefore, the change in chemotactic behavior following T-cell activation is due, in part, to the switch in

the expression of chemokine receptors, which occurs in response to antigenic stimulation. In a subsequent study, we found, using a monoclonal anti-FoxP3 antibody, that FoxP3⁺ T_{regs} are found in T–B area borders and within GCs [46], key areas where T-cell dependent activation of B cells occurs.

Antibody-secreting cells or plasma B cells, generated by T-dependent or -independent antigens, migrate to bone marrow or mucosal sites, such as intestinal lamina propria [72–76]. IgM- or IgG-producing B cells express CXCR4 and preferentially migrate to the red pulp (spleen), medulla (lymph nodes) or bone marrow, where CXCL12 is highly expressed [77]. For migration of IgA-producing B cells to the small intestine, the CCR9 and CCL25 chemokine receptor–chemokine pair is thought to be important [78–81]. T_{regs} are widely found at tissue sites of plasma B cells, including bone marrow [82] and intestine [70]. T_{regs} are highly enriched in human bone marrow [82], and approximately 40% of the CD4⁺ T cells in mouse bone marrow are Foxp3⁺ T cells [83]. Using chimeric non-obese diabetic-severe combined immunodeficient (NOD-SCID) mice, it was demonstrated that human T_{regs} migrate to mouse bone marrow [82]. Granulocyte colony-stimulating factor (G-CSF) is a potent mobilizer of bone-marrow cells, including hematopoietic stem and progenitor cells. It was demonstrated that G-CSF mobilizes marrow T_{regs} to the peripheral-blood system by decreasing CXCL12 expression in marrow. CXCL12 is implicated in recruiting and retaining CXCR4⁺ cells in bone marrow [65,84], and most Foxp3⁺ T cells highly express CXCR4 [83]. The exact function of T_{regs} at sites of plasma B cells is unknown. T_{regs} could regulate the function of plasma B cells because they can directly suppress B cells [46]. Migration of FoxP3⁺ T cells to various tissues sites of B cells is summarized in FIGURE 1.

FoxP3⁺ cells regulate B-cell response

Suppression of T cells by T_{regs} has been extensively studied *in vitro* and *in vivo*. As T cells provide positive signals for activation and differentiation of B cells into antibody-secreting cells, it is likely that suppression of T-cell function would lead

to suppression of B-cell function. Using a T-cell-dependent B-cell Ig-production system driven by pokeweed mitogen *in vitro* [50], it was demonstrated that CD4⁺CD25⁺ T_{regs} were

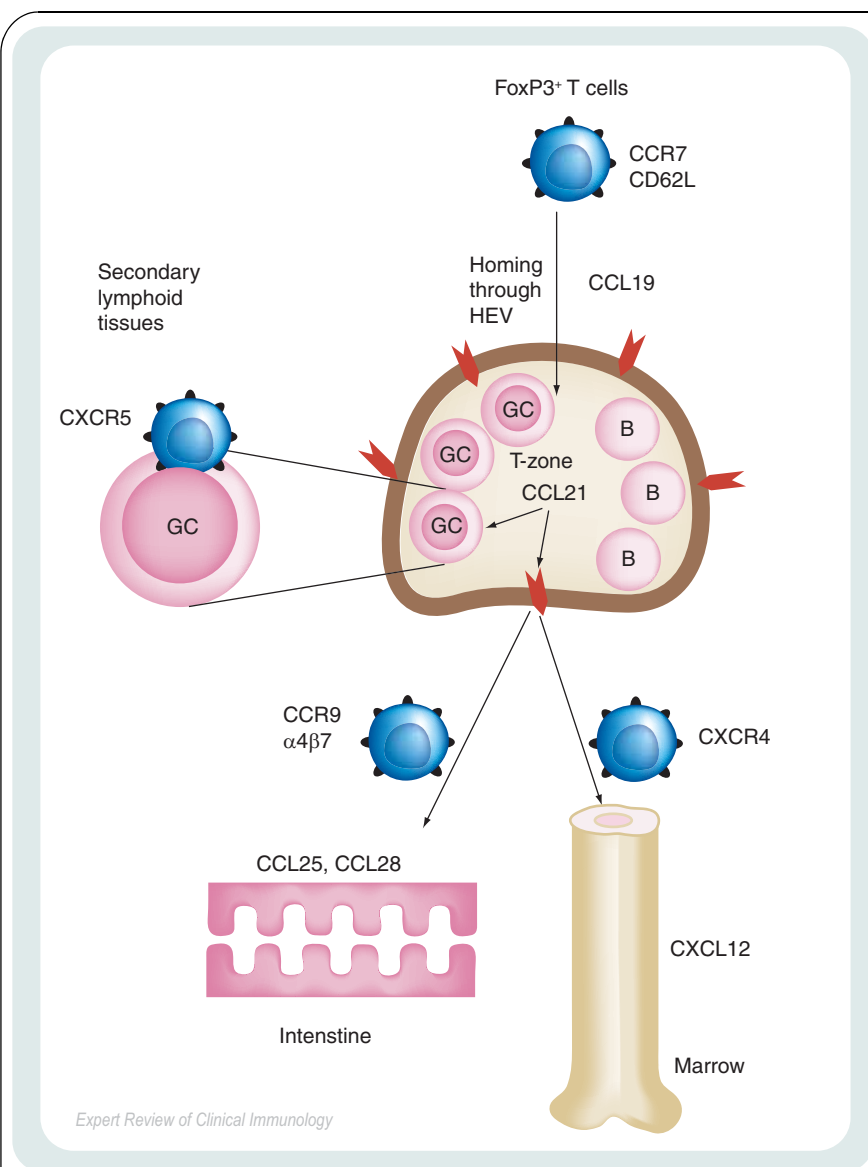


Figure 1. Migration of FoxP3⁺ T cells to tissue sites of humoral immune responses. Generally, most FoxP3⁺ T_{regs} express CD25, cytotoxic T-lymphocyte antigen-4, glucocorticoid-induced tumor-necrosis-factor receptor, CD44 and tumor growth factor-β. Furthermore, some FoxP3⁺ T cells variably express CD62L, CD103 and a number of chemokine receptors. This suggests that multiple FoxP3⁺ T-cell subsets exist with heterogeneous expression patterns of trafficking receptors. Largely, FoxP3⁺ T cells can be divided into secondary lymphoid tissue-homing and nonlymphoid tissue-homing subsets. Secondary lymphoid tissue homing T_{regs} express CCR7, CD62L and CXCR4, while nonlymphoid tissue-homing T_{regs} express other chemokine receptors (CCR2–6, CCR8–10 and CXCR3–6) and adhesion molecules (E/P-selectin ligands). FoxP3⁺ T cells that are made in thymus emigrate and circulate in the blood system. These FoxP3⁺ T cells are secondary lymphoid tissue-homing T_{regs}. CCR7 would guide their migration into the T-zones of secondary lymphoid tissues. Upregulation of CXCR5 on T_{regs} occurs upon activation by antigens. This would position CXCR5⁺ T_{regs} into the B-cell zones or T–B zone border. FoxP3⁺ T cells suppress both Th cells and B cells and limit the production of antibodies to self-antigens, alloantigen and foreign antigens. Among the nonlymphoid tissue homing FoxP3⁺ T cells, some express α4β7 and CCR9 and migrate to the gut, while others express CXCR4 at high levels and migrate to bone marrow, where antibody secreting cells are enriched.

able to suppress mouse B cells and their production of Igs. This suppression was mediated through TGF- β 1, but not IL-10, produced by CD4⁺CD25⁺ T_{regs}. In this regard, T_{regs} produce or express soluble or membrane-bound TGF- β 1 [50].

T_{regs} can suppress GC T cells and T-cell-induced B-cell responses, such as Ig production, survival and expression of AID [71]. However, it was unclear which cell type was suppressed by T_{regs} in the system. It could be T cells, B cells or both. In a subsequent study, we were able to demonstrate that FOXP3⁺ T_{regs} can directly suppress B-cell response in the absence of Th cells [46]. As the same FOXP3⁺ regulatory subset is able to suppress Th cells independently of B cells, T_{regs} appear to suppress both B cells and Th cells. At molecular levels, FOXP3⁺ T_{regs} can directly inhibit AID expression and Ig class-switch recombination in B cells. The role of T_{regs} in T-cell-dependent B-cell antibody response has been demonstrated *in vivo* in mice [85]. In this study, antibody depletion of CD25⁺ cells lead to significantly increased antigen-specific antibody production and affinity maturation, but had relatively minor impact on T-independent antibody responses. The mechanisms by which human T_{regs} suppress B cells or Th cells are still unclear. Neutralization of TGF- β and/or IL-10 had only marginal abolishing effects on the suppressive activity of human T_{regs} on B cells [46].

In addition to the roles of TGF- β and IL-10, the potential roles of granzymes, CTLA4-indoleamine 2,3-dioxygenase (IDO) and hemeoxygenase (HO)-1 have been suggested to explain the suppressive activity of T_{regs} in mice and humans [23,86]. CTLA-4 appears to mediate some of the suppressive activity of T_{regs} on target cells [67,87]. CTLA-4 would activate B7 on target cells, such as DCs, and the signal generated by the B7–CTLA4 induces expression of IDO, an enzyme involved in the catabolism of the amino-acid tryptophan [88]. Depletion of tryptophan would suppress activation and function of target cells. HO-1 is another factor that may mediate the suppressive function of T_{regs}. HO-1 is an enzyme that converts heme to carbon monoxide, free iron and biliverdin (a precursor of bilirubin) [89]. Carbon monoxide is the product of HO-1 that has anti-inflammatory effects [90] and suppresses IL-2 production [91]. HO-1 is induced by a variety of stimuli in T cells, including FoxP3 expression [92].

T_{regs} may also kill Th cells or B cells to suppress their functions. It was demonstrated that human T_{regs} express granzyme A and display perforin-dependent cytotoxicity against T cells, monocytes and DCs [93], while mouse T_{regs} express granzyme B and kill target cells [94]. In this regard, mouse CD4⁺CD25⁺ T cells can kill antigen-presenting B cells and decrease B-cell antibody response in a manner dependent on perforin and granzymes [95]. However, other T-cell subsets also have cytotoxicity, and it is unclear whether T_{regs} are better than other T-cell subsets, such as cytotoxic T cells or non-FoxP3⁺ memory/effector CD4⁺ T cells in killing target cells. It remains to be determined if the cytotoxicity of T_{regs} is really important *in vivo* or it is just an *in vitro* phenomenon.

As an indirect mechanism, T_{regs} can significantly diminish the antigen priming process in Th cells to suppress the expression of B-cell helping signals. Using an intravital multiphoton

microscopy technique that allows imaging live immune cell interaction occurring deep in lymph nodes, it has been demonstrated that T_{regs} suppress the interaction of antigen-specific T cells and DCs [96]. In another intravital multiphoton microscopy study, it was observed that T_{regs} and non-FoxP3⁺ T cells do not significantly contact with each other during active suppression [97]. In this situation, the preferential interaction partner of T_{regs}, of course, was DCs bearing antigens. One caveat with the interpretation of the intravital study results is that events occurring deep (>300 μ m) in the tissues cannot be visualized. Another limitation is that rarely occurring events (e.g., interaction between T_{regs} and B cells in GCs) are difficult to be tracked.

Whatever the mechanism of suppression, the important function of FoxP3⁺ T cells in regulation of humoral immune response and maintenance of self-tolerance has been well demonstrated. Depletion of T_{regs} by monoclonal anti-CD25 antibody resulted in the production of autoantibodies [98,99]. T_{regs}, activated in the presence of IL-2 and T-cell receptor agonists, lose their suppressive function, leading to autoantibody (antiparietal antibodies) production and gastritis in nude mice [99]. Depletion of T_{regs} in mice led to accelerated formation of GCs and B cell proliferation upon immunization [98]. Neutralization of the chemokine CCL4, which attracts T_{regs}, induced formation of autoantibodies to double-stranded DNA (dsDNA), myeloperoxidase and cardiolipin [98]. In the presence of T_{regs}, B cells that express the B-cell receptor that are specific for dsDNA and other self-antigens become anergic (i.e., unresponsive) in normal mice. However, this B-cell anergy can be reversed by the helping signals of Th cells, and this loss of B-cell anergy can be prevented by CD4⁺CD25⁺ T_{regs} by suppressing the Th cells [100]. Taken together, the information provided in this section supports the important roles of FoxP3⁺ T cells in regulation of humoral immunity to self- and nonself-antigens. Regulation of B-cell responses by FoxP3⁺ T cells is summarized in FIGURE 2.

Impacts of FoxP3⁺ cells on diseases of the humoral immune system

T_{regs} are required for maintenance of tolerance in humoral immune responses and are implicated in many diseases of the humoral immune system. Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by inappropriate regulation of self-reactive B and T cells. In pediatric SLE patients, the frequency of CD4⁺CD25⁺ T_{regs} was significantly decreased in patients with active SLE compared with normal controls and patients with inactive SLE, and was inversely correlated with serum anti-dsDNA antibody levels [101]. Collagen-induced arthritis (CIA) is a systemic antibody-mediated disease and a popular model of rheumatoid arthritis. In a mouse CIA model, CD25⁺ cell depletion led to a significantly more severe disease with higher antibody titers against collagen than that of control mice [102].

Another convincing evidence for the important role of T_{regs} in humoral immunity came from a mouse strain that does not have functional FoxP3 (scurfy mice) [103]. The mice have a

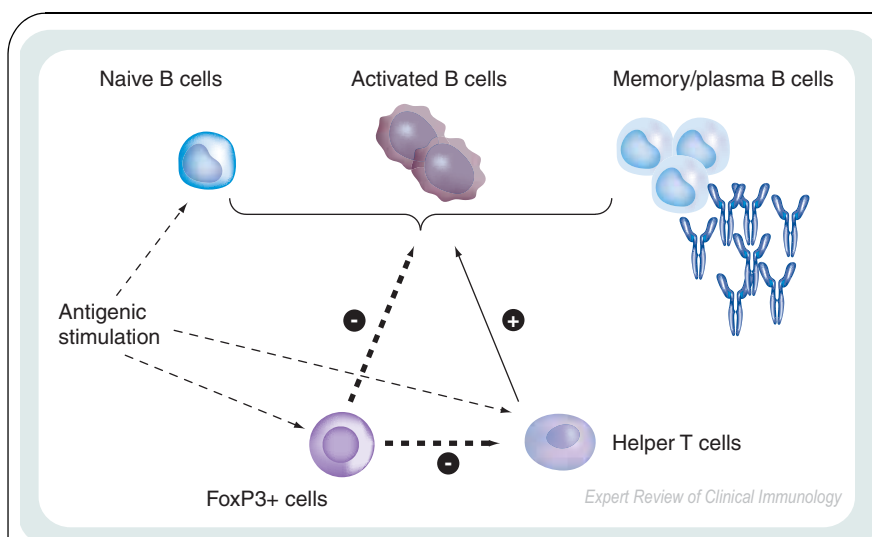


Figure 2. Regulation of antibody response by FoxP3⁺ T cells. Protein antigens, such as self-antigens, would activate B cells and Th cells. Activated autoreactive B cells in the presence of Th cells would lead to production of autoantibodies and autoimmunity. However, in the presence of FoxP3⁺ T cells that are reactive to self-antigens, activation of autoreactive B cells and Th cells would be suppressed, leading to abortive response to self-antigens. In many autoimmune diseases involving aberrant production of autoantibodies, the number and activity of FoxP3⁺ T cells are severely reduced. Activation of Th cells and B cells in the absence of optimal FoxP3⁺ cell activity leads to massive production of Th2 cytokines that, in turn, amplifies the production of autoantibodies. On the other hand, excessive FoxP3⁺ cell activity may lead to insufficient antibody response to pathogens. Actually, tumor cells and some pathogens increase the numbers of FoxP3⁺ T cells to avoid host immune responses toward themselves.

to scurfy mice was generated [109]. These FoxP3 mutant mice develop hyper-Th2 response, hyperimmunoglobulinemia E, eosinophilia and allergic airway inflammation. In a mouse model of autoimmune response to chromatin, T_{regs} were efficient in suppression of B cell production of anti-chromatin antibodies [110]. In this study, the authors demonstrated that T_{regs} decreased the expression levels of costimulator molecule ICOS on Th cells and inhibited late response in formation of autoantibody, but did not suppress early responses, such as initial activation of and follicular entry by Th or B cells.

T_{regs} play critical roles in regulation of humoral immune response not only to self-, allo- and tumor antigens, but also to foreign antigens, including pathogens in certain conditions. An important question is why T_{regs} suppress immune response to pathogens. More often than not, vigorous immune responses are required to clear pathogens, and therefore, suppression could lead to uncontrolled infection that is harmful to hosts. It is well established that infection with certain pathogens is linked to the onset of

frameshift mutation in the *FoxP3* gene located in the X chromosome and express a FoxP3 protein lacking the fork-head DNA domain [104]. Scurfy mice develop a distinctive external phenotype (scaliness in the skin, crusting of the eyelids, ears and tail, runting, reddening and swelling of the genital papilla) and die at the age of 2–4 weeks. Scurfy mice have a severe lymphoproliferative disease evidenced by marked splenomegaly, hepatomegaly, enlarged lymph nodes, severe anemia and leukocytosis in a manner similar to the animals deficient in TGF-β1 or CTLA-4 [105,106]. As the consequence of the unregulated immune response, concentrations of cytokines, such as IL-4, -6, -7 and TNF-α, are greatly increased in these mice. T cells in scurfy mice are overly responsive to the signals generated from ligation of TCR and CD28 and are extremely active in helping B cells [107]. By contrast, T cells of FoxP3-overexpressing transgenic mice poorly express CD40 ligand and are limited in helper function for T-cell-dependent antibody production [26]. All of the autoimmune diseases in scurfy mice were prevented by transfer of bone marrow or CD4⁺CD25⁺ T cells from normal mice [108], emphasizing the important role of FoxP3⁺ T cells in regulation of B cells and other autoimmune responses.

It is thought that Th2 cells play important roles in inducing a hyperimmune response to allergens, promoting IgE production, allergy and asthma. Th2 cells can be suppressed by T_{regs}, and therefore, T_{regs} are believed to regulate the humoral immune responses leading to allergy. A FoxP3 mutant mouse strain similar

specific autoimmune diseases [111,112]. FoxP3⁺ T cells may prevent emergence of autoimmunity following infection and they can suppress hyperimmune responses to pathogens. Alternatively, pathogens would take advantage of the regulatory cells to evade immune responses to themselves. *Pneumocystis carinii* is an opportunistic pathogen and causes pneumonia in lymphopenic mice. Although CD4⁺CD25⁺ T cells, when injected into mice, induce immune response to *P. carinii* and severe pneumonia, CD4⁺CD25⁺ T_{regs} did not induce lethal pneumonia and, actually, prevented the development of the disease induced by CD4⁺CD25⁺ T cells [13]. This is a good example where T_{regs} are required to prevent hyperimmune responses to pathogens. *Litomosoides sigmodontis* are a parasite that infects the thoracic cavity of mice and there is a dramatic increase in T_{regs} at the site of infection [113]. Depletion of T_{regs} or inactivation of their suppressive function by antibodies to CD25 and GITR increased antigen-specific immune responses and killing of the parasites, suggesting that parasites can exploit T_{regs} to suppress antiparasitic humoral immune response. However, T_{regs} did not alter the primary or secondary IgG response to *Streptococcus pneumoniae*, where vigorous antibody response to the pathogen is desirable [114].

It is unclear how T_{regs} achieve this level of selectivity in suppression of immune responses. One possible mechanism is to modulate the suppressive activity of T_{regs} by Toll-like receptor (TLR) ligands. The TLR 4 ligand lipopolysaccharide (LPS) was able to increase the activity of T_{regs} by tenfold and control naive

CD4 T cell-dependent wasting disease in mice [115]. TLR2-deficient mice are more resistant than wild-type mice to *Candida* infection, possibly due to reduced T_{reg} activity [116]. TLR2 agonists increase the survival and proliferation of T_{regs} and, consistently, T_{regs} are decreased in numbers in TLR2-deficient mice. The TLR5 ligand flagellin enhances the suppressive function and *FOXP3* expression of human T_{regs} [117]. All of these reports support the positive roles of TLR ligands in enhancing the activity of T_{regs} . In certain situations, such as immune tolerance to cancer cells, however, TLR ligands can negatively regulate the suppressive activity of T_{regs} and help break the immune tolerance [118]. Therefore, TLR ligands can both positively and negatively regulate the activity of T_{regs} . As TLR ligands originate from pathogens and normal microflora, the type of pathogens and their molecular constituents could determine the levels of regulatory activity of FoxP3^+ T cells. Using the same logic, normal microflora could further modulate the immune response to pathogens. Other factors that can control the activity of T_{regs} are cytokines (e.g., enhancement by TGF- β and reversible suppression by IL-2) and surface costimulation receptors (e.g., CD28, CTLA4 and GITR) [119,120]. Therefore, there are a number of factors that can control the regulatory activity of FoxP3^+ T cells and this would help achieve appropriate levels of immunity to pathogens without breaking immune tolerance to self-antigens.

Expert commentary & five-year view

FoxP3^+ T cells are programmed to migrate to the tissue sites where B cells are enriched and humoral immune responses take place. These tissue sites are found in secondary lymphoid tissues (B-cell zone, T-cell zone, T-B border, marginal zones of spleen or medulla of lymph nodes), the intestine (lamina propria and Peyer's patches) and bone marrow. Chemokine receptors and adhesion molecules regulate migration of T_{regs} to T- or B-cell sites of secondary lymphoid tissues (CD62L, CCR7, CXCR4 and CXCR5), the intestine (CCR9 and $\alpha 4\beta 7$) and bone marrow (CXCR4, E/P-selectins and VLA-4). FoxP3^+ T cells, upon activation by antigens, suppress B cells and Th cells to decrease humoral immune response to self and foreign antigens. As B cell response in the absence of T-cell response is relatively weaker than that requires T-cell help, suppression of T helper cells than of B cells by T_{regs} would have a greater impact on overall humoral immunity.

The suppressive activity of FoxP3^+ T cells is highly regulated by TLR ligands, cytokines and costimulators, so that their suppressive activity is specifically directed toward auto- and hyper-immune responses initiated by foreign antigens, but not toward necessary immune response to clear pathogens. It remains to be determined how specific is the suppressive activity of FoxP3^+ T cells for self- versus nonself-antigens. Although many possible mechanisms have been proposed, it is unclear with which mechanisms FoxP3^+ T cells suppress humoral immune responses in various situations *in vivo*. Furthermore, it is unknown whether FoxP3^+ T cells also suppress marginal zone B cells or B-1 cells, which mediate T-cell-independent humoral immune response. More studies in terms of humoral immune response to T-dependent versus T-independent antigens need to be performed to clearly assess the extent of the role of FoxP3^+ T cells in regulation of humoral immune responses.

Acknowledgements

This work was supported, in part, from grants from American Heart Association, NIH-NIAID (AI063064) and Sidney Kimmel Foundation to CHK.

Key issues

- Specificity of FoxP3^+ T cells in the regulation of humoral immune responses to self-antigens versus foreign antigens, such as pathogens.
- Mechanisms of suppression of humoral immune responses by FoxP3^+ T cells.
- Target-cell specificity of FoxP3^+ T cells for different B-cell subsets (e.g., B-2, B-1 and marginal-zone B cells).
- Specificity of FoxP3^+ T cells in T-dependent versus T-independent antigens.
- Positive and negative regulations of FoxP3^+ T-cell suppression of humoral immunity.
- Clinical application of FoxP3^+ T cells for the regulation of autoantibody responses.
- Identification of trafficking receptors required for FoxP3^+ T-cell migration to various B-cell sites.

References

- 1 Sakaguchi S. Naturally arising CD4^+ T_{regs} for immunologic self-tolerance and negative control of immune responses. *Ann. Rev. Immunol.* 22, 531–562 (2004).
- 2 Baecher-Allan C, Viglietta V, Hafler DA. Human $\text{CD4}^+\text{CD}25^+$ T_{regs} . *Semin. Immunol.* 16, 89–98 (2004).
- 3 Bach JF, Francois Bach J. T_{regs} under scrutiny. *Nat. Rev. Immunol.* 3, 189–198 (2003).
- 4 Bluestone JA, Abbas AK. Natural versus adaptive T_{regs} . *Nat. Rev. Immunol.* 3, 253–257 (2003).
- 5 Shevach EM, Piccirillo CA, Thornton AM, McHugh RS. Control of T cell activation by $\text{CD4}^+\text{CD}25^+$ suppressor T cells. *Novartis Found. Symp.* 252, 24–36; discussion 36–44, 106–114 (2003).
- 6 Rudensky A. Foxp3 and dominant tolerance. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 360, 1645–1646 (2005).
- 7 Thompson C, Powrie F. T_{regs} . *Curr. Opin. Pharmacol.* 4, 408–414 (2004).
- 8 Wood KJ, Sakaguchi S. T_{regs} in transplantation tolerance. *Nat. Rev. Immunol.* 3, 199–210. (2003).
- 9 Chai JG, Xue SA, Coe D et al. T_{regs} derived from naive $\text{CD4}^+\text{CD}25^-$ T cells by *in vitro* Foxp3 gene transfer, can induce transplantation tolerance. *Transplantation* 79, 1310–1316 (2005).
- 10 Jaeckel E, von Boehmer H, Manns MP. Antigen-specific Foxp3-transduced T-cells can control established type 1 diabetes. *Diabetes* 54, 306–310 (2005).

- 11 Shimizu J, Yamazaki S, Sakaguchi S. Induction of tumor immunity by removing CD25⁺CD4⁺ T cells: a common basis between tumor immunity and autoimmunity. *J. Immunol.* 163, 5211–5218. (1999).
- This is an early study to demonstrate the potential role of FoxP3⁺ regulatory cells in regulation of antitumor immunity.
- 12 Gallimore A, Sakaguchi S. Regulation of tumour immunity by CD25⁺ T cells. *Immunology* 107, 5–9 (2002).
- 13 Hori S, Carvalho TL, Demengeot J. CD25⁺CD4⁺ T_{regs} suppress CD4⁺ T cell-mediated pulmonary hyperinflammation driven by *Pneumocystis carinii* in immunodeficient mice. *Eur. J. Immunol.* 32, 1282–1291 (2002).
- 14 Mills KH. T_{regs}: friend or foe in immunity to infection? *Nat. Rev. Immunol.* 4, 841–855 (2004).
- 15 Baum J, Liebermann G, Frenkel EP. The effect of immunologically induced lymphopenia on antibody formation. *J. Immunol.* 102, 187–193 (1969).
- 16 Baker PJ, Barth RF, Stashak PW, Amsbaugh DF. Enhancement of the antibody response to type 3 pneumococcal polysaccharide in mice treated with antilymphocyte serum. *J. Immunol.* 104, 1313–1315 (1970).
- 17 Kerbel RS, Eidinger D. Variable effects of anti-lymphocyte serum on humoral antibody formation: role of thymus dependency of antigen. *J. Immunol.* 106, 917–926 (1971).
- 18 Katz DH. The allogeneic effect on immune responses: model for regulatory influences of T lymphocytes on the immune system. *Transplant. Rev.* 12, 141–179 (1972).
- 19 Gershon RK, Kondo K. Infectious immunological tolerance. *Immunology* 21, 903–914 (1971).
- 20 Katz DH, Benacerraf B. The regulatory influence of activated T cells on B cell responses to antigen. *Adv. Immunol.* 15, 1–94 (1972).
- 21 Okumura K, Herzenberg LA, Murphy DB, McDevitt HO. Selective expression of H-2 (i-region) loci controlling determinants on helper and suppressor T lymphocytes. *J. Exp. Med.* 144, 685–698 (1976).
- 22 Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α -chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 155, 1151–1164. (1995).
- 23 von Boehmer H. Mechanisms of suppression by suppressor T cells. *Nat. Immunol.* 6, 338–344 (2005).
- The first study to demonstrate that most FoxP3⁺ regulatory cells express CD25.
- 24 Sakaguchi S. Naturally arising Foxp3-expressing CD25⁺CD4⁺ T_{regs} in immunological tolerance to self and non-self. *Nat. Immunol.* 6, 345–352 (2005).
- 25 Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299, 1057–1061. (2003).
- One of the several reports to demonstrate that enforced expression of FoxP3 can convert conventional T cells into regulatory cells.
- 26 Kasprlowicz DJ, Smallwood PS, Tyznik AJ, Ziegler SF. Scurfin (FoxP3) controls T-dependent immune responses *in vivo* through regulation of CD4⁺ T cell effector function. *J. Immunol.* 171, 1216–1223 (2003).
- 27 Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4⁺CD25⁺ T_{regs}. *Nat. Immunol.* 4, 330–336 (2003).
- 28 Schubert LA, Jeffery E, Zhang Y, Ramsdell E, Ziegler SF. Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. *J. Biol. Chem.* 276, 37672–37679 (2001).
- An early study to demonstrate that FoxP3 can act as a transcription repressor.
- 29 Bettelli E, Dastrange M, Oukka M. Foxp3 interacts with nuclear factor of activated T cells and NF- κ B to repress cytokine gene expression and effector functions of T helper cells. *Proc. Natl Acad. Sci. USA* 102, 5138–5143 (2005).
- Demonstrates that FoxP3 can interact with and regulate the function of the nuclear factor of activated T cells.
- 30 Szabo SJ, Sullivan BM, Peng SL, Glimcher LH. Molecular mechanisms regulating Th1 immune responses. *Ann. Rev. Immunol.* 21, 713–758 (2003).
- 31 Kretschmer K, Apostolou I, Hawiger D, Khazaie K, Nussenzweig MC, von Boehmer H. Inducing and expanding regulatory T cell populations by foreign antigen. *Nat. Immunol.* (2005).
- 32 Zheng SG, Wang JH, Gray JD, Soucier H, Horwitz DA. Natural and induced CD4⁺CD25⁺ cells educate CD4⁺CD25⁺ cells to develop suppressive activity: the role of IL-2, TGF- β , and IL-10. *J. Immunol.* 172, 5213–5221 (2004).
- 33 Jonuleit H, Schmitt E, Schuler G, Knop J, Enk AH. Induction of interleukin 10-producing, nonproliferating CD4⁽⁺⁾ T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J. Exp. Med.* 192, 1213–1222 (2000).
- 34 Mahnke K, Qian Y, Knop J, Enk AH. Induction of CD4⁺CD25⁺ T_{regs} by targeting of antigens to immature dendritic cells. *Blood* 101, 4862–4869 (2003).
- 35 Min WP, Zhou D, Ichim TE et al. Inhibitory feedback loop between tolerogenic dendritic cells and T_{regs} in transplant tolerance. *J. Immunol.* 170, 1304–1312 (2003).
- 36 Verhasselt V, Vosters O, Beuneu C, Nicaise C, Stordeur P, Goldman M. Induction of FOXP3-expressing regulatory CD4^{pos} T cells by human mature autologous dendritic cells. *Eur. J. Immunol.* 34, 762–772 (2004).
- 37 Yamagiwa S, Gray JD, Hashimoto S, Horwitz DA. A role for TGF- β in the generation and expansion of CD4⁺CD25⁺ T_{regs} from human peripheral blood. *J. Immunol.* 166, 7282–7289 (2001).
- Demonstrates the role of tumor growth factor (TGF)- 1 in induction of human FoxP3⁺ cells *in vitro*.
- 38 Zheng SG, Gray JD, Ohtsuka K, Yamagiwa S, Horwitz DA. Generation *ex vivo* of TGF- β -producing T_{regs} from CD4⁺CD25⁺ precursors. *J. Immunol.* 169, 4183–4189 (2002).
- 39 Fu S, Zhang N, Yopp AC, Chen D et al. TGF- β induces Foxp3⁺ T-regulatory cells from CD4⁺CD25⁺ precursors. *Am. J. Transplant.* 4, 1614–1627 (2004).
- 40 Skapenko A, Kalden JR, Lipsky PE, Schulze-Koops H. The IL-4 receptor α -chain-binding cytokines, IL-4 and IL-13, induce forkhead box P3-expressing CD25⁺CD4⁺ T_{regs} from CD25⁺CD4⁺ precursors. *J. Immunol.* 175, 6107–6116 (2005).
- 41 Polanczyk MJ, Carson BD, Subramanian S et al. Cutting edge: estrogen drives expansion of the CD4⁺CD25⁺ regulatory T cell compartment. *J. Immunol.* 173, 2227–2230 (2004).
- 42 Gregori S, Casorati M, Amuchastegui S, Smirldo S, Davalli AM, Adorini L. T_{regs} induced by 1 α ,25-dihydroxyvitamin D3 and mycophenolate mofetil treatment mediate transplantation tolerance. *J. Immunol.* 167, 1945–1953 (2001).
- 43 Baratelli F, Lin Y, Zhu L et al. Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4⁺ T cells. *J. Immunol.* 175, 1483–1490 (2005).
- 44 Vieira PL, Christensen JR, Minaee S et al. IL-10-secreting T_{regs} do not express Foxp3 but have comparable regulatory function to naturally occurring CD4⁺CD25⁺ T_{regs}. *J. Immunol.* 172, 5986–5993 (2004).

- 45 Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. Regulatory T cell lineage specification by the forkhead transcription factor Foxp3. *Immunity* 22, 329–341 (2005).
- 46 Lim HW, Hillsamer P, Banham AH, Kim CH. Cutting edge: direct suppression of B cells by CD4⁺CD25⁺ T_{regs}. *J. Immunol.* 175, 4180–4183 (2005).
- The authors demonstrated the function of FoxP3⁺ cells in direct suppression of B cells and antibody production.
- 47 Fontenot JD, Rudensky AY. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. *Nat. Immunol.* 6, 331–337 (2005).
- 48 Roncador G, Brown PJ, Maestre L *et al.* Analysis of FOXP3 protein expression in human CD4⁺CD25⁺ T_{regs} at the single-cell level. *Eur. J. Immunol.* 35, 1681–1691 (2005).
- The first report on generation of monoclonal antibody to FOXP3 for detection of human FOXP3⁺ cells.
- 49 O'Garra A. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity* 8, 275–283 (1998).
- 50 Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4⁺CD25⁺ T_{regs} is mediated by cell surface-bound transforming growth factor β . *J. Exp. Med.* 194, 629–644 (2001).
- Demonstrates the role of TGF-1 in the effector functions of FoxP3⁺ cells and started the big controversy that followed the publication.
- 51 Zhang X, Izikson L, Liu L, Weiner HL. Activation of CD25⁺CD4⁺ T_{regs} by oral antigen administration. *J. Immunol.* 167, 4245–4253 (2001).
- 52 Groux H, O'Garra A, Bigler M *et al.* A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389, 737–742 (1997).
- 53 Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 265, 1237–1240 (1994).
- 54 Levings MK, Gregori S, Tresoldi E, Cazzaniga S, Bonini C, Roncarolo MG. Differentiation of Tr1 cells by immature dendritic cells requires IL-10 but not CD25⁺CD4⁺ Tr cells. *Blood* 105, 1162–1169 (2005).
- 55 Gray D, Bergthorsdottir S, van Essen D, Wykes M, Poudrier J, Siepmann K. Observations on memory B-cell development. *Semin. Immunol.* 9, 249–254. (1997).
- 56 Parker DC. T cell-dependent B cell activation. *Ann. Rev. Immunol.* 11, 331–360 (1993).
- 57 McHeyzer-Williams MG, McHeyzer-Williams LJ, Fanelli Panus J. Antigen-specific immunity. Th cell-dependent B cell responses. *Immunol. Res.* 22, 223–236 (2000).
- 58 Noelle RJ, Ledbetter JA, Aruffo A. CD40 and its ligand, an essential ligand-receptor pair for thymus-dependent B-cell activation. *Immunol. Today* 13, 431–433 (1992).
- 59 Lipsky PE, Attrep JF, Grammer AC, McIlraith MJ, Nishioka Y. Analysis of CD40-CD40 ligand interactions in the regulation of human B cell function. *Ann. NY Acad. Sci.* 815, 372–383 (1997).
- 60 Sharon R, McMaster PR, Kask AM, Owens JD, Paul WE. DNP-Lys-ficoll: a T-independent antigen which elicits both IgM and IgG anti-DNP antibody-secreting cells. *J. Immunol.* 114, 1585–1589 (1975).
- 61 McHeyzer-Williams LJ, Driver DJ, McHeyzer-Williams MG. Germinal center reaction. *Curr. Opin. Hematol.* 8, 52–59 (2001).
- 62 Wolniak KL, Shinall SM, Waldschmidt TJ. The germinal center response. *Crit. Rev. Immunol.* 24, 39–65 (2004).
- 63 Kim JR, Lim HW, Kang SG, Hillsamer P, Kim CH. Human CD57⁺ germinal center-T cells are the major helpers for GC-B cells and induce class switch recombination. *BMC Immunol.* 6, 3 (2005).
- 64 Kim C. Chemokines and their receptors in hematopoietic cell development and function. In: *Chemokines, Chemokine Receptors, and Disease*. Schiebert L (Ed.), 115–142 (2005).
- 65 Kim CH. The greater chemotactic network for lymphocyte trafficking: chemokines and beyond. *Curr. Opin. Hematol.* 12, 298–304 (2005).
- 66 Lim HW, Broxmeyer HE, Kim CH. Regulation of trafficking receptor expression in human FOXP3⁺ T_{regs}. *J. Immunol.* 177(2), 840–851 (2006).
- Comprehensively characterized the regulation of trafficking receptor expression by human FOXP3⁺ cells.
- 67 Annunziato F, Cosmi L, Liotta F. Phenotype, localization, and mechanism of suppression of CD4⁺CD25⁺ human thymocytes. *J. Exp. Med.* 196, 379–387. (2002).
- 68 Iellem A, Mariani M, Lang R. Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by CD4⁺CD25⁺ T_{regs}. *J. Exp. Med.* 194, 847–853. (2001).
- 69 Iellem A, Colantonio L, D'Ambrosio D. Skin-versus gut-skewed homing receptor expression and intrinsic CCR4 expression on human peripheral blood CD4⁺CD25⁺ suppressor T cells. *Eur. J. Immunol.* 33, 1488–1496 (2003).
- 70 Rieger K, Loddenkemper C, Maul J *et al.* Mucosal FOXP3⁺ T_{regs} are numerically deficient in acute and chronic GvHD. *Blood*(2005).
- 71 Lim HW, Hillsamer P, Kim CH. T_{regs} can migrate to follicles upon T cell activation and suppress GC-Th cells and GC-Th cell-driven B cell responses. *J. Clin. Invest.* 114, 1640–1649 (2004).
- 72 Benner R, Meima F, van der Meulen GM, van Muiswinkel WB. Antibody formation in mouse bone marrow. I. Evidence for the development of plaque-forming cells in situ. *Immunology* 26, 247–255 (1974).
- 73 Chaperon EA, Selner JC, Claman HN. Migration of antibody-forming cells and antigen-sensitive precursors between spleen, thymus and bone marrow. *Immunology* 14, 553–561 (1968).
- 74 O'Connor BP, Gleeson MW, Noelle RJ, Erickson LD. The rise and fall of long-lived humoral immunity: terminal differentiation of plasma cells in health and disease. *Immunol. Rev.* 194, 61–76 (2003).
- 75 Cyster JG. Homing of antibody secreting cells. *Immunol. Rev.* 194, 48–60 (2003).
- 76 Kunkel EJ, Butcher EC. Plasma-cell homing. *Nat. Rev. Immunol.* 3, 822–829 (2003).
- 77 Hargreaves DC, Hyman PL, Lu TT *et al.* A coordinated change in chemokine responsiveness guides plasma cell movements. *J. Exp. Med.* 194, 45–56 (2001).
- 78 Kunkel EJ, Campbell JJ, Haraldsen G *et al.* Lymphocyte CC chemokine receptor 9 and epithelial thymus-expressed chemokine (TECK) expression distinguish the small intestinal immune compartment: epithelial expression of tissue-specific chemokines as an organizing principle in regional immunity. *J. Exp. Med.* 192, 761–768 (2000).
- 79 Bowman EP, Kuklin NA, Youngman KR *et al.* The intestinal chemokine thymus-expressed chemokine (CCL25) attracts IgA antibody-secreting cells. *J. Exp. Med.* 195, 269–275 (2002).
- 80 Pan J, Kunkel EJ, Gossler U *et al.* A novel chemokine ligand for CCR10 and CCR3 expressed by epithelial cells in mucosal tissues. *J. Immunol.* 165, 2943–2949 (2000).

- 81 Kunkel EJ, Kim CH, Lazarus NH *et al.* CCR10 expression is a common feature of circulating and mucosal epithelial tissue IgA Ab-secreting cells. *J. Clin. Invest.* 111, 1001–1010 (2003).
- 82 Zou L, Barnett B, Safah H *et al.* Bone marrow is a reservoir for CD4⁺CD25⁺ T_{regs} that traffic through CXCL12/CXCR4 signals. *Cancer Res.* 64, 8451–8455 (2004).
- 83 Lee JH, Kang SG, Kim CH. FoxP3⁺ T cells undergo conventional first switch to lymphoid tissue homing receptors in thymus but accelerated second switch to non-lymphoid tissue homing receptors in secondary lymphoid tissues. *J. Immunol.* 178 (2007) (In Press).
 - Describes the two trafficking receptor switches in FoxP3⁺ cells during their generation and antigen priming *in vivo*.
- 84 Kim CH, Johnson R. Trafficking potentials of unconventional T cell subsets. *Curr. Med. Chem. Anti Inflamm. Anti Allergy Agents* 3, 321–330 (2004).
- 85 Eddahri F, Oldenhove G, Denanglaire S, Urbain J, Leo O, Andris F. CD4⁺ CD25⁺ T_{regs} control the magnitude of T-dependent humoral immune responses to exogenous antigens. *Eur. J. Immunol.* 36, 855–863 (2006).
- 86 Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat. Rev. Immunol.* 4, 762–774 (2004).
- 87 Manzotti CN, Tipping H, Perry LC *et al.* Inhibition of human T cell proliferation by CTLA-4 utilizes CD80 and requires CD25⁺ T_{regs}. *Eur. J. Immunol.* 32, 2888–2896 (2002).
- 88 Grohmann U, Orabona C, Fallarino F *et al.* CTLA-4-Ig regulates tryptophan catabolism *in vivo*. *Nat. Immunol.* 3, 1097–1101 (2002).
- 89 Otterbein LE, Soares MP, Yamashita K, Bach FH. Heme oxygenase-1: unleashing the protective properties of heme. *Trends Immunol.* 24, 449–455 (2003).
- 90 Sato K, Balla J, Otterbein L *et al.* Carbon monoxide generated by heme oxygenase-1 suppresses the rejection of mouse-to-rat cardiac transplants. *J. Immunol.* 166, 4185–4194 (2001).
- 91 Pae HO, Oh GS, Choi BM *et al.* Carbon monoxide produced by heme oxygenase-1 suppresses T cell proliferation via inhibition of IL-2 production. *J. Immunol.* 172, 4744–4751 (2004).
- 92 Choi BM, Pae HO, Jeong YR, Kim YM, Chung HT. Critical role of heme oxygenase-1 in Foxp3-mediated immune suppression. *Biochem. Biophys. Res. Commun.* 327, 1066–1071 (2005).
- 93 Grossman WJ, Verbsky JW, Barchet W, Colonna M, Atkinson JP, Ley TJ. Human T regulatory cells can use the perforin pathway to cause autologous target cell death. *Immunity* 21, 589–601 (2004).
 - The first report on the cell-killing activity of FOXP3⁺ regulatory T cells *in vitro*.
- 94 Gondek DC, Lu LF, Quezada SA, Sakaguchi S, Noelle RJ. Cutting edge: contact-mediated suppression by CD4⁺CD25⁺ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. *J. Immunol.* 174, 1783–1786 (2005).
- 95 Zhao DM, Thornton AM, DiPaolo RJ, Shevach EM. Activated CD4⁺CD25⁺ T cells selectively kill B lymphocytes. *Blood* 107, 3925–3932 (2006).
- 96 Tadokoro CE, Shakhar G, Shen S *et al.* T_{regs} inhibit stable contacts between CD4⁺ T cells and dendritic cells *in vivo*. *J. Exp. Med.* 203, 505–511 (2006).
- 97 Tang Q, Adams JY, Tooley AJ *et al.* Bluestone JA. Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice. *Nat. Immunol.* 7, 83–92 (2006).
- 98 Bystry RS, Aluvihare V, Welch KA, Kallikourdis M, Betz AG. B cells and professional APCs recruit T_{regs} via CCL4. *Nat. Immunol.* 2, 1126–1132. (2001).
 - Provided evidence that the FoxP3⁺ cell recruitment is important for their effector function in regulation of humoral immunity.
- 99 Takahashi T, Kuniyasu Y, Toda M *et al.* Immunologic self-tolerance maintained by CD25⁺CD4⁺ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int. Immunol.* 10, 1969–1980. (1998).
- 100 Seo SJ, Fields ML, Buckler JL *et al.* The impact of T helper and T regulatory cells on the regulation of anti-double-stranded DNA B cells. *Immunity* 16, 535–546 (2002).
- 101 Lee JH, Wang LC, Lin YT, Yang YH, Lin DT, Chiang BL. Inverse correlation between CD4⁺ regulatory T-cell population and autoantibody levels in paediatric patients with systemic lupus erythematosus. *Immunology* 117, 280–286 (2006).
- 102 Morgan ME, Suttmuller RP, Witteveen HJ *et al.* CD25⁺ cell depletion hastens the onset of severe disease in collagen-induced arthritis. *Arthritis Rheum* 48, 1452–1460 (2003).
- 103 Russel W, Russel L, Gower J. Exceptional Inheritance of a sex-linked gene in the mouse explained on the basis that the X/O sex-chromosome constitution is female. *Proc. Natl Acad. Sci. USA* 45, 554–560 (1959).
- 104 Brunkow ME, Jeffery EW, Hjerrild KA *et al.* Disruption of a new forkhead/winged-helix protein, scurfy, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat. Genet.* 27, 68–73 (2001).
- 105 Shull MM, Ormsby I, Kier AB *et al.* Targeted disruption of the mouse transforming growth factor- β 1 gene results in multifocal inflammatory disease. *Nature* 359, 693–699 (1992).
- 106 Waterhouse P, Penninger JM, Timms E *et al.* Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science* 270, 985–988 (1995).
- 107 Clark LB, Appleby MW, Brunkow ME, Wilkinson JE, Ziegler SF, Ramsdell F. Cellular and molecular characterization of the scurfy mouse mutant. *J. Immunol.* 162, 2546–2554 (1999).
- 108 Smyk-Pearson SK, Bakke AC, Held PK, Wildin RS. Rescue of the autoimmune scurfy mouse by partial bone marrow transplantation or by injection with T-enriched splenocytes. *Clin. Exp. Immunol.* 133, 193–199 (2003).
- 109 Schmidt-Weber CB, Blaser K. The role of the FOXP3 transcription factor in the immune regulation of allergic asthma. *Curr. Allergy Asthma Rep.* 5, 356–361 (2005).
- 110 Fields ML, Hondowicz BD, Metzgar MH *et al.* CD4⁺ CD25⁺ T_{regs} inhibit the maturation but not the initiation of an autoantibody response. *J. Immunol.* 175, 4255–4264 (2005).
- 111 Oldstone MB. Molecular mimicry, microbial infection, and autoimmune disease: evolution of the concept. *Curr. Top. Microbiol. Immunol.* 296, 1–17 (2005).
- 112 Penninger JM, Bachmaier K. Review of microbial infections and the immune response to cardiac antigens. *J. Infect. Dis.* 181(Suppl. 3), S498–S504 (2000).
- 113 Taylor MD, LeGoff L, Harris A, Malone E, Allen JE, Maizels RM. Removal of regulatory T cell activity reverses hyporesponsiveness and leads to filarial parasite clearance *in vivo*. *J. Immunol.* 174, 4924–4933 (2005).
 - One of the early studies that demonstrated the roles of FoxP3⁺ T cells in evasion of the immune system by pathogens.
- 114 Lee KS, Sen G, Snapper CM. Endogenous CD4⁺ CD25⁺ T_{regs} play no apparent role in the acute humoral response to intact *Streptococcus pneumoniae*. *Infect. Immun.* 73, 4427–4431 (2005).

- 115 Caramalho I, Lopes-Carvalho T, Ostler D, Zelenay S, Haury M, Demengeot J. T_{regs} selectively express Toll-like receptors and are activated by lipopolysaccharide. *J. Exp. Med.* 197, 403–411 (2003).
- Demonstrates the role of Toll-like receptor ligands in regulation of the FoxP3⁺ T-cell biology.
- 116 Netea MG, Suttmuller R, Hermann C *et al.* Toll-like receptor 2 suppresses immunity against *Candida albicans* through induction of IL-10 and T_{regs}. *J. Immunol.* 172, 3712–3718 (2004).
- 117 Crellin NK, Garcia RV, Hadisfar O, Allan SE, Steiner TS, Levings MK. Human CD4⁺ T cells express TLR5 and its ligand flagellin enhances the suppressive capacity and expression of FOXP3 in CD4⁺CD25⁺ T regulatory cells. *J. Immunol.* 175, 8051–8059 (2005).
- 118 Yang Y, Huang CT, Huang X, Pardoll DM. Persistent Toll-like receptor signals are required for reversal of regulatory T cell-mediated CD8 tolerance. *Nat. Immunol.* 5, 508–515 (2004).
- 119 Zheng Y, Manzotti CN, Liu M, Burke F, Mead KI, Sansom DM. CD86 and CD80 differentially modulate the suppressive function of human T_{regs}. *J. Immunol.* 172, 2778–2784 (2004).
- 120 Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S. Stimulation of CD25(+)CD4(+) T_{regs} through GITR breaks immunological self-tolerance. *Nat. Immunol.* 3, 135–142 (2002).
- Identifies glucocorticoid-induced tumor-necrosis factor receptor as a potential regulatory receptor for FoxP3⁺ cells.

Affiliation

- Chang H Kim, PhD
Laboratory of Immunology and Hematopoiesis,
Department of Pathobiology, Purdue Cancer
Center, Purdue University, West Lafayette,
IN 47907, USA
Tel.: +1 765 494 0976
Fax: +1 765 494 9830
chkim@purdue.edu