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Cutting Edge: Plasmacytoid Dendritic Cells Provide Innate Immune Protection against Mucosal Viral Infection In Situ

Jennifer M. Lund, Melissa M. Linehan, Norifumi Iijima, and Akiko Iwasaki

Plasmacytoid dendritic cells (pDCs) are well known for their ability to recognize and respond to a variety of viruses (1). The pDCs recognize viral genomic nucleic acids of dsDNA viruses (2–5) and ssRNA viruses (6–8) via TLR9 and TLR7, respectively, in the acidified endosomes without becoming infected themselves. The importance of pDCs in innate defense against parenteral virus infections in vivo has been demonstrated by several recent studies (9–11). However, the role of pDCs in providing the first line of defense upon natural mucosal viral infection at the local sites remains unknown. Furthermore, although pDCs do not directly activate naive lymphocytes upon immunization (12, 13), their role in the initiation of adaptive immunity against viruses by activating conventional DCs (cDC) has been implicated (14, 15).

Despite their well-known ability to circulate systemically and to enter lymph nodes (LNs) and other lymphoid organs (16–20), the capacity of pDCs to survey the peripheral tissues and to secrete type I IFNs at the local site of infection is less well understood. pDCs have been detected in the liver, lungs (17, 21), and the Peyer’s patches (22, 23). In the lungs, pDCs have been shown to prevent Th2-mediated asthmatic reactions to inhaled noninfectious Ags (24). However, the migration of pDCs to the lamina propria of mucosal tissues and their ability to provide antiviral protection is unknown. The homing capacity of pDCs to the genital mucosal tissues is expected to be crucial in the defense against sexually transmitted viruses such as HIV type 1 and HSV-2.

In this study, we examined the role of pDCs in both the innate defense against genital HSV-2 challenge, as well as the generation of adaptive immunity. We focused specifically on the role of pDC in the development of Th1 responses, which is required for providing protection against genital herpes (25, 26). Furthermore, we examined the importance of innate recognition of HSV-2 via TLR9. Although pDCs recognize HSV-2 exclusively via TLR9 (4), many other cell types express TLR9 and respond to dsDNA viruses (11). Thus, comparison of the innate antiviral immunity in pDC-depleted and TLR9-deficient mice allowed us to dissect the importance of pDCs and non-pDCs in providing protection to HSV-2 challenge in a TLR9-dependent manner.

Materials and Methods

Animals

Female B6129PF2/J (wild type (WT); The Jackson Laboratory) and Tlr9tm1Aki (F2 generations of mixed 129P2/OlaHsd, 129X1/SvJ, and C57BL/6 background) (27) mice were bred in the Yale animal facility. All procedures used in this study complied with federal guidelines and institutional policies of the Yale Animal Care and Use Committee.

Virus and infection

For HSV-2 infection, mice were infected intravaginal (ivag) with 104 PFU of WT (186syn +) HSV-2 (28) as previously described (29). Disease severity was assessed using a pathology scoring system as previously described (30). Disease severity was assessed using a pathology scoring system as previously described (30). For some

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3 Abbreviations used in this paper: pDC, plasmacytoid DC; cDC, conventional dendritic cell; LN, lymph node; DLN, draining LN; ivag, intravaginal; p.i., postinfection; WT, wild type; mPDCA, murine plasmacytoid DC Ag; KO, knockout; PAMP, pathogen-associated molecular pattern.
experiments pDCs were depleted by i.p. injection of 200 μg of either rat IgG (isotype control; Jackson ImmunoResearch) or 120G8 (16) (AbCys S.A.) starting 3 days before infection and every other day thereafter until the conclusion of the experiments. Similar results were obtained when anti-murine plasmacytoid DC Ag 1 (mPDCA-1) (Milenyi Biotec) was used (data not shown). For other experiments, NK cells were depleted three times at −3, −1, and 1 day post-infection (p.i.) by i.p. injection of 200 μg in 200 μl of anti-NK1.1 (eBioscience). Vaginal washes were collected at various time points and assayed for virus titer on Vero cells. The levels of murine IFN-α and IL-12p40 in vaginal washes were measured by ELISA as described (4).

Vaginal cell staining

Vaginas were harvested from mice and treated for 1 h at 37°C with 2 U/ml dispase II (Roche Applied Sciences). Tissues were then minced and treated with 0.02% EDTA/0.05% trypsin (Invitrogen Life Technologies) for 30 min at 37°C. Cells were filtered to obtain single cell suspensions and stained with anti-mPDCA-1 (Milenyi Biotec), anti-CD11c (BD Biosciences), anti-CCR5 (eBioscience), and anti-CXCR3 (R&D Systems) Abs. Leukocytes were gated based on forward and side scatter properties, and live cells were gated based on 7-aminoactinomycin D exclusion.

Cell preparation and stimulation

CD4+ T cells (105) were cultured with either CD11c+ DCs (5 × 104) or APCs (2 × 105) for 3 days with or without exogenous viral Ags and cytokine production was assessed by ELISA as previously described (29).

Results and Discussion

pDCs provide protection from genital HSV-2 infection

To investigate the role of pDCs in antiviral defense against a physiological mucosal infection, we used a well-established murine model of genital herpes (31). To examine the requirement for pDCs in innate protection against ivag HSV-2 challenge, pDCs were depleted by systemic injection of the pDC-specific Ab 120G8 (16) before infection. In accordance with previous studies (16, 24, 32, 33), injection of 120G8 resulted in ~80% pDC depletion (data not shown). In parallel, we examined the importance of innate recognition of HSV-2 via TLR9 in vivo, in providing innate resistance to mucosal viral challenge. The requirement for TLR9 recognition of HSV-2 (4) and murine CMV (10) for the production of serum IFN-α secretion subsequent to i.v. challenge has been demonstrated. Because pDCs exclusively utilize TLR9 to detect HSV-2 (4), if pDCs are the main cell type required to provide innate protection against HSV-2 genital infection, pDC-depleted and TLR9-deficient mice are expected to share similar clinical outcomes. Indeed, pDC-depleted and TLR9-deficient mice displayed very similar survival curves that were significantly worse than WT pDC-sufficient mice (Fig. 1A). Further, TLR9 knockout (KO) and pDC-depleted mice exhibited similar pathology scores that were consistently worse than WT pDC-sufficient mice (Fig. 1B). These data demonstrated the importance of pDCs, and specifically their capability for HSV-2 recognition via TLR9, in providing innate protection from mucosal HSV-2 challenge. Consistent with this notion, pDCs and TLR9 were required for the suppression of viral replication at the site of infection, as evidenced from the vaginal wash viral titers (Fig. 1C). Compared with the WT pDC-sufficient mice, increased leukocyte infiltration, and more severe inflammation and tissue destruction were observed in pDC-depleted and the TLR9 KO mice 6 days p.i. (Fig. 1D). On average, TLR9 KO mice had more severe tissue destruction and inflammation compared with pDC-depleted mice. Therefore, pDCs, and the ability to recognize HSV-2 via TLR9, are required for prolonged survival, protection from severe pathology, and reduced virus titer at the infection site following genital HSV-2 challenge.

pDCs’ innate antiviral defense is associated with their local secretion of type I IFNs but not IL-12

Next, we examined the mechanism by which pDCs mediate antiviral innate protection against HSV-2 challenge. To this end, we measured local secretion of IFN-α and IL-12 in mice sufficient or deficient for pDCs or TLR9. Analysis of cytokines from the vaginal washes revealed that WT pDC-sufficient mice produced IFN-α at the site of infection starting on day 2 p.i., and its levels remained high through the 3rd day (Fig. 2A). In contrast, both WT pDC-depleted and TLR9 KO mice produced...
only very low levels of vaginal IFN-α in the first 4 days of infection (Fig. 2A), thereby demonstrating that pDCs are the predominant cell type producing IFN-α in the vagina following ivag HSV-2 infection in a TLR9-dependent manner. Although pDCs can secrete IL-12 in response to HSV-2 in vitro (4), other cell types such as cDCs are known to respond to HSV-1 and secrete IL-12 in vitro (3). Upon examination of the vaginal washes, WT pDC-sufficient mice were found to produce high levels of IL-12p40, and this response peaked at day 3 p.i. (Fig. 2B). However, in contrast to the IFN-α, WT pDC-depleted mice were also able to mount this robust IL-12p40 response (Fig. 2B). Moreover, pDC secretion of IFN-α is always coupled to IL-12 in response to HSV-2 ex vivo (data not shown), eliminating the possibility that residual pDCs contribute to the observed IL-12. Thus, a non-pDC cell type is responsible for producing the majority of the IL-12 at the site of infection following vaginal HSV-2 infection. Interestingly, IL-12p40 production was completely abrogated in the TLR9 KO mice (Fig. 2B), indicating that the non-pDC cell type, such as cDCs, recognizes HSV-2 infection in a TLR9-dependent manner and secretes IL-12. However, IL-12 secretion in the absence of pDCs was not sufficient to provide protection from genital herpes disease (Fig. 1). In addition, depletion of NK cells did not affect survival, pathology, or viral replication following HSV-2 challenge (unpublished observations), indicating that the pDC-mediated protection does not rely on NK cells. These data demonstrated that pDCs and TLR9 are both required for the robust vaginal IFN-α response (Fig. 2A) and suppression of local viral replication (Fig. 1C).

**pDC recruitment to the peripheral site of infection**

The observation that pDCs were important for innate defense against mucosal HSV-2 challenge prompted us to examine their recruitment to the site of infection. Because the viral pathogen-associated molecular patterns (PAMPs) associated with HSV-2 following ivag infection are restricted to the vaginal mucosa and are not detectable in the draining lymph node (DLN) (29), for pDCs to exert their anti-viral effects through TLR9, they must somehow come in contact with viral infection in the peripheral mucosa. Therefore, we examined the presence of the pDCs in the vaginal tissues both at steady state and following HSV-2 infection. These results revealed that a low frequency of pDCs was present in the vagina of naive mice at steady state (Fig. 3). The pDCs in the vagina exhibited the expected phenotype, in that they expressed intermediate levels of CD11c (9, 34) and high levels of mPDCA-1. As early as 1 day p.i., the pDC frequency at the site of infection doubled, and continued to increase for up to 4 days p.i. (Fig. 3A). mPDCA-1^-CD11c^ cDCs were also found in the vagina at steady state but increased following ivag HSV-2 infection (Fig. 3A). Therefore, pDCs were found to be present in the genital tissues of mice before the virus infection, and they were further recruited to the site of infection along with cDCs (29). Because pDCs have been shown to utilize chemokine receptors, CXCR3 (20) and CCR5 (35), we analyzed the expression levels of these receptors on the vagina-recruited pDCs at 3 days p.i. These data demonstrated that both CCR5 and CXCR3 were expressed by the recruited pDCs (Fig. 3B). In contrast, splenic pDCs predominantly expressed CXCR3 but not CCR5. Since the ligands for these chemokine receptors are highly expressed in the vaginal tissue upon infection (data not shown), these receptors might be important for pDC recruitment to the vaginal tissue.

**pDCs are not required for adaptive Th1 immunity in the DLN following genital HSV-2 infection**

In the final set of experiments, we investigated the requirement for pDCs in the generation of the protective Th1 response in the DLN subsequent to infection. Mice were depleted of pDCs before and during infection, and at 2, 3, and 4 days p.i., the IFN-γ response from CD4^+ T cells from the DLN was assessed. CD4^+ T cells from the DLN of ivag HSV-2 infected intact and pDC-depleted mice secreted similar levels of IFN-γ in response to HSV-2 Ag beginning at 3 days p.i. ex vivo (Fig. 4A). Thus, pDCs were not required for the Th1 differentiation in the DLN following ivag HSV-2 infection. Although normal Th1 differentiation was observed in the absence of pDCs, it was formally possible that pDC depletion resulted in an impairment of the LN cDCs in Ag presentation in vivo (15). To examine the potential effects of pDC depletion on Ag presentation by cDCs, DLN cDCs from pDC-sufficient or pDC-depleted mice were isolated at 2, 3, and 4 days following ivag HSV-2 infection. These cDCs were used to re-stimulate HSV-2-primed CD4^+ T cells, and IFN-γ secretion from CD4^+ T cells was assessed. The cDCs isolated from the DLN of the pDC-sufficient or pDC-depleted mice were found to present in vivo-derived viral peptides and induce similar IFN-γ secretion from CD4^+ T cells at all time points tested (Fig. 4B). Furthermore, in the presence of exogenous HSV-2 Ag, cDCs isolated from pDC-sufficient and pDC-depleted mice induced similar levels of IFN-γ secretion from HSV-2-primed CD4^+ T cells (Fig. 4C). Thus, pDCs were neither required to help the LN cDCs for their Ag-presenting capacity, nor for any other aspects of the generation of adaptive Th1 immunity in the DLNs following genital HSV-2 infection. These results differ from those published by Yoneyama et al. (15), in which they...
The authors have no financial conflict of interest.

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