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Mini-Review—The Rabies Virus

The role of immune responses in the pathogenesis of rabies

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In the absence of treatment, infection with a variety of rabies virus strains most often results in a lethal outcome. This can be averted by prompt immunization following exposure demonstrating that the development of anti-rabies viral immunity prior to extensive infection of neurons is protective. Otherwise it might be expected that immune clearance of the virus would result in neurological sequelae. Thus, the capacity of a rabies virus to induce a protective immune response is a major, negative determinant of its pathogenicity and highly pathogenic rabies viruses have characteristics that avoid triggering protective immune responses. On the other hand, there is evidence that certain aspects of immunity may contribute to the pathogenesis of rabies under certain circumstances. The relationship between rabies virus and the immune system of the host is the focus of this review. *Journal of NeuroVirology* (2005) **11,** 88–92.

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Introduction

Historically, bite exposure of humans to rabies virus (RV) was once believed to be invariably fatal. This view was changed by the demonstration by Louis Pasteur in 1885 that prompt vaccination of an individual following exposure could prevent the development of clinical rabies and its lethal outcome. To this day, postexposure treatment of rabies continues to be based on the Pasteur principle of active vaccination against the virus, now supplemented with the passive administration of RV-neutralizing antibodies. The possibility that bite exposure to RV in humans may not always result in death has only been examined once, inadvertently, in a group of individuals bitten by a rabid wolf in Iran in the 1950s. In this instance, 12 of the 24 individuals bitten on the face and neck and 6 of 8 of those bitten on the extremities survived without treatment (Gremliza, 1953). These observations support the hypothesis that the protective immune response to RV is the primary, negative determinant of rabies pathogenesis, and must be avoided for RV infection to be lethal. Evidence supporting this concept will be reviewed in "Protective immunity is the primary, negative determinant of rabies pathogenesis." Contrasting with the protective role of immunity, it has been well established that immune mechanisms, even those generally thought to be protective, often have pathological attributes depending on the extent of the infection when immune effectors come into play. This is likely to be particularly the case for reactions occurring in nervous tissue. The potential contribution of immunity to the pathogenesis of rabies is discussed in "Antiviral immune mechanisms can contribute to rabies pathogenesis."

Protective immunity is the primary, negative determinant of rabies pathogenesis

The fact that an anti-RV immune response elicited after exposure to the virus is capable of preventing rabies suggests that the pathogenicity of a particular RV strain may depend upon its ability to spread without inducing a protective immune response. A key element of this may be the neurotrophism of

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the virus, as central nervous system (CNS) tissues are naturally sequestered from the immune system. Nevertheless, other aspects of the nature of the RV clearly have a major impact on the capacity to induce a protective antiviral immune response, the nature of which has been extensively studied (e.g., Kaplan *et al*, 1975; Xiang *et al*, 1995; Hooper *et al*, 1998; Irwin *et al*, 1999). For example, the immunogenicity of RV strains in animal models is inversely related to the level of rabies glycoprotein that they express (Morimoto *et al*, 1999; Faber *et al*, 2002). Although this is not surprising as the viral glycoprotein is the target for most RV-neutralizing antibodies, rabies glycoprotein has also proven to be a strong inducer of the apoptosis of infected cells, evidently an immunogenic process in rabies (Morimoto *et al*, 1999; Faber *et al*, 2002; Pulmanausahakul *et al*, 2002; Préhaud *et al*, 2003). The contribution of virus-induced apoptosis to the immunogenicity of rabies virus infection is revealed by the fact that a RV engineered to express proapoptotic cytochrome *c* is more immunogenic without increased glycoprotein expression (Pulmanausahakul *et al*, 2002). It is also possible that the rapid death of infected cells, somewhat paradoxically because of increased local neuropathy, may result in less overall morbidity and mortality by interfering with optimal virus replication and spread. Not only could the neural architecture required for virus spread be disrupted but RV particles would also be made available for neutralization by antibody. Thus the amount of glycoprotein made during an infection by highly pathogenic RV strains may be limited to not only reduce its availability as an antigen but also to diminish its contribution to immune stimulation through the induction of neuronal apoptosis. If this is the case, the induction of certain T-cell functions and neutralizing antibody should be reduced in response to infection with RV expressing low levels of glycoprotein. A comparison of immune responses induced by infection of normal mice with CVS-N2c, a highly pathogenic RV strain that expresses low glycoprotein levels, and CVS-B2c, a relatively apathogenic strain that expresses high glycoprotein levels (Morimoto *et al*, 1998, 1999), provides support of this possibility. Infection of mice with CVS-N2c most often results in their death despite the generation of RV-specific T cells and a significant, but non-neutralizing, antibody response (Figure 1). In contrast, infection of similar mice with CVS-B2c elicits both RV- and glycoprotein-specific T cells as well as an antibody response with a higher proportion of neutralizing antibody, and is nonlethal (Figure 1). Notably, a highly pathogenic variant of RV associated with the silver-haired bat also does not induce apoptosis in the brains of experimentally infected mice (Xan *et al*, 2001). Conceivably, a reduction in the immunostimulatory attributes of highly pathogenic RV strains may be related to their lesser impact on the expression of major histocompatibility complex (MHC) class II, an important element of CD4 T-cell recognition of viral antigen,

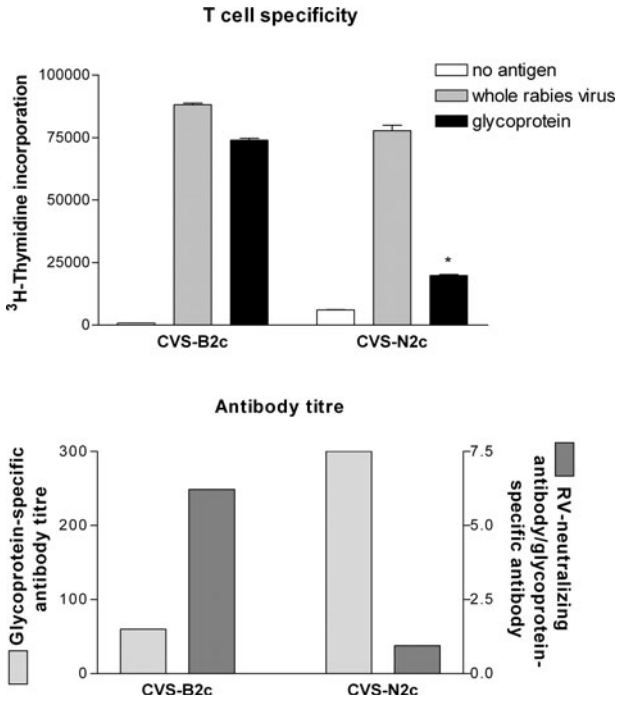

Figure 1 RV-specific immune responses differ in mice infected with CVS-B2c and CVS-N2c, RV strains dissimilar in their levels of glycoprotein expression and host pathogenicity. Mice (8- to 10- week-old-female, C3H/HeJ; 5 per group) were infected intramuscularly (gastrocnemius) with 10^5 focus-forming units (ffu) of the less pathogenic CVS-B2c or highly pathogenic CVS-N2c (see Morimoto *et al*, 1998, 1999, for characterization of these viruses). At 48 h post infection, T cells from lymph nodes draining the site of inoculation and spleen cells were cultured at a 2.5:1 ratio in alpha-MEM supplemented with 0.6% normal mouse serum, and stimulated with either whole UV-inactivated ERA-RV or isolated rabies ERA-glycoprotein. Extensive antigenic cross-reactivity between the infecting and ERA-RV strains was previously confirmed in that immunization with inactivated ERA-RV–elicited T cells and antibodies capable of recognizing both CVS-B2c and CVS-N2c (not shown). At 96 h of culture, replicate wells containing 2.5×10^6 cells were pulsed for 4 h with ^{3}H-thymidine and its incorporation into newly synthesized DNA measured as previously detailed (Kean *et al*, 2000). The results, shown in the upper panel, demonstrate that the response to glycoprotein is significantly lower ($P < .001$ by the *t* test) in mice infected with CVS-N2c. The lower panel shows the ERA-glycoprotein–specific serum antibody titre at 6 days post infection, determined by ELISA (Hooper *et al*, 1998) as the dilution of antibody giving an optical density higher than that of nonimmune plus 4 times the standard deviation. The ratio of the neutralizing antibody titre, determined by the rapid focus-forming inhibition test using ERA-RV (Hooper *et al*, 1998), to the total glycoprotein-specific response is also shown.

in the infected brain (Irwin *et al*, 1999). Infection of mice with an avirulent RV strain (RV194-2) induced MHC class II expression in the brain whereas the pathogenic CVS did not, correlating with the ability of the animals to survive the infection (Irwin *et al*, 1999).

In addition to aspects of RV replication that may limit the induction of protective anti-RV immunity, there are processes mediated by certain pathogenic RV strains that evidently interfere with immunity in general. Progressive RV infection has

long been known to down-regulate cell-mediated immune function (Wiktor *et al*, 1977; Hirai *et al*, 1992). A tumor necrosis factor (TNF)-α p55 receptor–dependent process has been implicated in this mechanism as infection with a highly neurotropic rabies virus strain (CVS) is less immunosuppressive in mice lacking this receptor by comparison with controls (Camelo *et al*, 2001). This results in a briefly prolonged survival of CVS-infected, TNF-α p55 receptor knockout mice that may be attributed to increased antiviral immunity (Camelo *et al*, 2000). In this regard, there is speculation that the apoptosis of T cells invading the CNS of RV-infected mice may reduce the antiviral response (Baloul and Lafon, 2003). *In vitro* evidence that RV-induced apoptosis is regulated by Bcl-2 (Thoulouze *et al*, 2003) suggests the possibility that the differential induction of Bcl-2 in neurons versus T cells during infection with diverse RV strains may dictate the contribution of apoptosis to infection with a particular RV.

Antiviral immune mechanisms can contribute to rabies pathogenesis

The clearance of most viral infections is at least in part dependent upon destruction of the infected cells, generally by CD8 T cells. In rabies, where neurons are infected, this would undoubtedly cause some pathology. This is evidently the case for the paralytic form of rabies which is rare in humans but can be readily induced in animal models (e.g., Iwasaki *et al*, 1977). In these models, neuronal destruction and paralysis is dependent upon an immune response and is associated with CNS inflammation (Iwasaki *et al*, 1977; Sugamata *et al*, 1992; Weiland *et al*, 1992). T-cell depletion experiments have demonstrated that the pathogenesis of paralytic rabies in mice is dependent upon the CD8 T-cell subset (Weiland *et al*, 1992).

Unlike animals with the encephalitic form of rabies, mice that develop paralytic rabies often produce significant levels of rabies-specific antibody and can sometimes clear the infection (Iwasaki *et al*, 1977; Weiland *et al*, 1992), suggesting that, in this case, there is a close relationship between the immune mechanisms that clear the infection and cause damage. A CNS inflammatory response is evidently central to the rapid clearance of attenuated RV from CNS tissues (Hooper *et al*, 1998). Nevertheless, the inflammatory component of the antiviral response has long been known to have pathological consequences (e.g., Iwasaki *et al*, 1977), which likely include the cytotoxic effects of CD8 T cells (Weiland *et al*, 1992) and free radicals produced by cells of the monocyte lineage (Hooper *et al*, 1995). In addition, there is evidence that rabies nucleoprotein has superantigen-like properties that activate a large subset of T cells and can stimulate responses to unrelated antigens (Astoul *et al*, 1996). Conceivably this could not only exacerbate an inflammatory response to RV but also contribute to the induction of autoimmune encephalomyelitis, which is infrequently seen following immunization with Semple brain tissue–derived rabies vaccine (Piyasirisilp *et al*, 1999). Thus, if an immune response to rabies develops either inappropriately (i.e., low neutralizing antibody levels and a bias towards cell-mediated immunity) or after the infection has spread sufficiently, immune-mediated CNS tissue damage would be expected to be extensive. In this case, immune mechanisms rather than the virus, per se, may kill the subject. The nature of the virus is a major contributor to immunopathology as strains differ in their immunostimulatory properties, as discussed above (e.g., Irwin *et al*, 1999; Morimoto *et al*, 1999; Baloul and Lafon, 2003). However, the route of infection is also important in this regard. For example, administration of RV in the extremities, like the mouse footpad, is more likely to result in peripheral nerve damage leading to paralytic rabies, and some prospect of survival, as opposed to the lethal encephalitic disease that follows intracerebral infection with the same strain (e.g., Sugamata *et al*, 1992). The likelihood that paralytic rabies is immune mediated is supported by studies demonstrating that this form of disease only occurs in immunocompetent animals (Iwasaki *et al*, 1977; Smith *et al*, 1982; Sugamata *et al*, 1992). Moreover, the extent of immune-mediated apoptosis of infected neurons and by stander cells in the CNS is greater in animals that become paralyzed (Galelli *et al*, 2000).

The prospect that immunity can contribute to the pathogenesis of rabies is also supported by what has been termed the "early death" phenomenon, where inadequately immunized mice occasionally die more rapidly of rabies than unvaccinated controls. Insight into the contribution of the immune response to the accelerated death of RV-infected animals comes from classic experiments in which immunocompromised mice were found to survive RV infection for longer periods of time than normal animals (Prabhakar and Nathanson, 1981; Smith *et al*, 1982). This is despite the fact that immunosuppression increased the overall mortality rate, where possible (Smith *et al*, 1982). Clinical signs of rabies and death were precipitated in immunosuppressed mice when immune serum was administered or the immune response to rabies recovered, suggesting that antiviral antibody can contribute to rabies immunopathogenesis (Prabhakar and Nathanson, 1981; Smith *et al*, 1982). It should be understood that infection of mice with deficits in diverse aspects of immunity with different RV strains does not always lead to a prolongation of survival. Together with the types of immune effectors elicited, the temporal relationships between the rates of virus invasion, replication and spread in the CNS, and the time required for the activation of an antiviral immune response, likely determine the extent of immune-mediated CNS tissue damage. It is therefore not surprising that the few individuals and animals

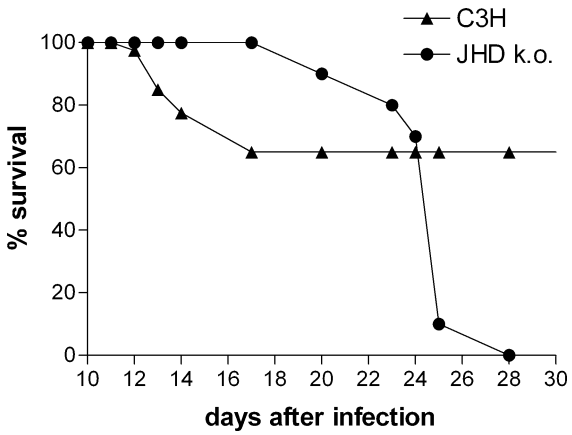

Figure 2 CVS-F3 infection of young immunocompetent mice. Mice, 6 to 8 weeks of age (C3H/HeJ, male:female 1:1, $n = 40$; JHD k.o., male:female 1:1 $n = 10$) were infected intranasally with 10^5 focus-forming units (ffu) of CVS-F3. The mice were examined daily for mortality, which is presented as the % survival over time. No mice died of rabies after 28 days following infection.

that survive RV infection after showing clinical signs of rabies most often have significant sequelae reflecting neurological damage caused by the clearance of the virus from the CNS.

The delicate balance between protective immunity and immunopathogenesis is also apparent when young C3H mice (6 to 8 weeks old) are infected intranasally with CVS-F3, an attenuated RV strain that is apathogenic for normal adult mice (>8 weeks old) when administered via the same route. As shown in Figure 2, although most young C3H mice clear the infection and survive, a significant proportion of these mice die beginning around 12 days after infection. In contrast, mice lacking the ability to produce antibody (JHD k.o.) despite retaining some capacity to mediate a T-cell response against the virus all succumb to the infection, but at least 8 days later than the C3H mice that die (Figure 2). Given that mice incapable of clearing the virus survive considerably longer, in this case the immune response to CVS-F3 must be responsible for the incomplete mortality seen with the C3H mice.

Although the pathological aspects of immune reactivity in the CNS are sufficient to explain the contribution of immune responses to rabies pathogenesis, there is another potential contribution related to immune function that has not been examined in great detail. This is the possibility that elements of either the adaptive or innate immune response may either directly or indirectly promote the replication and spread of the virus. For example, immune mediators may contribute to the induction of growth factors and cell functions conducive to virus replication as several of these factors appear to be up-regulated over the same time period in the RV-infected mouse brain (Prosniak *et al*, 2001). Finally, several RV strains, including those associated with the silver-haired bat that are known to infect humans without overt exposure, are capable of replicating in a wide variety of cells *in vitro* (Ray *et al*, 1995; Morimoto *et al*, 1996; Thoulouze *et al*, 1997). Evidence that cells of the immune system can be infected (Ray *et al*, 1995; Thoulouze *et al*, 1997) suggests the possibility that such cells could transport the virus from poorly innervated areas to locations that are highly innervated, such as the lymph nodes, thereby facilitating spread of RV to the CNS. This could explain how RV can enter the nervous system when introduced via organ transplants, as has recently occurred (CDC, 2004).

Conclusions

Although RV infection is invariably lethal in the absence of a protective immune response, elements of the immune response can contribute to the pathogenesis of rabies. Lethal disease can be prevented if an appropriate anti-RV immune response develops in a timely fashion. In some cases, functionally significant immunopathology is associated with clearance of the virus, causing paralysis for example. However, the immune response to RV, whether or not it is protective under other circumstances, can also accelerate lethal disease, particularly if the infection has reached vital areas of the CNS. This could be the result of cell- or antibody-mediated destruction of infected cells as well as occur through the action of cytotoxic free radicals elaborated by inflammatory cells. Thus the route of infection, the inherent capacity of a RV strain to spread to the CNS and replicate without triggering a protective immune response, as well as the timing, specificity, and nature of the immune response all dictate the outcome of exposure.

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