Massive Apoptosis in Lymphoid Organs in Animal Models for Primary and Secondary Progressive Multiple Sclerosis

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The mechanism(s) responsible for generating the different forms of multiple sclerosis, primary progressive (PP) and secondary progressive (SP) versus relapsing-remitting (RR), is not well understood. Using myelin oligodendrocyte glycoprotein (MOG)₉₂₋₁₀₆, we have established animal models that mimic the different types of multiple sclerosis. A.SW mice develop PP or SP-experimental allergic encephalomyelitis (EAE) with large areas of demyelination and high titers of MOG antibody whereas SJL/J mice develop RR-EAE with perivascular T cells and mild demyelination. In A.SW progressive EAE, we found atrophy of the thymus, spleen, and lymph nodes with depletion of T and B cells and massive apoptosis, as demonstrated by immunohistochemistry, terminal dUTP nick-end labeling, and DNA agarose gel electrophoresis. To test whether lymphoid apoptosis itself contributes to disease progression, we injected SJL/J mice with apoptotic thymocytes. Injection of apoptotic cells resulted in greater than 20% of mice developing SP-EAE with ataxia. SJL/J mice with SP-EAE had large areas of demyelination, high MOG antibody titers and atrophic lymphoid organs. Spleen cells from mice with progressive EAE produced less interferon- γ than those from RR-EAE when stimulated with mitogen. We suggest that induction of lymphoid apoptosis alters the balance of Th1 versus Th2 immune responses and increases MOG antibody production, leading to exacerbation of demyelination and subsequent disease progression. (*Am J Pathol* 2005, 167:1631–1646)

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS).¹ The spectrum of clinical disease for MS is diverse. Based on either the presence or absence of relapses and remissions or

progression of neurological deficits, the clinical course of MS can be categorized into four forms: relapsing-remitting (RR), primary progressive (PP), secondary progressive (SP), and progressive relapsing (PR).² RR-MS is defined by relapses with full recovery or with sequelae. In contrast, PP-MS continuously progresses from the onset. RR disease is often followed by progression (SP-MS). Because the clinical, epidemiological, immunological, and pathological findings in PP-MS are notably different from those described for other forms of MS,^{3,4} the question arises whether PP-MS and RR-MS are two distinct disease entities. Magnetic resonance imaging studies indicate that compared to RR-MS and SP-MS, PP-MS patients experience 1) fewer gadolinium-enhancing and T2-weighted magnetic resonance imaging lesions, 2) more abnormalities in the normal appearing white matter, and 3) more atrophy of the spinal cord.^{5,6} Less inflammation is also seen in PP-MS as shown by a pathological study.⁷ However, the mechanism(s) of these differences and transition between the forms are not well understood.

Myelin oligodendrocyte glycoprotein (MOG) is a minor component of CNS myelin and is expressed on the myelin sheath and on the surface of oligodendrocyte(s).⁸ Using the encephalitogenic peptide MOG₉₂₋₁₀₆, we have established animal models that mimic different forms of MS in two strains of *H-2^s* mice, SJL/J and A.SW.⁹ We induced experimental allergic encephalomyelitis (EAE) in the presence or absence of supplemental *Bordetella pertussis*. Although SJL/J mice developed RR-EAE whether *B. pertussis* was given or not, A.SW mice developed PP-EAE without *B. pertussis* supplementation and SP-EAE with *B. pertussis* supplementation. These models indicate that a single encephalitogen can induce RR-, PP-, or SP- forms of demyelinating disease in hosts with immunologically different humoral immune responses. Histologically, SJL/J mice developed mild perivascular

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demyelinating disease with T-cell infiltration, whereas A.SW mice developed large plaque-like areas of demyelination with immunoglobulin (Ig) deposition and neutrophil infiltration, but with minimal T-cell infiltration. In A.SW mice with PP-EAE, high titers of circulating MOG antibody were detected and the anti-MOG IgG2a/IgG1 ratio correlated with survival periods of the mice.⁹ These results suggest that, in progressive EAE, production of myelinotoxic antibodies could lead to progressive forms of disease with early mortality. Interestingly, it was noted that mice with progressive EAE had large numbers of apoptotic cells in certain lymphoid organs.

Apoptotic elimination of immune cells is important for controlling immune responses under physiological and pathological conditions. Recently, apoptotic cells (bodies) have been shown to elicit immune responses: 1) uptake of apoptotic cells by macrophages resulted in the enhanced secretion of interleukin (IL)-10 and transforming growth factor- β and decreased secretion of tumor necrosis factor- α , IL-1 β , and IL-12;^{10–12} 2) intravenous injection of syngeneic apoptotic cells into mice induced polyclonal autoantibody production with IgG deposition in the glomeruli;¹³ and 3) autoimmune disease developed in mice with impaired clearance of apoptotic cells in the spleen.¹⁴

Here, we extend our previous findings with the various animal models of MS.⁹ SJL/J mice with RR-EAE have enlargement of draining regional lymph nodes. We found paradoxically that A.SW mice with progressive EAE have atrophic spleen, thymus, and lymph nodes. The numbers of T and B cells were dramatically decreased and massive apoptosis was detected in the atrophic spleens of animals with progressive disease. We hypothesize that the apoptosis within lymphoid organs itself modulates anti-self-immune responses. To test this hypothesis apoptotic thymus cells were injected into SJL/J mice with RR-EAE. We found ~20% of the mice developed SP disease with large demyelinating lesions and high titers of MOG antibody. Spleen cells from mice with SP-EAE showed decreased interferon (IFN)- γ production with sustained IL-4 production.

Materials and Methods

EAE Induction and Analysis

Female SJL/J and A.SW mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously at the base of the tail with 100 nmol of MOG_{92–106} peptide (DEGGYTCF-FRDHSYQ) (Core Facility, University of Utah Huntsman Cancer Institute, Salt Lake City, UT)^{15,16} in complete Freund's adjuvant containing 2 mg/ml of *Mycobacterium tuberculosis* H37 Ra (Difco Laboratories, Detroit, MI) with or without intravenous injection of 5×10^9 *B. pertussis* cells (Michigan Department of Public Health, Lansing, MI). Mice were weighed six times per week and observed for clinical signs for 4 to 6 months after injection. We have previously defined and described the various patterns of RR-, PP-, and SP-EAE.⁹ These are based on the criteria and definitions as proposed by Lublin and Reingold² for

the different forms of MS. Briefly, RR-EAE is an animal having a single or multiple episodes of disease without continuous disease progression. Animals with RR-EAE did not usually die from their disease. Animals having PP-EAE continually progress with increasing clinical scores and would die from the disease if not euthanized. SP-EAE initially starts out as RR-EAE and then converts to a progressive disease with increasing clinical scores and animals succumb to their disease if not euthanized. Scoring for grading EAE is described below.

Classical EAE signs were scored according to the following criteria:¹⁷ 0, no clinical disease; 1, loss of tail tonicity; 2, mild hind leg paresis; 3, moderate hind leg paralysis; 4, complete paraplegia; and 5, quadriplegia, moribund state or death. A second clinical phenotype, ie, an ataxic form of EAE, was scored according to the following criteria:^{9,18} 1 or 2, mice turned their heads or bodies to one side slightly (score 1) or obviously (score 2); 3, mice continuously rolled by twisting their bodies or rotated laterally in a circle; 4, mice could not stand but would lay on their sides with or without rolling; and 5, moribund state or death.

Induction and Transfer of Apoptotic Cells

Apoptosis of thymocytes was induced by γ -irradiation (600 rads) using a ¹³⁷Cs irradiator.^{13,19} The irradiated thymocytes were incubated in tissue culture medium at 37°C for 4 hours to allow apoptotic changes to occur. During the disease course, groups of SJL/J mice were given 10^7 apoptotic cells in 200 μ l of phosphate-buffered saline (PBS) per injection intravenously during the induction phase of MOG-induced EAE (two injections, 0 and 1 week after injection) or during the effector phase of MOG-induced EAE (four or five injections, 1 to 3 months after injection), or weekly (9 or 10 injections, 0 weeks to 3 months after injection). Control mice were given nonirradiated spleen cells, PBS, or no injection.

Histology

Mice were euthanized with halothane when moribund, or after the 4- to 6-month observation period. Animals were perfused with PBS, followed by a 4% paraformaldehyde solution. Organs were harvested and fixed in paraformaldehyde for 1 week at 4°C. Brains were divided into five coronal slabs and spinal cords into 10 to 12 transverse slabs, and tissues were embedded in paraffin. Four- μ m-thick tissue sections were stained with Luxol fast blue to visualize the extent of demyelination. Histological scoring was performed as previously described.⁹ For scoring of spinal cord sections, each spinal cord section was divided into quadrants: the anterior funiculus, the posterior funiculus, and each lateral funiculus. Any quadrant containing meningitis or demyelination was given a score of 1 in that pathological class. The total number of positive quadrants for each pathological class was determined, then divided by the total number of quadrants present on the slide and multiplied by 100 to give the percent involvement for each pathological class. Spleens were also

harvested, weighed, embedded in paraffin, and stained with hematoxylin and eosin. Mast cells were identified by the intense metachromatic staining of their cytoplasmic granules using a 0.25% cresyl violet acetate (Aldrich Chemical Co., Inc., Milwaukee, WI) solution.²⁰

Immunohistochemistry

T cells, B cells, macrophages, and plasma cells were detected using CD3 ϵ antibody (1:30 dilution, after trypsinization; Dako Corp., Carpinteria, CA),^{9,21,22} biotin-conjugated anti-mouse antibody CD45R/B220 (1:3000 dilution; PharMingen, San Diego, CA),^{9,23} F4/80 (Ly-71) (1:50 dilution; American Type Culture Collection, Rockville, MD),²⁴ and anti-mouse CD138 (syndecan-1) (clone 281-2, 1:6000 dilution; PharMingen),^{25,26} respectively. For detection of Bcl-2 and Bax expression, slides were stained with polyclonal rabbit anti-mouse Bcl-2 (1:1000 dilution, PharMingen) or polyclonal rabbit anti-Bax (1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) after a microwave treatment of 14 minutes in 0.01 mol/L sodium citrate buffer at pH 6.0. The slides were labeled using the avidin-biotin peroxidase complex technique with 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO) in 0.01% hydrogen peroxide (H₂O₂) in PBS. Sections were analyzed by Image-Pro Plus version 4.5.1 (Media Cybernetics, Inc., Silver Spring, MD).⁹

Agarose Gel Electrophoresis

For the visualization of DNA fragmentation, samples of spleens and inguinal lymph nodes were harvested and stored at -70°C. DNA was isolated by homogenizing the tissues in 2 ml of lysis buffer (10 mmol/L Tris-HCl, pH 7.5, 20 mmol/L ethylenediamine tetraacetic acid, 0.5% sodium dodecyl sulfate, 100 mmol/L NaCl), adding 0.1 mg/ml of proteinase K (Sigma Chemical Co.) and incubating for 18 hours at 55°C. The DNA was phenol/chloroform extracted and ethanol precipitated followed by incubation with 100 μ g/ml of RNase for 1 hour at 37°C.²⁷ DNA samples were electrophoretically separated for 2 hours at 50 V in a 1.8% agarose gel. Gels included molecular weight markers and DNA from irradiated thymocyte cultures, a positive control for internucleosomal DNA fragmentation. DNA was visualized by a UV transilluminator with ethidium bromide and the gels were photographed.

Terminal dUTP Nick-End Labeling (TUNEL)

To demonstrate DNA fragmentation as a histological hallmark of apoptosis, we used the TUNEL method as previously described.²⁸ Briefly, after proteinase K digestion, the sections were rinsed with double-distilled water and immersed in TdT buffer (30 mmol/L Tris, pH 7.2, 140 mmol/L sodium cacodylate, 1 mmol/L cobalt chloride). TdT (United States Biochemical, Cleveland, OH) and biotin-16-dUTP (Boehringer Mannheim, Indianapolis, IN) in TdT buffer were then added to the sections and incu-

bated at 37°C for 60 minutes. After removal of endogenous peroxidase by 30 minutes of incubation in 3% H₂O₂, the sections were visualized using the avidin-biotin peroxidase complex technique as described above.

Serum MOG Antibody Assay

Blood was collected from MOG-sensitized mice when sacrificed. We used an enzyme-linked immunosorbent assay (ELISA) to measure the level of serum MOG antibody as described previously,^{9,29} using 96-well flat-bottom Nunc-Immuno plates, MaxiSorp surface (Nalge Nunc Int., Rochester, NY). Plates were coated with MOG₉₂₋₁₀₆ peptide overnight. After blocking with 10% fetal bovine serum, serial dilutions of sera were added to the plates and incubated for 90 minutes. After washing, a peroxidase-conjugated anti-mouse IgG (H + L) (Life Technologies, Gaithersburg, MD), IgG1, IgG2b (Caltag Laboratories, Burlingame, CA), or IgG2c antibody (Southern Biotechnology Associates, Inc., Birmingham, AL)^{30,31} was added for 90 minutes. Immunoreactive complexes were detected with o-phenylenediamine dihydrochloride (Sigma Chemical Co.) and were read at 492 nm on a Titertek Multiskan Plus MK II spectrophotometer (Flow Laboratories, McLean, VA).

Cytokine Assay

Spleen and lymph node cells were cultured at 2×10^6 cells/ml in six-well plates (Corning Inc., Corning, NY) in the presence or absence of concanavalin A (5 μ g/ml, Sigma Chemical Co.). Culture supernatants were harvested 48 hours after stimulation. We measured IFN- γ and IL-4 using the ELISA system, OptEIA Set (PharMingen), according to the manufacturer's instructions.²⁹

Results

Atrophy of Lymphoid Organs in Mice with Progressive EAE

Generally, in EAE, draining regional lymph nodes enlarge after subcutaneous injection of an encephalitogen.^{32,33} SJL/J mice with RR-EAE had marked swelling of the inguinal lymph nodes with a normal size spleen and thymus whether *B. pertussis* was given or not. In contrast, in A.SW mice with progressive EAE, severe atrophy of the thymus, spleen, and lymph nodes was noted (Figure 1a). Spleen weights were significantly lower in A.SW mice than in SJL/J mice (Figure 1b; $P < 0.01$, analysis of variance). A.SW mice with PP-EAE had smaller spleens than those of A.SW mice with SP-EAE induced with *B. pertussis* supplementation (Figure 1b; $P < 0.01$, analysis of variance). No atrophy was seen in nonlymphoid organs in A.SW or SJL/J mice with EAE. The spleens of A.SW mice without EAE were slightly smaller than those of SJL/J mice without EAE [mean spleen weight (g) $\pm$ SEM: A.SW mice, 0.09 ± 0.01 ; SJL/J mice, 0.11 ± 0.01].

Figure 1. a: Lymphoid atrophy in A.SW mice. SJL/J mice with relapsing-remitting (RR)-EAE had swelling of draining lymph nodes with a normal size spleen and thymus (**left**). In contrast, A.SW mice with PP-EAE had severe atrophic lymph nodes, spleen, and thymus (**right**). **b:** Splenic atrophy in mice with progressive EAE. A.SW mice with PP- and SP-EAE showed a significant decrease in spleen weights compared with mice with RR-EAE (** $P < 0.01$). No atrophy was seen in spleens from SJL/J mice with RR-EAE, whether *B. pertussis* was given or not. In ultraviolet (UV)-irradiated SJL/J mice (SJL/UV), mice with SP-EAE had significantly smaller spleens than those with RR-EAE (** $P < 0.01$). All tissues were harvested during the chronic stage of EAE, more than 1 month after EAE induction. Shown are mean weights $\pm$ SEM of 5 to 13 mice for each group.

We have previously shown that ultraviolet (UV) irradiation induced SP-EAE in 20% of SJL/J mice sensitized with MOG₉₂₋₁₀₆.³⁴ Among the UV-irradiated mice, we found severe atrophy of the thymus, spleen, and lymph nodes only in mice with SP-EAE. UV-irradiated mice with SP-EAE had significantly smaller spleens than those with RR-EAE (Figure 1b; $P < 0.01$, analysis of variance). This indicates that these results were not strain-specific, because atrophy of lymphoid organs was characteristic of progressive EAE whether occurring in A.SW or SJL/J mice.

Microscopically in RR-EAE, the follicles in the lymph node and spleen were enlarged and varied considerably

in size and shape (Figures 2d and 3b). No involution of the thymus was seen (Figure 2b). Paracortical zones in lymph nodes and periaarterial lymphoid sheaths in the spleen were conspicuous, whereas germinal centers with tingebale-body macrophages¹⁴ were occasionally seen in some follicles. In the spleens of mice with progressive EAE, the white and red pulps were severely depleted of cells and trabeculae were conspicuous (Figure 3a). Areas of white pulp were small and red pulp contained polymorphonuclear cells, hemosiderin-laden macrophages, and mast cells, but few lymphocytes. In lymph nodes and thymus of most mice with progressive EAE, the architecture was indistinct and neither follicles nor paracortex was evident. Only in a few mice, did we observe relatively preserved architecture of the thymus and lymph nodes; the former had decreased cellularity in the cortex (Figure 2a), and the latter had fibrosed follicles (Figure 2c). Occasionally, mast cells with granules and plasma cells with Russell bodies³⁵ were seen in the remnants of the lymph nodes (Figure 2e) and the thymus. Mast cell granules were metachromatic (Figure 2f) and plasma cells were positive for CD138 (syndecan-1) (Figure 2g). No histological abnormalities were seen in lymphoid organs in age-matched A.SW mice without MOG sensitization (data not shown).

Immunoarchitecture of the Spleen Differs between Mice with RR-EAE versus PP- and SP-EAE

We investigated the immunoarchitecture of lymphoid organs between our EAE models using immunohistochemistry. We compared the number of CD3⁺ T cells in the periaarterial lymphoid sheath around a central artery of the spleen between the different forms of EAE. Although masses of CD3⁺ T cells were located around arterioles in RR-EAE (Figure 3d), the periaarterial CD3⁺ T-cell sheaths in mice with PP- and SP-EAE were small and markedly reduced (Figure 3c). Quantitative analyses revealed that A.SW mice had significantly fewer numbers of T cells in the periaarterial lymphoid sheath than those in the two SJL/J groups (Figure 4a, A.SW mice without *B. pertussis* supplementation, $P < 0.01$; A.SW mice with *B. pertussis* supplementation, $P < 0.05$, analysis of variance).

In RR-EAE, large B220⁺ B-lymphocyte follicles located adjacent to periaarterial T-cell regions in the spleen were prominent (Figure 3f). In contrast, in PP- and SP-EAE, B cells formed small clusters or narrow zones adjacent to T-cell areas (Figure 3e). The numbers of B cells in the white pulp were significantly lower in A.SW mice with or without *B. pertussis* supplementation than those in the two SJL/J groups (Figure 4b; $P < 0.01$, analysis of variance).

Using the macrophage-specific marker, F4/80,²⁴ we stained macrophages that were located in the red pulp of the spleen. Although the white pulp and/or the periaarterial lymphoid sheath in the spleen were well demarcated and circumscribed, the red pulp was not demarcated. Thus, the total numbers of T and B cells were countable in the white pulp, but the numbers of macrophages in the red pulp were not. Therefore, we compared the ratio of red pulp area to

Figure 2. Lymph node and thymus pathology in PP-EAE (**a, c, e–g**) versus RR-EAE (**b, d**). **a:** In the thymuses of mice with PP-EAE, the numbers of lymphocytes were decreased, resulting in lower cellularity of the cortex (Cx), compared to the medulla (M). **b:** No thymic involution was seen in RR-EAE; the cellularity was higher in the cortex than in the medulla. **c:** In the regional lymph nodes of mice with PP-EAE, lymphoid follicles were depopulated of lymphocytes and had fibrotic areas (**arrowheads**). **d:** Germinal centers were seen in the regional lymph nodes of mice with RR-EAE. **e:** In the lymph nodes of PP-EAE mice, there were increased numbers of plasma cells, some of which were distorted by eosinophilic globules, Russell bodies. Mast cells were also increased in number and stained blue. Mast cell granules showed metachromasia by cresyl violet (**f**) and plasma cells were visualized by immunohistochemistry against CD138 (syndecan-1) (**g**). Original magnifications: $\times 100$ (**a, b**); $\times 130$ (**c, d**); $\times 335$ (**e–g**).

total area between the different forms of disease to determine whether the amount of the red pulp (F4/80⁺ macrophages) was reduced in relation to the amount of total area. In PP- and SP-EAE, the area of the red pulp tended to be larger than that of the white pulp (Figure 3g). However, there was no statistical difference in the percentage of the red pulp area to the total area among mice with the different clinical disease courses, except for the percentage of the red pulp in SP-EAE mice (A.SW mice with *B. pertussis* supplementation), which was statistically higher than that in SJL/J mice with *B. pertussis* supplementation (Figures 3h and 4c; $P < 0.05$, analysis of variance). These results suggest that a significant decrease in spleen size in progressive EAE (Figure 1) cannot be attributed solely to the de-

pletion of cells in the white pulp; the cells in the red pulp would be expected to have been depleted as well, because no significant enlargement of the red pulp was seen in progressive EAE.

Plasma cells were detected using an antibody against syndecan-1 (CD138).²⁶ In naïve mice or mice with RR-EAE, small clusters of plasma cells were seen in the red pulp (data not shown). In mice with progressive EAE, we found large numbers of plasma cells in the red pulp (Figure 4d), suggesting that plasma cells were relatively resistant to the leukopenic insult.

In most mouse strains, very few if any mast cells can be detected in the spleen. However, mast cells in spleens of A-strain mice as well as A.SW mice can be readily

PP-EAE

RR-EAE

H. E.

T cells

B cells

**Macro-
phages**

Figure 4. Immunoarchitecture of the spleens of MOG-sensitized A.SW and SJL/J mice. A.SW and SJL/J mice were sensitized with MOG with (A.SWBP, cross-hatched bars; SJLBP, hatched bars) or without (A.SW, filled bars; SJL, open bars) *B. pertussis* supplementation. **a:** CD3⁺ T cells were enumerated in the periarteriolar lymphoid sheath (PALS) around central arteries. A.SW mice had significantly fewer numbers of CD3⁺ T cells than those of SJL/J mice. **b:** B220⁺ B cells were enumerated in the white pulp of the spleens. The white pulp of A.SW mice had significantly fewer numbers of B cells than those of SJL/J mice. **c:** Percentage of the area of the red pulp, which was positive for a macrophage marker, F4/80, to total area of the spleens. The red pulp area of A.SW mice was relatively larger than that of the white pulp. There was no statistical difference among groups, with the exception of the A.SWBP group versus the SJLBP group. Shown are means $\pm$ SEM of four to six spleens in each group. CD3⁺ T cells and B220⁺ B cells were enumerated in at least five T-cell regions and white pulp regions, respectively, per spleen. * $P < 0.05$; ** $P < 0.01$, analysis of variance. **d:** In the spleens of mice with progressive EAE, large numbers of plasma cells were seen in the red pulp (RP), but not in the white pulp (WP). Immunohistochemistry against syndecan-1. **e:** Metachromatic mast cells were preserved in the red pulp of the spleens of A.SW mice with progressive EAE. Cresyl violet stain. Spleens were harvested during the chronic stage of EAE, more than 1 month after EAE induction. Original magnifications: $\times 87$ (**d, e**); $\times 535$ (inset).

found.³⁶ Metachromatic mast cell granules are stained by cresyl violet. In A.SW mice with progressive EAE, we found large numbers of mast cells in the red pulp of the spleen (Figure 3e), suggesting that mast cells were also resistant to the leukopenic insult. In contrast, few if any mast cells were detected in naïve SJL/J mice or SJL/J mice with RR-EAE.

Apoptosis within Lymphoid Organs in Mice with Progressive EAE

We tested whether lymphoid atrophy in progressive EAE was caused by apoptosis. Electrophoresis of DNA from

the atrophic spleens and lymph nodes of A.SW mice with PP-EAE or SP-EAE showed a ladder pattern indicating DNA degradation, with bands corresponding to multiples of 180- to 200-bp subunits (Figure 5, a and b), a biochemical hallmark for apoptosis. In contrast, spleens and lymph nodes from naïve mice or mice with RR-EAE had only a high molecular weight DNA band. We were not able to test thymic DNA from animals with progressive EAE, due to extensive thymic atrophy.

Apoptosis of spleens in progressive EAE was confirmed by TUNEL staining. In mice with progressive EAE, we found a large number of TUNEL⁺ cells in the spleen (Figure 5c). Most TUNEL⁺ cells were seen in the red pulp

Figure 3. Severe atrophy and cell depletion of the spleens of MOG-sensitized A.SW mice with PP-EAE, compared with the spleens of SJL/J mice with RR-EAE. **a:** In PP-EAE, parenchymal cells were severely depleted in both the red (RP) and the white pulps (WP); the sizes of follicles were small. The capsule (Cap) was thickened and wrinkled and trabeculae (T) were conspicuous. **b:** In RR-EAE, a large T-cell area, periarteriolar lymphoid sheath, formed a mass around a central artery (*). B-cell areas also formed large follicles that exhibited germinal centers (gc). **c:** In PP-EAE, only small numbers of CD3⁺ T cells were seen around central arteries. **d:** In contrast, in RR-EAE, thick CD3⁺ cuffs surrounded the central arteries and CD3⁺ T cells were also scattered in the germinal center. **e:** In PP-EAE, B220⁺ B cells were also severely depleted and formed small masses adjacent to T-cell areas. **f:** In RR-EAE, a large number of B cells were localized mainly to the germinal center and mantle zone. **g** and **h:** The red pulp stained positive for a macrophage-specific marker, F4/80. No significant difference in the ratio of the area of the red pulp to the whole area was seen between PP-EAE (**g**) versus RR-EAE (**h**). Spleens were harvested during the chronic stage of EAE, more than 1 month after EAE induction. **a** and **b:** H&E staining. **c-h:** Immunohistochemistry against CD3 (**c, d**), B220 (**e, f**), and F4/80 (**g, h**). Original magnifications, $\times 50$. Scale bars, 200 μ m.

Figure 5. **a** and **b:** Agarose gel electrophoresis of DNA from spleens (**a**) and lymph nodes (**b**). DNA was analyzed by electrophoresis through a 1.8% agarose gel. **Lane 1**, molecular weight markers; SP-EAE, secondary progressive EAE; PP-EAE, primary progressive EAE; RR-EAE, relapsing-remitting EAE; No Tx, naïve mice; Thymocytes, irradiated thymocytes (positive control). The typical ladder patterning of nuclear DNA fragmentation was found in PP- and SP-EAE. **c** and **d:** Apoptosis was also detected in the spleens by the TUNEL method. **c:** In mice with progressive EAE, a large number of TUNEL⁺ nuclei were detected mainly in the red pulp (RP), while a few TUNEL⁺ nuclei were also seen in the white pulp (WP). TUNEL⁺ nuclei showed nuclear fragmentation and condensation (**inset**). **d:** No TUNEL⁺ nuclei were seen in RR-EAE. **e** and **g:** In the spleens of mice with progressive EAE, Bcl-2⁺ cells (**inset**) were observed in the white pulp (**e**), whereas a reverse pattern of staining was observed in immunohistochemistry for Bax; the white pulp was Bax-negative (**inset**) and Bax⁺ cells were observed in the red pulp (**g**). **f** and **h:** In the spleens of mice with RR-EAE, low levels of Bcl-2 were seen in the red pulp (**f**), whereas Bax⁺ cells were seen in the red pulp as well as in the center of the white pulp (**h**). All tissues were harvested from mice during the chronic stage of EAE, more than 1 month after EAE induction, or age-matched control mice. Original magnifications: $\times 76$ (**c**, **d**); $\times 560$ (**c**, **inset**); $\times 76$ (**e**–**h**); $\times 410$ (**e**, **g**, **inset**).

with a few TUNEL⁺ cells found in the white pulp. Some TUNEL⁺ cells had fragmented nuclei with condensation (Figure 5c, inset), whereas other cells having a macrophage appearance had phagocytosed apoptotic bodies within the cytoplasm. In contrast, very few TUNEL⁺ cells were detected in the spleen of normal mice and mice with RR-EAE (Figure 5d).

To examine additional apoptotic markers in progressive disease, we investigated the localization of Bcl-2, an anti-apoptotic regulator, and Bax, a proapoptotic regulator.^{37–39} In the spleens of naïve mice or mice with RR-EAE, low-level expression of Bcl-2 was found in cells of the red pulp, and no Bcl-2 expression was seen in cells of the white pulp (Figure 5f). Bax⁺ cells were seen in both

Table 1. Clinical Diseases of MOG-Immunized Mice with Apoptotic Cell Injections

Treatment*	Disease incidence [†]	SP-EAE/mice [‡]	Clinical score [§]
Control	7/9 (78%)	0/9 (0%)	2.1 ± 0.5
Induction	6/9 (67%)	1/9 (11%)	1.8 ± 0.6
Effector	7/10 (70%)	2/9 (22%)	2.6 ± 0.7
Weekly	9/10 (90%)	3/10 (30%)	2.6 ± 0.5

*Mice were sensitized with myelin oligodendrocyte glycoprotein (MOG)₉₂₋₁₀₆ peptide in complete Freund's adjuvant (CFA). Mice received apoptotic cells either during induction or effector phase, or weekly. Control mice received no apoptotic cells.

[†]Number of mice with clinical signs of relapsing remitting (RR)-EAE or secondary progressive (SP)-EAE/total number of mice examined (percentage). RR-EAE was defined as follows: clearly defined disease attacks with full recovery or with sequelae and residual deficit upon recovery; periods between disease relapses characterized by a lack of disease progression. Since the disease did not progress, no mice with RR-EAE died (mortality was 0% by the definition). SP-EAE was defined as follows: initial RR disease course followed by progression. When the mice had switched from RR-EAE to SP-EAE, the disease progressively worsened. Therefore, all mice with SP-EAE became moribund or died (mortality was 100%). We categorized mice as SP-EAE when mice became moribund or died.

[‡]Number of mice with SP-EAE/total number of mice examined (percentage).

[§]Values are means of maximum clinical scores of all mice, including both asymptomatic and symptomatic mice, in each group during the entire observation period ± SEM.

red and white pulp (Figure 5h). In contrast, in the spleens of mice with progressive EAE, up-regulation of Bcl-2 was seen in surviving cells around the central artery of the white pulp (Figure 5e) where few TUNEL⁺ nuclei were detected (Figure 5c). Also, Bax was up-regulated in the red pulp, and the remaining lymphocytes of the white pulp expressed only low levels of Bax protein (Figure 5g).

Conversion to SP-EAE by Injection of Apoptotic Thymocytes

Because apoptotic cells (bodies) are known to modulate immune responses,¹³ we hypothesized that a large number of apoptotic cells in lymphoid organs in mice with progressive EAE could result in modulation of the immune response, contributing to disease progression. MOG-sensitized SJL/J mice were injected with syngeneic apoptotic thymocytes weekly or during the induction or effector phases of EAE. Apoptosis of thymocytes was induced by γ -irradiation and confirmed biochemically by agarose gel electrophoresis (Figure 5, a and b) and annexin V-fluorescein isothiocyanate/propidium iodide staining by flow cytometry (54% of cells were annexin V⁺). Because nonirradiated thymocytes have been shown to contain $\geq 10\%$ apoptotic cells after isolation, spleen cells rather than thymocytes were used as a control for these experiments as demonstrated by Mevorach and colleagues.¹³ When control mice sensitized with MOG were given normal spleen cells or sham injected, mice developed RR-EAE; no mice died during the 4- to 6-month observation period (Table 1, Figure 6a). Regardless of treatment, mice with RR-EAE showed either a classic or an ataxic phenotype of EAE or both. The characteristic clinical feature of classic EAE is ascending paralysis, commencing at the tail and moving to affect

hind limbs with or without incontinence. Ataxic EAE initiates with tilting of the head or trunk, which progress to continuous axial rotation, and eventually leads to the mouse lying on one side, as we described for A.SW mice with PP-EAE and SP-EAE.^{9,34} The ataxic EAE is also known as atypical,⁴⁰ rotatory,⁴¹ axial rotatory,^{42,43} or non-classical EAE.⁴⁴ In contrast, 6 of 28 mice (21%) receiving apoptotic thymocytes, either during the induction or effector phase or weekly, developed SP-EAE (Table 1; Figure 6, b and c). Once disease progressed, all mice with SP-EAE had the ataxic phenotype.

Large Demyelinating Lesions with Markedly Fewer T Cells in SP-EAE versus RR-EAE

The neuropathology of mice that developed RR-EAE was essentially the same regardless of whether mice were injected with apoptotic cells or not. Similarly, the neuropathology of mice that developed SP-EAE after apoptotic cell injections was the same, regardless of the timing of the injections. The CNS tissues of RR-EAE were harvested during the late chronic stage, 4 to 6 months after EAE induction. At this late chronic stage in RR-EAE, there was no significant difference in neuropathology among mice, regardless of whether mice were in remission or relapse. Thus, we pooled the neuropathology data from all mice that developed RR-EAE, and compared it to data from mice that developed SP-EAE. In mice with RR-EAE, we found limited areas of demyelination with mononuclear cell infiltration in the meninges and perivascular spaces (Figure 7b). In contrast, mice with SP-EAE developed large demyelinating lesions with macrophages and polymorphonuclear cell infiltration (Figure 7a). The extent of meningitis and demyelination in spinal cords of mice with SP-EAE was significantly greater than that of mice with RR-EAE (Figure 8a, $P < 0.05$, t -test). Using immunohistochemistry against neurofilament protein,²² we confirmed relative preservation of axons in demyelinating areas in RR-EAE and SP-EAE, while axonal injury was seen in both types of EAE (data not shown).

To further characterize the two contrasting lesions, we compared CD3⁺ T-cell infiltration in the CNS of SP-EAE versus RR-EAE (Figure 7, c to h, and Figure 8b). In RR-EAE, T cells predominated in cellular infiltrates found in the meninges and perivascular spaces (Figure 7, f and h, and Figure 8b), and were also seen penetrating the CNS parenchyma in and around demyelinating lesions (Figure 7g). Conversely, the neuropathology seen in SJL/J mice with SP-EAE induced by injections of apoptotic cells was similar to that observed in MOG-sensitized A.SW mice with PP- or SP-EAE.⁹ In SP-EAE, T cells comprised a small portion of the cellular infiltrates in both meningeal and perivascular spaces, and only a few T cells were detected in parenchymal demyelinating lesions (Figure 7, c to e). The number of T cells in the meninges, perivascular spaces, and parenchyma of mice with SP-EAE were significantly lower than those of mice with RR-EAE (Figure 8b; $P < 0.01$, t -test).

Figure 6. Modulation of the clinical course of MOG-induced EAE by injections of apoptotic cells. Mice were sensitized with MOG without (**a**) or with injections (**arrows**) of apoptotic cells during effector phase (**b**) or weekly (**c**). Mice were observed for clinical signs (**left**) and weight change (**right**). **a:** In the control group without injection of apoptotic cells, mice developed RR-EAE. **b** and **c:** With injection of apoptotic cells during the effector phase of EAE or weekly, some mice developed RR-EAE (open circle), while other mice developed SP-EAE (filled triangle); mice initially showed a RR disease followed by progression. Regardless of treatment, mice with RR-EAE showed either a classical ascending paralysis or ataxic signs of EAE or both, while all mice had ataxic signs of EAE once disease progression developed (SP-EAE). Shown are clinical courses of two representative mice of each group in two independent experiments.

Splenic Atrophy in SJL/J Mice with SP-EAE Induced by Apoptotic Cell Infusion

All SJL/J mice with SP-EAE induced by apoptotic cell injections showed atrophy of the thymus, spleen, and lymph nodes. Among mice that received apoptotic cells,

spleens from SP-EAE mice were significantly smaller than those from RR-EAE mice [mean spleen weight (g) $\pm$ SEM: SP-EAE, 0.039 ± 0.007 ; RR-EAE, 0.128 ± 0.001 , $P < 0.001$, t -test]. Mice with RR-EAE showed swelling of regional lymph nodes, whether apoptotic cells were provided or not. There was no statistical difference in spleen

Figure 7. Neuropathology of MOG-sensitized SJL/J mice with SP-EAE (**a, c–e**) or with RR-EAE (**b, f–h**). **a:** The demyelinated plaque involved almost the entire segment of the spinal cord of a mouse with SP-EAE induced by weekly injections of apoptotic cells. Myelin, which was stained blue, was absent in the demyelinated plaque, and was preserved only in the corticospinal tract of the posterior funiculus and the central margin of the lateral funiculus (**arrowheads**). **b:** A small demyelinating lesion (**arrows**) was seen at the ventral root exit zone in the spinal cord of a mouse with RR-EAE. **c–e:** In SP-EAE, CD3⁺ T cells were sporadically seen in the meninges (**c**) and perivascular spaces (**e**), but very few were present in parenchymal vacuolar lesions (**d**). **f–g:** In RR-EAE, CD3⁺ T cells were predominant in cell infiltrates in the meninges (**f**) and perivascular spaces (**h**). T cells also infiltrated the parenchymal vacuolar lesions (**g**). Despite the fact that, in SP-EAE, the spinal cord shown was harvested from a mouse with the highest clinical score (score of 5) and had a large area of demyelination (**a**), T-cell infiltration in the lesions was minimal (**c–e**). In contrast, in RR-EAE, although the spinal cord was harvested from a mouse with a moderate clinical score (score of 3) and had a small area of demyelination (**b**), active T-cell infiltration was seen in the meninges, perivascular cuffing, and parenchyma (**f–h**). Spinal cords were harvested during the chronic stage of EAE, more than 1 month after EAE induction. **a** and **b:** Luxol fast blue stain. **c–h:** Immunohistochemistry against CD3. Original magnifications: $\times 20$ (**a, b**); $\times 160$ (**c, e, f, h**); $\times 250$ (**d, g**).

weights among mice with RR-EAE, whether they received apoptotic cells during the induction or effector phase, weekly, or received no injection.

High MOG Antibody Levels in Mice Injected with Apoptotic Cells

We tested whether serum MOG antibody titers correlated with clinical and pathological findings. As seen in Figure 9, the group given apoptotic cells weekly had higher serum anti-MOG IgG (H + L), IgG1, IgG2c (formerly IgG2a of *Igh-1b* allotype),^{31,45} and IgG2b responses than the other groups. Although mice with SP-EAE tended to have high levels of MOG antibody in their sera, no statistical difference was seen between RR-EAE versus SP-EAE because of a large range of IgG levels among individual mice (data not shown).

Decreased IFN- γ Production in Progressive EAE

We compared mitogen-induced production of IFN- γ versus that of IL-4 by spleen cells from SJL/J and A.SW mice with EAE (Figure 10). Regardless of whether mice were injected with apoptotic cells or not, high levels of IFN- γ

were detected in cultures of spleen cells from SJL/J mice with RR-EAE. In contrast, only low levels of IFN- γ were found in splenic cultures from SJL/J mice with SP-EAE induced by apoptotic cells. Similarly, although splenic cultures from A.SW mice sensitized with MOG produced high levels of IFN- γ before the onset of clinical disease (days 12 and 21), only low levels of IFN- γ were detected in splenic cultures from A.SW mice with PP-EAE or SP-EAE during the disease progression (Figure 10a). Interestingly, we found no difference in IL-4 production between mice with RR-EAE versus those with progressive EAE (Figure 10b).

Discussion

Here we demonstrated that disease progression in MOG-induced EAE was associated with massive cellular apoptosis in lymphoid organs and that apoptotic cell injection could also convert the RR disease course to the SP disease course. Although the precise mechanism remains to be clarified, induction of apoptotic bodies in itself could contribute to alterations of cytokine profiles leading to an increase in MOG antibody in progressive EAE. Uptake of apoptotic cells by macrophages and

Figure 8. a: Neuropathology scores of the spinal cord in MOG-sensitized mice with SP-EAE or RR-EAE. Mice were sensitized with MOG₉₂₋₁₀₆ followed by injections of apoptotic thymocytes. Mice with SP-EAE had higher meningeal and demyelination scores than mice with RR-EAE (* $P < 0.05$, *t*-test). Values are the means $\pm$ SEM of pooled results from two independent experiments. **b:** Percentage of T cells in total cellular infiltrates in mice with SP-EAE and RR-EAE. We detected T cells in brain and spinal cord sections using CD3 antibody. In RR-EAE, CD3⁺ T cells predominated in meningeal and perivascular infiltrates, and a significant number of T cells were also seen infiltrating into the CNS parenchyma. In SP-EAE, a significantly lower number of T cells were present in meninges, perivascular regions, and parenchyma, compared with those in RR-EAE (** $P < 0.01$, *t*-test). Spinal cords were harvested during the chronic stage of EAE, more than 1 month after EAE induction. Data represent the mean percentage of T cells in cell infiltrates (the number of CD3⁺ cells/total number of cells in infiltrate) $\pm$ SEM of CNS sections from 69 lesions in SP-EAE and 56 lesions in RR-EAE. Neuropathology scores of SP-EAE were pooled from SP-EAE mice injected with apoptotic cells during induction or effector phase or weekly. The scores of RR-EAE were pooled from mice that developed RR-EAE from all groups.

dendritic cells has been shown to induce a variety of immune responses, including altered cytokine profiles,¹⁰⁻¹² and an increase in antibody production.^{13,14}

Lymphoid depletion or immunosuppressive treatments can ameliorate EAE. However, under certain conditions, it can exacerbate EAE, leading to disease progression and

Figure 9. Serum MOG antibody responses in MOG-EAE mice with injections of apoptotic cells during induction or effector phase, weekly or no (control) injections. Weekly injection of apoptotic cells significantly increased anti-MOG total IgG (**a**) ($P < 0.01$ compared with the other groups), IgG1 (**b**) ($P < 0.01$, compared with induction and effector groups), IgG2c (**c**) ($P < 0.01$, compared with the induction group), and IgG2b responses (**d**) ($P < 0.05$, compared with control and induction groups). Values are mean optical density (OD) $\pm$ SEM of three independent experiments. Sera were collected during the chronic stage of EAE, more than 1 month after EAE induction. Each experimental group consisted of 14 to 18 mice. * $P < 0.05$; ** $P < 0.01$ by analysis of variance.

death in animals.^{46,47} Similarly, in MS, patients treated with total lymphoid irradiation had less of a functional decline than controls, but interestingly some patients showed disease progression after total lymphoid irradiation.⁴⁸ The contrasting outcomes due to immunomodulation treatment have also been observed in experimental animal models and human cases of systemic lupus erythematosus (SLE). Although thymectomy in mice prone to autoimmunity accelerates the autoimmune manifestations in NZB/W mice, the opposite effect is seen in MRL/lpr mice.⁴⁹ In humans, thymectomy alleviates symptoms in some patients with SLE, while exacerbating disease in others.⁴⁹

The protocols that induce a lymphopenic state, including thymectomy, cyclosporin A administration, and total lymphoid irradiation have been shown to result in the development of organ-specific immunity.⁵⁰ These procedures could selectively deplete regulatory T cells, such as CD4⁺CD25⁺ cells, but leave the autoreactive effector populations intact.⁵¹ Interestingly, defects in IFN- γ -mediated suppressor CD8⁺ T cells have been found in patients with progressive MS, but not with RR-MS.⁵² In the

a

b

Figure 10. IFN- γ (a) and IL-4 (b) levels in MOG-sensitized SJL/J mice with RR-EAE or SP-EAE and MOG-sensitized A.SW mice before (latent, no Tx) or after disease progression (progress, no Tx). SJL/J mice with sham injection (RR-EAE, no Tx) developed RR-EAE, while SJL/J mice injected with apoptotic cells developed either RR-EAE (RR-EAE, Apo i.v.) or SP-EAE (SP-EAE, Apo i.v.). The cytokine concentrations of the supernatants from concanavalin A-stimulated spleen cells were measured by an ELISA. **a:** IFN- γ production was low in mice with progressive-EAE, whereas high levels of IFN- γ production were detected in all groups of mice with RR-EAE. **b:** In contrast, no difference in IL-4 production was seen between RR-EAE mice versus SP-EAE mice.

atrophic spleen in progressive EAE, TUNEL-positive cells were mostly seen in the red pulp. At a glance, one might think that apoptosis occurred mainly in cells in the red pulp. However, not only the number of macrophages in the red pulp but also the numbers of T and B cells in the white pulp were significantly decreased in progressive EAE. This observation could be explained by the following scenario. T and B cells and macrophages died by apoptosis, leading to the reduction in the size of both white and red pulp regions in the spleen. Bcl-2⁺ cells resistant to apoptosis remained in the white pulp,

whereas Bax⁺ cells underwent apoptosis and were phagocytosed by macrophage in the red pulp.

Certain cell types are relatively resistant to apoptosis. In mice with progressive EAE, we found a relative preservation of plasma cells and mast cells. Preliminary results showed that significant numbers of NKT cells also existed in the spleen and lymph nodes of mice with progressive EAE (I. Tsunoda and R.S. Fujinami, unpublished data). This is consistent with the relative resistance of NKT cells to apoptosis as suggested by Seino and colleagues.⁵³ Reciprocal expression of Bcl-2 and Bax proteins in our model might contribute to survival for certain cell types.^{54,55}

Mast cells have been suggested to play a crucial role in inflammation and demyelination in MS and EAE⁵⁶ and to influence the severity of EAE, both from inside and outside the CNS.^{57,58} In A.SW mice with progressive EAE, we found a large number of mast cells in the lymphoid organs, whereas only a few mast cells were found in the CNS. Because mast cells produce immunomodulating molecules, including IL-4,⁵⁹ and are capable of antigen presentation, mast cells in the spleens of MOG-sensitized A.SW mice could play a variety of roles in disease progression; IL-4 could help antibody production by plasma cells that were also found in large numbers in the spleens of MOG-sensitized A.SW mice.

In the spleens of mice with progressive EAE, production of IFN- γ was decreased, while IL-4 levels were maintained in progressive EAE and RR-EAE. Although IFN- γ has been considered a pathogenic cytokine in the context of CNS inflammation, experimental data support the idea that IFN- γ can have a protective role in EAE.¹ Systemic administration of IFN- γ antibody exacerbated clinical signs both in acute and chronic forms of EAE whether induced by active immunization with spinal cord homogenate^{60–62} and myelin basic protein^{63,64} or by passive transfer of encephalitogenic T cells.^{63,64} Interestingly, treatment with IFN- γ antibody induced a progressive lethal disease even in resistant strains of mice.⁶⁴ Conversely, IFN- γ treatment ameliorated EAE in susceptible SJL/J mice injected with spinal cord homogenate.⁶⁰ Other studies using IFN- γ or IFN- γ receptor knockout mice also suggest that IFN- γ has a protective role in EAE.¹ During the latent period of our MOG-induced EAE, IFN- γ might also protect mice from disease progression by suppressing Th2-type cytokines and autoantibody production. However, during the disease course, we speculate that selective killing of Th1-type cells in lymphoid tissues and/or relative survival of Th2-type cells or uptake of apoptotic bodies by phagocytes might decrease the production of IFN- γ . This would favor autoantibody production, and lead to disease progression. Increased overall MOG antibody responses, particularly those of IgG1 seen in mice injected with apoptotic cells, also suggest that the injections enhance humoral immune responses by modulation of Th1 versus Th2 immune responses.

We detected high MOG antibody titers in EAE mice injected with apoptotic cells weekly or in mice with SP-EAE. This suggests a pathogenic role of MOG antibody. However, the antibody titers did not correlate with the

severity of demyelination or disease progression (data not shown). Some mice with RR-EAE had higher MOG antibody titers than those of mice with SP-EAE. This could be explained by: 1) deposition of MOG antibody in the CNS, leading to a decrease in antibody in the circulation; 2) a dominant intrathecal production of MOG antibody; or 3) an increase in MOG antibody responses as an epiphenomenon due to the imbalance of Th1/Th2 responses.

Autoimmune diseases have been associated with an increased incidence of splenic atrophy or hypoplasia.⁶⁵ Although it is rare, spleen atrophy with active humoral immune responses has been reported in patients with SLE.^{66,67} Atrophy of the thymic cortex has also been demonstrated in murine models of SLE.^{68,69} In EAE, animals usually have an enlargement of lymphoid organs, particularly the regional lymph nodes, despite substantial weight loss. Atrophy of lymphoid organs in EAE is rare and has been reported only in a few instances. In a marmoset monkey EAE model, a marked depletion of periarteriolar T cells with preservation of B cells and macrophages was found in the spleen.⁷⁰ In acute EAE, moderate atrophy of the spleen and thymus has been shown in Lewis rats immunized with spinal cord homogenate.^{71–73} In those EAE studies, stress or a rise in serum corticosterone was suggested to be a cause of the atrophy, and the atrophy was believed to contribute to down-regulation of clinical signs of acute EAE. However, the lymphoid atrophy seen in our progressive EAE model was remarkably severe, involving not only the thymus and spleen but also lymph nodes, and was associated with disease progression. Lymphoid pathology in our model is similar to that observed in acute fatal EAE in Lewis rats sensitized using brain homogenate by Hara and colleagues.⁷⁴ In their model, the thymus was severely atrophic and macrophages contained phagocytosed fragmented lymphocytes. The spleen weight was also decreased with atrophy of the white pulp. In the CNS, demyelination was severe, but no perivascular cell infiltration was observed. The authors suggested that autoreactive lymphocytes might recognize a common antigen shared between lymphocytes and oligodendrocytes⁷⁵ leading to demyelination in the CNS and atrophy of the thymus and spleen.

Molecular mimicry could contribute to the lymphocyte depletion in our model. MOG belongs to the Ig superfamily (IgSF).^{76,77} One of the consensus motifs, DXGXYX, present in other IgV (variable)-like domains of the IgSF, is located within MOG_{92–106}, the predicted IgSF β -strand F of MOG.⁷⁷ Thus, MOG-specific immune responses could recognize IgSF molecules on immune cells by molecular mimicry between MOG and other IgSF members.⁷⁸ Indeed, we found cross-reactivity of MOG antibody with whole peripheral blood protein preparations by ELISA (L.K. Peterson, I. Tsunoda, T. Masaki, and R.S. Fujinami, unpublished data). In this scenario, MOG antibody could bind immune cells and act as a lymphocytotoxic antibody. Lymphocytotoxic antibodies have been demonstrated in autoimmune diseases. For example, in SLE lymphocyte autoantibodies have been associated with disease flares and lymphopenia.⁷⁹

Induction of apoptosis in lymphoid organs has been observed in murine models of sepsis and patients with sepsis.^{54,80–83} Cytokines, humoral and endocrine factors, such as corticosteroid, Fas ligand, tumor necrosis factor- α , and IL-10, have been shown to increase apoptosis, but the precise mechanisms are still not fully understood.⁸³ In sepsis, apoptosis could be either detrimental by compromising host defenses or beneficial by regulating the inflammatory response. Interestingly, despite increased apoptosis in lymphoid organs, apoptosis of neutrophils seemed to be decreased, which is thought to be important in enhancing tissue injury in sepsis. This is similar to the pathology observed in our current progressive EAE model, in which we found massive apoptosis in lymphoid organs and high numbers of polymorphonuclear cell infiltration in the CNS.

Many organ-specific autoimmune diseases are believed to be mediated by Th1-type immune responses. However, as seen in our model and some other models,⁴⁷ autoantibody responses and/or Th2-type responses can contribute to disease progression. As in sepsis, apoptosis of lymphoid cells in organ-specific autoimmune diseases, including EAE, can be a double-edged sword. During the induction phase, Th2-type immune cells, including NKT and mast cells, would counteract disease initiation by Th1-type autoimmune cells. During the progressive phase, however, Th2-type immune cells might aid in autoantibody production leading to disease progression. Disease progression may be a conversion from an organ-specific autoimmune disease to a systemic one. Autoantibodies as well as autoreactive T cells contribute to pathology inside and out of the CNS.

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