Macrophages and Dendritic Cells in IRBP-Induced Experimental Autoimmune Uveoretinitis in B10RIII Mice

Hui-Rong Jiang, Lynne Lumsden, and John V. Forrester

PURPOSE. To investigate the characteristics of the mononuclear cell infiltrate in murine experimental autoimmune uveoretinitis (EAU).

METHODS. EAU was induced by immunization with bovine interphotoreceptor retinal binding protein (IRBP) in Freund’s complete adjuvant (subcutaneous injection) and pertussis toxin (intraperitoneal injection) in B10RIII mouse. Then animals were killed on days 7, 9, 12, 15, 20, 26, and 39 after immunization. Eyes were processed for hematoxylin and eosin staining to characterize the disease and to assess the severity and extent of the EAU. Single and dual immunohistochemical staining in various combinations with monoclonal antibodies against CD45, CD4, CD8, major histocompatibility complex (MHC) class II, CD11c, NLDC-145, and a variety of macrophage markers was performed.

RESULTS. The authors’ results showed that vitritis, vasculitis and perivasculitis, retinal detachment, and granuloma formation in retina and choroid were the predominant features of IRBP-induced B10RIII mice EAU. Immunohistologic results showed that CD4+ T cells and macrophages were the main infiltrating cells in retina and choroid throughout the entire course of the disease. MHC class II negative macrophages expressing antigens reacting with MOMA-2, F4/80, sialoadhesin, and CD11b were prominent during the peak phase of tissue damage in the retina and choroid. Dendritic cells (DCs) characterized by dual positivity for MHC class II and CD11c and negative for sialoadhesin appeared at time of disease onset and continued to be recruited during the inflammatory process. DCs at the site of inflammation were NLDC-145 weak and CD8 negative, indicating that they were of the myeloid rather than the lymphoid lineage.

CONCLUSIONS. The results suggest that EAU in B10RIII mice is initiated by local-infiltrating, dendritic antigen-presenting cells, whereas tissue damage is associated with sialoadhesin-positive, phagocytic nonantigen-presenting macrophages during the effector stage. (Invest Ophthalmol Vis Sci. 1999;40:3177-3185)

Experimental autoimmune uveoretinitis (EAU) is a photoreceptor-specific autoimmune disease inducible in several susceptible animal models with a variety of retinal autoantigens.1 EAU resembles some human posterior uveoretinitis syndromes, including sympathetic ophthalmia, Vogt–Koyanagi–Harada disease (VKH), sarcoidosis, Behçet’s disease, and birdshot retinochoroidopathy.2,3 To date, there are at least five autoantigens4 that are uveitogenic in experimental animals, but the most widely studied and characterized antigens are S-ag (S-Ag) and interphotoreceptor retinoid binding protein (IRBP). Both are major components of the outer segment photoreceptor cells that are presumed to be the primary target of the autoimmune attack in EAU.

The rodent (Lewis rat) EAU model is the most commonly used model so far for investigations of mechanisms and for immunomodulation studies relevant to human ocular inflammation. This model is characterized by an acute or hyperacute onset, followed by diffuse retinal damage and necrosis, exudative retinal detachment and massive cellular infiltration within the anterior and posterior segments of the eye. However, most forms of human uveoretinitis usually are chronic or relapsing, and therefore, the model of Lewis rat is not considered to be wholly ideal.2,5 In contrast, the disease in guinea pig and mouse more closely resembles human posterior uveoretinitis.6 However, immunohistochemical studies that can provide useful information relevant to mechanisms of disease have been limited in both the mouse7 and the guinea pig.8

Studies in rat models suggest that macrophages play an important role in generating tissue damage in the course of experimental autoimmune diseases, such as collagen-induced arthritis,9,10 nephritis,11,12 thyroiditis,13 and experimental allergic encephalomyelitis (EAE). In uveoretinitis, it was reported2 that macrophages are engaged in phagocytosis of rod outer segments (ROS). Moreover, a depletion study in rats showed that the blood-borne, activated macrophages are major effectors of tissue damage during EAU,14 probably under the control of antigen-specific T cells and tumor necrosis factor...
alpha (TNFα). In addition, tissue damage appears to be mediated at least in part by release of free radicals, particularly nitric oxide. Further investigation of macrophage subpopulations in some autoimmune diseases has shown that macrophages expressing distinct antigens function differently in the early or late stages of the diseases.

Macrophages also are considered to be potent antigen-presenting cells (APCs) in inflammatory autoimmune disease. However, recent interest in dendritic cells has centered on their essential role as initiators of disease. We have shown previously that DCs reside in normal choroidal tissue, particularly close to the retinal pigment epithelium (RPE) at the chorioretinal interface, and have suggested that these cells are responsible for the initiation of granulomatous lesions at the target photoreceptor site.

Previous studies have documented the numbers of macrophages and dendritic cells that infiltrate the choroid of the rat eye during EAU. However, no information on dendritic cell infiltration into the retina has yet been provided in any animal model of EAU. DCs are absent from the normal retina, and the nature of the APCs initiating retinal disease as opposed to choroidal inflammation has variously been attributed to microglia and a small population of perivascular macrophages, as has been suggested for the central nervous system parenchyma. The aim of this study therefore was to determine whether dendritic cells infiltrated the retina during the development of EAU and to evaluate differences in phenotype between DCs and macrophages.

**METHODS**

**Antigen**

IRBP was prepared as previously described. Briefly, interphotoreceptor matrix was loaded onto a concanavalin A (ConA)–Sepharose affinity chromatography column (Pharmacia, Uppsala, Sweden), and crude IRBP was eluted using Tris-Cl/0.15 mM NaCl/1mM CaCl/1mM MnCl/0.2 mM methyl-D-mannopyranoside, pH 7.5 (Sigma, Poole, UK). Further purification was achieved using a Sepharose high-performance chromatograph (Pharmacia) and mannose agarose affinity column (Sigma) to remove contaminating ConA. The purified IRBP was dialyzed against phosphate-buffered saline (PBS), and then the concentration of the IRBP was tested by Coomassie Protein Assay Reagent (Pierce, Chester, UK). The purified IRBP produced a single band on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (PAGE) and was aliquoted and stored at −20°C before use.

**Animals**

Inbred female and male B10RIII mice 10 to 12 weeks of age were obtained from the animal facility at the medical school, University of Aberdeen. The procedures adopted conformed to the regulations of the Animal License Act (UK) and to the ARVO Statement for the use of Animals in Ophthalmic and Vision Research.

**Immunization and Evaluation of Disease**

Mice were immunized subcutaneously (SC) with 100 μg IRBP emulsified with an equal volume of Freund’s complete adjuvant (CFA, H37Ra; Difco Laboratories, Detroit, MI) in a total volume of 300 μl. An additional intraperitoneal injection of 0.5 μg of purified Bordetella pertussis toxin (PTX, Strain Wellcome 28; Wellcome, Speywood, UK) in 250 μl was also given to each animal. Control mice were immunized with the same volume of PBS instead of IRBP in CFA and PTX.

Male animals were killed and their eyes (6 eyes at the early 6 time points and 4 eyes at the day 39) were removed at days 7, 9, 12, 15, 20, 26, and 39 after primary immunization. One eye from the early 6 time points, and four eyes from day 39 were fixed in 2.5% buffered glutaraldehyde and embedded in paraffin for standard hematoxylin and eosin staining. The remaining eyes were frozen in OCT immediately to obtain frozen sections. The intensity of uveoretinitis was evaluated histologically and was graded by independent observers. A slightly modified version of the customized histologic grading system established in this laboratory for rat EAU was used. This grading system permits a semiquantitative assessment of the severity and extent of both infiltrative and structural/morphologic changes of the uveoretinitis at various points throughout the course of EAU. Four additional male and female mice were immunized and killed on day 15 for a gender difference study.

**Antibodies and Immunohistology**

Sections from each eye were incubated with a panel of rat anti-mouse primary monoclonal antibodies (mAbs). The specificities of the mAbs as mouse leukocyte markers and the criteria for distinguishing macrophages from dendritic cells are listed in Table 1.

For immunostaining, 8-μm cryostat sections were first fixed with 100% acetone and then rehydrated in Tris-buffered solution (TBS), followed by incubation with the primary monoclonal antibodies diluted appropriately in TBS containing 1% bovine serum albumin for at least 1 hour. Samples were then treated with biotinylated rabbit anti-rat antibody containing 10% mouse serum, to avoid background staining. Samples were then incubated with streptavidin-alkaline phosphatase (AP) or streptavidin-horse radish peroxidase (HRP). All three were from DAKO Ltd (Cambridge, UK). Finally, the signal was visualized with substrates 3-aminolevulinic acid (Alcian Blue) for HRP, producing a red/brown color, and naphthol AS-BI phosphate with New Fuchsin in Tris buffer (pH 9.7) for AP, producing red color. Levamisole was added to the AP substrate to block endogenous alkaline phosphatase activity; all the chemicals were from Sigma. Both second and third antibodies were incubated with the tissue for thirty minutes. All procedures were at room temperature.

Dual fluorescent staining was used to investigate the co-expression of leukocyte markers on macrophages and APCs in the inflammatory tissues. Sections were prepared according to the above procedures for color staining, except that the streptavidin Texas red (TR) or streptavidin FITC was applied to each animal. Male animals were killed and their eyes (6 eyes at the early 6 time points and 4 eyes at the day 39) were removed at days 7, 9, 12, 15, 20, 26, and 39 after primary immunization. One eye from the early 6 time points, and four eyes from day 39 were fixed in 2.5% buffered glutaraldehyde and embedded in paraffin for standard hematoxylin and eosin staining. The remaining eyes were frozen in OCT immediately to obtain frozen sections. The intensity of uveoretinitis was evaluated histologically and was graded by independent observers. A slightly modified version of the customized histologic grading system established in this laboratory for rat EAU was used. This grading system permits a semiquantitative assessment of the severity and extent of both infiltrative and structural/morphologic changes of the uveoretinitis at various points throughout the course of EAU. Four additional male and female mice were immunized and killed on day 15 for a gender difference study.

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Macrophages and Dendritic Cells in Uveoretinitis

**RESULTS**

Characteristics of B10RIII Murine EAU

In a preliminary experiment, the peak of severe EAU was observed 15 days after the immunization, using the immunization protocol above. Accordingly, to study the development of the inflammatory changes and the infiltration of leukocyte subtypes into the retina, eyes were enucleated 7, 9, 12, 15, 20, 26, and 39 days after immunization. Histologically, no disease was seen in any control mice eyes (Fig. 1A), whereas the IRBP-immunized eyes showed typical features of EAU (Figs. 1B, 1C): retinal vasculitis and perivasculitis with mild-to-severe cuffing of infiltrating cells, marked invasion of large mononuclear cells in the photoreceptor layer and granuloma formation in the retina and choroid, local folds and exudative detachment of retina, and subretinal neovascularization. Mild-to-severe thickening of choroid also was seen frequently. At the peak of the disease, anterior chamber inflammatory cells and thickening and cellular infiltration in iris and ciliary body also were observed (not shown). During the resolution phase, epithelioid cells and multinucleate giant cells with intracellular melanin appeared in the different layers of the retina, particularly in the ROS layer (Fig. 1D). The ROS layer became partially or completely atrophic as did the neuronal layer and choroid at the late stages. In the very late stage of day 39 (Fig. 1E), the retina became largely degenerate and disorganized, being reduced to a few resident retinal cells and fibroblast-like cells. During the course of the disease, the patchy nature of the retinal inflammation was apparent and early infiltrative lesions with late fibrotic stages of disease were seen at the same time in the same eyes. This is similar to some human posterior uveoretinitis and other models of EAU.

Kinetics of IRBP-Induced B10RIII Murine Disease

Using the methods described above, we found that the incidence of IRBP-induced EAU was 96% (from 12 days to 39 days after immunization). This is in agreement with previous reports in murine EAU. However, we noted that male mice were slightly more susceptible (with 100% incidence and severity grade of 8.3) than female mice (with an incidence of 87.5% and disease severity grade of 6.5). IRBP-induced EAU first appeared on day 9 after the immunization, with 50% of the eyes showing small patchy folds and a few infiltrating cells in retina and choroid. The disease reached a peak by day 12 to day 15 with severe infiltration and structural damage. By three weeks (days 21 to 26) the disease remained active, but there was less inflammatory cell infiltration and more extensive retinal degeneration. Eventually by day 39, inflammation was minimal and the concomitant tissue necrosis was followed by reparative fibrosis and extreme thinning of the retina and choroid. At this stage the structure damage score was the main feature compared with the decreasing infiltrating score. Both the incidence and the intensity of the disease on the above time points were graded using a semiquantitative assessment system and are shown in Figure 2.

**Phenotype of Inflammatory Cells in B10RIII Mice EAU**

At the onset of the disease (day 9), CD45+ cells were observed in the peripheral retina and to some extent also in the central posterior retina (Fig. 1G). In addition, these cells comprised CD4+ T cells and MHC class II+ cells (Fig. 1F) which were mainly around the vessel in the ganglion cell layer, whereas MOMA-2+ (Fig. 1H) and CD8α+ (Fig. 1I) and CD11b+ macrophages were also seen round the vessels or in the other retinal layers between the ganglion cell layer and the ROS layer. Sialoadhesin + cells were absent at this stage of disease. Large numbers of inflammatory cells with the different markers were observed at the peak of the disease (Figs. 3A–3E). CD4+ cells were mainly distributed around the vasculature or in the granuloma, whereas CD8+ cells were only seen occasionally (Fig. 3F). In contrast, there was a marked increase of the number of monocytes and macrophages expressing MOMA-2, F4/80, CD11b, and sialoadhesin in all the inflammatory areas of the posterior segment as well as the anterior chamber angle, iris, and ciliary body. Some macrophages persisted in the retina, especially in the photoreceptor layer, throughout the late course of the disease.

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**TABLE 1. Anti-Mouse Monoclonal Antibodies and the Criteria for Distinguishing Macrophages from Dendritic Cells**

<table>
<thead>
<tr>
<th>Antibodies to Mouse Inflammatory Cells</th>
<th>Macrophages</th>
<th>Myeloid Dendritic Cells</th>
<th>Lymphoid Dendritic Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>F4/80 (C1:A3-1)</td>
<td>++/−</td>
<td>+/−</td>
<td>−</td>
</tr>
<tr>
<td>Sialoadhesin (3d6.112)</td>
<td>++/−</td>
<td>−/−</td>
<td>−</td>
</tr>
<tr>
<td>MOMA-2</td>
<td>+/−</td>
<td>++/−</td>
<td>+/−</td>
</tr>
<tr>
<td>CD11b (M1/70)</td>
<td>++/−</td>
<td>+/−/−</td>
<td>+/−</td>
</tr>
<tr>
<td>MHC class II (P7/7)</td>
<td>++/−</td>
<td>++/−/−</td>
<td>+/−</td>
</tr>
<tr>
<td>CD11c (N418)</td>
<td>++/−/−</td>
<td>+/−</td>
<td>+</td>
</tr>
<tr>
<td>NLDC-145</td>
<td>++/−</td>
<td>−/−</td>
<td>+</td>
</tr>
<tr>
<td>CD8α (53-6.7)</td>
<td>−/−</td>
<td>+/−</td>
<td>+</td>
</tr>
</tbody>
</table>

All the antibodies including CD45 (IBL-3/16) were from Scotec, except CD4 (L3T4, H129.19) and CD8α (53-6.7), which were from Pharmingen. −, no expression; +/−, no expression or only a small percentage of cells weakly expression; +, low or no expression; ++, expression; ++++, strong expression; ?, not known. Cell specificity data taken from ref. 28. The table indicates that macrophages normally stain with any of the first antibodies in the first column but need not necessarily stain with all these antibodies at the same time (see Results). Conversely dendritic cells (DCs) stain with the last three antibodies in the third column but also not necessarily inclusively because lymphoid and myeloid DCs stain differentially (see Results). MHC class II and CD11b may stain both DCs and macrophages, whereas a small population of (DCs) may also be positive for F4/80.
(Figs. 3B–3E). No clear difference was seen at the peak or late stages of the disease in the location, distribution, and morphology of cells stained with the various antimacrophage markers: MOMA-2, F4/80, sialoadhesin, and CD11b. It seems that although each of these four molecule labels certain subsets of the macrophages, the exact difference between them, especially the functional significance of these markers is unknown. Dendritic cells identified specifically as positive staining with MHC class II and CD11c were observed from day 9 and became more frequent at day 12 or day 15 (Fig. 3n). DCs were present in both retina and choroid, particularly related to granulomas. The numbers of DCs decreased in the late stages. The distribution of the different immune cells in the disease is shown in Table 2.

**Phenotypic Characterization of Macrophages and Dendritic Cells in EAU**

Dual fluorescent immunostaining was used to characterize macrophages and dendritic cells in this study of murine EAU. The majority of macrophages were CD11b, MOMA-2, F4/80+, and many were also sialoadhesin+ (Fig. 3g) but were MHC class II negative. However, we also found occasional CD11b, F4/80, and even MOMA-2+positive cells, which coexpressed MHC class II (Fig. 3j). This supports the previous findings in rat normal retina that a subpopulation of the MHC class II+ cells are ED2+ resident macrophages. Cells expressing high levels of MHC class II+ but lacking macrophage markers were deemed to be dendritic cells if they coexpressed CD11c (Figs. 3i–3n), and their appearance in the retina indicated that they
were newly recruited DCs, usually arriving through the retinal vessels and probably important in the initiation of the disease.

Double immunohistochemical staining further demonstrated that MHC class II$^1$ cells were NLDC-145$^{-}$ and CD8a$^{-}$ (Fig. 3i), both of which are specific markers for lymphoid DCs rather than myeloid DCs. Table 3 summarizes the colocalization data between MHC class II and the other phenotypes in the inflammatory cells in this study of murine EAU.

Because the sialoadhesin$^1$ macrophages were consistently negative for MHC class II, colocalization studies were performed to further characterize the macrophage subpopulations at the peak of disease (day 15). Sialoadhesin$^+$ macrophages were also consistently negative for CD11b (and CD11c, a dendritic cell marker). However, approximately 50% and 70% of F4/80$^+$ cells were dual-positive for sialoadhesin and CD11b, respectively. In addition, similar percentages of MOMA$^+$ cells were dual-positive for sialoadhesin and CD11b, respectively. In summary, therefore no coexpression of sialoadhesin was found with either MHC class II or with CD11b, but coexpression of any dual combination of F4/80, MOMA, and sialoadhesin or F4/80, MOMA, and CD11b was observed. This suggested that F4/80, MOMA are common macrophage markers, whereas MHC class II/CD11b and sialoadhesin are mutually exclusive macrophage differentiation markers.

**DISCUSSION**

The murine model of EAU was established 10 years ago$^6$ and is thought to be a more representative model for therapeutic approaches to human posterior uveitis. Genetic susceptibility and resistance to EAU,$^{29}$ identification of uveitogenic epitopes,$^{30}$ and other studies of tolerance in EAU by using anterior chamber associated immune deviation$^{31}$ or oral tolerance regimes$^{32–34}$ have been performed in this model. It was reported$^{29}$ that B10 background mice strains have the highest positive responses to IRBP-induced experimental autoimmune uveoretinitis, and the H-2r haplotype was the most susceptible strain of mice in IRBP-induced EAU. Our results confirmed previous findings$^{29,30}$ of the susceptibility of the IRBP induction in B10RIII mice. Moreover, we found that the time of onset was earlier in this model than previous reports of murine EAU.$^6,29$ By transferring S-Ag–specific T cells in rat, Prendergast et al.$^{35}$ found that the anterior segment inflammation occurs more rapidly than does retinal inflammation. This may be reflected in our results, whereby the onset time of this disease is much earlier than B10A murine EAU, which has minimal involvement of anterior segment.$^6$

The pathologic findings of the later phase of the disease show considerable similarity to the reports of human sympa-
thetic ophthalmitis, VKH syndrome, or Behçet’s disease. Multinucleated giant cells with pigment phagocytosis and proliferation of the retinal pigment epithelium together with glial cell hyperplasia containing phagocytosed uveal pigment were present in both the human atrophic retina and the current model of EAU (Fig. 1).

Experiments using CD4+ T-cell lines to adoptively transfer EAU in Lewis rats or mice and CD4+ T-cell depletion before inducing EAU in rats have successfully demonstrated a pivotal role for CD4+ T cells in the induction of EAU. MHC class II–expressing APCs are presumed to present autoantigens to CD4+ T cells, which then activate the effector cells such as macrophages, leading to tissue damage. Our study fits this model. However, the initial site of inflammatory cell infiltration into the eye remains controversial. There is evidence that supports the suggestion that the uveal tract is the initial site of EAU. Furthermore, the presence of a rich network of MHC class II (Ia)–expressing APCs in the uveal tract, and their absence from the neural retina suggests an active role by these cells in disease induction. However, our immunohistologic findings suggest that the disease also may be initiated from the vessels in the inner layer of retina, where Ia+ cells and CD4+ T cells accumulated first around the vessel and were seldom seen in the other parts of the retina at day 9 after immunization. By that time the T-cell–activated blood-borne macrophages expressing MOMA-2, F4/80, and/or CD11b moved directly to the target site of the inflammation, that is, the ROS layer (Fig. 1).

**FIGURE 3.** AP immunohistochemical staining of mouse EAU at peak (days 12–15) and late stages of the disease (days 20–39) with New Fuchs (A to F). (A) Large CD45+ cells in ROS layer; (B) peak of EAU (day 15), sialoadhesin-expressing cells in the granuloma in ROS layer (arrow); (C) large numbers of F4/80+ cells in EAU at peak of the disease (day 15); (D) MOMA-2+ cells in choroid and ROS layer, some showing melanin granules in the cytoplasm; (E) CD11b+ cells with intracellular melanin appearing to migrate from choroid through retina; (F) scattered CD8+ positive cells were only seen occasionally in mouse EAU (day 20). (g to n) Dual fluorescent immunohistochemical staining of inflammatory cells in mouse EAU tissue. (g and h) Dual staining for MHC class II (FITC, green) and sialoadhesin (TR, red). In (g) there is no coexpression of the antigens on the cells (MHC-II+ cells, arrow; sialoadhesin+ cells, arrowhead) in the inflammatory tissue; in (h) only, MHC class II–positive staining was detected on cells in the inner nuclear layer; (i) dual staining for MHC class II (FITC, green) and CD8α (TR, red), showing lack of coexpression on individual cells in mouse EAU tissue; (j) MHC class II (FITC, green) and CD11b (TR, red), showing double-positive dendritic-shaped cells (orange) in the inner nuclear layer of retina; (k, l, m) dual staining for MHC class II (green) and CD11c (red), showing coexpression of both antigens (brown) in the outer nuclear layer of retina.
Table 2. Phenotype of Inflammatory Cells at Different Times in the EAU Eyes

<table>
<thead>
<tr>
<th></th>
<th>CD45</th>
<th>CD4</th>
<th>CD8α</th>
<th>MHC-II</th>
<th>CD11c</th>
<th>MOMA-2</th>
<th>F4/80</th>
<th>Sialoadhesin</th>
<th>CD11b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset (P19)</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Peak (P12–15)</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Resolution (P20–26)</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Infiltration of inflammatory cells in the EAU eyes at various times. −, No cells; +, a few cells; ++, moderate numbers of cells positive; ++++, many cells positive; ++++, extensive cell positivity.

Table 3. Coexpression of Two Different Antibodies on Macrophages or Dendritic Cells in the Inflammatory Retina with Dual Staining

<table>
<thead>
<tr>
<th>Abs Used for Dual Staining</th>
<th>F4/80/ MHC II</th>
<th>Sialoadhesin MHC II</th>
<th>MOMA-2 MHC II</th>
<th>CD11b MHC-II</th>
<th>CD11c MHC-II</th>
<th>CD8α MHC-II</th>
<th>NLDC-145 MHC-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset (P19)</td>
<td>(+)</td>
<td>−</td>
<td>+/−</td>
<td>+</td>
<td>++</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Peak (P12–15)</td>
<td>(+)</td>
<td>−</td>
<td>+/−</td>
<td>+</td>
<td>++</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Resolution (P20–26)</td>
<td>+/−</td>
<td>−</td>
<td>+/−</td>
<td>+/−</td>
<td>++</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

−, No colocalisation; +/−, very few cells sharing colocalisation; (+), a few cells sharing colocalisation; +, some cells sharing colocalisation; ++, most cells sharing colocalisation.

the route of entry for DCs or how and where the DCs become primed to the autoantigen. One possible explanation is that DCs in the peripheral circulation may be recruited to sites around the retinal vessels via upregulated adhesion molecule expression and chemokine release by retinal endothelial cells induced by antigen-specific T cells that have been primed initially in the peripheral lymphoid tissues (i.e., systemic priming of autoreactive T cells has occurred for instance via cross-reactive antigen).

Macrophages are important at different stages of EAU and macrophage heterogeneity is well established.1 In addition to the normal populations of resident macrophages,45 we have observed in wholemount preparations that MOMA-2 positive macrophages are present in a perivascular location in normal B10RIII mice choroid (not shown). The role of infiltrating macrophages in the immunopathology of experimental allergic encephalomyelitis, both in directly mediating damage to the central nervous system and in attracting other cells to lesions, is well accepted44,45 and is supported by depletion studies.46 Similar results have been demonstrated in rat EAU.14 In these studies, the number of sialoadhesin-positive macrophages appeared to increase in the later stages of disease, whereas MOMA+ and F4/80 cells appeared at an earlier time point.18,19,45,46 In the present study, MOMA-2, F4/80+ cells occurred in the early stages of EAU, whereas sialoadhesin+, MHC class II negative cells were more prominent later, suggesting a non–antigen-presenting role for macrophages in EAU and perhaps a downregulatory role, as proposed in other systems for the subset of sialoadhesin-positive macrophages.47

The present study also indicates the relationship between macrophages, dendritic cells, and antigen presentation. Three populations of nonlymphoid mononuclear cells were observed: (a) MHC class II positive cells, which coexpressed some macrophage markers (F4/80, CD11b, and MOMA-2); (b) MHC class II positive cells, which were negative for all macrophage markers but included a population of cells coexpressing CD11c; and (c) MHC class II negative cells, which strongly expressed macrophage markers, especially sialoadhesin. Cells included in groups (a) and (b) were present at all stages of active inflammation, whereas cells of group (c) were present usually at later stages of the disease.

APCs present exogenous antigen, including autoantigen, via MHC class II, and the most potent of these is the myeloid dendritic cell.48 Myeloid dendritic cells in the mouse are frequently characterized on the basis of their coexpression of MHC class II and CD11c surface antigens.28 Accordingly, we believe that, of the three groups of cells detected in EAU, cells in group (b) represent dendritic cells. Similarly cells in group (c) represent macrophages and are not involved in antigen presentation because they lack MHC class II. Cells in group (a) thus represent an intermediate group of cells because they express surface markers common to both dendritic cells and macrophages.

Myeloid dendritic cells are derived from precursors in the bone marrow that circulate to the tissues via the blood stream.28 Depending on the cytokine milieu that they experience in the tissues, such precursor cells may differentiate into antigen-presenting dendritic cells or into phagocytic macrophages.48-51 We suggest that cells in group (a) above, identified from the earliest stages of EAU, represent early differentiating precursor cells, which may either (a) lose their macrophage markers and develop into mature dendritic cells engaged in antigen presentation and initiation of the immune responses, or (b) downregulate their MHC class II antigen and develop into phagocytic, sialoadhesin-positive, tissue-damaging macrophages. These observations also support the view that sialoadhesin is not involved in antigen presentation. Which route the precursor cells follow will be determined by the cytokine milieu at different stages of the inflammation, which would appear to be different from early to late stages of the disease. We thus believe that bone marrow–derived myeloid, but not lymphoid41,52 (Fig. 3i) dendritic cells are the major APCs in EAU and that macrophages do not function significantly as APCs but act rather as effector cells and scavengers.
References


