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The Susceptibility of Mice to Immune-Mediated Neurologic Disease Correlates with the Degree to Which Their Lymphocytes Resist the Effects of Brain-Derived Gangliosides

David N. Irani

SjL mice develop immune-mediated disorders of the central nervous system (CNS) when infected with certain neurotropic viruses or when immunized with myelin Ags. Other strains including BALB/c are more resistant to these diseases. During Sindbis virus-induced encephalitis, both mice are easily infected and elicit rapid mononuclear cell inflammation in the brain. However, only SJL mice develop immune-mediated paralysis; BALB/c mice remain asymptomatic. To understand how the same stimulus produces such divergent immunologic effects on the host, the present study investigated lymphocytes that were isolated from the brains of Sindbis virus-infected animals. Cells from the brains of SJL mice exhibited more proliferation, produced more IL-2, maintained a higher viability, and expressed less bax mRNA (a proapoptotic mediator) than did lymphocytes from the brains of BALB/c mice. Since the central nervous system is enriched in gangliosides that regulate T cell proliferation and IL-2 production in vitro, purified brain-derived gangliosides were tested on peripheral lymphocytes from both strains. These lipids had less of an effect on the mitogen-induced proliferation, IL-2 production, activation-induced cell death, and up-regulation of bax mRNA in lymphocytes from SJL mice compared with those from BALB/c mice. Thus, gangliosides may inhibit various T cell effector functions and induce T cell apoptosis to a greater degree in the brains of BALB/c mice compared with the brains of SJL mice. This relative deficiency in local lymphocyte regulation may enhance the susceptibility of SJL mice to immune-mediated neurologic disease. The Journal of Immunology, 1998, 161: 2746–2752.

Immunemediated disorders of the central nervous system (CNS) that are induced by neurotropic viruses or by challenge with myelin Ags have been developed in experimental animals as models for the human demyelinating disease multiple sclerosis (MS). The susceptibility of inbred rodents to these disorders is determined by genetic factors that segregate particular strains into discrete populations based on their likelihood of developing disease. In mice, susceptibility to experimental autoimmune encephalomyelitis (EAE) and Theiler’s murine encephalomyelitis virus-induced demyelinating disease has been linked to polymorphisms in both MHC and non-MHC genes (1–3). SJL mice are highly susceptible to both of these disorders, while BALB/c mice are relatively resistant. Although CD4 + T cells of the Th1 phenotype play a well-established role in the pathogenesis of such diseases (1, 4–6), the precise immunologic cascade of events that leads to neuropathologic injury and clinical symptoms in susceptible mice is unclear. Furthermore, the role of host gene products in modulating these events also remains poorly understood. In one system, however, both susceptible and resistant strains of mice developed similar myelin-reactive Th1 responses in lymphoid tissue and equivalent degrees of CNS inflammation in response to a myelin challenge (7). Since only the susceptible animals in these experiments manifested clinically severe disease, it was proposed that the efficacy of local immune effector function in the brain was one determinant of whether neurologic symptoms actually develop following the accumulation of self-reactive T cells in the CNS (7). In another model system, the properties of the brain itself appeared to inhibit local T cell effector function during a noninjurious inflammatory response (8). If these putative, local immunoregulatory effects serve a more generalized function within the brain, it would be of interest to determine whether and how this regulation is attenuated in a neurologically symptomatic host. As a result, further light could be shed on the pathogenesis of the immune-mediated diseases of the CNS as a whole.

When BALB/c and SJL mice are infected with the encephalitic alphavirus Sindbis virus (SV), both animals initiate similar CNS mononuclear cell inflammatory responses (9). This CNS inflammation results in the clearance of infectious virus from the brains and spinal cords of these two hosts with identical kinetics (9). Yet despite their equivalent antiviral responses, SJL mice develop prolonged CNS inflammation and immune-mediated paralysis during infection, while BALB/c mice terminate inflammation and remain asymptomatic throughout the disease (9). As a result, the present study was undertaken to explore how the same stimulus could elicit such divergent immunologic effects on the host. Attention was focused on lymphocyte function and survival in the brains of these animals, since previous data have shown that the proliferation and IL-2 production of T cells isolated from the CNS of SV-infected BALB/c mice were abrogated through a mechanism that involved the cells being exposed to inhibitory substances within the brain (8), and because apoptosis has recently been shown to be an important means of down-regulating pathologic inflammation in the brain (10, 11). The present data show that CNS lymphocytes...
from SJL mice perform several important effector functions more readily than do cells that have been isolated from the brains of BALB/c mice. These cells also appear to be less susceptible to apoptosis, possibly through a mechanism that involves limiting the expression of the proapoptotic gene, bax (12). Furthermore, parallel experiments show that peripheral lymphocytes from SJL mice are less susceptible than cells from BALB/c mice to the inhibitory effects that purified brain-derived gangliosides exert on these same T cell effector functions. These complex membrane glycosphingolipids are highly enriched in the brain and are known to inhibit both the proliferation and the production of Th1-associated cytokines in T cells that have been activated in vitro (13). While brain-derived gangliosides up-regulate bax mRNA expression in lymphocytes from both strains, they do so to a much lesser degree in cells from SJL mice. Taken together, these findings indirectly imply that the differences in the degree to which lymphocytes resist the immunoregulatory effects of CNS gangliosides may explain why the effector responses of lymphocytes that have been isolated from the brains of these two hosts are different. Whether the susceptibility of lymphocytes to the regulatory effects of gangliosides has anything to do with the risk of the host developing immune-mediated neurologic disease remains to be determined. However, since it is proposed that an increased responsiveness of lymphocytes to the local regulatory effects of CNS gangliosides may help to protect a host from such disorders, studying how these lipids exert their effects at a molecular level could shed further light on the pathogenesis of immune-mediated CNS disease.

**Materials and Methods**

**Animal manipulations**

BALB/cJ and SJL/J mice of 3 to 4 wk of age (The Jackson Laboratory, Bar Harbor, ME) were maintained as colonies according to institutional guidelines for animal care. All animal procedures were performed under methoxyflurane anesthesia. To induce encephalitis, mice were injected intra-cranially with 1000 plaque-forming units of SV that were suspended in 0.02 ml of HBSS (Life Technologies, Grand Island, NY). Some animals were injected i.v. with 50 mg/kg of 5-bromo-2’-deoxyuridine (BrdU) (Sigma Chemical Co., St. Louis, MO) at various stages of infection. These animals were euthanized several hours later and perfused extensively with HBSS; brain and spleen tissue was frozen at −70°C for subsequent immunocytochemical staining (see below). In other experiments, mononuclear cells were isolated from the brains of SV-infected animals for in vitro culture, FACS analysis, or the isolation of total cellular mRNA (see below). Spleen-derived lymphocytes from PBMCs and perfused brain tissue and uninfected animals of both strains for use as responder cells in various in vitro assays and for preparing brain tissue supernatants, respectively (see below).

**Immunocytochemical detection of BrdU-labeled cells in tissue sections**

As described above, 10-μ frozen sections of brain and spleen tissue were prepared from SV-infected animals that had been labeled in vivo with BrdU. The slides were stained with a biotinylated primary anti-BrdU Ab according to the manufacturer’s instructions using a commercial BrdU Staining Kit (Oncogene Research Products, Cambridge, MA). A total of 10 slides per animal from three BALB/c and three SJL mice were prepared and stained at each stage of infection. A single section of spleen from each animal was also stained to confirm that the BrdU had been adequately injected into all recipients. The slides of brain tissue were examined by light microscopy in a blinded manner, and the total number of BrdU-positive cells per area of inflammation was counted. A minimum of 25 inflammatory foci were examined for each host strain at each stage of infection. In this way, the mean and SD of the number of BrdU-positive cells per inflammatory focus was determined. While the average number and size of inflammatory foci tended to increase over time in SV-infected animals, there were no differences found in these parameters between BALB/c and SJL mice at each timepoint after the number of BrdU-positive cells had been counted and the slides unblinded (data not shown).

**Isolation of tissue-derived lymphocytes**

A complete description of the technique that was used to isolate inflammatory cells from neural tissue has been reported previously (14). Briefly, each perfused brain was gently homogenized in HBSS containing 0.1% collagenase D (Boehringer Mannheim, Indianapolis, IN) and 10 μg/ml of DNase I (Sigma). Tissue fragments were removed by sedimentation at unit gravity, and the remaining suspension was centrifuged over a modified density gradient that had been prepared by mixing 3 parts Ficoll-Paque (Pharmacia, Piscataway, NJ) with 1 part RPMI 1640 (Life Technologies). Debris was retained at the gradient interface, while intact cells were recovered from within the gradient medium. Spleen homogenates were centrifuged over Lympholyte-M murine density separation medium (Cedarlane Laboratories, Hornby, Canada), and viable lymphocytes were recovered from the gradient interface.

**Preparation of supernatants from brain tissue homogenates**

Homogenates of uninfected brain tissue from both strains of mice were prepared to compare the immunoregulatory properties of normal brain constituents in vitro. Each perfused brain was homogenized directly in 10 ml of RPMI 1640 containing 10% FCS (Life Technologies). Clarified supernatants were then centrifuged to sediment the homogenates at 15,000 × g for 10 min. These supernatants were used directly as 0 to 10% (v/v) solutions in vitro.

**In vitro culture experiments**

Isolated lymphocytes were washed in HBSS and resuspended in RPMI 1640 containing 10% FCS. Cells were typically cultured in 96-well microtiter plates (2 × 10^5 cells/well in a final volume of 200 μl) in media containing 50 ng/ml of PMA (Calbiochem, La Jolla, CA) and 2 μg/ml of ionomycin (Calbiochem). Quadruplicate wells were prepared for each condition in each experiment, and the mean and SD values of each measured value are shown in the figures.) For some of the brain-derived inflammatory cells, sterile [H]ThiDr (1 μCi/well, 5 Ci/mmol) (DuPont-New England Nuclear, Boston, MA) was added during the last 16 h of either 24-, 48-, or 72-h incubation. Plates were harvested for scintillation counting after 24 h as described above. In other plates, culture supernatants were collected after 24 and 48 h of stimulation for the measurement of IL-2 and IL-4 production using commercially available mouse cytokine ELISA kits (Endogen, Cambridge, MA). To insure that any differences could not be attributed to discrepancies in cell viability, readouts for both proliferation and cytokine production were normalized to the values that had been obtained per 10^5 viable cells counted at each timepoint.

Spleen cells from uninfected mice of both strains were stimulated as described above. In some assays, increasing concentrations of brain supernatant were added, and the amount of proliferation compared with untreated cells was measured by [H]ThiDr incorporation after 72 h of culture. In other experiments, various concentrations of purified brain-derived gangliosides (Calbiochem) were added to the culture media. The ganglioside preparations were >98% pure by TLC and contained 21% GM1, 40% GD1a, 16% GD1b, and 19% GT1b according to the manufacturer. Here, cellular proliferation was measured at 72 h by [H]ThiDr incorporation, IL-2 production was measured in culture supernatants at 24 and 48 h by ELISA (Endogen), cell viability was determined after 24, 48, and 72 h by trypan blue exclusion, and total cellular mRNA was extracted after 24, 48, and 72 h for RT-PCR analysis (see below). Again, readouts for both proliferation and cytokine production were normalized to values that had been obtained per 10^5 viable cells at each timepoint to insure that any differences could not be attributed to discrepancies in cell viability.

**FACS analysis of tissue-derived lymphocytes**

To look for evidence of apoptosis, FACS analysis was performed on brain-derived inflammatory cells that had been isolated from SV-infected animals. Here, cells were stained using a combination of FITC-annexin V and propidium iodide (PI) according to the protocol supplied by the manufacturer (R&D Systems, Minneapolis, MN). The percentage of necrotic cells (annexin V-positive, PI-negative) and apoptotic or necrotic cells (annexin V-positive, PI-positive) in each population was determined using a FACS Calibur flow cytometer (Becton Dickinson Immunocytometry Systems, Mountain View, CA).
RT-PCR quantitation of relative intracellular bax mRNA levels

Total cellular RNA was extracted from brain-derived lymphocytes that had been isolated directly from SV-infected animals or from peripheral lymphocytes that were cultured in the presence of brain-derived gangliosides for various intervals as described above to detect bax-specific mRNAs by RT-PCR and to quantitate their levels relative to the constitutively expressed glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. Briefly, 1 μg of total cellular RNA was reverse-transcribed using Moloney murine leukemia virus reverse transcriptase (Life Technologies) in a 25-μl reaction volume containing 50 mM Tris-HCl (pH 8.3), 20 mM KCl, 10 mM MgCl₂, 5 mM DTT, 1 μM of each deoxynucleotide triphosphate, and 20 μg/ml oligo(dT) for 40 min at 42°C. The reaction mixture was diluted 1/8 with distilled water after first-strand cDNA synthesis, and 10 μl of the diluted product was used in each PCR. PCRs contained 200 μM of each deoxynucleotide triphosphate, 1 μM of each specific primer, buffer as supplied by the manufacturer, and 2.5 U of Taq DNA polymerase (Boehringer Mannheim). The sequences for the primers and probes used in these experiments have been published elsewhere (15). PCR was performed at a cycle number that ensured that amplification was occurring in a linear range. A 9600 thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) was used for all of the PCRs. Serial dilutions of a positive control for bax and GAPDH were amplified at 25, 30, and 35 cycles generating standard curves to insure a fixed relationship between the initial RNA input and the densitometric readout. A portion of each PCR reaction product was electrophoresed through a 1.2% agarose gel and transferred to a Hybond-N membrane (Amersham, Arlington Heights, IL) using standard blotting techniques. Southern transfers were probed with labeled internal bax-specific oligonucleotides and visualized using the enhanced chemiluminescence chemiluminescent detection system (Amersham). Autoradiograms were scanned using a laser densitometer (Molecular Dynamics, Sunnyvale, CA). The relative amount of bax-specific mRNA was determined by dividing the measured intensity of each band with that of the GAPDH gene from the same sample, thus controlling for the amount of mRNA that was transcribed in each RT reaction.

Results

Inflammatory cells in the brains of BALB/c and SJL mice proliferate to variable degrees during acute viral encephalitis

Unlike BALB/c mice that remain asymptomatic, SJL mice develop immune-mediated paralysis during acute SV encephalitis (9). To understand how the same infection could result in such divergent immunologic effects on the host, qualitative differences between the CNS inflammatory response of these two strains were sought. In particular, the capacity of brain-derived lymphocytes to survive and exert their various effector functions was investigated, since a previous study has shown that both the proliferation and the IL-2 production of T cells that have been isolated from the CNS of SV-infected BALB/c mice are impaired (8). To determine whether similar local immunoregulatory effects were being exerted in the brains of SJL mice, a BrdU labeling technique was used to compare the proliferation of CNS inflammatory cells in these two strains during acute SV infection. These studies showed that, while most of the inflammatory cells infiltrating the brain were not proliferating (Fig. 1A), SJL mice had more BrdU-positive cells per inflammatory focus than did BALB/c mice (Fig. 1B). In contrast, BrdU staining was quantitatively similar in the spleens of both host strains at all stages of infection (data not shown). When lymphocytes were isolated from the brains of infected animals and cultured in vitro, those from SJL mice exhibited measurable [3H]TdR incorporation over time, while those from BALB/c mice did not (Fig. 1C). To insure that these in vitro results could not be explained by differences in the viabilities of these two populations, all Δcpm measurements were normalized to counts per 10⁵ viable cells. Together, these findings support the hypothesis that the inflammation in the brains of SJL mice is regulated differently than the inflammation in the brains of BALB/c mice during acute SV infection.

Brain-derived lymphocytes from SJL mice produce more IL-2 than do cells from BALB/c mice

To determine whether the lymphocytes isolated from the brains of SJL mice during SV infection could respond more readily in other ways than cells from the brains of BALB/c mice, the cytokine-producing capacity of these two populations was studied in vitro. In bulk

FIGURE 1. Proliferation of CNS inflammatory cells during acute SV encephalitis in BALB/c and SJL mice. While most of the cells in an inflammatory infiltrate from the brain of an SV-infected SJL mouse did not proliferate, a few cells incorporated BrdU into their DNA using an in vivo labeling technique (A) (immunoperoxidase (anti-BrdU) and hematoxylin, ×40 magnification). B. When quantitated over the full course of infection, more BrdU-positive cells per inflammatory focus were present in the brains of SJL mice than in BALB/c mice. C. Following the isolation of lymphocytes from the brains of infected animals and culture in vitro, cells from SJL mice incorporated more [3H]TdR per 10⁵ viable cells than did lymphocytes from the brains of BALB/c mice.
culture, lymphocytes from the brains of SJL mice produced more IL-2 per 10^5 viable cells than did lymphocytes from the brains of BALB/c mice (Fig. 2). In contrast, levels of the Th2-associated cytokine, IL-4, were not different between the two populations (data not shown). Thus, at least during SV infection, lymphocytes that were isolated from the brains of SJL mice appeared more capable of another response than did cells from the brains of BALB/c mice.

Lymphocytes from the brains of SJL mice exhibit less apoptosis and contain less bax mRNA than do cells from the brains of BALB/c mice

Apoptosis has recently been proposed as the mechanism that down-regulates inflammation in the brain during EAE remissions (10, 11). Furthermore, apoptotic signaling through the Fas/Fas ligand (FasL) pathway augments the development of this disease and may promote the death of oligodendrocytes in MS (7, 16, 17). When analyzed in the present system by flow cytometry, fewer lymphocytes from the brains of SJL mice exhibited annexin V binding, which is a cell membrane event that begins early in apoptosis, compared with cells from the brains of BALB/c mice (Fig. 3). There was also less total cellular DNA fragmentation found in SJL-derived cells compared with BALB/c-derived cells when analyzed on ethidium bromide-stained agarose gels (data not shown). It is likely that this decreased rate of lymphocyte cell death accounts for the prolonged inflammation that is found in the brains of SV-infected SJL mice (9). However, while lymphocytes from the brains of SJL mice appeared less susceptible to apoptosis, parallel FACS analyses showed that these cells did not express different levels of either Fas or FasL compared with cells from the brains of BALB/c mice (data not shown). As a result, the mechanism(s) through which these cells induce symptomatic neurologic disease in this setting remain unknown.

Nevertheless, to understand how lymphocytes from the brains of SJL mice were less susceptible to apoptosis than were cells from the brains of BALB/c mice, the relative expression of the mRNAs encoding various bcl-2 family members was measured in these two populations by RT-PCR. While bcl-2 and bcl-xL transcripts were not appreciably different between BALB/c- and SJL-derived cells (data not shown), there was significantly less bax mRNA found in the lymphocytes that had been isolated from the brains of SJL mice at multiple stages of SV infection (Fig. 4). This gene product is known to directly antagonize the protection that bcl-2 provides to cells and consequently can accelerate programmed cell death (12). Furthermore, the induction of bax in perivascular lymphocytes in the brain correlates.
incorporated cpm per 10^5 viable cells to insure that decreased cell viability of the brain supernatant-treated cells was also measured as proliferation in these assays was 22,850 cpm per 10^5 viable cells for the antiproliferative effects of the BALB/c brain supernatant. The baseline proliferation in these experiments was 24,150 cpm per 10^5 viable cells for the BALB/c cells and 44,100 cpm per 10^5 viable cells for the SJL cells. The proliferation of ganglioside-treated cells was also measured as incorporated cpm per 10^5 viable cells to insure that decreased cell viability did not contribute to the measurements of cell proliferation.

FIGURE 5. Conditioned media from brain tissue homogenates (“brain supernatants”) inhibit the mitogen-induced proliferation of lymphocytes from the spleens of BALB/c and SJL mice to different degrees. A. When compared with the proliferation of untreated cells (baseline proliferation), the proliferation of the BALB/c lymphocytes that had been treated with brain supernatants from BALB/c mice was more potently inhibited than was the proliferation of SJL cells that had been treated with SJL brain supernatants. B. When assay conditions were reversed, BALB/c cells were inhibited by SJL brain supernatant; SJL cells were more resistant to the antiproliferative effects of the BALB/c brain supernatant. The baseline proliferation in these assays was 22,850 cpm per 10^5 viable cells for the BALB/c cells and 48,675 cpm per 10^5 viable cells for the SJL cells. The proliferation of the brain supernatant-treated cells was also measured as incorporated cpm per 10^5 viable cells to insure that decreased cell viability did not contribute to the measurements of cell proliferation.

FIGURE 6. Purified brain-derived gangliosides inhibit the proliferation of BALB/c lymphocytes to a much greater degree than SJL lymphocytes. When compared with the proliferation of untreated cells (baseline proliferation), gangliosides inhibit the proliferation of lymphocytes from the spleens of BALB/c mice more potently than cells from SJL mice. The baseline proliferation in these experiments was 24,150 cpm per 10^5 viable cells for the BALB/c cells and 44,100 cpm per 10^5 viable cells for the SJL cells. The proliferation of ganglioside-treated cells was also measured as incorporated cpm per 10^5 viable cells to insure that decreased cell viability did not contribute to the measurements of cell proliferation.

To understand the basis for this difference, BALB/c brain supernatants were tested on SJL lymphocytes and vice versa. While SJL brain supernatant could easily inhibit the proliferation of BALB/c cells, SJL lymphocytes were relatively resistant to the antiproliferative effects of the BALB/c brain supernatant (Fig. 5B). This finding strongly suggests that the response of the cells, and not the content of the brain supernatant, accounts for the differences observed. Proliferation was calculated in these assays as incorporated cpm per 10^5 viable cells to eliminate the possibility that discrepancies in the viability of responder cells might explain this effect. When these in vitro data are extrapolated, it seems more likely that the greater numbers of inflammatory cells in the brains of SV-infected SJL mice could incorporate BrdU because they were more resistant to the antiproliferative effects of the substances found within the brain, rather than the local immunologic environment in the brains of these mice being intrinsically less capable of inhibiting lymphocyte proliferation.

Peripheral lymphocytes from SJL and BALB/c mice differ in their responses to purified brain-derived gangliosides

It has been shown that the inhibitory activity of BALB/c brain supernatant is due in large part to its abundant ganglioside content (8). Therefore, the effects of purified brain-derived gangliosides were compared in cultures of peripheral lymphocytes from BALB/c and SJL mice. Like their response to the whole brain supernatants, SJL lymphocytes were more resistant to the antiproliferative effects of the brain-derived gangliosides than were BALB/c cells (Fig. 6). Because these lipids also block the production of IL-2 by activated T cells through a mechanism that is separate from their antiproliferative effects (13), this event was examined in peripheral lymphocytes from these two host strains as well. When assayed in culture supernatants, brain-derived gangliosides inhibited the production of IL-2 by activated BALB/c lymphocytes; however, the production of IL-2 by SJL cells was not altered to any appreciable degree (Fig. 7). The divergent effects of CNS gangliosides on the proliferation and IL-2 production by these two lymphocyte populations could not be explained by any selective effects on cell viability, since both responses were carefully normalized to a fixed number of viable cells in each assay. This distinction is important, because the brain-derived gangliosides did in

with a down-regulation of inflammation during the later stages of EAE (11). The present data imply that a slower and/or less vigorous induction of bax mRNA in lymphocytes from the brains of SV-infected SJL mice may limit their apoptotic clearance from the CNS.

Peripheral lymphocytes from SJL mice resist the antiproliferative effects of clarified brain tissue homogenates

To explore whether different degrees of responsiveness to the local immunoregulatory effects exerted within the CNS might explain why lymphocytes from the brains of these two animals behaved differently in vitro, clarified supernatants that had been prepared from homogenates of normal brain tissue were added to cultures of peripheral lymphocytes that were stimulated in vitro. In assays of mitogen-induced proliferation, SJL brain supernatant had less of an inhibitory effect on the proliferation of SJL cells compared with the effect of equal concentrations of BALB/c brain supernatant on BALB/c cells (Fig. 5A).
fact accelerate the rate of activation-induced cell death in lymphocytes from BALB/c cells without affecting the viability of mitogen-stimulated SJL cells (Fig. 8). These lipids also caused a preferential induction of bax mRNA in BALB/c lymphocytes over time (Fig. 9). Taken together, the variable effects that brain-derived gangliosides exert on peripheral lymphocytes from BALB/c and SJL mice closely predict the degree to which CNS lymphocytes from these two mice respond.

**Discussion**

Multiple lines of evidence suggest that the brain has developed some intrinsic capacity to control inflammation to minimize the risk that nonrenewable cells such as neurons might incur immune-mediated damage over time. Certainly the paucity of MHC Ag expression in the CNS has long been proposed as one explanation for how local cellular immune reactivity is contained (18–20). Recently, brain-enriched gangliosides have been shown to inhibit the expression of both class I and class II molecules on astrocytes as one means of controlling MHC Ag expression in the CNS (21). Of all neural cells, MHC suppression is most complete on neurons (20, 22), which is consistent with their capacity to synthesize high levels of complex gangliosides compared with cells of both glial and nonneural origins (23–25). Since an intercellular transfer of polysialogangliosides from neurons to astrocytes can occur, neurons and the gangliosides that they produce may be important regulators of MHC expression in the brain as a whole (23, 26). This hypothesis is supported by the sharp decrease in membrane ganglioside content of astrocytes cultured in the absence of neurons over time (23, 26), the concomitant increase in both constitutive and inducible MHC Ag expression on astrocytes cultured in such a manner (19, 27, 28), and the direct, ganglioside-mediated suppression of MHC expression on astrocytes in vitro (21). Importantly, inducible MHC expression on astrocytes that have been derived from rodent brains is also known to be host strain-dependent (19, 29). Therefore, it seemed possible that other cell types from different host strains could vary in their susceptibility to the effects that are mediated by CNS gangliosides. In this study, peripheral lymphocytes from SJL mice are shown to be more resistant to the various effects that purified, brain-derived gangliosides exert compared with cells from BALB/c mice. Furthermore, lymphocytes that are isolated from the brains of SJL mice during acute viral encephalitis are less susceptible to apoptosis and respond more readily than do cells that have been isolated from the brains of BALB/c mice, possibly due to differences in their susceptibility to CNS gangliosides in vivo.

It is likely that multiple factors influence the susceptibility of different rodent strains to immune-mediated CNS disorders. However, one important variable is the capacity of lymphocytes to exert their effector functions within the brain; in one study, cells from mice that were deficient in either Fas or FasL could accumulate in the CNS but...
could not induce paralysis during EAE (7). A previous study using the current encephalitis model system showed that T cells that were isolated from the brains of SV-infected BALB/c mice had impaired effector responses resulting from their exposure to gangliosides within the brain (8). Since SJL mice develop prolonged inflammation and immune-mediated paralysis with acute SV infection, and BALB/c mice display resistance (9), the current experiments arose from the hypothesis that the lymphocytes recruited into the brains of these mice somehow resisted the local inhibitory effects of gangliosides, thereby retaining some capacity to induce neurologic disease. These data confirm that SJL lymphocytes resist the inhibitory effects of brain-derived gangliosides in vitro and exert more robust effector responses within the brain in vivo. However, these findings do not prove that any of these effects are in any way responsible for the induction of neurologic symptoms in SV-infected SJL mice. Furthermore, such results argue against a role for the Fas/FasL pathway as an effector of this process. Proof of a cause-and-effect relationship between these data and disease susceptibility will require either a more complete understanding of how gangliosides alter T cell function at the molecular level and the capacity to block their effects pharmacologically, or the generation of mice that are deficient in the enzymes that regulate the synthesis of CNS gangliosides or the gene product(s) that control lymphocyte responses to gangliosides. In lieu of such advances, however, improving the strength of the correlation between the susceptibility of lymphocytes to gangliosides in vitro and resistance to immune-mediated CNS disease in vivo will continue using peripheral lymphocytes from other susceptible and resistant rodent strains as well as from normal human controls and patients known to have MS. Furthermore, the study of other T cell responses in SV-infected BALB/c and SJL mice may clarify the mechanism(s) through which neurologic symptoms actually develop.

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