# nature REVIEWS

# IMMUNOLOGY

# **Regulatory T cells**

# Ethan Shevach and Todd Davidson

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  $T_{Req}$  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<sub>Rea</sub> 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<sub>Rea</sub> cell function may facilitate the generation of tumour immunity or enhance responses to weak vaccines.



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Document #29957 | Version 1.2.0

# **Development and phenotype of regulatory T cells**

Many T cell types have immune regulatory function, but the two most important T<sub>Reg</sub> 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<sub>Reg</sub> cell function. However, functional activated human FOXP3<sup>+</sup> T<sub>Reg</sub> cells express a unique pattern of cell surface markers that can facilitate their isolation. A third important type of T<sub>Reg</sub> 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 ( $T_{H}$ 1) or  $T_{H}$ 2 cell subsets. Other T cell subpopulations including natural killer T (NKT) 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



Lethal infection,

highly polarized

Autoimmune

T<sub>µ</sub>1 cell response

microenvironment

T<sub>Reg</sub> cell <u>B cell-derived</u>

L-6.

L-1.

IL-23

IL-17

in vitro

-OXP3- T<sub>1</sub>17 cell

signals in

Peyer's patch

FOXP3

T<sub>г⊔</sub> cell

FOXP3

T<sub>µ</sub>2 cell

 $\bigcirc$ 

FOXP3

IL-4, IL-5

IL-21

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

FOXP3<sup>+</sup> T<sub>Reg</sub> cells have been shown to influence the outcome of immune responses in several tissues. For example, in the intestine T<sub>Reg</sub> 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<sub>Reg</sub> 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<sub>Req</sub> cells can inhibit the antitumour immune response, thereby preventing tumour clearance. During infection T<sub>Reg</sub> 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<sub>Reg</sub> 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.



## Plasticity in the periphery

Under certain conditions, FOXP3<sup>+</sup> T<sub>Reg</sub> cells can downregulate their expression of FOXP3, lose suppressor functions and manifest some of the functions of conventional effector  $T_{H}1$ ,  $T_{H}2$ ,  $T_{H}17$ and  $T_{FH}$  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<sub>Rea</sub> cell-specific deletion of certain transcription factors that are shared between

 $T_{Reg}$  cells and effector cell subsets (for example, the  $T_{H2}^{RC9}$  cell-specific factor IRF4) results in impaired suppression of  $T_{H}2$  cell responses by the  $T_{Reg}$  cells.

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

Markers shared by FOXP3 <sup>+</sup> T <sub>Reg</sub> cells and conventional activated CD4 <sup>+</sup> T cells (mice and humans)		Markers preferentially expressed by activated mouse FOXP3 <sup>+</sup> T <sub>Reg</sub> cells FOXP3 Latent TGFβ	Markers specifically expressed by activated human FOXP3 <sup>+</sup> T <sub>Reg</sub> cells FOXP3 <sup>hi</sup>
CD25	CD127 <sup>low</sup>	CD103	Latent TGF <sup>β</sup>
GITR	CTLA4		GARP
CD45RB <sup>low</sup> (mice only)		Subpopulations of human FOXP3 <sup>+</sup> T <sub>Reg</sub> cell	ls CD121a (IL-1R1)
CD45RO (humans only)		CD45RA <sup>+</sup> FOXP3 <sup>low</sup> (naive)	CD121b (IL-1R2)
Folate receptor 4 (mice only)		CD45RA <sup>-</sup> FOXP3 <sup>hi</sup> (activated) CD45RA <sup>-</sup> FOXP3 <sup>low</sup> (cytokine-secreting)	

T<sub>µ</sub>1 cell

FOXP3

FOXP3

IFNγ, IL-17, IL-21

IFNγ

# **Mechanisms of action of FOXP3<sup>+</sup> regulatory T cells**



#### **Maintaining intestinal homeostasis**



### **Promoting tumour progression**



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The authors declare no competing financial interests.

Edited by Lucy Bird and Kirsty Minton; copyedited by Gemma Ryan; designed by Simon Bradbrook.

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#### **STEMCELL** Technologies

#### **Isolation of Regulatory T Cells**

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 Treqs 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. STEMCELL Technologies has developed optimized Treg isolation kits that address these specific challenges.

#### Our EasySep<sup>™</sup> Human CD4<sup>+</sup>CD127<sup>low</sup>CD25<sup>+</sup> Regulatory T Cell Isolation Kit allows for fast and easy isolation of highly purified, magnetic particle-free Treqs in under an hour from PBMCs or leukapheresis samples. First, CD25<sup>+</sup> cells are isolated by column-free immunomagnetic positive selection using EasySep<sup>™</sup> Releasable RapidSpheres<sup>™</sup>. Then, bound magnetic particles are removed from the EasySep<sup>™</sup>-isolated CD25<sup>+</sup> cells, and unwanted non-Tregs are targeted for depletion. Isolated Tregs express high levels of FOXP3, and are suitable for immediate downstream experiments, including flow cytometry, in vitro expansion or suppression assays.

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Abbreviations for poster entitled Regulatory T cells by Ethan Shevach and Todd Davidson

 $A_{2A}$ R, adenosine receptor 2A; CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; CTL, cytotoxic T lymphocyte; CTLA4, cytotoxic T lymphocyte antigen 4; GARP, glycoprotein A repetitions predominant (also known as LRRC32); GITR, glucocorticoid-induced TNF-receptor-related protein; IDO, indoleamine 2,3-dioxygenase; IEC, intestinal epithelial cell; IRF4, interferon-regulatory factor 4; LAG3, lymphocyte activation gene 3; M cell, microfold cell; PBMC, peripheral blood mononuclear cell; R, receptor; RA, retinoic acid; TCR; T cell receptor; T<sub>FH</sub> cell, T follicular helper cell; TGFβ, transforming growth factor-β; TLR, Toll-like receptor; T<sub>R</sub>1 cell, inducible regulatory type 1 T cell.