Mice Transgenic for Simian Immunodeficiency Virus nef Are Immunologically Compromised

Key Words
Simian immunodeficiency virus
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IgG1
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Abstract
An intact nef gene is essential for rapid development of immunodeficiency in human immunodeficiency virus and simian immunodeficiency virus infections. To assess the role of nef in the immune response, mice transgenic for SIV nef were constructed and the humoral and cellular immune response to herpes simplex virus type-1 (HSV-1), measured. Mice transgenic for SIV-mac239 nef exhibited a significantly increased mortality rate when challenged with HSV-1 and also showed unusual antibody kinetics in response to viral challenge. During a 32-week period following exposure to HSV, it was noted that IgG subclass titers continued to rise in the nef+ animals, while titers of nef- animals decreased. Additionally, following secondary challenge with HSV, nef- mice had a significantly greater rise in HSV-neutralizing antibody titers than nef+ mice. A decreased proliferative response to the T cell mitogen, PHA, was noted in the nef+ animals. These results suggest that the presence of nef+ is sufficient to induce immune dysfunction.

Introduction
In 1994, AIDS became the leading cause of death among men 25–40 years of age in the US [3] and it is estimated that 30–40 million people worldwide will contract this disease by the year 2000 [25]. Due to its pandemic nature and the severity of the immunodeficiency syndrome caused by infection, viruses capable of causing AIDS or an AIDS-like syndrome have received considerable attention. Two of these viruses, simian immunodeficiency virus (SIV) which causes an AIDS-like disease in primates [13], as well as human immunodeficiency virus (HIV) itself have been extensively studied. The functions of the genes that make up these viruses have been characterized in an attempt to fully understand the mechanism of pathogenesis as well as possible points in the replication cycle for therapeutic intervention.

One of these genes, nef, is found in all human and simian AIDS viruses [23], indicating a strong selection pressure for the advantageous presence of this gene. Early studies indicated that this gene repressed transcription of the virus, thus it was given the name, negative factor [1, 24]. Nef is nonessential for replication of the virus in tissue culture [18]. However, nef is essential for HIV and
SIV pathogenesis in vivo [8, 14, 33]. Nef has been associated with at least four specific functions: downmodulation of CD4 [2, 10] and MHC I [16, 20, 29], alteration of T cell signaling [19, 24, 31, 32], and the enhancement of viral infectivity [4, 21]. A strong association between the development of immunodeficiency accompanied by disease progression and the presence of nef has been suggested through studies with SIV in animals as well as humans infected with HIV. When primates were infected with SIV with specific mutations in nef, the onset of AIDS was delayed or inhibited [14]. Studies with HIV isolated from humans who have been infected with the virus for at least 10 years but show no signs of disease (i.e. long-term nonprogressors) revealed that the virus in these individuals had deletions in the nef gene [8, 15].

Nef's pathogenic potential may, in part, be related to in vivo replication of the virus. Infection of macaques with SIV containing a functional nef gene results in high lymphoid tissue virus titers, whereas animals infected with a virus deleted in nef have low viral loads [14]. These findings indicate that nef plays a crucial role in natural infections.

Additionally, using a transgenic mouse model containing SIV nef, Kestler [pers. commun.] has shown that nef alone may alter the immune status in these animals since 25% of these animals, but not control wild-type, developed tumors by 12 months of age. Also, when monkeys, which had been inoculated 2 years previously with a non-disease-producing strain of SIV containing a 182-bp deletion in the nef gene, were challenged with a virulent strain of the virus, they were protected and developed no clinical signs of immunodeficiency [6].

Because of the protective effect of mutated nef in the animal model, the existence of long-term nonprogressors infected with HIV with a mutated nef, the increased incidence of tumors in mice transgenic for nef, and the profound effect that SIV and HIV have on the immune system, the studies reported here were designed to examine the effect of nef alone on the immune system. For this purpose, mice transgenic for SIV nef were exposed to herpes virus (HSV) and animal mortality and the humoral as well as cellular responses to the virus examined. Our results indicate that nef+ animals, when compared to nef− animals, were significantly more susceptible to the lethal effects of the virus, mounted a secondary humoral immune response that was less vigorous in its neutralizing ability than in controls, and their cellular immune response had a reduced ability to respond to a T cell mitogen.

Methods

Cell Culture and Virus

African green monkey kidney cells (Vero) and NIH Swiss mouse embryo cells (NIH3T3) were obtained from the American Type Culture Collection, Rockville, Md. Vero cells were propagated in Medium 199 supplemented with 5% fetal bovine serum, 0.075% NaHCO₃, and 50 μg/ml gentamycin sulfate. NIH3T3 cells were propagated in Dulbecco's modified Eagle medium supplemented with 10% bovine calf serum, 0.15% NaHCO₃, and 50 μg/ml gentamycin sulfate. HSV type 1 (HSV-1) used in these studies was isolated from a patient and was shown to be pathogenic in mice [9]. Vero cells were used to prepare HSV-1 pools and in plaque assays. NIH3T3 cells were infected with HSV-1 to prepare viral antigen.

Transgenic Mice

Transgenic mice used in these studies were generated at the National Cancer Institute, Bethesda, Md. by M. Gonda and G. Tobin and the Cleveland Clinic Foundation by J. Salkowitz and H. Kestler. The transgenic strain was constructed on the inbred FVB background [34]. Zygotes were microinjected with a nef expression vector which contained the complete open reading frame of the nef gene from a molecularly cloned strain of SIV mac 239 [13] which had been fused to the human cytomegalovirus immediately early enhancer/ promoter.

The nef+ transgenic mice were identified by extracting DNA from a 1-cm piece of tail and amplifying a nef-specific region by PCR using primers 0SJ47 5'(CTGGAGATCTGCGACAGACTCT) and 0SJ48 (TTCTCTCTTCAGTGGGTTC)3'. Control wild-type FVB mice are referred to as nef−. All mice were housed in microisolator cages with sterile water, food and bedding.

Mortality Studies

Control nef−, as well as transgenic nef+ mice were infected intraperitoneally with HSV-1. Mice which were 6 weeks of age were infected with 1 × 10⁶ plaque-forming units (PFU) of HSV-1, while mice greater than 28 weeks of age were infected with 5 × 10⁶ PFU of HSV-1. The animals were monitored daily for viral effects.

Immunization Studies

A group of nef+ or nef− mice 28 weeks of age were injected with 5 × 10⁶ PFU of HSV-1 in the right rear footpad. The second viral challenge (1 × 10⁷ PFU) was administered subcutaneously 35 days later. A third viral challenge of 1 × 10⁷ PFU was given intraperitoneally 36 weeks after the first injection of HSV-1. Blood was drawn from the orbital sinus of all animals 18 days after the first injection, 7 days after the second injection and 1 week before and 2 weeks after the third injection.

Neutralization Antibody Titer

Serial 2-fold dilutions of immune or control serum were mixed with an equal volume of a virus solution containing 500 PFU of HSV-1. The serum/virus mixture was incubated 1 h at room temperature prior to measuring residual infectious virus by the plaque assay [26]. The reciprocal of the immune-serum dilution, which caused a 50% reduction, relative to control serum, in the number of plaques determined by regression analysis, was the serum titer.