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Transcriptional Analysis of the Guinea Pig Mucosal Immune Response to Intravaginal Infection with Herpes Simplex Virus Type 2

Ronald L. Veselenak[a,b], Gregg N. Milligan[a,b,c], Aaron L. Miller[b], Richard B. Pyles[a,b,c], Nigel Bourne[a,b,c,*]

[a] Department of Microbiology and Immunology, University of Texas Medical Branch, 301 University Blvd, Galveston, Texas, USA 77555
[b] Department of Pediatrics, University of Texas Medical Branch, 301 University Blvd, Galveston, Texas, USA 77555
[c] Sealy Center for Vaccine Development, University of Texas Medical Branch, 301 University Blvd, Galveston, Texas, USA 77555

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ABSTRACT

Genital herpes infection in guinea pigs closely models human infection but tools for immune characterization are limited. Immunity to HSV infection at the vaginal epithelial surface was characterized in guinea pigs using PCR-based array analysis of vaginal swab samples. IFNγ was one of the most significantly upregulated genes throughout the infection and over 40% of genes with significantly altered expression were linked to IFNγ based on INTERFEROME analysis. IFNγ transcripts and biologically active IFNγ at the genital mucosa were confirmed by RTPCR and IFNγ reporter cells. Gene ontology analysis revealed activation of many biological processes related to genital immunity shared by humans and mice demonstrating the similarities of the local immune response to primary genital HSV-2 infection in guinea pigs and other established models. This transcription-based array will be useful for dissection of immunity during reactivation from latency, an infection outcome that is not well recapitulated by other animal models.

1. Introduction

Worldwide it is estimated that more than 536 million people are infected with herpes simplex virus type 2 (HSV-2), the leading cause of genital herpes making this one of the most prevalent sexually transmitted infections (Looker et al., 2008). Initial genital HSV-2 infection can result in painful skin or mucosal lesions, but may also be asymptomatic. During the initial infection virus moves to the ganglia of innervating neurons and a lifelong latent infection is established (Shin and Iwasaki, 2013; Whitley and Roizman, 2001). Periodic reactivation from this latent virus pool results in episodic recurrent lesions and more frequently shedding of virus into the genital tract without clinical signs (Schiffer et al., 2009; Tronstein et al., 2011). Virus shed asymptomatically in this way is believed to be a major source of transmission to susceptible partners (Schiffer et al., 2014; Tronstein et al., 2011) and complicates efforts to control the spread of the disease. In addition, genital HSV-2 infection has been shown to increase the risk of HIV acquisition by over three-fold in both men and women (Freeman et al., 2006). Thus, genital HSV-2 infection represents a significant worldwide public health concern.

There are currently no licensed prophylactic vaccines to prevent genital herpes, or for therapeutic vaccines to reduce recurrent disease and transmission. Further, although suppressive antiviral therapies exist for the treatment of genital herpes, studies have shown that not even high-dose regimens are completely effective in abrogating HSV-2 transmission (Johnston et al., 2012). Thus, more effective methods to prevent and control genital herpes are clearly needed.

Much of our understanding of local immune responses to genital HSV-2 infection derives from studies in mice for which a wide variety of immune reagents are available. However, genital HSV-2 infection of mice does not accurately reflect the self-limiting vesiculo-ulcerative disease experienced by humans. Murine infection is frequently lethal and surviving animals do not experience spontaneous reactivation of latent virus. By contrast, genital HSV-2 infection of guinea pigs results in a self-limiting vulvovaginitis with neurologic and urologic complications that closely mirror those found in human disease. In addition, guinea pigs, like humans, undergo spontaneous, intermittent reactivation of virus that may manifest as episodic erythematous and/or vesicular lesions on the perineum or as asymptomatic recurrences characterized by shedding of virus from the genital tract. Thus, the guinea pig model of genital HSV-2 infection has many advantages over the mouse. However, the ability to study the developing immune response

* Corresponding author.
E-mail addresses: rlvesele@utmb.edu (R.L. Veselenak), gnmillig@utmb.edu (G.N. Milligan), aamiller@utmb.edu (A.L. Miller), rbpyles@utmb.edu (R.B. Pyles), nibourne@utmb.edu (N. Bourne).

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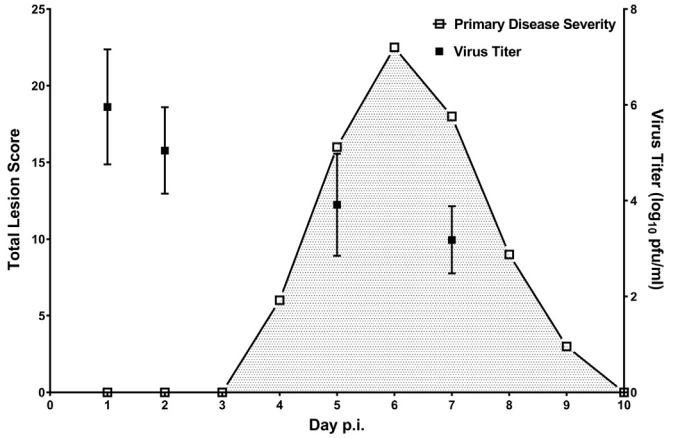

Fig. 1. Course of vaginal virus replication and genital skin disease during primary genital herpes in guinea pigs. Female Hartley guinea pigs (N = 18) were inoculated intravaginally with 6.0 log_{10} pfu HSV-2. Virus titers (Mean ± SD; ■) in vaginal swab samples collected from all animals were highest on Day 1 p.i. subsequently decreasing through the observation period. Genital skin lesions (□) were first detected on Day 4 p.i. increased in severity until Day 6 p.i. after which they began to heal resolving by Day 10 p.i.

has been limited due to the paucity of immune reagents and assays available in this species. To address this problem we and other investigators have been working to develop new immune assays and reagents for use in the guinea pig. In the studies described here we used a recently developed custom guinea pig PCR array (gpArray) (Veselenak et al., 2015) to measure vaginal changes in expression of gene targets including cytokines, chemokines, and cell-specific surface markers throughout primary genital herpes infection in the guinea pig.

2. Results

2.1. Gene Expression Following Genital Challenge with HSV-2

Eighteen outbred Hartley guinea pigs were inoculated intravaginally with HSV-2. All of the animals developed genital infection as confirmed by recovery of virus from vaginal swabs collected on the first 2 days post-inoculation (p.i.). Fig. 1 shows that vaginal virus titers were highest on day 1 p.i. (5.96 ± 1.2 log_{10} pfu/ml), dropped significantly on day 2 p.i. (5.05 ± 0.9 log_{10} pfu/ml; $P < 0.05$, Student's t test) and continued to decrease on following days sampled so that no virus was detected in vaginal swabs on day 10 p.i. Fifteen of the eighteen animals developed vesiculo-ulcerative primary genital skin disease with lesions being detected beginning on day 4 p.i., peaking in severity on day 6 p.i. and resolving by day 10 p.i. (Fig. 1).

We characterized the host immune response at the vaginal epithelium during the primary HSV-2 infection in the animals using a 96 gene PCR-based gpArray that included genes for cytokines, chemokines and cell surface markers for immune cell subsets (Veselenak et al., 2015). Vaginal swab samples were collected from all 18 animals on multiple days through primary infection and compared to pre-challenge samples collected from the same guinea pigs so that each animal served as its own control. To characterize the cell populations present at the vaginal mucosal surface, cells obtained from vaginal swabs taken prior to infection and on days 2 and 6 p.i. were collected by cytospin and stained with a differential stain. Epithelial cells were the predominant cell type present in swabs from uninfected animals (Fig. 2). A large inflammatory infiltrate with occasional epithelial cells comprised vaginal swab cells obtained at day 2 p.i. whereas the vast majority of cells obtained at day 6 p.i. were immune infiltrate cells. The infiltrating immune cell populations included both mononuclear and polymorphonuclear inflammatory cells on both days p.i. The data were initially analyzed as a heat map showing changes in gene expression measured by the ΔΔCt

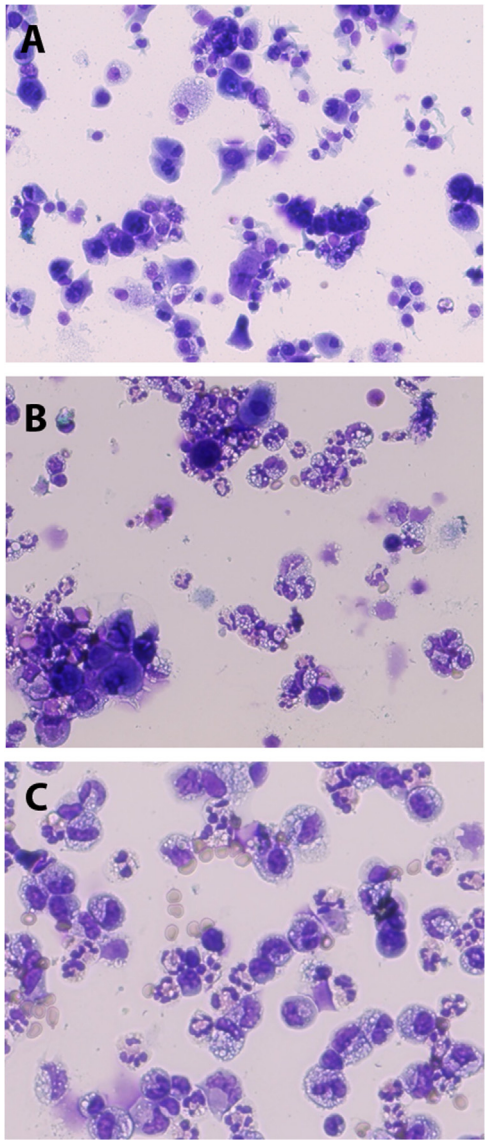

Fig. 2. Changes in the cellular composition of vaginal swab samples during primary genital herpes in guinea pigs. Cell preparations obtained from vaginal swab samples collected A) Before virus challenge showing predominantly epithelial cells. B) Day 2 p.i. occasional epithelial cells are again seen but with a large inflammatory cell infiltrate and C) Day 6 p.i. immune infiltrate cells represent the majority of cells seen. Cell morphologies consistent with granulocyte, monocyte/macrophage, and natural killer cell/ lymphocyte populations were present on both days p.i.

value (Supplemental Fig. 1). Subsequently we analyzed the data generated looking at the number of genes with significantly altered expression ($p < 0.05$, Student's t test) on each day tested grouped by fold change (Fig. 3). The number of genes showing significantly altered expression was greatest on day 1 p.i. (N = 45). Subsequently, the number remained relatively constant on days 3, 5, and 7 (39, 36, and 43 genes, respectively) but decreased to 30 on day 10 at the time when the genital skin disease had resolved. Additionally, over 50% of the genes in which expression was significantly changed were downregulated on all of the days examined except day 10.

Nine genes had significantly altered expression throughout the entire primary infection period. Three of these (IFNγ, CXCL10 and CXCL11) were up-regulated while six (superoxide dismutase [SOD1], CCL20, TLR-6, type I interferon receptor 1 [IFNAR1], bactericidal/ permeability-increasing protein [BPI], and IL-16) were down-regulated. The changes in expression for the top 25 differentially expressed genes