Cellular Immunology 278 (2012) 45–54

Contents lists available at SciVerse ScienceDirect

Cellular Immunology

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

A distinct tolerogenic subset of splenic IDO$^+$CD11b$^+$ dendritic cells from orally tolerized mice is responsible for induction of systemic immune tolerance and suppression of collagen-induced arthritis

Min-Jung Park [a,1], Kyung-Su Park [a,b,1], Hyun-Sil Park [a], Mi-La Cho [a], Sue-Yun Hwang [c], So-Youn Min [a], Mi-Kyung Park [a], Sung-Hwan Park [a,b], Ho-Youn Kim [a,b,*]

[a] The Rheumatism Research Center, Catholic Research Institute of Medical Science, The Catholic University of Korea, Banpo-dong, Seocho-gu, Seoul 137-701, South Korea
[b] Center for Rheumatic Disease, Division of Rheumatology, Department of Internal Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Banpo-dong, Seocho-gu, Seoul 137-701, South Korea
[c] Graduate School of Bio&Information Technology, Institute of Genetic Engineering, Hankyong National University, Ansung 456-749, South Korea

ARTICLE INFO

Article history:
Received 27 March 2012
Accepted 29 June 2012
Available online 10 July 2012

Keywords:
Oral tolerance
Indoleamine-2,3-dioxygenase
Spleen
Dendritic cell
Regulatory T cell
Collagen-induced arthritis

ABSTRACT

In oral tolerance, locally instigated tolerance in the gut propagate to systemic tolerance. In order to investigate the mechanism, we analyzed indoleamine 2,3–dioxygenase (IDO) expression in splenic dendritic cell (DC) subsets and tested whether DCs suppress collagen-induced arthritis (CIA) by inducing regulatory T cells (Tregs). The proportion of IDO-expressing cells was higher in the CD11b$^+$ subset of splenic DCs from orally tolerized CIA mice. These DCs suppressed type II collagen-specific T cell proliferation and promoted Treg induction from CD4$^+$CD25$^-$ T cells using transforming growth factor-β. These DCs also increased the expression of cytotoxic T lymphocyte antigen-4 and programmed death-1 on Tregs. When adoptively transferred, spenic IDO-expressing CD11b$^+$ DCs from tolerized animals suppressed the development of arthritis, increased the Treg/Th17 cell ratio, and decreased the production of inflammatory cytokines in the spleen. Taken together, a distinct subset of splenic IDO$^+$CD11b$^+$DCs is responsible for the systemic immune regulation in oral tolerance.

1. Introduction

Oral administration of antigen suppresses the immune response to the fed antigen by stimulating the gut-associated lymph tissue (GALT) such as Peyer's patches (PPs), lamina propria, and mesenteric lymph nodes (mLNs) [1–4]. Dendritic cells (DCs) are the major player in this type of immune suppression and DCs in the GALT display tolerogenic characteristics. However, little is known about the biological characteristics of DCs in the spleen and how they exert their systemic effect after induction of oral tolerance.

Regulatory T cells (Tregs) play an important role in immune regulation and in preventing autoimmunity, and are indispensable to the development of oral tolerance [5,6]. Tregs are usually induced by DCs in GALT [7]. We reported previously that the DCs with the CD11b$^+$ phenotype in PPs can induce CD4$^+$ T cells to differentiate into antigen-induced Tregs in orally tolerized mice with collagen-induced arthritis (CIA) [8]. However, the biological characteristics of the major tolerance-inducing DC subsets in the spleen, and whether these cells exert their regulatory function under inflammatory conditions such as CIA remain unknown.

DC populations in each specific lymphoid tissue possess distinct characteristics that reflect the disparity in their immunological environments [9]. Each lymphoid organ is thought to prime different helper T cell responses. For example, freshly isolated DCs from PPs, especially the CD11b$^+$ myeloid subset, produce IL–10 and induce the differentiation of Th2-type cells, whereas splenic DCs induce mainly Th1 cytokines [10,11]. However, injection of splenic DCs purified from ovalbumin-fed mice transfers the immune suppression of oral tolerance toward this antigen [12]. These seemingly contradictory results may be explained by more detailed characterization of tolerogenic DCs. We have demonstrated that among DCs from PPs, the subset expressing the immune-regulatory enzyme

Abbreviations: APCs, antigen-presenting cells; CIA, collagen-induced arthritis; CII, type II collagen; DCs, dendritic cells; ELISA, enzyme-linked immunosorbent assay; GALT, gut-associated lymph tissue; IDO, indoleamine 2,3-dioxygenase; MFI, mean fluorescence intensity; mLN, mesenteric lymph node; 1-MT, 1-methyltryptophan; pDCs, plasmacytoid dendritic cells; PPs, Peyer's patches; Th17, T helper 17 cells; Tregs, regulatory T cells.

* Corresponding author at: Rheumatism Research Center, Catholic Institute of Medical Science, 505 Banpo-Dong, Seocho-Ku, Seoul 137-040, South Korea. Fax: +82 2 599 4287.
E-mail address: ho0919@catholic.ac.kr (H.-Y. Kim).

[1] These two authors have contributed equally to this study.

0008-8749/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cellimm.2012.06.009

indoleamine 2,3–dioxygenase (IDO) is crucial for the induction of type II collagen (CII)-mediated oral tolerance and suppression of arthritis in a CIA mouse model [13]. We were interested in identifying the subset of splenic DCs mainly responsible for the IDO production, Treg differentiation and consequent induction of systemic immune tolerance.

The potential role of IDO in immune suppression first gained attention because of its involvement in Treg induction [14]. IDO is an enzyme that catabolizes tryptophan and produces several metabolic products including kynurenine, which inhibits the proliferation of effector T cells [15]. One subset of GALT DCs that expresses IDO is the $CD103^+$ population, which was reported to induce $Foxp3^+$ Treg differentiation and to inhibit IL–17 production [16]. The same study showed that deregulation of IDO activity caused impaired oral tolerance and increased intestinal inflammation. IDO has also been linked to the tolerogenic properties of plasmacytoid DCs (pDCs) isolated from tumor-draining lymph nodes [17]. By contrast, IDO-expressing cells are found only in the mLNs and not in the spleens of normal mice under physiological conditions [18]. In the CIA mouse model, the incidence and severity of CIA was significantly higher in mice treated with 1–methyl tryptophan (1–MT), a chemical inhibitor of IDO [19]. In another study, IDO-deficient mice had a higher incidence of arthritis and exacerbated disease severity compared with IDO-competent mice [20]. These results emphasize the importance of IDO to immune tolerance. However, studies of IDO have examined its local expression only, for example in the gut or a tumor, and the pattern of IDO expression in the peripheral immune system under inflammatory conditions is unknown.

To understand the biological characteristics of tolerogenic DCs in peripheral lymphoid organs, we examined the characteristics of IDO-expressing DC subsets in the spleens of orally tolerized CIA mice. We focused on whether the IDO^+ DCs subset from tolerized CIA mice can promote Treg differentiation and thereby regulate the immune response. We found that a splenic subset of IDO-expressing $CD11b^+$ DCs is a major player in the immune regulation in response to experimental arthritis after induction of oral tolerance.

2. Materials and methods

2.1. Mice

Male DBA1/J mice (SLC, Inc., Shizuoka, Japan), 7 weeks of age, were maintained under specific pathogen-free conditions and fed standard laboratory mouse chow (Ralston Purina, St. Louis, MO) and water *ad libitum*. All mice were treated according to the regulations of the Catholic Ethics Committee of the Catholic University of Korea.

2.2. Preparation of CII

Bovine CII was kindly provided by Professor Andrew Kang of the University of Tennessee. CII was extracted in its native form from the articular cartilage of fetal calf and was purified as described previously [21].

2.3. Induction of oral tolerance and arthritis in DBA/1 mice

The mice used in this study were divided into three groups: wild-type, tolerized (CII-fed) CIA, and untolerized (saline-fed) CIA mice. To induce CIA, DBA1/J mice were injected in the base of the tail with 100 μg of CII emulsified in complete Freund's adjuvant (CFA). The tolerized group was fed 100 μg of bovine CII using an oral Zonde needle (Natsume, Tokyo, Japan) every 2 days for 2 weeks, beginning 2 weeks before the tail injection of CII to induce CIA. Mice in the untolerized CIA group were fed an equal volume of saline instead of CII through the same administration schedule.

2.4. Cell isolation

Mononuclear cells from the spleens were incubated with anti-mouse CD11c-conjugated microbeads (Miltenyi Biotec, Auburn, CA) and subjected to positive selection through magnetic-activated cell sorting. The DCs obtained were incubated with anti-CD11c and anti-CD11b after FcR blocking. $CD11b^+$ and $CD11b^-$ DC subsets were sorted on the basis of their expression of CD11c and CD11b using a Vantage FACSorter (BD Biosciences). The purity of the sorted DCs was > 95%.

To isolate $CD4^+CD25^-$ T cells, mononuclear cells from the spleens of tolerized CIA mice were stained with a mixture of anti-CD4 and –CD25 monoclonal antibodies (mAbs) (BD Pharmingen, San Diego, CA) and sorted. The purity of the sorted $CD4^+CD25^-$ cells was 95–99% as evaluated by flow cytometry.

2.5. Flow cytometry

Single mononuclear cells were prepared from the spleens of each group of mice, stained with mAbs to CD11c, CD11b, CD8α, CD19, pDC, MHC II, CD80, CD86, programmed death ligand 1 (PD–L1), and PD–L2 after FcR blocking, permeabilized, and fixed with CytoPerm/CytoFix (BD Pharmingen) as instructed by the manufacturer. Cells were stained further with rabbit anti-IDO polyclonal antibody (Transgenic Inc., Kobe, Japan), followed by PE-conjugated goat anti-rabbit Ig, and then subjected to flow cytometric analysis (FACSCalibur, BD Biosciences, San Jose, CA). Rabbit IgG was used as the corresponding isotype antibody control.

To identify Tregs, expanded T cells were stained first with mAbs to CD4, CD25, Inducible costimulator (ICOS), Glucocorticoid induced TNF related (GITR), and Programmed death-1 (PD–1), and then with mAbs to cytotoxic T lymphocyte antigen 4 (CTLA–4) and Foxp3 using the regulatory T Cell Staining Kit (eBioscience, San Diego, CA). Events were collected and analyzed with FlowJo software (TreeStar).

2.6. Confocal microscopy

Spleens were removed 5 weeks after tail injection of CII and were snap-frozen in liquid nitrogen and stored at −80 °C. Tissue sections (7 mm) of spleens were fixed in 4% paraformaldehyde and stained using FITC-labeled anti-CD11b mAb (BD Biosciences, San Diego, CA), biotinylated anti-IDO mAb (BD Biosciences, San Jose, CA), streptavidin–Cy3 in PBS, and allophycocyanin-labeled anti-CD11c mAb (BD Biosciences). After overnight incubation at 4 °C, stained sections were analyzed by confocal microscopy (LSM 510 Meta; Carl Zeiss, Göttingen, Germany).

2.7. Measurement of the CII-specific T cell proliferative response

Mice were euthanized 5 weeks after CII injection in the tail. $CD11b^+$ DCs (1×10^4 cells) isolated from splenic mononuclear cells of CII-fed tolerized or saline-fed untolerized CIA mice were cultured for 3 days with irradiated antigen-presenting cells (APCs) (1×10^5 cells) and CII–reactive $CD4^+$ T cells (1×10^5 cells) obtained from the spleens of untolerized CIA mice. Cells were pretreated with 1–MT (250 μM), an IDO–specific inhibitor, 2 h before CII stimulation. Eighteen hours before the termination of culture, 0.5 μCi of [^{3}H] thymidine (New England Nuclear, Boston, MA) was added to each well. Cells were harvested onto glass fiber filters and counted in a Matrix-96 direct ionization counter (Packard Instrument Co., Downers Grove, IL). Data are presented as the mean cpm of triplicate cultures.