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NKT cell activation by local α-galactosylceramide administration decreases susceptibility to HSV-2 infection

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ABSTRACT

NKT cells are a subgroup of T cells, which express a restricted TCR repertoire and are critical for the innate immune responses to viral infections. Activation of NKT cells depends on the major histocompatibility complex-related molecule CD1d, which presents bioactive lipids to NKT cells. The marine sponge derived lipid αGalCer has recently been demonstrated as a specific agonist for activation of human and murine NKT cells. In the present study we investigated the applicability of αGalCer pre-treatment for immune protection against intra-vaginal HSV-2 infection. We found that C57BL/6 WT mice that received local pre-treatment with αGalCer prior to intra-vaginal HSV-2 infection had a lower mean disease score, mortality and viral load in the vagina following infection, compared to mice that did not receive αGalCer pre-treatment. Further, we found increased numbers of CD45 and NK1.1 positive cells in vaginal tissue and elevated levels of IFN-γ in the vaginal tissue and in vaginal fluids 24 h after αGalCer pre-treatment. Collectively our data demonstrate a protective effect of αGalCer induced activation of NKT cells in the innate immune protection against viral infection.

Introduction

NKT cells are a subgroup of T-cells, which express the NK cell surface marker NK1.1 and a restricted TCR repertoire recognizing glycolipids and self-peptides presented by the major histocompatibility complex class 1-related glycoprotein CD1d (Fowlkes et al., 1987; Makino et al., 1995; Issazadeh-Navikas, 2012; Liu et al., 2011a). Besides being expressed on professional APCs a recent report has shown that CD1d is also expressed on epithelial cells in the human vagina (Kawana et al., 2008). CD1d can present exogenously administered glycolipid antigens such as αGalCer, but will also present endogenously produced immune activating glycolipids in response to Toll-like receptor signalling (Koch et al., 2005; Zhou et al., 2004). Activated NKT cells release considerably high amounts of cytokines, IL-4 and IFN-γ (Matsuda et al., 2008; Kronenberg and Gapin, 2002; Stetson et al., 2003) in particular, and can induce immune activation of other cell types such as macrophages, neutrophils, NK, B, and T cells (Van Kaer et al., 2013; Taniguchi et al., 2003; Carnaud et al., 1999; Eberl and MacDonald, 2000). In addition, NKT cells have cytolytic activities, which depend on the expression of granzyme B, perforin, and FasL (Wu et al., 2009).

αGalCer is a naturally occurring glycolipid first discovered in the marine sponge *Agelas Mauritianus* (Natori et al., 1993). Initially, αGalCer was demonstrated to have tumour growth inhibiting activities and to stimulate lymphocytic proliferation (Kobayashi et al., 1995; Natori et al., 1994). The immune activating properties of αGalCer were furthermore shown to increase resistance to virus infections and to have adjuvant effects for induction of protective immunity (Johnson et al., 2002; van Dommelen et al., 2003; Ho et al., 2008; Kakimi et al., 2000; Lindqvist et al., 2009). These properties depend on CD1d presentation of αGalCer to NKT cells (Kawano et al., 1997; Kobayashi et al., 1996).

Abbreviations: αGalCer, α-galactosylceramide; CXCL10, C-X-C motif chemokine 10; FCS, fetal calf serum; HSV-1, herpes simplex virus 1; HSV-2, herpes simplex virus 2; IMDM, Iscove's modified Dulbecco's medium; HIV, human immunodeficiency virus; KO, knock out; MEM, Eagle's minimal essential medium; mRNA, messenger ribonucleic acid; IFN-γ, interferon-γ; IL-4, interleukin-4; NK cell, natural killer cell; NKT cell, natural killer T cell; p.i., post infection; PBS, phosphate buffered saline; RT-PCR, reverse transcription polymerase chain reaction; WT, wild type.

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HSV-2 belongs to the family *Herpesviridae* and replicates in permissive cells of epithelial linage causing mucocutaneous lesions as seen in human genital herpes infection (Greenwood, 2012). Following primary infection of epithelial cells HSV-2 establishes lifelong latency in sensory neurons (Gupta et al., 2007). Reactivation from the latent sensory neurons causes recurrent symptoms such as genital lesions, but it can also be asymptomatically and only result in viral shedding (Gupta et al., 2007). In addition, HSV-2 is considered a major risk factor for acquisition of HIV (Freeman et al., 2006).

The role of NKT cells in immune protection against HSV infections has been studied using CD1d KO mice and NKT/NK cell deficient IL-15 KO mice. Both CD1d KO mice and mice lacking NKT/NK cells have impaired clearance of HSV-1 and HSV-2 and develop a more severe disease when infected with these viruses (Grubor-Bauk et al., 2003; Ashkar and Rosenthal, 2003). Furthermore, it has been demonstrated that intra-vaginal immunization with HSV-2 glycoprotein combined with αGalCer generates potent IFN-γ and HSV-2 glycoprotein specific lymphoproliferative responses in the genital lymph nodes (Lindqvist et al., 2009). Finally, it has been shown that HSV-1 affects the presentation of αGalCer to NKT cells by impairing the CD1d recycling from the lysosome to the plasma membrane (Yuan et al., 2006). In this study we investigated if activation of NKT cells by local application of αGalCer affected the susceptibility to vaginal HSV-2 infection in mice. We found that a single intra-vaginal pre-treatment with αGalCer lowered the mean disease score and vaginal viral load and increased overall survival when mice were subsequently challenged with HSV-2. αGalCer pre-treatment furthermore increased the number of CD45 and NK1.1 positive cells in vaginal tissue and the levels of IFN-γ in vaginal tissue and in vaginal fluids. In addition we report that CD1d KO mice, displayed a higher disease score than WT mice after intra-vaginal HSV-2 infection. In conclusion, we have demonstrated that activation of NKT cells by local administration of αGalCer provides increased immune protection against vaginal HSV-2 infection. Further, the immune protection was likely caused by a αGalCer induced production of IFN-γ in and recruitment of leukocytes to the vaginal tissue.

Materials and methods

Ethics

All animal experiments were approved by Danish Government authorities (The Ministry of Food, Agriculture and Fisheries) and comply with Danish law.

Animals

C57BL/6 mice were purchased from Taconic M&B, Denmark. The CD1d KO mice and their wild type littermates in C57BL/6 background were provided by Shohreh Issazadeh-Navikas, Neuroinflammation Unit, Biotech Research and Innovation Centre, University of Copenhagen, Denmark. Mice were housed in the animal facility at Aarhus University in accordance with institutionally approved protocols.

Reagents

Synthetic αGalCer (KRN7000) purchased from Avanti Polar Lipids was dissolved in PBS with 33% DMSO and 0.5% Tween20 to a concentration of 0.33 μg/ml. As "mock" solution we used PBS with 33% DMSO and 0.5% Tween20.

IFN-γ and CXCL10 levels were measured in vaginal washes using DuoSet ELISA Development kits (R&D Systems) in accordance with the manufacturers recommendations.

The murine intra-vaginal model

Mice were pre-treated with Depo-Provera (Pfizer) injected subcutaneously. 5 days later mice were anesthetized with isoflurane (Baxter) and inoculated intra-vaginally with αGalCer solution or with HSV-2. From here on three different setups were used:

(1) 1 and 2 days after αGalCer treatment or HSV-2 infection vaginal fluids were collected by pipetting IMDM in and out of the vagina. Washes was put on dry ice immediately and stored at −70 °C until analysis. Unstimulated controls in αGalCer experiments received PBS with 33% DMSO and 0.5% Tween20 (mock).
(2) 1 day after αGalCer treatment the mice were sacrificed by cervical dislocation, the vaginas extracted and prepared for flow cytometry or mRNA extraction.
(3) 1 day after αGalCer treatment the mice were inoculated intravaginally with HSV-2. Mice were monitored and scored daily for disease symptoms and survival. Disease severity was scored as: (0) healthy, (1) genital erythema, (2) moderate genital inflammation, (3) purulent genital inflammation and/or bad general condition (lack of activity, pilo erection), (4) hind limb paralysis and/or severe bad general condition. When reaching score 4 mice were sacrificed by cervical dislocation. Uninfected controls received PBS (vehicle). In separate experiments vaginal fluids were collected on day 1 and 2 p.i. as described under (1).

Flow cytometry

Following extraction of the vaginal tissue single cells suspensions were made by cutting the tissue into small pieces and suspending them in collagenase/dispase (Roche) and DNase 1 (Roche). Enzyme activity was stopped by PBS with 0.02% EDTA. Cell suspensions were filtered trough a 70 μm pore size mesh followed by a 40 μm pore size mesh to removed debris. Cells were pelleted by centrifugation, resuspended and blocked with mouse immunoglobulin G (Jackson ImmunoResearch) and anti-mouse CD16/32 (eBioscience). For surface staining we used anti-CD45 (BD Biosciences) and anti-NK1.1 (BD Biosciences). To identify live cells we used LIVE/DEAD® Fixable Dead Cell Stain Kits (Invitrogen). Flow-Count beads (Beckman Coulter) were added immediately before analysis. Flow cytometry was performed using a FACSAria III or LSR Fortessa (BD Biosciences). Data were analyzed using FlowJo version 10.0.7.

IFN-α/β bio-assay

IFN-α/β was measured using a L929 cell-based bioassay. Samples from vaginal washes were plated in successive 2-fold dilutions. Murine IFN-α (R&D Systems) was used as positive control. Samples were UV treated to ensure that virus was inactivated. L929 cells in MEM supplemented with 5% FCS were added. Samples were incubated for 24 h. Vesicular stomatitis virus was added and plates were incubated for 48 h. Plates were analyzed, and the dilution were 50% of the cells were alive was defined as having contained 1 U/ml IFN-α/β.

Vaginal viral titres

For intra-vaginal infection we used HSV-2 strain 333. Viral titres in the vaginal washes from day 1 and 2 were determined by a Vero cell-based plaque assay. Vero cells in MEM supplemented with 5% FCS were plated on Petri dishes and incubated 24 h. Samples in serial dilutions were added. After 1 h. MEM supplemented with 5% FCS and 0.2% human immunoglobulin (CLS Behring) were added.