Analysis of the Cellular Infiltrate in the Iris during Experimental Autoimmune Encephalomyelitis

Alex F. de Vos,1,2 Andrew D. Dick,3 Jan Klooster,4 Lidy Broersma,1 Paul G. McMenamin,5 and Aize Kijlstra1,6

PURPOSE. Previous studies have shown that experimental autoimmune encephalomyelitis (EAE) and anterior uveitis (AU) develop in Lewis rats immunized with myelin basic protein (MBP). The purpose of this study was to characterize the dynamics, distribution, and phenotype of infiltrating cells in the iris during EAE-associated AU.

METHODS. Lewis rats were immunized with MBP emulsified in complete Freund’s adjuvant (CFA) or with CFA alone. Cellular infiltration of the iris was analyzed at various time points by immunohistochemistry of wholemounts, flow cytometry, and immunoelectron microscopy, by using monoclonal antibodies specific for monocytes/macrophages (ED1), T lymphocytes (R73, W3.25, OX8), T-cell activation markers (OX39, OX40), granulocytes (3A10), major histocompatibility complex (MHC) class II (OX6), and neurofilament (2H3).

RESULTS. MBP-immunized rats showed development of characteristic monophasic EAE, followed, after resolution of paralysis, by mild self-limited AU. Initially, focal infiltrates of round MHC class II+ and ED1+ cells were found in the iris. During the course of AU, the midiris became massively infiltrated with ED1+ monocytes-macrophages, R73+ T cells, granulocytes (HIS48+), and MHC class II+ cells. The influx of T cells consisted of CD4+ and CD8+ cells, of which only a small fraction (<14 and 11%, respectively) expressed activation markers. The infiltrating cells accumulated in proximity to myelinated and nonmyelinated nerve bundles and in the vicinity of blood vessels in the iris. No evidence was found for demyelination or nerve degradation. Neither EAE nor AU developed in CFA-treated control rats.

CONCLUSIONS. These data show that EAE-associated AU is characterized by a transient mixed cellular infiltrate consisting of monocytes-macrophages, granulocytes, and CD4 and CD8 T cells. The preferential accumulation of inflammatory cells in the vicinity of nerve fibers suggests that AU in this model may result from autoreactivity to nerve antigens. (Invest Ophthalmol Vis Sci. 2000;41: 3001–3010)

Experimental autoimmune encephalomyelitis (EAE) is an acute, CD4+ T-cell-mediated inflammatory disease of the central nervous system (CNS), which mirrors some aspects of multiple sclerosis (MS). Similar to MS, EAE is accompanied by several ocular changes, including optic neuritis,1–5 retinal vasculitis,6,7 and uveitis.8,9 Recently, it was found that Lewis rats immunized with myelin-basic protein (MBP) or certain peptides of MBP in adjuvant developed a mild self-limited form of anterior uveitis (AU) in addition to EAE.10,11 The intraocular inflammation induced by MBP was characterized by mononuclear cell infiltration of the iris and the anterior chamber. A substantial number of CD4+ cells in the iris expressed the Vβ8.2 T-cell receptor (TCR),11,12 a characteristic marker of encephalitogenic T cells in the rat. Moreover, infiltrating T cells, isolated from the eye, proliferate in vitro in a specific manner in response to MBP.12 Adoptive transfer of activated T cells specific for MBP to naïve Lewis rats also induced EAE-associated AU;10,11 underlining a central role of T cells in this experimental uveitis model.

The pathogenic mechanisms underlying EAE-associated AU are still unclear. The initiation of a CD4+ T-cell-mediated autoimmune inflammatory response in the eye requires presentation of the target antigen by major histocompatibility complex (MHC) class II+ antigen-presenting cells (APCs). The rat iris contains a rich plexus of sensory, sympathetic, and parasympathetic nerves,13 of which some are myelinated and contain MBP.14 These myelinated nerves in the iris could thus provide the target antigen for activated encephalitogenic T cells. Various potential types of APC are present in the iris. The iris contains a rich network of MHC class II+ dendritic cells,15,16 which possess strong antigen-presenting capacity after cytokine-induced maturation in vitro.17 Moreover, a dense network of macrophages is present in the iris stroma,18,19 which may present antigen to previously activated T cells. Together, these findings suggest that AU may be initiated in a

From the 1Department of Ophthalmology–Immunology and the 4Department of Morphology, Netherlands Ophthalmic Research Institute, Amsterdam; the 2Department Ophthalmology, University of Aberdeen, United Kingdom; the 5Department of Anatomy and Human Biology, University of Western Australia, Perth; the 6Department Ophthalmology, University of Amsterdam, The Netherlands.

2Current address: Department of Immunology, Erasmus University Rotterdam, The Netherlands.

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Corresponding author: Alex F. de Vos, Department of Immunology, Erasmus University Rotterdam, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands. devos@immu.fgg.eur.nl

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direct manner by encephalitogenic T cells. Evidence against this notion, however, was provided by Verhagen et al., who found that in most cases AU starts after remission of paralysis. Moreover, disruption of the blood-brain barrier and blood-ocular barrier does not abolish the lag period between the onset of EAE and AU.

The purpose of the present study was to characterize the inflammatory cell infiltrate in the iris during the course of EAE by means of immunohistochemistry, flow cytometry, and electron microscopy. In view of the mild uveitis in this model, we used immunohistochemistry of iris wholemounts to determine the phenotype and distribution of inflammatory cells. Previously, we have shown the considerable advantage of this technique over immunohistochemistry of tissue sections in studies on resident immune cells in the iris and in studies on ocular changes in the models of endotoxin-induced uveitis (EIU), experimental autoimmune uveoretinitis (EAU), and experimental melanin protein–induced uveitis (EMIU). Flow cytometry was performed to further analyze the phenotype and density of inflammatory cells in the anterior uvea. Electron microscopy was used to provide information about the ultrastructure of infiltrating cells and of the site at which they accumulated. Our results show that EAE-associated AU in Lewis rats was characterized by accumulation of various inflammatory cells in proximity with myelinated and nonmyelinated nerve fibers in the iris.

METHODS

Animals

Specific pathogen-free female inbred Lewis rats (Harlan, Zeist, The Netherlands), 6 to 8 weeks of age, were used in this study. All animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the guidelines of the local Animal Care and Ethics Committee of the Royal Netherlands Academy of Sciences.

Purification of MBP and Induction of EAE

MBP was purified from guinea pig brain according to the method of Brostoff and Mason, with a final cation-exchange chromatography step (CM-Sephadex; Pharmacia Biotech, Uppsala, Sweden), according to the instructions of the manufacturer. Analysis of purified MBP by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed a single band with a molecular weight of approximately 20 kDa.

Rats were injected on the dorsal surface of one hind foot with 25 μg MBP emulsified (1:1) in complete Freund’s adjuvant (CFA), containing 4 mg/ml Mycobacterium tuberculosis (Difco, Detroit, MI). Control rats were injected with 50 μl of saline-CFA emulsion. After immunization, rats were examined for weight loss, paralysis, and ocular changes using a slit lamp. A pilot experiment revealed that MBP-immunization induces EAE and AU with kinetics similar to those described earlier by Verhagen et al.

Immunohistochemistry of Iris Wholemounts and CNS

Before whole-body perfusion (to remove cells from the vessels) rats were injected with 500 IU of intravenous heparin. Rats (n = 3 per group) were killed at several time points after immunization (days 0, 10, 14, 20, 25, 31, and 40) for single-staining immunohistochemistry and days 20, 25, and 31 for double-staining immunohistochemistry) by an overdose of pentobarbital and flushed through the left ventricle of the heart with 250 ml cold phosphate-buffered saline (PBS) and 250 ml cold 4% paraformaldehyde. Iris–ciliary body (ICB) complexes were dissected from the eyes, as previously described and immediately used for immunohistochemistry. Spinal cords and optic nerves were removed, immersed in 20% sucrose in PBS overnight at 4°C and frozen in optimal temperature cutting compound (OCT; Tissue-Tek; Miles, Elkhart, IN).

Immunohistochemistry of wholemounts was performed as previously described (McMenamin) using the following primary monoclonal antibodies (Table 1): ED1 (anti-CD68; lysosomal antigen present in monocytes, macrophages, and most dendritic cells), R73 (anti-αβ TCR), OX8 (anti-CD8), HIS48 (recognizing granulocytes), 2H3 (anti-165-kDa neurofilament), and OX6 (anti-MHC class II). All monoclonal antibodies were obtained from Serotec (Oxford, UK), except ED1 (kindly provided by Christine D. Dijkstra) and 2H3 (Developmental Studies Hybridoma Bank, Iowa City, IA). Immunolabeled cells were visualized by horseradish peroxidase–conjugated goat anti-mouse immunoglobulins (Dako, Glostrup, Denmark) and 3-aminoo-9-ethylcarbazole (AEC) as substrate (red reaction product). For double-labeling studies, biotinylated ED1 (kindly provided by Ed Döpp) and R73 (Serotec) were used in conjunction with streptavidin-conjugated alkaline phosphatase (Dako) and fast blue as a chromogen (blue reaction product). Immunohistochemical analysis of spinal cord and optic nerve sections was performed in a similar manner with monoclonal antibodies ED1, R73, and OX6.

A semiquantitative scoring system was used to determine the dynamics of cell infiltration of the iris during EAE. The density of immunopositive infiltrating cells was scored as 0 (no cells, normal), 1 (single or few focal infiltrates), 2 (<50% of the iris infiltrated), or 3 (>50% of the iris infiltrated).

Electron Microscopy

Ultrastructural analysis of infiltrating monocytes and T cells in the iris was performed by immunoelectron microscopy (EM). Rats (n = 2 per group) were killed on days 24 and 26 after MBP immunization. Immunohistochemistry of iris wholemounts was performed as described earlier with monoclonal antibodies ED1 and R73 (Table 1) and diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO) as a substrate. The DAB reaction product was intensified by a gold-substituted silver peroxidase method. Iris wholemounts were postfixed in 1%...
osmium tetroxide, 1% potassium ferricyanide in 0.1 M cacodylate buffer (pH 7.4), dehydrated in ethanol, and flat embedded in epoxy resin. Ultrathin sections, cut perpendicular to the iris, were stained with uranyl acetate and lead citrate and analyzed with a transmission electron microscope (model 201; Philips, Eindhoven, the Netherlands).

Flow Cytometry
Rats (n = 2–7 at each time point) were killed at days 14, 21, 26, 28, 34, and 38 after immunization and perfused with cold PBS, as described earlier. Subsequently, the ICB complex was microscopically dissected from enucleated eyes and dissociated in RPMI 1640 containing collagenase D (1 mg/ml; Boehringer-Mannheim, Mannheim, Germany), DNase I (0.1 mg/ml; Boehringer-Mannheim), and 10% fetal calf serum at 37°C for 60 minutes. Cells were washed twice with cold 1% bovine serum albumin (BSA), 0.1% NaN₃, and PBS (FACS buffer; Becton Dickinson, Mountain View, CA) before immunolabeling.

Two- and three-color flow cytometry was performed as previously described. All reagents and incubations were kept at 4°C. Mouse monoclonal antibodies (Table 1) specific for rat cell surface antigens were OX39 (anti-CD25-interleukin [IL]-2 receptor), OX40 (anti-activation marker on CD4+ T cells), biotinylated OX1 (anti-CD45-leukocyte common antigen), W3/25 (anti-CD4), OX8 (anti-CD8), and phycoerythrin-conjugated R73 (anti-αβTCR) were all obtained from Serotec. Unconjugated anti-human factor-I IgG1 (OX21; Serotec), biotinylated anti-tri nitro pherol IgG1 (PharMingen, San Diego, CA), and phycoerythrin-conjugated mouse IgG1 (Serotec) were used as isotype controls. Unconjugated antibodies were detected with fluorescein-isothiocyanate (FITC)-labeled goat anti-mouse immunoglobulins (Sigma), and biotinylated antibodies were detected with streptavidin-Cy5 (PharMingen). Flow cytometry was performed (FACScan; Becton Dickinson, Mountain View, CA), and 10,000 cells were analyzed. Regions for analysis were set according to leukocyte forward- and side-scatter characteristics, backgating to the leukocyte gate, or isolation of the leukocyte population using a third color.

RESULTS
EAE and Inflammation of the CNS
To induce EAE-associated AU, Lewis rats were immunized with MBP in adjuvant. In all MBP-immunized rats, typical monophasic EAE developed with transient abdominal paralysis and weight loss. EAE was first noted on day 11 after immunization, peaked at day 15, and resolved by day 20. Immunohistologic analysis revealed an influx of ED1+, R73-, and OX6-positive cells in the spinal cord and optic nerve (data not shown), as described by others. Infiltrating cells were observed around blood vessels and in the parenchyma of the CNS at day 15 after immunization. Despite complete remission of the clinical signs of EAE, infiltrating ED1+ and R73+ cells were found in the spinal cord until day 31 after immunization. EAE or CNS infiltration was not observed in CFA-immunized control rats.

Inflammation of the Anterior Uvea during EAE
EAE-associated AU was examined clinically and in detail by immunohistochemistry. Slit lamp biomicroscopy revealed AU in MBP-immunized rats between days 14 and 40, as previously described. All rats (n = 27) killed at day 25 or later showed development of AU. The ocular inflammatory response was characterized by signs of miosis and by white infiltrates in the iris stroma, which were most prominent from days 25 through 31 after immunization.

Immunohistochemistry of wholemounts was performed to further analyze the dynamics, composition, and severity of the cellular infiltration in the iris. In the iris of CFA-injected control rats, a dense regular network of dendriform and pleomorphic ED1+ and OX6+ cells was found (Figs. 1A, 1I), consistent with previous descriptions. No evidence of infiltrating cells was found in the iris of these control rats during the entire experiment (until day 40 after immunization). Similar to untreated rats, the iris of CFA-injected rats contained only a few round ED1+ monocytes-macrophages, R73+ T cells, and HIS48+ granulocytes, scattered throughout the tissue (Figs. 1A, 1D, 1G, 1J).

During the course of EAE, there was a marked influx within the iris of all cell types investigated. Figure 2 shows the dynamics of ED1-, R73-, HIS48- or OX6-positive cell infiltration of the iris, as determined by immunohistochemistry and the density of these infiltrating cells in the iris.

Infiltration of ED1+ cells was first detected at day 14 after immunization (in one of three rats). The inflammation was characterized by a focal accumulation of a small number of round and pleomorphic cells. At day 20, inflammatory foci were detected in all rats. With time, the number and size of these foci increased. Infiltrates of round ED1+ cells were distributed unequally throughout the iris. The majority were found within the midiris, and a few were found in the peripheral ciliary part of the iris. The sphincter region was devoid of infiltrating cells. At days 25 and 31, the number of ED1+ cells further increased. Infiltrates in the midiris became more diffuse, whereas infiltrates in the periphery appeared to form cuffs (Figs. 1B, 1C). The distribution of the ED1+ cells suggested an accumulation in the vicinity of nerve fibers, which was visible after partial closure of the diaphragm of the microscope (to cause an interference-like image). Some round ED1+ cells were found around or attached inside blood vessels. By day 40 after immunization, the number and distribution of ED1+ cells had returned to normal, and only a few foci were detected (in two of three rats).

Monoclonal antibody R73, specific for the αβTCR, was used to detect infiltrating T cells. Infiltration of round R73+ cells was observed in the iris from day 20 (in two of three rats) through to day 31 (in all rats), but returned to normal by day 40 after immunization. Infiltrating R73+ cells, similar to ED1+ cells, were found in the midiris, and a few cells were present in the periphery (Figs. 1E, 1F). The largest number of infiltrating R73+ T cells was found on day 31 after immunization.

Infiltrating HIS48+ cells were found from days 20 through 31 after immunization (in six of nine rats), at the time of massive ED1+ and R73+ cell infiltration. The characteristic cellular staining suggested that the HIS48+ cells were granulocytes and not erythroid cells. Similar to ED1- and R73-positive cells, HIS48+ cells were found predominantly in the midiris (Figs. 1H, 1I).

Focal infiltrates of round OX6+ cells were detected at day 20 (in one of three rats) and day 14 (in two of three rats), before the influx of R73+ T cells. Infiltration of OX6+ cells increased dramatically at later time points (Figs. 1K, 1L). At day 20, resident OX6+ cells obtained a more pleomorphic shape.
Figure 1. Influx of inflammatory cells in the iris of Lewis rats 25 days after immunization with CFA (A, D, G, J) or MBP (B, C, E, F, H, I, K through N). Iris wholemounts were stained with monoclonal antibody ED1 (A, B, C), R73 (D, E, F), HIS48 (G, H, I), OX6 (J, K, L), ED1 in combination with 2H3 (M), or R73 in combination with 2H3 (N). Magnification, (A, B, D, G, H, J, K) ×36; (C, F, I, L) ×115; (M, N) ×105.
Because of the high density of cells in the infiltrates, it was not possible to determine the shape of these cells at later time points (Fig. 1L). Many round or pleomorphic cells could be observed inside blood vessels. The distribution of OX6\(^+\) cells paralleled the pattern of ED1\(^+\) cells—that is, infiltrating cells were found mainly in the midiris and few in the periphery. At day 40, the number of OX6\(^+\) cells had declined compared with the number on day 31. At this time, a large number of pleomorphic OX6\(^+\) cells was present in the midiris, scattered throughout the tissue, and foci of OX6\(^+\) cells were occasionally detected.

**Localization of Inflammatory Cells in the Iris**

To determine the distribution of infiltrating cells in relation to the iris nerves, double staining was performed on iris wholemounts with ED1 or R73 in combination with a monoclonal antibody (2H3) specific to neurofilaments. In the peripheral ciliary part of the iris, where nerves mainly have a radial direction, infiltrating ED1\(^+\) and R73\(^+\) cells were found along thick bundles of nerve fibers and occasionally along small nerve bundles (Figs. 1M, 1N), but not along single nerve fibers. Morphologically, infiltrating cells formed perineuronal cuffs. At sites where thick nerve bundles crossed large vessels, perineuronal sheaths were interrupted, and perivascular infiltrates were observed (Fig. 1M). In the midiris, small nerve bundles and single nerve fibers formed an irregular circular plexus. In this area, inflammation was diffuse and not obviously perineural. A plexus of thin nerve fibers was also detected in the sphincter region, but inflammatory cells were not present in this area.

Transmission electron microscopy (TEM) was performed on ED1- or R73-immunostained iris wholemounts to examine the sites of inflammation and the ocular changes at an ultrastructural level. Nonmyelinated and electron-dense myelinated nerves could be distinguished by TEM. Sections were cut out of areas with accumulations of inflammatory cells in the peripheral region of the iris. ED1\(^+\) cells displayed typical labeling of multiple lysosomal compartments (Figs. 3A, 3B), characteristic of activated monocytes-macrophages. Most ED1\(^+\) cells had indented nuclei, multiple small protrusions, numerous lysosomes, mitochondria and vesicles, and a well-developed Golgi apparatus (Figs. 3A, 3B). Occasionally, ED1\(^+\) cells contained secondary lysosomes, containing membranous material (Fig. 3B).

R73\(^+\) cells were labeled predominantly at the cell surface (Figs. 3C, 3D, 3E). Two types of R73\(^+\) cells were detected in the iris. One type had a high nucleus-to-cytoplasm area ratio and no or few organelles (Figs. 3C, 3D), indicative of resting

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**Figure 2.** Dynamics of ED1\(^+\) (A), R73\(^+\) (B), HIS48\(^+\) (C) and OX6\(^+\) (D) cells in the iris after MBP immunization. The density of cells was scored in a semiquantitative manner as 0 (normal), 1 (single or few focal infiltrates), 2 (<50% of the iris infiltrated), and 3 (>50% iris infiltrated). Each dot represents an iris of one rat.
FIGURE 3. Immunoelectron microscopy of infiltrating ED1⁺ (A, B) and R73⁺ (C through E) cells in the iris 26 days after MBP immunization. ED1⁺ cells are characterized by lysosomal staining (A, B) and R73⁺ cells by membrane staining (C through E). Both cell types accumulate near blood vessels in proximity with the nerves. (+), Myelinated nerves; arrowheads, nonmyelinated nerves. (D) High-power view of the lymphocyte in (C). Note the vesicles in some nonmyelinated nerves. L, vascular lumen; g, Golgi apparatus; m, mitochondrion. Bar, (A, C) 2 µm; (B, D, E) 1 µm.
lymphocytes. The other type of infiltrating R73+ cell had a large cytoplasm with multiple mitochondria, a well-developed Golgi apparatus and endoplasmic reticulum (Fig. 3E). Large protrusions were found on both types of R73+ cells.

Both infiltrating ED1+ and R73+ cells were observed in the stroma of the iris in proximity with myelinated and nonmyelinated nerve bundles (Figs. 3A through 3E) and in the vicinity of blood vessels (Figs. 3A, 3C). No evidence was found for nerve degradation or demyelination.

Most vascular endothelial cells had a normal appearance. At the site of intravascular or extravasated cells, however, endothelial cells possessed numerous small protrusions at the luminal surface (Fig. 3C). Signs of high endothelium-like venules were not found.

**Characteristics of Inflammatory T Cells in the Anterior Uvea**

The density and phenotype of infiltrating T cells in the iris during EAE was analyzed in a separate experiment using flow cytometry. For these experiments, the ICB was isolated and digested with collagenase to obtain a single-cell preparation. Few leukocytes were found in the ICB of CFA-treated control rats (Fig. 4A) or untreated rats (3.2% ± 1.0% of total ICB cells; mean ± SD). In accordance with the immunohistochemistry results, flow cytometry revealed that cellular infiltration of the iris began after remission of the clinical signs of EAE (Fig. 4B). At 28 days after immunization, the highest number of infiltrating OX1+ (CD45+; leukocyte) cells was found in the iris (19.9% ± 6.3% of total ICB cells). At day 21 the cellular infiltrate consisted predominantly of leukocytes not expressing R73 (αβTcR), whereas at later time points an almost equal number of R73+ and R73− leukocytes was found in the inflamed iris.

The majority of infiltrating αβTCR+ (R73+; Table 2) cells in the iris was CD4+ (Table 2). At the peak of disease (day 28 after immunization), 7.8% ± 4.5% of the total number of ICB cells were CD4+αβTCR+ cells, whereas 2.5% ± 1.5% were CD8+αβTCR+ cells. Only a minor proportion of the T cells expressed OX39 (CD25/IL-2 receptor [IL-2R]) or OX40, both markers for T-cell activation. Expression of OX39 was detected on approximately 10% to 14% of the CD4+αβTCR+ cells in the iris at the peak of disease. In most rats, OX40 was expressed on a smaller number of CD4+αβTCR+ cells than was OX39. Expression of OX39 was detected on a small number of CD8+αβTCR+ cells in the iris. At day 28 after immunization, only 2% of the CD8+αβTCR+ T cells expressed OX39.

**Discussion**

The present data show that AU developing after EAE was characterized by a massive infiltrate of monocytes-macrophages, T lymphocytes, and granulocytes. Furthermore, the infiltration of T cells began after the influx of monocytes-macrophages and consists of both CD4+ and CD8+ cells, of which only a small fraction expressed activation markers. The cellular infiltrates accumulated around both myelinated and nonmyelinated nerves according to TEM, but neither demyelination nor destruction of the iris was observed. These results are consistent with the findings of Verhagen et al. but differ slightly from those of Shikishima et al. and Adamus et al., who found a more vigorous response involving the iris, ciliary body, trabecular meshwork, and anterior chamber of the eye. Moreover, EAE and intraocular inflammation coincided in the latter studies. The discrepancy between these results may be attributable to the use of different rat strains, antigen preparations, dose of antigen, and adjuvant. Our observation that the cellular infiltration in the iris was focal is clearly distinct from the massive infiltration throughout the whole iris observed during EAU, EIU, and EMIU, when analyzed by similar methods.

Although it is plausible that EAE-associated AU is initiated after antigen presentation on MHC class II+ resident APCs in the iris to trace numbers of activated CD4+ T cells, the nature of the early APC events at the onset of EAE-associated AU is largely unknown. The iris contains extensive networks of MHC class II+ dendritic cells and macrophages. Although iris dendritic cells have strong antigen-presenting capacity for naive T cells after cytokine-induced maturation, they may be poor APCs in situ in the eye. Recently, it was found that resident macrophages isolated from the iris were more efficient APCs for activated primed T cells than dendritic cells. In the present study, resident dendritic ED1+ cells and OX6+ cells in the iris revealed little alteration in shape before the onset of EAE-associated AU, suggesting little or no activation of these cells. Infiltrating monocytes-macrophages (round ED1+ and OX6+ cells) appear to play an important role in the augmentation of EAE-associated AU. They were observed in the inflammatory foci in the iris before the influx of R73+ T cells, and their number increased dramatically during the course of AU. Macrophages are essential effector cells in EAE, EAU, and EIU. In EAE, specific elimination of macrophages produced both a significant suppression of the clinical signs of disease and a marked inhibition of CNS inflammation. In the present model, similar depletion experiments may reveal the role of iris infiltrating macrophages, as APCs or effectors or regulatory cells.

Only a small number of the infiltrating CD4+ T cells in the iris during EAE-associated AU were activated as reflected by CD25 expression (<14%) or OX40 expression (<9%). The latter is slightly lower than the result of Adamus et al., but this may be attributable to the removal of intravascular cells by whole-body perfusion before the isolation of the iris performed in the present study. These findings are in line with a number of studies of EAE. In the CNS of rats with EAE, only a minority of total T cells express CD25 or OX40, characterized by a massive infiltration of activated infiltrating T cells in the iris to trace numbers of activated CD4+ T cells, the nature of the early APC events at the onset of EAE-associated AU is largely unknown. The iris contains extensive networks of MHC class II+ dendritic cells and macrophages. Although iris dendritic cells have strong antigen-presenting capacity for naive T cells after cytokine-induced maturation, they may be poor APCs in situ in the eye.
cells with increased cytoplasm, shown by Shikishima et al., could not be confirmed in the present study. These authors also described adherent inflammatory cells on the luminal surface of the vascular endothelial cells and entrapment of cells in small vessels. The discrepancy between these results is most likely caused by the addition of *Bordetella pertussis* to the emulsion used for immunization by Shikishima et al. *B. pertussis* augments vascular permeability by increasing the sensitivity of endothelial cells to mast cell–derived vasoactive amines, and enhances EAE and EAU.

Several findings suggest that EAE-associated AU may result directly from MBP-specific T cells that target MBP in the iris. First, MBP is present in the myelin sheath of thick nerve fibers in rat iris. Second, T cells accumulate in proximity to myelin-
reactive T cells. Immunization with MBP or MBP peptides produces iridocyclitis, indicating that the antigen specificity of the uveitogenic T cells in this model can be determined. This animal model may help to elucidate the immunopathogenic mechanisms underlying this form of AU.

In summary, this study describes the characteristics of anterior uveitis in Lewis rats. The results show that infiltrating T cells accumulated in the vicinity of nonmyelinated nerve fibers in the iris before the onset of EAE-associated AU. This suggests that AU may result from autoreactivity to nerve antigens. Whether a similar response is involved in AU associated with MS has yet to be determined.

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References


Table 2. Flow Cytometry Analysis of Infiltrating T Lymphocytes in the Iris and Ciliary Body During EAE

<table>
<thead>
<tr>
<th>Time (d)</th>
<th>n</th>
<th>CD4⁺ T Cells⁺ (% of Total ICB Cells)</th>
<th>CD25/IL-2R⁺ Cells⁺ (% of CD4⁺ T Cells)</th>
<th>OX40⁺ Cells⁺ (% of CD4⁺ T Cells)</th>
<th>CD8⁺ T Cells⁺ (% of Total ICB Cells)</th>
<th>CD25/IL-2R⁺ Cells⁻ (% of CD8⁻ T Cells)</th>
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<td>Control 1§</td>
<td>2</td>
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<td>6.7 ± 0.6</td>
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<td>8.3 ± 11.8</td>
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<tr>
<td>Control 2</td>
<td></td>
<td>3</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>ND</td>
<td>0.1 ± 0.2</td>
</tr>
<tr>
<td>14</td>
<td>2</td>
<td>0.2 ± 0.1</td>
<td>7.8 ± 3.9</td>
<td>ND</td>
<td>0.1 ± 0.2</td>
<td>0 ± 0</td>
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<tr>
<td>21</td>
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<td>13.6 ± 7.2</td>
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<tr>
<td>38</td>
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ND, not determined.
⁺ CD4⁺ T cells were OX39⁻ cells.
⁻ CD25/IL-2R⁺ cells were OX39⁻ cells.
§ Control 1 consisted of normal Lewis rats.
|| Control 2 consisted of CFA-immunized rats killed at day 28.


