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Journal of Reproductive Immunology 74 (2007) 114–123

JOURNAL OF REPRODUCTIVE IMMUNOLOGY

www.elsevier.com/locate/jreprimm

Role of IFN-α/β signaling in the prevention of genital herpes virus type 2 infection

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Received 10 April 2006; received in revised form 22 June 2006; accepted 25 September 2006

Abstract

This study has shown that IFN-α/β signaling is crucial for combating primary herpes simplex virus type 2 (HSV-2) infection and for responding to immunotherapy using ligands to TLR3, 7 and 9, but not for vaccine-induced immunity. Both genital viral replication and the disease progression were enhanced in HSV-2-infected mice lacking the IFN-α/β receptor (IFN-α/βR−/−). IFN-α/βR−/− mice were, however, able to mount a normal HSV-2-specific Th1 response and acquired sterilizing immunity following vaccination. Anti-viral treatments using agonists to TLR3, 7 and 9 by administration of synthetic dsRNA, imiquimod and oligonucleotides containing unmethylated CpG motifs, respectively, were strongly dependent on IFN-α/β receptor signaling for their efficacy. Even though all treatments had a weak impact on local vaginal viral replication in infected IFN-α/βR−/− animals, they did not affect disease progression or mortality in these animals as opposed to wild type controls where all three treatments reduced viral replication as well as disease severity and mortality. Lack of IFN-α/βR signaling also blocked production of IFN-γ and TNF-α in response to TLR9 activation. These studies have shown that IFN-α/β receptor signaling is important for multiple events in the anti-viral defense.

Keywords: Interferon; Toll-like receptors; Herpes simplex virus type 2; Cytokines; Protection

1. Introduction

Herpes simplex virus type 2 (HSV-2) is a sexually transmitted pathogen infecting human genital tract mucosa and is the most common cause of genital ulcer disease in humans (Kinghorn, 1994). HSV-2 infects the genital epithelium and can, following vaginal replication, be transmitted to the central nervous system *via* uptake and retrograde transport in sensory neurons. The virus may establish latency in infected ganglia and can therefore give rise to lifelong infection (Richards et al., 1981; Whitley, 2002).

IFN-α/β represent the first line of defense against most viral infections and also affect the subsequent immune response towards a so-called Th1 profile, *i.e.* a response characterized by high production of IFN-γ and generation of cytotoxic T-cells. IFN-α/β are induced in the vast majority of cells in response to viral infections, and often through interaction between viral nucleic acid and specific receptors, *e.g.* TLRs, present in the exposed cell. IFN-α/β act through binding to the IFN-α/β receptor (IFN-α/βR) which enhances production of IFN-α/β and induces activation of several anti-viral pathways in the cell (Malmgaard, 2004). Deficient IFN-α/βR signaling will thus affect both the amount of IFN-α/β produced and the ability to inhibit viral replication. The most prominent TLRs involved in induction of IFN-α/β are TLR3, 7 and 9, which are activated by double-stranded RNA, single-stranded RNA and unmethylated CpG-rich

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0165-0378/$ – see front matter
doi:10.1016/j.jri.2006.09.002

DNA, respectively (Alexopoulou et al., 2001; Doyle et al., 2002, 2003; Krug et al., 2004; Lund et al., 2003).

IFN-α/β have important roles in the early host response against genital HSV-2 infection by repressing viral replication and thus disease progression. Recombinant IFN-α1 or plasmid DNA encoding IFN-α1 administered topically to mice can limit HSV-2 infection in the vagina and also cornea (Harle et al., 2001; Noisakran et al., 1999) in a process that, in outbred ICR mice, requires both CD4+ and CD8+ T-cells (Noisakran and Carr, 2000) and in inbred C57BL/6 mice, CD4+ T-cells that secrete IFN-γ (Carr and Noisakran, 2002). Treating patients with recurrent genital HSV infection with topical IFN-α leads to a more rapid cessation of viral shedding and fewer recurrences (Shupack et al., 1992). The importance of endogenous IFN-α/β production in innate defense against HSV-2 has been shown in mice deficient in IFN-α/βR where there is an accelerated vaginal viral replication (Murphy et al., 2003). This is somewhat surprising since the HSV-2 vhs protein inhibits IFN-α/β-mediated anti-viral resistance (Murphy et al., 2003). However, *in vitro* studies have shown that mouse-strain differences in HSV-2 susceptibility are linked to amounts of IFN-α/β that different strains can produce in response to HSV-2 (Ellermann-Eriksen et al., 1986). The mechanism of IFN-α/β action on HSV replication is not fully known (Ellermann-Eriksen, 2005). Several IFN-α/β systems are involved in anti-viral defense, including, *e.g.* PKR (Al-Khatib et al., 2004), OAS (Al-Khatib et al., 2004) and PML bodies (Chee et al., 2003). PKR is the dominant anti-HSV-2 pathway activated by the IFN-α1 pathway (Carr et al., 2005) and mice lacking functional PKR have an increased viral replication, both *in vivo* and *in vitro* (Khabar et al., 2000; Leib et al., 2000).

HSV is a potent inducer of IFN-α. Different forms and different strains of HSV can induce IFN-α to different extents and through different pathways (Rong et al., 2003). Plasmacytoid dendritic cells and macrophages produce high levels of IFN-α in response to HSV-2. The IFN-α produced by these cells is mediated through either binding of genomic DNA from HSV-2 to TLR9 (Lund et al., 2003; Malmgaard et al., 2004) or *via* binding of the virus to either the mannose receptor (Milone and Fitzgerald-Bocarsly, 1998; Rong et al., 2003) or chemokine receptors CCR3 and CXCR4 (Ankel et al., 1998).

IFN-α/β production can be initiated also by synthetic ligands to toll-like receptors (TLR), *e.g.* binding of poly I:C (polyinosinic–polycytidylic acid salt; synthetic dsRNA) to TLR3, imiquimod (an imidazoquinoline compound) to TLR7 and CpG-ODN (oligonucleotides containing unmethylated CpG motifs) to TLR9 (Alexopoulou et al., 2001; Diebold et al., 2004; Hemmi et al., 2002). Administration of these synthetic agonists to rodents can be used to treat HSV-2 infection. In mice, pretreatment with locally, but not systemically, administered poly I:C protects animals from HSV-2 infection (Ashkar et al., 2004). Imiquimod treatment reduces HSV-2 replication in the vaginal mucosa and spinal cord of guinea pigs (Miller et al., 1999). Treatment with CpG-ODN protects mice from genital HSV-2 infection, and this is associated with induction of the Th1 cytokines IFN-γ, IL-12 and IL-18 (Harandi et al., 2003; Pyles et al., 2002). Whether these treatments require type I interferons for their efficacy is, however, not known.

In this study, we sought to elucidate the requirements for IFN-α/β signaling in control of a primary genital HSV-2 infection, in development of protective Th1-immunity against genital HSV-2 infection, and for the efficacy of anti-viral treatments utilizing known TLR agonists. For this purpose, we used mice lacking the receptor for IFN-α/β in a mouse model of genital HSV-2 infection (McDermott et al., 1984; Parr et al., 1994), and treated the animals with poly I:C, imiquimod and CpG-ODN.

2. Materials and methods

2.1. Mice

Female 6–14-week-old mice were used for all experiments. Naïve SV129 mice (IFN-α/βR+/+ mice) and IFN-α/βR−/− mice (IFN type I receptor knockout mice on a SV129 background; kind gift from Maries van den Broek) (Muller et al., 1994) were used for all experiments. Mice were kept in ventilated cages under pathogen-free conditions at the Department of Experimental Biomedicine, Göteborg University, Sweden. These studies were approved by the ethical committee for animal experiments, Göteborg, Sweden.

2.2. Chemicals

The CpG-ODN 1668 5′> TCC ATG ACG TTC CTG ATG CT <3′ and the GpC-ODN equivalent 5′> TCC ATG AGC TTCC CTG ATG CT <3′ with phosphorothioate backbones were synthesized by Scandinavian Gene Synthesis AB, Köping, Sweden. Imiquimod acetate (imiquimod) was purchased from Sequoia Research Products Ltd., Oxford, UK, and synthetic double-stranded RNA, polyinosinic–polycytidylic acid salt