Journal of Autoimmunity 75 (2016) 39–49

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

IDO-orchestrated crosstalk between pDCs and Tregs inhibits autoimmunity

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ARTICLE INFO

Article history:
Received 17 May 2016
Received in revised form
7 July 2016
Accepted 10 July 2016
Available online 26 July 2016

Keywords:
Regulatory T cells
Plasmacytoid dendritic cells
Indoleamine 2,3-dyoxygenase
Antigen presentation
Tolerance
Experimental autoimmune encephalomyelitis

ABSTRACT

Plasmacytoid dendritic cells (pDCs) have been shown to both mediate and prevent autoimmunity, and the regulation of their immunogenic versus tolerogenic functions remains incompletely understood. Here we demonstrate that, compared to other cells, pDCs are the major expressors of Indoleamine-2,3-dioxygenase (IDO) in steady-state lymph nodes (LNs). IDO expression by LN pDCs was closely dependent on MHCII-mediated, antigen-dependent, interactions with Treg. We further established that IDO production by pDCs was necessary to confer suppressive function to Tregs. During EAE development, IDO expression by pDCs was required for the generation of Tregs capable of dampening the priming of encephalitogenic T cell and disease severity. Thus, we describe a novel crosstalk between pDCs and Tregs: Tregs shape tolerogenic functions of pDCs prior to inflammation, such that pDCs in turn, promote Treg suppressive functions during autoimmunity.

1. Introduction

Plasmacytoid dendritic cells (pDCs) are important sensors of non-self-nucleic acids derived from bacteria or viruses and are crucial mediators of innate anti-microbial responses through the production of inflammatory cytokines and type-I IFNs [1,2]. In addition, pDCs have been implicated in the development of several autoimmune diseases, including lupus, psoriasis, multiple sclerosis (MS) and type-1 diabetes [3–7]. Following abnormal release of self-DNA during inflammatory processes, pDCs are activated through TLR signalling and subsequently produce type-I IFN [8]. Importantly, a few years ago, the notion emerged that pDCs act not only as innate sensors but can also function as *bona fide* antigen (Ag) presenting cells (APCs) and directly impact T cell responses [9]. It was shown that pDCs capture and process Ags [10], and load antigenic peptides onto MHC class I (MHCI) [11] and MHC class II (MHCII) molecules [12–14]. The modulation of Ag-presenting pDC functions led to important consequences on T cell immunity, the outcome being highly dependent on the cytokine microenvironment [15].

Many studies, including those investigating oral tolerance and allograft models, suggest that steady-state Ag-presenting pDCs exclusively promote T cell tolerance [16–18]. Although the nature of the factors controlling distinct pDC functions remains to be established, once activated, pDCs exhibit both immunogenic and tolerogenic functions. For example, using mice exhibiting a specific loss of MHCII expression by pDCs, we showed that CpG-B activated

Abbreviations used: Ag, antigen; cDCs, conventional dendritic cells; BM, bone marrow; CNS, central nervous system; cTECs, cortical thymic epithelial cells; dLN, draining lymph node; EAE, experimental autoimmune encephalomyelitis; IDO, indoleamine-2,3-dyoxygenase; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; pDCs, plasmacytoid dendritic cells.

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http://dx.doi.org/10.1016/j.jaut.2016.07.004

pDCs present Ag and promote effector Th17 cell differentiation, a property that can be exploited for anti-tumor vaccines [19]. Pro-pathogenic Ag-presenting pDCs were similarly described in a mouse model of atherosclerosis in which pDCs induced pathogenic Th1 cells [20]. In addition, BST-2 mediated specific Ag delivery to CpG-activated pDCs led to cytotoxic T lymphocyte (CTL) and Th1 cell differentiation and triggered protective immunity against viral infection and tumor growth [21]. In contrast, in the context of EAE, Ag targeting to pDCs via Siglec-H promoted $CD4^+$ T cell anergy and inhibited CNS inflammation [22]. We previously demonstrated that in EAE, pDCs present myelin Ags on MHCII molecules to induce the expansion of suppressive Tregs, a phenomenon correlated with disease amelioration [23].

Indoleamine 2,3-dioxygenase (IDO) is an immunomodulatory enzyme involved in the initial and the rate-limiting step of tryptophan catabolism. Upon inflammation, IDO production has been shown to compromise T cell proliferation, promote T cell anergy and Tregs [24–26]. Depending on the experimental context, IDO can be induced either by IFN-γ, IFN-α/β, or TGF-β. CTLA-4 binding to cell-surface expressed costimulatory molecules promotes IDO production by pDCs through IFN-γ or IFN-α/β signalling [27–30]. Furthermore CD200-Ig binding to his cognate receptor induces IDO in an IFN-α/β dependent signalling pathway [31]. Both IFN-γ and IFN-α/β pathways result in IDO^+ immunosuppressive effects which are closely dependent on the catalytic activity of the enzyme.

CTLA-4-binding also promote IDO in tumor contexts, but the enzyme has reveal activity in only a minor DC subpopulation expressing the marker CD19, but none of the pDC classical markers [32,33]. IDO enzymatic functions in tumor dLN-sorted pDCs have been correlated to *in vitro* Treg differentiation and suppressive functions [24,34]. More recently, Pallotta and colleagues described that IDO^+ pDCs induced long-lived Tregs by using a TGF-β-dependent pathway distinct from the catalytic activity of the enzyme. In mouse a model of skin delayed-type hypersensitivity, they shown that whereas IFN-γ-dependent IDO enzymatic activity in pDCs leads to T cell anergy, TGF-β induced IDO phosphorylation results in increased Treg frequencies [35].

It is so far unknown whether IDO expression in naïve pDCs pre-exists, and how it would be regulated in steady-state LNs. In contrast, recent work has implicated IDO expression in pDC immunoregulatory functions, including Treg induction, in inflamed LNs. Furthermore, IDO production by tumor-associated pDCs has been correlated to *in vitro* Treg-mediated suppression. However, the nature of the cells expressing IDO, as well as the impact on Treg functions in chronic inflammatory diseases, such as autoimmune disorders, remain undetermined.

Here we show that in steady-state lymph nodes (LNs), IDO is highly expressed by pDCs compared to other LN resident cells. We further established that IDO expression is positively regulated in steady-state pDCs following MHCII-mediated interactions with Tregs. During autoimmune disorders, such as EAE, IDO expression by MHCII competent pDCs is mandatory to confer suppressive functions to pDC-induced Tregs. IDO-competent Ag-presenting pDCs promote Tregs that inhibit autoimmune effector T cell responses in LNs, resulting in reduced disease severity. Therefore, we have identified a bidirectional interaction between pDCs and Tregs that favours self-tolerance.

2. Materials and methods

2.1. Mice

All mice had a pure C57BL/6 background and were bred and maintained under SPF conditions at Geneva medical school animal facility and under EOPS conditions at Charles River, France or at the National Institutes of Health, Bethesda, US. DEREG [36], Ubiquitin-eGFP [37], pIII + $IV^{-/-}$ [38], $IDO^{-/-}$ [39], BDCA2-DTR [40], MARILYN $Rag2^{-/-}$, OTII $Rag2^{-/-}$ [41], AND $Rag2^{-/-}$ [42], SMARTA $Rag1^{-/-}$ [43], $Rag2^{-/-}$, Scurfy [44], CD45.1 (Charles River, France), and 2D2 [45] mice have been previously described. WT C57BL/6 mice were purchased from Harlan laboratories (France) or Taconic (US). All procedures were approved by and performed in accordance with the guidelines of the animal research committee of Geneva or of the NIH.

2.2. Generation of BM chimeric mice

BM chimeric mice were generated as described [19]. Briefly, BM cells were recovered from tibia and femurs of donor mice. 5 to 7×10^6 cells were injected intravenously into sub-lethally irradiated recipient mice (two consecutive doses of 450 cGy). Reconstitution was assessed by analysing blood cells by flow cytometry after 6–8 weeks. For mixed BM chimeras, CD45.2 WT eGFP and CD45.2 pIII + $IV^{-/-}$ BM cells were simultaneously transferred into irradiated CD45.1 WT recipient mice in a 1:1 ratio.

2.3. EAE experiments

Active EAE was induced by immunizing mice, subcutaneously in both flanks, with 100 μg of MOG_{35-55} peptide (MEVGWYRSPFSRVVHLYRNGK, Biotrend) emulsified in incomplete Freund's adjuvant (BD Diagnosis) supplemented with 500 μg/ml *Mycobacterium tuberculosis* H37Ra (BD Diagnosis). At the time of immunization and 48 h later, mice also received 300 ng of pertussis toxin (Sigma-Aldrich) into the tail vein. Mice were monitored daily for disease clinical symptoms, and blindly scored as follows. 1, flaccid tail; 2, impaired righting reflex and hind limb weakness; 3, complete hind limb paralysis; 4, complete hind limb paralysis with partial fore limb paralysis; 5, moribund.

For passive EAE induction, encephalitogenic $CD4^+$ T cells were generated *in vitro* from LN and spleen cells of 2D2 mice as described [46]. $1–2 \times 10^6$ total cells were injected *i.p.* into recipient mice. Mice received 67 ng of pertussis toxin at the day of cell injection and 48 h later. Mice were monitored daily for disease clinical symptoms as described above.

Adoptive transfers of Treg cells were performed as follows. WT → WT, pIII + $IV^{-/-}$ → WT and $IDO^{-/-}$ → WT chimeric mice were immunized or not with MOG_{35-55} + CFA. $CD4^+$ $CD25^{hi}$ T cells were harvested from total skin LNs (naïve) or dLNs (day 10 after EAE immunization) and $1–5 \times 10^5$ $CD4^+$ $CD25^{hi}$ T cells were injected intravenously into tail vein of recipient mice. EAE was induced by active immunization the day after Treg transfer. In some experiments, CD45.1 mice were used as recipients, and Treg migration was assessed in dLNs at day 3 and in dLNs and SC at day 15 after EAE immunization.

Adoptive transfers of pDCs were performed as follows. $1.2–1.5 \times 10^6$ BM derived pDCs loaded with 10 μg/mL of MOG_{35-55} were injected intravenously into tail vein of recipient mice, and EAE was induced by active immunization the day after.

In some experiments, EAE mice were treated *i.p.* at indicated time points with DT (100 ng/mouse for BDCA2-DTR and 1 μg/mouse for DEREG).

2.4. Ex vivo cell isolation

Treg cells were isolated from total skin LNs of naïve mice or from dLNs of EAE mice (day 10 after immunization). LNs were scratched and LN cells were subjected to $CD4^+$ T cell enrichment using $CD4^+$ T cell isolation kit (Miltenyi biotec). $CD4^+$ $CD25^{hi}$ Treg cells were next sorted using a MoFlowAstrios (Beckman Coulter).