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Differential Regulation and Function of Fas Expression on Glial Cells

Sung Joong Lee,* Tong Zhou,† Chulhee Choi,* Zheng Wang,† and Etty N. Benveniste2*  

Fas/Apo-1 is a member of the TNF receptor superfamily that signals apoptotic cell death in susceptible target cells. Fas or Fas ligand (FasL)-deficient mice are relatively resistant to the induction of experimental allergic encephalomyelitis, implicating the involvement of Fas/FasL in this disease process. We have examined the regulation and function of Fas expression in glial cells (astrocytes and microglia). Fas is constitutively expressed by primary murine microglia at a low level and significantly up-regulated by TNF-α or IFN-γ stimulation. Primary astrocytes express high constitutive levels of Fas, which are not further affected by cytokine treatment. In microglia, Fas expression is regulated at the level of mRNA expression; TNF-α and IFN-γ induced Fas mRNA by ~20-fold. STAT-1c and NF-κB activation are involved in IFN-γ- or TNF-α-mediated Fas up-regulation in microglia, respectively. The cytokine TGF-β inhibits basal expression of Fas as well as cytokine-mediated Fas expression by microglia. Upon incubation of microglial cells with Fasl-expressing cells, ~20% of cells underwent Fas-mediated cell death, which increased to ~60% when cells were pretreated with either TNF-α or IFN-γ. TGF-β treatment inhibited Fas-mediated cell death of TNF-α- or IFN-γ-stimulated microglial cells. In contrast, astrocytes are resistant to Fas-mediated cell death, however, ligation of Fas induces expression of the chemokines macrophage inflammatory protein-1β (MIP-1β), MIP-1α, and MIP-2. These data demonstrate that Fas transmits different signals in the two glial cell populations: a cytotoxic signal in microglia and an inflammatory signal in the astrocyte. The Journal of Immunology, 2000, 164: 1277–1285.
human fetal astrocytes as well as adult astrocytes and was up-regulated by several proinflammatory cytokines such as IL-1, IL-6, IFN-γ, and TNF-α (24). In addition, the fetal astrocytes underwent apoptosis when treated with agonistic anti-Fas Ab. It has been suggested that the susceptibility of human astrocytes to Fas-mediated apoptosis is dependent on cell passage and other microenvironmental factors; only late passage astrocytes are sensitive to Fas-induced apoptosis and the presence of IFN-γ enhances apoptosis (25). Apoptosis in microglia has not been well studied compared with that in astrocytes. In several studies using immunohistochemistry in EAE brain, microglial cells were positively stained by the TUNEL assay more frequently than astrocytes (16). Recently, Fas-mediated apoptosis was reported in cultured microglia upon TNF-α or IFN-γ stimulation (26).

In this study we have investigated the regulation and function of Fas expression using mouse astrocytes and microglia. We examined the influence of several pro- and anti-inflammatory cytokines (IFN-γ, TNF-α, TGF-β) on Fas expression in glial cells as well as the functional outcome of Fas ligation on these cells. Herein, we report that Fas expression is differentially modulated by cytokines on astrocytes and microglia, and Fas ligation transmits distinct signals in these two glial cell types.

Materials and Methods
Primary mouse astrocytes, microglia, and the microglial cell line EOC13
Primary glial cell cultures were established from neonatal CD1 as well as C57BL/6/lpr mouse cerebra as previously described (27). Cells were cultured in DMEM, high glucose formula supplemented with glucose to a final concentration of 6 g/L, 2 mM glutamine, 0.1 mM nonessential amino acid mixture, G418 (10 μg/mL), 0.1% gentamicin, and 10% FBS (HyClone, Logan, UT). After 2 wk in primary culture, oligodendrocytes and microglia were separated from astrocytes by mechanical dislodgment. Microglia-enriched cultures were obtained by incubating the detached cells in six-well plates and changing the medium after 1 h to remove nonadherent cells. Microglia were also prepared from CD1 STAT-1−/− mice (provided by Dr. David E. Levy, New York University School of Medicine, New York, NY) (28). Astrocyte cultures were routinely >95% positive for glial fibrillary acidic protein, a specific marker for astrocytes, and primary microglial cultures were >95% positive as assessed by immunostaining for the Mac-1 surface Ag. The microglial cell line EOC13 was derived from C3H/HeJ CH-2k mice using a nonviral immortalization procedure as previously described (29). This CSF-1-dependent cell line is B7.1−, Mac-1−, CD45−, and class 1 MHC− as well as phagocytic. The EOC13 cell line was maintained in DMEM complete medium (2 mM glutamine, 10% heat-inactivated FBS, and 20% LADMAC-conditioned medium, which contains CSF-1) as previously described (27).

Reagents and plasmids
Recombinant murine IFN-γ was purchased from Genzyme (Boston, MA), human TNF-α was obtained from R&D Systems (Minneapolis, MN), and rat TNF-α was purchased from BioSource International (Camarillo, CA). Hamster anti-mouse Fas Ab (Jo2), PE-conjugated hamster anti-mouse Fas Ab (Jo2), hamster anti-mouse Fas ligand Ab (MFL3), hamster anti-mouse ICAM-1 Ab (3E2), and hamster anti-TNF IgG were purchased from Pharmingen (San Diego, CA). The NF-κB kinase MEKK (MEK) inhibitor U0126 (5-[4-ethylphenyl]−2-(3-morpholinyl)−1,4−benzodiazepin−2−one) was purchased from Promega (Madison, WI). Murine Fas cDNA in the pcDNA1.1 plasmid was generated by PCR as previously described (30). The plasmid was cut with XhoI, and the 500-bp fragment containing the C-terminal half of mouse Fas cDNA was subcloned into pcDNA3 at an XhoI restriction enzyme site. After linearization with HindIII, this construct (pcDNA3-mouse Fas) was used for in vitro transcription to generate a 420-bp antisense RNA probe. A PGEM-4Z vector containing a fragment of mouse IFN-γ (corresponding to bp 223−343) inserted at the poly linker sites EcoRI/KpnI was linearized with EcoRI. In vitro transcription of this plasmid with T7 RNA polymerase generated a 270-bp antisense RNA probe.

Analysis of Fas protein expression by immunofluorescence flow cytometry
Primary astrocytes were plated at 5 × 10³ cells/well into six-well plates (Costar, Cambridge, MA) and treated with IFN-γ or TNF-α in the absence or the presence of TGF-β1 for various time periods. The cells were trypsinized, washed with PBS, incubated with 20 μg/mL of PE-conjugated anti-Fas Ab (Jo2) for 1 h at 4°C, washed, then fixed in a final volume of 100 μL of 1% parafomaldehyde. The cells were then analyzed on the FACSCalibur (Becton Dickinson, Mountain View, CA). Total fluorescence intensity was calculated as the mean fluorescence intensity × percentage of positive cells. Negative controls were incubated with isotype-matched Ab. For the analysis of primary microglia or EOC13 cells, cells were treated as described above, then scraped to detach from the well and incubated with 50 μL of 2.4G2 supernatant (which contains rat anti-mouse FcγR Ab) supplemented with 10% normal mouse serum for 30 min at 4°C before addition of PE-conjugated anti-Fas Ab.

RNA isolation and RNase protection assay (RPA)
Total cellular RNA was isolated from confluent monolayers of EOC13 cells or astrocytes that were incubated with cytokines or anti-Fas Ab, respectively, as previously described (31). Briefly, cells were washed once in PBS and lysed directly in the culture dish. RNA was extracted with guanidinium isothiocyanate and phenol, and precipitated with ethanol. Ten micrograms of total cellular RNA was analyzed by RPA using an RPA kit (Ambion, Austin, TX) as previously described (31). Total RNA was hybridized with both mouse Fas and GAPDH riboprobes (2.5 × 10³ cpm) at 42°C overnight in 20 μL of 40 mM PIPES (pH 6.4), 80 μM dideoxynucleotide mixture, 400 mM NaOAc, and 1 μM EDTA. The hybridized mixture was treated with RNase A/T1 (1/100 dilution in 200 μL of RNase digestion buffer) at 37°C for 30 min, and then analyzed by 5% denaturing (8 M urea) PAGE. The protected fragments of the Fas and GAPDH riboprobes are 374 and 212 nucleotides in length, respectively. Quantification of protected RNA fragments was performed by scanning with the PhosphorImager (Molecular Dynamics, Sunnyvale, CA), and values for Fas mRNA were normalized to GAPDH mRNA levels for each experimental condition. The linearized mouse chemokine multigene probe set mC5-5 (catalogue no. 45026P) was purchased from PharMingen. The mC5-5 was in vitro transcribed with T7 RNA polymerase to produce antisense chemokine RNA probes as previously described (32).

51Cr release assay
Fas-mediated microglial cell lysis was measured by the 51Cr release assay. Fas-expressing effector cells were generated by transfection of Fasl-containing adenovirus vector into macrophages from Fas-deficient lpr mice as previously described (33). EOC13 cells were incubated with medium alone or different cytokines for 40 h and radiolabeled by incubation with 20 μCi of 51Cr (1 μCi) (Amersham, Arlington, TX) for 1 h. After washing the cells three times, 51Cr-labeled EOC cells (1 × 10⁵) were incubated with an equivalent number of effector cells. The release of 51Cr in the supernatant was assessed 8 h later using a gamma counter. For each condition, Fas-L-negative macrophages were used as a negative control. 51Cr release was measured, and values were used as spontaneous release. Maximum 51Cr release was measured from each positive control in which cells are lysed in 1% Triton X-100. The percentage of specific cell death rate was calculated as (experimental 51Cr release − spontaneous release)/(maximum 51Cr release − spontaneous release).

Results
Fas expression by microglia and astrocytes
We initially analyzed cell surface Fas expression on primary mouse astrocytes and microglia by FACS analysis. Both cell types were incubated in medium alone or with TNF-α or IFN-γ for 40 h before staining. On astrocytes, Fas is constitutively expressed and is not significantly modulated by treatment with TNF-α or IFN-γ (Fig. 1). In primary mouse microglia, Fas is expressed at a low level compared with that of astrocytes, and expression is up-regulated upon TNF-α or IFN-γ treatment (~5-fold induction; Fig. 1).

Because Fas expression was regulated on microglia by the cytokines TNF-α and IFN-γ, we further investigated cytokine regulation of Fas expression using the microglial cell line, EOC13. EOC13 is a transformed microglial line that exhibits numerous...
Cytokine regulation of Fas mRNA expression involves STAT-1

Characteristics of primary microglia (27, 29). Surface Fas expression on unstimulated or cytokine-stimulated EOC13 cells was regulated in a comparable manner to that of primary microglia (Fig. 2A). Kinetic analysis demonstrated that IFN-γ up-regulated Fas expression with slightly different kinetics compared with those of TNF-α (Fig. 2B). TNF-α up-regulated Fas expression by ~6–12 h after stimulation, with a peak at 36 h, whereas IFN-γ most actively induced Fas within a 24- to 36-h period, with expression still increasing at 40 h (Fig. 2B).

Next, Fas mRNA expression was examined in EOC13 cells upon cytokine stimulation. Constitutive Fas mRNA was barely detectable (Fig. 3, lanes 1 and 7), but was induced by TNF-α or IFN-γ in a time-dependent manner (lanes 2–6 and 8–12). TNF-α induced Fas mRNA expression peaked at 6 h, while IFN-γ-induced Fas mRNA expression was optimal at 12 h of stimulation. These data demonstrate that Fas is regulated by the cytokines TNF-α and IFN-γ at the mRNA level.

Cytokine regulation of Fas mRNA expression involves STAT-1α and NF-κB

TNF-α- or IFN-γ-mediated Fas up-regulation has been reported in several other cell types (34). However, the transcriptional regulatory mechanisms and/or the transcription factors involved in Fas expression have been only recently reported (35). It is well known that the IFN-γ receptor transmits signals through the JAK-STAT pathway to induce transcription of various target genes (for review, see Ref. 36). Therefore, we investigated the involvement of STAT-1α in IFN-γ-mediated Fas expression using microglia from STAT-1α-deficient mice. As shown in Fig. 4A, IFN-γ-induced Fas expression was severely impaired in STAT-1α-deficient mouse microglia, while TNF-α mediated Fas up-regulation was intact, demonstrating that STAT-1α is critical for IFN-γ mediated Fas induction. TNF-α binding to its receptor rapidly activates the transcription factor NF-κB in many cell types (for review, see Ref. 37). To further test the involvement of NF-κB activation in TNF-α-induced Fas expression, the activation of NF-κB was suppressed using the specific inhibitor, SN50. This oligopeptide contains the nuclear localizing signal found in NF-κB and specifically blocks nuclear translocation of NF-κB (38). SN50 inhibited TNF-α-induced Fas mRNA expression by ~60%, while having no effect on IFN-γ-mediated Fas expression (Fig. 4B). These data clearly demonstrate that NF-κB activation is required for TNF-α-mediated Fas expression in microglial cells.

Regulation of Fas expression by TGF-β

Previous studies from our laboratory have shown that TGF-β exerts a strong anti-inflammatory effect on microglia by antagonizing the effect of proinflammatory cytokines such as TNF-α and IFN-γ. Hence, we tested the effect of TGF-β on cytokine-induced Fas expression on EOC13 cells. FACS analysis demonstrated that TGF-β slightly inhibited the constitutive expression of Fas. In addition, TGF-β inhibited TNF-α-mediated Fas expression by ~60% and IFN-γ-mediated Fas expression by ~70% in EOC13 cells (Fig. 5). We also tested the inhibitory effect of TGF-β on Fas expression in primary mouse microglia and found comparable inhibitory effects (data not shown). Regulation of Fas mRNA expression was also tested upon TGF-β stimulation (Fig. 6). TGF-β
alone had no effect on Fas mRNA expression (lane 4), whereas it inhibited TNF-α-induced Fas mRNA expression by ~50% (compare lanes 2 and 5) and IFN-γ-induced Fas expression by ~45% (compare lanes 3 and 6). These data demonstrate that the inhibitory effect of TGF-β on Fas expression is mediated at the mRNA level.

Functional significance of Fas expression by microglial cells

Fas transmits apoptotic signals into susceptible target cells (39). We tested whether the Fas receptor on the surface of microglia could induce cell death upon ligation with FasL. FasL-expressing macrophage cells from the lpr mouse that were transiently transfected with FasL cDNA were used. This cell line expresses FasL on the cell surface and induces apoptosis in Fas-bearing target cells (33). EOC13 cells were labeled with 51 Cr and incubated with either FasL or lpr macrophages or FasL-untransfected control cells, then 51 Cr released in the medium was measured. Without any stimulation, ~20% of EOC13 cells underwent Fas-mediated cell death upon Fas ligation. This cell death rate increased to ~60% when cells were stimulated with either TNF-α or IFN-γ (Fig. 7A), indicating that TNF-α and IFN-γ potentiate Fas-mediated cell death in EOC13 cells. This cytokine-mediated potentiation of microglial cell death was almost completely inhibited when cells were incubated with TGF-β (Fig. 7A). In addition, incubation with TGF-β alone inhibited the basal level of cell death of EOC13 cells (Fig. 7A). The specificity of cell death was demonstrated by addition of Abs specific for Fas or FasL in the coculture. Abs against either Fas or FasL completely blocked the potentiation of cell death by TNF-α stimulation (Fig. 7B). Likewise, the IFN-γ-induced cell death was completely blocked by Abs against Fas or FasL (data not shown). However, neither anti-ICAM-1 nor isotype control Ab (hamster IgG) inhibited Fas-induced cell death (Fig. 7B).

Ligation of Fas on astrocytes induces inflammatory chemokine expression

Recently, several studies have reported that Fas can transmit inflammatory signals depending on the cell type (25, 40). Because primary mouse astrocytes were completely resistant to Fas-mediated cell death in the 51 Cr release assay (data not shown), we examined whether Fas on astrocytes has an alternative function, i.e., transduction of inflammatory signals. Specifically, we examined whether Fas ligation on astrocytes affected chemokine expression (Fig. 8). Astrocytes incubated with anti-mouse Fas Ab (Jo2) for 1 h up-regulated the mRNA expression of MIP-1β by ~3-fold, MIP-1α by ~2-fold, and MIP-2 by ~2-fold (Fig. 8A, lane 2) compared with that in the control sample (lane 1) in which cells were incubated with isotype-matched Ab. The addition of anti-hamster IgG Ab (as a secondary Ab) with anti-Fas Ab did not further enhance anti-Fas Ab-induced chemokine expression (lane 4), suggesting that anti-Fas Ab alone is sufficient to transduce inflammatory signals. As well, the addition of secondary Ab alone...
did not affect chemokine expression (lane 3). Considering the abundant expression of the proinflammatory cytokine TNF-α in the CNS during EAE pathogenesis (41), it is likely that Fas transmits signals in the presence of TNF-α during the disease process. To test possible concerted effects of Fas-mediated signals and TNF-α, we stimulated cells with anti-Fas Ab in the absence or the presence of TNF-α (Fig. 8B). TNF-α alone induced mRNA expression of MIP-1α and -β by about 2-fold (lane 3). Furthermore, in the presence of TNF-α, cross-linking of Fas resulted in a synergistic effect on MIP-1β expression (~7-fold induction) and an additive effect on MIP-1α expression (~4-fold induction; lane 4). Possible additive/synergistic effects between Fas cross-linking and TNF-α on MIP-2 expression were not obvious, because TNF-α-induced MIP-2 expression was so strong (Fig. 8B, lane 3). To test the specificity of Fas signaling, we used primary astrocytes isolated from lpr mice that are deficient in functional Fas expression (Fig. 8C). Although TNF-α-mediated chemokine induction was intact (lane 3), anti-Fas Ab-induced chemokine expression was completely abolished in lpr astrocytes (compare lanes 1 and 2). As well, addition of anti-Fas Ab plus TNF-α showed neither a synergistic nor an additive effect on chemokine expression in lpr astrocytes compared with TNF-α alone (lanes 3 and 4). These data clearly demonstrate that anti-Fas Ab generates an inflammatory signal through binding to the Fas molecule on astrocytes. To test
Ligation of Fas on astrocytes induces chemokine expression. A, Primary mouse astrocytes were incubated with hamster anti-TNP (0.5 μg/ml), hamster anti-Fas Ab (1 μg/ml), or isotype control Ab (1 μg/ml) for 30 min, then stimulated with either hamster anti-TNP Ab (1 μg/ml; lane 1), anti-Fas Ab (1 μg/ml; lane 2), or anti-hamster IgG Ab (0.5 μg/ml; lane 3), or anti-Fas plus anti-hamster IgG Ab (lane 4) for 1 h. Total RNA was prepared from each sample and used for multiprobe RPA with the chemokine probe set mCK-5. The name of each protected mRNA signal is denoted in the middle. Results are representative of five experiments. B, Astrocytes were stimulated with either hamster anti-TNF-α Ab (1 μg/ml; lane 1), anti-Fas Ab (1 μg/ml; lane 2), TNF-α (50 ng/ml; lane 3), or anti-Fas Ab plus TNF-α (lane 4) for 4 h. Total RNA was used for chemokine multiprobe RPA. Results are representative of three experiments. C, Astrocytes from IFN-γ mice were stimulated as described and used for chemokine multiprobe RPA. Results are representative of two experiments. D, EOC13 cells were stimulated with medium alone (lanes 1 and 2) or TNF-α (50 ng/ml; lanes 3 and 4) for 40 h, then incubated with either anti-Fas Ab (1 μg/ml; lanes 2 and 4) or isotype control Ab (1 μg/ml; lanes 1 and 3) for 1 h and used for chemokine multiprobe RPA. Results are representative of three experiments. E, Primary mouse astrocytes were incubated with varying concentrations of U0126 (lanes 3–5), olomoucine (OL; lanes 6–8), or SB202190 (SB; lanes 9–11) for 30 min, then stimulated with either hamster anti-TNF-α Ab (1 μg/ml; lane 1) or anti-Fas Ab (1 μg/ml; lanes 2–11) for 1 h. Total RNA was prepared from each sample and used for chemokine multiprobe RPA. Results are representative of two experiments.

FIGURE 8. Ligation of Fas on astrocytes induces chemokine expression. A, Primary mouse astrocytes were incubated with hamster anti-TNP...
suggested a species-specific differential sensitivity of astrocytes to TNF-α stimulation of Fas expression.

In distinction to astrocytes, microglia express low basal levels of Fas, which are further up-regulated by TNF-α or IFN-γ. The RPA data demonstrate that Fas expression is regulated by cytokines at the mRNA level. Interestingly, we observed that cytokine induction of Fas mRNA expression is higher than that of Fas protein expression, suggesting the possibility of different regulatory mechanisms at the post-transcriptional and/or translational level. Studies using STAT-1α-deficient microglia and the specific NF-κB inhibitor SN50 prove that STAT-1α and NF-κB activation are required for IFN-γ- and TNF-α-mediated Fas induction in microglia, respectively. In HeLa cells, CCAAT/enhancer-binding protein β (C/EBPβ) is responsible for influenza virus-infection mediated Fas up-regulation (44). In addition, NF-κB and C/EBP consensus elements are suggested to be involved in rat Fas expression (45). Recently, STAT-1α involvement in IFN-γ-induced Fas expression in the human colon carcinoma cell line HT29 was reported (35).

To date, the transcriptional regulatory mechanism of Fas expression by TNF-α has not been reported. Within a 1-kb region of the mouse Fas promoter, five NF-κB consensus elements have been found (46). Further investigation of which NF-κB element(s) is critical for TNF-α induction of Fas and which isoforms of NF-κB are induced to bind to the elements is warranted. IFN-γ activation of STAT-1α leads to STAT-1α binding to IFN-γ activation sequence (GAS) elements in the promoter regions of IFN-γ inducible genes (for review, see Ref. 36). Interestingly, a GAS element is not present in the 1-kb promoter region of the mouse Fas gene (46). Considering the relatively late induction of Fas mRNA by IFN-γ, it is possible that transcription factors are induced/activated by STAT-1α to ultimately transcribe Fas. As well, a GAS element may be present in other regions of the Fas promoter.

In our study of the function of Fas expression on microglial cells, we observed that incubation of microglia with Fasl-bearing cells induced cell death. This Fas-mediated microglial cell death was further increased by TNF-α or IFN-γ stimulation, consistent with a previous report (26). Interestingly, microglial cell death was not observed when cells were incubated with anti-Fas Ab alone or with anti-hamster IgG Ab as a secondary Ab (data not shown). These results suggest that engagement of Fas by membrane-bound Fasl is necessary for subsequent cell death. Although the 51Cr release assay used in our study does not differentiate cell death through apoptosis vs necrosis, in conjunction with data from previous reports (16, 26), our data argue that microglia undergo Fas-mediated apoptosis by Fasl-bearing cells.

Furthermore, we have found that TGF-β exerts a strong anti-apoptotic effect by inhibiting surface Fas expression on microglia. TGF-β has differential inhibitory mechanisms on the apoptotic process depending on the cell type under study. TGF-β protects dendritic cell precursors from apoptosis by reducing Fas expression (47). In contrast, TGF-β decreases apoptosis of human T cells by inhibiting FasL expression, with no apparent effect on Fas expression (48). These findings in conjunction with the results from this study indicate that TGF-β uses different pathways to inhibit the apoptotic process. It has been reported that TNF-α or IFN-γ potentiate microglial cell death partly by down-regulating anti-apoptotic molecules, such as Bcl-2 and Bcl-xL (26). In this regard, we tested the effect of TGF-β on mRNA expression of Bcl family members, including antiapoptotic members (Bcl-w, Bcl-xL, and Bcl-2) as well as proapoptotic members (Bak, Bax, and Bad). In these experiments TGF-β slightly down-regulated mRNA expression of all of the above-mentioned Bcl family members (data not shown), indicating that TGF-β-mediated inhibition of microglial cell death does not involve down-regulation of proapoptotic molecules or up-regulation of antiapoptotic molecules. Rather, TGF-β inhibits microglial cell death by inhibiting surface Fas expression.

TGF-β is a strong immunosuppressive cytokine, and its expression is elevated in various diseases, including MS (49). In the CNS, astrocytes, microglia, and oligodendrocytes can be activated to express TGF-β (50–52). In EAE, TGF-β expression is prominent during the recovery phase (53) and has been proposed to contribute to disease recovery (54, 55). The immunosuppressive function of TGF-β on microglia has been reported in our laboratory as well as others. TGF-β inhibits IFN-γ-induced class II MHC and CD40 expression by microglia (27, 56, 57). As well, TGF-β inhibits TNF-α, IL-1, IL-6, and IL-12 production by microglia (56, 58). These results in conjunction with our findings that TGF-β inhibits cell death of microglia suggest that TGF-β expression at the recovery phase of EAE can protect microglia from cell death as well as inhibit their ability to function as an APC within the CNS. Given that microglia can exert protective effects in the CNS, such as neurotropin production (59, 60) and regeneration of neurons (61), preservation of these cells may be viewed as beneficial at certain stages of disease.

Compared with microglia, the function of Fas molecules on astrocytes has been controversial. In one report mouse astrocytes were shown to be susceptible to Fas-mediated apoptosis in vitro (17). Apoptotic astrocytes have been detected in the white matter of Thélier’s virus-infected mice as well (62). However, no apoptotic astrocytes have been detected in brains from EAE-induced mice (16). In our study we could not detect astrocyte cell death based on the 51Cr release assay (data not shown). It is not clear why astrocytes, which express high constitutive levels of Fas, are resistant to Fas-mediated cell death. Recently, it was proposed that susceptibility to Fas-mediated apoptosis of human astrocytes varies depending on the cell passage state and also on microenvironmental factors, such as the presence of cytokines (25). In that study the resistance of early passage fetal astrocytes to Fas-mediated apoptosis correlated with the expression of Fas-associated phosphatase-1 (FAP-1), a tyrosine phosphatase interacting with the C-terminal region of Fas (63). FAP-1 inhibits Fas-mediated apoptosis upon overexpression and is highly expressed in tissues and cell lines that are resistant to Fas-mediated apoptosis (64). Interestingly, we detected mRNA expression of FAP, a mouse homologue of human FAP-1, in mouse astrocytes but not in microglia (data not shown), which may explain the relative resistance of astrocytes to Fas-mediated cell death compared with microglia.

Based on our in vitro data as well as the in vivo data reported by Bonetti et al. (16), we argue that mouse astrocytes do not undergo Fas-mediated apoptosis during EAE pathogenesis. To the contrary, we propose that Fas expressed on astrocytes can facilitate inflammatory responses by inducing the expression of chemokines such as MIP-1α, MIP-1β, and MIP-2. MIP-1α and -β are members of the C-C chemokine family that function as chemoattractants primarily for monocytes and T lymphocytes (for review, see Ref. 65). MIP-2 is a functional mouse homologue of human IL-8, a potent chemoattractant for neutrophils (for review, see Ref. 65). The importance of chemokine expression in EAE induction has been previously reported (for review, see Ref. 65). In the mouse model of EAE, production of MIP-1α in the CNS correlated with development of severe clinical disease, and administration of anti-MIP-1α Ab inhibited EAE induction (66). Similarly, MIP-1β expression was detected in rat brain upon EAE induction (67). It has been noted that CNS infiltration of activated immune cells is ameliorated in EAE-induced lpr mice (14). Considering the critical role of chemokines in recruitment of activated immune cells to the CNS, it is quite possible that the lack of Fas-mediated induction of
chemokines by astrocytes is partly responsible for the resistance of lpr mice to EAE induction. Furthermore, we found that Fas-mediated signaling exerts a synergistic effect with TNF-α for MIP-1β mRNA expression. This suggests that during EAE pathogenesis, when TNF-α expression in the CNS is prominent, signaling through Fas on astrocytes can enhance MIP-1β expression. In our study we could not detect MIP-1β expression at the protein level due to the lack of a sensitive mouse MIP-1β detection system. However, in another study we have found that Ab cross-linking of Fas on human astrocytes can induce inflammatory chemokine and cytokine expression at both the mRNA and protein levels (C. Choi, X. Xu, J. W. Oh, S. J. Lee, and E. N. Benveniste, manuscript in preparation). To date, CNS-infiltrating activated T cells, microglia, and astrocytes have been reported to express FasL (16, 68, 69). However, it is not clear which cell type(s) contributes to the ongoing inflammatory response by engaging Fas on astrocytes in vivo. A preliminary analysis of the signaling cascades activated by Fas ligation on astrocytes suggests that the MAPK pathway is involved. Future studies on the signaling pathways activated through Fas on astrocytes may enable us to interrupt Fas-facilitated inflammatory responses in the CNS.

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