Immunology Letters 172 (2016) 21–28

Contents lists available at ScienceDirect

Immunology Letters

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

Intrinsic features of the CD8α⁻ dendritic cell subset in inducing functional T follicular helper cells

Changsik Shin[a,1], Jae-A Han[a,1], Bongseo Choi[a], Yoon-Kyoung Cho[a,b], Yoonkyung Do[a], Seongho Ryu[c,*]

[a] School of Life Sciences, Ulsan National Institute of Science and Technology, UNIST-gil 50, Ulsan 44919, Republic of Korea
[b] Center for Soft and Living Matter, Institute for Basic Science (IBS), UNIST-gil 50, Ulsan 44919, Republic of Korea
[c] Soonchunhyang Institute of Medi-bio Science (SIMS), Soonchunhyang University, Chonan-Si, Chungcheongnam-do 31538, Republic of Korea

ARTICLE INFO

Article history:
Received 26 October 2015
Received in revised form 6 January 2016
Accepted 31 January 2016
Available online 2 February 2016

Keywords:
Dendritic cell subset
T follicular helper cell
Localization
Cytokine
Cellular immunity
Humoral immunity

ABSTRACT

T follicular helper (Tfh) cells, a true B cell helper, have a critical role in enhancing humoral immune responses. However, the initial differentiation of Tfh cells by dendritic cells (DCs), the most potent antigen presenting cells, has not been clearly understood, particularly in the knowledge of the two major conventional dendritic cell subsets, CD8α⁺ DCs or CD8α⁻ DCs. Here we demonstrated that the localization of CD8α⁻ DCs in the marginal zone (MZ) bridging channels is closely associated with the induction of CXCR5⁺CCR7^{low} Tfh cells. We also showed that the major source of IL-6 for inducing Tfh cells is provided from the activated CD4⁺ T cells induced by CD8α⁻ DCs, and IL-6 directly secreted from the DC subsets seems minor. CD8α⁻ DCs were superior in inducing functional Tfh cells over other antigen presenting cells including B cells. We here observed the unknown intrinsic features of the DC subsets, suggesting the potential of utilizing the CD8α⁻ DC subset as therapeutic vaccine for the regulation of humoral immune responses.

1. Introduction

T follicular helper (Tfh) cells are identified as the true B cell helper and have been noted for their capability of modulating T cell dependent humoral immune responses [1,2]. Since the role of CXCR5 in Tfh cells was reported, leading its CXCL13 dependent migration into the germinal center (GC) [3,4], several discrete features of Tfh cells such as high expressions of ICOS and PD1 or low expression of CCR7, transcription factor Bcl6, and major cytokine IL-21 were identified [5]. In addition, the clinical importance of Tfh cells involved in humoral immunity related diseases such as autoimmunity and humoral immunodeficiency was reported [6], and thus the understanding the initiation of the Tfh cell differentiation is urgent and crucial. Although many researches have actively identified specific features of Tfh cells, how naïve CD4⁺ T cells differentiate into Tfh cells is not clearly understood.

Dendritic cell (DC), the most potent antigen presenting cell (APC), is the key modulator in inducing several CD4⁺ effector T cell subsets [7]. Two major conventional DC subsets in the secondary lymphoid organs are defined by the expression of CD8α, either CD11c⁺CD8α⁺ DCs expressing the endocytic receptor DEC-205 or CD11c⁺CD8⁻ DCs expressing the endocytic receptor DCIR2 [8,9]. We recently reported that the conventional CD8α⁻ DC subset is superior over CD8α⁺ DCs in inducing functional Tfh cells both *in vitro* and *in vivo*, generating efficient antigen specific humoral immune responses [10]. CD8α⁻ DCs were very effective in enhancing the number of antigen specific Tfh cells, the formation of germinal centers, and the quality and quantity of antibody titers against human pathogenic antigens such as *Yersinia pestis* LcrV, HIV Gag and Hepatitis B surface antigen [10]. We also reported the significance of ICOSL and OX40L expressed on CD8α⁻ DCs via the highly enhanced non-canonical NF-κB signaling pathway in inducing functional Tfh cells [10]. However, several important features such as the proximal location of the two DC subsets to antigen specific CD4⁺ T cells *in vivo*, cytokines secreted from the DC subsets and the potential involvement of other APCs, majorly B cells, in the induction of Tfh cells have not been addressed. Thus, the aim of the present study was to examine the undescribed intrinsic features of

* Corresponding author at: Soonchunhyang Institute of Med–bio Sciences (SIMS), Chonan–Si 31538, Republic of Korea.
E-mail address: ryu@sch.ac.kr (S. Ryu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.imlet.2016.01.009

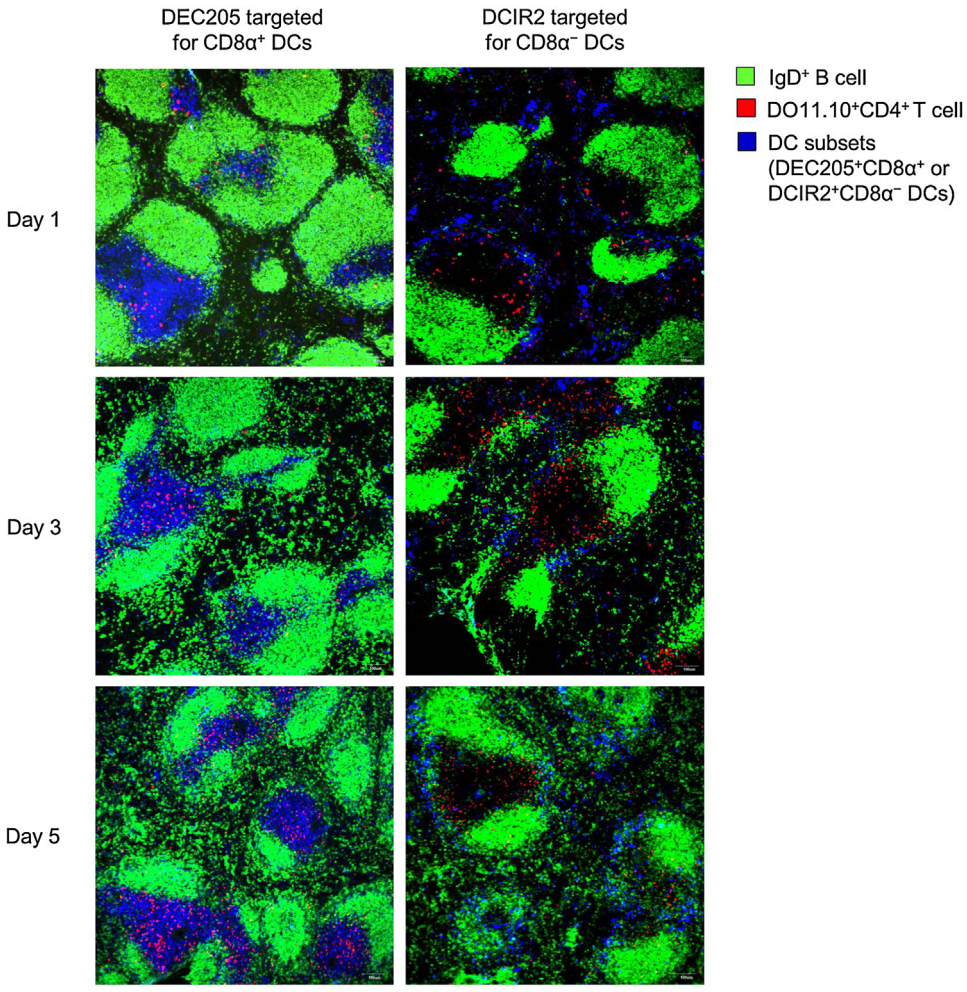

Fig. 1. $CD4^+$ T cells primed by $CD8\alpha^-$ DCs localized in the MZ bridging channels. OVA specific DO11.10$^+$CD4$^+$ T cells were adoptively transferred to naive BALB/c mice at day −1, and were immunized subcutaneously (s.c.) either with αDEC:OVA ($CD8\alpha^+$ DC targeting) or αDCIR2:OVA ($CD8\alpha^-$ DC targeting) conjugated monoclonal antibodies (mAbs) in the presence of poly (I:C) at day 0. At each indicated time point after the immunization, immunohistochemical staining of spleen sections from each group was prepared and detected by fluorescence confocal microscopy. IgD, green; DO11.10, red; DEC205 (for $CD8\alpha^+$ DCs) or DCIR2 (for $CD8\alpha^-$ DCs), blue. Data are representative of three or more independent experiments ($n > 10$ per group). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the two DC subsets in the initiation of Tfh cell differentiation by utilizing DC subset targeting strategy and purified DC subsets.

2. Materials and methods

2.1. Mice

BALB/c (H-2^d) and C57BL/6 (H-2^b) mice were purchased from Taconic, and OT-II and DO11.10 Thy 1.1 mice were purchased from The Jackson Laboratory. All mice were maintained under specific pathogen-free conditions and used at 6–8 weeks approved by Ulsan National Institute of Science and Technology Institutional Animal Care and Use Committee (approval number: UNISTIACUC-12-006-A).

2.2. Dendritic cell preparation

Total number of splenic dendritic cells was increased by Fms-like tyrosine 3 ligand (Flt3L) as previously described [9]. In brief, 5×10^6 cells of Flt3L-melanoma cells were subcutaneously (s.c.) injected to naïve C57BL/6 mice. After 10–14 days, the expanded splenic $CD11c^+$ DCs were enriched with positive magnetic-activated cell sorting (MACS; Miltenyi Biotec, GmbH, Bergisch Gladbach, Germany) and were further sorted into the two DC subsets, $CD3^-B220^-CD11c^+CD8\alpha^+$ or $CD3^-B220^-CD11c^+CD8\alpha^-$ DCs by MoFlo XDP (Beckman Coulter, Brea, CA, USA). To analyze cytokines or surface marker expressions in/on the two DC subsets, the purified each DC subset was stimulated either with 25 μg/ml poly (I:C) or 100 ng/ml LPS for 12 or 24 h *in vitro*.

2.3. $CD4^+$ T cell preparation

OVA-specific transgenic $CD4^+$ T cells from the lymph nodes and the spleen of OT-II or DO11.10 Thy1.1 mice were negatively isolated using hybridoma supernatant cocktail of rat-anti mouse −CD8 (2.43), −MHC class II (T1B120), −Mϕ (F4/80), −B220 (RA3-6B2), and −NK cell (NK1.1) antibodies followed by depletion with dynabeads sheep anti-rat IgG (Invitrogen, Carlsbad, CA, USA).

2.4. $CD4^+$ T cell priming by the DC subsets in vitro

Naive OVA-specific $CD4^+$ T (3×10^5) cells purified from OT-II mice were co-cultured either with sorted $CD8\alpha^+$ or $CD8\alpha^-$ DCs (each 0.3×10^5 DCs, 1:10 ratio of DC to T cells) per well in the round bottom 96 well plate for 3 days in the presence of 25 μg/ml poly (I:C) or 100 ng/ml LPS with 2 μM OVA peptide (a.a. 323–339) (Genscript, Piscataway, NJ, USA). Then, cytokines in supernatants were detected by ELISA.