Human Immunology 70 (2009) 313-320

Contents lists available at ScienceDirect

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

Allostimulatory activity of bone marrow-derived plasmacytoid dendritic cells is independent of indoleamine dioxygenase but regulated by inducible costimulator ligand expression

Bridget L. Colvin [a], Tina L. Sumpter [a], Daisuke Tokita [a], Jennifer Salati [a], Andrew L. Mellor [b], Angus W. Thomson [a,c,*]

[a] *Starzl Transplantation Institute and Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213, USA*
[b] *Immunotherapy Center, Medical College of Georgia, Augusta, Georgia 30912, USA*
[c] *Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213, USA*

ARTICLE INFO

Article history:
Received 6 January 2009
Accepted 29 January 2009
Available online 3 February 2009

Keywords:
Dendritic cells
Indoleamine dioxygenase
T cells
DNAX-activating protein of 12 kDA
Inducible costimulator ligand

ABSTRACT

We investigated the role of two key immunoregulatory molecules, indoleamine dioxygenase (IDO) and inducible costimulator ligand (ICOSL), in determining the function of bone marrow (BM)-derived plasmacytoid (p)DC, which offer the potential for therapy of allograft rejection. pDC generated from BM of wild-type (WT) or IDO knockout (KO) C57BL/6 mice were used to stimulate T-cell proliferation and interferon-γ (IFN-γ) production in response to alloantigen (alloAg) via the direct or indirect pathways. In some experiments, pDC were first activated by exposure to CpG $\pm$ CTLA4Ig for IDO induction via B7 ligation. Although IDO KO pDC induced enhanced T-cell responses compared with WT pDC, the use of the IDO inhibitor 1-methyltryptophan (1-MT) demonstrated that the inferior stimulatory capacity of WT pDC was not caused by the production of functional IDO, even under IDO-inducing conditions. The DNAX-activating protein of 12 kDa (DAP12), which inhibits functional IDO expression, was expressed in BM-pDC. DAP12 silencing increased the T-cell stimulatory capacity of WT pDC, but only in the presence of 1-MT. Compared with WT pDC, activated IDO KO DC expressed much lower levels of ICOSL. Moreover, when ICOSL was blocked on WT pDC, T-cell proliferation resembled that induced by IDO KO pDC, and interleukin (IL)-10 secretion in MLR was markedly decreased. These findings implicate ICOSL-induced IL-10, but not IDO in the regulation of BM-derived pDC function.

1. Introduction

Dendritic cells (DC) are rare, yet ubiquitous, bone marrow (BM)-derived antigen (Ag)-presenting cells that induce and regulate innate and adaptive immunity [1–3]. Two principal DC subsets have been identified—"conventional" myeloid DC (mDC; $CD11c^+CD123^-$ in humans; $CD11c^+$ $CD8\alpha^-$ $CD11b^+$ in mice) and plasmacytoid DC (pDC; $CD11c^-CD123^+$ in humans; $CD11c^{intermediate}$ $B220^+$ in mice [4,5]). Both subsets exhibit immunostimulatory [3,4,6,7] and tolerogenic [8–10] functions that reflect their site of origin/isolation, Ag-presenting properties [11], exposure to specific stimuli/inhibitors, and maturation state at the time of T-cell interaction.

Following viral stimulation, pDC rapidly produce large amounts of type-1 interferons (IFNs) that activate conventional DC to prime virus-specific T cells [12]. However, pDC can also regulate autoimmune reactivity [13,14], impede antitumor responses [15], mediate oral tolerance [16], and suppress allograft rejection [17–19] and acute graft-versus-host disease [10]. Thus, infusion of donor BM-derived pDC can prolong murine heart allograft survival in an Ag-independent manner [17], whereas pDC that have acquired alloAg and migrated to host lymphoid tissue induce alloAg-specific regulatory T cells (Treg) and promote transplant tolerance [18]. Understanding the mechanisms that underlie these observations is key to the therapeutic targeting of pDC and the potential use of *in vitro*-propagated BM-derived pDC (BM-pDC) in cell-based therapies.

The inducible costimulator (ICOS)/ICOS ligand (L) (B7RP-1) costimulatory pathway has been implicated in the regulation of T-cell responses initiated by pDC [20]. Whereas ICOS–ICOSL interaction stimulates T cells in the context of mDC [21], ICOSL expression on human pDC correlates with diminished T-cell responsiveness and increased interleukin (IL)-10 production [20]. ICOS–ICOSL interaction has also recently been shown to expand T helper cell type-2 immunity [22] and the size of $Foxp3^+$ Treg and $CD62L^{lo}CD44^{hi}$ effector-memory CD4+ T-cell populations via modulation of DC maturation [23].

The comparatively poor allostimulatory capacity of BM-pDC may also be regulated by the expression of indoleamine dioxygenase (IDO). Functional expression of IDO by DC subsets (elicited by cytotoxic T-lymphocyte Ag [CTLA4]/CTLA4Ig [24,25],

* Corresponding author.
E-mail address: thomsonaw@upmc.edu (A.W. Thomson).
The first and second authors contributed equally to this work.

0198-8859/09/$32.00 - see front matter © 2009 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.humimm.2009.01.021

IFN-γ [26,27], or CD200Ig [28]), however, results in the breakdown of tryptophan, the rarest essential amino acid necessary for T-cell proliferation. Tryptophan catabolism induces activated T-cell apoptosis [26,29], suppresses T-cell proliferation in response to self-peptide in delayed-type hypersensitivity reactions [30,31] or in mixed leukocyte reactions (MLR) [24], and activates Treg [32]. IDO expression by physiologic DC from mouse spleen is tightly regulated and is responsive to IFN type I and type II in a rare but distinctive subset of pDCs [24,33].

The role of ICOSL or IDO in the regulation of T-cell reactivity initiated by *in vitro*-propagated BM-pDC has not been evaluated. Our aim was to investigate the roles of these molecules in the regulation of alloreactive T-cell proliferation and cytokine production by mouse BM-pDC. The findings suggest that IDO expression by BM-pDC is negatively regulated by DNAX-activating protein of 12kDA (DAP12), a transmembrane signaling adapter that inhibits IDO in murine CD8α^+ DC [34]. In contrast, the comparatively poor T-cell allostimulatory capacity of WT BM-pDC appears to reflect their expression of ICOSL.

2. Subjects and methods

2.1. Animals

Six- to eight-week-old C57BL/6 (B6; $H2^b$) and BALB/cByJ (BALB/c; $H2^d$) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained in the specific pathogen-free Central Animal Facility of the University of Pittsburgh School of Medicine. C57BL/6 $IDO^{-/-}$ (IDO knockout [KO]; $H2^b$) mice, generated at the Medical College of Georgia (Augusta, GA) were bred at the University of Pittsburgh. Experiments were conducted under an Institutional Animal Care and Use Committee-approved protocol and in accordance with National Institutes of Health guidelines. The animals were fed a diet of Purina rodent chow (Ralston Purina, St. Louis, MO) and received tap water *ad libitum*.

2.2. Media and reagents

The DC poietin recombinant (r) human fms-like tyrosine kinase 3 ligand (Flt3L) (CHO cell derived) was a gift from Amgen (Seattle, WA). Mouse recombinant (r) granulocyte–macrophage colony-stimulating factor and r human IL-4 were gifts from Schering-Plough (Kenilworth, NJ). Complete medium (CM) was composed of RPMI 1640 (BioWhittaker, Walkersville, MD) supplemented with 10% v/v fetal calf serum (Nalgene, Miami, FL), nonessential amino acids, L-glutamine, sodium pyruvate, penicillin–streptomycin, and 2-mercaptoethanol (all from Life Technologies, Gaithersburg, MD). The Toll-like receptor (TLR) 9 ligand CpG-B, certified endotoxin free, was obtained from Coley Pharmaceuticals (Wellesley, MA). Monoclonal antibodies (mAbs) used for flow cytometry were anti-CD11c (HL3, CyChrome conjugated) (eBiosciences, San Diego, CA), anti-CD45R/B220 (RA3-6B2; FITC conjugated), and PE-conjugated anti-IA^b (AF6-120.1), anti-CD19 (1D3), and anti-CD86 (GL1) (BD PharMingen, San Diego, CA). PE-conjugated anti-B7-H1 (MIH5), anti-B7-DC (TY25), and anti-ICOSL (B7RP-1) (HK5.3) were from eBioscience. Isotype-matched control Igs were from BD PharMingen. CTLA4Ig was a gift from Bristol-Myers Squibb Pharmaceutical Research Institute (Candiac, Quebec, Canada).

2.3. Generation and purification of BM-derived pDC and mDC

DC were generated and purified from freshly isolated BM as described for pDC and mDC [17,35,36], with minor modifications. Briefly, for pDC propagation, BM cells were cultured for 8 days in complete medium in 200 ng/ml Flt3L. On day 4, 50% of the supernatant was replaced with fresh, cytokine-containing medium. On day 8, the cells were enriched for $B220^+$ cells by incubation with anti-mouse B220-coated immunomagnetic beads (Miltenyi Biotec, Auburn, CA), according to the manufacturer's instructions, for 15 min at 4°C and then positively selected by passage through a paramagnetic column (Miltenyi Biotec), yielding a highly enriched (>90%) $CD11c^+B220^+$ population. In some experiments, DC were then activated by overnight culture (18 hours) in granulocyte–macrophage colony-stimulating factor (20 ng/ml) and CpG (2 μg/ml).

2.4. AlloAg pulsing of DC and MLR cultures

Pulsing of B6 DC with Ag was performed as described [37]. Briefly, bead-purified pDC were incubated with cell-free BALB/c splenocyte lysate at a DC:splenocyte equivalent ratio of 1:10 for 24 hours at 37°C. Graded numbers of γ-irradiated (20 Gy) pDC (or control mDC) were then used as stimulators in 72-hour MLR, with nylon–wool column-enriched normal syngeneic (B6) T cells as responders (10^5/ml) in 96-well, round-bottom plates, as described [38]. In allo-MLR, BALB/c T cells were used as responders. Where indicated, neutralizing anti-ICOSL mAb (10 μg/ml) was added to BM-pDC for 30 minutes prior to the addition of T cells. During the final 18 hours, wells were pulsed with 1 μCi [^{3}H]thymidine (Perkin Elmer Life Sciences/NEN, Woodbridge, Ontario, Canada) and radioisotope incorporation was determined using a β-scintillation counter. Results are expressed as the mean cpm $\pm$ 1 SD of 3–6 replicate wells. In some experiments, T cells were collected and stained for CD4 and intracellular Foxp3, as described [39].

2.5. Cytokine quantitation

IFN-γ, IL-4, and IL-10 were quantified by enzyme-linked immunosorbent assay (ELISA) using commercial kits from Biolegend (San Diego, CA) and following the manufacturer's recommended procedures. The detection limits were 4 pg/ml for IFN-γ and IL-4 and 30 pg/ml for IL-10.

2.6. 1-Methyl-D-tryptophan (1-MT)

1-MT (Aldrich, Milwaukee, WI) was added to cultures at a final concentration of 100 or 200 μM, as described [25,40].

2.7. Transfection of pDC with DAP12 small interfering RNA (siRNA) and confirmation of gene silencing by reverse transcription-polymerase chain reaction (RT-PCR)

pDC were collected from 7- or 8-day-old BM cultures and plated at 5×10^5 cells/500 μl CM overnight. They were then transfected using GeneSilencer siRNA Transfection Reagent (Genlantis, San Diego, CA) following the manufacturer's instructions with either DAP12 siRNA (400 μM) or Silencer Negative Control No. 1 (both from Ambion, Foster City, CA). After 6 hours, the cells were brought to 1.0 ml with CM. In some experiments, pDC were pulsed with alloAg, as described above. Twenty-four hours after transfection, the pDC were collected for use in MLR or to confirm efficient gene silencing by conventional RT-PCR for DAP12 (F: 5′-TGGTGCCTTCTGTTCCTTCC-3′; R: 5′-TTGTTTC-CGGGTCCCTTCC-3′) [41] or β-actin (F: ATGGATGACGATATCGCT; R: ATGAGGTAGTCTGTCAGGT).

2.8. Statistical analysis

Statistical analysis was performed using the two-tailed Student *t* test. Differences between groups were considered significant at $p < 0.05$. Results are expressed as means $\pm$ 1 SD.

3. Results

3.1. IDO knockout (KO) BM-pDC are more potent stimulators of naive T-cell proliferation and IFN-γ production in response to alloAg than wild-type (WT) BM-pDC

DC do not express IDO constitutively and an exogenous signal such as IFN type I or type II is required to induce DC to acquire suppressive functions via IDO [25–27,42]. Thus, to establish a base-