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The Biology of FoxP3: A Key Player in Immune Suppression during Infections, Autoimmune Diseases and Cancer

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Abstract

The Transcription factor FoxP3 belongs to the forkhead/winged-helix family of transcriptional regulators and shares general structural features with other FoxP family members. FoxP3 functions as a master of transcription for the development of regulatory T-cells (Treg cells) both in humans and in mice. Natural genetic mutations of FoxP3 that disrupt its function in humans result in an autoimmune syndrome called Immune Polyendocrinopathy, Enteropathy, X-linked (IPEX) and in mice, its deletion causes the Scurfy phenotype, with similar pathology. The finding that FoxP3 is required for the development and function of Tregs has led to an explosion of research in determining its regulation and function in the immune system. Understanding the biological properties of FoxP3 has a wide range of implications for immune tolerance, autoimmune disorders, inflammation and immune response to infectious diseases and cancer.

Introduction

The Immune system has evolved sophisticated mechanisms to mount effective protective immune responses and to limit damage to the host by tightly regulating its potentially harmful side effects. A specialized cell type within the immune system called regulatory T-cells (Tregs) is instrumental in preventing immune responses against self-antigens and dampening immune activation to nonself antigens. These regulatory T-cells were initially defined by high expression of the IL-2 receptor alpha chain (CD25) and were found to be part of the $CD4^+$ helper T-cell subset. Treg cells were then shown to express and require the transcription factor FoxP3, which also became a defining factor for their biology.

The Discovery of FoxP3

The forkhead family transcription factor Foxp3 was shown to be critically important for the development and function of regulatory T-cells.[1,2] FoxP3 was first identified as the culprit mutant gene responsible for the spontaneous scurfy mutation in mice and the human syndrome called Immunedysregulation Polyendocrinopathy Enteropathy, X-linked, or IPEX.[3,4] Both of these genetic defects resulted in death of animals and humans.

In 2003 it was discovered that FoxP3 is expressed in 5–10% of peripheral $CD4^+$ T-cells in mice and 1–5% in humans. FoxP3 expression was shown to be sufficient for murine Treg cell development and function as revealed by studies using ectopic expression of FoxP3 in otherwise conventional T-cells.[2] In humans, ectopic overexpression of FoxP3 in naive T-

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cells was also shown to differentiate these cells into Treg mimics in vitro,[5,6] although the role of FoxP3 turned out to be more complex in the human system, as discussed below.

Tregs are functional only when activated though their T-cell receptors (TCR) and are derived from the thymus, where they are selected based on their positive affinity for self antigens.[7] Thus, Tregs can recognize self antigens to suppress self-reactive T-cells once they migrate to the periphery. Although Tregs require antigenic stimulation for their suppressive function, they are hyporesponsive to in vitro TCR activation.[8,9] Upon stimulation, Treg cells fail to efficiently flux calcium, display impaired proliferative capacity and produce reduced levels of proinflammatory cytokines, such as IL-2 and IFNγ, when compared to effector T-cells.[7,9,10]

How Tregs exert their suppressive function is not fully characterized; however a number of mechanisms have been identified or proposed.[11] Treg cells constitutively express several surface markers, including CD25, GITR and CTLA-4.[7] However these molecules are also present on activated conventional T-cells. The discovery of FoxP3 was monumental in this regard, as it served to define Treg cells both genetically and phenotypically through protein expression.

Functional and Structural Features of FoxP3

In humans, FoxP3 maps to the Xp 11.23-Xql 3.3 locus.[4] FoxP3 has 11 exons, which encode a 431 amino acid protein.[12] Murine FoxP3 is 86% similar to the human protein.[12] FoxP3 shares a structural scaffold with FoxP 1, FoxP2 and FoxP4. It has the greatest percent homology with FoxP1.[13,14] Similar to other members of the family, FoxP3 has a forkhead domain at the C-terminus, which is responsible for DNA binding, a leucine zipper like domain, which mediates oligomerization and a zinc finger motif with unknown function.[13,15] At the N-terminus, FoxP3 contains a proline rich region, while other FoxP proteins have a glutamate rich poly Qregion.[13] The N-terminus is thought to be the repressor domain.[13] Most of the IPEX mutations map to the Forkhead domain of FoxP3. The proline rich repressor domain and the leucine zipper domain are also mutated in several IPEX patients, albeit at a lower frequency.[15] Missense mutations within the Forkhead, leucine zipper and repressor domains also cause IPEX sydnrome,[15,16] suggesting the necessity of all three domains for proper function of this transcription factor. Other Foxp3 mutations in IPEX patients include C-terminal elongation due to loss of a stop codon and a point mutation in the polyadenylation site, which affects mRNA stability.[16] The latter mutation in the polyA site is interesting because it was identified in a multigenerational family in which some affected males lived well into the first decade and one even into the third decade of life,[12] indicating an intermediate IPEX severity due to low levels of mRNA translation. Also, the deaths associated with these ‘intermediate IPEX patients’ occurred after infection or immunization[16] highlighting the importance of intact Treg function during an immune response. A similar phenotype is seen in ‘FILIG’ mice, which have attenuated expression of FoxP3. These mice display uncontrolled lymphocyte proliferation, but the disease severity is lower in FILIG mice compared to scurfy mice,[17] which completely lack FoxP3 expression due to a frameshift mutation.[18]

Forkhead Domain

The FoxP family of proteins is unique in that the Forkhead domain lies at the C-terminal end, whereas the other Fox family members have an N-terminal Forkhead domain.[16] The Forkhead domains of the 4 FoxP family members share a >90% similarity.[14] In FoxP3, this domain extends from exon 9 to exon ll.[16] The Forkhead domain contains a putative nuclear localization sequence (NLS) at the C terminal end.[16] It is also responsible for binding the DNA targets of FoxP3 and for binding Nuclear Factor of Activated T-cells (NFAT).[19,20]

Cofractionation experiments in FoxP3 transfected and activated T-cells found that FoxP3 associates with both a high molecular weight and a low molecular weight complex. The former contains chromatin remodeling factors, while the latter is associated with FoxPl and NFAT.[13] Although the interaction with FoxPl has not been characterized, the interaction with NFAT has been localized to the Forkhead domain.[19]

NFAT is a transcription factor activated by the calcium flux that occurs when all T-cells are activated. Together with another protein called AP-1, the NFAT complex binds to promoters of cell activation genes, such as IL-2 and CD25. The FoxP3 Forkhead domain binds to NFAT, as well as the AP-1 target DNA sequence. Thus FoxP3 effectively blocks AP-1 activity by stealing its binding partner and by occupying its position on the DNA. Using a ChIP assay, the NFAT-FoxP3 complex was shown to bind to the promoters of IL-2, CD25 and CTLA-4.[19] Interestingly, acetylation of FoxP3 in the Forkhead region was also shown to enhance FoxP3 binding to the IL-2 promoter,[21] suggesting that the Forkhead domain can undergo posttranslational modification to modulate its function.

The Forkhead domain of FoxP3 also has numerous DNA binding sites. A genome wide analysis using microarray on the nuclear fraction from mouse $CD4^+CD25^+$ cells and a CHIP assay found that FoxP3 binds at 1,276 regions throughout the mouse genome.[22] FoxP3 binding sites were substantially enriched within 10 kb of the 5′ untranslated region of genes, correlating with the position of promoter regions, as would be expected from a transcription factor. The list of FoxP3 binding targets that are up or downregulated in $FoxP3^+$ cells confirms that FoxP3 can act as both an activator and a repressor.[22] Histone H3 modifications are common at FoxP3 binding sites, indicating that chromatin remodeling occurs during FoxP3 activity. This is probably a result of the ability of the N-terminal region of FoxP3 to recruit chromatin-remodeling factors. It was also revealed that FoxP3 bound genes were mosdy involved in TCR signaling, cell communication and transcriptional regulation. These profiles support the notion that FoxP3 is involved in regulating TCR mediated signals intracellularly, can promote the expression of genes with intercellular effector functions and contributes to genetic programming and cell development.[22]

Leucine Zipper Domain

Leucine zipper and zinc finger domains are both traditionally known as protein-protein interaction domains, which have the potential to bind DNA.[16] The leucine zipper is known to be indispensable for FoxP3 function based on two IPEX patient missense mutations. Although the function of the zinc finger domain of FoxP3 is not currently established, the leucine zipper is responsible for oligomer formation. FoxP3 can form homo-oligomers and can also form a heterodimer with FoxP 1. In fractionation experiments, FoxP 1 was found in the low molecular weight complex with FoxP3 and NFAT.[13] In addition, recombinant FoxP3 raised in either bacterial or mammalian cells, forms homotetramers. The IPEX E251 mutation of FoxP3 eluted as a monomer, indicating that compromising the oligomer formation could be disrupting protein function.[16]

Forkhead- Leucine Zipper Linker Region

The region that bridges the Forkhead and leucine Zipper domains in FoxP3 (aa 278–336) binds to the Acute Myeloid Leukeamia-1 (AML-1) /Runt Related transcription factor (RUNX-1) protein, specifically, in the C-terminal repressor domain. AML-1 binds upstream of the IL-2 gene, acting as a promoter enhancer. FoxP3 is shown to block this enhancement and FoxP3 mutations that attenuate binding to AML-1, result in increased IL-2 production. Furthermore, these mutations impair the expression of Treg phenotype markers and some Treg functions.[23]

N-Terminal Proline Rich Repressor Domain

Analysis of ChIP and microarray experiments show that FoxP3 directly binds only 6% of the genes that it regulates.[22] This could be because FoxP3 binds to the promoters of genes that in turn control other genes or because DNA binding is not the only mechanism by which FoxP3 alters gene expression. Indeed, DNA binding activity alone probably does not account for the indispensable activity of FoxP3 in regulatory T-cells, as Tregs also express FoxPl, which has 90% similarity to FoxP3 in the Forkhead domain.[14] In other studies, it was noted that the N-terminus of the protein is also important in interaction of FoxP3 with NFAT and its function.[19] Thus it is conceivable that the N-terminal domain of FoxP3 is a major distinguishing factor between the function of FoxP3 and the other members of the family.[13]

The N-terminal proline rich region has crucial function in binding to chromatin remodeling factors that are necessary for FoxP3 transcriptional activity. As mentioned above, fractionation experiments with FoxP3 overexpressing cells showed that FoxP3 associates with both a high and a low molecular weight complex in cells.[13] The high molecular weight complex is composed of chromatin remodeling factors.[13] Specifically, it was found that TIP60, a histone acetyltransferase, binds to the N-terminal proline rich region of FoxP3.[24] TIP60 acetylates FoxP3 in Tregs and a TIP60 mutant, deficient in the ability to acetylate (HAT domain mutated) cannot promote transcriptional repression. This interaction was thought to be necessary for repression of FoxP3 target genes as assessed through IL-2 production, because repression of IL-2 does not occur in TIP60 knockdown cells.[6] In addition, TIP60 recruits a histone deacetylase called HDAC7.[13] Histone deacetylases remove acetyl groups from histone tails, which in turn encourages high-affinity binding of histones to DNA. Therefore, HDAC7 could be preventing transcriptional access, consistent with a model of FoxP3 mediated repression of some target genes. Indeed, HDAC7 is also found in complex with FoxP3 during coimmunoprecipitation experiments. Mutating the N terminal proline rich region abolishes the coimmunoprecipitation of FoxP3 and HDAC7 and abolishes the transcriptional repressor function of FoxP3.[13] However, it was also shown that treating Treg cells with a broad based HDAC inhibitor increased their suppressive function.[21] This effect however, may be the result of HDAC regulation of the FoxP3 gene itself, as HDAC inhibitor treatment also resulted in increased expression of FoxP3 in the cells. In addition, FoxP3 binding to the promoters of cytokines IL-2 and IFNγ was shown to deacetylate histone H3, inhibiting chromatin remodeling and effectively blocking transcription.[25]

Multiple Isoforms and Subcellular Localization

In contrast to the murine version, human FoxP3 has two isoforms, which are called FoxP3a and FoxP3b. FoxP3a is full-length protein and FoxP3b is a splice variant lacking exon 2. Interestingly, in activated $CD4^+CD25^+$ cells, FoxP3a can be found in both the nucleus and the cytoplasm, FoxP3b is only found in the nucleus.[14] Exon 2 has a nuclear export signal (NES), thus FoxP3b is not properly exported to the cytoplasm after activation due to lack of an NES.[14] The implications of a cytoplasmic export in human cells is not clear since mouse FoxP3 appears to be only localized to the nucleus.[26]

It was also reported that expression of full length FoxP3a results in a more unresponsive T-cell phenotype as compared to the FoxP3b isoform. Human cells expressing only FoxP3b have an intermediate Treg phenotype in terms of curbed proliferative capacity and dampened cytokine secretion.[27] However, in other reports, both isoforms were shown to possess a similar capacity to induce Tregs and to suppress T-cell activation.[6,28,29] The region encoded by exon 2 is also thought to be critical for the association of Foxp3 with transcription factors retinoic acid related orphan receptor alpha RORα[30] and RORγt,[28]

which are master transcription factors for development of a proinflammatory T-cell subset called Th 17.

FoxP3 Regulation and Function

Role of FoxP3 in Development and Function of Tregs

It is now well-established that FoxP3 is required for development of Treg cells both in humans and mice. However, it is not fully clear whether FoxP3 expression alone is sufficient to program conventional T-cells into bona fide Tregs, especially in the human system. Ectopic expression of FoxP3 in $CD4^+CD25^-$ non-Treg cells produced a regulatory phenotype, as these cells exhibited suppressive activity in vitro and also protected the host mice from autoimmune diseases in several adoptive transfer models.[1,2,31] In humans, ectopic overexpression of FoxP3 in naive T-cells was also shown to differentiate these cells into Treg mimics in vitro.[5,6] However in microarray experiments the gene expression profile between natural Tregs and FoxP3 ectopically expressing cells in mice were found to be different;[32] specifically, there are genes upregulated in Tregs that are not under the control of FoxP3. In experiments utilizing FoxP3 knock-out/GFP knock-in mice, it was found that some Treg characteristics and marker genes are present even in the absence of FoxP3.[33,34] Taken together, these results suggest there may be other important factors required along with FoxP3, in the development of Treg lineage cells.

Cell Extrinsic Regulation of FoxP3

The cytokine TGFβ induces FoxP3 expression in $CD4^+CD25^-$ cells.[41] In mice, TGFβ induced FoxP3 programs cells with Treg characteristics and the ability suppress T-cell activation, these cells are sometimes referred as iTreg, or inducible Treg.[42] Peripheral, but not thymic Tregs were found to be reduced in eight to ten day old TGFβ1−/− mice and Tregs deficient in TGFβ Receptor II were also poorly maintained in the periphery, suggesting TGFβ's critical role in peripheral Treg maintenance.[43,44] Human $CD4^+CD25^-$ T-cells upregulate FoxP3 upon activation in the presence of TGFβ.[41] However, in human cells, such induction does not confer suppressive function.[37,38] It is possible that FoxP3 has a second role in human cells, in mediating hyporesponsiveness of $CD4^+CD25^-$ T-cells in vivo.[45] Recently, a molecule called GARP was shown to be specifically expressed on Tregs and can potentially be used to differentiate between $FoxP3^+$ bona fide Tregs and TGFβ-induced FoxP3 expressing cells.[46]

The downstream signaling cascade leading to FoxP3 induction is not yet clearly established; however several key players have been identified. In keeping with conventional TGFβ signaling, Smad3 has been identified as necessary for FoxP3 induction.[47] Stat5, which functions downstream of IL-2 signaling, binds the FoxP3 promoter similarly to NFAT, which is activated after TCR triggering.[48,49] These findings are consistent with the requirement of IL-2 and TCR activation for Treg function. Signaling through the Notch receptor/trancription factor pathway may also be involved in FoxP3 expression, as pharmacological inhibition of Notch 1 blocks FoxP3 induction.[47] Another signaling protein important in cellular survival called Akt has been established as a repressor of novel FoxP3 induction, although it cannot reverse already established FoxP3 expression.[50] Phosphoinositide 3-kinase and downstream signaling molecule mTOR can also antagonize FoxP3 expression;[51] in fact, the mTOR inhibitor Rapamycin promotes FoxP3 expression both in vitro and in vivo and has been used therapeutically in IPEX patients.[52–54]

It was recently reported that the Vitamin A metabolite retinoic acid (RA) could promote FoxP3 expression in T-cells.[55] RA is present in the gut and produced by antigen-presenting cells such as macrophages, which have the necessary metabolic enzymes.[55,56] It is possible that RA may play a role in establishing oral tolerance to ingested food and to the vast

microbiome that inhabits the human gut. In fact, dietary vitamin A has been known for over twenty years to protect against autoimmunity in mice.[57] It was also suggested that RA enhances stability of FoxP3 induced by TGFβ.[58]

Epigenetic and Posttranslational Regulation of Foxp3

As discussed in the structural section, FoxP3 is subject to posttranslational modification in its N-terminal repressor domain by TIP60. FoxP3 can also be acetylated in the Forkhead domain and optimal Treg repressor function is dependent on this acetylation, as it allows binding to the IL-2 promoter.[21] The administration of HDAC inhibitors therefore positively regulates FoxP3 activity.[21]

Evidence also exists that FoxP3 may regulate itself through positive feedback. During analysis of mice genetically modified to replace FoxP3 with GFP at the FoxP3 locus (FoxP3^{-}GFP knock-in mice), FoxP3^{-}GFP^{+} T-cells downregulated GFP over time, while the majority of the FoxPV^{+}/ GFP^{-} cells maintained FoxP3 expression,[33,41] indicating that FoxP3 presence promotes further transcription at the FoxP3 locus. A positive feedback loop for FoxP3 expression is also supported by the findings that FoxP3 obstructs development of other helper T-cell subsets.[17] Recent research has suggested a role for epigenetic chromatin patterning in this process. Specifically, demethylation occurs near the FoxP3 promoter in naturally occurring Tregs.[59] Methylation of DNA is a mechanism to limit access to transcriptional proteins and demethylation would be predicted to relieve this restriction. Using azacytidine, a DNA methyl transferase inhibitor, FoxP3 expression was induced stably in cells that do not physiologically express it, including conventional T-cells.[60] Furthermore, demethylation at the FoxP3 locus was a faithful marker of natural Tregs and neither transiendy FoxP3 expressing cells nor TGFβ induced FoxP3^{+} cells were demethylated at this locus.[59] The stable expression that demethylation at the FoxP3 gene locus confers may also contribute to a positive feedback mechanism in which FoxP3 promotes its own synthesis, thus maintaining abundant and sustained levels in the cell.

Recent studies have confirmed the link of chromatin remodeling to the regulation of FoxP3 and have provided mechanistic insight into cell extrinsic mechanisms in this process. An enhancer region upstream of the FoxP3 gene together with Smad3 and NFAT are required for histone acetylation at the enhancer, thus opening up the region for transcription.[61] As several Smads are involved in TGFβ signaling, this may also help to explain the TFGβ-mediated induction of FoxP3 expression.[61] The T-cell cytokine IL-4 was also found to inhibit FoxP3 induction, through transcription factor STAT6, which was shown to bind to the silencer region in the vicinity of FoxP3 and inhibit chromatin remodeling at the locus.[62] Interestingly, RA reduced STAT6 binding to the silencer region, relieving the inhibition and enhancing histone acetylation.[62] Another cytokine, IL-6, can promote methylation at the FoxP3 locus, silencing its transcription.[63] Epigenetic control of the FoxP3 locus may therefore be critical in understanding complex regulation of FoxP3 gene expression.

Role of FoxP3 in Cancer

As FoxP3^{+} Tregs mainly function to eliminate self-reactive lymphocytes, they can be potentially detrimental to the immune response against tumors. Because most tumor-associated antigens are recognized as self, they are more likely to activate Tregs rather than effector T-cells capable of mounting an immune response. In addition, tumor cells often acquire the ability to secrete cytokines such as TGFβ, which induces FoxP3 expression in T-cells. Indeed, high levels of FoxP3^{+} cells have been detected in the tumor environments of many cancers and strategies to eliminate them to block their tumor protective effects are in development.

Foxp3$^+$ T-cells are also actively recruited to tumor sites. In a model of human ovarian cancer, it was found that a chemokine called CCL22 is released by cells in the tumor microenvironment and specifically recruits Tregs.[64] Several groups have shown that in various tumor models in mice and man, natural Tregs are present and proliferating in the tumor tissue.[65–68] TGFβ, which is often produced by tumor cells,[70] is favored to be the inducer Treg proliferation in these tumor microenvironments.[69]

It is also known that tumors can induce expression of FoxP3 in conventional T-cells. In addition to TGFβ, indoleamine 2,3-dioxygenase (IDO) can contribute to this induction. An IDO inhibitor abolishes conversion of conventional CD4$^+$ cells to Treg in the A20 lymphoma model[71] and IDO expression by human leukemia cells correlates with the number of FoxP3$^+$ cells in the blood. Tumor resident antigen-presening cells such as plasmacytoid dendritic cells can also produce IDO.[72] Both TGFβ and IDO inhibitors are under investigation to override tumor mediated immune suppression.[69]

FoxP3 expression by non-T-cells may also have an important role in development of certain malignancies such as breast cancer. For example, mice that are heterozygous for FoxP3, have increased incidence of breast cancer development. Furthermore, human breast cancer cells that express the HER/*neu* markers of aggressive malignancy, downregulate FoxP3 in breast tissue.[73] In fact, FoxP3 was found to repress transcription of SKP2, a breast cancer oncogene.[74] Loss of FoxP3 in non-T-cells therefore may lead to more aggressive tumor growth. Thus Foxp3 expression is a double-edged sword in cancer.

FoxP3 in Infectious Diseases

Parasitic Infections

Recent observations have further demonstrated that FoxP3$^+$ Tregs may influence the immune response to many microbes. One of the first observations on the role of Tregs during infection was made with the parasitic pathogen *Leishmania major*.[75,76] When Tregs were removed from the site of infection, the animals could better discard the infection.[75] However, further studies showed that in certain strains of mice Tregs actually held the cutaneous infection in check, which otherwise would result in progressive lesions.[76] A similar picture was observed in adoptive transfer of Treg depleted cells into SCID mice, which developed more severe infections than those that also received the Treg subset. From these studies it is clear that Tregs could play a useful role in Leishmania pathogenesis, although too much Treg response also diminishes the immunity to the pathogen resulting in chronic disease. Similarly, in Malaria, increased Tregs were detected in the peripheral blood, where *Plasmodium falciparum* resides on red blood cells. A positive correlation between FoxP3$^+$ T-cells and growth rate of the parasite was observed.[77]

Viral Infections

Several viral infections, especially those that persist, may perturb the immune response,[78,79] which can result in increased susceptibility to other infections, tumors or even autoimmunity.[80] Tregs have recently been implicated in mediating functional impairment of CD8$^+$ T-cells during persistent retroviral infection.[81] Other instances of viral infections wherein the Treg response acts to the detriment of the host are recognized. For example, in HSV infection of mice, the magnitude of both CD8 and CD4 responses against the virus were elevated two to three fold if mice were depleted of Treg cells prior to the infection.[82] In chronic hepatitis C infection, Tregs can curb liver damage.[77] Tregs are also expanded in mice persistently infected with Friend retrovirus, suggesting that they may contribute to immunosuppression in the absence of T-cell depletion in chronic viral infections.[83]

HIV Infection

Another viral infection where FoxP3$^+$ T-cells may have a critical dual role is HIV infection. The ability of HIV to establish a persistent infection is critically dependent on T-cell activation signals.[84] Indeed, a chronic state of hyperactivation is a hallmark of HIV infection.[85] Consequendy, this state of chronic immune activation combined with the direct destruction of CD4$^+$ T-cells by HIV leads to a profound immunodeficiency characterized by progressive deterioration in immune function.[86] FoxP3$^+$ T-cells were found to be highly susceptible to HIV infection both in vitro[5] and in vivo.[87] It is possible that the loss of FoxP3$^+$ T-cells in turn could potentially result in hyperactivity of conventional T-cells due to the lack of regulation by Tregs, thereby creating more T-cell targets for HIV. In a mouse model reconstituted with a human immune system to study HIV pathogenesis, FoxP3$^+$ Treg cells were preferentially infected and depleted.[88] When these mice were depleted of their Tregs during acute infection, HIV infection was reduced.[88] Conversely, if Tregs are specifically activated by HIV during the earlier stages of infection, this could have a suppressive effect on the protective immune response against the virus.[89–91]

Foxp3 may also play a direct role in facilitating HIV transcription in infected T-cells. HIV gene transcription is dependant on endogenous host cell factors such as NFAT and NFκB.[92] FoxP3 was shown to enhance NFκB binding the HIV LTR, increasing HIV-transcription in these cells.[93] Abrogating FoxP3 binding to NFκB prevented this enhancement. However, other groups found that FoxP3 suppressed gene expression from the HIV LTR,[94,95] FoxP3 and FoxP3$^+$ T-cells thus play a multifaceted role during HIV infection.

Foxp3 in Transplantation Tolerance

FoxP3$^+$ Tregs are partly responsible for maintaining peripheral tolerance to self in the body and could be invoked to suppress immune responses to foreign antigens. This would be particularly important in a not fully matched organ transplantion, which can result either in rejection of the transplanted tissue or an immune response by the donor called graft-versus-host disease (GVHD). It is conceivable for example to educate donor Tregs to recognize allogeneic antigens from the transplated host and transfer these along with the transplant tissue. This would presumably suppress donor effector T-cells from attacking the host, thus preventing GVHD. Patients with chronic GVHD indeed show diminished FoxP3$^+$ T-cell numbers and low dose IL-2 therapy is currendy being explored as an approach to induce FoxP3 and promote Treg survival in these patients.[96]

Alternatively, if FoxP3 expression can be induced by host cells after transplantation, this could also help to establish tolerance and complement immune-suppressive therapies. There is evidence to support that the Treg response does not need to be specific to transplant tissue and can prevent immune activation by bystander suppression.[97] Indeed, higher levels of FoxP3 mRNA detected in the urine of renal transplant patients and higher Treg cells correlated with reduced graft rejection.[97] In this regard, the immune-suppressive drug rapamycin could have dual function both by dampening immune responses and by selectively inducing FoxP3$^+$ Tregs.[97]

FoxP3 in Autoimmune Diseases

Disruption of FoxP3 function leads to severe autoimmunity both in humans and mice, highlighting the critical importance of this transcription factor in preventing unwanted immune response against self. Here we will review some of the experimental autoimmune models in mice, where FoxP3$^+$ Tregs were shown to play a crucial role.

Multiple Sclerosis

Experimental autoimmune encephalomyelitis (EAE) is a syndrome of inflammation of the Central Nervous System (CNS), which is used as a mouse model for human multiple sclerosis (MS) disease, also caused by autoimmune response to myelin.[98] EAE is typically induced by myelin injection or by transferring myelin-reactive $CD4^+$ cells to susceptible mice. Early experiments done before the discovery of FoxP3 showed that $CD4^+CD25^+$ T-cells transferred from healthy mice could protect susceptible mice against EAE.[99] It was then determined that $FoxP3^+$ Treg cells were responsible for this protection in an antigen (myelin) specific or bystander fashion.[98] In humans, analysis of blood samples and spinal fluid from MS patients also shows evidence of Treg perturbation.[98]

Inflammatory Bowel Diseases

The murine colitis model is used to gain insight into ways to control human autoimmune diseases of the intestine, such as ulcerative colitis and Crohn's disease. In this model, immune deficient mice are populated with naive $CD4^+$ T-cells, which causes severe intestinal inflammation. Mice that receive $CD4^+FoxP3^+$ T-cells are cured of the disease within weeks and it was shown that Treg cells migrated to the colon, which is the site of inflammation.[98]

Type I Diabetes

Type I diabetes, or diabetes mellitus, is an autoimmune syndrome in which the insulin producing beta cells in the pancreas are attacked by the immune system. Neonatal diabetes mellitus is characteristic of IPEX patients with FoxP3 mutations. A broad study with Type 1 diabetes patients showed that (GT)n microsatellite polymorphisms in the FoxP3 gene were also associated with the disease.[100] Another study correlated a lower FoxP3 mRNA level with Type I diabetes patients.[101] In a mouse model of Type I diabetes called nonobese diabetic (NOD), $FoxP3^+$ T-cells decreased as the disease progressed.[102] The main culprit in this mouse model appears to be increased beta cell specific effector T-cells that are also resistant to suppression by $FoxP3^+$ T-cells;[103,104] there is no defect in the generation or maintenance of Tregs, indicating that FoxP3 function is intact.[105,106] However, when beta cell specific Tregs from diabetic mice were expanded in vitro and transferred back to diseased mice, the diabetes regressed.[107] In alternative experiments T-cells specific to pancreatic beta cells were genetically manipulated to express FoxP3, which also caused regression of disease when transferred to diabetic mice.[107]

Emerging and Potential Therapeutic Intervention

$Foxp3^+$Tregs or Foxp3-programmed T-cells have a vast array of functions and roles in human diseases (Table 1). Thus, Foxp3 is potentially a significant target for therapeutic approaches against these diseases. On the one hand enhancing $FoxP3^+$ Tregs could be useful in the treatment of autoimmune syndromes, inflammatory disorders, transplantation and complications from chronic infections. On the other hand attenuating the $FoxP3^+$ Treg responses would be beneficial in enhancing antitumor immunity, responses to acute infections and boosting the potency of vaccines.

Although the prospect of targeting a transcription factor is generally avoided because of the widespread and often unforeseen activities of transcriptional regulators, FoxP3 has been shown to be relatively specific to the immune system and associated primarily with immune activation. Several questions remain to be answered in order to manipulate FoxP3 or FoxP3 expressing cells during human diseases. First, how can we induce FoxP3 in specific cell types? It is possible that the signaling pathways used by TGFβ to induce FoxP3 can be exploited to develop pharmacological agonists to induce FoxP3 expression. Conversely, in

conditions such as cancer or acute infectious diseases it may be desirable to dampen FoxP3 expression to amplify the immune response.

Second, how can we generate antigen-specific FoxP3$^+$ Tregs and direct them to the sites of inflammation? It may be possible to identify certain epitopes of antigens that preferentially stimulate Tregs versus effector T-cells. Reverse approaches to exclude these epitopes in vaccines would boost immune response to antigens. Migration of T-cells to tissues is largely dependent on their chemokine receptor expression profiles. Increased knowledge in this field has revealed various biological agents such as cytokines that can program cells to express given chemokine receptors and target them to sites of infection or inflammation. Future approaches to genetically manipulate T-cells to ectopically express FoxP3, forced expression of TCRs specific to antigens of interest or specific chemokine receptors on bona fide Treg could also be powerful cellular treatment options in controlling chronic immune activation or inflammation.

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Table 1

Function and role of Foxp3 in diseases

Disease	Role of Foxp3	References
IPEX syndrome	Natural genetic mutations in Foxp3 causes autoimmune syndrome in humans	3,4
Ovarian cancer	$Foxp3^+$ Tregs recruited to the site of tumor	64
Breast cancer	Breast cancer cells that express the HER/*neu* markers of aggressive malignancy, downregulate FoxP3 in breast tissue	73
Breast cancer	FoxP3 represses transcription of SKP2, a breast cancer oncogene	74
Malaria	A positive correlation between $FoxP3^+$ T-cells and the growth rate of *plasmodium falciparum* was observed	77
HSV infection	The magnitude of immune responses against the virus elevated if mice were depleted of $Foxp3^+$ T-cells prior to the infection	82
Hepatitis C infection	Foxp3 expressing Tregs can curb liver damage	77
HIV infection	$FoxP3^+$ T-cells were found to be highly susceptible to HIV infection	87,88
HIV infection	FoxP3 shown to enhance NFκB binding the HIV LTR, increasing HIV-transcription in infected cells	93
GVHD	In chronic GVHD $FoxP3^+$ T-cell numbers are reduced, they could potentially be protective against the disease	96
Renal transplant rejection	Higher levels of FoxP3 mRNA detected in the urine of renal transplant patients correlated with reduced graft rejection	97
Multiple sclerosis	$FoxP3^+$ Treg cells are responsible for protection of mice against EAE, the model of multiple sclerosis in mice	98
Type l diabetes	Polymorphism in the FoxP3 gene is associated with the disease, lower FoxP3 mRNA level in Type I diabetes patients	100,101