# CHAPTER 2

# FOXP3 and Its Role in the Immune System

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## Abstract

FOXP3 is a member of the forkhead transcription factor family. Unlike other members, it is mainly expressed in a subset of $CD4^+$ T-cells that play a suppressive role in the immune system. A function of FOXP3 is to suppress the function of NFAT and NFκB and this leads to suppression of expression of many genes including IL-2 and effector T-cell cytokines. FOXP3 acts also as a transcription activator for many genes including *CD25*, *Cytotoxic T-Lymphocyte Antigen 4 (CTLA4)*, *glucocorticoid-induced TNF receptor family gene (GITR)* and *folate receptor 4*. $FOXP3^+$ T-cells are made in the thymus and periphery. The $FOXP3^+$ T-cells made in the thymus migrate to secondary lymphoid tissues and suppress antigen priming of lymphocytes. Antigen priming of naïve $FOXP3^+$ T-cells and naïve $FOXP3^-$ T-cells leads to generation of memory $FOXP3^+$ T-cells which are efficient in migration to nonlymphoid tissues. Memory $FOXP3^+$ T-cells are, therefore, effective in suppression of effector T-cell function, while naïve $FOXP3^+$ T-cells are adept at suppressing the early immune responses in lymphoid tissues. Both naïve and memory $FOXP3^+$ T-cells are required for effective maintenance of tolerance and prevention of autoimmune diseases throughout the body. Many factors such as cytokines and noncytokine factors regulate the generation of $FOXP3^+$ T-cells. For example, retinoic acid, produced by the dendritic cells and epithelial cells in the intestine, works together with TGF-β1 and promotes generation of small intestine-homing $FOXP3^+$ T-cells by upregulating the expression of FOXP3 and gut homing receptors. $FOXP3^+$ T-cells can be produced in vitro from autologous naïve T-cells and, therefore, have great therapeutic potentials in treating a number of inflammatory diseases and graft rejection.

## Introduction

FOXP3 is one of the most extensively studied members of the FOX family which is defined by a common DNA-binding domain (DBD) termed the forkhead box or winged helix domain.[1] FOXP3 receives a lot of attention because of its clear role in generation of immune suppressor T-cells. The function of FOXP3 in programming the gene expression to make suppressor T-cells is attributed to its transcription regulation activity.[2] Its major targets include NFAT and NFκB, key transcription factors that mediate antigen receptor signals. FOXP3 suppresses the function of these transcription factors but induces expression of many other genes through mechanisms that are incompletely understood at this stage. Our body is making $FOXP3^+$ T-cells in both the thymus and periphery. $FOXP3^+$ T-cells play important roles in limiting the activation of immune cells in response to infection.[3-6] They play important roles also in prevention of autoimmune diseases. It appears that some pathogens and cancer cells have been evolved to utilize $FOXP3^+$ T-cells to avoid

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*Forkhead Transcription Factors: Vital Elements in Biology and Medicine*, edited by Kenneth Maiese.

immune responses because FOXP3+ T-cells would effectively suppress antipathogen and anticancer cell-specific immune responses.[7,8] While FOXP3 is mainly expressed by T-cells, it is also expressed by epithelial cells in certain organs such as thymus and mammary glands.[9] In these organs, FOXP3 seems to play completely different roles.[9,10] This monograph is to provide general information on the structure and function of the FOXP3 gene and protein and on the immunological roles of the cells expressing FOXP3.

## Structure and Function of FOXP3

The human FOXP3 gene is composed of 11 exons and is present in the p arm of the X chromosome (Xp11.23, Fig. 1).[11,12] The translation of the FOXP3 protein starts from the middle of the second exon. The mouse gene is at X 2.1 cM, a location comparable to that of the human gene. It is also called JM2 (human) or scurfin (mouse). FOXP3 is a 48 kD protein composed of 431 amino acids. The FOXP3 protein has four distinctive domains: forkhead (FH) domain, leucine zipper, zinc-finger and the proline-rich repressor domain.[4] The C-terminal forkhead domain consists of ~100 amino acids and forms a DNA binding domain. FOXP3 binds genes containing the forkhead binding motif.[13,14] The forkhead domain is required also for nuclear localization of FOXP3. The role of the zinc-finger domain is unknown. The leucine zipper domain is thought to mediate dimerization or tetramerization of the transcription factor.[15] The N-terminal repressor domain (amino acids 1-193) is composed of two subdomains. The first subdomain (amino acids 1-105) is

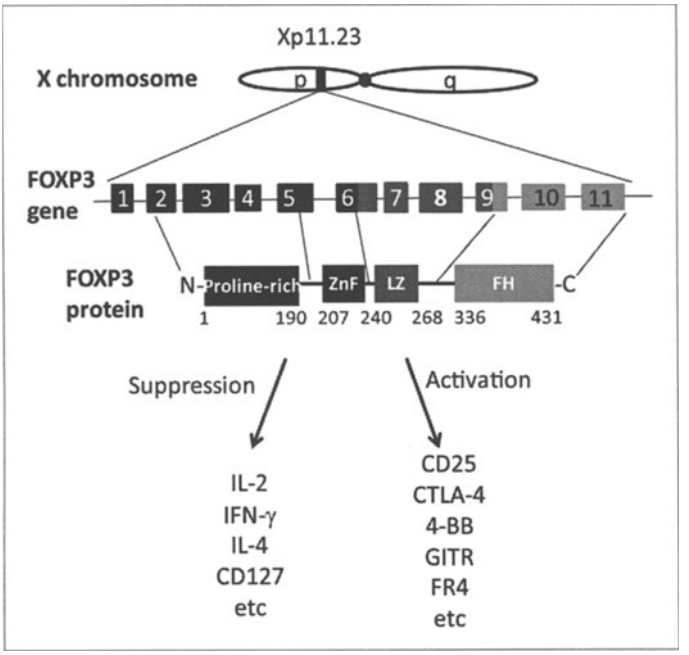


Figure 1. The structure of the FOXP3 gene and protein. The human FOXP3 gene is located on the p arm of chromosome X (Xp11.23). The FOXP3 gene is composed of 11 exons. There are four identifiable domains in the FOXP3 protein. The N-terminal proline-rich domain is involved in suppression of NFκB and NFAT. The leucine zipper domain is required for dimerization or tetramerization. The C-terminal forkhead domain has a nuclear localization sequence and a DNA binding domain. FOXP3 functions to induce the expression of many genes such as CTLA-4, FR4 (folate receptor 4),[142] GITR and CD25 and to suppress the expression of other genes such as IL-2,IL-4 and IFN-γ. It has been reported that expression of 700-1000 genes is regulated by FOXP3 either directly or indirectly.

involved in general transcriptional repression by FOXP3 and the second subdomain (N-terminal 106- to 190-aa proline-rich region) is involved in suppression of NFAT and NFκB-mediated transcription.[15,16] The second half of the domain mediates the association of the FOXP3 protein with key transcriptional regulators such as Tat-interactive protein 60 kDa (TIP60)[17] and class II histone deacetylases (HDAC7).[18] Mutations have been found in the forkhead domain, leucine zipper domain and repressor domain of the FOXP3 gene of human IPEX patients.[19-21]

FOXP3 can bind forkhead DNA binding elements in many genes including IL-2, CTLA4, GITR and CD25.[22] FOXP3 decreases IL-2 expression but increases the expression of CTLA4, GITR and CD25. Thus, FOXP3 acts as a transcriptional activator and repressor. Studies using Chip-on-Chip (chromatin immunoprecipitation) revealed that 700-1100 genes are regulated either positively or negatively by FOXP3.[23,24] Most of the genes would be indirectly regulated as the result of T-cell differentiation rather than as the consequence of direct FOXP3 binding. For suppression of NFAT, the N-terminal repressor domain is required.[16] FOXP3 and NFAT cooperatively bind to the antigen receptor response element (AREE2) within the IL-2 promoter in a manner similar to the binding of AP-1 and NFAT.[25] Some amino acid residues in the forkhead domain are important for this interaction. FOXP3 also interacts with NFκB and suppresses its activity.[16]

## Expression of FOXP3

FOXP3 is most highly expressed by a subset of $CD4^+$ T-cells, commonly called $CD4^+CD25^+$ regulatory T-cells.[5,26] Expression of the FOXP3 gene is more tightly regulated in mouse T-cells compared to human T-cells.[27] In human T-cells, simple T-cell activation induces FOXP3 at a low but detectable level. In addition, some $CD8^+$ T-cells also express FOXP3 and function as regulatory T-cells.[28] In the mouse thymus, FOXP3 expression is detected on a small subset of CD4 and CD8 double positive T-cells and CD4 single positive T-cells.[29-31] In humans, however, small numbers of double negative thymocytes also express FOXP3.[32] Expression of FOXP3 is important for T-cells to gain the suppressive function. In mice, FOXP3 over-expression by retroviral gene transfer was sufficient to generate suppressive T-cells.[33,34] Again, there is a species difference in this regard that enforced FOXP3 expression in human T-cells by itself was not sufficient to turn regular T-cells into suppressor T-cells.[35]

While normal T-cell receptor (TCR) activation would not efficiently induce $FOXP3^+$ T-cells, premature termination of TCR signaling and inhibition of phosphatidyl inositol 3-kinase (PI3K) p110a, p110d, protein kinase B (Akt), or mammalian target of rapamycin (mTOR) effectively induced FOXP3 expression.[36] FOXP3 expression is regulated at both genetic and epigenetic levels. NK-cells, for example, don't express FOXP3 but do express it when they are treated with 5-aza-2'-deoxycytidine, a DNA methylation inhibitor.[37] Complete demethylation of CpG motifs as well as histone modifications are found on the conserved region of the FOXP3 promoter in $FOXP3^+$ cells but not $FOXP3^-$ T-cells.[38] Methylation at the FOXP3 promoter can block the binding of transcription factors such as cyclic-AMP response element binding protein (CREB)/activating transcription factor (ATF) which are involved in activation of the FOXP3 promoter.[39] TGF-β1 induces FOXP3 expression in T-cells undergoing T-cell receptor activation.[40,41] The transcription factors Smad3 which mediates TGF-β1 signaling and NFAT which mediates the T-cell receptor activation signal are required to induce FOXP3 expression.[42] Interestingly, these TGF-β1-induced $FOXP3^+$ T-cells are not heavily methylated on their FOXP3 promoter locus compared to natural $FOXP3^+$ T-cells.[43] However, another group reported more complete methylation at the FOXP3 promoter locus in induced $FOXP3^+$ T-cells, suggesting that the methylation in the in vitro-induced $FOXP3^+$ T-cells varies depending on the culture condition.[44] Induced $FOXP3^+$ cells, generated in vivo from naïve T-cells, exhibited more complete methylation on the FOXP3 locus and thus natural and fully differentiated-induced $FOXP3^+$ T-cells are indistinguishable in methylation at the FOXP3 locus.[45]

In addition to TGF-β1, IL-2 promotes the generation of induced $FOXP3^+$ T-cells. Consistently, the intracellular signaling mediators of IL-2 such as STAT5a and STAT5b play positive roles in

expression of FOXP3.[37,46] It has been reported that IL-4 suppressed, while STAT6 (a mediator of IL-4 signaling) gene deletion enhanced, the TGFβ1-induced expression of FOXP3.[47] TGF-β1 can enhance FOXP3 expression but suppress the expression of FOXP3 when IL-6 is present. Indeed, TGF-β1 and IL-6 induce different effector T-cells called "Th17 cells", which can characteristically produce IL-17.[48,49] In line with this, TGF-β1 treatment increases acetylated FOXP3 on the chromatin but IL-6 down-regulates FOXP3 binding to the chromatin in the presence of TGF-β1.[50]

## Ontogeny and Migration of FOXP3$^+$ Cells

FOXP3$^+$ T-cells are generated in thymus as naïve FOXP3$^+$ cells and in periphery as induced FOXP3$^+$ cells (Fig. 2). The naïve FOXP3$^+$ T-cells, generated in thymus, express CD62L and CCR7 and migrate to secondary lymphoid tissues.[31] CD62L would mediate rolling and CCR7 triggers integrin-mediated firm adhesion on endothelial cells.[51-56] This trafficking receptor phenotype is retained as long as the FOXP3$^+$ T-cells do not encounter antigens in the secondary lymphoid tissues.[31] Unlike the naïve FOXP3$^+$ T-cells, the induced FOXP3$^+$ T-cells have heterogeneous memory/effector type trafficking receptors.[31] It is thought that memory FOXP3$^+$ T-cells and induced FOXP3$^+$ T-cells are similar to each other in trafficking receptor phenotype and suppressive function. Some induced FOXP3$^+$ T-cells express gut homing receptors such as CCR9 and α4β7.[31] These receptors allow the migration of the T-cells into the small intestine.[57-63] Some FOXP3$^+$ T-cells express CXCR5 and migrate into B-cell follicles including germinal centers.[64]

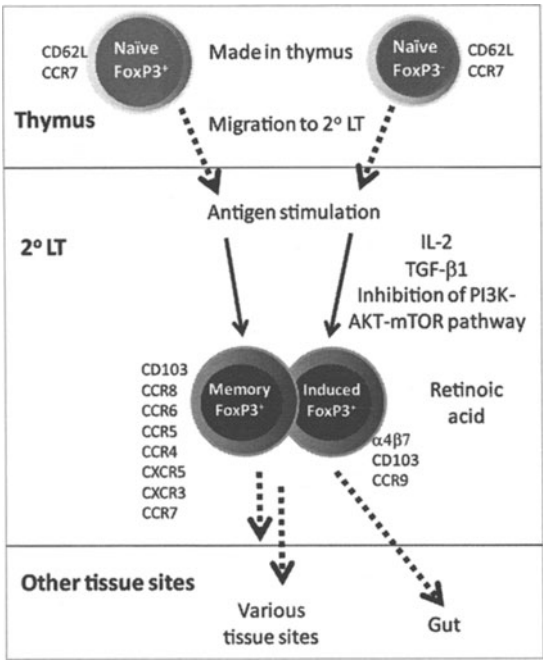


Figure 2. Generation and trafficking of FOXP3$^+$ T-cells. FOXP3$^+$ T-cells are made in the thymus at the double negative stage (human), double positive stage and single positive stage (human and mice). The thymus emigrating FOXP3$^+$ T-cells have the naïve T-cell phenotype in trafficking behavior and migrate to secondary lymphoid tissues. The migration of natural FOXP3$^+$ T-cells into secondary lymphoid tissues is to regulate the antigen priming of lymphocytes and to undergo antigen priming themselves. Antigenic stimulation of naïve FOXP3$^+$ T-cells changes their homing behavior for migration to various nonlymphoid tissues. Antigen priming of naïve T-cells also drives the conversion of FOXP3$^-$ naïve T-cells into FOXP3$^+$ T-cells. Certain factors such as TGF-β1 and retinoic acid play important roles in promotion of this event.

Some other FOXP3$^+$ T-cells express CCR8, a skin-homing related receptor.[31] In general, induced or memory FOXP3$^+$ T-cells highly express CD103, CCR4, CCR6, CXCR3, CXCR4 and CXCR6.[31,65] CCR4 is required for successful suppression of inflammation by FOXP3$^+$ T-cells. In a heart transplantation model, recruitment of FOXP3$^+$ cells to the allograft tissue is dependent on CCR4.[66] The CCR4-dependent recruitment of FOXP3$^+$ T-cells is required for effective induction of tolerance with tolerizing strategies such as CD154 mAb therapy. Scurfy mice reconstituted with CCR4-deficient FOXP3$^+$ cells develop severe inflammatory diseases in the skin and lungs.[67] Another chemokine receptor CCR7 appears to be important for FOXP3$^+$ T-cell migration to the T-cell area of lymphoid tissues. CCR7-deficient FOXP3$^+$ cells fail to migrate into the lymph nodes and suppress antigen-induced T-cell responses.[68]

The induction mechanism of gut homing FOXP3$^+$ T-cells has been elucidated. In 2007, six groups reported that retinoic acid has the function of triggering the expression of FOXP3 in T-cells undergoing activation.[69-76] Retinoic acid induces chromatin reorganization by inducing histone acetylation in the FOXP3 promoter. Retinoic acid alone can generate human FOXP3$^+$ T-cells but TGF-β1 is required at least at a suboptimal level to induce retinoid-induced mouse FOXP3$^+$ T-cells.[77] Retinoic acid is produced from retinol by dendritic cells and epithelial cells in the intestine.[78] Therefore, the intestinal microenvironment provides the signal to induce gut homing FOXP3$^+$ T-cells. This role of retinoic acid is thought to be important for inducing tolerance in the gut by generating FOXP3$^+$ T-cells that would suppress potentially harmful immune responses in the intestine. It has been well-established that immune responses to commensals can cause inflammatory bowel diseases such as Crohn's disease and ulcerative colitis.[79,80] It is thought that retinoic acid functions to prevent inflammatory bowel diseases by promoting the immune tolerance in the intestine. Another function of retinoic acid is to suppress the differentiation of naïve T-cells into Th17 cells in vitro. This could also promote the immune tolerance in the intestine by suppressing Th17 cells.[69,73,74,81] However, the function of retinoic acid in suppression of Th17 cells has not been confirmed in vivo. This may be because of the fact that retinoic acid production in vivo is tightly regulated that retinoic acid would not be available at the high concentrations (100-1000 nM) used in vitro in demonstration of the suppression of Th17 cells.

Another trafficking receptor that is potentially important for FOXP3$^+$ regulatory T-cells is CD103.[65,82,83] CD103 is the alpha subunit of the integrin αEβ7, which serves as the ligand for E-cadherin. It is unclear how CD103 functions in terms of the suppressive function of FOXP3$^+$ T-cells. It was proposed that CD103$^+$ FOXP3$^+$ T-cells are memory cells and they are more efficient in suppression of inflammation in the joints.[65] One caveat with this is that there are many CD103$^-$ memory FOXP3$^+$ T-cells as well. Thus, CD103 is not a universal marker for all memory FOXP3$^+$ T-cells. In suppression of graft-versus-host disease (GVHD), however, CD62L$^+$ FOXP3$^+$ T-cells are more efficient than CD62L$^-$ memory FOXP3$^+$ T-cells.[84,85] Therefore, it is not memory FOXP3$^+$ T-cells that are always more efficient than naïve FOXP3$^+$ T-cells in suppression of inflammation. Whether a FOXP3$^+$ T-cell subset is effective or not effective in suppression of inflammation in a certain tissue would be determined by the migration ability of the FOXP3$^+$ T-cells to the major tissue site of initiation or amplification of the inflammation. In other words, naïve FOXP3$^+$ T-cells or their migration into lymphoid tissues is important if initiation and amplification of the inflammatory disease occur in the lymphoid tissues. Otherwise, memory FOXP3$^+$ T-cells or their migration to effector sites would be important for suppression of inflammation at effector sites (Fig. 3).

## Mechanisms of Suppression Mediated by FOXP3$^+$ T-Cells

TGF-β1 is implicated in the suppressive function of FOXP3$^+$ T-cells. Nakamura et al reported that spleen CD4$^+$CD25$^+$ T-cells produce soluble TGF-β1.[86] In their study, the TGF-β1 concentration in the culture supernatant of CD4$^+$CD25$^+$ T-cells reached ~2 ng/ml, which is a concentration sufficient to suppress target T-cells. Moreover, CD4$^+$CD25$^+$ T-cells expressed surface TGF-β1 as the latency associated protein. Neutralizing anti-TGF-β1 abrogated the suppressive activity of CD4$^+$CD25$^+$ T-cells. Piccirillo et al, however, reported that neutralization of TGF-β1 was not

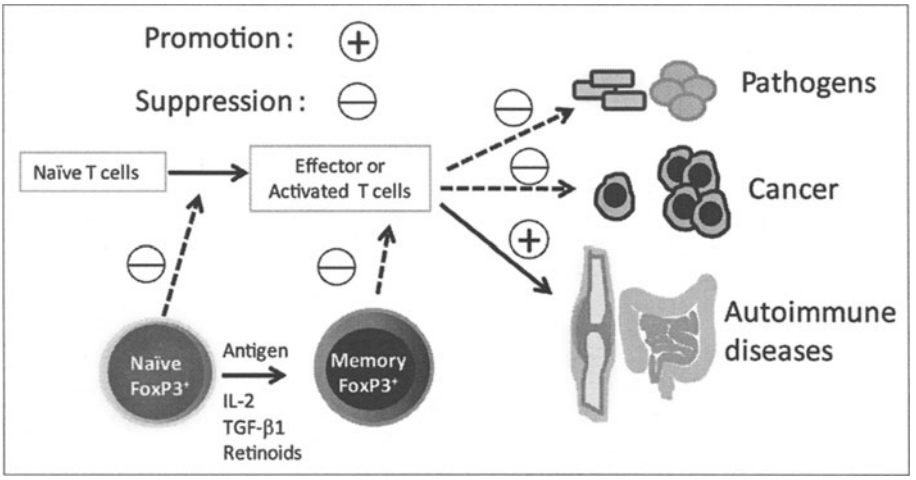


Figure 3. Immune regulatory functions of FOXP3+ T-cells. Naïve FOXP3+ T-cells have the tissue tropism for secondary lymphoid tissues while memory FOXP3+ T-cells have diverse tissue tropisms for nonlymphoid tissues (e.g., gut versus other tissues). Therefore, naïve T-cells are designed to suppress the immune responses in secondary lymphoid tissues perhaps to limit the activation of various immune cells. This would be important to prevent the generation of autoimmune effector T- and B-cells. Memory FOXP3+ T-cells can migrate to nonlymphoid tissues. Depending on the site of antigen priming, some can migrate to the gut, while others migrate to different tissue sites. Therefore, memory FOXP3+ T-cells can suppress the potentially inflammatory activity of effector lymphocytes in diverse peripheral tissues. It is thought that FOXP3+ T-cells can suppress harmful autoimmune responses but can be utilized by tumors and pathogens to delay beneficial immune responses.

able to abrogate the suppressive effect of $CD4^+CD25^+$ T-cells.[87] Similarly, Smad3 (−/−) T-cells and the T-cells that cannot receive the TGF-β1signaling were suppressed by $CD4^+CD25^+$ T-cells. In their study, TGF-β1 (−/−) $CD4^+CD25^+$ T-cells were able to suppress target T-cells. This group also performed an in vivo study through which they found that the suppression of autoimmune gastritis by $CD4^+CD25^+$ T-cells was not reversed by anti-TGF-β1. A caveat with this study is that in vivo neutralization would not always work and thus this data does not prove lack of a role for TGF-β1 in vivo. Mamura et al provides evidence that compromises the results of the two reports.[88] Adoptive transfer of TGF-β1 (−/−) splenocytes into TGF-β1 (+/+) Rag2 (−/−) mice induced an autoimmune inflammatory disease and cotransfer of TGF-β1 (−/−) $CD4^+CD25^+$ T-cells partially ameliorated the disease. However, this suppression was weaker compared to that by wild type $CD4^+CD25^+$ T-cells, suggesting that $CD4^+CD25^+$ cells may suppress target T-cells in both TGF-β1-dependent and independent manners. Using a dextran sodium sulfate (DSS)-induced colitis mouse model in conjunction with a model with impaired TGF-β1-signaling by overexpressing a truncated version of the TGF-β Type II receptor in T-cells, Huber et al reported that transfer of wild-type but not transgenic $CD4^+CD25^+$ T-cells was found to suppress colitis in wild-type mice.[89] Unlike $CD4^+CD25^+$ T-cells from wild type mice, $CD4^+CD25^+$ T-cells from TGF-β1(−/−) mice did not protect recipient mice from colitis in T-cell induced SCID mice.[90] In contrast, a different group reported that $CD4^+CD25^+$ cells from either TGF-β1 (+/+) or TGF-β1 (−/−) mice can suppress the incidence and severity of colitis.[91] It was notable, however, that $CD4^+CD25^+$ cells from TGF-β1 (+/+) mice were always more efficient than the $CD4^+$ $CD25^+$ cells from TGF-β1 (−/−) mice in suppression of inflammation. These authors observed that anti-TGF-β1 neutralization exacerbated effector-T cell induced colitis and claimed that $CD4^+CD25^+$ T-cells are able to suppress intestinal inflammation by a mechanism not requiring Treg cell-derived TGF-β1. One

problem with this claim is that FOXP3$^+$ T-cells can be induced following naïve T-cell transfer. Overall, it appears that TGF-β1 has certain roles in the suppressive function of FOXP3$^+$ T-cells but the degree of contribution may depend on the type of disease and immune responses. This implies that there are TGF-β1-independent mechanisms of suppression.

Indeed, there are a number of candidate mechanisms that could mediate the suppressive function of FOXP3$^+$ T-cells. FOXP3$^+$ T-cells highly express CTLA-4 and CTLA-4 can suppress antigen presenting cells through the cognate CTLA4-B7 interaction.[92,93] The CTLA4-B7 interaction triggers the expression of indoleamine 2, 3-dioxygenase (IDO). IDO converts tryptophan to kynurenine, 3-hydroxyanthranilic acid, picolinic acid and quinolinic acid and thus is an enzyme that depletes tryptophan required for proliferation and function of immune cells.[94] Certain regulatory T-cells express cytotoxic molecules such as granzyme A and granzyme B, which can kill target cells in perforin-dependent and independent mechanisms.[95-97] FOXP3$^+$ T-cells express also heme oxygenase (HO)-1, an enzyme that produces carbon monoxide.[98,99] The suppressive function of human CD4$^+$CD25$^+$ T-cells was blocked in the presence of an HO-1 inhibitor, suggesting a role of carbon monoxide in the suppressive function of FOXP3$^+$ T-cells.[98]

## Role of FOXP3$^+$ T-Cells in Suppression of Diseases

Immune dysregulation, polyadenopathy, enteropathy and X-linked inheritance (IPEX) patients develop various clinical symptoms. Most patients suffer from systemic autoimmune diseases evidenced by severe acute enteritis, Type I diabetes, elevated serum IgE and eczema.[19-21] The patients variably have also hypothyroidism, anemia, thrombocytopenia, neutropenia and autoantibodies. The exact phenotype is thought to be determined by the type of mutations in the FOXP3 gene because partially functional FOXP3 can be made with certain types of mutations. Also, other factors such as genetics and environmental factors can affect the progression of the disease. Scurfy mice are a mouse version of human IPEX.[100,101] Male scurfy mice with the scurfy mutation in the X chromosome develop runting, exfoliative dermatitis, hypergammaglobulinemia and severe anemia.[100,101] In a manner similar to IPEX patients, scurfy mice die young at around 3 weeks of age. The phenotypes of IPEX patients and scurfy mice clearly show that autoimmune responses play central roles in developing the disease. FOXP3 is mainly expressed by CD4$^+$ T-cells and therefore, this suggests that FOXP3$^+$ T-cells play important roles in prevention of the autoimmune disease. The scurfy symptom can be prevented by adoptive transfer of FOXP3$^+$ T-cells,[102,103] further supporting the role of these cells in prevention of the disease.

Because FOXP3$^+$ T-cells can suppress many types of immune cell such as CD4$^+$ T-cells, CD8$^+$ T-cells, CD1d-restricted NKT cells, monocytes/macrophages, naïve/memory B-cells, dendritic cells and NK cells,[104-110] they have the potential to suppress a wide spectrum of immunological diseases. This is indeed true in animal models that FOXP3$^+$ T-cells can either prevent or suppress existing immunological diseases such as experimental autoimmune encephalomyelitis (EAE), inflammatory bowel disease (IBD), diabetes, collagen-induced arthritis, lupus, autoimmune gastritis and allergy.[111-118] Similarly, FOXP3$^+$ T-cells can effectively suppress allogeneic immune responses leading to graft rejection and graft-versus-host disease.[119-121] Infection is a type of diseases that are different from autoimmune diseases and the suppressive function of FOXP3$^+$ T-cells may be disadvantageous for the hosts during infection. In infection, some pathogens can suppress immune responses by expanding FOXP3$^+$ T-cells.[122-125] FOXP3$^+$ T-cells are perhaps required to terminate immune responses and prevent over-active immune responses which could lead to autoimmune diseases. However, excessive expansion of FOXP3$^+$ T-cells could deter clearance of pathogens by the immune system. Cancer is yet another class of diseases. Most tumor types including colorectal cancer, head and neck cancer, hepatocellular carcinoma, breast cancer, pancreas adeno caricinoma, melanoma, cervical carcinoma, gastrointestinal tract cancer, lung cancer, ovarian cancer, leukemia and lymphoma have increased numbers of tumor-infiltrating FOXP3$^+$ T-cells.[126-137] It is unclear if these cells are induced within the tumor or immigrated into tumors. What seems clear is that these tumor-associated FOXP3$^+$ T-cells have the potential to suppress antitumor immune responses.

## Functions of FOXP3 in Nonhematopoietic Cells

FOXP3 appears to have a role in thymic epithelial cells. The scurfy mutation in the *FOXP3* gene causes diminished proliferation of double negative thymocytes and thymic atrophy.[9] Interestingly, FOXP3 is expressed also by nonimmune cells such as epithelial cells in mammary glands, prostate and lungs.[10,138] The function of FOXP3 in the epithelial cells is largely unknown but FOXP3-deficient mammary gland cells are more prone to become cancerous.[10] It is possible that FOXP3 would regulate the expression of certain oncogenes in these cells. HER-2/ErbB2 oncogene and S-phase kinase-associated protein 2 (SKP2, a component of the E3 ubiquitin ligase SKP1-Cul1-Fbox complex) are such oncogenes that are implicated in FOXP3-mediated suppression of cell proliferation in mammary gland cells.[10,139] FOXP3 functions to down-regulate the expression of ERB2 and SKP2.[10] Therefore, FOXP3 appears to play a potentially important role in regulation of the proliferation of epithelial cells in certain organs. Although the role is unclear, FOXP3 is expressed also by some tumor cells.[140,141]

## Concluding Remarks

The significance of FOXP3 in regulation of the immune system is well-established. FOXP3 functions as a transcription activator and suppressor and programs the gene expression program in T-cells in a direction to promote immune tolerance. The detailed mechanisms for the gene expression regulation by FOXP3 remain to be determined but it appears to modulate the function of major transcription factors and to change the chromosomal conformation. A plethora of information is available regarding the immune regulatory function of FOXP3$^+$ T-cells. The data clearly support the clinical application potential of FOXP3$^+$ T-cells in suppression of inflammation and prevention of immunological diseases. Control of immunological diseases can be achieved either through increasing the numbers of FOXP3$^+$ T-cells for suppression of immune cells or decreasing the numbers for promoting immune responses. Autoimmune diseases can be treated by utilizing the former method, while cancer and control of infection can be achieved by adopting the latter method. FOXP3$^+$ T-cells can be prepared in vitro by culturing naïve CD4$^+$ T-cells in the presence of TGF-β1 and IL-2 or various other agents that can turn on the expression of FOXP3. The migratory and functional properties of FOXP3$^+$ T-cells can be altered by using homing receptor inducers such as retinoic acid or by gene therapy. This would make them more efficient in migration to target tissues and to control diseases. It is expected that FOXP3-based therapies would be actively utilized in treating human patients in the near future.

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