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DNA IMMUNIZATION AND CENTRAL NERVOUS SYSTEM VIRAL INFECTION

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I. VIRUS INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

Mild central nervous system (CNS) symptoms such as headache and drowsiness can result from systemically elevated cytokine levels, and therefore are common in many virus infections, even in the absence of infection of the CNS. In this chapter we shall consider only those viruses that are known to infect the CNS.

A. Poliovirus

Poliovirus, a member of the picornavirus family and *Enterovirus* genus, was a major scourge in the earlier part of the twentieth century. As the genus name indicates, the virus replicates in the gastrointestinal tract; as such, it is usually transmitted by the fecal-oral route. Viremia is common, but the vast majority of infections remain asymptomatic. CNS infection is quite unusual, and is initiated either as a result of the viremia or, more rarely, by neural spread. The virus infects the anterior horn motor neurons of the spinal cord, causing poliomyelitis (from the Greek *polios* plus *myelos*—"inflammation of the gray marrow"), the disease for which the virus is named. Loss of these cells results in paralysis, often of a lower limb. In some cases, the infection ascends the cord to cause paralysis of upper limbs, and in the most extreme cases the infection reaches the junction of the spinal cord and the brain, resulting in paralysis of the muscles of respiration (bulbar palsy) and requiring that the victim be placed in an "iron lung." The development, in the mid-1950s, of killed and live polio vaccines massively reduced the frequency of this infection, and of the associated disease, and a program directed by the World Health Organization aims to eradicate poliovirus within the next few years. If successful, this will be the second virus (following smallpox) to have been exterminated by vaccination. The pathogenesis of the murine "equivalent" of poliovirus—Theiler's virus—has been extensively studied, and will be described later in this chapter.

B. Herpesviruses

Herpesviruses are probably the commonest viruses to infect neuronal tissue. Herpes simplex virus (HSV) and varicella-zoster virus (VZV) establish latent infections in the dorsal root ganglia of the peripheral nervous system and, particularly in the immunocompromised, can reactivate and disseminate to cause encephalitis or, more commonly, vesicular eruptions in the skin area innervated by the infected neurons—leading to cold sores/fever blisters (HSV) or shin-

gles (VZV). Epstein-Barr virus (EBV), the cause of infectious mononucleosis, is also associated with a rare encephalitis (Andersson *et al.*, 1999; Schiff *et al.*, 1982).

C. Measles Virus

Measles virus is a negative-stranded RNA virus, and a member of the morbillivirus genus. Infection by this virus most commonly results in the characteristic rash, which is immunopathological, being mediated not by direct viral cytotoxicity, but instead by the T cell response of the host. However other organs are frequently affected, and giant cell pneumonia can be lethal. CNS infection is infrequent, but meningitis and encephalitis can occur. Postinfectious encephalomyelitis occurs in ~1/1000 cases, usually occurring within weeks of infection; however, it is difficult to detect virus in the CNS, and it has been suggested that the observed perivenular demyelination is immune-mediated (Gendelman *et al.*, 1984). A rare complication (~1 in 2×10^6 cases) is subacute sclerosing panencephalitis (SSPE), in which measles virus RNA and protein persist in glial cells and neurons, leading to CNS dysfunction and death.

D. Lentiviruses

In the 1950s, the Icelandic virologist Bjorn Sigurdsson carried out epidemiological studies of the sheep diseases rida (Sigurdsson, 1954a) (more commonly known by the English term "scrapie") and visna, and suggested that they were infectious in origin, but had an incubation period much longer than that of "standard" viruses (Sigurdsson, 1954b). These led him to propose a new category of virus, the "slow virus." Slow viral diseases were observed in many other species, including humans; the CNS disease kuru, first described in Papua New Guinea, had an incubation period measured in years. For many years this curious and clinically defined category of viruses remained devoid of molecularly characterized members, but eventually it became clear that the grouping encompassed several very different agents. Some of these agents were relatively standard viruses, but other agents—including the agent of scrapie—were refractory to categorization until Stanley Prusiner's groundbreaking identification of prions, which are described in Section I,I below. One of the first-characterized slow viruses was a retrovirus that caused visna; indeed, the long incubation period gave this virus group its name (lentiviruses; from the Latin word *lentus*, "slow"). Human immunodeficiency virus (HIV) is a lentivirus and, in common with all of this group, disease

often does not appear until many years postexposure. The later stages of HIV infection are frequently characterized by encephalitis and dementia (Fox *et al.*, 1997; Moller *et al.*, 1988; Wiley *et al.*, 1991).

E. Rabies Virus

This virus, a member of the rhabdovirus family, kills ~40,000 people annually, and is transmitted through the saliva of infected animals, usually by bites. The virus may undergo local replication at the site of inoculation, perhaps in muscle cells, but a key feature is its subsequent centripetal spread to the CNS within neuronal axons. Viremia is not a prominent feature, and disease can be prevented by physical or chemical interruption of axonal transport (Ceccaldi *et al.*, 1989; Tsiang, 1979). On reaching the CNS, the virus spreads within the brain (often resulting in Negri body formation, especially in the hippocampus); however the detectable histological damage is often less extensive than might be expected, given the severity of the neurological and behavioral symptoms observed (the term "rabies" is derived from the Latin term for "madness"). After CNS infection has been established, the virus may spread centrifugally to various tissues, including the salivary glands, from which it is secreted into the saliva.

F. Arenaviruses

This family includes a variety of human pathogens, some of which cause hemorrhagic fevers (Lassa, Junin, and Machupo viruses). The prototype of the family, lymphocytic choriomeningitis virus (LCMV), infects humans more frequently than is often realized. The virus is rodent-borne, and recent surveys in Baltimore indicated that 9% of house mice and 4.7% of humans were seropositive (Childs *et al.*, 1991; Childs *et al.*, 1992). The spectrum of human disease ranges from subclinical infection to fatal meningoencephalitis, and the virus is teratogenic, leading to hydrocephalus (Larsen *et al.*, 1993). The immunobiology and pathogenesis of LCMV infection have been extensively studied, and the use of this model to evaluate DNA immunization will be described later in this chapter.

G. Arboviruses

Arboviruses (Arthropod-borne viruses) are important human pathogens causing, for example, yellow fever and dengue hemorrhagic fever. In the United States, the primary clinical manifestation of arboviral disease is encephalitis. Many viruses, from several different viral families, are implicated; most are mosquito-borne, but some are

transmitted by ticks. The most commonly diagnosed arboviral encephalitis in the United States is that caused by the flavivirus St. Louis encephalitis (SLE) virus. However, we cannot underestimate the capacity of viruses to enter new ecological niches. An outbreak of viral encephalitis in New York in September 1999 was initially ascribed to SLE, but subsequently was attributed to West Nile virus, which had not previously been identified in this country. Other arboviral encephalitides include those caused by western equine and Venezuelan equine encephalitis viruses (alphaviruses) and the California group of encephalitis viruses (bunyaviruses).

H. Miscellaneous Viral Encephalitides

Borna disease virus (BDV) is the prototype of a new family of negative-stranded RNA virus (Briese *et al.*, 1994; de la Torre, 1994). This virus causes an immune-mediated encephalitis in many species (Planz *et al.*, 1995; Stitz *et al.*, 1991). Infectious BDV has not been identified in humans, but antibodies, proteins, and nucleic acids have been identified in the sera and/or CNS of patients suffering from certain psychiatric disorders (Bode *et al.*, 1995; de la Torre *et al.*, 1996a; de la Torre *et al.*, 1996b), raising the intriguing possibility that some psychoses may be virus-induced; a chapter of this book is devoted to BDV neurotropism and its consequences. Also described elsewhere in this volume are coronavirus infections of the CNS. Finally, viral diseases of the CNS include progressive multifocal leukoencephalopathy (PML), caused by the papillomavirus JC. More than 80% of humans carry antibodies specific for this virus, but PML usually is seen only in immunosuppressed patients; consequently, the incidence of PML has increased in parallel with HIV infection (Dorries, 1998; Gordon and Khalili, 1998; Jensen and Major, 1999; Weber and Major, 1997).

I. Prions

As mentioned above, these agents have an extremely protracted incubation period. The prion protein (PrP) was first identified as part of the protease-resistant material proposed by Prusiner as a protein-only infectious agent responsible for scrapie, and for other transmissible spongiform encephalopathies (TSEs). This heretical notion engendered justified skepticism among many experts, but it has resisted numerous challenges, and the evidence in its favor is now very strong. The PrP gene encodes a cellular protein, expressed on many cell types including neurons and cells of the immune system, whose normal function remains uncertain. Mice lacking this gene (PrPko

mice) have few detectable CNS abnormalities (Bueler *et al.*, 1992; Kuwahara *et al.*, 1999). Expression of this gene is a prerequisite for host susceptibility to TSE agents, and PrP^{ko} mice are resistant to challenge (Bueler *et al.*, 1993; Prusiner *et al.*, 1993). This host protein can exist in at least two conformations, which are distinguished by their sensitivity to protease; the protease-sensitive form appears to be the “normal” conformer, present in normal hosts, and not causing disease, while the protease-resistant form appears to be infectious. Prion replication and infectivity appear to be determined by the ability of the “pathogenic” conformer to initiate conformational changes in its “normal” counterpart; this is the key to prion diseases, and underpins both the replication of the agent in an infected host, and its transmission in the absence of a nucleic acid. Such conformational changes have recently been demonstrated in tissue culture studies (Bessen *et al.*, 1995; Kocisko *et al.*, 1994; Kocisko *et al.*, 1995). The TSEs, then, are transferred to the new host when the misfolded protein uses the normal proteins of the new host as substrates for production of abnormal conformers. The genes permitting the replication of this novel form of infectious pathogen are therefore provided by the unwitting victim. They are unusual, as they contain no nucleic acid genome. Instead, prions are infectious proteins that are encoded by the host’s own PrP gene. This protein can exist in at least two conformations; one is normal (and seems to serve some function in the CNS), while the other is abnormal. The abnormal conformer, which is infectious, appears able to act as a template, causing its normal siblings to convert to abnormality. The accumulation of abnormal conformers leads to spongiform encephalopathy, the histological hallmark of prion diseases. The historical relationship between “slow viruses” and prions has often resulted in the inclusion of prions in virological textbooks—indeed, they are the topic of two chapters in this volume—but their radically different (a) coding strategy (as a host gene), (b) mode of replication (by directed misfolding of a self-protein into an abnormal conformer), and (c) mechanism of infection (as an infectious protein) surely render them unique. Despite their questionable membership in the virus taxon, we mention them here because they cause CNS diseases, and because recent studies suggest that immunization may modify other CNS diseases characterized by abnormal deposition of self-proteins.

J. CNS Diseases That May (or May Not) Be of Viral Origin

The causes of certain CNS diseases remain unknown. For example, multiple sclerosis (MS) is a degenerative disease of the CNS, which is characterized by demyelination. The clinical and histological features

of MS can vary from relapsing–remitting disease to chronic progression. What role might viruses play? First, MS may be the result of a persistent virus infection, acting to drive a chronic immune response. Over the past several decades, various viruses have been advanced as the cause of MS but, to date, none of these suggestions has withstood further analysis; as a result, enthusiasm for this hypothesis has perhaps diminished. However, while it is unlikely that MS results from persistent infection by a known virus, it is possible that it is caused by a virus which has yet to be identified. While it may be tempting to think that, at the millennium, we have identified all microbes and their associated infections, it has been estimated that only ~0.4% of extant bacteria have been cataloged, and new viruses continue to be identified. Indeed, even entire virus families have been discovered in the past decade (e.g., the *Bornaviridae*, mentioned above). No animal other than humans develops MS. This is not true of other autoimmune diseases. For example, humans and other animals develop diabetes, arthritis, and thyroiditis. Thus, MS could be caused by a microbe whose host range is tightly restricted to humans. However, a second, more popular, hypothesis implicates autoimmunity; MS, like several other autoimmune diseases, it is commoner in women than in men. A number of ideas have been advanced to explain virus-induced autoimmunity (recently reviewed in Oldstone, 1998; and in Whitton and Fujinami, 1999); these include the release, from infected cells, of sequestered host proteins which then act as autoantigens (see the chapter on “epitope spreading” in this book). Consistent with this, antibodies and T cells specific for CNS self-antigens are detectable in the CNS of MS patients, but not of healthy individuals. It is thought that MS may be initiated by an infection with one virus, but that subsequent infections—with unrelated viruses—might “boost” the immune response against the released CNS self-antigen. This proposition is supported by an apparent association between a relapse of MS and recent infection. However, one could argue that relapses are caused instead by a transient immunosuppression, accompanying virus infection, with a resulting reactivation of an unidentified persistent or latent virus. Thus, while the pathogenesis of MS remains uncertain, many researchers feel that viruses play some role in the initiation and maintenance of the disease; a chapter in this volume is devoted to this important topic.

II. ANTIVIRAL IMMUNE RESPONSE

To evaluate the role of DNA immunization in protecting against viral infection of the CNS, and against the related diseases, we must

first consider how the immune system recognizes viruses, and virus-infected cells, and how it deals with these challenges. This topic has recently been summarized (Whitton and Oldstone, 2000).

A. Overview

The immune response to virus infection is divided into two components, the innate response and the adaptive response, which are serially expressed in partially overlapping temporal phases. Soon after the host is first infected with a virus, the innate immune response is activated. Many cells secrete interferons α and β , while natural killer (NK) cells (and, rarely, activated macrophages) secrete interferon- γ (IFN γ). (IFN γ is also an important effector molecule released by T cells during the antigen-specific phase of the immune response; this is described in more detail below.) Upon exposure to these cytokines, noninfected cells are rendered resistant to virus infection; the interferons therefore limit the ability of the virus to spread locally. Meantime the NK cell population expands, usually peaking approximately 3–4 days postinfection. These cells cannot specifically detect virus-infected cells, instead being triggered by a combination of 2 factors: poorly characterized stimulatory molecules, and the absence of class I major histocompatibility complex (MHC) molecules. As the innate response wanes, so the adaptive response expands. The adaptive response differs from the innate in two key ways. First, the adaptive response is *antigen-specific*; it recognizes specific structures (usually proteins, but occasionally carbohydrates and glycolipids) on viruses or on virus-infected cells. Second, the adaptive response exhibits *memory*; antigen-specific cells are maintained long after the infection is cleared, and these memory cells permit a more rapid and elevated response if the host is reexposed to the antigen. Antigen-specific memory forms the cornerstone of vaccination; a vaccine induces memory cells specific for the appropriate antigen(s), and these cells respond rapidly, should the host encounter the related pathogen. This chapter is devoted to vaccination, and so we shall focus below on the adaptive immune response. All antigen-specific immunity relies on lymphocytes, of which there are two types: B lymphocytes (which produce antibodies) and T lymphocytes.

B. How Antibodies Recognize Viruses and Virus-Infected Cells

Antibodies recognize antigen through regions of hypervariable sequence. Crystallographic analyses of the antibody–antigen union indicate that the union is more “hand in glove”—in which components

can, to some extent, alter their conformation to accommodate one another—than “lock and key,” in which both elements are fixed, with each being unable to modulate to the other (Arevalo *et al.*, 1993; Rini *et al.*, 1992). As a rule, when playing their part in host immunity, antibodies recognize intact proteins. Thus, antibodies can interact with bacteria and viruses, as well as with viral proteins (most often glycoproteins) expressed on the surface of infected cells.

C. How Antibodies Control Virus Infections

Viruses are obligate intracellular parasites, but they are (usually) not transmitted in association with cells but, rather, as free infectious particles. Since antibodies can recognize free viruses, it is easy to see how antibodies can play a major role in controlling virus infection, by inactivating the virus before it can enter the cell. Antibodies play an extremely important part in antiviral immunity. Indeed, in many cases, the administration of specific antibodies can confer complete protection against subsequent challenge with the relevant virus, and passively transferred antibody remains an important component of medical treatment of patients exposed to certain viruses (e.g., rabies). Antibodies neutralize viruses in a number of ways: (1) they may bind to the part of the virus that interacts with its cell-surface receptor, preventing virus attachment to the cell; (2) they may agglutinate many infectious particles into a single “clump,” thus reducing the number of cells that will become infected; (3) viruses may activate complement (directly; or indirectly, via antibodies), releasing chemotactic factors such as C5a and C3q. Note that intact antibodies are not a prerequisite for antiviral effectiveness; Fab fragments specific for Respiratory Syncytial Virus (RSV) F glycoprotein, when instilled into the lungs of infected mice, were therapeutically effective (Crowe *et al.*, 1994). Such approaches hold promise, particularly in the light of recent advances in technologies that allow the rapid production of antibodies of any desired specificity (Barbas *et al.*, 1991; Kang *et al.*, 1991).

There are five different classes of antibody, immunoglobulin (Ig)A, IgG, IgM, IgD, and IgE, each with different functional attributes. During natural infection, most viruses gain entry via respiratory or enteric mucosal surfaces. It is therefore not surprising that mucosal immunity and, in particular, secretory IgA, plays an important role in control of viral infections (Ogra and Garofalo, 1990). The pentameric, decavalent IgM molecule is produced early after virus infection, is usually independent of T cell help, and acts as the initial antibody-mediated systemic antiviral response. Later in infection, and on secondary exposure, most IgM-producing cells switch to produce IgG of the same antigen speci-

ficity. IgG₁ is the major complement-binding and opsonizing antibody in humans (Spiegelberg, 1990), and complexing of viruses with IgG will also facilitate their Fc receptor-mediated phagocytosis by monocytes macrophages and by polymorphonuclear leukocytes.

Even after cell entry, antibodies can exert effects on virus infection by interacting with viral proteins (most often glycoproteins) on the surface of infected cells, lysing the infected cell in association with complement, or modulating the intracellular viral replication (Fujinami and Oldstone, 1979). Relevant to this chapter, it has been suggested that neuronal virus infections can be eradicated by antibodies, without damaging the neurons (Levine *et al.*, 1991); the mechanism for this remains undefined. However, many viruses delay glycoprotein expression until late in the infective cycle, when viral maturation may have occurred, and at this point the antibody-mediated effects may be biologically inconsequential. How can a host detect a virus-infected cell early in the infection process, thus maximizing its immunological advantage? Here, antibodies are less effective, being limited by their recognition requirements, whereas T cells play a critical role, as detailed below.

D. How T Cells Recognize Viruses and Virus-Infected Cells

T cells can be categorized by the surface marker proteins (CD4 or CD8) that they express. The majority of CD8⁺ cells are cytotoxic T lymphocytes (CTLs), although some CD8⁺ cells are nonlytic and exert their antiviral effects by cytokine release (Levy *et al.*, 1996), while most CD4⁺ cells are helper cells that secrete cytokines to assist B cell maturation, and perhaps to aid a developing CD8⁺ T cell response. T cells recognize antigens via a cell surface heterodimer, the T-cell receptor (TcR). This molecule is structurally reminiscent of the Fab portion of an antibody molecule, but the nature of T cell recognition differs from that of antibody recognition in one critical aspect: while antibodies recognize antigen in isolation, T cells react to antigen in the form of a short peptide presented by a host glycoprotein encoded in the MHC. There are two major classes of MHC molecule (class I and class II), and there is a close relationship between the class of MHC/peptide complex recognized by a T cell and the surface marker (CD8 or CD4) borne by the T cell. MHC class I molecules are the "classical" molecules associated with graft rejection (the phenomenon that gave the MHC its name); they are expressed on most somatic cells, and they interact with T cells bearing the CD8 surface marker. In contrast, MHC class II molecules have a much more restricted expres-

sion, being found only on specialized antigen-presenting cells (e.g., macrophages, B lymphocytes, dendritic cells), and they interact with T cells carrying the CD4 surface marker. At the target cell surface, class I and class II molecules are similar in overall structure. The class I heterodimer comprises the class I heavy (H) chain closely complexed with a non-MHC-encoded protein, β_2 -microglobulin (β_2 M). The class II heterodimer consists of two similar chains, α and β . Both class I and class II form a structure graphically described as a Venus fly trap, the groove of which binds an antigenic peptide, in a sequence-specific manner, and presents it on the cell surface for the perusal of T cells (Bjorkman *et al.*, 1987a; Bjorkman *et al.*, 1987b). Although superficially similar, the two types of MHC/peptide complex differ in how they reach the cell surface. MHC class I is optimized to present intracellular antigen, while MHC class II presents antigen captured from the extracellular milieu. Thus, when T cells distinguish between a peptide/class I complex and a peptide/class II complex, they are really discriminating on the basis of the source of the peptide—was that peptide derived from protein made within the cell, or from protein taken up from the extracellular spaces?

The MHC class I pathway is vital for recognition of virus-infected cells. Viral proteins are synthesized and degraded within the cell, and the resulting peptides are transported to the endoplasmic reticulum, where they encounter empty MHC class I molecules. Peptides with sufficient affinity for particular MHC alleles bind in the groove; β_2 M attaches to, and stabilizes, the complex; and the trimolecular structure travels to the cell membrane, to be screened by the CD8⁺ T cells of the host; these cells, as the effector arm of the antiviral T cell response, therefore assume great significance in antiviral immune responses. One major advantage of this arrangement is that CD8⁺ T cells can recognize almost any viral protein (as long as it contains a peptide sequence that can be presented by MHC class I). Therefore, even proteins expressed at the beginning of the viral life cycle, and limited to the cytosol, are vulnerable to degradation and MHC class I presentation. In this way, the host can identify and eradicate infected cells at a very early stage, long before viral maturation can occur. For example, the major CTL response to human cytomegalovirus (HCMV) is directed to a protein expressed immediately on infection; a similar situation exists for VZV and HSV. Any defect in the MHC class I antigen-processing pathway may result in the infected cell's being unable to present viral peptide on the cell membrane, which in turn would render the virus "invisible" to CD8⁺ T cells.

E. How T Cells Control Virus Infections

Antibodies are important in limiting the number of infected cells, and in clearing virus from the host, but CD8⁺ T cell responses play a critical role in the control of many virus infections. These cells have been extensively characterized in animal models, and the results equate well with those obtained in human studies. Although the role of CD8⁺ T cells in controlling primary virus infection has been recognized for some time, the importance of these cells in vaccine-induced protective immunity is often disregarded. Many studies have shown that vaccine-induced CD8⁺ T cell responses, in the absence of vaccine-induced antibody responses, are sufficient to confer solid protective immunity against a subsequent virus challenge. For example, in the LCMV mouse model, recombinant vaccines containing "minigenes" that encode isolated LCMV CTL epitopes as short as 11 residues can confer protection against normally lethal doses of challenge virus, and different epitopes can be linked on a "string of beads" to protect on several MHC backgrounds (An and Whitton, 1997; An and Whitton, 1999; Whitton *et al.*, 1993). No LCMV-specific antibody responses are induced by these vaccines, which proves that protective effects can be mediated by cellular immune responses. CD8⁺ T cells are also important in the control of human viral diseases. EBV infects and transforms human B lymphocytes, and the control of this cell population appears to be managed in large part by virus-specific CD8⁺ cells. Indeed, some immunosuppressed individuals, lacking such cells, may develop EBV⁺ lymphomata (Rickinson *et al.*, 1992). Marked CD8⁺ T cell responses have also been found against influenza virus, measles virus, mumps, respiratory syncytial virus, human immunodeficiency virus, and other agents.

Two major effector mechanisms underlie the *in vivo* antiviral effects of virus-specific CD8⁺ T cells: cell lysis and cytokine release. Most virus-specific CD8⁺ T cells can lyse infected target cells, and thus justify the name CTL. CTLs contain the protein perforin (Podack *et al.*, 1988), which is released on contact with an infected cell, and self-assembles into transmembrane pores that penetrate the cytoplasmic membrane of the target cell—leading to cell death. Transgenic mice with a dysfunctional perforin gene are much less effective at controlling infection by some (though not all) viruses (Kagi *et al.*, 1994a; Kagi *et al.*, 1994b; Walsh *et al.*, 1994). Furthermore, virus-specific CD8⁺ T cells can induce apoptotic lysis when the Fas ligand (FasL) protein, expressed on the T cell membrane (Suda and Nagata, 1994), interacts with Fas protein on the infected cell, initiating a signaling cascade that ends in target cell death (Shresta *et al.*, 1998; Welsh *et al.*, 1990;

Zychlinsky *et al.*, 1991). CD8⁺ T cells release antiviral cytokines. Many CD8⁺ T cells release high levels of cytokines—for example, interferon- γ (IFN γ) and tumor necrosis factor- α (TNF α). Mice lacking the IFN γ receptor have increased susceptibility to several infections, despite apparently normal CTL and Th responses (Huang *et al.*, 1993). It has been cogently argued that a major role of the TcR/MHC/peptide interaction is simply to hold CD8⁺ T cells in the immediate proximity of virus-infected cells, thus focusing cytokines on the infected cell (Ramsey *et al.*, 1993; Ruby and Ramshaw, 1991); and convincing data from mice persistently infected with LCMV (Oldstone *et al.*, 1986; Tishon *et al.*, 1995), from hepatitis B virus (HBV) transgenic mice (Guidotti and Chisari, 1996; Guidotti *et al.*, 1996), and from HBV-infected primates (Guidotti *et al.*, 1999) have shown that viral materials can be eradicated *in vivo* from neurons (Oldstone *et al.*, 1986; Tishon *et al.*, 1995) and from hepatocytes (Guidotti and Chisari, 1996; Guidotti *et al.*, 1996), in the absence of cytolysis.

The availability of these two T cell effector mechanisms allows us to consider how virus infections might ideally be handled by the host. In the following scenarios we shall consider two interacting variables: first, the pathogenicity of the virus; and second, the resilience of the infected organ. Consider a cell infected by a highly lytic virus. Intuitively, it may seem that the host should attempt to lyse the doomed cell; after all, the cell will die soon, and early lysis may benefit the host, by destroying a virus “factory” and thus preventing release of infectious particles. In many organs, this is precisely what happens. Often, the cells that are lysed are later replaced; for example, the regenerative power of the liver is legendary (it has been estimated that 10^9 hepatocytes are produced daily to replenish cells lost during HBV infection [Nowak *et al.*, 1996]). Furthermore, even if tissue regeneration is incomplete, most host organs are sufficiently functionally redundant so as to allow the host to tolerate loss of a significant proportion of the organ mass; for example, we can tolerate loss of ~90% of kidney function before suffering signs and symptoms of renal failure. However, what of a tissue which can neither regenerate, nor function appropriately, if some of its components are lost? In such a tissue, it would not make sense for the host to lyse infected cells; it would be better to take the risk that the virus is lytic than to consign the cell to certain immunopathological death. Since the host presumably cannot foresee the lytic capacity of an infectious agent, it faces a dilemma—should it kill an infected cell (beneficial in most tissues, for both lytic and nonlytic viruses), or should it instead secrete cytokines, allowing the infected cell to survive (possibly beneficial, for nonlytic infections

in organs that have minimal regenerative capacity, and in which the host cannot tolerate cell loss)? To render such a choice meaningful, the CD8⁺ T cell response would have to be able to mount responses that were nonlytic in nature, thus providing the capacity for a cytokine-mediated antiviral effect, while permitting survival of the infected cell. There is some evidence for the existence of nonlytic CD8⁺ T cells (Blackbourn *et al.*, 1994; Levy *et al.*, 1996).

III. CENTRAL NERVOUS SYSTEM AS A HAVEN FOR VIRUSES

Many DNA and RNA viruses establish infection in the CNS, and often in neurons. Why should this be the case? The most likely reason is that the CNS, and its cells, are immune privileged—they are less open to immune surveillance than most other organs or cell types. Intuitively, this makes sense. CNS neurons are nondividing cells, and have historically been considered irreplaceable. Although some recent findings indicate that neurons and their pathways may be more resilient and plastic than previously thought, it is clear nevertheless that the host can ill afford to lose such vital cells. Were neurons to be as accessible as most somatic cells following virus infection, they would be susceptible to lysis by virus-specific CTLs, if sufficient class I/peptide complexes were expressed (as discussed below). Perhaps to limit this destruction, evolution has rendered neurons less open to immune surveillance.

A. Blood–Brain Barrier

Much of the CNS resides behind the blood-brain barrier, which resists passage of most cells, and even of many molecules. As a result, the CNS is biochemically and cellularly distinct from other organs. (The blood–brain barrier is the topic of another chapter in this volume.)

B. CNS Cells are Not Easily Recognized by Antigen-Specific T Cells

When analyzed *in vitro*, neurons show minimal transcription or surface expression of class I MHC, although this is inducible by IFN- γ (Joly *et al.*, 1991; Lampson *et al.*, 1983; Lampson and Fisher, 1984; Neumann *et al.*, 1995). Furthermore, neurons differ from most cell types in failing to express several other components of the class I antigen presentation pathway (e.g., β_2m and the TAP transporters) (Joly and Oldstone, 1992). Interestingly, IFN- γ upregulates these mole-

cules, along with the class I heavy chain, leading to cell-surface expression of peptide/MHC complexes; in contrast, TNF α upregulates transcription of class I, but not of various accessory molecules, and therefore, there is no increase in cell surface class I/peptide expression following exposure to this extremely toxic cytokine (Neumann *et al.*, 1997). We have recently shown that different populations of virus-specific CD8⁺ T cells can selectively express IFN γ or TNF α (Slifka and Whitton, 2000); it is tempting to suggest that interactions between infected neurons and particular subpopulations of virus-specific T cells in the CNS might secrete TNF α , but not IFN γ , thereby permitting the eradication of virus, without causing extensive disruption of the immune recognition status of neighboring CNS cells. Furthermore, we have recently analyzed the regulation of cytokine synthesis by antigen-specific CD8⁺ T cells, and have shown it to be exquisitely sensitive to antigen contact (Slifka *et al.*, 1999). Even at the height of infection, cytokine synthesis is turned off in the vast majority of the virus-specific CD8⁺ T cells; transcription of cytokine mRNA begins immediately upon antigen contact, and cytokine production terminates instantly upon antigen disengagement. Thus, CD8⁺ T cells produce cytokines only when they are in direct contact with the appropriate peptide/MHC complex. One can speculate that there may be an evolutionary advantage of this arrangement in the CNS. Cytokines such as IFN- γ upregulate cell-surface expression of class I MHC, which may be disadvantageous to the host; if T cells produced IFN- γ in a promiscuous manner, this might lead to the display of MHC complexes on cells throughout the CNS, so the tight regulation of cytokine production ensures that this risk is minimized.

Of course, *in vitro* results may not reflect the normal status and responsiveness of neurons *in vivo*. However, *in vivo* studies have shown that, under normal circumstances, CNS neurons—whether they lie within or outwith the blood–brain barrier—exhibit low-to-undetectable levels of MHC class I and β_2m (Lampson and Hickey, 1986; Whelan *et al.*, 1986).

C. CNS Environment May Suppress T Cell Activity

Gangliosides—glycosphingolipids—have long been thought to modulate the immune response mounted by NK and T cells (Bergelson *et al.*, 1989; Bergelson, 1993; Bergelson, 1995), and recent work (discussed in another chapter) indicates that these molecules may contribute to an immunosuppressive milieu in the CNS during virus infection (Irani *et al.*, 1996; Irani, 1998). It is therefore possible that activated virus-

specific T cells which enter the CNS are functionally impaired by interactions with these complex lipids, which are abundant in this tissue.

IV. VACCINATING AGAINST VIRUS-INDUCED CNS DISEASES: AN INTRODUCTION TO TWO MOUSE MODELS

A. Vaccinating Against CNS Viral Diseases

Vaccines are designed not to prevent infection, but to diminish the frequency and severity of disease. Immunizing against virally induced CNS diseases does not necessarily require the induction of immunity within the CNS itself. Perhaps the best example is polio vaccine, which induces a strong antibody-mediated mucosal immunity. If the vaccinee ingests virally contaminated material, these antibodies either prevent enteric infection, or else radically reduce the level to which the virus can replicate in the gastrointestinal tract. This has two benefits. First, the infected individual is less likely to develop a severe viremia, which in turn greatly diminishes the risk of CNS infection and disease. Second, the infected host will excrete less virus, thus reducing the risk of infection for his or her susceptible neighbors. Thus, polio vaccines can protect the individual and the community against poliomyelitis, without inducing CNS-specific immune responses in the vaccinee. Indeed, none of the currently available vaccines against the diseases reviewed in Section I of this chapter are known to induce responses in the CNS; all of them work by inducing systemic immunity, which limits infection or viral replication/dissemination. In many ways this is encouraging, for it implies that, to be successful, a vaccine does not have to overcome the immune privilege present in a healthy CNS.

B. Two Mouse Models of CNS Virus Infection and Disease

Having argued that the CNS is an immune-privileged site, we must now acknowledge that this privilege is incomplete. Indeed, this is implicit in the fact that virus infections can result in encephalitis; the inflammatory response (particularly the presence of virus-specific lymphocytes) is unequivocal evidence that any immune privilege has been breached. Animal models have revealed much about the immune responses that take place in the CNS. Here we shall describe two models, the LCMV and Theiler's virus, which are studied by our laboratories; these virus infections allow us to demonstrate different facets of the immune response in the CNS.

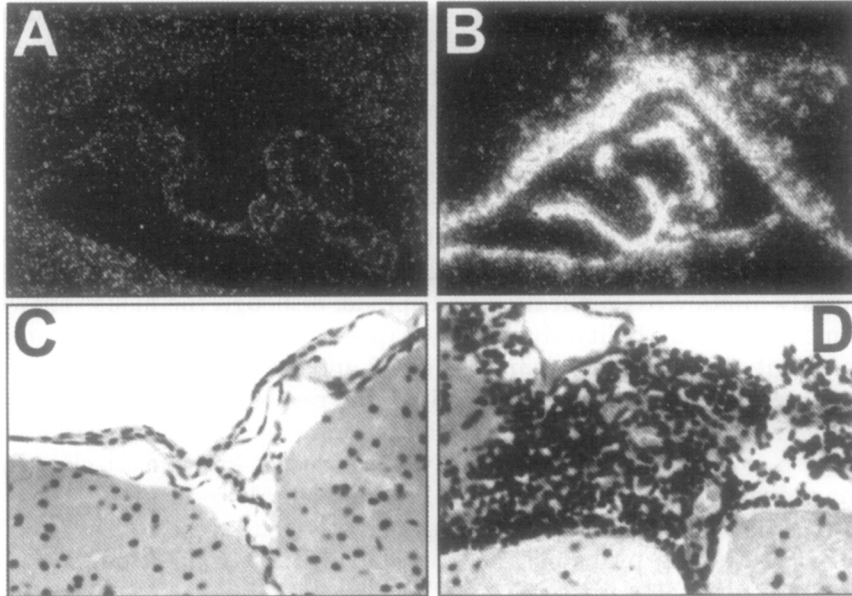


FIG 1. Lymphocytic choriomeningitis 6 days after intracranial LCMV inoculation, and virus distribution in the choriomeninges. (A and C) Brain sections from uninfected control mice; (B and D) from a BALB/c mouse 6 days after intracranial LCMV infection (infectious dose: 2 pfu). Sections were evaluated using either *in situ* hybridization to detect LCMV (A, B), or by H&E staining to evaluate inflammatory changes (C, D).

1. LCMV

This arenavirus causes aseptic meningitis in humans and mice. In the mouse model, choriomeningitis is most consistently achieved by intracranial inoculation of a low dose of virus (~0.2–2 pfu [plaque-forming units] in 20–50 μ l). The mice appear essentially normal for ~5 days; on the sixth day they become ill (ruffled fur, hunched posture, reduced mobility), and they die between days 7 and 8. Analysis of the cerebrospinal fluid reveals a massive lymphocytic infiltration (as shown in Fig. 1), which is dominated by CD8⁺ T cells whose depletion permits the mouse to survive (Dixon *et al.*, 1987). Therefore, lethal LCM is a good example of CD8⁺ T cell-mediated immunopathology. Although the lethal outcome is CTL-dependent, these same cells can confer protection against infection and disease (Allan and Doherty, 1985). Indeed, as stated above, a vaccine encoding a single CTL epitope can protect against subsequent intracranial LCMV challenge (Klavinskis *et al.*, 1989; Whitton *et al.*, 1993). This apparent anomaly is explained by con-

sidering the relative kinetics of virus infection and of the immune response. In a previously naive mouse, the virus replicates in the original infected cell and, in the absence of an established CTL response, is free to disseminate throughout the choriomeninges. By the time that the virus-specific CTL response has amplified to a meaningful level, the choriomeningeal cells are heavily infected, as shown in Fig. 1B. The CTL response is therefore intense and extensive (Fig. 1D), and results in death of the host. In contrast, if the mouse has been successfully vaccinated to induce epitope-specific CTL, the accelerated CTL response quickly limits virus replication and spread; although the mouse shows some signs of morbidity around day 4 (presumably the result of a mild meningitis), the virus is cleared by day 7 and the animal makes a complete recovery. The kinetics of the immune response are crucial; if the response induced is too low, the disease may, in fact, be exacerbated (Oehen *et al.*, 1991). Surprisingly, we still do not know precisely why the naive animals succumb to LCMV challenge. Mice lacking the perforin gene survive, despite mounting a strong virus-specific CD8⁺ T cell response leading to histological choriomeningitis (Kagi *et al.*, 1994a; Walsh *et al.*, 1994); this indicates that abrogation of the CD8⁺ T cells' lytic activity is sufficient to prevent death, even in the presence of an infiltrate. It is hypothesized—but not proven—that death results from perforin-mediated destruction of the choroid plexus, which leads to dysregulation at the blood/CSF interface.

2. Theiler's Virus Infection of CNS

Theiler's murine encephalomyelitis virus (TMEV) is a single-stranded positive-sense RNA virus. These viruses can be separated into two general groups, depending on neurovirulence. Highly neurovirulent strains include the GDVII and FA viruses. As little as 5 pfu injected intracranially causes a massive infection of the limbic system, particularly the hippocampus (Fig. 2A), and can kill a mouse in 7 days. Neurons die by apoptosis (Tsunoda *et al.*, 1997). This is contrasted with infection of mice with the less neurovirulent strains, DA, WW, and BeAn viruses, which leads to an acute polioencephalomyelitis that is followed by a chronic inflammatory demyelinating disease. An interesting feature of this less virulent infection is that the CNS distribution of lesions and virus alters as the infection transits from the acute phase to the chronic phase. During the acute phase of infection in susceptible mice, viral antigens and RNA are found mostly in neurons of the gray matter. Inflammation is also exclusively present in the gray matter. In contrast, during the chronic phase, virus-infected cells, and inflammation accompanied by demyelination, are primarily detected in the white

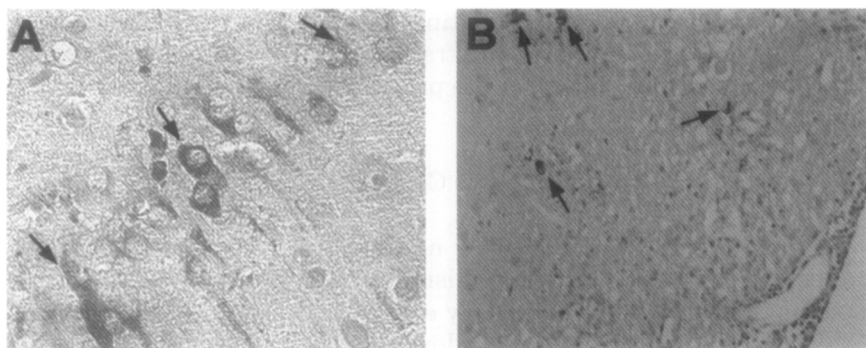


FIG 2. Acute and chronic infections with Theiler's virus. (A) Acute disease during Theiler's virus infection. Hippocampal neurons are shown to contain viral proteins. Magnification: $\times 200$. (B) Root entry zone from a mouse chronically infected with Theiler's virus. Magnification: $\times 100$. Inflammation and viral antigen-positive cells are present in close association.

matter of the spinal cord (Fig. 2B) (Yamada *et al.*, 1991). It is still not clear whether the astrocyte, oligodendrocyte, microglial cell, or macrophage (or a combination thereof) is the primary site of virus persistence. Resistance to chronic Theiler's virus disease maps to the MHC class I H-2D region (reviewed in Yamada *et al.*, 1991). MHC class I-restricted CD8⁺ CTLs, specific for VP1 and VP2 capsid proteins, are found in resistant mice, indicating that MHC class I-restricted virus-specific CD8⁺ CTLs are important in clearance of infection. Since MHC class I expression is upregulated in the CNS during Theiler's virus infection, it has been hypothesized that the CTL response eliminates virus during the acute phase. Neurons are infected during the early acute phase of infection, but during this phase MHC class I molecules are expressed only in glial and endothelial cells, not in neurons (Altintas *et al.*, 1993; Lindsley *et al.*, 1992). Therefore, in resistant mice, CTL may play a role in clearing virus from macrophages and/or glial cells, resulting in protection of these mice from the chronic stage. Tolerance induction of mice in regard to myelin did not alter the development of inflammatory demyelinating lesions characteristic of Theiler's mouse encephalomyelitis (Lang *et al.*, 1985). However, tolerance induction in regard to Theiler's virus prevented the development of clinical disease including inflammation and demyelination, which suggests that chronic immunopathogenic disease was directed against virus antigens persisting in the CNS (Karpus *et al.*, 1995). Some of the clinical and pathological features mimic the human demyelinating disease, MS. It appears that both CD4⁺ and CD8⁺ T cells contribute to the TMEV-

induced inflammatory demyelinating disease. Therefore, infection of mice with the less neurovirulent strains of TMEV has been used as an experimental animal model for the progressive forms of MS.

V. DNA VACCINES AND CNS VIRAL INFECTIONS

DNA vaccination is a relatively new entrant in the vaccine sweepstakes, but is viewed with optimism, for a number of reasons. This topic has been reviewed (Donnelly *et al.*, 1997; Hassett and Whitton, 1996; Liu *et al.*, 1997), but the following advantages of DNA vaccines should be noted. First, introduction of the encoded proteins into the MHC class I pathway induces good CD8⁺ T cell responses. Second, in most cases, proteins also should encounter the MHC class II pathway, and B cells, thus inducing CD4⁺ T cell and antibody responses. Third, the space limitation of most potential viral vectors does not apply to DNA vaccines, since many different plasmids could be contained in a single vaccine "cocktail." Fourth, it is possible to manipulate the immune response induced—for example, by directing plasmid-encoded proteins to selectively induce CD8⁺ T cells (Rodriguez *et al.*, 1997; Rodriguez *et al.*, 1998)—or to enhance induction of CD4⁺ T cells (Rodriguez and Whitton, unpublished data). Fifth, DNA vaccines should be safe, and easy to produce cheaply, in quantity, and at a high level of purity. These benefits have led many laboratories to evaluate DNA vaccines in a number of animal models, including several involving viruses that infect the CNS.

A. DNA Vaccines Against LCMV

DNA vaccines encoding the nucleoprotein (NP) from LCMV can confer protection against the normally lethal intracranial challenge (Yokoyama *et al.*, 1995; Zarozinski *et al.*, 1995), and can prevent the establishment of persistent infection (Pedroza Martins *et al.*, 1995). Protection is CTL-mediated and does not depend on the induction of antiviral antibodies. The vehicle (saline, or lipid-associated) and the route of administration are important in determining the level of induced immunity (Yokoyama *et al.*, 1996; Yokoyama *et al.*, 1997). The LCMV model has allowed the demonstration of the exquisite flexibility of DNA vaccines. If the LCMV NP gene is fused to the host protein ubiquitin, the resulting protein is targeted for very rapid intracellular degradation; a plasmid encoding this ubiquitin–NP fusion induces enhanced protection against intracranial challenge (Rodriguez *et al.*, 1997), perhaps because it increases the precursor frequency of NP-specific CTLs (Rodriguez *et al.*, 1998). In

addition, the LCMV model has been used to study neonatal DNA immunization. A single inoculation, within hours of birth, is sufficient to induce protective immunity, even in the presence of maternal antibodies (Hassett *et al.*, 1997), and these responses are long-lived and remarkably abundant; as long as 1 year post-DNA immunization, 1–2% of the animal's CD8⁺ T cells are NP-specific (Hassett *et al.*, 2000). Note that all of these studies employed “peripheral” immunization; none of them attempted to induce responses within the CNS. However, these data indicate that, following intracranial inoculation of virus, the DNA-vaccine-induced CTL can enter the CNS and limit LCMV replication and dissemination.

B. DNA Vaccines Against Theiler's Virus

To investigate the utility of DNA vaccines against Theiler's virus, cDNAs encoding the viral capsid proteins, VP1, VP2, and VP3, were constructed (Tolley *et al.*, 1999). Susceptible SJL/J mice were vaccinated intramuscularly one, two, or three times with the DNA vaccines. Mice were then infected with Theiler's virus, and clinical and pathological features of disease were followed. Interestingly, mice vaccinated with cDNA encoding VP2 were partially protected from clinical and pathological disease. In addition, VP3 vaccination was somewhat able to ameliorate clinical disease in infected mice. VP4 vaccination also protects mice from demyelinating disease (Tsunoda and Fujinami, unpublished). In contrast, mice vaccinated with cDNA encoding VP1 had a more severe clinical disease and enhanced histopathology as compared to nonvaccinated mice. There was no relationship between the antiviral antibody titers and the extent or course of disease. Thus, different outcomes were observed, depending on the viral antigen included in the vaccine.

C. DNA Vaccines Against Other Viruses that Cause CNS Disease

DNA vaccines have been shown to be effective against several of the agents reviewed in section I. In rabies, in a mouse model, immunization with plasmids encoding the rabies glycoprotein conferred complete protection against subsequent viral challenge (Ray *et al.*, 1997; Xiang *et al.*, 1994; Xiang *et al.*, 1995); protection was also seen in mice immunized as neonates (Wang *et al.*, 1997), confirming the efficacy of neonatal DNA immunization as demonstrated in the LCMV model. Recently, DNA immunization of *Cynomolgus* monkeys was shown to completely protect against subsequent challenge, and to generate levels of antibodies comparable to those induced by the standard human diploid cell vaccine

(Lodmell *et al.*, 1998). These data suggest that DNA immunization may have a future in higher primates, such as the readers of this chapter. In measles, there is ample evidence showing that DNA vaccines can induce measles-specific humoral and cell-mediated immunity (Cardoso *et al.*, 1996). Furthermore, neonates are an important target for measles vaccination, and DNA immunization at this age induces measles-specific CTL (Martinez *et al.*, 1997). Although there is no widely used small animal model for measles-induced postinfectious encephalomyelitis or SSPE, intracranial measles virus inoculation can cause encephalitis, and this disease is abrogated by prior DNA immunization with a plasmid-encoding measles nucleoprotein (Hsu *et al.*, 1998). Recently, a transgenic mouse line has been developed that expresses measles virus receptor in neurons (Rall *et al.*, 1997), and provides the opportunity to evaluate the effects of measles-specific immune responses in the CNS (Lawrence *et al.*, 1999). DNA vaccines are also effective (in animal models) in combating various arboviral encephalitides, including St. Louis encephalitis (Konishi *et al.*, 1998; Phillpotts *et al.*, 1996), Japanese encephalitis (Ashok and Rangarajan, 1999; Konishi *et al.*, 1999; Lin *et al.*, 1998), La Crosse encephalitis (Schuh *et al.*, 1999) and Murray Valley encephalitis (Colombage *et al.*, 1998).

D. DNA Vaccines Against Autoimmune Diseases of the CNS

Several virus-induced CNS diseases may be explained by their triggering of autoimmunity. Experimental autoimmune encephalomyelitis (EAE) is a well-characterized CNS disease induced by the administration of certain CNS proteins (or epitopes from these proteins). We have shown that peripheral immunization with recombinant vaccinia viruses or plasmid DNAs encoding these CNS proteins or epitopes can radically alter the susceptibility of the host to EAE (Barnett *et al.*, 1993; Barnett *et al.*, 1996; Tsunoda *et al.*, 1998; Wang *et al.*, 1999)—thus establishing the potential for vaccination against autoimmune phenomena. However, vaccination with plasmid DNA alone can potentiate both EAE and the Theiler's virus demyelinating disease, most likely due to the immunostimulatory CpG motifs contained in the bacterial DNA (Tsunoda *et al.*, 1999).

E. DNA Vaccines Against Prion Diseases

TSEs are rare in humans, and at present the major medical interest probably comes from the risk of interspecies transfer to humans; it is hypothesized that a number of unusual cases of CJD in young Britons

resulted from their having been exposed to products from cattle carrying bovine spongiform encephalopathy (Will *et al.*, 1996). Although this problem may have been partially addressed by the culling of infected herds, the investigation of interspecies transfer remains important, as the pooled offal and rendering products, although no longer fed to animals directly in the human foodchain, in some cases are used to make other products to which some of us are intimately exposed (cosmetics, for example). Thus, despite the rarity of TSEs, the prospect of being able to immunize against them is exciting.

Most infectious agents stimulate antigen-specific host immune responses. Current dogma suggests that host immunity plays little or no role in the pathogenesis of TSE; indeed, since the infectious protein is host-encoded, it might be expected that no immune response would be mounted and, consistent with this, a mouse TSE agent inoculated into normal mice appears to induce a very limited immune response. Thus, it might appear pointless to pursue the idea of vaccinating against "self"-proteins. However—possibly relevant to the immunological modification of prion diseases—Alzheimer's disease may result from CNS deposition of the misfolded β -amyloid protein, and immunization with this protein's precursor fragment (which is, of course, a self-protein) slows the development of the characteristic neuropathological changes (Schenk *et al.*, 1999), raising the possibility that immune responses to PrP might alter the disease course. Furthermore, steroids appear to reduce susceptibility to TSE (Outram *et al.*, 1974), and recent findings indicate that CD8⁺ T cell infiltration may occur as an early indicator of TSE (Betmouni *et al.*, 1996), although the antigen-specificity of these T cells was not defined. DNA immunization offers a promising tool for evaluating the relevance of prion-specific immune responses, because it induces CD8⁺ T cells, and may even be able to overcome a "nonresponder" status of the host (Schirmbeck *et al.*, 1995). In fact, DNA immunization of PrPko mice does indeed induce anti-PrP antibodies, but T cell responses were not pursued (Krasemann *et al.*, 1996). Might DNA immunization protect against disease (a vaccine against interspecies transfer?), or might it exacerbate disease by priming for immunopathology? Such studies are under way in one of our laboratories (JLW).

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