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Pathological effect of IL-17A-producing TCRγδ$^+$ T cells in mouse genital mucosa against HSV-2 infection

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ABSTRACT

Interleukin (IL)-17A is a cytokine that plays an important role in infectious, autoimmune, and inflammatory diseases. In this study, we found that TCRγδ$^+$CD4$^-$CD8$^-$ T cells, but not TCRαβ$^+$CD4$^+$ T cells, are the primary producers of IL-17A in the genital tract of female mice in the steady-state condition. High mRNA levels of IL-17A and RORγt were determined in TCRγδ$^+$ T cells isolated from mouse genital tract but lacked detectable expression of IFNγ, T-bet, and FoxP3. IL-17A production by genital TCRγδ$^+$ T cells was maintained after intravaginal vaccination with cholera toxin or avirulent herpes simplex virus type (HSV)-2 186 syn ΔTK strain. Of note, the deaths of IL-17A$^{-/-}$ mice were significantly delayed after intravaginal HSV-2 infection compared with wild-type mice. Further, genital TCRγδ$^+$ T cells continued to produce comparable amounts of IL-17A after antibiotic treatment. These results imply that genital IL-17A-producing TCRγδ$^+$ T cells constitutively exist at steady state and that they play a pathogenic effect against HSV-2 infection and are not affected by microflora, unlike conventional Th17 cells.

1. Introduction

Interleukin (IL)-17A was initially cloned from activated T cells with 57% homology to the protein encoded by T lymphotropic herpesvirus (HSV) saimiri gene 13 and named CTLA-8 [1,2]. To date, six IL-17 members (A, B, C, D, E, and F) have been identified in the mouse family [3]. Of these, IL-17F has the highest homology with IL-17A; both are mainly produced by activated CD4$^+$ T cells that are classified as Th17 cells [4–6]. The orphan nuclear receptor RORγt is a key transcription factor that induces transcription of the genes encoding IL-17A and IL-17F in naïve CD4$^+$ T helper cells and is required for their expression in response to IL-6 and TGF-β, the cytokines known to induce IL-17 [7].

IL-17A exerts proinflammatory effects by induction of TNF-α, IL-1β, IL-6, CXC chemokines, and other chemoattractants, recruiting neutrophils and other immune cells [8]. IL-17A is thought to have a protective role in host defense against extracellular pathogens such as fungi and bacteria, while it has pathological aspects in organ-specific autoimmune diseases [9]. Several studies have addressed the relationship between IL-17 and viral infections [10–13]; however, its exact role in modulating immune response against viral infection in the mucosal site, which is the first line of defense, has not been fully elucidated.

Although CD4$^+$ T cells are major sources of IL-17A upon antigen stimulation, current studies show that IL-17A can be produced by innate lymphocytes such as NK cells, NKT cells, and TCRγδ$^+$ T cells [14,15]. TCRγδ$^+$ T cells, which constitute only a minor subset in the systemic compartments, are disproportionately enriched at mucosal tissues with a restricted repertoire of the variable region of the T cell receptor [16]. The role of TCRγδ$^+$ T cells has been elucidated in epithelial homeostasis and recruitment of inflammatory cells to sites of tissue damage [17,18]. Although TCRγδ$^+$ T cell-deficient mice are resistant to *Listeria monocytogenes* challenges, they show substantial alterations in patterns of immunopathology [19–21]. In contrast, TCRγδ$^+$ T cells have a protective role against HSV type 1 infection in a mouse model [22].

In the present study, we found that TCRγδ$^+$CD4$^-$CD8$^-$ T cells are the major population of IL-17A-secreting cells in the female genital tract of mice at the steady state. TCRγδ$^+$ T cells in the genital tract exhibit different expression profiles of cytokines and transcription factors compared to those from spleen. Here, we attempted to examine cytokine production by genital tract TCRγδ$^+$ T cells in response to vaginal vaccination with toxin- or viral-based vaccines and antibiotic treatment. Of note, TCRγδ$^+$ T cells did not produce IFNγ and maintained IL-17A production under all experimental conditions. These findings suggest that IL-17A-producing TCRγδ$^+$ T cells in the genital tract have unique characteristics.

Abbreviations: AhR, arylhydrocarbon receptor; CT, cholera toxin; HSV-2, herpes simplex virus type 2; MNCs, mononuclear cells; Th17, T helper type 17; MPA, medroxyprogesterone 17-acetate.

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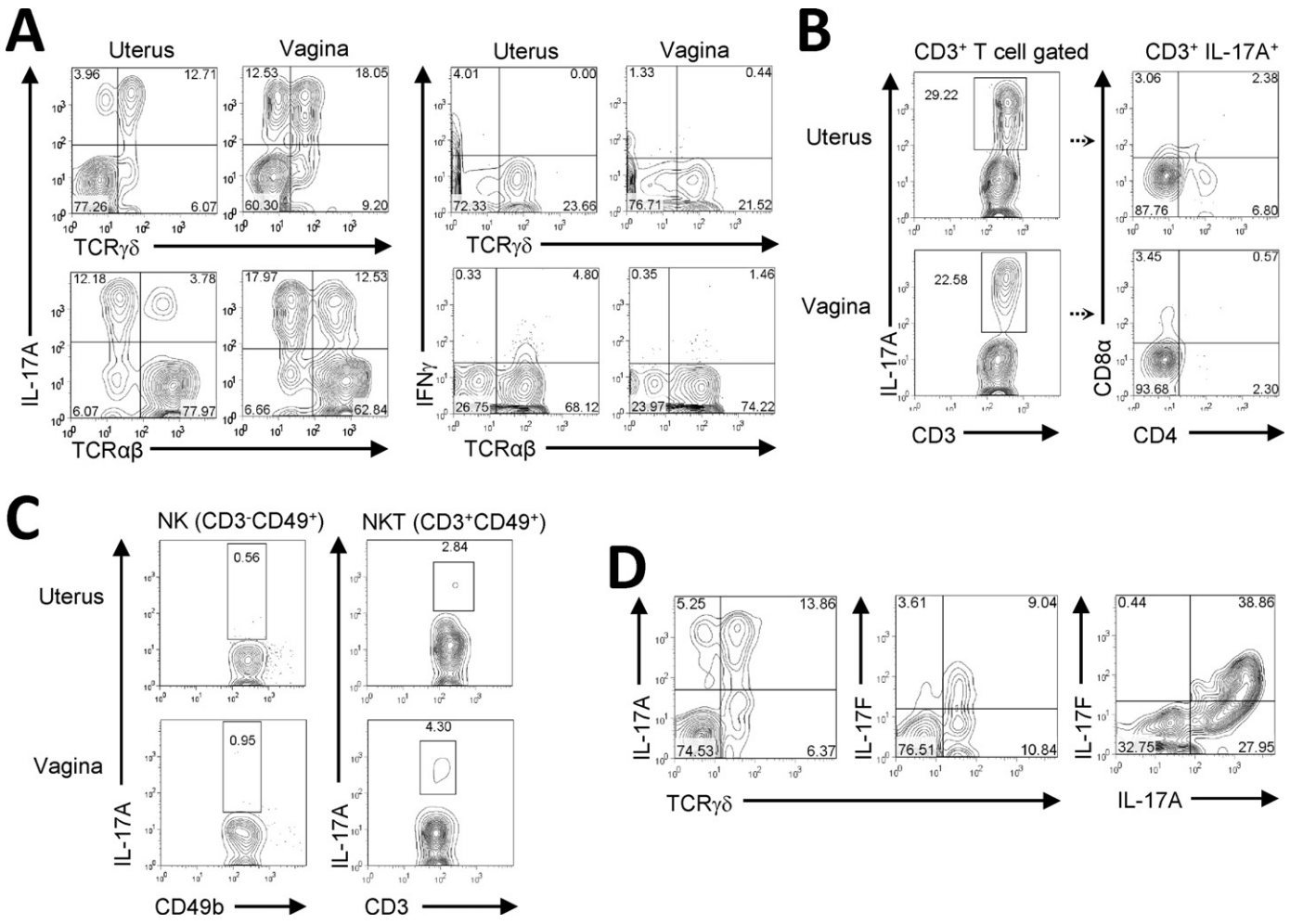

Fig. 1. Identification of major IL-17A-producing cells in female mouse genital mucosa. Single cells were obtained from pooled uterus and vaginal specimens from 6- to 8-week-old BALB/c mice using collagenase/dispase treatment followed by Percoll gradient centrifugation. Cells were stimulated with PMA and ionomycin for 4 h. (A) Cells were stained for CD3ε, TCRβ, TCRγδ, and IL-17A and gated on CD3ε$^+$ cells by FACS analysis. Cells were stained for CD3ε, CD4, CD8α, and either IL-17A (left panel) or IFNγ (right panel). (B) IL-17A$^+$ populations on CD3ε$^+$ T cells (box, left panel). Boxed populations (IL-17A$^+$ CD3ε$^+$ T cells) show CD4 and CD8α expression (right). (C) IL-17A-producing NK cells (CD3ε$^-$CD49b$^+$ gated, left panel) and NKT cells (CD3ε$^+$CD49b$^+$ gated, right panel). (D) IL-17A or IL-17F production by pooled uterus and cervico-vaginal cells. Cells were stained for CD3ε, TCRγδ, IL-17A, and IL-17F and gated on CD3ε$^+$ cells (left and middle panels) or TCRγδ$^+$ cells (right panel).

2. Materials and methods

2.1. Mice

Six- to eight-week-old female C57BL/6 and BALB/c mice were purchased from Charles River Laboratories (Orient Bio Inc., Sungnam, Korea). IL-17A$^{-/-}$ mice were provided by Dr. Yoichiro Iwakura (Tokyo University, Tokyo, Japan). All mice were maintained under specific pathogen-free conditions in the experimental facility at the International Vaccine Institute (Seoul, Korea) where they received sterilized food and water ad libitum. When cholera toxin (CT) or avirulent HSV was administered, the mice were subcutaneously pretreated with medroxyprogesterone 17-acetate (MPA) (Sigma, St. Louis, MO) to synchronize female hormone cycles. CT or virus was administered via vagina in a 20-μl volume adjusted with PBS. All experiments described were approved by appropriate institutional animal care and use committees.

2.2. Virus

HSV-2 186 syn and HSV-2 186 syn ΔTK [23] were kindly provided by Dr. David Knipe (Harvard Medical School, Boston, MA). Both wild-type and ΔTK of HSV-2 186 strain were propagated on Vero cells [24]. Viral stocks were prepared using serum-free media, VP-SFM (Invitrogen, Carlsbad, CA) and titered on the Vero cells.

2.3. Isolation of mononuclear cells (MNCs) from genital tract

For isolation of MNCs, each uterus and cervico-vaginal tissue was removed and chopped with scissors. The tissue was then incubated with RPMI 1640 containing 10% bovine calf serum, antibiotics (penicillin and streptomycin), 0.1 mg/ml of DNaseI (Sigma), and 2 mg/ml of collagenase/dispase (Roche Diagnostics, Mannheim, Germany) and harvested twice after stirring for 30 min at 37 °C. The isolated cells were pooled and separated on a 40/75% discontinuous Percoll gradient (Pharmacia, Piscataway, NJ) and centrifuged at 600 × *g* at 25 °C for 20 min. Cell layers for MNCs were recovered and suspended in complete RPMI 1640. Cells were restimulated with PMA (50 ng/ml) and ionomycin (750 ng/ml) (BD Pharmingen, San Diego, CA) in the presence of Golgi-Plug (Invitrogen) for 4 h to enable intracellular measurement of IL-17A and IL-17F.

2.4. Flow cytometric analysis

Single cell suspensions were pre-incubated with anti-FcRII/III mAb (2.4G2; BD Pharmingen). Anti-mouse CD3ε-PerCP (145-2C11;