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Intravaginal infection with herpes simplex virus type-2 (HSV-2) generates a functional effector memory T cell population that persists in the murine genital tract

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ABSTRACT

Although the female genital tract is the main portal of entry for sexually transmitted infections in women, we still have limited understanding of the generation, maintenance and characteristics of memory T cells in the local tissue. Here, we utilized a mouse model of intravaginal HSV-2 infection and tetramers against the immunodominant HSV glycoprotein B epitope recognized by CD8+ T cells to examine the generation, maintenance and characteristics of anti-HSV memory T cells in the genital tract following acute infection. Our results show that the highest percentage of HSVgB-specific CD8+ T cells was found in the genital tract compared to the spleen or iliac lymphnode. Indeed, although the actual number of CD8+ T cells contracted following viral clearance, approximately one quarter of the CD8+ population that remained in the genital tissue was HSVgB-specific. Memory gB-tetramer + CD8 T cells in the genital tract were positive for CD127 and KLRG1 and negative for CD62L and CCR7, thus confirming that HSV-specific CD8 cells were effector memory T cells that lack the capacity for homing to lymphoid tissues. Functionally, both memory CD8+ and CD4+ HSV-specific populations in the genital tract produced IFNγ when stimulated *in vitro* and CD4+ cells also produced TNFα. Genital HSVgB-specific memory T cells expressed tissue-homing integrins CD103 (αE integrin) and CD49a (VLA-1 or α1 integrin). Our findings suggest that HSV-specific memory T cells are retained in the genital tract, poised to act as an early line of defense against future virus encounter.

1. Introduction

It is well established in genital HSV-2 infection that T cells contribute to the clearance of both virus and herpetic lesions through the production of gamma interferon (IFNγ) and in part by cytolytic mechanisms (Milligan et al., 2004; Dobbs et al., 2005). In addition to viral clearance, memory CD8+ T cells control herpes virus reactivation and latency (Liu et al., 2000; Van Lint et al., 2005). Persistence of HSV-specific CD8+ T cells at the site of virus replication has been reported in both HSV-1 infections in mice (Van Lint et al., 2005) as well as genital herpes infections in humans where they are believed to play an important role in immune control of viral latency and reactivation (Zhu et al., 2007). In genital infections with other sexually transmitted pathogens such as the simian/human immunodeficiency virus (SHIV), persistence of anti-viral T cells at the vaginal mucosa is also correlated with protection (Genesca et al., 2008, 2009). Despite the relevant role of genital T cells in mediating immunity against sexually

Abbreviations: gB-tet, HSV-2 glycoprotein B H-2K^b tetramer; HSV-2, herpes simplex virus type 2; IFNγ, interferon gamma; iLN, iliac lymph node; IVAG, intravaginal; TEM, T effector memory; TK-HSV-2, thymidine kinase deficient HSV-2.

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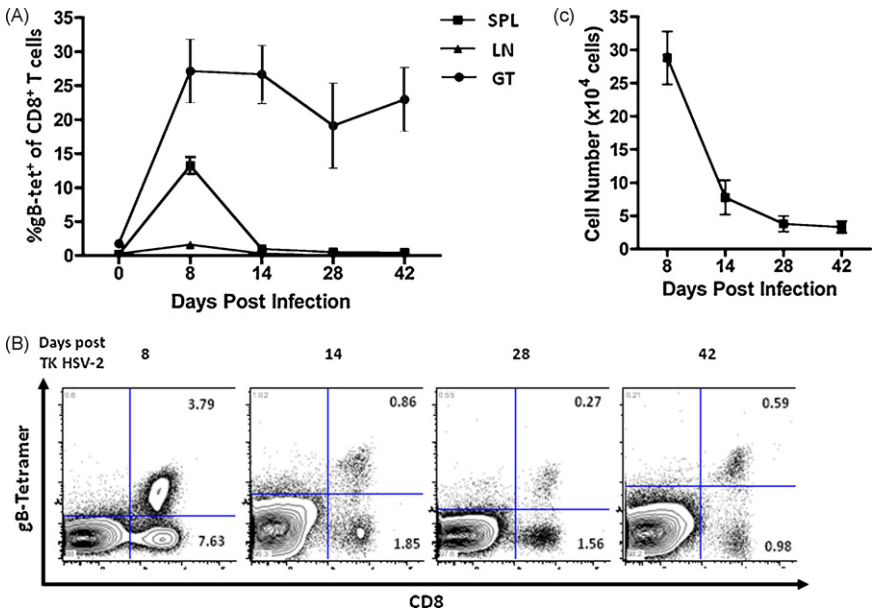

Fig. 1. HSVgB-specific CD8+ T cells persist at high frequency in the genital tract after IVAG infection with TK-HSV-2. (A) The frequency of HSV-specific CD8+ T cells in the genital tract (GT), iliac lymph nodes (LN), and spleen (SPL) as indicated by percent gB-tet+ of total CD8+ T cells in each tissue. (B) Dot-plot of representative graph of gB-tet+ CD8+ T cells in the genital tract for each time point. (C) Total number of gB-tet+ CD8+ T cells in the genital tract over 42 days post-infection. $N = 5$ at each time point.

transmitted infections (STI), these lymphocyte populations remain poorly characterized.

Effector memory T (TEM) cells lack the expression of lymphoid homing markers such as CCR7 and CD62L and are found at mucosal and peripheral tissues such as the lung, gut, and skin (Sallusto et al., 1999; Masopust et al., 2001). These memory T cells of the periphery express integrins that allow for homing and trafficking into tissue and mucosa surfaces. Both CD103 (αE integrin) and VLA-1 (α1 integrin) are found on memory T cells that reside in tissues that include nerves (Gebhardt et al., 2009), lung airway (Ray et al., 2004), gut (Cepek et al., 1994), and skin (Pauls et al., 2001). It is unclear whether memory T cells in the genital tract also express these markers for tissue homing.

Genital herpes (HSV-2) is one of the most common sexually transmitted infections (STI) in the world; affecting on average 17.2% of adults in the US, with higher prevalence in females (23% vs. 11%) (Xu et al., 2006). This life-long infection is characterized by periods of virus reactivation, which can result in the formation of lesions at the genital epithelium, and quiescence when latency is established within nerves. There is currently no efficacious vaccine for the prevention of genital herpes infection (Stanberry et al., 2002). Success in future STI vaccine endeavors will likely depend on further understanding of the generation and nature of protective immune responses at the genital mucosa.

Using a mouse model for intravaginal (IVAG) HSV-2 infection, we identified a population of HSV-specific TEM cells that reside in the genital tract of mice after clearance of IVAG HSV-2 infection. These cells expressed the mucosal homing integrins CD103 and VLA-1. Genital memory CD8+ T cells produced IFNγ while memory CD4+ T cells produced both IFNγ and TNFα when stimulated *in vitro* with HSV antigens.

2. Materials and methods

2.1. Mice

Five to six week-old-female C57BL/6 mice were purchased from Charles River Laboratories (St. Constant, QC, Canada). A 12 h light and 12 h dark cycle was used in the maintenance of mouse colonies. All animal research was conducted in accordance with the Animal Research Ethics Board (AREB) of McMaster University. Mice were injected subcutaneously with 2 mg of Depo-Provera (Upjohn, Don Mills, ON, Canada) 5 days prior to IVAG infection.

2.2. Virus infection

Mice were anesthetized with ketamine (150 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally (IP) and inoculated intravaginally with 10^5 pfu of attenuated thymidine kinase deficient herpes simplex virus type 2 (TK^- HSV-2) in a 10 μl volume.

2.3. Tissue harvest and lymphocyte isolation

Genital tracts were removed and processed individually. Tissues were first minced using a scalpel blade followed by enzymatic digestion with collagenase A (Roche, Laval, QC) in 10% RPMI at a concentration of 1.67 mg/ml at 37 °C, while shaking for 2 h. The resulting single-cell suspension was filtered through a 40 μm nylon mesh to remove tissue debris and washed either with 0.2% bovine serum albumin (BSA) in PBS prior to staining for flow cytometry or 10% RPMI for *in vitro* stimulation. Single-cell suspensions were prepared from spleen and iliac lymph nodes by pressing tissue through nylon mesh. ACK (ammo-