

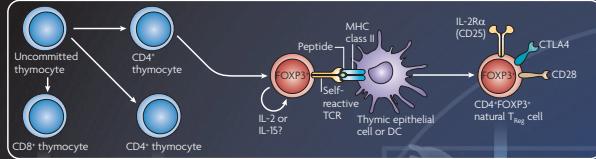
To avoid immune-mediated pathology and unrestricted clonal expansion of responder T cells, the immune system has subsets of T cells, known as regulatory T ( $T_{reg}$ ) cells, that are dedicated to mediating immune suppression. The most important subset of  $T_{reg}$  cells expresses the transcription factor forkhead box P3 (FOXP3). Both mice and humans with genetic deficiencies of FOXP3 develop severe abnormalities of immune homeostasis.  $T_{reg}$  cells modulate the immune response in numerous settings, including autoimmune disease, allergy, microbial infection, tumour immunity,

organ transplantation, foetal-maternal tolerance and even obesity. Defects in  $T_{reg}$  cell function may be an important factor in the development of autoimmunity or in the failure to control immunopathology, whereas overactive  $T_{reg}$  cell function may contribute to the suppression of tumour immunity. Enhancement of  $T_{reg}$  cell function either pharmacologically or by cell-based therapy may prove to be an adjunct to the treatment of autoimmunity, whereas deletion or inactivation of  $T_{reg}$  cell function may facilitate the generation of tumour immunity or enhance responses to weak vaccines.

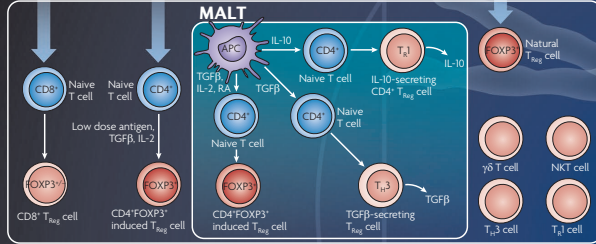
Development and phenotype of regulatory T cells

Many T cell types have immune regulatory function, but the two most important  $T_{reg}$  cell subsets express the transcription factor FOXP3 and develop in the thymus or can be induced in peripheral sites including the mucosa-associated lymphoid tissue (MALT). Although expression of FOXP3 is considered a useful marker for these cell subpopulations in mice, FOXP3 expression may also be induced in human T cells that lack  $T_{reg}$  cell function. However, functional activated human FOXP3<sup>+</sup>  $T_{reg}$  cells express a unique pattern of cell surface markers that can facilitate their isolation. A third important type of  $T_{reg}$  cell secretes the immunosuppressive cytokine interleukin-10 (IL-10) and may develop from conventional CD4<sup>+</sup> T cells by activation in the presence of IL-10 or may develop from T helper 1 ( $T_H1$ ) or  $T_H2$  cell subsets. Other T cell subpopulations including natural killer (NK) T cells,  $\gamma\delta$  T cells and CD8<sup>+</sup> T cells can also exert potent suppressor functions in certain settings. Although both human and mouse CD8<sup>+</sup> T cells can be induced to express FOXP3, a suppressive function for these cells *in vivo* has yet to be clarified.

Thymus

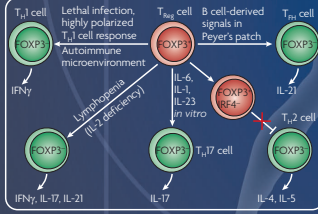


Periphery



Plasticity in the periphery

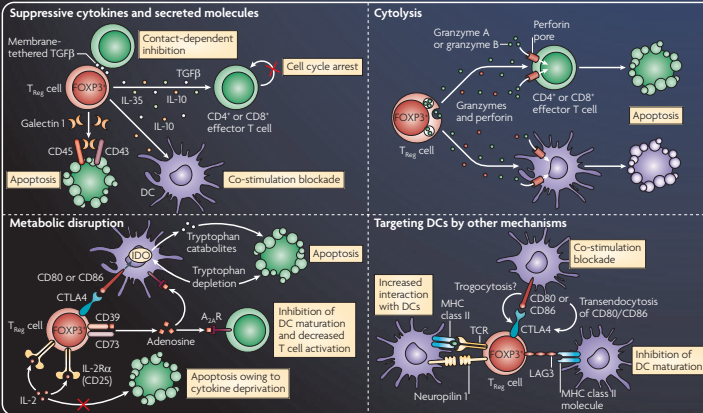
Under certain conditions, FOXP3<sup>+</sup>  $T_{reg}$  cells can downregulate their expression of FOXP3, lose suppressor functions and manifest some of the functions of conventional effector  $T_H1$ ,  $T_H2$ ,  $T_H17$  and  $T_H1$  cell subsets. The key causes of this loss of FOXP3 expression include inflammatory environments with high levels of cytokines that are normally involved in the induction of effector T cells, such as IL-6 and interferon- $\gamma$  (IFN $\gamma$ ). In addition,  $T_{reg}$  cell-specific deletion of certain transcription factors that are shared between  $T_{reg}$  cells and effector cell subsets (for example, the  $T_H2$  cell-specific factor IRF4) results in impaired suppression of  $T_H2$  cell responses by the  $T_{reg}$  cells.



Phenotypic markers of FOXP3<sup>+</sup> regulatory T cells

<p>Markers shared by FOXP3<sup>+</sup> <math>T_{reg}</math> cells and conventional activated CD4<sup>+</sup> T cells (mice and humans)</p> <p>CD25 GITR CD45R0<sup>low</sup> (mice only) CD45RO (humans only) Folate receptor 4 (mice only)</p>	<p>Markers preferentially expressed by activated mouse FOXP3<sup>+</sup> <math>T_{reg}</math> cells</p> <p>FOXP3 Latent TGFβ CD103</p> <p>Subpopulations of human FOXP3<sup>+</sup> <math>T_{reg}</math> cells</p> <p>CD45RA<sup>+</sup>FOXP3<sup>+</sup> (naive) CD45RA<sup>+</sup>FOXP3<sup>+</sup> (activated) CD45RA<sup>+</sup>FOXP3<sup>+</sup> (cytokine-secreting)</p>	<p>Markers specifically expressed by activated human FOXP3<sup>+</sup> <math>T_{reg}</math> cells</p> <p>FOXP3<sup>+</sup> Latent TGFβ GARP CD121a (IL-1R) CD121b (IL-1R2)</p>
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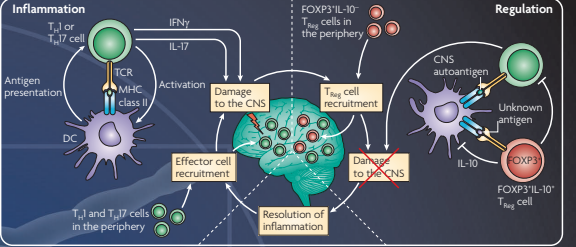
Mechanisms of action of FOXP3<sup>+</sup> regulatory T cells



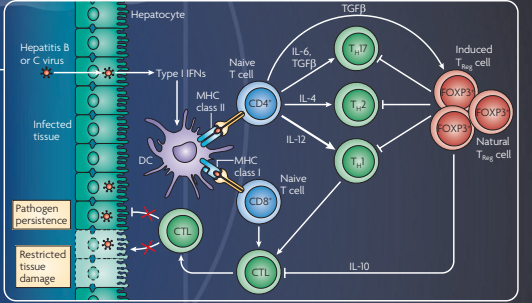
Function of FOXP3<sup>+</sup> regulatory T cells

FOXP3<sup>+</sup>  $T_{reg}$  cells have been shown to influence the outcome of immune responses in several tissues. For example, in the intestine  $T_{reg}$  cells have a key role in maintaining tissue homeostasis by inhibiting the overactivation of dendritic cells (DCs) and effector T cells. In the case of autoimmunity, such as that depicted in the central nervous system (CNS),  $T_{reg}$  cells can have a beneficial effect by short circuiting the inflammatory loop of T cells and antigen-presenting cells (APCs). This same general mechanism can have negative consequences in the setting of a tumour, in which  $T_{reg}$  cells can inhibit the antitumour immune response, thereby preventing tumour clearance. During infection  $T_{reg}$  cells carry out a delicate balancing act: preventing immunity would lead to the inability to clear the pathogen, whereas unrestrained immunity would lead to unwanted immune-mediated tissue destruction. In each of the examples shown, we focus on the role of FOXP3<sup>+</sup>  $T_{reg}$  cells, although interactions between multiple immune cell types and indeed different types of regulatory T cell are also likely to be important in the regulation of immune responses.

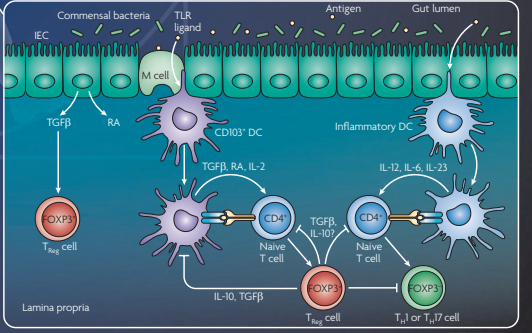
Preventing autoimmunity



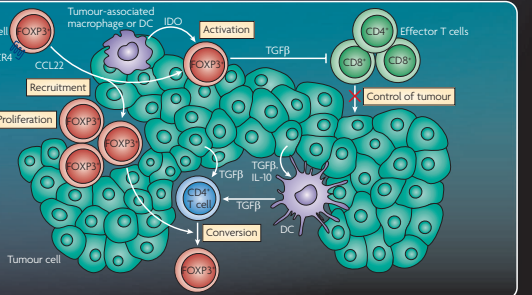
Establishing chronic infection



Maintaining intestinal homeostasis



Promoting tumour progression



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The authors declare no competing financial interests.  
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**STEMCELL Technologies**  
Regulatory T cells (Tregs) comprise only a small fraction of total CD4<sup>+</sup> T cells in human peripheral blood and mouse spleen, and therefore must be highly enriched to evaluate their suppressive function and therapeutic potential. Since Tregs lack a unique cell surface marker and often share phenotypic similarities with activated T cells, isolation of highly purified Tregs is typically difficult and time consuming, often requiring multiple steps. To meet the needs of Treg researchers, STEMCELL Technologies has developed a full range of optimized Treg isolation kits that address these specific challenges. Our Complete Kits for the isolation of Tregs provide the fastest and easiest method to isolate highly purified human Tregs directly from whole blood or buffy coat in just two steps. CD4<sup>+</sup>CD127<sup>low</sup> or CD4<sup>+</sup>CD127<sup>low</sup>CD49d<sup>+</sup> T cells are pre-enriched by RosetteSep<sup>®</sup>, which combines a normal Ficoll<sup>™</sup> density centrifugation step with an antibody-mediated specific cell enrichment procedure. CD25<sup>low</sup> T cells are then positively selected from the pre-enriched cells using our column-free immunomagnetic cell separation system, EasySep<sup>®</sup>, or our fully automated cell separator, RoboSep<sup>®</sup>. For researchers working with human PBMCs or mouse spleen samples, our EasySep<sup>®</sup> kits provide a fast, easy and gentle method to isolate highly purified Tregs. Positively selected cells express high levels of FOXP3 and are suitable for immediate downstream experiments, including flow cytometry, *in vitro* expansion or suppression assays. For more information about our complete range of cell isolation products, or to request a free sample, please visit [www.stemcell.com](http://www.stemcell.com).

or CD4<sup>+</sup>CD127<sup>low</sup>CD49d<sup>+</sup> T cells are pre-enriched by RosetteSep<sup>®</sup>, which combines a normal Ficoll<sup>™</sup> density centrifugation step with an antibody-mediated specific cell enrichment procedure. CD25<sup>low</sup> T cells are then positively selected from the pre-enriched cells using our column-free immunomagnetic cell separation system, EasySep<sup>®</sup>, or our fully automated cell separator, RoboSep<sup>®</sup>. For researchers working with human PBMCs or mouse spleen samples, our EasySep<sup>®</sup> kits provide a fast, easy and gentle method to isolate highly purified Tregs. Positively selected cells express high levels of FOXP3 and are suitable for immediate downstream experiments, including flow cytometry, *in vitro* expansion or suppression assays. For more information about our complete range of cell isolation products, or to request a free sample, please visit [www.stemcell.com](http://www.stemcell.com).

Starting sample	Phenotype of cells	STEMCELL Cell Isolation Kit	STEMCELL Catalog number
Human	CD4 <sup>+</sup> CD25 <sup>+</sup> T cells	Complete Kit for Human CD4 <sup>+</sup> CD25 <sup>+</sup> T Cells	15862
	CD4 <sup>+</sup> CD127 <sup>low</sup> CD25 <sup>+</sup> T cells	Complete Kit for Human CD4 <sup>+</sup> CD127 <sup>low</sup> CD25 <sup>+</sup> Regulatory T Cells	15863
	CD4 <sup>+</sup> CD127 <sup>low</sup> CD45RO <sup>+</sup> CD25 <sup>+</sup> Regulatory T cells	Complete Kit for Human CD4 <sup>+</sup> CD127 <sup>low</sup> CD45RO <sup>+</sup> CD25 <sup>+</sup> Regulatory T Cells	15864
PBMC	CD4 <sup>+</sup> CD25 <sup>+</sup> T cells	EasySep <sup>®</sup> RoboSep <sup>®</sup> Human CD4 <sup>+</sup> CD25 <sup>+</sup> T Cell Isolation Kit	18062
	CD4 <sup>+</sup> CD127 <sup>low</sup> T cells	EasySep <sup>®</sup> RoboSep <sup>®</sup> Human CD4 <sup>+</sup> CD127 <sup>low</sup> T Cell Enrichment Kit	19321
	CD4 <sup>+</sup> CD127 <sup>low</sup> CD45RO <sup>+</sup> T cells	EasySep <sup>®</sup> RoboSep <sup>®</sup> Human CD4 <sup>+</sup> CD127 <sup>low</sup> CD45RO <sup>+</sup> Regulatory T Cell Enrichment Kit	19322
Mouse	CD4 <sup>+</sup> CD25 <sup>+</sup> T cells	EasySep <sup>®</sup> Mouse CD4 <sup>+</sup> CD25 <sup>+</sup> Regulatory T Cell Isolation Kit	19782