Cellular & Molecular Immunology (2015) 12, 521–524

www.nature.com/cmi

RESEARCH HIGHLIGHT

The IL-33/ST2 axis is specifically required for development of adipose tissue-resident regulatory T cells

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Cellular & Molecular Immunology (2015) **12,** 521–524; doi:10.1038/cmi.2015.49; published online 17 August 2015

Currently, T cells dedicated to mediating immunosuppression and homeostasis are called regulatory T (Treg) cells. Treg cells are closely associated with infection, allergy, autoimmunity, tumor immunity, fetal–maternal tolerance, and organ transplantation. Treg cells exert their immunosuppressive functions by directly killing or inhibiting T cells and antigen-presenting cells (dendritic cells, macrophages, and B cells) and by producing suppressive cytokines such as TGF-β, IL-10, IL-35, and galectin-1. However, the classification and phenotypes of Treg cell subsets remain largely unclear. The discovery of tissue-resident Treg cells with transcriptional, phenotypic, and functional heterogeneity renders Treg cell subsets more enigmatic. Research regarding tissue-resident Treg cells has become a hot topic. Due to the particular distribution, distinct developmental process and antigenic specificity of various tissue-resident Treg cells, their roles also go beyond the canonical functions of the immune system in health and disease[1,2]. The best example is the visceral adipose tissue Treg (VAT-Treg) cell subset, which prevents obesity-associated inflammation and preserves insulin sensitivity and glucose tolerance; thus, this subset of cells is closely associated with obesity, type 2 diabetes and metabolic cardiovascular disease[3,4]. In a recent issue of *Nature Immunology*, Vasanthakumar *et al.* revealed a critical role and mechanism of the IL-33/ST2 axis in the development and maintenance of VAT-Treg cells[5].

The classification and phenotypes of Treg cell subsets are extremely complex and confusing. Listing all of the subsets that have been discussed in the literature is difficult. To recognize VAT-Treg cells better, the following basic information regarding how to group Treg cell subsets that have frequently appeared in published papers must be understood (Table 1).

$Foxp3^+$/$Foxp3^-$ Treg cells. Although Foxp3 (the transcription factor forkhead box P3) is also expressed by activated conventional T cells, Foxp3 is generally considered the most important marker of Treg cells and plays acritical role in the development and function of Treg cells. However, the presence of $Foxp3^-$ Treg cells such as $Foxp3^-$ type 1 regulatory T (Tr1) cells is also well known[6].

$CD4^+$/$CD8^+$ Treg cells. Treg cells can be divided into $CD4^+$ and $CD8^+$ subsets based on their expression. $CD4^+Foxp3^+$ Treg cells have been dominantly investigated; $CD8^+$ Treg cells are currently being addressed[7].

Regulatory natural killer T (NKTreg) cells and γδ Treg cells. NKTreg cells and Treg cells with γδ TCRs can exert suppressive functions in certain settings[8].

Natural Treg (nTreg) and induced Treg (iTreg) cells. Compared to nTreg (thymically derived or naturally occurring Treg) cells, iTreg cells are induced peripherally *via* post-thymic maturation. Both $Foxp3^-$ Tr1 cells and $Foxp3^+$ Th3 cells are included in iTreg cells[9].

Thymus-derived Treg (tTreg), peripherally derived Treg (pTreg), and induced Treg cells in cell culture (iTreg)[10]. tTreg and pTreg cells partly overlap with the above-described nTreg and iTreg cells.

Follicular Treg (T_{FR}) cells. $CD4^+Foxp3^-Bcl\text{-}6^+CXCR5^+ICOS^+CD19^-$ and $PD\text{-}1^+GITR^-$ follicular helper T (T_{FH}) cells are essential for germinal center formation and B-cell activation/differentiation into plasma cells. In contrast, the $CD4^+Foxp3^+Bcl\text{-}6^+CXCR5^+ICOS^+CD19^-$ and $PD\text{-}1^+GITR^+$ T_{FR} cells within the germinal center can inhibit T_{FH} cell function and naïve T- and B-cell activation[11].

Central Treg (cTreg), effector Treg (eTreg), and tissue-resident Treg cells. Drs. Liston and Gray classified the peripheral $CD4^+Foxp3^+$Treg cells into cTreg (also referring to as 'resting' or 'naive' Treg), eTreg (or activated Treg), and tissue-resident Treg cells[12]. The majority of Treg cells in the circulation and secondary lymphoid organs belong to the cTreg cell subset that expresses $CD62L^{high}CCR7^+$ or $CD45RA^{high}CD25^{low}$, while a minor fraction of the Treg cells are eTreg cells that express $CD62L^{low}CCR7^{low}CD44^{high}$ $CD103^+KLRG1^+$ (killer cell lectin-like receptor subfamily G member 1-positive) or $CD45RA^{low}CD25^{high}$. The transcription factor Blimp-1 (B lymphocyte-induced maturation protein-1) is a key player in late B-cell and conventional

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Received: 9 May 2015; Accepted: 11 May 2015

Table 1 Enigmatic classification and phenotypic heterogeneity of regulatory T cell subsets

Treg cell subset	Development	Major phenotype	Effector
Foxp3$^+$/Foxp3$^-$ Treg	Variety	Foxp3$^+$… or Foxp3$^-$…	Variety
CD4$^+$/CD8$^+$ Treg[7]	Variety	CD4$^+$Foxp3$^{+/-}$… or CD8$^+$Foxp3$^{+/-}$…	Variety
NKTreg and γδ-Treg[8]	Variety	Markers of NKT or γδ-T cells	IL-10/TGF-β
nTreg/iTreg[9]			
nTreg (natural Treg)	In thymus	CD4$^+$Foxp3$^+$CD25$^{int/hi}$, CD127^{lo}CTLA4$^+$GITR$^+$	IL-10/TGF-β Direct contact Granzyme B
iTreg (induced Treg)			
Tr1	In periphery	CD4$^+$Foxp3$^-$CD25$^-$, CD25$^{lo\text{-}var}$CD45RB^{lo}	IL-10
Th3	In periphery	CD4$^+$Foxp3$^+$CD25$^+$, CD25$^{lo\text{-}var}$CD45RB^{lo}	TGF-β
tTreg/pTreg/iTreg[10]			
tTreg (thymus-derived)	In thymus	CD4$^+$Foxp3$^+$…	Variety
pTreg (peripherally derived)	In periphery	CD4$^+$Foxp3$^+$, from Foxp3$^-$ T-conventional cells	Variety
iTreg (induced in cell culture)	In cell culture	CD4$^+$Foxp3$^+$…	Variety
T_{FR} (follicular Treg)[11]	In germinal center	CD4$^+$Foxp3$^+$Bcl-6$^+$CXCR5$^+$ICOS$^+$CD19$^-$, PD-1$^+$GITR$^+$	Direct contact IL-10/TGF-β
cTreg/eTreg/tissue-resident Treg[12]			
cTreg (central Treg)	Located in circulation and secondary lymphoid organs	CD4$^+$Foxp3$^+$Blimp-1$^-$CD62L^{hi}CCR7$^+$, CD45RA^{hi}CD25^{lo}	Variety
eTreg (effector Treg)	Located in circulation, secondary lymphoid organs, various tissues	CD4$^+$Foxp3$^+$Blimp-1$^+$CD62L^{lo}CCR7^{lo}, D44^{hi}CD103$^+$KLRG1$^+$, CD45RA^{lo}CD25^{hi}	Variety
Tissue-resident Treg[1,2]	Located in various tissues	Phenotype of eTreg …	Variety

T-cell differentiation. Blimp-1 is also expressed on all eTreg cells and is required for IL-10 production. The phenotypes of Blimp-1$^+$CD62L^{low}CCR7^{low} and Blimp-1$^-$CD62L^{high}CCR7$^+$ are thereby recognized as markers for distinguishing eTreg from cTreg cells[5,13], whereas Blimp-1$^+$ eTreg cells are derived from Blimp-1$^-$ cTreg cells in response to specific stimuli[13]. Treg cells that reside in non-lymphoid tissues for long-term are characterized as tissue-resident Treg cells. Most tissue-resident Treg cells dominantly express the phenotypes of eTreg cells[1,5]. However, tissue-specific factors that promote the development of specialized tissue-resident eTreg cells from cTreg cells in each tissue need to be further determined.

IL-33 plays a crucial role in inflammatory, allergic, infectious, and autoimmune diseases. IL-33 binding to its receptor, which is composed of IL-33Rα (ST2) and IL-33Rβ (IL-1RAcP), is involved in the differentiation and functions of various immunocytes including Th2 cells, CD8$^+$ T cells, group 2 innate lymphoid cells (ILC2), NK cells, mast cells, basophils, and eosinophils. Recently, IL-33 was shown to be essential for VAT-Treg cell development and maintenance[5], thereby linking IL-33 to obesity and associated diseases. As outlined in Figure 1, CD4$^+$Foxp3$^+$Treg cells were grouped into three subsets of cTreg, eTreg, and tissue-resident Treg cells, and how the IL-33/ST2 axis specifically controls VAT-Treg cell development was revealed via three major approaches[5].

In the first approach (Figure 1a), RNA sequencing (RNA-seq) confirmed that the transcriptional profiles of cTreg and eTreg cells from spleen and lymph nodes of *Blimp1*^{GFP} reporter mice are distinct from each other. The differential expression of some crucial molecules on eTreg and VAT-Treg cells was also verified by flow cytometric analyses. Typically, PPAR-γ (the known transcription factor essential for VAT-Treg cell development)[14], ST2 and IL-10 are markedly expressed by VAT-Treg cells.

In the second approach (Figure 1b), wild-type (WT), ST2-deficient (*Il1rl1*$^{-/-}$), IL-33-deficient (*Il33*$^{-/-}$), Myd88-deficient (*Myd88*$^{-/-}$), BATF-deficient (*Batf*$^{-/-}$) and IRF4-deficient (*Irf4*$^{-/-}$) mice were used to reveal the crucial roles of these molecules in VAT-Treg cell development and how PPAR-γ and ST2 expression was upregulated. IL-33 is known to signal through the adaptor protein MyD88, while IRF4 and BATF are T cell-associated transcriptional regulators. Compared to WT mice, the deficiencies of ST2, IL-33, Myd88, BATE, and IRF4 greatly reduced VAT-Treg cells, while the numbers of cTreg or eTreg cells in spleen, lymph nodes, and some other tissues were moderately affected or unaffected. Sufficient and convincing data have demonstrated that (i) the IL-33/ST2 axis is specifically required for VAT-Treg cell proliferation and differentiation both *in vitro* and *in vivo*, (ii) VAT-Treg cell differentiation requires MyD88-mediated signaling downstream of IL-33, and (iii) BATF and IRF4 are indispensable transcriptional regulators for VAT-Treg cells to express PPAR-γ and ST2. As outlined in Figure 2, TCR stimulation *via* a specific antigen *in vivo* or *via* anti-CD3/anti-CD28 *in vitro* induced BATF and IRF4 expression, which triggered PPAR-γ and ST2 expression by simultaneously binding *Pparg* (encoding PPAR-γ) and *Il1rl1* (encoding ST2) loci. The requirement for TCR signalling in VAT-Treg cell development was also

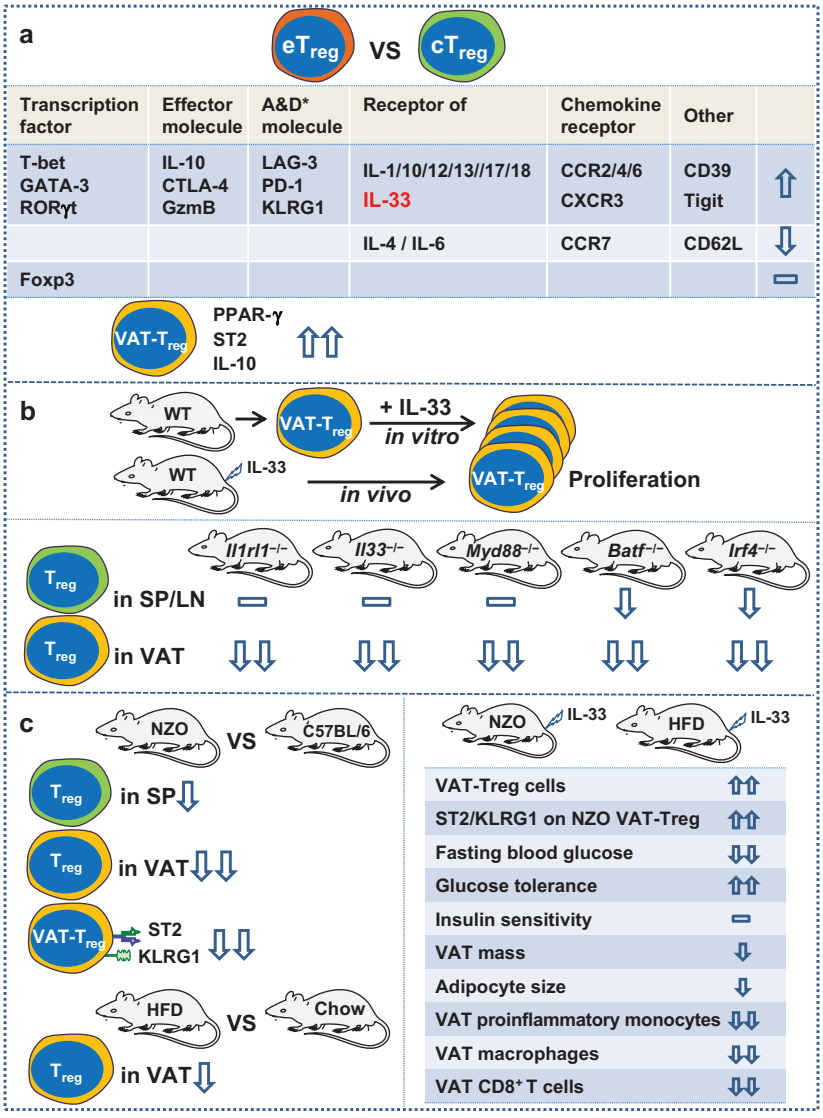

Figure 1 The major experimental approaches revealing the specific requirement of the IL-33/ST2 axis for VAT-Treg cell development. Cells: cTreg, central Treg; eTreg, effector Treg; VAT-Treg, visceral adipose tissue Treg. Mice: WT, wild-type; *Il1rl1*$^{-/-}$, ST2-deficient; *Il33*$^{-/-}$, IL-33-deficient; *Myd88*$^{-/-}$, Myd88-deficient; *Batf*$^{-/-}$, BATF-deficient; *Irf4*$^{-/-}$, IRF4-deficient; NZO; New Zealand obese; Chow, fed with normal chow; HFD, high fat diet-induced obese mouse. *A&D molecules, activation- and differentiation-associated molecules; GzmB, granzyme B; SP, spleen; LN, lymph nodes; CD39, ectonucleotidase; Tigit, co-inhibitory molecule.

confirmed by detecting the expression of Nur77 and TCF7, two transcription factors that are up- and downregulated, respectively, in response to TCR signaling.

In the third approach (Figure 1c), the crucial role of IL-33 in VAT-Treg cell development and maintenance was further tested by IL-33 administration into New Zealand obese (NZO, genetically obese) mice and high fat diet-induced obese mice (HFD mice). Compared to control C57BL/6 mice, NZO mice displayed early onset of obesity and hyperglycemia, as well as severely reduced numbers of VAT-Treg cells[14,15]. The VAT-Treg cells from NZO mice expressed extremely low levels of ST2 and KLRG1. Compared to control mice fed with normal chow, HFD mice also displayed a reduced number of VAT-Treg cells and abnormal metabolic parameters. IL-33 administration in NZO and HFD mice rescued VAT-Treg cell numbers, restored ST2 and KLRG1 expression on NZO VAT-Treg cells, improved metabolic parameters including fasting blood glucose concentration and glucose tolerance, decreased VAT mass and adipocyte size, and suppressed the obesity-associated inflammation in VAT with reduced VAT proinflammatory monocytes, macrophages, and CD8$^+$ T cells. However, IL-33 administration did not affect insulin sensitivity in NZO and HFD mice.

Taken together, the three approaches outlined above demonstrate that the IL-33/ST2 axis plays an essential role in the development of VAT-Treg cells in both the mouse and human. These findings provide new insight into the differential mechanism of specialized Treg cell subsets. Thus, IL-33 has potential future uses in treating obesity and obesity-associated metabolic cardiovascular

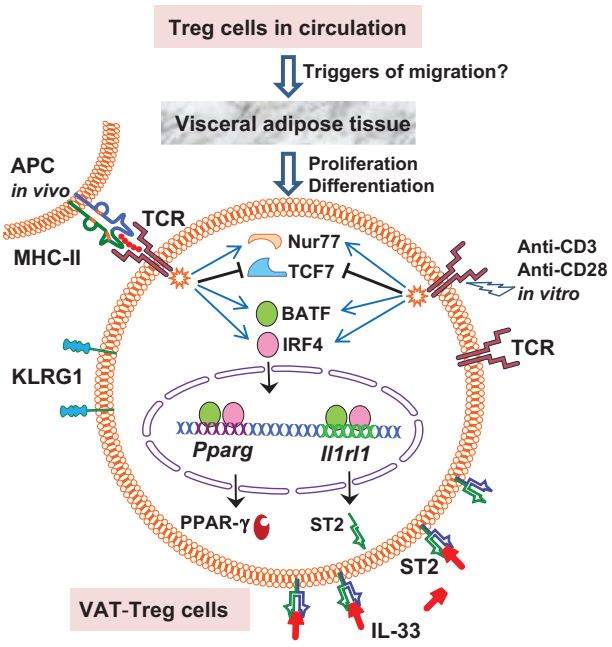

Figure 2 Crucial roles of TCR signals, the transcriptional regulators BATF and IRF4, and the IL-33/ST2 axis in VAT-Treg cell proliferation and differentiation from cTreg or eTreg cells. APC, antigen-presenting cells; *Pparg* and *Il1rl1*, encoding PPAR-γ and ST2, respectively; Nur77 and TCF7, transcription factors.

diseases. Further studies are required to determine the triggers of cTreg or eTreg cell migration into adipose tissue. The antigenic specificity of VAT-Treg cells remains to be further investigated. In addition, clarifying the causality between obesity and the reduced Treg cell number in adipose tissue and uncovering the underlying mechanisms are important. Whether obesity-associated inflammation results in the reduced VAT-Treg cell number in adipose tissue or whether the reduction of VAT-Treg cell number causes the obesity-associated inflammation remains to be determined.

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