VIRUS-INDUCED IMMUNOPATHOLOGY

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I. Introduction

The burgeoning field of viral immunology has been heavily influenced by the lifestyle set by infection of mice with lymphocytic choriomeningitis virus (LCMV). Studies of this virus provided vital clues to the understanding of immunological tolerance (Burnet and Fenner, 1949). T-cell recognition (Doherty and Zinkernagel, 1974), the concept of high-dose paralysis (Hotchin, 1971) [now more popularly termed "immune exhaustion," Moskophidis *et al.* (1993)], and the notion that tissue injury may result from an immune response to virus-infected cells (Buchmeier *et al.*, 1980) or to immune complexes composed of viral protein and antibody (Oldstone and Dixon, 1969). Virus-induced immunopathology likely operates to some degree in many, perhaps even all, animal virus infections. In fact, a degree of immunopathology may be the tariff the body must pay to eliminate infections by most agents, even those which are highly cytopathic. With many noncytolytic viruses it is only because of the immune response against them that any discernable disease occurs. The prime example is set by LCMV, but the mechanism(s) operative in this infection do not repre-

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sent the full gamut of cellular and molecular events involved in virus-induced immunopathologies. In this brief review, some principal examples of immunopathogenesis are discussed, accompanied by speculations about management. Indeed, the control of such viral infections requires the use of therapeutic vaccines and immune modulators that suppress the development of lesions, since prophylactic vaccines may not always succeed in preventing infection.

II. IMMUNOPATHOLOGICAL LESIONS WHICH PRIMARILY INVOLVE CD8⁺ T CELLS

Infection of mice with LCMV provides the hallmark example of a CD8⁺ T-cell-mediated immunopathology (Buchmeier et al., 1980). Strains of LCMV differ in tissue tropism and virulence, but all are largely noncytolytic and the virus fails to cause overt tissue damage (Hotchin, 1971). Such damage only occurs after the development of an immune response that primarily involves T cells of the CD8⁺ subset (Doherty et al., 1990; Kagi et al., 1995). Early clues that lesions in LCM are immunopathological came from the observation that the disease only occurred in immunocompetent animals. Accordingly, tolerized as well as immunosuppressed mice failed to exhibit the characteristic choriomeningitis (Burnet and Fenner, 1949; Buchmeier et al., 1980). Later evidence came from adoptive transfer experiments with T-cell subtypes, the use of monoclonal antibodies to selectively deplete various cell types in vivo, and very recently the use of gene knockout and transgenic mice (Kagi et al., 1995; Baenziger et al., 1986; Leist et al., 1987).

The evidence provides a clear-cut conclusion: tissue damage occurring in LCMV infection involves the essential participation of T cells of the CD8⁺ lineage. However, the exact mechanisms by which CD8⁺ T cells cause the inflammatory tissue damage remains unresolved. For example, in cerebral infections with LCMV, a significant recruitment and extravasation of immunoinflammatory cells to the sites of viral replication occurs in the meninges, ependymal membranes, and choroid plexus of the brain (Buchmeier et al., 1980). Only a minor proportion of cells in the inflammatory reactions are LCMV antigen-specific CD8⁺ T cells, yet these are essential for the inflammation to proceed (Doherty et al., 1990). One hypothesis contends that the tissue damage results from direct cytotoxicity by CD8⁺ cytotoxic T lymphocytes (CTL) to virus-infected cells (Kagi et al., 1995). This idea is supported by the observation that knockout mice unable to generate perforin do not express disease (Kagi et al., 1995). However, in brains

with lesions no evidence of overt cellular pathology is present histo-
logically (Walker *et al.*, 1975). Alternatively, although the primary step
may involve recognition by specific CD8⁺ T cells, the bulk of tissue
damage may result from the release of several proinflammatory cyto-
kines from the activated inflammatory cells recruited to the site by
factors released from CD8⁺ T cells (Campbell *et al.*, 1994a).

Cytokines such as IL-1, IL-6, TNFα/β, IFNα, and IFNγ have been
advocated as likely proinflammatory participants (Campbell *et al.*,
1994b; Sandberg *et al.*, 1994). Moreover, the actual cellular injury
could well involve the generation of nitric oxide from IFNγ-activated
macrophages recruited to the site (Campbell *et al.*, 1994a). Contrary to
this hypothesis, however, is the recent evidence that lesions and dis-
ease susceptibility occur normally in IFNγ knockout mice (Campbell,
1995) and that attempts to ablate nitric oxide production do not
ameliorate LCM (Campbell *et al.*, 1995). In LCMV-induced disease, the
most intensively studied virus-induced immunopathology, one must
conclude that the cellular and especially the molecular events leading
to tissue damage remain a poorly understood immunoinflammatory
bouillabaisse.

Several other viral infections also appear to induce disease by inter-
acting with CD8⁺ T cells. At least two human diseases provide candi-
date examples. These are acute hepatitis caused by hepatitis B virus
(HBV) and myocarditis induced by Coxsackie B virus (CBV). A third
candidate is certain lesions caused by HIV (Pantaleo *et al.*, 1993). With
the former two examples, excellent murine animal models are avail-
able, and these have helped considerably to depict a role of CD8⁺ T cells
in their pathogenesis. Particularly informative have been studies on
hepatitis B virus infection using the artificial system of transgenic
mice expressing various gene constructs of HBV (Chisari and Ferrari,
1995). These investigations reveal that CD8⁺ T cells are principally
involved as mediators of viral clearance as well as immunopathology,
and that a variety of cellular and molecular events are at play (Ando *et
al.*, 1993). It seems reasonable to assume that similar CD8⁺ T-cell-
mediated events occur in human HBV-induced hepatitis, but this
remains to be shown.

Transgenic mice that express HBV envelope antigens in their
hepatocytes remain normal. However, they readily develop lesions
which resemble human acute viral hepatitis if given adoptive transfers
of CD8⁺ MHC class I restricted HBV-specific cytotoxic T lymphocytes
(Chisari and Ferrari, 1995). Sequential analysis of the basic system
reveals that the lesions progress in a predictable stepwise fashion. The
earliest detectable step involves direct attachment of CTL to HBV

antigen-positive hepatocytes, with the latter being killed by apoptosis. The widely scattered apoptotic hepatocytes in the transgenic mouse livers are reminiscent of acute viral hepatitis lesions in humans (Ando *et al.*, 1994a). Given the nature of apoptosis, death of such hepatocytes is unlikely to release agonists which drive the inflammatory response. However, after the initial apoptotic phase, antigen-nonspecific inflammatory cells such as neutrophils and monocytes are recruited, and these cause far more hepatic cell damage than do CTL and the damage zone extends way beyond the sites of CTL-mediated apoptosis (Ando *et al.*, 1993). Such events are presumed to be mediated by cytokines, particularly IFNγ, probably released by CTL, which can be directly cytotoxic to hepatocytes that express abundant levels of HBV surface antigen (Guidotti *et al.*, 1995).

In some transgenics in which hepatocytes retain high levels of HBV surface antigen, necrosis can be massive, and the mice die of hepatitis (Guidotti *et al.*, 1995). This step can be prevented by prior administration of neutralizing antibody to IFNγ or by depletion of macrophages. Consequently, in the HBV transgene model the immunopathology is initiated and likely orchestrated mainly by CD8⁺ T cells, but the principal immunopathological effects appear to be mediated nonspecifically by cytokines and recruited cells such as macrophages. This pattern of events is more commonly found in CD4⁺ T-cell-mediated immunopathology (discussed subsequently). What is of particular interest in the HBV transgene model is that when the transgene is expressed in tissues such as the kidney or brain, no disease ensues in these organs following the subsequent intravenous adoptive transfer of HBV-specific CTL (Ando *et al.*, 1994b). To cause damage in such organs, which compared to the liver have blood vessels that impede the escape of T cells, requires that the CTL be placed directly into the organs. Such observations indicate that the induction of immunopathology to a virus infection requires not only that the agent be present but that it be available for recognition by CD8⁺ T cells. In the HBV transgene model, as in LCM, the molecular mechanisms of disease expression await further elucidation.

The murine CBV model of immunopathology has received less investigation than LCMV and HBV, and CBV is far more cytolytic than either LCMV or HBV and can alone cause tissue damage (virological pathology) (Woodruff, 1980). The CBV type 3 strain causes myocardial disease, although the extent of this syndrome is subject to numerous variables of virus and host (Chow *et al.*, 1991). Recently, knockout mice were used to follow the pathogenesis of CBV-3-induced myocarditis, which occurs in mice that survive the acute disease (Hanke *et al.*,

1995). A clear role for CD8$^+$ T-cell function was observed. Accordingly, myocarditis was severe in CD4$^+$ knockout mice but only minimal in animals deficient for a CD8$^+$ T-cell response because of β2M knockout (Hanke *et al.*, 1995). Moreover, the severe disease in CD4$^+$ knockout mice was abrogated by *in vivo* depletion of CD8$^+$ T cells with specific monoclonal antibodies. Such data clearly implicate a major role for CD8$^+$ T cells in the immunopathogenesis of CBV-3-induced myocarditis, but the molecular mechanism of tissue damage remains to be established. Some favor the notion that TNFα and IL-1β are involved, since if such cytokines are administered to infected mice the myocarditis is exacerbated (Lane *et al.*, 1992). Furthermore, TNFα is readily demonstrable in inflammatory cells at the site of cardiac lesions (Hanke *et al.*, 1995).

Great interest and alarm recently came from the outbreak of a rapidly progressive influenza-like, often fatal illness in several previously healthy persons in the Four Corners area of the United States (Nichol *et al.*, 1993). The outbreak was associated with a previously unrecognized agent now named Sin Nombre Virus (SNV) (Eliott *et al.*, 1994). The pulmonary histopathology in patients dying of the disease appeared consistent with an acute immunopathological response to virus-infected cells in the lung (Zaki *et al.*, 1995). Prominent among the inflammatory cells were CD8$^+$ T lymphocytes and it seems possible that such cells may be primary mediators of the pathogenesis of the emerging infectious disease. The results of ongoing studies on the pathogenesis of SNV infection should prove intriguing.

The viral disease whose mechanism of pathogenesis is under the most intensive investigation is, of course, HIV. Unfortunately, suitable murine models of HIV pathogenesis are lacking, and so it remains difficult to assess experimentally the viewpoint that HIV pathogenesis involves CD8$^+$ T cells (Pantaleo *et al.*, 1993; Zinkernagel and Hengartner, 1994). Initially, such cells are considered to play a protective role. However, this defense function is imperfect and virus usually persists in numerous cell types without causing their destruction. Conceivably, destruction of antigen-presenting cells as well as CD4$^+$ T cells which harbor virus by antigen-specific CD8$^+$ T cells may contribute to immunosuppression (Zinkernagel and Hengartner, 1994). The idea that viruses cause immunosuppression by a CD8$^+$ T-cell-mediated immunopathological reaction against infected cells of the immune system is clearly evident in LCMV infection of mice (Mims and Wainwright, 1968; Jacobs and Cole, 1976, Odermatt *et al.*, 1991). Depending on numerous variables affecting both the virus and infected host, a marked immune suppression and resultant enhanced suscepti-

bility to other agents are observed. Indeed, some have strongly advo-
cated that the pathogenesis of immune suppression in HIV infection
might best be understood by studying LCMV infection in mice
(Zinkernagel and Hengartner, 1994). In LCMV infection, suppression
results from an antiviral CD8$^+$ CTL response against infected antigen-
presenting cells such as macrophages and perhaps dendritic cells
(Jacobs and Cole, 1976). For a more complete discussion on the im-
munopathogenesis of HIV infection, other reviews are recommended
(Pantaleo et al., 1993; Zinkernagel and Hengartner, 1994).

III. IMMUNOPATHOLOGICAL REACTIONS PRIMARILY INVOLVING CD4$^+$ T LYMPHOCYTES

Of the two principal types of $\alpha\beta$TCR T cells, the CD4$^+$ T-cell subset is
usually considered to participate in effector activities more by generat-
ing an abundance of cytokines than do CD8$^+$ T lymphocytes. Such cyto-
kines express a variety of activities that include recruitment and
activation of nonspecific effector cells (Meltzer and Nacy, 1989). CD4$^+$ T
cells can mediate direct effects such as cytotoxicity (Kolaitis et al.,
1990), but likely a more common function in vivo is to conduct an
inflammatory reaction (Meltzer and Nacy, 1989). Such responses are
usually termed delayed-type hypersensitivity (DTH) reactions. These
represent accumulations of numerous cell types. Only a minority of
cells are lymphocytes, and these include the antigen-specific CD4$^+$ T
cells. The majority of cells are neutrophils and mononuclear cells, and
these cells, especially the latter, are assumed to be the principal pur-
veyors of the immunoprotective and tissue-damaging effects (Meltzer
and Nacy, 1989). These damaging effects are numerous and include
several proteolytic enzymes, reactive radicals of oxygen and nitrogen, and
perhaps some cytokines such as TNFα (Laskin and Pendino, 1995).

Inflammatory reactions instigated by CD4$^+$ T cells vary in cellular
composition and the nature of the chemical activities generated. In
part, this reflects the fact that CD4$^+$ T cells express different profiles of
cytokines and perhaps other signaling molecules such as chemokines
(Mossman and Coffman, 1989). Usually, CD4$^+$ T cells are divided into
two functional subsets: Th1 T cells producing principally IFNγ, IL-2,
and TNFβ, and Th2 cells, which mainly produce IL-4, IL-5, and IL-10
(Mosmann and Coffman, 1989; Paul and Seder, 1994). The former cell
types largely marshal inflammatory responses dominated by mononu-
clear cells and neutrophils. Th2-mediated inflammatory responses
have more eosinophils and basophils; these are uncommon responses

to viral infections, although they are likely responsible for the alveo-litis which occurs in respiratory syncytial virus (RSV) infection (Alwan *et al.,* 1994). The Th1-organized DTH reactions are considered common responses to viruses, and in some situations these are chronic and tissue-damaging. Such reactions occur in response both to cytolytic viruses such as measles (Johnson *et al.,* 1978) and herpes simplex virus (HSV) (Doymaz and Rouse, 1992) and to viruses which are noncyto-destructive. Against cytolytic viruses the DTH response may be consid-ered primarily protective unless, as discussed subsequently, the infec-tion causes the exposure of self-derived determinants which in turn perpetuate the response.

A. Virus-Induced Immunopathology Orchestrated Mainly by Type 1 Cytokine-Producing CD4⁺ T Cells

In the case of persistent viruses that are minimally cytopathic, the CD4⁺-mediated DTH reaction to them, with its accompanying tissue damage, must be considered as largely immunopathological. Examples of agents which largely follow this script in the mouse include Theiler's murine encephalomyelitis virus (TMEV) (Miller and Karpus, 1994), mouse coronavirus (Fleming *et al.,* 1993), and Semliki Forest virus (SFV) (Mokharian and Swoveland, 1987). These persistent virus infec-tions affect the central nervous system (CNS) of rodents and induce late inflammatory responses that involve the white matter, causing demyelination [reviewed in Fazakerley and Buchmeier (1993)]. Such viruses are of particular interest since the cause of a common demye-linating disease of humans, multiple sclerosis (MS), remains elusive, and involvement of viruses is suspected (Kurtzke, 1993). Despite sev-eral candidates, however, no single agent is currently accepted as a cause of MS (Waksman, 1995), but the idea that viruses can trigger MS as well as *several other autoimmune diseases remains a viable hypoth-esis* (Theofilopoulos, 1995). The many viruses associated with immune-mediated inflammatory diseases in the CNS of rodents include mouse coronavirus, SFV, and the picornavirus TMEV. In each instance the outcome of infection is markedly affected by virus strain, host geno-type, and the dose and route of infection. As these variables apply to TMEV the topic has been comprehensively reviewed by Brahic *et al.* (1991), and for mouse coronavirus by Fazakerly and Buchmeier (1993). In all three viruses, immune-mediated disease appears associated only with persistent noncytolytic infection, but the actual immune mecha-nisms at play are still in dispute. In the case of TMEV, maybe the best-understood of these infections, overwhelming evidence points to a

crucial role for CD4$^+$ T cells of the Th1 subtype (Miller and Karpus, 1994). Such cells are assumed to organize inflammatory responses that consist largely of monocytic phagocytes which mediate bystander demyelination. Accordingly, monocytes were demonstrated to strip neurons of their myelin lamellae *in vitro* (Dal Canto and Lipton, 1975), but exactly how this is accomplished biochemically is unclear. Candidate mechanisms likely include oxyradical production, nitric oxide, and TNFα released from IFNγ-activated macrophages. Other cytokines such as IL-6 might also play a role, since overexpression of this cytokine as well as some others in the brain causes neuropathologic consequences (Campbell *et al.*, 1993). Evidence also exists for direct destruction of oligodendroglial cells, the cellular source of myelin, by the cytokine TNFβ generated by CD4$^+$ T cells of the Th1 subtype (Miller and Karpus, 1994).

In the TMEV system, compelling evidence indicates that CD4$^+$ T cells of the Th1 subtype act as the essential cell type that organizes the demyelination, at least in the susceptible SJL mouse (Miller and Karpus, 1994). Data indicate that disease severity correlates temporally with the development of MHC class II restricted DTH reactions and Th1-dependent IgG2a antibody production (Peterson *et al.*, 1992). In addition, disease development is suppressed by anti-CD4 or anti-Ia treatment (Gerety *et al.*, 1994). More persuasive evidence for a vital role of CD4$^+$ Th1 cells came from experiments which showed that mice exposed to a modified form of TMEV antigens, which resulted in selective Th1 anergy (tolerance), had delayed and diminished disease (Karpus *et al.*, 1995). In addition, such mice had reduced Th1 cytokine production but elevated levels of Th2 cytokines. The tolerized mice also had minimal DTH reactions, elevated Th2-dependent IgG1 antibody responses and reduced numbers of CD4$^+$ T cells in the CNS. In TMEV, although virus may persist in oligodendroglial cells, most virus appears to be present in brain macrophages (Clatch *et al.*, 1985). Such cells express high levels of MHC class II in inflamed brains, and these cells likely act as the principal activators of the CD4$^+$ virus-specific T cells (Miller *et al.*, 1995). Indeed, macrophages isolated from brains with TMEV demyelination readily stimulate CD4$^+$ TMEV antigen reactive lines *in vitro* (Miller, 1995). Moreover, the disease is potentiated if CD4$^+$ class II restricted Th1-type TMEV-specific clones are adoptively transferred to infected mice (Gerety *et al.*, 1994). In conclusion, current results in the TMEV system clearly point to an immunopathological disease involving principally CD4$^+$ T cells of the Th1 phenotype. These cells, upon recognition of antigens, drive a bystander demyelinating lesion mediated by cytokine-activated mononuclear phagocytes.

Our current understanding of the cellular and molecular pathogene-

sis of mouse coronavirus and SFV lags behind the TMEV system. Several observations are consistent with the notion that the demyelination occurring in the white matter in both diseases results from T-cell-mediated immune responses to persistent noncytolytic virus infection of glial cells [reviewed in Fazakerley and Buchmeier (1993)]. Although one suspects that the mechanisms at play in mouse coronavirus and SFV resemble those occurring in TMEV and primarily involve CD4+ Th1 T-cell-mediated reactions, this idea has yet to be proven.

Whether or not any human demyelinating disease involves mechanisms akin to those discussed above for TMEV in the SJL mouse remains moot. Demyelination occurs occasionally as a sequel to measles infection and vaccination (Johnson *et al.*, 1978), and although an immune-mediated pathogenesis is suspected, viral antigens have not been demonstrated in the demyelinating lesions (Johnson *et al.*, 1984). However, measles virus might induce an autoimmune demyelination by a "hit and run" mechanism such as is suggested to occur in HSV-induced stromal keratitis, described subsequently. Postinfection demyelination also occurs very occasionally after infections with vaccinia, varicella, rubella, mumps, influenza, and EBV (Fazakerley and Buchmeier, 1993), but possible immune mechanisms at play have not been elucidated. There is considerable interest in the fact that HIV causes an inflammatory disease of the CNS (Spencer and Price, 1992). However, this occurs only in individuals who are markedly immunosuppressed and have very few circulating lymphocytes. The virus affects macrophages and these become MHC class II+ (Kure, 1990). There is no evidence of T-cell involvement, but it may be that the pathogenesis involves overproduction of the cytokine TNFα from macrophages occurring because of the paucity of CD4+ T cells, which normally produce inhibitors of macrophages such as IL-10 (Tyor *et al.*, 1995). However, demyelination certainly appears to have an immune pathogenesis in visna, an HIV-related virus infection of sheep (Narayan and Clements, 1989). Actually, the lesions of visna closely resemble those of TMEV-induced disease. The T cells in the inflammatory lesions appear to mediate their inflammatory effects by elaborating the ovine equivalent of gamma interferon (Narayan, 1989).

B. Virus-Induced Immunopathology Orchestrated Mainly by Type 2 Cytokine-Producing CD4+ T Cells

Of the viruses which commonly cause disease in humans, RSV infection provides the best example of a disease that likely has a CD4+ T-cell-controlled immune-mediated pathogenesis (Graham *et al.*, 1991). The virus persists in the body for a short time only and is minimally

cytopathic (Herman, 1990). When disease occurs, it usually manifests as the virus is being eliminated and involves many symptoms which mimic allergic reactions. Indeed, reports of the presence of IgE and eosinophil breakdown products in nasal secretions were consistent with an allergic pathogenesis (Welliver *et al.*, 1981), but such reports seem not to have been confirmed. Recently, several groups have studied RSV in mouse models and have shown a clear role for T cells as mediators of immunopathology (Alwan *et al.*, 1994; Graham *et al.*, 1991; Connors *et al.*, 1992). Although RSV replicates poorly in most mouse strains, a role for T cells in disease expression is well-established. For instance, lesions are minimal in immunosuppressed mice (Graham *et al.*, 1991), but become severe in immunocompetent virus-infected mice given adoptive transfers of RSV antigen-specific activated T cells (Alwan *et al.*, 1994). The most effective disease-producing cell transfers are CD4$^+$ T cells that express a Th2 cytokine profile (Alwan *et al.*, 1994). Interestingly, in the RSV system the different viral proteins appear to induce T cells of different cytokine-producing phenotypes (Alwan *et al.*, 1993). In fact, whereas the G protein of RSV induces the immunopathologic CD4$^+$ T cells with a type 2 cytokine profile, other proteins such as the F protein induce both CD4$^+$ and CD8$^+$ T cells, but such cells mediate protection rather than pathology (Anderson and Heilman, 1995). Moreover, these protective cells are largely IFNγ-producing and are considered type 1 cells. Exactly how the CD4$^+$ Th2 cells mediate the pathological immune reactions in the mouse lung is not known, but the prominence of eosinophils in the lesions indicates that such cells may participate in the tissue damage (Anderson and Herman, 1995).

With RSV infection in humans, it is well known that past efforts at vaccination led occasionally to augmented disease (Kapikian *et al.*, 1969). Using a mouse model which mimics this situation, Graham *et al.* (1993) have demonstrated that CD4$^+$ T cells of the type 2 cytokine-producing profile appear responsible for vaccine-augmented reactions. In addition, others showed that inhibition of type 2 cytokines with specific anticytokine antibodies eliminated the enhanced pulmonary pathology (Connors *et al.*, 1992). Taken together, the observations on murine RSV infections indicate a pathological role for type 2 cytokine-producing CD4$^+$ T cells. This makes RSV an unusual, possibly unique, viral agent whose pathogenesis mimics a pattern of events found more commonly in parasitic infections (Sher and Coffman, 1992). In the RSV system, it seems likely that shifting the immune response to a type 1 cytokine-producing cell dominance, as occurs by immunization with live virus (Anderson and Heilman, 1995), or perhaps better still by immunization with minimal vaccines that solely induce Th1 responses, would be a beneficial approach to prevent immunopathologic disease

following infection. Accordingly, in RSV infection, which commonly infects and causes repeated disease in children, the aim is to exploit Th1 memory maximally.

C. T-Cell-Mediated Immunopathogenesis in Cytolytic Virus Infections

The majority of viral infections that induce lesions with an immune-mediated component are persistent and minimally cytopathic. However, some highly cytodestructive viruses do induce immunopathic lesions at least in certain locations. Infection of the eye with HSV provides such an example (Doymaz and Rouse, 1992). Another might be rashes caused by viruses such as measles. Interestingly, measles virus rashes usually fail to occur in immunosuppressed patients (Enders, 1962), consistent with the notion that the lesions in immunocompetent individuals involve an immune reaction.

Ocular infection with HSV is one of the most common causes of vision impairment in the United States, with around 300,000 new cases of infection annually (Mader and Stulting, 1992). Lesions caused by HSV are usually confined to the corneal epithelium, but virus always enters the sensory nerve fibers that innervate sites of infection and passes to the trigeminal ganglion where a nonproductive infection (latency) occurs (Roizman and Sears, 1987). Periodically, virus reactivates and travels back to the cornea where replication occurs and an inflammatory reaction results in the underlying stroma. Repeated episodes of such recrudescence in the stroma ultimately result in opacity (Doymaz and Rouse, 1992). Stromal disease likely represents an immunopathological reaction set off by viral infection, since the disease responds to treatment with corticosteroids (Baum, 1995).

Most of our knowledge of the pathogenesis of herpetic stromal keratitis (HSK) has come from studies in animal models, particularly the mouse (reviewed in Doymaz and Rouse, 1992). Susceptible mouse strains routinely develop HSK after primary infection, but spontaneous reactivated disease is rare. The disease in mice is clearly immunopathological and primarily involves CD4^{+} T cells of the Th1 subset (Niemialtowski and Rouse, 1992; Henricks et al., 1992). Thus, HSK in the mouse represents a DTH reaction in the corneal stroma. Evidence that CD4^{+} T cells orchestrate the inflammation has come from experiments showing that virus-infected athymic and SCID mice, or mice selectively depleted of CD4^{+} T cells, fail to express HSK (Newell et al., 1989; Mercadal et al., 1993). Moreover, lymphocytes isolated from the inflamed corneas of mice with HSK are predominantly CD4^{+} T cells, and these produce mainly type 1 cytokines except during disease re-

mission, when type 2 cytokines become prominent (Niemialtowski and Rouse, 1992; Babu *et al.*, 1995a). With HSK, although several lines of evidence point to an immunopathological reaction organized by CD4$^+$ T cells, the target antigens which drive the response remain unknown.

Interestingly, virus replication and the bulk of viral antigen expression occur in the corneal epithelium, whereas the inflammatory reaction occurs in the stroma (Mitchell *et al.*, 1994). Furthermore, evidence of viral gene and antigen expression is absent by the time the invasion by CD4$^+$ T cells and the recruited nonspecific inflammatory cells occurs (Babu *et al.*, 1995b). HSK can also be induced in SCID mice reconstituted with populations of CD4$^+$ T cells from HSV-naive donors, and recipient animals develop HSK before virus-specific immunity is detectable (Mercadal *et al.*, 1993). Such observations indicate that the HSV infection may cause the expression of some secondary agonist, such as a self-peptide derived from the immune-sequestered cornea, which drives the immune inflammatory response (Avery *et al.*, 1995). In consequence, HSK may represent an autoimmune inflammatory response set off by HSV which more or less acts as a "hit and run" agent.

Further evidence that HSK may represent an autoimmune inflammatory response was provided recently by the Foster group (Avery *et al.*, 1995). In their system the difference in HSK susceptibility between two congenic mouse strains is known to be controlled by an allotypic variation in an immunoglobulin gene (Jayaraman *et al.*, 1993). Mice expressing the IgH^b allele are resistant, whereas congenic animals expressing the IgH^d allele are sensitive. By inducing tolerance to IgH^b-expressing Ig in susceptible mice, HSK fails to develop. The absence of HSK was interpreted to mean that IgH^b-derived peptides provide tolerance to the target autoantigens recognized in the disease (Avery *et al.*, 1995). This concept was additionally supported by data showing that CD4 Th1-type clones specific to the peptide could transfer HSK to athymic recipients, just as could virus-immune T cells. The observations on HSK pathogenesis collectively indicate that HSV infection may be an example of a viral agent that can cause immune inflammatory disease by triggering an autoreactive response. Other examples and possible mechanisms of virus-induced autoimmune inflammatory responses are briefly discussed in the next section.

IV. Immune Inflammatory Responses Involving Antibody

Most examples of immunopathological responses to viruses involve T lymphocytes and the role of antibody in immunopathology is an almost neglected topic. However, there are at least two widely accepted

examples in which humoral mechanisms account for the immuno-
pathogenesis of viral lesions. These are immune complex/complement-
dependent lesions and antibody-dependent enhancement of viral infec-
tion. The latter phenomenon probably accounts for the pathogenesis of
dengue hemorrhagic fever (DHF) (Kurane and Ennis, 1994) and
coronavirus-induced infectious peritonitis, a common viral disease in
the domestic cat (Trautwein, 1992). In DHF, which only occurs in
persons with existing antibody at the time of infection, the syndrome is
assumed to be a sequel to the facilitated infection of F_c receptor-bearing
cells such as macrophages which take up virus–antibody complexes.
The infected cells respond by producing an abundance of proinflamma-
tory cytokines and stimulate CD4$^+$ and CD8$^+$ T cells to do the same
(Kurane and Ennis, 1994). The resultant "cytokine storm" and other
chemical mediators released are assumed to trigger the plasma leak-
age and hemorrhage in DHF. One group contends that vascular damage
results from the effects of a novel cytokine produced by stimulated
CD4$^+$ T cells, termed "cytotoxic factor" (Mukerjee and Chaturvedi, 1995).

Virus-induced immunopathology resulting from the entrapment in
tissues of complement-activating immune complexes was first de-
scribed for LCMV infection (Oldstone and Dixon, 1969). Immune com-
plex disease results only if complexes are generated in excess as can
only happen if the virus is not eliminated efficiently by the immune
response. This might occur if agents replicate continuously in sites
beyond effective access by protective T cells or if some protective com-
ponent of the immune response is dysfunctional or exhausted because
of overstimulation. Immune exhaustion due to overwhelming antigen
exposure has been shown most convincingly to occur in transgenic
systems, and involves CD8$^+$ T cells (Moskophidis *et al.,* 1993). In
human viral disease, immune-complex-induced lesions have been ob-
served, but only in the case of HBV infection has viral antigen been
demonstrated to form part of the complexes (Chisari and Ferrari,
1995). However, immune-complex-mediated lesions in the joints and
kidney are reasonably common in humans and it is possible that other
viral agents may occasionally be involved.

V. VIRUSES AND AUTOIMMUNITY

The idea that viruses might trigger autoimmune responses has been
popular for some time, but there is little solid evidence to support the
notion at least for any human autoimmune disease. The subject has
received several recent reviews (Theofilopoulos, 1995; Sercarz *et al.,*
1993; Lehmann *et al.,* 1993; Lanzavecchia, 1995) and so only a few

points will be made. The oldest, simplest, and possibly most likely mechanism is that virus replication in an anatomically sequestered tissue releases autoantigens which activate self-reactive lymphocytes. This could be the principal mechanism at play in keratitis caused by HSV, since the avascular cornea can be considered unavailable to surveillance by the immune system. A similar explanation might apply to a common sequel to TMEV-induced demyelination in SJL mice wherein animals often develop a late response to myelin components such as PLP (Miller *et al.*, 1995).

A more sophisticated derivation of the antigen release concept is the unveiling of cryptic determinants. This idea, made popular recently by the excellent reviews of Sercarz (Sercarz *et al.*, 1993; Lehmann *et al.*, 1993), provides a more compelling explanation for virus-induced autoimmunity occurring in nonsequestered tissues. Examples might include Coxsackie virus-induced myocarditis (Huber and Lodge, 1984), and may explain why animals previously infected in the brain with persistent agents such as SFV become far more susceptible to the subsequent induction of Experimental Allergic Encephalomyelitis (EAE) when exposed to myelin antigens (Mokharian and Swoveland, 1987).

The essence of the cryptic self-hypothesis is that viral infection leads to the expression and altered presentation of determinants that are molecularly sequestered from the immune system and therefore do not induce tolerance (Lanzavecchia, 1995). The molecular unmasking and presentation could have a variety of causes. These include the possibility that viruses or induced cytokines, or even complexes between viruses and antibodies (Simitsek *et al.*, 1995), may modulate the expression or activity of proteases in APC which might result in the generation and presentation of previously cryptic peptides (Elson *et al.*, 1995). Another possibility is that the induction of abundant cytokines from infected or bystander cells might alter the surface expression of host proteins so that they become autoreactive. Some support for this idea comes from the observation of transgenic mouse models constructed to overexpress certain cytokines. Aberrant inflammatory reactions occur frequently (Campbell *et al.*, 1993; Gieger *et al.*, 1994). In one example, $IFN\gamma$ overexpression in the mouse retina gave rise to retinitis, which interestingly became more intense following HSV infection of the eye (Gieger *et al.*, 1994). Such data are consistent with the notion that viruses may trigger autoimmunity in some instances by causing a cytokine storm. There is, however, no well-accepted example of this effect occurring under natural circumstances.

A further mechanism by which cryptic determinants become unveiled was reported by Salemi *et al.* (1995). It was shown that if $CD4^+$ T

cells are exposed to HIV gp120, CD4$^+$ is taken up more abundantly. In consequence, previously cryptic determinants on CD4 become exposed and these stimulate autoreactive T cells. Conceivably, such cells could account in part for the depletion of activated CD4$^+$ T cells in AIDS.

Another hypothesis used to explain how viruses might break tolerance and induce autoimmunity is the molecular mimicry hypothesis (Oldstone, 1989). This hypothesis has its enthusiasts, but currently a well-documented example of a natural autoimmune viral disease that results from infection by viruses that act as molecular mimics is lacking. The hypothesis states that viruses share determinants with self-tissues, and the effective immune response generated to the viral determinant spills over to the host and an autoreactive response occurs. There are numerous examples of shared peptide sequences between viral and host proteins. For example, several peptides derived from viral sequences were shown to stimulate T-cell clones derived from MS patients (Wucherpfennig and Strominger, 1995). These data were interpreted to support the notion that viruses are involved in the etiology of MS via a molecular mimicry mechanism. However, as discussed before, no single known virus is currently accepted as an initiating factor in MS (Waksman, 1995). For an enthusiastic viewpoint about molecular mimicry as it relates to virus-induced autoimmunity, the article of Wucherpfennig and Strominger (1995) is recommended. It is also worth noting that the molecular mimicry hypothesis has been advocated to explain aspects of the pathogenesis of HIV infection (Silvestris *et al.*, 1995).

Additional hypotheses have been advanced to explain how viruses might trigger autoimmunity. Included among them is the possibility that viral proteins which express superantigen activity might activate normally quiescent autoreactive clones of T cells (Scherer *et al.*, 1993). As with other hypotheses to explain virus-induced autoimmunity, widely accepted actual examples in natural viral diseases are not at hand.

VI. CONTROL OF VIRUS-INDUCED IMMUNE INFLAMMATORY DISEASE

The adage that an ounce of prevention is worth a pound of cure is certainly true in the field of viral pathogenesis. Preventing viral infection or manipulating immune processes during the initial phases of infection is far more successful than attempting to counteract pathological events once underway. With virus-induced immunopathologies, we are usually faced with a chronic tissue-damaging response to antigens that are being constantly replenished from a persistent replicat-

ing agent. The therapeutic challenge is either to remove or to neu-
tralize the agonists which drive the inflammation or to redirect the
symphony of events occurring so that tissue damage is minimized or
ablated. Few viruses are subject to inhibition by drugs and some of
them have strategies that hide them from the chemical attack. Herpes
simplex virus provides the best example of this scenario: the virus is
susceptible to several antiviral drugs during the replication phase but
to none during latency.

Most virus-induced immunopathologies are orchestrated by T cells
of one type or another. Such T cells usually recognize viral antigens
although rarely is the antigen's identity known, particularly in outbred
animals. However, one approach worth pursuing is to prevent specific
antigen recognition by pathogenic T cells. The experimental induction
of immunological tolerance to offending antigens is clearly the most
desirable way to control any immune-mediated pathogenesis, but even
when the culpable antigens are known, success is hard to achieve and
maintain. Moreover, conceptually there are several forms of tolerance
(Matzinger, 1994). These include (i) deletion of T cells specific for a
particular MHC–peptide combination, (ii) induction of T cells that sur-
vive in a form that is hyporesponsive to antigen (anergy), and (iii) T-cell
survival in a form that responds strongly to a particular stimulus but
in way which differs from the standard response. This latter state,
which is often termed "immune deviation," currently represents the
most likely practical way to control viral immunopathology.

Immune deviation is an old concept originating from studies on DTH
by Geoffery Asherson in the 1960s, which showed that exposure of
guinea pigs to antigen by various routes selectively inhibited the DTH
response (Asherson and Stone, 1965). Immune deviation is now better
understood at a mechanistic level. It has its basis in the fact that
subsets of T cells, both CD4$^+$ and CD8$^+$, exist which have different
functional activities and that many of the cytokines they produce cross-
regulate each subset (Paul and Seder, 1994; Coffman *et al.*, 1991; Croft
et al., 1994). Administration of antigen by various nonsystemic routes,
e.g., may induce responses dominated by type 2 cytokine-producing
cells which serve to down-regulate the induction of the type 1 cytokine
producers that normally appear after systemic exposure (Ridgway
et al., 1994; Powrie and Coffman, 1993). Other means of achieving im-
mune deviation include the use of analogue peptides for induction
(Sette *et al.*, 1994) or the use of reagents which influence the micro-
environment of antigen-activated T cells (Bluestone, 1995; Linsley,
1995). Regarding the latter, it is now evident that the cytokine or
costimulator microenvironment in tissues during T-cell activation can

profoundly influence the outcome in terms of the functional set of T cells that differentiates (Linsley, 1995). For example, in an environment dominated by IL-4, the CD4$^+$ subset induced from uncommitted precursors is usually of the Th2 phenotype (Paul and Seder, 1994). Such an effect operating during induction of the immune response to TMEV would diminish the induction of lesion-inducing CD4$^+$ Th1 T cells. This result has, in fact, been reported (Karpus *et al.*, 1995). Thus, exposure of mice to viral antigen coupled to syngeneic spleen cells with ethylcarbodiimide (ECDI) abrogates the normal Th1 T-cell response and shifts the response to one dominated by Th2 T cells (Karpus *et al.*, 1995). This procedure not only prevents the demyelinating disease after subsequent TMEV infection but can suppress lesion severity in infected animals, at least if given not later than 2–3 weeks after infection.

Approaches also exist which favor the induction of Th1 cells, a scenario likely to be beneficial in minimizing the pathology associated with RSV infection. Th1-enhancing procedures include administering or promoting the production of the cytokine IL-12 (Manetti *et al.*, 1993) as well as manipulating the costimulator environment with agents which block CD28 stimulation, such as CTLA-Ig (Linsley, 1995). Recently, a surprising observation was reported which achieves a result similar to IL-12 potentiation. Administering the Schiff-base-forming molecule tucaresol along with antigen led to the accentuation of a CD4$^+$ Th1 response (Rhodes *et al.*, 1995). The mechanism of action is unknown but probably involves the bypass of a costimulator pathway which normally activates Th2-like responses (Shearer, 1995). There is some evidence that the costimulator B7-2 on APC is responsible for Th2 activation (Manetti *et al.*, 1993) and that tucaresol may inhibit the B7-2 stimulus in some way (Shearer, 1995).

All of the aforementioned approaches may achieve immune deviation, but they are usually successful only if used during the induction phase of an immune response. Reversing a given pattern of events by immunomodulators once fulminant lesions are present is a challenging problem. Possibly coming closest to this objective is the success being achieved using the oral tolerance approach to suppress certain experimental autoimmune diseases and perhaps even the human diseases multiple sclerosis and rheumatoid arthritis (Chen *et al.*, 1995). Thus, by feeding antigen, clinical disease expression is minimized. This effect works best using a low-dose antigen regimen which seemingly induces a bystander suppressor-type effect mediated by TGFβ and perhaps other cytokines (Friedman and Weiner, 1994). At higher oral tolerizing doses, the mechanism of tolerance induction appears to be T-cell deletion or anergy (Friedman and Weiner, 1994). This situation is probably

less desirable than a bystander suppressor effect, since in natural diseases the specific antigens involved are rarely if ever known with certainty. It remains to be seen if oral tolerance or any immune deviation scheme will successfully control an established virus-induced immunopathological lesion.

There are other immunomodulatory strategies that might succeed in arresting the advance of an inflammatory lesion and turn the tide to permit repair and recovery to occur. These include interfering with the effector function of lymphocytes and nonspecific inflammatory cells. Strategies include the use of cytokine receptor antagonists, particularly against the proinflammatory cytokines IL-1 and TNFα (Klein and Brailly, 1995). The approach has shown promise in certain model systems, but from a practical viewpoint it is not convenient since a continual administration of the antagonist is required. However, a successful formulation might come from combining proinflammatory cytokine arrest with the simultaneous use of agonists which achieve lymphocyte reeducation. As regards the latter, it is pertinent to note the intriguing observations of the Chisari laboratory showing that immunization of HBV transgenic mice with a DNA vaccine may curtail transgene expression (Chisari, 1995). In other viral immunopathologies, disease remission may be associated with the expression of the cytokine IL-10 (Babu *et al.*, 1995b; Tumpey *et al.*, 1994). It seems likely that the use of DNA vaccines, particularly those encoding regulatory cytokines, may prove useful to manipulate the immune inflammatory state. One report already attests to this possibility (Rogy *et al.*, 1995).

VII. CONCLUSIONS

The induction of an immune response which succeeds in eliminating virus-infected cells and extracellular virus is the common outcome of a viral infection. Removing infected cells usually engenders some tissue damage because of a concomitant inflammatory response, but this is not an unreasonable price to pay to control infection by highly cytolytic agents. If the agent is either noncytopathic or minimally so, destroying functionally intact cells may be, however, an undesirable consequence. This is especially true if cellular destruction is massive or occurs in an organ or tissue which is intolerant to damage, such as the cornea of the eye or the brain. Several viruses cause damage to the brain by immunopathological mechanisms, yet the same agents may cause insignificant lesions in other tissues either because function is retained or the damage is repaired rapidly. In Table I, a compilation is presented of

TABLE I

SOME EXAMPLES OF VIRUS-INDUCED IMMUNOPATHOLOGY

Likely principal mechanism involved	Virus	Reference[a]
CDB⁺ T-cell-mediated	LCMV	Doherty *et al.* (1980)
	Hepatitis B	Chisari and Ferrari (1995)
	Coxsackie B	Hanke *et al.* (1995)
	HIV	Zinkernagel and Hengartner (1994)
	Sin Nobre Virus	Zaki *et al.* (1995)
CD4⁺ T-cell-mediated	Theilers virus	Miller and Karpus (1994)
(type 1)	Mouse coronavirus	Fleming *et al.* (1993)
	Semliki Forest Virus	Mokharian and Swoveland (1987)
	Measles	Johnson *et al.* (1978)
	HSV	Doymaz and Rouse (1992)
	Visna	Narayan and Clements (1989)
(type 2)	Respiratory syncytial	Alwan *et al.* (1993)
Antibody-mediated	Dengue	Kurane and Ennis (1994)
	Feline infectious peritonitis	Trautwein (1992)

[a] These are good source references and are not meant to reflect the primary discoveries of the phenomenon.

some viral examples in which at least some of the lesions have an immunopathological pathogenesis.

The most common mechanism of lesion development in virus-induced immunopathology involves T cells. Usually, it seems that when $CD8^+$ T cells act as the controlling cell type, lesions are acute and the outcome is decided quickly. The classic example is provided by LCM in mice. The newest candidate may turn out to be SNV infection in humans. Lesions orchestrated primarily by $CD4^+$ T cells can be either acute or long-lasting. Curiously, in the LCMV example, if $CD8^+$ T cells are removed from the scene, immunopathological responses may still occur and these involve $CD4^+$ T cells (Doherty *et al.*, 1993; Fung-Leung *et al.*, 1991). Such responses are far more chronic and of lower grade than those mediated by $CD8^+$ T lymphocytes. One possible sequel to chronic inflammatory responses to viruses is that autoreactive inflammatory reactions are initiated and an autoimmune disease occurs. Many mechanisms by which viruses trigger autoimmunity have been conceived but all lack concrete examples, at least with respect to human autoimmune disease.

For some viral agents involved in immunopathological lesions, a clear picture of the cellular events and chemical mediators that participate in tissue damage is available. Rarely, however, is the biochemistry of the actual tissue damage fully understood. Conceivably, such knowledge will accrue from the ever-expanding array of *in vivo* models, particularly those which succeed in changing upon demand the expression of some molecular or cellular event. The practical bonus of such knowledge should be the generation of various approaches that will manage lesions and minimize their clinical significance. The challenge to practical viral immunology is to move from the secure territory of viral prophylaxis to the still alien field of lesion immunomodulation.

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