REGULATION OF T CELL RESPONSES DURING CENTRAL NERVOUS SYSTEM VIRAL INFECTION

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I. INTRODUCTION

T cells can serve a variety of functions as part of the host immune response during central nervous system (CNS) viral infection. They can participate directly in viral clearance from the brain or they can promote the survival of the host without exerting any direct effect on virus replication. T cell responses elicited during CNS viral infection may sometimes actually cause neuropathological injury above and beyond what is induced by the pathogen itself. Such variability in the effects on outcome may in large part be dictated by the nature of the specific pathogen, but host factors also exert quantitative and qualitative effects on the types of T cell responses elicited during disease. When considering the various roles that T cells may play during CNS viral infections, one important and interesting issue is whether and

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how different T cell responses are regulated within the brain. After briefly reviewing how T cells may contribute to the pathogenesis of neurologic disease, we will discuss specific examples of how individual T cell effector functions can be regulated during CNS viral infections. Although our current understanding of the mechanism(s) that underlie such regulatory events is limited, further characterization of these processes will be relevant in neurovirology as well as in the broader fields of basic and clinical neuroimmunology.

II. EFFECTOR FUNCTIONS OF T CELLS DURING CNS VIRAL INFECTION

A. T Cells Facilitate Recruitment of Other Inflammatory Cells into CNS

Only a small number of T cells infiltrate the brain under normal circumstances; this paucity of immune surveillance of baseline is one of several reasons why the CNS has often been characterized as an "immunologically privileged" site. Yet during the course of CNS viral infection (whether restricted to the meninges, leading to an influx of inflammatory cells into the cerebrospinal fluid (CSF), or following viral spread to the brain and spinal cord, leading to actual parenchymal inflammation), the processes that normally exclude immune elements from the CNS are overcome and inflammatory cells rapidly enter these tissue compartments (brain, CSF, meninges). Parenchymal inflammation during viral encephalitis is most common around blood vessels in the form of perivascular infiltrates. Such an extravasation of cells from the bloodstream into the perivascular space, and then into the parenchyma itself, is likely to depend on specific molecular interactions between circulating immune cells and the cerebrovascular endothelium (Griffin et al., 1992; Irani and Griffin, 1996). In several well-studied experimental paradigms, CD4+ and CD8+ T cells, NK cells, and γδ+ T cells constitute many of the earliest inflammatory cells that infiltrate the brain during CNS viral infection (Moench and Griffin, 1984; Williamson et al., 1991; Irani and Griffin, 1991; Griffin et al., 1992). The proportion of these earliest-appearing T cells that are specific for viral antigens is not known, but it is likely that these virus-specific cells release inflammatory mediators, thereby contributing to subsequent inflammatory cell recruitment into the brain. For example, when T cell-deficient athymic nude mice are infected with the encephalitic alphavirus, Sindbis, animals develop markedly less perivascular inflammation, and recruitment of virus-specific B cells into the CNS decreases as compared to immunologically normal hosts (Hirsch and Griffin, 1979; Tyor et al., 1989).

Likewise, during murine lymphocytic choriomeningitis virus (LCMV) infection, activated, CD8$^+$, virus-immune T cells are the principal regulators of how much inflammation subsequently develops in the CSF of infected animals (Doherty *et al.*, 1988). Thus, T cells play an important role in facilitating the recruitment of other inflammatory cell types into the CNS during viral infection, thereby determining the overall level of inflammation.

B. T Cells Promote Clearance of Viruses from Brain

T cell-mediated lysis of infected cells has been demonstrated to be an important mechanism of viral clearance from tissues other than the CNS (Yap *et al.*, 1978; Dharakul *et al.*, 1990; Young *et al.*, 1990; Munoy *et al.*, 1991, Ando *et al.*, 1994). Both CD4$^+$ and CD8$^+$ virus-specific cytotoxic T lymphocytes (CTLs) have been described. The cytolytic actions of these T cells require that compatible major histocompatibility complex (MHC) class II and class I molecules, respectively, are expressed on virally infected target cells. Yet within the CNS, overall MHC expression is restricted, with glial cells expressing more of these molecules than neurons (Mauerhoff *et al.*, 1980; Wong *et al.*, 1984; Joly *et al.*, 1991). The paucity of MHC expression has been proposed as one means by which viruses may escape immune recognition and persist in the brains of infected hosts. Nevertheless, these limitations can be overcome and T cell-mediated clearance of viruses from the CNS has been demonstrated in a number of experimental systems. Following the inoculation of the JHM strain of mouse hepatitis virus (JHMV) into susceptible mice, for example, an acute encephalomyelitis is elicited with robust infection of astrocytes, oligodendrocytes, and, to a lesser degree, neurons. Using an adoptive transfer strategy, viral clearance from the brains of these hosts has been shown to be mediated by an *H2-D*-restricted, virus-specific CD8$^+$ T cell population (Sussman *et al.*, 1989). Interestingly, these CD8$^+$ CTLs have an absolute requirement for CD4+ T cells that appear to support their local survival within the brain (Stohlman *et al.*, 1998).

Likewise, during persistent LCMV infection of mice, the adoptive transfer of H-2-compatible, virus-immune T cells results in clearance of infectious virus from many tissues including the brain (Oldstone *et al.*, 1986). Most notable in this particular model system were tissue-specific differences in the apparent mechanism of viral clearance; this process took much longer in the CNS as compared to other organs but occurred without any histological evidence of neuronal destruction, while clearance from other organs involved the actual cytolysis of infected cells (Oldstone *et al.*, 1986; Tishon *et al.*, 1993). Whether this

is the result of organ-specific differences in levels of MHC antigen expression is unclear, but viral clearance without the actual destruction of infected neurons, a nonrenewable cell population, would certainly be advantageous to the host. There now is convincing evidence in other systems that the antiviral actions of CD8+ T cells within the CNS depend on their local production of soluble mediators such as interferon-γ (IFN-γ), and not on direct perforin-mediated cytolysis of target cells (Kündig et al., 1993; Lin et al., 1997). In sum, while more than one mechanism may underlie their effects, it is clear that T cells can promote viral clearance from the CNS in many situations.

C. T Cells Can Protect Hosts from Lethal CNS Viral Infections and May Promote Neuronal Survival

The adoptive transfer of primed T cells can protect naive mice from a variety of otherwise lethal viral infections (Zinkernagel and Welsh, 1976; Yap et al., 1978; Jacoby et al., 1980; Sethi et al., 1983; Stohlman et al., 1986; Stohlman et al., 1995). In some situations, transferred T cells play an active role in suppressing virus replication, serving as the primary means through which they protect their hosts. In other cases, however, no effect on virus replication is seen and protection has to be explained through another mechanism, such as causing a change in the cell tropism of the virus or inhibiting virus-induced cell death. There are several notable examples where virus-immune T cells serve in this capacity during CNS viral infection. In the JHMV model, for example, a virus-specific, CD4+ T cell clone was found to protect mice from an otherwise lethal viral challenge without actually decreasing the amount of infectious virus in the brain (Stohlman et al., 1986). Protective T cells had to be administered intracerebrally in this system; their beneficial effect also depended on the recipient's own immune system as it could be ablated by pretreating the host with cyclophosphamide (Stohlman et al., 1986).

In more recent experiments using an otherwise lethal strain of Sindbis virus, animals preimmunized against nonstructural viral proteins were highly protected against permanent paralysis and death (Gorrell et al., 1997). This protection was not associated with either reduced virus replication in the brain or altered virus tropism (neurons of the brain and spinal cord remained the principal target of infection), nor was it due to an augmented humoral response against structural viral proteins that could also protect lethally infected animals (Stanley et al., 1986; Gorrell et al., 1997). Instead, this nonstructural protein-mediated protection proved to be a function of immune T cells and occurred by promoting the survival and functional recovery of virus-

infected neurons (Gorrell *et al.*, 1997). Because virus-induced neuronal apoptosis is believed to be the cellular substrate underlying the neurovirulence of Sindbis virus *in vivo* (Lewis *et al.*, 1996), it was suggested that this particular form of protection occurred through local T cell cytokine release within the CNS early during infection that somehow interrupted apoptotic signaling in infected neurons. Although the mechanism of protection in this model remains incompletely understood, such a finding highlights the fact that T cells may also serve to promote the survival of target cells within the brain during CNS viral infection, until other immune elements can control virus replication and achieve viral clearance.

D. T Cells Can Induce Immune-Mediated Damage

In several well-characterized animal models of CNS viral infection, part of the elicited T cell response actually contributes to the pathology and adverse outcome of disease. Neurotropic LCMV infection of adult mice is perhaps the premier example of this phenomenon; the resulting fatal choriomeningitis that develops is mediated by the same virus-specific CD8+ T cells that participate in viral clearance (Cole *et al.*, 1972; Mims and Blanden, 1972; Buchmeier *et al.*, 1980; Oldstone *et al.*, 1986). In another model system, virus-specific CD4+ T cells clearly mediate the immunopathology seen in adult rats infected with Borna disease virus (BDV) and can actually transfer disease to another host in an MHC class II–restricted manner (Richt *et al.*, 1989). Finally, during chronic Theiler's murine encephalomyelitis virus (TMEV) infection that occurs when virus is not fully cleared from the CNS following acute encephalitis, CD4+ T cells, initially directed against viral antigens but later including T cells specific for various myelin epitopes, cause destruction of oligodendrocytes and produce demyelination (Lipton and Dal Canto, 1976; Pope *et al.*, 1996, Miller *et al.*, 1997). Other experimental examples also exist; there is no lack of evidence that T cells can sometimes induce immune-mediated damage to the CNS during viral infection.

III. REGULATION OF T CELL RESPONSES DURING CNS VIRAL INFECTION

A. T Cell Responses in Brain versus the Periphery During CNS Viral Infection: Selective Recruitment, Preferential Retention, or Local Regulation

Many arguments have been used to characterize the CNS as an "immunologically privileged" tissue: the restrictive nature of the

blood–brain barrier that normally excludes circulating immune elements; the paucity of MHC antigen expression that minimizes local T cell reactivity; the lack of a formal lymphatic drainage system that limits immune exposure to antigens present in the CNS; and even the constitutive expression of inhibitory cytokines, such as transforming growth factor-β (TGF-β), in the brain that may serve local immunoregulatory functions. Yet despite all these apparent obstacles, vigorous immune responses are rapidly mounted inside the CNS to a variety of stimuli including local viral infection. The various roles that T cells may serve during these infections have been briefly reviewed, but whether and how particular T cell responses are regulated during CNS viral infection are less clear. One way to examine whether such regulation takes place is to characterize local T cell responses in the brain and compare them to responses that are elicited in peripheral immune compartments. Differences found between T cell responses inside and outside the CNS may then be explained in several ways including: (a) the preferential recruitment of specific subpopulations of effector T cells from the circulation into the brain; (b) a selective retention of T cell subpopulations within the CNS, once the blood–brain barrier has been traversed; and/or (c) the local alteration of T cell effector function within the CNS microenvironment. Since the molecular mechanisms that underlie the processes of T cell recruitment into, and retention within, the CNS are only now beginning to emerge, and since it is unclear to what degree the brain may actively influence local T cell effector function, details regarding how T cell responses may be regulated during CNS viral infection are correspondingly incomplete. Nevertheless, specific examples showing that individual T cell responses (CTL activity, proliferation, cytokine production, and apoptosis) differ between CNS and peripheral immune compartments provide preliminary evidence that regulation of T cell responses during viral infections of the nervous system can and does occur.

B. CTL Activity in Brain

Extensive characterizations of virus-specific CTL responses that develop on either side of the blood–brain barrier of mice with viral encephalitis have been undertaken in several experimental systems. Such studies serve as important illustrations of how this particular T cell function may be regulated during CNS viral infection. During acute JHMV infection, for example, several groups have found that the virus-specific CD8+ CTLs present in the brain are of restricted antigenic specificity (they respond mainly to the viral nucleocapsid protein but not to the spike, membrane, or hemagglutinin–esterase proteins);

CTLs with this same nucleocapsid specificity are not found, or are present at much lower levels, in lymphoid organs outside the CNS, including cervical lymph nodes (Stohlman et al., 1993; Castro et al., 1994). Such differences in specificity suggest that either these particular T cells are present at very low levels in the periphery and get rapidly and selectively recruited into the CNS or that they are actually generated locally within the brain. Some preliminary studies that address this question now suggest that JHMV-specific cytotoxic T lymphocyte precursor (CTLp) are, in fact, primed within cervical lymph nodes before migrating into the CNS (C. Bergmann and S. Stohlman, unpublished observations). This implies that these T cells possess some property that makes them particularly capable of entering or being retained within the brain. Whether priming in cervical lymph nodes (rather than in the spleen or other lymph node chains) causes their preferential recruitment into the CNS is not clear. Existing evidence, however, points to cervical lymph nodes as a site where antigen accumulates following its inoculation into the CNS and where the virus-specific CTLp are generated following the intracerebral inoculation of mice with neurotropic LCMV (Harling-Berg et al., 1989; Lynch et al., 1989).

In the chronic phase of JHMV infection that occurs as a result of incomplete viral clearance from glial cells such as oligodendrocytes, the fine antigen specificity of brain-derived CTLs is even more restricted than during the acute phase (Marten et al., 1999). Thus, while CTLs from chronically infected animals recognize the same viral nucleocapsid antigens as T cells found in the CNS during the acute illness, they are much less tolerant of amino acid mutations in the specific epitope peptides (Marten et al., 1999). This "focusing" of the antigenic specificity of local CTL over time during JHMV infection has been proposed to occur through a preferential selection (survival or even selective expansion) within the brain of CTLs with higher T cell receptor affinities. The forces behind such a selection process are not well understood, but they may in part relate to the fact that the supply of MHC-bound viral peptides becomes extremely limited as viral load diminishes and cellular tropism narrows in the brain over time. Whether such a focusing event is more likely to occur in the sequestered environment of the CNS (where complete viral clearance is more difficult to achieve relative to other tissues) is not known.

In a murine model of neurovirulent influenza virus infection, activated, virus-specific CTLs were found to persist in the brain long after viral clearance had been achieved (Hawke et al., 1998). In this paradigm, it was proposed that these T cells were not actively recirculating into and out of the brain since the systemic spread of infection was minimal. Instead, it seemed more likely that CTLs were being acti-

vated and retained within the CNS by the ongoing expression of MHC class I–viral peptide complexes that were simply below the threshold of detection (Hawke et al., 1998). Importantly, the selective retention of influenza-specific CTLs in the lungs of mice inoculated intranasally with the same virus was not found (CD8+ T cells persisted within this tissue well after viral clearance had been achieved but had lost their capacity to lyse virus-infected targets), leading to the conclusion that factors which promote long-term CTL activation are present in the brain but not in other tissues (Hawke et al., 1998).

It is provocative to consider that persistent viral RNA itself, in the absence of detectable virus replication, may provide enough of a stimulus to activate selected CD8+ T cell effector functions in the CNS. While such a local T cell response may in fact be the reason why virus replication remains at undetectable levels, by definition it contributes to a chronic inflammation in the brain. Such an experimental finding then makes CNS viral persistence a much more plausible basis for explaining how immune-mediated neurologic diseases such as multiple sclerosis may be triggered. Indeed, the process of local antigenic focusing noted among T cells during the transition from acute to chronic JHMV infection may in part serve to reduce the risk that cross-reactive or even autoreactive CTLs evolve among the cells that persist within the brain. Such an evolution can occur (although, in this case, mediated by CD4+ T cells); CNS autoimmunity has now been shown to develop in mice persistently infected with TMEV through an immunologic process referred to as "epitope spreading" (Miller et al., 1997). Nevertheless, the long-term retention of activated, but nonproliferating, CTLs in the brain may provide a necessary component of immunity that is important for this "immunologically privileged" tissue (Hawke et al., 1998).

C. T Cell Proliferation in CNS During Viral Infection

The ex vivo proliferative capacity of brain-infiltrating T cells has been investigated in various experimental models of CNS inflammation. In many of these studies, the goal has been to characterize the antigenic specificity of T cells within the CNS as compared to those obtained from various extracerebral sites. In experimental autoimmune encephalomyelitis (EAE), for example, the reactivity of brain-derived T cells to various myelin antigens has been examined in efforts to try to understand how demyelination that may accompany this disorder can occur. During CNS viral infection, questions regarding the frequency of virus-reactive T cells in the brain are common, and in cases where virus-induced inflammation leads to immunopathology

within the CNS, the issue of whether these T cells cross-react with neural antigens has also been examined. In the present context, however, examining such studies may serve to help understand how another T cell response (i.e., clonal expansion) may potentially be regulated during CNS viral infection.

T cells appear to be recruited nonspecifically into the CNS during chronic TMEV infection in susceptible SJL mice, at least as measured by a polymerase chain reaction (PCR)-based assay that determines T cell receptor (TcR)β-chain diversity among T cells from the spleens and brains of infected hosts (Musette et al., 1995). By extending this molecular strategy, however, it was possible to demonstrate that populations of T cells in the CNS expressing certain TcR β-chains were markedly expanded while others were not; such an expansion did not occur among splenic T cells, suggesting that it was due to local T cell proliferation in the brain (Musette et al., 1995). While this local CNS expansion was assumed to be antigen-driven, it was not possible to determine the specificity of this event because brain-derived T cells were not actually being cultured in vitro. Other studies, however, have shown that CD4+ T cells isolated from the CNS of TMEV-infected animals proliferate in vitro to nearly the same degree as splenic T cells when stimulated with viral antigens (Pope et al., 1996). By themselves, these data would suggest that local regulation of T cell proliferation does not occur to any degree during CNS viral infection.

Other experimental results, however, suggest that this may not always be the case. In the murine model of neurovirulent influenza virus infection discussed above, proliferation among the activated, CD8+ T cells that persisted in the brains of infected animals, following viral clearance, was examined. Using an in vivo 5-bromo-2'-deoxyuridine (BrdU) incorporation technique, these investigators showed that despite their activated phenotype and their preserved ability to lyse virus-infected targets in vitro, the vast majority of these CD8+ T cells were not proliferating locally within the brain (Hawke et al., 1998). Although an explanation for this finding was not offered in this report, the effect appeared specific to the CNS because many splenic T cells from these animals were actively dividing throughout disease (Hawke et al., 1998). The effect also seemed to be related to how long the T cells had been retained within the brain (Hawke et al., 1998).

In a murine alphavirus encephalitis model using an avirulent strain of Sindbis virus, T cells isolated from the brains of infected BALB/c animals did not proliferate ex vivo in response to either viral antigens or mitogenic lectins (Irani et al., 1997). Notably, virus-induced immunopathology or neurologic sequelae are not induced following

infection in these hosts. In fact, many of these brain-derived T cells appeared to have arrested in the cell cycle despite the fact that peripheral lymphocytes from infected mice showed no defect in proliferation (Irani et al., 1997). The same virus inoculated into SJL mice, however, elicited more intense and prolonged CNS inflammation as well as immune-mediated paralysis, despite equally effective viral clearance from the brain (Mokhtarian et al., 1989; Irani, 1998; Rowell and Griffin, 1999). One notable difference between the inflammatory responses in the brains of these two hosts was an increased proliferative capacity of brain-infiltrating T cells in the SJL mice (Fig. 1). Such a difference, although not proven to be related to the immune-mediated CNS damage observed in SJL mice, was attributed to intrinsic properties of the T cells and not something different about the local environment of the brain that was more permissive for local T cell proliferation in this mouse strain (Irani, 1998). Nevertheless, if T cells in SJL mice are more capable of proliferating within the brain than T cells within BALB/c mice following the same CNS viral infection, it is possible that a defect in local T cell regulation during this infection may predispose these hosts to immune-mediated neurologic injury. In this sense, deficient local regulation of certain T cell effector functions may actually contribute to the pathogenesis of disease in CNS viral infection.

D. Local T Cell Cytokine Production in Brain

Another important T cell effector function that occurs during CNS viral infection is the local production of cytokines within the brain. Like the T cell responses already discussed (CTL activity, proliferation), this process may also be controlled within the CNS in certain disease situations. However, regulation of T cell cytokine production during CNS viral infection must be considered in light of the fact that these mediators exert pleiotropic effects in different model systems; some cytokines (notably IFN-γ) may contribute directly to the control of virus replication in the brain (Kündig et al., 1993), while others may serve immunoregulatory functions such as promoting B cell survival and differentiation in the CNS (Tyor and Griffin, 1993; Wesselingh et al., 1994), and still others may act on infected neural cells in such a way that alters the cellular response to infection (Doherty et al., 1989; Gorrell et al., 1997). Despite these differences, examining some of these studies may reveal how this T cell effector response can be regulated during CNS viral infection.

During acute Sindbis virus encephalitis in mice, mRNAs encoding a number of cytokines are produced within the brain (Wesselingh et al.,
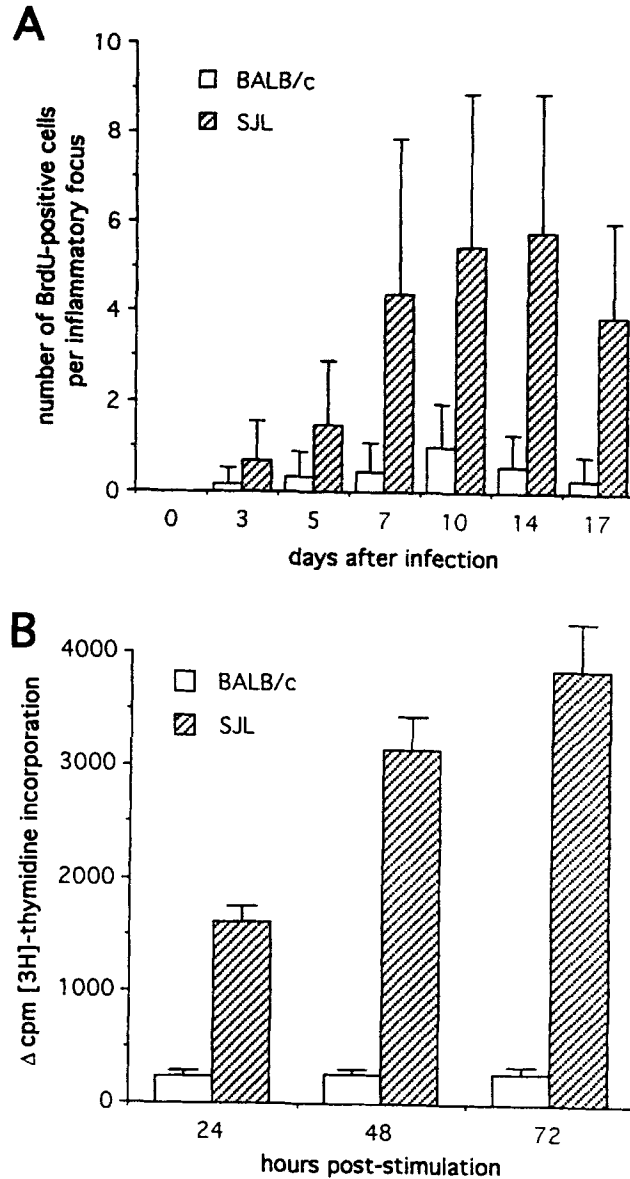
FIG 1. Proliferation of CNS inflammatory cells during acute Sindbis virus encephalitis in BALB/c and SJL mice. Using an *in vivo* BrdU labeling technique, more proliferating cells were found within the brains of SJL mice than those of BALB/c mice (A). Similarly, T cells isolated from the brains of infected SJL mice incorporated more tritiated thymidine per 10^5 viable cells than did T cells derived from the brains of BALB/c mice (B). Adapted with permission from Irani, 1998.

1994). While some (e.g., TNF-α, interleukin-1β [IL-1β] IL-6) are upregulated in advance of any histologic evidence of inflammation, suggesting production by intrinsic neural cells, most (e.g., IFN-γ) parallel the degree of brain parenchymal inflammation and are markedly reduced in the brains of severe combined immunodeficient (SCID) mice as compared to immunocompetent control animals (Wesselingh et al., 1994). Quantitation of transcripts encoding T cell–derived cytokines in this disease suggests a predominant Th2-type response (high IL-4 and IL-10, lower IFN-γ, minimal IL-2) (Fig. 2). Since antibodies specific for the viral glycoproteins are the primary effectors of viral clearance from infected neurons (Levine et al., 1991), and since virus-specific antibody-secreting B cells are retained in the CNS of animals for months after infectious virus has been cleared (Tyor et al., 1992), it seems likely that factors which help to enrich the environment of the brain with Th2-type cytokines facilitate these virus-specific antibody responses.

How the local T cell population becomes skewed toward a Th2-type response is not known. Since activated T cells traffic into the brains of Sindbis-infected animals nonspecifically (Irani and Griffin, 1996), regulation of local cytokine production may be exerted within the CNS after T cells have accumulated at that site (Irani et al., 1996; Irani et al., 1997). Evidence to support this hypothesis comes from the observation that once T cells have crossed the blood–brain barrier and entered the perivascular and parenchymal compartments of the CNS, it is the virus-immune cells that are selectively retained within the brains of infected animals (Fig. 3). When T cells are adoptively transferred into the circulation of infected mice, those that traffic into, and are retained within, the brains selectively downregulate their expression of Th1-type cytokines (principally IL-2) over time (Irani et al., 1997). This supports the hypothesis that, at least during acute Sindbis virus encephalitis, the brain can exert an active regulatory influence over the cytokine production by infiltrating T cells. How this regulation occurs is not known, but certain glycolipids (gangliosides) that are abundant on the external cell membranes of neural cells selectively inhibit Th1- but not Th2-type cytokine production by T cells activated in vitro (Fig. 4). Thus, during some viral infections of the CNS, T cell cytokine production can be regulated locally in the brain by lipid molecules that selectively inhibit the production of Th1-type cytokines. The net effect of this regulation is to enhance the relative levels of Th2-type cytokines. Others have also suggested that the CNS is intrinsically a "Th2-type" environment that naturally supports humoral immune responses and suppresses cellular responses (Cserr and Knopf, 1992).
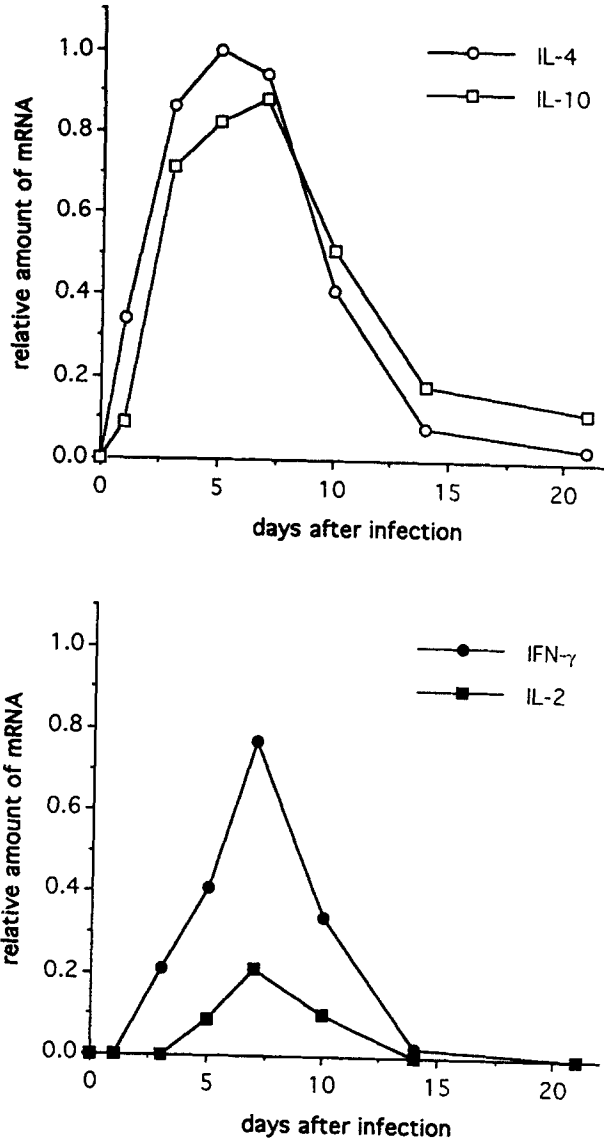
FIG 2. Quantitation of T cell cytokine mRNA levels present in the brain parenchyma of mice with acute Sindbis virus encephalitis. Individual cytokine transcripts were quantitated by RT-PCR relative to the constitutively expressed glyceraldehyde-3-phosphate dehydrogenase gene. Th2-type cytokines were present at higher levels. Adapted with permission from Wesselingh *et al.*, 1994.
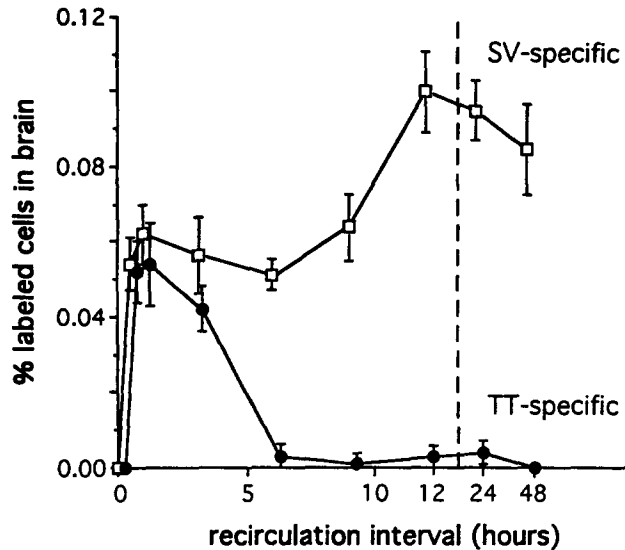
FIG 3. Kinetics of lymphocyte entry into the CNS of mice with Sindbis virus encephalitis. Fluorescently labeled T cells specific either for Sindbis virus (SV-specific) or tetanus toxoid (TT-specific) were inoculated intravenously into infected recipients. Inflammatory cells were then isolated from the brains of mice at various intervals and the percent of labeled cells present in each isolate was measured by flow cytometry. While T cells of either specificity rapidly entered the brain to equivalent degrees, virus-specific T cells were preferentially retained within this tissue while nonspecific T cells disappeared. Adapted with permission from Irani and Griffin, 1996.

E. Control of T Cell Survival Within CNS

In EAE, lymphocytes that infiltrate the brain may undergo apoptosis during the remission phase of disease (Schmeid et al., 1993; Bauer et al., 1995; Bonetti et al., 1997). Lymphocytic inflammation may also be cleared via an apoptotic process from the CNS of animals recovering from both acute coronavirus- and alphavirus-induced encephalitis (Barac-Latas et al., 1995; Irani, 1998). Based on these observations, it has been proposed that the induction of T cell apoptosis locally within the brain may be a generalized mechanism through which the brain terminates many inflammatory responses. Conversely, the persistence of CNS inflammation during viral encephalitis may in part result from a relative lack of apoptosis among brain-infiltrating T cells (Irani, 1998). In this situation, were myelin-reactive T cells to gain entry into the CNS and not be subjected to a process that normally downregulates inflammation, autoimmune injury could ensue. Examining how the survival of brain-infiltrating T cells is controlled during CNS viral
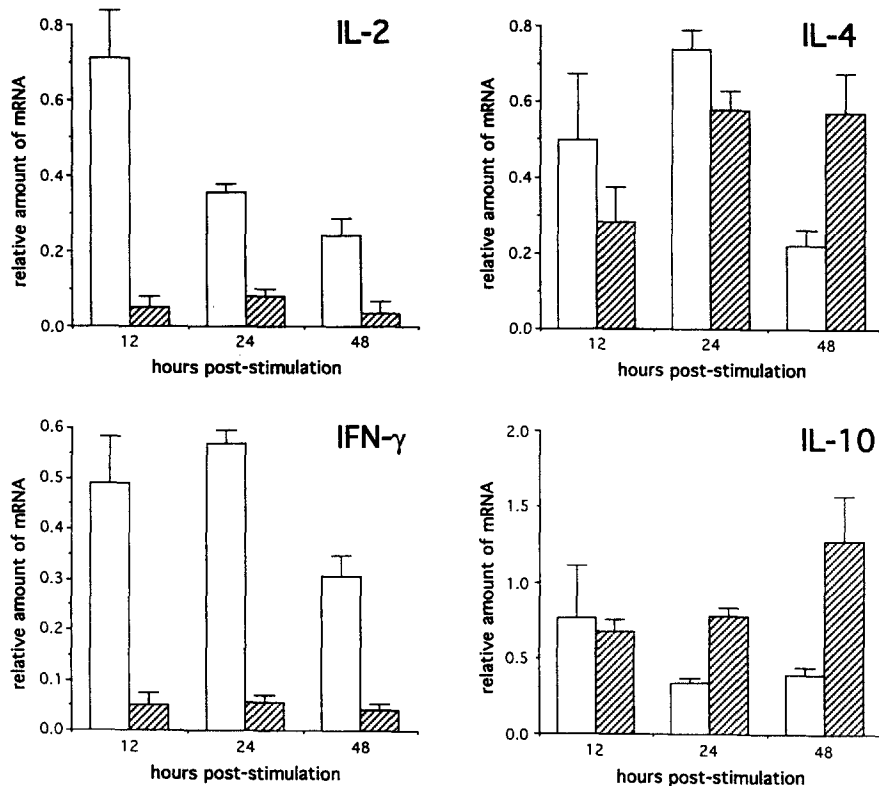
FIG 4. Quantitation of cytokine mRNA production by murine T cells stimulated in the absence (open bars) or presence (hatched bars) of normal brain-derived gangliosides. Individual cytokine transcripts were quantitated by RT-PCR relative to the constitutively expressed glyceraldehyde-3-phosphate dehydrogenase gene. Gangliosides selectively inhibited the accumulation of Th1-type cytokine mRNAs. Adapted with permission from Irani *et al.*, 1996.

infection may serve as yet another example of how T cell responses can be regulated during these diseases.

Irrespective of the experimental system being examined, there is a paucity of information regarding the control of T cell apoptosis in the brain. In animals with EAE, it appears that only T cells that have actually infiltrated the brain parenchyma undergo apoptosis; those present in the meninges or in the perivascular space seem to escape apoptotic destruction (Schmeid *et al.*, 1993; Bauer *et al.*, 1995). This finding implies either that brain-infiltrating T cells are exposed to something in the CNS environment that induces apoptosis or that they no longer have access to survival factors that inhibit their apoptotic cell death.

Any number of potential mechanisms to explain this finding may be invoked: aberrant antigen presentation without costimulatory signals that are necessary for optimal T cell activation; downregulation of T cell survival factors such as IL-2; and/or local exposure to proapoptotic mediators such as TGF-β, Fas ligand (FasL), and possibly others.

In the context of CNS viral infection, T cell apoptosis in the brain has been investigated during acute Sindbis virus encephalitis. BALB/c and SJL mice initiate comparable CNS mononuclear cell inflammatory responses following this infection, which lead to viral clearance from the brain with identical kinetics (Mokhtarian et al., 1989; Irani, 1998; Rowell and Griffin, 1999). Yet despite these equivalent antiviral host immune responses, CNS inflammation is more intense, persists much longer, and may actually contribute to paralysis in SJL mice, while cellular infiltrates disappear in a predictable manner from the brains of BALB/c mice that remain asymptomatic throughout disease (Mokhtarian et al., 1989; Irani, 1998; Rowell and Griffin, 1999). When T cells isolated from the brain parenchyma of these two hosts at late stages of infection are examined ex vivo, those from BALB/c mice are more frequently apoptotic, suggesting that this process contributes to the rate at which local immune responses are terminated (Fig. 5). Brain-derived T cells from BALB/c mice also express significantly higher intracellular levels of the proapoptotic mediator, Bax (Fig. 6), and peripheral T cells from these hosts are much more susceptible to apoptosis induced in vitro, following incubation with brain tissue extracts (Fig. 7). These data imply that substances present in the brain may directly induce T cell apoptosis, perhaps through the induction of proapoptotic mediators such as Bax, and that T cells from different strains of mice show variable susceptibilities to this apoptotic stimulus. Indeed, T cells from SJL mice, a host that is uniquely susceptible to immune-mediated CNS disease, show a particular resistance to local apoptosis in the brain. While it remains to be proven whether and how the brain directly controls the survival of infiltrating lymphocytes in such a tissue-specific manner, it is attractive to speculate that such a mechanism exists and that a defect in this process may contribute to CNS autoimmunity.

Other host factors also contribute to the survival of brain-infiltrating T cells during CNS viral infection. For example, following the inoculation of JHMV into susceptible mice, virus-specific CD8+ CTLs within the brain have an absolute requirement for CD4+ T cells that primarily serve to support their survival in this tissue compartment (Stohlman et al., 1998). Thus, lymphocyte apoptosis in the brains of JHMV-infected mice that have been depleted of CD4+ T cells is significantly increased (Stohlman et al., 1998). While specific survival factors
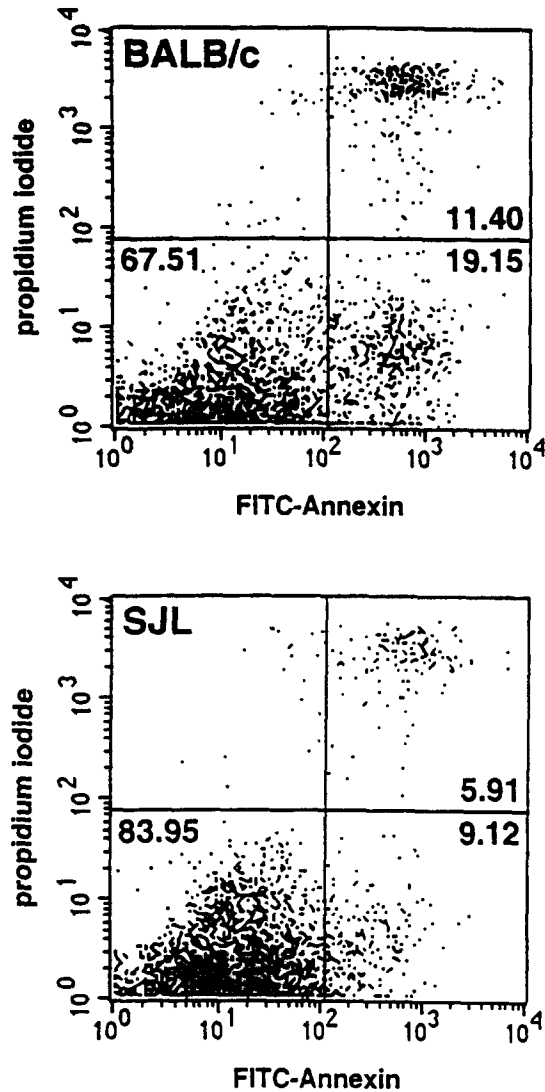
FIG 5. Flow cytometric analysis shows that a greater percentage of cells isolated from the brains of Sindbis virus-infected BALB/c mice are preapoptotic (annexin V-positive, propidium iodide-negative) or nonviable (annexin V-positive, propidium iodide-positive) as compared to cells recovered directly from the brains of infected SJL mice. Adapted with permission from Irani, 1998.
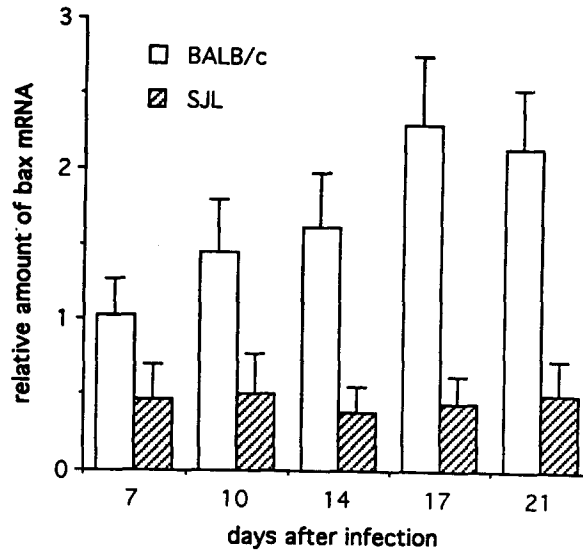
FIG 6. By RT-PCR, Bax-specific mRNAs are more abundant in T cells isolated from the brains of Sindbis virus-infected BALB/c mice as compared to T cells derived from the brains of infected SJL mice. Bax-specific transcripts were quantitated by RT-PCR relative to the constitutively expressed glyceraldehyde-3-phosphate dehydrogenase gene. Adapted with permission from Irani, 1998.

generated directly or indirectly through the actions of CD4+ T cells that prevent local CD8+ T cell apoptosis in the brain were not identified, their existence is strongly suggested. Such factors would be assumed to work in direct opposition to any proapoptotic influence exerted by the CNS microenvironment; it seems likely that the overall process that controls T cell survival within the CNS involves weighing the relative contributions of multiple pro- and anti-death influences. Such influences may in fact be integrally entwined as the brain may regulate the survival of infiltrating T cells, not by directly inducing their death, but by controlling how T cells produce their own survival factors (see Section III, D). Understanding these events will be crucial to improving our ability to gain pharmacologic control over pathologic, T cell–driven inflammation in the brain.

IV. CONCLUDING REMARKS

Studies in a number of experimental systems have shown that T cells can serve many different functions as part of the host immune
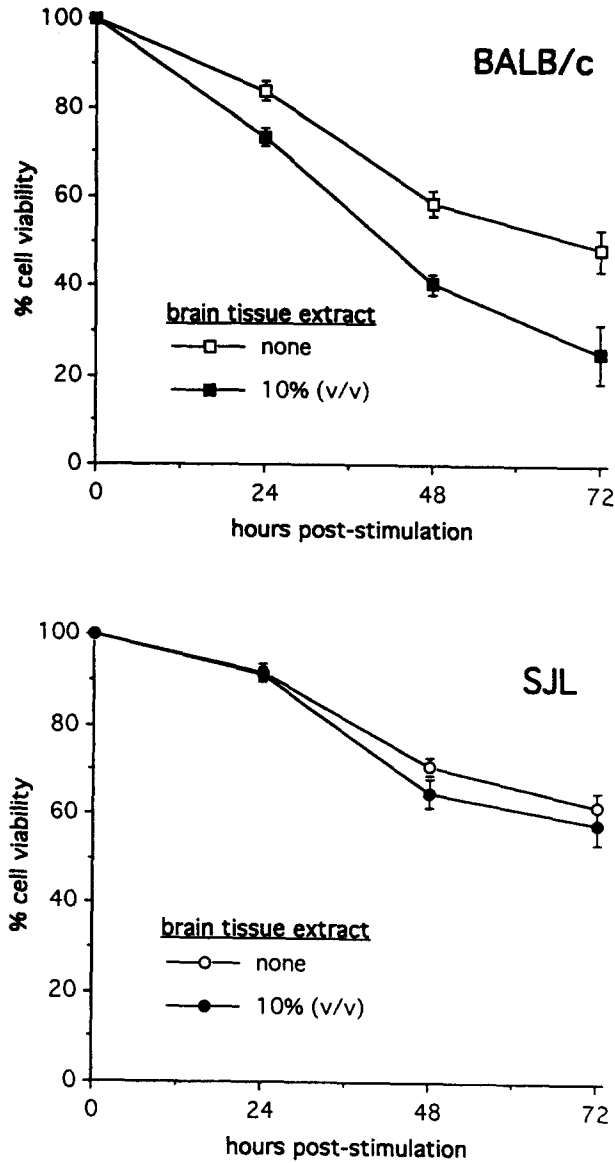
FIG 7. Tissue extracts prepared from the brains of uninfected BALB/c or SJL mice accelerate the activation-induced cell death of mitogen-stimulated peripheral T cells from BALB/c mice to a greater degree than T cells from SJL mice. Cell viability was measured in these experiments by using trypan blue exclusion. Adapted with permission from Irani, 1998.

response to CNS viral infection. Of particular interest here is how individual T cell responses may be actively regulated within the CNS during these diseases. We propose that such immunoregulatory events occur in CNS viral infection and we have focused on the control of local T cell responses within the brain, to support the idea that such regulation does occur. Our current understanding of the mechanisms that underlie such regulatory events is limited, but further study of these processes may provide broader insight into how pathologic T cell responses within the CNS, following a variety of stimuli, may or may not occur. An improved understanding of these regulatory events should be of interest to the entire neuroimmunology community.

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