Comparative Immunology, Microbiology and Infectious Diseases xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

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

Phenotypic and functional analysis of CD1a+ dendritic cells from cats chronically infected with feline immunodeficiency virus

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ARTICLE INFO

Article history:
Received 30 January 2015
Accepted 29 July 2015

Keywords:
Feline immunodeficiency virus
Dendritic cells
CD1a
Regulatory T cells

ABSTRACT

Numerous studies suggest dendritic cell (DC) dysfunction is central to the dysregulated immune response during HIV infection; however, in vivo studies are lacking. In the present study we used feline immunodeficiency virus (FIV) infection of cats as a model for HIV-1 infection to assess the maturation and function of dendritic cells, in vivo and in vitro. We compared CD1a+ DC migration, surface phenotype, endocytosis, mixed leukocyte reaction (MLR) and regulatory T cell (Treg) phenotype induction by CD1a+ cells isolated from lymph nodes of FIV-infected and control cats. Results showed that resident CD1a+ DC in lymph nodes of chronically FIV-infected cats are phenotypically mature, can stimulate normal primary T cell proliferation, override Treg suppression and do not skew toward Treg induction. In contrast, FIV infection had deleterious effects on antigen presentation and migratory capacity of CD1a+ cells in tissues.

1. Introduction

Feline immunodeficiency virus (FIV) is a naturally occurring pathogenic lentivirus that infects domestic and non-domestic cats causing an immunodeficiency syndrome similar to HIV infection of people [1]. In addition to well-studied deficiencies in the adaptive immune response, innate immune defects caused by FIV have also been described [1,2]. We have previously shown that FIV-infected cats have a delayed and impaired response to subcutaneous challenge with the opportunistic intracellular bacterial pathogen, *Listeria monocytogenes* (Lm) [2]. Specifically, the immune defect is apparent in the first few days after bacterial challenge and is characterized by greater replication and delayed clearance of Lm. Failure of NK cell responses against Lm challenge during chronic FIV infection has been shown to correlate with the delayed control and clearance of this opportunistic pathogen [3]. However, it is still unclear whether qualitative or quantitative dendritic cell (DC) defects might underlie this phenomenon.

Dendritic cells play a key role in sensing Lm in tissues and respond by migrating to local lymph nodes (LN) to elicit effective innate and adaptive immune responses. Several myeloid DC (mDC) subpopulations reside in the skin including Langerhans cells (LC) in the epidermis and at least two migratory DC populations in the dermis. Although all three populations express CD1a, they are functionally distinguishable [4]. An impaired response of one or all of these dendritic cell subsets could explain innate immune defects observed in FIV-infected cats. Several studies have shown that Treg from FIV-infected cats are constitutively activated and strongly suppress T cell proliferation [5–7]. Treg have been shown to suppress anti-viral responses during HIV, SIV, and FIV infection [8–10]. Plasmacytoid dendritic cells (pDC) have been shown to induce Treg, but whether mDC could directly induce Treg differentiation is still unclear [11]. Helios is a transcription factor thought to be required for fully suppressive Treg in humans [12] and is expressed at higher levels in HIV positive individuals on antiretroviral therapy with suppressed viremia [13]. Glycoprotein A repetitions predominant (GARP) binds TGF-β to the cell membrane and this complex is increased on activated Treg from FIV-infected cats [14]. Furthermore, Treg expressing the GARP/TGF-β complex have been shown to convert T helper cells to the Treg phenotype [15]. Whether mDC are involved in induction of these two functional Treg markers has not been examined in chronically FIV infected cats.

In the present study, we explored whether DC abnormalities in FIV-infected cats might underlie innate immune defects. We compared CD1a+ cell migration, surface phenotype, endocytosis, mixed leukocyte reaction (MLR) and Treg phenotype induction by CD1a+ cells isolated from lymph node (LN) of FIV-infected and control cats.

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http://dx.doi.org/10.1016/j.cimid.2015.07.003
0147-9571/

2. Materials and methods

2.1. Animals and viral inoculum

Fifteen female specific-pathogen-free (SPF) cats were purchased from Liberty Labs (Liberty, NY). Cats were housed and maintained in accordance with the Association for the Assessment of Laboratory Animal Care standards and Institutional Animal Care and Use Committee guidelines. Cats were inoculated with 5×10^5 cell-associated FIV-NCSU$_1$ [16] by intravenous administration at 20 weeks of age. Virus inoculum was prepared as previously described [17] and FIV infection was confirmed [3]. Cats used in these studies had been infected for 2 years.

2.2. In vivo dendritic cell migration assay

The migration of epidermal DC was induced by application of 100 μl of 8 mg/ml FITC dissolved in 1:1 v/v acetone:dibutyl phthalate onto the skin cranial to the metatarsal footpad. Popliteal LN biopsies were performed on anesthetized cats 18 or 36 h after FITC application. LN were processed and single cells were obtained as previously described [18]. A minimum of 2×10^6 freshly isolated cells was labeled with the following antibodies for flow cytometric analysis. Anti-CD1a (Fe1.5F4) was provided by Dr. Peter Moore of the University of California at Davis [19] and was conjugated with APC using a kit from Prozyme (Hayward, CA). Anti-CD80 (B7.1.66) was provided by Dr. Mary Tompkins of North Carolina State University [20] and was conjugated with PE using a kit from Prozyme (Hayward, CA). Anti-MHC II was purchased from AbD Serotec (PG8J-9B; Raleigh, NC). Anti-mouse IgG2a-PE-Cy7 (Jackson ImmunoResearch, West Grove, PA) was used as the secondary Ab for MHCII staining. Cells were analyzed using an LSRII flow cytometer with FACSDiva software (BD Biosciences, San Jose, CA) and data were analyzed with FlowJo software (Treestar, OR). At least 1×10^6 gated lymphocyte events were collected per sample.

2.3. TRITC-dextran uptake assay

Cells from popliteal LN were pulsed with TRITC-dextran (1 mg/ml, ICN Biomedicals, OH) at 37 °C for 15 min. Cells were then washed 3 times with cold phosphate buffered saline pH 7.4 (PBS) containing 5% fetal bovine serum (FBS) and 0.1% sodium azide, stained for CD1a, and subjected to flow cytometric analysis.

2.4. DC stimulated proliferation and Treg suppression (MLR assay)

A single cell suspension was prepared from peripheral LN. CD1a+ cells were first enriched using anti-CD1a magnetic beads (Miltenyi Biotec, San Diego, CA) then purified on a MoFlo Cell Sorter (Beckman Coulter, Brea, CA). Purity of the CD1a+ cells was determined by flow cytometry. CD4+CD25+ Treg (suppressor cells) were isolated from the CD1a− population by MoFlo Cell Sorter as previously described [21]. CD4+CD25− (responder) T cells were prepared from a clinically healthy, unrelated cat and were purified by MoFlo Cell Sorter. The responder cells were washed in PBS and stained with 2.5 μM CellTrace Violet proliferation marker (Life Technologies, Carlsbad, CA) for 30 min in the dark at 37 °C. Labeling was quenched by addition of 10% FBS in PBS, followed by a wash with complete medium (RPMI 1640 medium supplemented with 15% FBS, 4 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 55 μM β-mercaptoethanol). Responder cells were added to a 96-well round-bottom polypropylene plate at 4×10^4 cells/well with 1×10^4 allogeneic DC, then CD4+CD25+ suppressor cells were added to yield suppressor:responder cell ratios from 1:4 to 1:32. Responder cells alone or with allogeneic suppressor cells were cultured with 2 μg/ml concanavalin A (ConA) as a proliferation control. Cells were incubated for 6 days at 37 °C, 5% CO_2 and then analyzed on an LSR II flow cytometer (BD Biosciences, San Jose, CA). Suppression of proliferation was calculated as (% proliferation of CD4+CD25− cells − % proliferation of CD4+CD25− cells with CD4+CD25+ cells)/% proliferation of CD4+CD25− cells.

2.5. DC induced Treg markers

CD4+ cells were purified from the clinically healthy, unrelated cat by MoFlo Cell Sorter, stained with CellTrace Violet, and added to a 96-well polystyrene plate at 2×10^5 cells/well with about 4×10^4 purified DC from FIV infected and control study cats. After 6 days in culture at 37 °C, 5% CO_2, cells were stained for CD25-FITC [21] and GARP (Plato-1; Enzo, NY) for 20 min, washed with PBS, stained with IgG2b-PE (Jackson ImmunoResearch) for 20 min, then washed and permeablized by eBioscience FOXP3 staining buffer for 25 min. After washing twice, cells were stained with FOXP3-PE-Cy7 (FJK-16s; eBioscience, San Diego, CA) and Helios-APC (22F6; eBioscience) for 1 h and were analyzed on an LSR II flow cytometer.

2.6. Statistical analysis

Data were compared by Mann–Whitney test using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA) with significance defined as p less than or equal to 0.05.

3. Results

3.1. Feline DC migration is significantly reduced in chronically FIV-infected cats

Previous studies have shown that migration of feline DC from skin to regional LN can be quantified by painting a solution containing FITC onto the skin [22]. We used this approach to determine whether DC migration is compromised in chronically FIV-infected cats. Flow cytometric analysis of FITC+ CD1a+ cells (recently migrated CD1a+ cells) was used to quantify the frequency in the lymph nodes. No significant differences in absolute numbers of total cells in LN were observed between FIV-infected and SPF-control cats. However, the relative percentages of recently migrated CD1a+ cells were significantly lower in the chronically FIV-infected cats at 18 h post FITC painting ($1.2\% \pm 0.2\%$ vs $5.8\% \pm 2.4\%$, $p < 0.05$) while no difference was observed at the 36 h time point (Fig. 1A and B).

3.2. Recently migrated DC from FIV-infected cats express lower levels of costimulatory molecules

To determine the maturation status of the DC, we assessed the expression of MHCII and CD80 as representative DC costimulatory molecules. The percentages of DC expressing costimulatory molecules were similar in FIV-infected and SPF-control cats (data not shown). However, the levels of MHCII and CD80 expression as measured by mean fluorescence intensity (MFI) in recently migrated DC were significantly lower ($p < 0.05$) at 18 h post FITC painting in FIV-infected cats as compared to SPF-control cats (MHCII MFI of 3833 ± 580 vs $10{,}803 \pm 3749$ and CD80 MFI of $10{,}032 \pm 2014$ vs $55{,}935 \pm 23{,}596$) (Fig. 2A and B). Similar trends were seen at 36 h post FITC painting but differences did not achieve statistical significance (Fig. 2C). FITC− CD1a+ cells (resident CD1a+ cells) expressed comparable levels of costimulatory markers between the two groups of cats (Fig. 2B and C).

Please cite this article in press as: L. Zhang, et al., Phenotypic and functional analysis of CD1a+ dendritic cells from cats chronically infected with feline immunodeficiency virus, Comp Immunol Microbiol Infect Dis (2015), http://dx.doi.org/10.1016/j.cimid.2015.07.003