Archivum Immunologiae et Therapiae Experimentalis, 2000, **48**, 381–388
PL ISSN 0004-069X

Review

Evidence that Fas and FasL Contribute to the Pathogenesis of Experimental Autoimmune Encephalomyelitis

BONNIE N. DITTEL*

Section of Immunobiology, Yale School of Medicine, New Haven, CT, USA

Abstract. The well established and characterized animal model for the human demyelinating autoimmune disease multiple sclerosis (MS) is known as experimental autoimmune encephalomyelitis (EAE)[29]. EAE is clinically characterized by focal areas of inflammation and demyelination and an infiltrate composed of large numbers of lymphocytes and macrophages, often found in a perivascular localization but also throughout the central nervous system (CNS)[24]. Active immunization of mice with several different protein components of myelin, including myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG), are capable of eliciting an immune response resulting in the quintessential symptoms of EAE: ascending paralysis involving the tail and then the limbs. Depending on the mouse strain and myelin antigen utilized, the disease course can be acute or chronic relapsing, characterized by a rapid onset of hind limb weakness that commonly progresses to paralysis, followed by spontaneous remission starting 7–10 days after the initial appearance of symptoms. EAE can also be induced passively by the adoptive transfer of *in vitro* activated $CD4^+$ T cell clones or lines, typically of the Th1 phenotype, into irradiated susceptible recipients[2]. The mechanisms involved in the cellular pathogenesis leading to paralysis and demyelination have been extensively studied and are primarily mediated by $CD4^+$ T cells of the Th1 phenotype, with specificity for myelin antigens. Following activation, Th1 CD4 T cells produce in abundance the inflammatory cytokines TNF-α, IFN-γ and lymphotoxin α (LT-α, also know as TNF-β). IFN-γ production is highly correlated with encephalitogenicity and may contribute to disease by up-regulation of adhesion molecules on endothelial cells, facilitating migration of lymphocytes into the CNS; by induction of major histocompatibility complex (MHC) class I and MHC class II molecules on astrocytes, microglial cells and brain endothelium, facilitating antigen (Ag) presentation in the CNS; and by activation of macrophages, leading to production of nitric oxide, a potent cytotoxic molecule. TNF-α and LT-α are both members of the TNF family of molecules and cause cell death by apoptosis following interaction with their counter-receptors, the TNFR1 and TNFR2, leading to a cascade of proteolytic events culminating in the blebbing of the cytoplasmic membrane, nuclear condensation and DNA fragmentation[5, 26]. Consequently, the production of TNF-α and LT-α by Th1 clones has been correlated with encephalitogenic potential[30] and antibodies (Abs) to both prevents EAE upon transfer of encephalitogenic clones[31]. Even though substantial evidence exists for the role of inflammatory cytokines in the pathogenesis of EAE, other mechanisms of myelin destruction are thought to exist. To date, many reports have implicated a role for the cell death-inducing ligand pair Fas and Fas-ligand (FasL).

Key words: Fas; Fas ligand; experimental autoimmune encephalomyelitis.

* Correspondence to: Dr Bonnie N. Dittel, Blood Research Institute, P. O. Box 2178, Milwaukee, WI 53201-2178, USA, tel.: +1 414 937 38 65, fax: +1 414 937 62 84, e-mail: bdittel@bcsew.edu

Fas and FasL in CD4$^+$ T Cell-Mediated Cellular Cytotoxicity

In addition to effector functions of CD4$^+$ T cells mediated through the production of cytokines, CD4$^+$ T cells also have the capacity to mediate the cytotoxicity of appropriate targets. This cytotoxicity was recently shown to be primarily mediated by the interaction of Fas on the target cell with the FasL on the CD4$^+$ T cell, leading to the apoptotic cell death of the Fas-expressing cell[1, 15, 17, 18, 22]. Fas (APO-1, CD95) is a member of the TNF-receptor superfamily[19] and was first identified with the use of a monoclonal antibody (mAb), which induced apoptosis upon binding to some tumor cells[39, 42]. FasL has also recently been cloned and is a type II membrane protein with homology to members of the TNF family[37]. Resting CD4$^+$ T cells do not express FasL on the cell surface, but activation either by antigen (peptide/MHC class II) or nonspecifically with anti-CD3, ConA or PMA/ionomycin leads to the rapid appearance of cell surface FasL[13, 15–17, 36]. Using Th1, Th2 and Th0 clones bearing identical T cell receptors (TCR)[10], we were able to demonstrate up-regulation of FasL following stimulation with PMA/ionomycin on all three types of Th clones (Fig. 1). Our findings are consistent with other reports demonstrating that some Th2 clones can express FasL (Fig. 1)[15, 16, 36]. Although cytotoxicity mediated by Fas/FasL is primarily a function of Th1 and Th0 cells, several reports have described killing by Th2 cells[21, 36]. Utilizing the same Th0, Th1 and Th2 clones, we demonstrate antigen-specific killing of a target cell by all three clones (Fig. 2). At least one

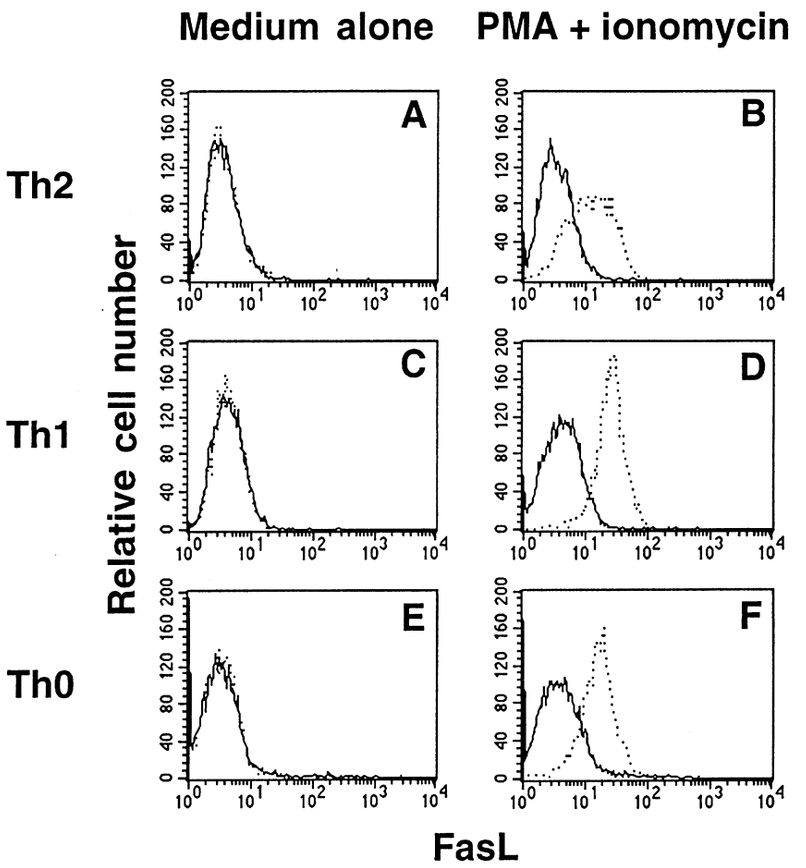

Fig. 1. Expression of FasL after activation with PMA/ionomycin and antigen stimulation. Expression of cell surface FasL was analyzed by a three-step indirect immunofluorescence staining and flow cytometry. The D10. TCR Th2 (A, B), Th1 (C, D) and Th0 (E, F) clones were incubated with anti-FasL (10 μg/ml) (PharMingen, San Diego, CA) followed by a second incubation with anti-mouse IgG-biotin and concluded with a third incubation with strepavidin-PE. Antibody incubations were carried out on ice with two washes following each step and the cells were fixed in 1% paraformaldehyde and analyzed using CellQuest on a FACScan® (Becton Dickinson, Mountain View, CA). Negative controls consisted of cells incubated with anti-mouse IgG-biotin followed by strepavidin-PE. Expression of the FasL was measured after a 6-hour incubation at 37°C with medium alone or in the presence of PMA (10 ng/ml) and ionomycin (1 mM) in six-well microtiter plates. Histograms represent fluorescence intensity on the horizontal axis and relative cell number on the vertical axis. The background staining is depicted in each histogram as a solid line and FasL specific staining is shown as a dotted line

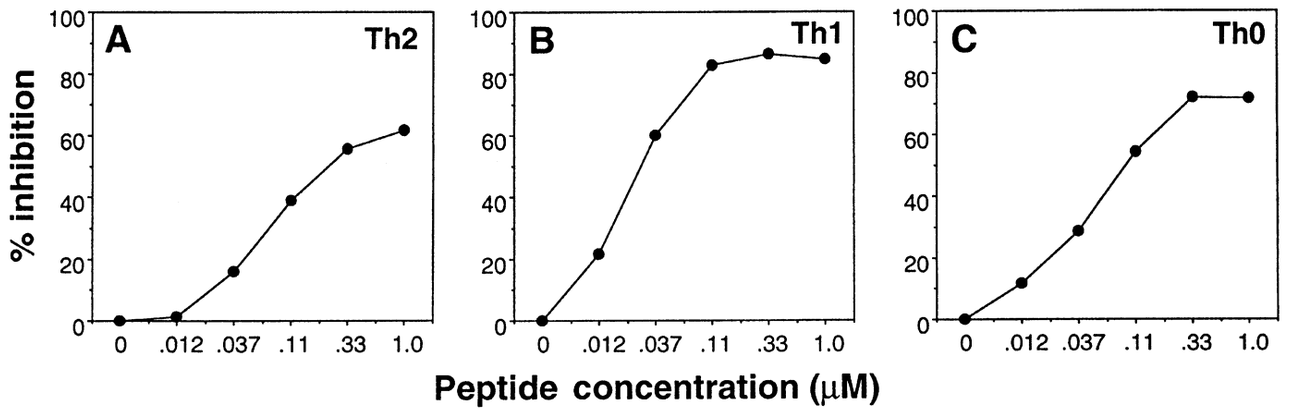

Fig. 2. Antigen specific cytotoxicity by the D10. TCR Th2, Th1 and Th0 clones stimulated with the antigenic peptide CA134-146 from the antigen hen egg conalbumin to which the D10. TCR is restricted in the context of I-A^k. Mitomycin-c inactivated D10. TCR clones were cocultured with CH27 cells (I-A^k) in the presence of 1:3 dilutions of CA134-146 from 0.012 to 1 μM. After 16–18 h the cultures were pulsed with 1 μCi [^{3}H] TdR and harvested after an additional 4 h of incubation. Data for the Th2 clone is shown in A, for the Th1 clone in B and for the Th0 clone in C. Data is presented as % inhibition of CH27 growth calculated as follows: (1 – CPM in presence of peptide: CPM in absence of peptide) × 100]

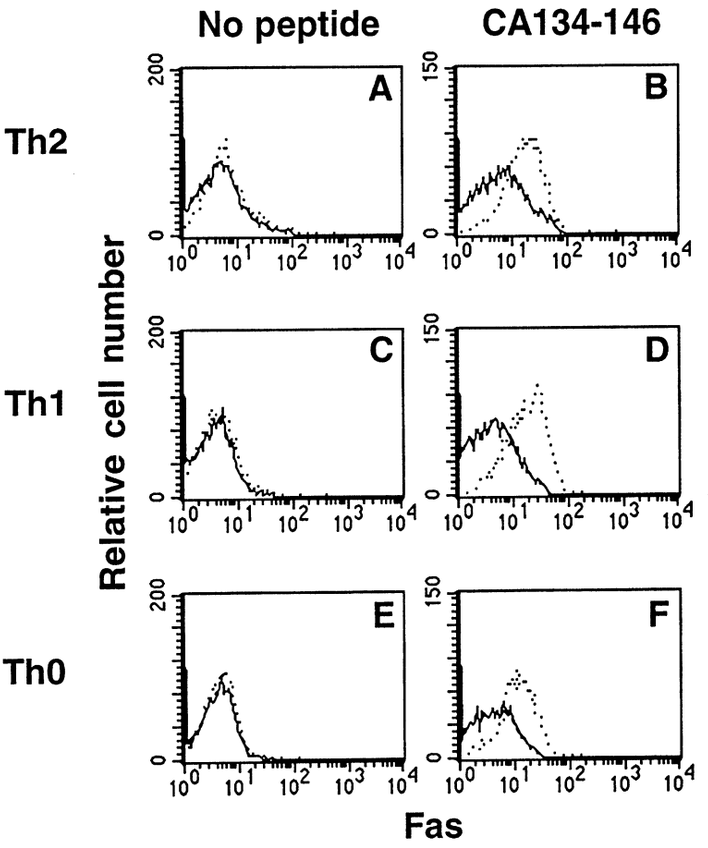

Fig. 3. Up-regulation of cell surface Fas following antigen stimulation. The D10. TCR Th2 (A, B), Th1 (C, D) and Th0 (E, F) clones were stimulated with 100 μM CA 134-146 (second column) or with medium alone (first column) using B10.BR spleen cells as APC. After 48 h, Fas was detected by direct immunofluorescence with anti-mouse Fas-PE (PharMingen, San Diego, CA). Histograms represent fluorescence intensity on the horizontal axis and relative cell number on the vertical axis. The background staining is depicted in each histogram as a solid line and Fas specific staining is shown as a dotted line

report has shown that Th2 cells can be encephalitogenic under specific conditions[20]. Thus, although EAE is primarily thought to be a consequence of pathogenicity mediated by Th1 cells, the role for Th2 cells either in pathogenicity, or in protection/recovery due to production of Th2 cytokines, such as IL-4 and IL-10, has yet to be firmly established[7, 14].

The potential importance for Fas/FasL-mediated cell death in the regulation of homeostasis within the immune system is demonstrated by dysregulation of T cell populations leading to lymphadenopathy, splenomegaly and autoimmune disorders[22, 26]. In addition to T cells killing target cells in an antigen-specific manner, several reports have shown that activated Th1 cells expressing FasL can lyse Fas-expressing targets in the absence of TCR ligation[13, 16, 36]. This nonspecific killing has been shown by several groups to include fratricidal T cell killing[16, 35] and T cell suicide[4, 8, 16, 22]. Since Fas is expressed by both Th1 and Th2 cells (Fig. 3), self-reactive T cell populations in EAE could potentially be controlled either in the periphery or in the CNS by promoting death of Th1 or Th2 cells following up-regulation of FasL, leading to cell death by fratricide and/or suicide.

Fas Expression in the CNS is Important for the Development of EAE

The role of Fas/FasL interactions in EAE have been facilitated by the presence of mice carrying autosomal recessive mutations in the genes encoding Fas (lpr) and FasL (gld)[26]. Fas is disrupted in lpr mice by an endogenous retrovirus insertion carrying a poly(A) adenylation signal on the long terminal repeats, leading to truncation of the mRNA[26]. In gld mice, the gene encoding FasL has a point mutation in the cytoplasmic region of the protein that disrupts interaction with Fas[26]. Several independent reports suggested a role for the cell death-inducing molecules Fas and FasL in the pathogenesis of EAE[23, 32, 40]. In these studies, mice deficient in Fas or FasL were immunized with myelin antigens and assessed for clinical signs of EAE. The study by SABELKO et al.[32] utilized guinea pig (gp) MBP-immunized B10.PL-lpr mice and showed almost complete protection from the paralytic clinical symptoms of EAE. Of interest was the similar T cell infiltrate observed in the spinal cords of Fas-deficient mice and wild-type (wt) mice, suggesting that Fas and FasL do not contribute to the process of T cell migration into the CNS. Several groups investigated active EAE induction using MOG35-55 in C57BL/6 mice carrying the lpr or gld mutations with contrasting results. WALDNER et al.[40] reported almost complete protection for EAE in both B6-gld and B6-lpr mice, with wt mice exhibiting a relapsing-remitting disease course. Similarly, a report by OKUDA et al. [28] also showed protection in C57BL/6-lpr mice immunized with MOG35-55. In contrast, the study performed by MALIPIERO et al.[23] showed only partial protection in disease severity, with lpr and gld mice exhibiting a monophasic disease course and the wt mice exhibiting a chronic disease course. The discrepancies in these two studies may be reflected by the more aggressive disease induction protocol employed by the Malipiero group, using a higher concentration of pertussis toxin and boosting with Ag 1 week following primary Ag challenge. The protection reported by the Waldner group may partially reflect disease induced in older male mice that are less susceptible to disease than female mice.

The relative role of Fas/FasL in the pathogenesis of EAE seems to be affected either by the antigen used or by the strain of mouse the experiments are being carried out on, or both. This is evident from a recent study by SUVANNAVEJH et al.[38] in which EAE induced in SJL mice deficient in Fas following active immunization with a peptide from PLP resulted in chronic progressive disease of greater severity as compared to the relapsing-remitting disease course observed in their heterozygote littermates. Although no differences were observed in the acute phase of disease, the chronic disease in the SJL-lpr mice was accompanied by an increase in mononuclear cells infiltrating the spinal cord[38]. These data suggest that Fas/FasL play a role in recovery from EAE in the SJL mouse, presumably by eliminating the self-reactive T cell population in the CNS.

The active induction of EAE in gld or lpr mice as performed in the above studies does not definitively reveal the cell populations in which the presence of Fas and FasL are important in EAE. To test whether Fas and FasL expression are important on the T cell or in the CNS, we performed adoptive transfer experiments to passively induce EAE, targeting the deficiency in Fas and FasL to the transferred T cells, the recipient or both[9]. These experiments revealed that B10.PL-lpr mice deficient in Fas were protected against EAE induction following adoptive transfer of an activated Th1 T cell line generated from a TCR transgenic mouse specific for a peptide of MBP comprised of the first 11 amino acids (Ac1-11). In comparison, B10.PL mice adoptively transferred with the same T cell line succumbed to disease with symptoms appearing around day 8 and peaking around day 15 with an acute mono-

phasic disease course characterized by hind limb paresis and paralysis[9]. Recovery from disease symptoms was observed around day 24 following T cell transfer[9]. We attribute the protection observed in the Fas deficient recipients to a blockage of Fas-induced cell death because of the absence of functional Fas expression in the CNS. This conclusion is supported by the reverse experiment, showing no disease protection when Fas-deficient T cells are transferred into wt recipients. Thus, protection was only observed when Fas was deficient in the CNS. The role for Fas expression in the CNS in EAE was also demonstrated by SABELKO-DOWNES et al.[33] using similar adoptive transfer procedures.

Surprisingly, we saw no protection when Fas-deficient T cells were transferred into Fas-deficient recipients[9]. We attribute this result to the loss of homeostatic regulation of the transferred T cell population by Fas and FasL[34]. Thus, the absence of Fas on the transferred T cell population may disrupt cell death of the activated T cells, allowing the T cells to accumulate in sufficient numbers to cause EAE by a mechanism that is clearly unrelated to Fas/FasL. To examine the possibility that the lack of protection is due to accumulation of the Fas-deficient T cells, we measured the percentage of transgenic T cells in the $CD4^+$ T cell population in the spleens and lymph nodes 7 and 14 days following adoptive transfer (Table 1). On day 7 following transfer of wt T cells into Fas-deficient recipients, 26% of the splenic $CD4^+$ T cell population were transgenic. This number was reduced to 16% when the T cells were transferred into an lpr recipient. These data suggest that the transferred T cell population is eliminated in the periphery, perhaps by host Fas-deficient lymphocytes, which have been reported to express enhanced levels of FasL[6]. In contrast, the transfer of Fas-deficient T cells into either wt or lpr mice resulted in elevated numbers of transgenic T cells, with more than 80% of the $CD4^+$ splenocytes being of donor origin (Table 1). As shown in Table 1, similar results were observed in lymph node CD4 T cell populations. Interestingly, on day 14 the percentage of transgenic T cells in the mice that received wt T cells increased, while the mice transferred with lpr T cells had reduced numbers of transgenic T cells. Overall, these data suggest that the lack of protection following transfer of lpr T cells into lpr recipients may partially be due to the accumulation of T cells in the periphery, generating a greater pool of T cells with encephalitogenic potential.

Interestingly, in the study by SABELKO-DOWNES et al.[33], protection from EAE was observed when lpr T cells were transferred into lpr recipients. This group also examined preferential elimination of transferred wt T cells into lpr recipients and saw no difference as compared to controls on days 3 and 5[33]. However, to conduct this analysis the authors used allogeneic T cells and did not assess the levels of Fas on the cell surface of the transferred T cell population. The discrepancy between our study and that of SABELKO-DOWNES et al.[33] could be attributed to the differences in generation of the T cell lines. In our study, the T cells were fully differentiated Th1 effector cells, having been rested for 7–10 days before reactivation and transfer[9]. In the SABELKO-DOWNES et al.[33] study, the T cells were stimulated with antigen for 4 days before transfer with no rest. This activation protocol may have been too short in duration to allow differentiation of the naïve T cells into polarized Th1 effectors cells. In addition, the authors did not analyze the cytokine profiles of the transferred cells. In our study, we showed that the transferred T cell populations expressed high levels of the Th1 inflammatory cytokines IFN-γ, TNF-α and LT-α and low levels of the Th2 cytokines IL-4, IL-5 and IL-10[9]. If high levels of IL-4 and IL-10 were produced by the transferred T cell population in the SABELKO--DOWNES et al.[33] study, this could have contributed to protection, as both these cytokines have been implicated in protection from EAE.

Table 1. Percentage of $CD4^+$ cells that express the MBP TCR transgene in the spleen and lymph nodes of B10.PL and B10.PL-1pr mice adoptively transferred with MBP-wt or MBP-lpr T cells

Adoptive transfer[a]	Day 7		Day 14	
	spleen (%)	lymph node[b] (%)	spleen (%)	lymph node (%)
MBP-wt into B10.PL	26[c]	52	31	25
MBP-wt into lpr	16	35	28	26
MBP-lpr into B10.PL	81	83	37	37
MBP-lpr into lpr	83	92	40	43

[a] 1 x 10^6 $CD4^+$ T cells isolated from MBP TCR transgenic mice with or without the lpr mutation were i. v. transferred into irradiated (600 rads) B10.PL or B10.PL-lpr mice 4 days following antigen specific activation.

[b] Lymph node cells include cells from the popliteal, auxiliary and inguinal lymph nodes.

[c] The percentage of total $CD4^+$ T cells isolated from the spleen or lymph nodes of B10.PL or B10.PL-lpr recipient mice that express the MBP TCR as assessed by staining with a mAb specific for Vβ8.2, the TCRβ chain expressed by the MBP TCR.

FasL Expression on the Encephalitogenic T Cell Population is Important in the Regulation of EAE

We also examined the role of FasL in the CNS and on the transferred T cell population. In agreement with SABELKO-DOWNES et al.[33], we found that the expression of FasL on the transferred T cell population is important for the development of EAE. This was demonstrated by protection from EAE when FasL was deficient on the transferred T cells[9, 33], while no protection was observed when wt T cells were transferred into gld recipients, of interest is the enhanced disease observed under these experimental conditions[9, 33]. We observed this enhancement as an earlier onset of disease and a longer, more severe disease course, suggesting that FasL expression in the recipient animal is involved in the regulation of the encephalitogenic T cell population[9]. This hypothesis was more fully examined by SABELKO-DOWNES et al.[33], who also observed more severe EAE of longer duration in gld recipients by examining spinal cord infiltrates in wt and gld mice. Infiltrates during the early/acute phase of disease (day 23) were similar in the two groups of mice[33]. However, differences were observed during the late/chronic stage of disease, with diminished infiltration in wt mice as compared to clusters of $CD4^+$ cells observed in the gld recipients[33]. The observation of groups of T cells remaining late after transfer suggests that the FasL deficient host is unable to effectively eliminate the infiltrating encephalitogenic T cells.

Although Fas/FasL have been shown to be involved in the pathogenesis of EAE, no role for either molecule has been demonstrated in the migration of T cells into the CNS. This was demonstrated following active induction of EAE in lpr mice where disease was suppressed, but the infiltration observed into the CNS 10 days following immunization was comparable to wt mice[32]. However, reduced levels of apoptotic cells were observed in the lesions of the lpr mice, again suggesting a role for Fas in the apoptosis of cells in the CNS. The cells undergoing apoptosis in the CNS have yet to be definitively identified, but a logical target cell would be the myelin-producing oligodendrocyte. Fas expression has been generally demonstrated in the CNS in patients with MS[11] and specifically on the oligodendrocyte population[12].

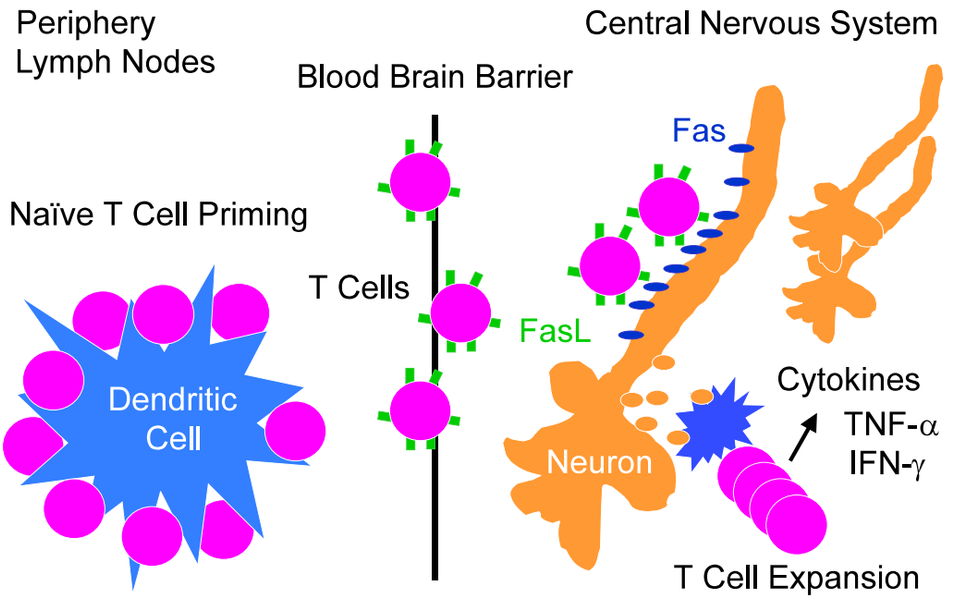

Fig. 4. Model for the role of Fas and FasL in the pathogenesis of EAE in the CNS. Priming of naïve $CD4^+$ T cells (light purple) with self-antigen in the peripheral lymph nodes by dendritic cells as antigen-presenting cells results in activated Th1 T cells expressing FasL (green) with the capacity to migrate across the blood-brain barrier. The first self-antigen-specific T cells to enter the CNS secrete inflammatory cytokines, up-regulating adhesion molecules and MHC class II molecules on endothelium and resident microglial cells in the CNS and potentially Fas (blue) on oligodendrocytes. The self-antigen-specific T cells expressing FasL interact with Fas expressed by oligodendrocytes that form a layer of myelin (orange) around the neuronal axon, causing cell death of the oligodendrocytes. Myelin antigens released following the apoptotic death of oligodendrocytes are taken up by antigen-presenting cells (possibly microglia), processed and presented to the infiltrating T cells. The T cells are reactivated, resulting in renewed proliferation leading to an expansion of Th1 T cells, allowing for local accumulation of the Th1 cytokines (TNF-α, IFN-γ and LT-α) at high levels. This local accumulation of cytokine production leads to a full-blown inflammatory response and could also result in further apoptotic cell death of oligodendrocytes through the TNFRs

Conclusion

The evidence accumulating from multiple laboratories using different mouse strains, thus utilizing different myelin antigens, has clearly demonstrated a role for Fas/Fas ligand not only in the pathogenesis of EAE in the CNS, but in the regulation of the encephalitogenic T cell population as well. In keeping with the subtle differences in the data from various laboratories, a mechanism for the role of Fas and FasL in EAE is shown in Fig. 4. As shown in Fig. 4, the early pathogenesis in the CNS is most likely mediated by a small number of $CD4^+$ T cells that express FasL. We found that the adoptive transfer of a large number of T cells could circumvent the protection observed in Fas-deficient mice[41]. FasL expressed by activated T cells infiltrating the CNS would interact with Fas on oligodendrocytes, presumably up-regulated by IFN-γ[25, 26] produced by the Th1 encephalitogenic T cells, leading to apoptotic death of oligodendrocytes. This early cell destruction would then lead to a full inflammatory response and the recruitment of additional autoreactive T cells, macrophages and other cell types to the site of immune attack, forming an inflammatory lesion. The ensuing myelin destruction would provide ample Ag for the continued activation and expansion of the autoreactive T cell pool. As the autoreactive Th1 cells accumulate, the local production of the pathogenic Th1 cytokines, IFN-γ, TNF-α and LT-α, would also increase, resulting in continued apoptotic death of oligodendrocytes and demyelination. Macrophages in the lesion may contribute to oligodendrocyte destruction by the production of nitric oxide[3]. The escalating inflammatory response could then be dampened by any number of proposed mechanisms, including T cell apoptosis by Fas/FasL and the emergence of a Th2 response[27].

References

1. ASHANY D., SONG X., LACY E., NIKOLIC-ZUGIC J., FRIEDMAN S. M. and ELKON K. B. (1995): Th1 $CD4^+$ lymphocytes delete activated macrophages through the Fas/APO-1 antigen pathway. Proc. Natl. Acad. Sci. USA, **92**, 11225–11229.
2. BARON J. L., MADRI J. A., RUDDLE N. H., HASHIM G. and JANEWAY C. A. JR. (1993): Surface expression of α4 integrin by CD4 T cells is required for their entry into brain parenchyma. J. Exp. Med., **177**, 57–68.
3. BOGDAN C. (1998): The multiplex function of nitric oxide in (auto)immunity. J. Exp. Med., **187**, 1361–1365.
4. BRUNNER T., MOGIL R. J., LA FACE D., YOO N. J., MAHBOUBI A., ECHEVERRI F., MARTIN S. J., FORCE W. R., LYNCH D. H., WARE C. F. and GREEN D. R. (1995): Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. Nature, **373**, 441–444.
5. CLEMENT M.-V. and STAMENKOVIC I. (1994): Fas and tumor necrosis factor receptor-mediated cell death: similarities and distinctions. J. Exp. Med., **180**, 557–567.
6. CHU J. L., RAMOS P., ROSENDORFF A., NIKOLIC-ZUGIC J., LACY E., MATSUZAWA A. and ELDON K. B. (1995): Massive upregulation of the Fas ligand in lpr and gld mice: implications for Fas regulation and the graft-versus-host disease-like wasting syndrome. J. Exp. Med., **181**, 393–398.
7. CUA D. J., HINTON D. R. and STOHLMAN S. A. (1995): Self--antigen-induced Th2 responses in experimental allergic encephalomyelitis (EAE)-resistant mice. Th2-mediated suppression of autoimmune disease. J. Immunol., **155**, 4052–4059.
8. DHEIN J., WALCZAK H., BAUMLER C., DEBATIN K.-M. and KRAMMER P. H. (1995): Autocrine T-cell suicide mediated by APO-1/(FAS/CD95). Nature, **373**, 438–441.
9. DITTEL B. N., MERCHANT R. M. and JANEWAY C. A. JR. (1999): Evidence for Fas-dependent and Fas-independent mechanisms in the pathogenesis of experimental autoimmune encephalomyelitis. J. Immunol., **162**, 6392–6400.
10. DITTEL B. N., SANT' ANGELO D. B. and JANEWAY C. A. JR. (1997): Peptide antagonists inhibit proliferation and the production of IL-4 and/or IFN-gamma in T helper 1, T helper 2, and T helper 0 clones bearing the same TCR. J. Immunol., **158**, 4065–4073.
11. DOWLING P., SHANG G., RAVAL S., MENONNA J., COOK S. and Husar W. (1996): Involvement of the CD95 (APO-1/Fas) receptor/ligand system in multiple sclerosis brain. J. Exp. Med., **184**, 1513–1518.
12. D'SOUZA S. D., BONETTI B., BALASINGAM K. B., CASHMAN N. R., BARKER P. A., TROUTT A. B., RAINE C. S. and ANTEL J. P. (1996): Multiple sclerosis: Fas signaling in oligodendrocyte cell death. J. Exp. Med., **184**, 2361–2370.
13. EL-KHATIB M., STANGER B. Z., DOGAN H., CUI H. and JU S.-T. (1995): The molecular mechanism of FasL-mediated cytotoxicity by $CD4^+$ Th1 clones. Cell. Immunol., **163**, 237–244.
14. FALCONE M. and BLOOM B. R. (1997): A T helper cell 2 (Th2) immune response against non-self antigens modifies the cytokine profile of autoimmune T cells and protects against experimental allergic encephalomyelitis. J. Exp. Med., **185**, 901–907.
15. HAHN S., GEHRI R. and ERB P. (1995): Mechanism and biological significance of CD4-mediated cytotoxicity. Immunol. Rev., **146**, 57–79.
16. HAHN S., STALDER T., WERNLI M., BURGIN D., TSCHOPP J., NAGATA S. and ERB P. (1995): Down-modulation of $CD4^+$ T helper type 2 and type 0 cells by T helper type 1 cells via Fas/Fas-ligand interaction. Eur. J. Immunol., **25**, 2679–2685.
17. HANABUCHI S., KOYANAGI M., KAWASAKI A., SHINOHARA N., MATSUZAWA A., NISHIMURA Y., KOBAYASHI Y., YONEHARA S., YAGITA H. and OKUMURA K. (1994): Fas and its ligand in a general mechanism of T-cell-mediate cytotoxicity. Proc. Natl. Acad. Sci. USA, **91**, 4930–4934.
18. HENKART P. A. (1994): Lymphocyte-mediated cytotoxicity: Two pathways and multiple effector molecules. Immunity, **1**, 343–346.
19. ITOH N., YONEHARA S., ISHII A., YONEHARA M., MIZUSHIMA S., SAMESHIMA M., HASE A., SETO Y. and NAGATA S. (1991): The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell, **66**, 223–243.
20. LAFAILLE J. J., VAN de KEERE F., HSU A. L., BARON J. L., HAAS W., RAINE C. S. and TONEGAWA S. (1997): Myelin basic

protein-specific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease. J. Exp. Med., **186**, 307–312.
21. LANCKI D. W., HSIEH C. S. and FITCH F. W. (1991): Mechanisms of lysis by cytotoxic T lymphocyte clones. Lytic activity and gene expression in cloned antigen-specific $CD4^+$ and $CD8^+$ T lymphocytes. J. Immunol., **146**, 3242–3249.
22. LYNCH D. H., RAMSDELL F. and ALDERSON M. R. (1995): Fas and FasL in the homeostatic regulation of immune responses. Immunol. Today, **16**, 569–574.
23. MALIPIERO U., FREI K., SPANAUS K.-S., AGRESTI C., LASSMANN H., HAHNE M., TSCHOPP J., EUGSTER H.-P. and FONTANA A. (1997): Myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis is chronic/relapsing in perforin knockout mice, but monophasic in Fas- and Fas ligand-deficient lpr and gld mice. Eur. J. Immunol., **27**, 3151–3160.
24. MARTIN R. and MCFARLAND H. F. (1995): Immunological aspects of experimental allergic encephalomyelitis and multiple sclerosis. Crit. Rev. Clin. Lab. Sci., **32**, 121–182.
25. NAGATA S. and GOLSTEIN P. (1995): The Fas death factor. Science, **267**, 1449–1456.
26. NAGATA S. and SUDA T. (1995): Fas and Fas ligand: lpr and gld mutations. Immunol. Today, **16**, 39–43.
27. O'GARRA A., STEINMAN L. and GIJBELS K. (1997): $CD4^+$ T-cell subsets in autoimmunity. Curr. Opin. Immunol., **9**, 872–883.
28. OKUDA Y., BERNARD C. C. A., FUJIMURA H., YANAGIHARA T. and SAKODA S. (1998): Fas has a crucial role in the progression of experimental autoimmune encephalomyelitis. Mol. Immumol., **35**, 317–326.
29. OLSSON T. (1995): Critical influences of the cytokine orchestration or the outcome of myelin antigen-specific autoimmunity in experimental autoimmune encephalomyelitis and multiple sclerosis. Immunol. Rev., **144**, 245–268.
30. POWELL M. B., MITCHELL D., LEDERMAN J., BUCKMEIER J., ZAMVIL S. S., GRAHAM M., RUDDLE N. H. and STEINMAN L. (1990): Lymphotoxin and tumor necrosis factor-α production by myelin basic protein-specific T cell clones correlates with encephalitogenicity. Int. Immunol., **2**, 539–554.
31. RUDDLE N. H., BERGMAN C. M., MCGRATH K. M., LINGENHELD E. G., GRUNNET M. L., PADULA S. J. and CLARK R. B. (1990): An antibody to lymphotoxin and tumor necrosis factor prevents transfer of experimental allergic encephalomyelitis. J. Exp. Med., **172**, 1193–1200.
32. SABELKO K. A., KELLY K. A., NAHM M. H., CROSS A. H. and RUSSELL J. H. (1997): Fas and Fas ligand enhance the pathogenesis of experimental allergic encephalomyelitis, but are not essential for immune privilege in the central nervous system. J. Immunol., **159**, 3096–3099.
33. SABELKO-DOWNES K. A., CROSS A. H. and RUSSELL J. H. (1999): Dual role for Fas ligand in the initiation of and recovery from experimental allergic encephalomyelitis. J. Exp. Med., **189**, 1195–1205.
34. SINGER G. G., CARRERA A. C., MARSHAK-ROTHSTEIN A., MARTÍNEZ C. and ABBAS A. K. (1994): Apoptosis, Fas and systemic autoimmunity: the MRL-lpr/lpr model. Curr. Opin. Immunol., **6**, 913–920.
35. SU M. W.-C., WALDEN P. R. and EISEN H. N. (1993): Peptide-induced destruction of $CD8^+$ cytotoxic T lymphocytes is due to fratricide. J. Immunol., **151**, 658–667.
36. SUDA T., OKAZAKI T., NAITO Y., YOKOTA T., ARAI N., OZAKI S., NAKAO K. and NAGATA S. (1995): Expression of the Fas ligand in cells of T cell lineage. J. Immunol., **154**, 3806–3813.
37. SUDA T., TAKAHASHI T., GOLSTEIN P. and NAGATA S. (1993): Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. Cell, **75**, 1169–1178.
38. SUVANNAVEJH G. C., DAL CANTO M. C., MATIS L. A. and MILLER S. D. (2000): Fas-mediated apoptosis in clinical remissions of relapsing experimental autoimmune encephalomyelitis. J. Clin. Invest., **105**, 223–231.
39. TRAUTH B. C., KLAS C., PETERS A. M. J., MATZKU S., MOLLER P., FLAK W., DEBATIN K. M. and KRAMMER P. H. (1989): Monoclonal antibody-mediated tumor regression by induction of apoptosis. Science, **245**, 301–305.
40. WALDNER H., SOBEL R. A., HOWARD E. and KUCHROO V. K. (1997): Fas- and FasL-deficient mice are resistant to induction of autoimmune encephalomyelitis. J. Immunol., **159**, 3100–3103.
41. WONG F. S., DITTEL B. N. and JANEWAY C. A. JR. (1999): Transgenes and knockout mutations in animal models of type 1 diabetes and multiple sclerosis. Immunol. Rev., **169**, 93–100.
42. YONEHARA S., ISHII A. and YONEHARA M. (1989): A cell-killing monoclonal antibody (anti-fas) to a cell surface antigen co-downregulated with the receptor of tumor necrosis factor. J. Exp. Med., **169**, 1747–1756.

Received in March 2000
Accepted in April 2000