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Non-pathogenic Sindbis virus causes hemorrhagic fever in the absence of alpha/beta and gamma interferons

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Abstract

The role of interferon-gamma (IFNγ) in antiviral innate immune responses during acute alphavirus infection is not well defined. We examined the contribution of IFNγ to the protection of adult mice from Sindbis virus (SB)-induced disease by comparing subcutaneous infection of mice lacking receptors for either IFNα/β (A129), IFNγ (G129) or both (AG129) to normal mice (WT129). While neither G129 nor WT129 mice exhibited clinical signs of disease, infection of A129 or AG129 mice was fatal with AG129 mice succumbing more rapidly. Furthermore, AG129 mice developed signs of viral hemorrhagic fever (VHF), including extensive hepatocellular damage, inflammatory infiltrates in multiple organs and vascular leakage, which were significantly delayed and/or partially ameliorated during fatal A129 infections. We conclude that: (i) IFNα/β is the primary mediator of innate immunity to SB infection, however; (ii) IFNγ is directly antiviral *in vivo*, acting before the adaptive immune response appears and; (iii) development of VHF may involve viral suppression of both IFNα/β and IFNγ responses.

Keywords: Alphavirus; Sindbis; Interferon; Viral hemorrhagic fever

Introduction

Among the mosquito-borne viruses in the *Alphavirus* genus, family *Togaviridae*, are a number of significant human pathogens that cause explosive outbreaks of febrile illness associated with polyarthritis and occasional hemorrhagic manifestations of varying severity (Enserink, 2006; Laine et al., 2004; Posey et al., 2005). These viruses include chikungunya, Ross River, o'nyong nyong and the Sindbis group viruses, Karelian fever and Okelbo. Although these viruses are causative agents of emerging infectious disease, their biology is poorly understood and neither effective therapeutics, nor licensed vaccines are available for protection of humans.

In nature, the prototypic alphavirus, *Sindbis virus* strain AR339 (SB), is maintained by mosquito-vectored transmission between avian reservoir hosts and, unlike several closely related alphaviruses, it is not believed to be a significant human pathogen (Griffin, 2001). Using this non-pathogenic virus to probe the host's immune response, we seek to elucidate virulence mechanisms employed by arboviral pathogens and explore the premise that virulent arboviruses antagonize and/or evade antiviral mechanisms to which SB is sensitive. When cDNA clone-derived, wild-type SB virus strain TR339 (Klimstra et al., 1998; McKnight et al., 1996) is administered to newborn mice by subcutaneous inoculation, mimicking the bite of an infected mosquito, a rapidly fatal infection ensues reminiscent of systemic inflammatory response syndrome (SIRS; Conti et al., 2004) with uncontrolled pathogen replication and systemic

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hyperinflammatory induction of interferon alpha/beta (IFNα/β), interferon gamma (IFNγ), tumor necrosis factor alpha (TNFα) and interleukin (IL)-6 (Klimstra et al., 1999). The absence of encephalomyelitis in this infection model (Klimstra et al., 1999) is consistent with febrile disease manifestations of arthritogenic alphaviruses in young humans.

Similar to other SB strains (Griffin, 1976; Johnson et al., 1972; Trgovcich et al., 1999), the severity of TR339-induced disease is intriguingly age-dependent (Ryman et al., 2007b), such that TR339 infection of an adult mouse is swiftly controlled by the host's immune system with little or no pathologic damage and induction of long-lasting, protective immunity (Ryman et al., 2000). Although a variety of host-encoded factors have been demonstrated to influence SB virulence and disease (e.g., Griffin et al., 1983; Johnston et al., 2001; Levine et al., 1996; Lewis et al., 1999; Liang et al., 1998), type I IFN has emerged as the critical mediator of defense in naive animals against infection by SB (Byrnes et al., 2000; Gil et al., 2001; Labrada et al., 2002; Ryman et al., 2000, 2002) and, to a lesser extent, other alphaviruses (Muller et al., 1994; White et al., 2001; Aguilar et al., 2005). In mice which lack a subunit of the IFNα/β receptor (A129) and are, therefore, defective in IFNα/β-mediated signaling and IFNα/β-stimulated gene (ISG) upregulation, normally benign TR339 infection becomes rapidly fatal (Ryman et al., 2000), highlighting the pivotal protective role of early IFNα/β responses and the necessity for antagonism/evasion by the pathogen. Absence of IFNα/β activity allows extensive replication in dendritic cells (DCs) which migrate to the lymph nodes draining the inoculation site (DLN) followed by viremic dissemination to myeloid-lineage cells in spleen, liver and other tissues. Infection of these cells correlates with the dysregulated induction of a hyperinflammatory cytokine cascade and rapid mortality. However, if A129 mice are "immunized" by infection with attenuated, cell culture-adapted, heparan sulfate (HS)-binding SB strains (Byrnes et al., 2000; Ryman et al., 2000), subsequent challenge with virulent virus is subclinical, from which we infer that acquired immune response mediators remain operational.

Whereas the ability to produce type I IFN is shared by most cell types, type II IFN (IFNγ) is primarily secreted by activated natural killer (NK) cells, T_H1-biased $CD4^+$ T cells, $CD8^+$ T and NKT cells, with a lesser contribution from DCs and macrophages. IFNγ mediates immunoregulatory effects on both innate and adaptive immune responses by inducing antiviral effectors, activating macrophages and microglia and upregulating antigen presentation machinery. T cells serve to protect non-renewable cells in the central nervous system (CNS) from several neurotropic viruses (Chesler and Reiss, 2002; Keogh et al., 2003) via IFNγ-dependent, non-cytolytic downregulation of viral replication. Furthermore, IFNγ controls levels of hepatitis B virus replication in the liver and mediates clearance by T cells (Guidotti and Chisari, 1999; Guidotti et al., 2002). Intriguingly, IFNγ has recently been implicated in the control of acute infection by other mosquito-borne viruses, dengue and West Nile viruses (Shresta et al., 2004; Shrestha et al., 2006) and acts in concert with antibody to prevent reactivation of SB infection in the CNS after initial clearance (Burdeinick-Kerr et al., 2007).

Here, we have evaluated the role of IFNγ in amelioration of acute SB-induced disease following subcutaneous inoculation, encouraged by interesting, but conflicting observations. We found previously that PKR/RNase L-dependent and independent antiviral activities against SB were potently induced by IFNα/β-mediated or IFNγ-mediated priming of DCs, independently of one another (Ryman et al., 2002). Paradoxically, high-level induction of IFNγ in infected A129 mice appeared to provide little protective effect against lethal disease (Ryman et al., 2000), although Gil et al. demonstrated that fatal infection of AG129 mice by a cell culture-adapted, attenuated SB strain required 100-fold less inoculum than A129 mice (Gil et al., 2001). To determine whether or not IFNγ has antiviral activity *in vivo* in the presence or absence of the type I IFN response, mice lacking either the IFNγ receptor (G129), the IFNα/β receptor (A129) or both receptors (AG129) were infected subcutaneously with TR339 and the pathogenesis of infection was examined.

Absence of IFNγ activity alone resulted in a minor, but not consistently significant, increase in viral burden early during the course of infection; however, both WT129 and G129 mice were protected from clinical disease signs by a functioning IFNα/β response. Mice with combined deficiency in IFNα/β and IFNγ responses exhibited an even more rapid spread of virus infection and more intense hyperinflammatory disease and rapid death as compared with deficiency in the IFNα/β response alone. Moribund AG129 mice exhibited severe pathologies of liver and lymphoid tissues and evidence of vasculopathy, typically associated with VHF in humans (Bray, 2005). These findings are striking in that an avirulent virus can be converted to a virus that causes VHF manifestations by the combined elimination of IFN responses. This result may implicate antagonism of or resistance to IFNγ, as well as IFNα/β, as underlying causes of VHF for some viruses and may provide a mouse model system in which to further study mechanisms of HF development and control.

Materials and methods

Cell lines

Baby hamster kidney (BHK-21: ATCC CCL-10) and L929 murine fibrosarcoma cell lines were maintained in alpha minimal essential medium (αMEM), supplemented with 10% donor calf serum (DCS), 2.9 mg/ml tryptose phosphate, 0.29 mg/ml L-glutamine, 100 U/ml penicillin and 0.05 mg/ml streptomycin (37 °C; 5% CO_2).

Mice

Mice with null mutations in the IFNα/β receptor (A129), the IFNγ receptor (G129) or both the IFNα/β and the IFNγ receptors (AG129) and congenic 129/Sv/Pas mice (WT129) were bred and housed in the Animal Resource Center at Louisiana State University Health Sciences Center (Shreveport, LA) under specific pathogen-free conditions. All procedures were carried out in accordance with the guidelines of the