CHAPTER 17

Host Range and Tissue Tropisms: Antibody-Dependent Mechanisms

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The classic concept of viral infection involves an initial interaction between determinants on the surface of a virus and cellular receptor binding sites of appropriate viral specificity, followed by stages of penetration, uncoating, and replication of the viral genome. With most infections, antiviral antibody will inhibit the initial binding step, and in so doing, will reduce viral infectivity. However, in certain cell–virus interactions, the presence of antiviral antibody increases infectivity, a phenomenon which is known as *antibody-dependent enhancement,* or ADE, of viral infectivity [1–4]. The explanation for this apparent paradox is that this type of infection is mediated, not by specific viral receptors, but by cellular receptors for components of the immune system that function as accessory viral receptors by binding infectious complexes of antiviral antibody with virus, this unusual binding step being followed by later replicative stages. ADE is thus conditional upon the presence on host cells of the necessary accessory receptors, but it is also determined by the nature of the infecting virus, by the specificity of the antiviral antibodies, and by the conditions under which the cells, virus, and antibody are brought together.

Cellular Receptors Mediating ADE

There are two principal classes of receptors for components of the immune system, namely, cellular receptors for the Fc portion of immunoglobulin molecules (Fc receptors, or FcR), and receptors for components of the complement system (complement receptors, or CR). FcR are present on a

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variety of immunocompetent cells, and CR are found on an even wider range of cell types [5]. Both classes are heterogeneous, both are variably expressed at the cell surface and may be inducible following viral infection or injury [6].

The concept that FcR might be capable of mediating ADE was first put forward by Halstead and his associates [2,7] in the context of hemorrhagic dengue and the dengue shock syndrome. Using preparations of primary peripheral blood white cells of human or primate origin, they were able to demonstrate substantially enhanced yields of dengue virus in the presence of subneutralizing concentrations of antiviral IgG, but not IgM or $F(ab')_2$ fragments of antibody. Enhancement was associated with cells of the monocyte–macrophage lineage, which are rich in FcR. However, the experimental system was difficult, probably because of heterogeneity in the primary cells being used. The substitution of continuous lines of macrophage-like cells for the primary cell cultures greatly simplified experimentation, and made possible a number of studies on the mechanism underlying ADE [3,8]. Mouse macrophages are now well characterized and are known to have at least two FcR, readily distinguishable by a monoclonal antibody, 2.4G2, which binds strongly to the trypsin-resistant FcRII. In a model system involving the mouse macrophage line P388D1, the flavivirus West Nile virus (WNV) and anti-WNV antibodies, pretreatment of cells with 2.4G2 antibody blocked ADE, clearly implicating FcRII in this enhancement [8]. Further evidence for the role of FcR has come from the use of hybrid cells, ADE being demonstrable only in those hybrids that contain FcR [9] and from observations that aggregated IgG will block enhancement [10].

Complement is well known to potentiate the neutralization of viruses by antiviral antibody, and complement also has a direct virolytic effect upon some enveloped viruses. It is, therefore, somewhat surprising to discover that a combination of antiviral IgM with complement can mediate enhanced viral replication. The evidence for this comes from the same WNV model system in P388D1 cells, which are known to carry CR as well as FcR, and in which two quite distinct pathways for ADE are possible [11,12]. The first pathway, which can result in 20- to 100-fold enhancement, involves FcR and antiviral IgG, and is independent of the presence of complement. The second pathway, which can result in 10- to 20-fold enhancement, involves CR and antiviral IgM with complement and is abolished by heat treatment or cobra venom treatment of the complement source. As previously noted, the monoclonal antibody 2.4G2, which binds to FcRII, blocks the IgG-dependent ADE, but has no effect upon the CR-mediated ADE, which is IgM dependent. Conversely, a different monoclonal antibody, M1/70, with specificity for CR3, blocks IgM enhancement, but has no effect upon FcR-mediated IgG enhancement. Although the two enhancement pathways have been separated under these experimental conditions, it is quite possible that the two mechanisms operate synergistically when both immunoglobulin classes are present, as may be the case early in the immune response to viral infection.

Japanese workers have reported substantial enhancement produced in BHK-21 cells infected with the alphavirus Getah virus, or the flavivirus, Japanese encephalitis virus, in the presence of appropriate antiviral antisera [13]. However, we have been unable to confirm these observations in a number of experiments in BHK-21 cells infected with Getah, West Nile, or Bunyamwera viruses, although all three viruses are capable of producing significant ADE when the same virus–antibody mixtures were tested in P388D1 cells.

Viruses Producing ADE

Dengue and West Nile viruses are both members of the genus flavivirus, in the family Togaviridae, and ADE has also been reported with Murray Valley encephalitis, Japanese encephalitis, Kunjin, Uganda S, yellow fever, and Zika viruses in the same genus, and with the alphaviruses Getah, Semliki forest, Sindbis, and Western equine encephalitis viruses in the same family [1,2,4,11,13]. The family Bunyaviridae contains four genera, in each of which at least one virus has been shown to produce ADE. Thus, in the Bunyavirus genus, Bunyamwera, Batai, Maguari, Lokern, California encephalitis, Tahyna, Trivittatus, and a number of recombinant viruses have all shown ADE [14]. It therefore seems probable that ADE is a general phenomenon as far as all viruses in the families Togaviridae and Bunyaviridae are concerned.

In other families, studies have been limited to relatively few viruses, but ADE has been reported with the following: rabbit pox virus [1], family Poxviridae; rabies virus and fish rhabdoviruses, family Rhabdoviridae [15]; Reovirus type 3, family Reoviridae; murine cytomegalovirus, family Herpesviridae [16]; and feline infectious pancreatitis virus, family Coronaviridae [17]. Failure to detect ADE has been reported with only two viruses, Mengo virus, family Picornaviridae, and herpes simplex virus, family Herpesviridae [4].

Without doubt the list of viruses showing ADE will be extended as further viruses are tested.

Antibody Specificity of ADE

Are enhancing antibodies distinct from the antibodies measured by neutralization (N), hemagglutination inhibition (HI), complement fixation (CF), or any other serological test? Quantitatively, enhancing antibodies are detectable at substantially lower serum concentrations than are N, HI, or CF antibodies. In a study involving a range of different flavivirus antisera, there was some correlation between enhancing antibody titers and cross-reactivity detected by HI, but little correlation between enhancing and N-antibody titers [18]. Studies with monoclonal antibodies provide more definitive evi-

dence. Three monoclonal antibodies with specificity directed against the envelope glycoprotein of WNV were all potent at producing ADE, but only one had significant N activity, and a different one had substantial activity against the viral hemagglutinin [19]. These, and other studies with monoclonal antibodies [10,20] support the view that any antibody with specificity against a virion surface epitope, but not antibodies against internal nucleocapsid antigens, would be expected to produce ADE, provided the antibody retained an intact Fc-terminus, and provided the host cells carried FcR or CR of appropriate receptivity. It is worth noting that the same monoclonal antibody could enhance viral replication when tested in one macrophage cell line, but fail to enhance in a second macrophage line that carried fewer FcR, or FcR of inappropriate IgG subclass specificity.

To date, there are no reports of ADE produced by IgA or IgE antibodies.

In addition to the heterogeneity of antibodies with respect to viral antigens, there are also some interspecies constraints that can affect ADE. Thus, while antiviral antibodies prepared in avian species will produce ADE when tested in avian cells, such avian antibodies will not produce ADE when tested in mammalian cells [1]. There are major differences in the strength of binding of IgGs of different mammalian species to mouse FcR [21], and the species homology, or lack of it, between the source of antiviral IgG and the host cell providing the FcR, can materially affect ADE. While these considerations may have profound effects in experimental procedures, they are obviously not relevant to the pathogenesis of viral infections *in vivo*.

Temporal Relationships

Prolonged pre-incubation of a virus–antibody mixture favors neutralization of infectivity, whereas enhancement is more likely if mixtures are applied to appropriate FcR-bearing cells without pre-incubation [1,4]. Similarly, pre-incubation at 37°C favors neutralization, whereas serum–virus mixtures that have been held in the cold are more likely to enhance infectivity.

ADE *in vivo*

Is ADE simply an interesting laboratory artifact or do the mechanisms underlying ADE contribute to viral pathogenesis in nature? Much of the laboratory work on ADE has stemmed from studies on dengue and dengue hemorrhagic fever [2,7], but there are other viral infections in which ADE may contribute to disease. The rabies "early death" phenomenon [22] may be a manifestation of ADE, as may a similar antibody-dependent early death in cats infected with the virus of feline infectious peritonitis, a member of the family Coronaviridae [17]. The severe reactions seen in some children who have been vaccinated against measles and respiratory syncytial viruses and

have subsequently suffered natural infections with these agents may be attributable to the absence of antibodies against the fusion factor in these viruses, but it is possible that non-neutralizing antibodies may potentiate infection of FcR-bearing cells in the respiratory tract, or of endothelial cells. Since it is known that several viruses in the family Herpesviridae are capable of inducing FcR [23], sequential infections, first with a herpesvirus, and shortly after with a different virus, may result in enhanced replication of the second with consequent disease. Other stimuli that activate macrophages can enhance expression of FcR [24], and may thus also contribute to ADE. Whether or not CR are also inducible by viral infection is not known. Much remains to be done to explore the contribution of CR, and other cell surface receptors, such as the mannose receptor, to viral pathogenesis.

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