Available online at www.sciencedirect.com
ScienceDirect
Immunology Letters 108 (2007) 174–178
Immunology Letters
www.elsevier.com/locate/

Short communication

Dendritic cell–T cell interactions: CD8αα expressed on dendritic cells regulates T cell proliferation

Li Hong [a,b], Tonya J. Webb [a,b], David S. Wilkes [a,b,c,*]

[a] *Center for Immunobiology, Indiana University School of Medicine, Indianapolis, IN, USA*
[b] *Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA*
[c] *Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN, USA*

Received 24 October 2006; received in revised form 30 November 2006; accepted 9 December 2006
Available online 8 January 2007

Abstract

Expression of the CD8αα homodimer has been used to differentiate lymphoid (CD8α$^+$) from myeloid (CD8α$^-$) dendritic cells (DCs). We have reported that CD8α$^+$ and CD8α$^-$ DCs have differential abilities to stimulate proliferation in allogeneic T cells. However, no specific function has been attributed to DC-derived CD8α. The current study examines the hypothesis that CD8αα expression on DCs regulates DC-induced T cell activation. CD8α$^-$ transduced bone marrow-derived DCs were more potent stimulators of T cell proliferation, and produced significantly greater quantities of IL-12 in co-culture with T cells. LCK, a kinase whose expression is reported to be T cell-restricted and known to bind to the cytoplasmic tail of CD8αβ in T cells, was detected readily in primary CD8α$^+$ splenic DCs and at greater levels than CD8α$^-$ DCs from the same tissues. LCK also co-precipitated with CD8α on immunblots strongly suggesting its role in CD8α$^+$ DC-induced T cell activation. Collectively, these data show that CD8α expressed on DC may not only be a lineage/maturation marker but also contribute to DC function.

Keywords: CD8α; Dendritic cells; Lymphoid

1. Introduction

Dendritic cells (DC) are the most potent antigen presenting cells in all tissues. Reports from our laboratory and others show unique functions for tissue DC that express CD8α [1–3]. The CD8αα homodimer differentiates lymphoid (CD8α$^+$) from myeloid (CD8α$^-$) murine DC, and is distinct from the CD8αβ heterodimer expressed on T cells. Both CD8αα and CD8αβ molecules are able to bind MHC class I, and CD8αβ interacts with MHC class I which contributes to activation of CD8$^+$ T cells. However, there are no reports indicating that DC-derived CD8αα may have a role in DC-induced T cell activation. Instead, CD8αα on DC has been regarded as a cell lineage marker rather than a molecule that could contribute to functional differences between myeloid and lymphoid DC [4]. We have recently reported that compared to CD8α$^+$ DCs, CD8α$^-$ lung DC have differential abilities to stimulate proliferation in allogeneic T cells [1]. In the current study we investigated the role of CD8a molecules in DC-induced T cell responses. Moreover we asked if CD8 molecules expressed on DC utilized the downstream signaling molecule, Lck, also known to be used in T cell signaling.

2. Materials and methods

2.1. Mice

Female C57BL/6 mice (6–8 weeks) were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN) and Jackson Laboratories (Bar Harbor, ME). Mice made genetically deficient in CD8α (B6.129S2-*Cd8a*$^{+m1MAK}$) and wild type litter mates (8–10 weeks of age) were purchased from the Jackson Laboratory. Mice were euthanized by intramuscular injection of a ketamine cocktail (79.3% Ketaject, 17.5% atropine, and 3.2% acepromazine). Mice were housed in specific-pathogen free facilities in the Laboratory Animal Resource Center at Indiana

* Corresponding author at: Center for Immunobiology, Indiana University School of Medicine, Van Nuys Medical Sciences Building-224, 635 Barnhill Dr., Indianapolis, IN 46202-5120, USA. Tel.: +1 317 278 7020; fax: +1 317 278 7030.
E-mail address: dwilkes@iupui.edu (D.S. Wilkes).

0165-2478/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.imlet.2006.12.003

University School of Medicine. All experiments were performed in accordance with procedures approved by Indiana University School of Medicine Animal Use and Care Committee.

2.2. Isolation of splenic dendritic cells

Splenic mononuclear cells were isolated using a modified procedure that has been previously reported [5]. Spleens were harvested from C57BL/6 mice and processed into single cell suspensions. Erythrocytes were lysed by hypotonic shock in 0.84% NH_4Cl. The remaining cells were washed twice with cRPMI, and then resuspended in the same medium. Anti-CD11c microbeads (Miltenyi Biotec) were used to isolate splenic DC from the mononuclear cell populations. Purity of all cells was >90%. Flow cytometry using the following fluorochrome-conjugated anti-mouse antibodies: FITC CD11c, PE CD80, PE CD86, PE CD40, and PE I-A^b (Pharmingen, San Diego, CA) revealed that splenic DC were identical to those reported in our prior studies [5].

2.3. Isolation of bone marrow-derived dendritic cells

BMDC were generated as previously described [6] with modifications. Bone marrow cells were obtained from C57BL/6 mice by flushing femoral cells with a 23-gauge needle with RPMI. Low-density mononuclear bone marrow cells were isolated by Ficoll and centrifuged. Cells (5×10^6) were cultured in 10 cm tissue dishes with the fresh CRPMI with recombinant GM-CSF (100 ng/ml) and IL-4 (100 ng/ml). Media was changed every 2–3 days and replaced with fresh media supplemented with GM-CSF (100 ng/ml) and IL-4 (100 ng/ml). On day 11, the adherent cells were stained with anti-CD11C-PE antibody and analyzed by flow cytometry. All cells were utilized for assays on day 12.

2.4. Cell lines

D10.G4.1 T cell hybridomas were purchased from ATCC and were cultured in cRPMI supplemented with 10% T cell stimulatory media (Sigma) and IL-1 (10 pg/ml) (BD Pharmingen, San Diego, CA). The DC2.4 and FSDC dendritic cell lines were generously provided by Dr. Kenneth Rock and Dr. Cheong-Hee Chang, respectively, and have been previously described [7,8].

2.5. Mixed lymphocyte reaction (MLR)

DC were gamma-irradiated (2000 cGy) and used as stimulators and Balb/c $CD90^+$ pure splenic T cells (I-a^d) (3×10^5), r D10.G4.1 T cells (1.5×10^5) [9] were used as responders. The D10.G4.1 cell line has been reported to proliferate vigorously in response to I-a^b [9]. $CD90^+$ and $CD4^+$ T cells were isolated using magnetic beads (Miltenyi Biotec) as reported [5]. These cells were co-cultured in a 37 °C, 5% CO_2 incubator in triplicate wells in 96-well flat bottom plates. After 2 days, the cells were pulsed with 0.5 μCi/well of [^{3}H] thymidine for 18 h, and proliferation determined as the mean ^{3}H incorporation in triplicate cultures.

2.6. Immunoblotting

Splenic DC were washed with 10 ml ice-cold HBSS, then lysed with 2× SDS-PAGE loading dye (4% SDS, 100 mM Tris–HCl, pH 6.8, 20% glycerol, 2% (w/v) 2-mercaptoethanol, and 0.1% bromphenol blue). The cell lysates were then resolved on a 10% SDS-PAGE gel (Bio-Rad) and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA). Anti-LCK antibodies (Santa Cruz) were used to detect LCK by western blotting. Also the lysates from $CD11c^+CD8\alpha^+$ and $CD11c^+CD8\alpha^-$ cells were immunoprecipitated (IP) with anti-CD8α antibody (BD Pharmingen) then western blotted with anti-LCK antibody (Santa Cruz). The blots were developed using chemiluminescence before exposure on film.

2.7. Transduction of bone marrow (BM) cells

(A) *Preparation of retrovirus*. Phoenix Eco cells (∼80% confluent) were plated on 10 cm tissue culture dish 10 ml of DMEM (high glucose) with 10% FCS. Then the plasmids (MIEG3, MIEG3CD8α) were added (10 μg DNA/dish) using Lipofectamine reagent. Eighteen hours later the medium was changed to DMEM containing 10 mM sodium butyrate and 10% FCS. Then, 8 h later, the cells were gently washed twice with warmed PBS, and 10 ml fresh DMEM with 10% FCS was added and the plates were incubated at 32–37 °C. Two to four days later the supernatant was collected every 24 h, filtered with 0.45 μm filter, and stored at −80 °C. Freshly thawed supernatant was used for transduction. (B) *Transduction of bone marrow cells*. To set up for transfection, non-tissue culture six-well plates were incubated with RetroNectin 50 μl (1 mg/ml) (TakaRa BIO) overnight at 4 °C. The next day supernatant was decanted and the wells were blocked with 2% BSA in PBS for 30 min at room temperature and wash twice with PBS. Next, prestimulated cells (2×10^6 cells/well) were incubated with either MIEG3 or MIEG + CD8α retroviral supernatant. After incubation at 37 °C overnight, transductions are repeated on the same plates the next day. Transduction efficiencies were assessed by determining the proportion of EGFP positive cells using FACS analyses 48 h after the transduction. After 48 h, both suspension cells and adherent cells were treated with Cell Dissociation Buffer and then culture in the fresh CRPMI with GM-CSF (100 ng/ml) and IL-4 (100 ng/ml). On day 8, the GFP positive cells are sorted by FACS and place in culture again in CRPMI with GM-CSF and IL-4. On day 11, the cells are stained with anti-CD11C-PE and anti-CD8α antibody and analyzed by flow cytometry. On day 12, then set up experiment: MLR (mixed lymphocyte reaction) and IL-12 production.

2.8. ELISA

IL-12 (p70) production from the supernatants of co-cultured cells was determined by standard ELISA. The standard was obtained from BD Pharmingen (Cat. No. 554592). The purified 9A5 antibody (Cat. No. 554658) and biotinylated C17.8 anti (Cat. No. 554476) were purchased from BD Pharmingen as well.