BTLA$^+$ Dendritic Cells: The Regulatory T Cell Force Awakens

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The role of dendritic cells (DCs) in the induction of antigen-specific tolerance mediated by extrathymic regulatory T (Treg) cells remains incompletely defined. In this issue of *Immunity*, Jones et al. (2016) show that BTLA$^+$DEC205$^+$CD8$^+$CD11c$^+$ DCs efficiently induce peripheral Treg cells via the engagement of HVEM, a receptor for BTLA.

A long time ago, in the periphery far, far away from the thymus, it was described that in absence of pro-inflammatory signals, antigen presentation by dendritic cells (DCs) induced T cell tolerance. DCs are highly heterogeneous, comprised of many distinct subsets, with some subpopulations having specialized suppressive or tolerogenic functions. Indeed, in the steady state, antigen delivery to DEC205$^+$ DCs is an efficient approach to induce peripheral tolerance (Hawiger et al., 2001) and demonstrates that DEC205$^+$ DCs possess tolerogenic properties.

Peripheral T cell tolerance is maintained by several distinct mechanisms including deletion, anergy, and extrinsic suppression by regulatory T cells. Indeed, the conversion of T cells by DCs into FoxP3$^+$ expressing regulatory Treg cells is one of the most powerful tolerogenic mechanisms in the periphery. Different mechanisms involved in this conversion have been described, from secretion by DCs of soluble factors such as transforming growth factor-β (TGF-β) or interleukin-10 (IL-10) to expression of tolerogenic molecules such as programmed death ligand-1 (PDL-1) or indoleamine 2, 3 deoxygenase (IDO). However, it is likely that other molecular interactions contribute to the generation of antigen-specific Foxp3$^+$ Treg cells in vivo. Further, the definition of which steady-state DC populations or other DC populations contribute to Treg cell induction remains incompletely defined.

In a first episode in 2004, Hawiger et al. demonstrated that targeting an encephalitogenic oligodendrocyte peptide (MOG) to DEC205$^+$ DCs induces peripheral T cell tolerance that prevents autoimmune experimental acute encephalomyelitis (EAE) (Hawiger et al., 2004). Tolerance was dependent on DC induction of CD5 expression by activated T cells. The specific role of CD5 in peripheral Treg cells was unclear until the authors demonstrated in a second episode (Henderson et al., 2015) that CD5 is required for induction of antigen-specific Treg cells.

Although these two studies identified DEC205$^+$ DCs as a major antigen-presenting cell population in the induction of Treg cells and highlighted the role of CD5 in peripheral Treg cell development, the precise subpopulation of DEC205$^+$ DCs and the molecular mechanisms by which DEC205$^+$ DCs induced CD5 on Treg cells was still unknown.

In this last episode, Jones et al. (2016) addressed these two important questions. They first demonstrated that tolerance was achieved in the experimental autoimmune encephalomyelitis (EAE) model when myelin oligodendrocyte glycoprotein (MOG) peptide was delivered with a recombinant chimeric antibody to DEC205$^+$ DCs, but not when antigen was targeted to all CD11c$^+$ DCs. Further, de novo conversion of FoxP3$^+$ Treg cells from MOG-specific T cells was observed only when MOG antigen was targeted to DEC205$^+$ DCs. Similar results were obtained with OVA antigen and OVA-specific T cells, demonstrating that Treg cell induction by DEC205$^+$ DCs was not restricted to a specific T cell receptor (TCR). Therefore, although the vast majority of DCs are CD11c$^+$, only a distinct subset of DCs could induce Treg cells, and the Treg cell-inducing effect was not dominant since targeting to all DCs was ineffective. In fact, antigen delivery to all CD11c$^+$ DCs not only failed to differentiate T cells into Treg cells but also failed to convert T cells into effector T helper 1 (Th1) or Th17 cells, indicating a transient T cell unresponsiveness that failed to alter a subsequent antigenic challenge. These results suggest that other CD11c$^+$ DC subsets may contribute to tolerance via anergy or agnosia but not via active suppression by Treg cell induction.

To identify precisely the DEC205$^+$ DC population that preferentially induced Treg cells, the authors used *Batf3*$^{-/-}$ mice, characterized by a reduced number of DEC205$^+$CD8$^+$ DCs. In these mice, targeting antigen to DEC205$^+$ DCs failed to induce a high proportion of Treg cells. Conversely, in *Irf4*$^{-/-}$ mice, where DEC205$^+$CD8$^+$ DCs are increased, antigen delivery to CD11c$^+$ DCs was associated with a higher proportion of Treg cells. The authors concluded that Treg cell conversion in the periphery was directly linked to the proportion of DEC205$^+$CD8$^+$ DCs among all CD11c$^+$ DCs.

Importantly, B- and T-lymphocyte attenuator (BTLA), an immunoglobin domain superfamily protein, is specifically

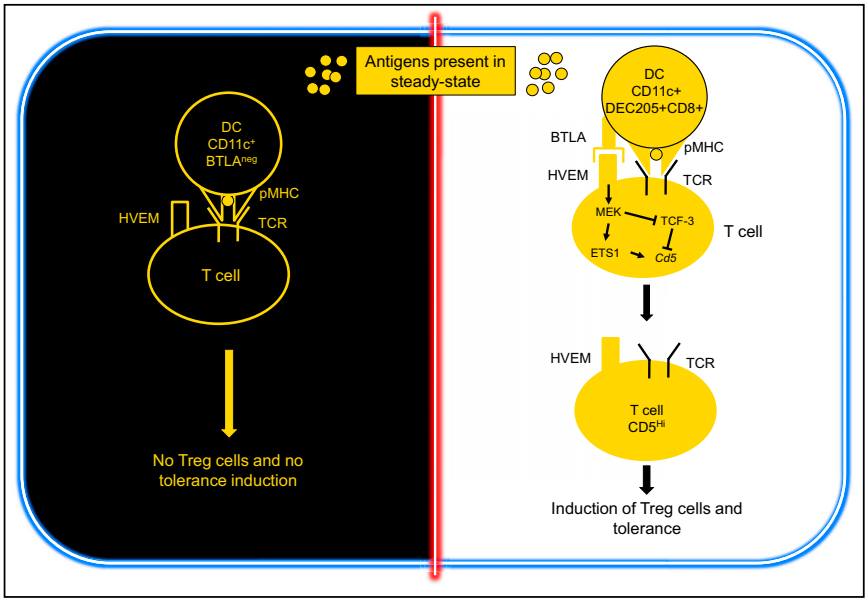

Figure 1. BTLA$^+$ DCs Promote CD5 Expression on T Cells that Instruct Treg Cell Differentiation

Antigen presentation in steady state by BTLA$^-$CD11c$^+$ DCs fails to upregulate CD5 expression in T cells and does not alter the subsequent immune response (left). BTLA$^+$DEC205$^+$CD8$^+$CD11c$^+$ DCs specifically mediate the upregulation of CD5 expression in T cells via HVEM engagement and instruct Treg cell differentiation (right). HVEM engagement increases phosphorylation of MEK. MEK increases the expression of the *Cd5*-positive regulator ETS1 and inhibits the expression of the *Cd5*-negative regulator TCF-3.

expressed in DEC205$^+$CD8$^+$ DCs. Therefore the authors speculated that DCs required BTLA to induce Treg cells. Indeed, an anti-BTLA blocking antibody that prevents BTLA binding to its ligand, the herpes virus entry mediator (HVEM), dramatically decreased Treg cell conversion. Similarly in *Btla*$^{-/-}$ recipient mice, Treg cell induction was reduced. Anti-BTLA abrogated DC-induced tolerance in the EAE model. BTLA expression by the DEC205$^+$ DC population was required for T cell upregulation of CD5 that preceded FoxP3 induction. Cross-linking of HVEM also upregulated surface expression of CD5 on naive, FoxP3$^-$ T cells. HVEM engagement increased phosphorylation of mitogen-activated protein kinase (MAPK) kinase (MEK) that was associated with increased expression of the positive regulator of *Cd5* expression (ETS1) and the downregulation of TCF-3, a negative transcriptional regulator of *Cd5* (Figure 1). Finally, engagement of HVEM maintained Treg cell conversion even in the presence of IL-4 and IL-6, and this effect was dependent on CD5.

The role of CD5 in Treg cell induction and maintenance is intriguing in the present context. CD5 is a transmembrane glycoprotein and a member of the highly conserved superfamily of scavenger receptor cysteine-rich (SRCR). The nature of the endogenous CD5 ligands is still an open question and so the physiological role of CD5 ligation remains an active area of investigation. CD5 is expressed on all T cells and is a well-established negative regulator of TCR signaling. In naive peripheral T cells, CD5 expression is induced by TCR signaling. CD5^{hi} T cells express TCR that bind with higher affinity to self-peptide-major histocompatibility complex molecules (Smith et al., 2001), and high CD5 surface expression identifies Treg cells (Josefowicz et al., 2012). Henderson et al. (2015) showed that iTreg cell induction from CD5^{hi} polyclonal T cells is more efficient than from CD5^{lo} T cells and that CD5^{hi} Treg cells are refractory to counter regulatory signals mediated by effector cytokines such as interleukin-4 (IL-4), IL-6, and interferon-γ (IFN-γ). Interestingly, inhibition of the nutrient sensor mTOR restores induction of Treg cells in the absence of CD5, suggesting that CD5 regulates cytokine activation of the mTOR pathway.

In this study, Jones et al. (2016) identified the BTLA-HVEM axis as a major mechanism for induction of peripheral Treg cells in the steady state. BTLA and HVEM are expressed by T lymphocytes, B lymphocytes, and antigen-presenting cells. Previous studies have demonstrated a tolerogenic role for BTLA expressed by T cells rather than by DCs. Thus, engagement of BTLA on the T cell surface with HVEM or with agonistic antibody is associated with lower T cell activation and favors Treg cell induction (Uchiyama et al., 2014). Moreover, BTLA deficiency in T cells renders mice resistant to oral tolerance and increases susceptibility to EAE. It is also important to note that the BTLA-HVEM pathway is not limited to inhibitory signaling in T lymphocytes, as it may enhance T cell effector function (Steinberg et al., 2013), and BTLA$^+$CD8$^+$ DCs deliver positive signals through HVEM expressed on T cells (Flynn et al., 2013). Therefore, BTLA-HVEM signals and functions are highly complex and probably context dependent. Thus, in other inflammatory settings, other tissues, or with other DC subsets, this molecular interaction may or may not be germane for Treg cell induction.

The present report highlights important questions for future investigations. The protection induced by BTLA$^+$ DCs is demonstrated here in an EAE model, though it is not known whether such specificity for protective effects is also observable with other disease models, other tissues, or other inflammatory contexts. It was demonstrated previously that antigen delivery in the steady state to langerin$^+$DEC205$^+$ migratory DCs induced antigen-specific Treg cells more potently than lymphoid-resident DCs (Idoyaga et al., 2013). However, the contribution of BTLA and relationship of that DC subset to the one described here is unknown. In the present study, the analysis of BTLA expression and the ex vivo co-cultures were performed with CD8$^+$DEC205$^+$ DCs obtained from either lymph nodes or the spleen (which lacks migratory DCs), so the origin, the relative distribution of splenic- or lymph node-resident DEC205$^+$CD8$^+$ DCs, and their respective contribution to Treg cell induction are not yet resolved.

The report by Jones et al. (2016) defines BTLA$^+$DEC205$^+$CD8$^+$ DCs as a new tolerogenic antigen-presenting cell subpopulation. These cells may offer new hope for the induction of peripheral tolerance in

immune-mediated diseases that can fight the dark side of pathogenic T cell troopers and prevent them from striking back.

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HIV Broadly Neutralizing Antibodies: Taking Good Care Of The 98%

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In this issue of *Immunity*, Huang et al. (2016) describe an exceptionally broad and potent neutralizing antibody to HIV. This antibody, N6, is capable of neutralizing up to 98% of global isolates with a potent median IC_{50} of 0.04 μg/mL, making it the current "best-in-class" for bNAbs targeting the CD4 binding site.

Developing an HIV vaccine is among the most difficult challenges in modern medicine. The challenge is due, in part, to the virus being a molecular escape artist, capable of continually generating mutants that are resistant to immune responses. In addition to this high molecular diversity, the HIV surface spikes, which are the sole target of protective neutralizing antibodies, are coated in a high density of self-sugars that derive from the host glycosylation machinery and are therefore poorly recognized by the immune system. In this tug of war between the human immune system and the virus, antibodies must not only pursue escape viruses, but must also puncture through the glycan shield to reach the protein surface of the spikes (Burton and Hangartner, 2016).

Despite the formidable defenses, prolonged exposure to HIV in some infected individuals results in the generation of antibodies that meet both of the challenges of epitope variability and glycan shielding. These are the so-called broadly neutralizing antibodies (bNAbs), which are able to recognize a diversity of HIV isolates. Biochemical, structural, and virological studies have revealed that bNAbs recognize relatively conserved regions on HIV Env, avoid variable regions, and accommodate, avoid, and/or directly bind sugars that surround the critical epitopes.

In the study presented by Huang et al. (2016) in this issue of *Immunity*, the authors describe an antibody called N6, which is a new member of the VRC01 class of bNAbs that target the CD4 binding site of gp120 subunits of the HIV surface spike. VRC01-class antibodies characteristically derive from the V_H1-2*02 heavy chain gene and harbor a rare L-CDR3 that is five amino acids in length (Zhou et al., 2015). The V_H1-2*02 heavy chain mimics CD4 in how it binds the receptor binding site, which explains the generally high neutralization breadth of this class of antibodies, hovering between 80% and 90% of global isolates. Although extraordinary in neutralization breadth, this class of antibodies typically exhibits modest potency as compared to bNAbs targeting some other epitopes on the surface spike. N6, however, is exceptional: this monoclonal antibody neutralizes up to 98% of a large panel of global isolates of different subtypes or clades at a median IC_{50} of 0.04 μg/mL (Figure 1). Such high potency rivals bNAbs such as PGT121 and PGT145, which target the V3-glycan and V2-apex epitopes at the top of the surface spike and are known for being exceptionally potent (Walker et al., 2011). Importantly, N6 also shows minimal autoreactivity by a number of measurements, favoring this antibody for use in therapeutic or prophylactic modalities. On the basis of this