IFN-γ and LPS-Mediated IL-10–Dependent Suppression of Retinal Microglial Activation

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PURPOSE. Human retinal microglia (MG) express constitutively major histocompatibility complex (MHC) class II molecules and have thus been highlighted as potential immunocompetent antigen-presenting cells (APCs). This study was undertaken to characterize microglial expression of coaccessory molecules and the functional changes in antigen expression, cytokine production, migration, and phagocytosis after proinflammatory stimulation.

METHODS. Fresh donor retinal MG were obtained and isolated using a percoll density gradient technique. Phenotypic characteristics used for isolating rodent microglia were applied and modified. Coaccessory molecule expression and intracellular cytokine production were assessed using three-color flow cytometric analysis in both freshly isolated and interferon (IFN)γ-lipopolysaccharide (LPS)–stimulated MG. Using five-millimeter retinal explants in culture, microglial migratory behavior, changes in cell surface antigen expression and phagocytic activity were documented.

RESULTS. MG could be clearly defined by the flow cytometric phenotype CD45lowCD11b+MHC class II+CD86lowCD40low. Freshly isolated MG showed mannose receptor–mediated uptake of dextran-FITC. MG migrated from explants, were adherent, and upregulated MHC class II expression. After IFNγ-LPS stimulation of single-cell suspension of MG isolates, MHC class II expression was reduced, with an increase occurring in MG intracellular interleukin (IL)-10 and IL-10 production. Microglial migration from explants was reduced after IFNγ-LPS stimulation.

CONCLUSIONS. These results highlight both phenotypic and behavioral characteristics that support an antigen-processing and -presenting capability of freshly isolated MG. However, proinflammatory stimulation with IFNγ-LPS induces an IL-10–mediated downregulation of cell surface antigen expression and loss of migratory and phagocytic activity. Therefore, although equipped to act as APCs, MG are able to rapidly modulate their own function and behavior and as a result may have the potential to limit inflammation. (Invest Ophthalmol Vis Sci. 2000;41:2613–2622)
(EAE).

Although MG activation is not likely to be responsible for disease onset, the role of MG as cytotoxic effector cells is central to the pathologic changes observed in such disorders. As a result of such studies, CNS MG activation has been characterized by increased expression of MHC class II and B7,

expression of Fc and complement receptor, and generation of reactive oxygen species.

However, functionally, species differences are seen. For example, presentation of alloantigen by mouse CNS MG is limited to CD8+ T cells. Moreover, there are distinct differences between freshly isolated CNS MG and cultured MG characterizing functions, exemplified by differing proliferative capacity and cytokine responsiveness. Furthermore, in contrast to cultured MG, freshly isolated adult MG are not mitotically active and do not proliferate in response to granulocyte macrophage–colony-stimulating factor (GM-CSF) or monocyte (M)-CSF because of the paucity of suitable tissue and low numbers of purified retinal MG obtained from retina for functional T-cell assays, there are few data to describe APC function or compare and contrast function with CNS MG. Therefore, the study’s purposes were to isolate and characterize phenotype of human retinal MG by FC analysis and determine the correlation of the response of freshly isolated MG to proinflammatory stimuli with phenotype, costimulatory molecule expression, intracellular cytokine production, migration, and phagocytic activity.

**Methods**

Donor tissue was received with consent and in accordance with the tenets of the Declaration of Helsinki (after removal of corneas for transplant) from the Amsterdam Eye Bank or the Manchester Eye Bank or from the Aberdeen University Pathology Department after autopsy. Postmortem times varied according from 4 to 76 hours. Viability of MG preparations were poor if postmortem time was greater than 48 hours or donor age was more than 70 years (see the Results section).

Eyes were stored at 4°C until MG isolation was undertaken. For each experiment a minimum of two eyes (single donor) to a maximum of six eyes were used. There was no effect on cell surface expression when donors were combined. Using Mann–Whitney analysis, P < 0.05 was considered significant.

**Monoclonal Antibodies**

Mouse monoclonal antibodies (mAbs) for human surface markers (see Table 1) were supplied by PharMingen–Becton Dickinson (San Diego, CA). mAbs were used either in purified form or directly conjugated to either fluorescein isothiocyanate (FITC), R-phycocerythrin (PE), or biotin as required. Biotin antibodies were labeled by streptavidin-allophycocyanin (SA–APC; PharMingen) for subsequent FC detection. PE-conjugated mouse or rat mAbs against the human cytokines interleukin (IL)-2, IL-4, and IL-10; FITC-conjugated interferon (IFN)–γ; and tumor necrosis factor (TNF)–α mAbs were also supplied by PharMingen.

**Isolation of Microgla**

Eyes were dissected by removal of the iris, lens, and vitreous. The vitreous was mechanically removed, including stripping the hyaloid face (if required), with pipette. After removal of the retina, the tissue and any remnants of posterior hyaloid face were disrupted mechanically by passing the vitreous through a metal sieve (250 μm). The resultant single-cell suspension was washed twice in 1% bovine serum albumin (BSA)–phosphate-buffered saline (PBS) followed by enzyme treatment with collagenase (5 U/retina) and DNase (15 U/retina; Boehringer–Mannheim, Mannheim, Germany) for 30 minutes at 37°C. During the course of digestion, cells were gently agitated by use of a sterile pipette. After further washing, MG were further purified by passing over a percoll density gradient, as previously described. Cell viability was determined by trypan blue exclusion. In some experiments, samples were further purified by positive selection against CD11b using labeled magnetic microbeads (Miltenyi Biotech, Auburn, CA).
were resuspended in buffer (2 mM EDTA/0.5% BSA-PBS) and incubated with MACS CD11b microbeads for 15 minutes at 4°C. Cells were then washed in buffer and resuspended in 500 μl for magnetic separation. The sample was pipetted onto the column, and the negative cells washed through. CD11b-positive cells were then flushed from the column and collected according to the manufacturer’s instructions. Approximately 75% MG purity was obtained.

**FC Analysis**

MG isolated as described were labeled with mAbs for cell surface antigen expression or for intracellular cytokine production. Specific mAbs were added at optimized concentrations (10 μl undiluted/10^6 cells for surface antigen mAbs, and 1:25 dilution per 25 μl per test dilution for intracellular mAbs) after blocking of Fc receptors by use of 10% heat inactivated (HI) normal human serum and 10% normal mouse (or rat) serum. Cells were meticulously washed in buffer (FACS buffer, containing 1% BSA-PBS and 10 mM NaNO₃; Becton Dickinson) between steps to minimize background staining. MG were analyzed directly ex vivo or incubated overnight in glucose-enhanced tissue culture medium (TCM, containing 5% FCS, RPMI, and penicillin-streptomycin), with or without IFNγ (100 U/ml) and lipopolysaccharide (LPS; 5 μg/ml). All mAbs and buffers were maintained at 4°C, and all incubations were performed on ice. Negative controls were isotype matched, and nonspecific cytokine staining was determined by the use of recombinant cytokine blocks. Before intracellular staining, cells were incubated for 4 hours with GolgiStop (PharMingen), containing monensin, to allow the accumulation of intracellular cytokines. A fixation and permeabilizing step using Cytofix–Cytoperm (PharMingen) was performed before addition of specific cytokine antibodies, as described. A permeabilizing step was also required during staining for Ki67. Cell acquisition was performed on FACScalibur (Becton Dickinson) after background fluorescence and forward- and side-scatter parameters were set. Analysis was performed by computer (CellQuest).

**IL-10 Assay**

Single-cell intracellular cytokine secretion was assessed on MG isolates. MG were preincubated for 1 hour in TCM alone or TCM containing neutralizing anti-IL10 antibody (5 ng/ml) or human recombinant IL-10 (100 U/ml). Cells were then incubated overnight with IFNγ-LPS as described earlier. Surface antigen expression was determined by three-color FC analysis and recorded as mean fluorescence intensity (MFI). Levels of IL-10 within the supernatants of duplicated MG cultures were determined using a human IL-10–capture enzyme-linked immunosorbent assay (ELISA) kit (OptEIA; PharMingen). Standards were prepared from stock human recombinant IL-10 by serial dilution steps, as recommended. Supernatants were not diluted. From three individual experiments, assays were repeated to confirm results. Absorbance was read at 450 nm (corrected for 570 nm) within 30 minutes of stopping the final reaction with 2 N H₂SO₄. Concentrations of IL-10 were determined by computer (Biolinx2 software; Dynex Technologies, VA).

**Retinal Explants**

Eyes were dissected as before and the retina placed into a sterile petri dish containing RPMI. Retinal discs (5 mm in diameter) were removed using a 5-gauge needle and placed into 24-well plates (1 disc/well) in triplicate with TCM or TCM containing IFNγ (100 U/ml) and LPS (5 μg/ml), with or without neutralizing IL-10, or recombinant human IL-10 (100 U/ml). Each of the wells was observed daily. Supernatants were removed over days 1 through 4 for ELISA estimation of cytokine production. Three groups of cells were observed within the wells: nonmigrating cells within the explant tissue (expant cells); migratory nonadherent cells within the supernatant (supernatant cells), and cells that had both migrated from the tissue and become adherent (adherent cells). Each cell type was harvested from the triplicate wells, and cells were pooled to achieve adequate cell numbers for FC analysis to compare differences in cell type, distribution, and proliferation. Explant cells were prepared by passing the tissue through a 250-μm metal sieve to obtain a single-cell suspension. Adherent cells were removed by 10-minute treatment on ice with 2 mM EDTA. In addition, to investigate the ability of MG to pinocytose, a final concentration 2 mg/ml of dextran-FITC (Sigma-Aldrich, Poole, UK) was added to the wells at 37°C for 1 hour before removing the cells. Uptake was determined by FC analysis and confirmed using cytoxin preparations and fluorescence microscopy. Mannose receptor (ManR)–dependent pinocytosis was blocked by prior incubation with final concentration of 0.5 mg/ml of the receptor inhibitor mannan (Sigma-Aldrich) at 37°C for 20 minutes.

**Immunohistochemical Analysis**

Donor eyes were cut into small sections consisting of sclera, choroid, and retina and fresh frozen in optimal temperature cutting compound (OCT; Miles, Elkhart, IN). Alternatively, the retina was first removed from the choroid and then fresh frozen in OCT. Serial sections were cut, air-dried, and acetone fixed. Blocking with normal horse serum and for endogenous avidin and biotin prevented background staining. The primary antibody was mouse anti-human CD11b (PharMingen 1:20). The secondary antibody, a biotin-labeled horse anti-mouse IgG (Vectorstain; Vector, Burlingame, CA), was added and visualized using streptavidin and biotinylated horseradish peroxidase complex (sABC) and diaminobenzidine tetrahydrochloride (DAB), according to the manufacturer’s instructions. The process was then repeated using the required second primary antibody at previously established optimal dilutions (CD45 1:50, MHC class II 1:100, CD40 1:25, CD86 1:50, and CD68 1:100). The secondary biotin-labeled antibody was this time visualized using sABC and an alkaline phosphatase–anti-alkaline phosphatase (AAPA) substrate, according to the manufacturer’s instructions. Negative controls were IgG isotype matched. Sections were lightly counterstained in dilute hematoxylin. For fluorescence staining, primary antibodies were labeled using goat anti-mouse FITC (Serotec, Oxford, UK) and sections counterstained using π mounting fluid (Apoptag Kit; Oncor, Gaithersburg, MD).

**RESULTS**

After percoll separation, a semipure population of MG was obtained from the interface. Confirmation of MG isolation was obtained by three-color FC analysis based on an established MG cell surface phenotype of CD11b^+CD45^low. A 75% pure population of MG was further achieved after positive selection.
against CD11b, using magnetically labeled microbeads. The ability to obtain a pure population using microbead purification decreased with increase in donor age and postmortem time (data not shown), and therefore these cells were not used for functional analysis. Either donor age of more than 70 years or postmortem time of more than 48 hours resulted in poor cell viability after overnight culture (>60% death). In the remaining samples deemed adequate for further experimental analysis, cell viability was well maintained independent of donor age or postmortem time. After isolation (postpercoll gradient separation), apoptosis or death as assessed by FC was seen in only 30% of retinal cells (annexin V/propidium iodide) and further purification of single cell isolates with microbeads enriched viable MG population in experiments to 75% purity. In addition, throughout the experiments there was no apparent correlation between donor age and effects of subsequent IFNγ-LPS stimulation or any differences in cell surface antigen expression, whether MG was isolated from a single donor or multiple donors.

**Directly Isolated Ex Vivo MG Express CD86 low and CD40 low**

Noncultured, directly isolated retinal MG, similar to CNS MG, have been shown to possess an in vivo phenotype of CD45/CD11b+10,20 By using such a phenotype (Fig. 1A), costimulatory molecule expression on directly isolated (resting) ex vivo MG was further determined by three-color FC analysis. Although MG were MHC class II–positive, expression varied from donor to donor, as has been described for human CNS MG.20 FC data represent results from repeated experi-
ments of both single and pooled donors. MG expressed constitutively low levels of CD86 and CD40. (Fig. 1B; Table 2). Resting MG also expressed CD11c, CD4, CD1a, CD54, and CD25 but in all MG preparations tested were negative for Fas ligand (FasL; n = 3). Low levels of intracellular IL-4, IFNγ, IL-2, and IL-10 were detected. No TNFα was seen. Dual immunohis-

tochemistry of serial sections (8 μm thick) of human retina by both fluorescence and APAAP detection confirmed FC pheno-
type. In addition, morphology revealed that MG were present in three areas: perivascular, juxtavascular, and the inner retinal parenchyme (Fig. 2).

**IFNγ-LPS Stimulation Mediated an IL-10–Dependent Reduction in Surface Antigen Expression**

In subsequent experiments, freshly isolated retinal MG were stimulated for 16 hours with IFNγ-LPS in vitro, after which cell surface expression was determined by three-color FC analysis. By means of annexin V and propidium iodide (PI) staining, no increase in levels of apoptotic cells within the media (annexin V+ PI− cells) was observed after stimulation (results not shown). Figure 1B shows the downregulation of cell surface antigen expression after IFNγ-LPS stimulation. Combining results of repeated experiments over a 2-year period confirmed statistically the individual experimental results we obtained throughout (Figs. 1, 3). As shown in Table 2, there was a significantly reduced expression of MHC class II and CD40. Levels of CD86 had also declined, but the reduction did not achieve statistical significance. Concomitant with the observed change in cell surface expression, MG increased expression of intracellular IL-10 (IL-10 MFI of 21.3 ± 5.4 resting MG and 46.4 ± 9.9, P < 0.024; Table 2).

Although IFNγ expression remained unchanged, IL-2, IL-4, and TNFα were not detected after stimulation. After the observation of increased IL-10 production concomitant with a downregulation of cell surface expression—particularly, costimulatory molecule (CD40 and CD86) expression—experiments were designed to determine whether the phenotypic shift was IL-10 mediated. Mixed retinal cell populations containing MG (at 5% of 5 × 10⁵ retinal cells/ml) were preincubated for 1 hour in media containing only TCM or TCM plus neutralizing IL-10 antibody (anti-IL-10 mAb). IFNγ (100 U/ml) and LPS (5 μg/ml) were then added to each of the samples and incubated for a further 16 hours. Cells were harvested and phenotype assessed by three-color FC analysis as described earlier. Confirming previous experiments, downregulation of surface antigen expression occurred after IFNγ-LPS stimulation. Figure 3 (representative of confirmatory experiment) shows that, in contrast, MG in mixed retinal cell population that had been preincubated with anti-IL-10 mAb prevented downregulation of CD86 or CD40 expression (Fig. 3A, right and left). Furthermore, MHC class II downregulation was enhanced after addition of exogenous IL-10 (Fig. 3B).

**Effect of IFNγ-LPS Stimulation on MG Cell Migration**

Resting MG are reported to have a low rate of division and a very low turnover within tissue after development. Whether they migrate within tissue or proliferate when stimulated remains controversial. The following experiments were designed to investigate where, and under what conditions, MG migrate from retinal tissue, by using an explant culture. When explants (5 mm) were seeded in 24-well plates in differing media (TCM, TCM plus IFNγ-LPS, TCM plus rIL-10, both with and without anti-IL-10 mAb), resident retinal cells migrated from the tissue. At 24 hours, cells appeared rounded, but with increasing culture time cells clustered and formed processes while adhering to the plate (Fig. 4). Migration was reduced when explants were treated with either IFNγ-LPS or rIL-10, while exhibiting less clustering and process formation

**Figure 2.** Immunohistochemical analysis of MG cell surface expression. In all cases, CD11b was visualized by DAB, and the subsequent surface marker by APAAP. (A) Dual CD11b-CD86–positive MG, both within the parenchyma (large arrow) and juxtavascularly (small arrow). (B) CD11b-CD45 dual-stained parenchymal MG. (C) Juxtavascular CD11b-CD40 MG (arrow). (D) CD11b-HLA-DR-positive juxtavascular MG (small arrow) and parenchymal MG (large arrow). (E) Single HLA-DR FITC-stained retina, counterstained with PI mounting fluid. Small arrow: positive staining surrounding a retinal vessel; large arrow: HLA-DR-positive cell within the inner retina. Magnification, (A, C, and E) ×300; (B) ×625; (D) ×150.

### Table 2. Surface Expression MFI and IL-10 Levels after IFNγ-LPS Stimulation

<table>
<thead>
<tr>
<th>MFI*</th>
<th>n†</th>
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<tr>
<td>MHC Class II</td>
<td></td>
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<tr>
<td>Direct</td>
<td></td>
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<tr>
<td>IFNγ-LPS</td>
<td>919.5 ± 172.3</td>
<td>6</td>
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<tr>
<td>CD86 Direct</td>
<td>77 ± 39.4</td>
<td>4</td>
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<tr>
<td>IFNγ-LPS</td>
<td>57.8 ± 18.21</td>
<td>5</td>
</tr>
<tr>
<td>CD40 Direct</td>
<td>21.25 ± 9.707</td>
<td>4</td>
</tr>
<tr>
<td>IFNγ-LPS</td>
<td>100.7 ± 22.45</td>
<td>3</td>
</tr>
<tr>
<td>IL-10 Direct</td>
<td>55.67 ± 9.77</td>
<td>3</td>
</tr>
<tr>
<td>IFNγ-LPS</td>
<td>21.29 ± 5.4</td>
<td>7</td>
</tr>
<tr>
<td>IL-10 Direct</td>
<td>46.4 ± 9.96</td>
<td>5</td>
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* MFI was determined by FC assay. Data are means ± SEM.
† Number of experiments in which the number of donor eyes varied from 2 to 6.
In these experiments, MG were shown to comprise approximately 2% to 3% of cell population of normal human retina. After migration, MG (CD45<sup>low</sup>CD11b<sup>+</sup> cells) were more prevalent in adherent cell population, accounting for 2.28% (mean of two experiments) of the population by day 3 (Fig. 5). This is consistent with previous observations of MG-adherent behavior in culture. Although the percentage of MG remaining in the explant was not correspondingly reduced, such a discrepancy may be accounted for by the overall decrease in total number of cells within the tissue. Furthermore, there was no evidence of MG proliferation as determined by Ki-67 expression (data not shown). Figure 6 shows the mean cell surface expression from two experiments. Migratory MG, either in supernatant or adherent showed increased expression of MHC class II and CD86 compared with MG remaining in explant. In two further confirmatory experiments, during days 1, 2, and 4 of the explant culture, supernatant was gently removed from test wells of TCM alone and TCM+FNγ-LPS and frozen for later ELISA estimation of IL-10 production. The ELISA kit (OptEIA; PharMingen) used had a minimum detection level of 7.8 pg/ml and maximum of 500 pg/ml. Consistent with results acquired from FC in which the MFI of labeled intracellular IL-10 increased after the addition of FNγ-LPS to the medium, levels of IL-10 increased in wells after FNγ-LPS stimulation. By day 2 of culture, levels had increased from undefinable to 101.6 pg/ml (mean of duplicate tests) and increased again to 105.3 pg/ml by day 4. Wells that consisted of complete medium also showed an increase from undefinable to 65.3 pg/ml over days 1 and 2, but IL-10 was again undetectable by day 4.

**ManR-Mediated Pinocytosis of Dextran-FITC by MG**

FC analysis of MG that had been incubated overnight showed they have the ability to pinocytose dextran-FITC. The extent of uptake by CD45<sup>low</sup>CD11b<sup>+</sup> MG was depicted by the MFI of FITC levels within the cells. Comparison between each of the three cell categories (explant, nonadherent, and adherent) showed that the cells present within the explant had the highest uptake of dextran. In confirmatory experiments, MG uptake of dextran was inhibited by 45% after preincubation of MG with mannan, a ManR inhibitor (Fig. 7). Nonmigrating, nonadherent, and adherent MG all lost the ability to take up dextran–FITC after 24 hours of culture, when in all cases detectable levels of fluorescence declined below background levels.
DISCUSSION

Within the retina, as in the CNS, there are two types of monocyte-derived cells, MG and PVCs, distinguishable by extent of expression of surface antigens CD45 and CD11b. The question of whether these separate subsets of cells subserve the same or differing functions during inflammation remains unresolved. For example, during autoimmune-mediated destruction of retinal photoreceptors in EAU, both antigen-specific T cells and nonspecific leukocytes infiltrate target tissue through inner retinal vessels. Activation of antigen-specific T cells requires presentation of antigen at the site. Given the high constitutive MHC class II expression and proximity of PVCs to extravasating T cells crossing the blood–retinal barrier, it is most likely that these cells act as the local APCs of the retina, akin to their role in the CNS. However, as these data infer, the role of MG within the retina is complex, not solely conferring a putative capacity to present antigen. Using a novel method of FC isolation of MG as previously described for rodent and human CNS MG and rodent retinal MG, we further investigated the function of directly ex vivo human retinal MG.

These results have confirmed previous immunohistochemical reports that resting human retinal MG possess the phenotype requisite for APC function. Human retinal MG express, albeit at low levels, MHC class II, B7.2 (CD86), and CD40. Our initial approach was to investigate MG isolated within a single cell suspension of retinal cells, including ganglion and neuronal cells, Müller cells, and endothelium, in an attempt to retain a retina-like environment during further functional studies. In both experimental models of autoimmune disease and graft-versus-host disease, CNS and retinal MG upregulate MHC II expression, although in human CNS, increased expression was observed as a rather leaky, nonspecific marker of activation.

However, in these experiments, when MG were stimulated with IFNγ-LPS, there was a reduced expression of co-stimulatory molecules with a concomitant increase in the production of an inhibitory cytokine IL-10. Why should a cell's
expression of molecules, inferring a potential capability to present antigen, suppress its own ability to do so? These results indicate that through surrender of their costimulatory ability, MG can alter and modulate the immune response in the eye. Instead of perpetuating the immune cascade, MG may have potential to prevent the functional activation of T cells by their omission of costimulation. Anergy or apoptosis thus occurs, limiting effector T-cell responses in the local retinal environment. CNS MG migrate and proliferate in response to an antigen-specific T-cell infiltrate, the interaction of which results in T-cell apoptosis. Similar indirect evidence exists within the retina. Apoptosis of T cells occurs during EAU and has also been observed within the retina of patients with uveitis. In addition, T-cell apoptosis within retina is reduced when apoptotic signals are blocked.

A possible immunomodulatory role of IL-10 in the retina was supported in this series of experiments. We observed that not only did intracellular IL-10 production increase after IFNγ-LPS stimulation, but also that a neutralizing anti-IL-10 mAb prevented downregulation of costimulatory molecule expression on MG despite IFNγ-LPS stimulation. Within the eye, other data show that IL-10 is central for maintaining and inducing anterior chamber–associated immune deviation (ACAID), and ocular APC function. In addition, during uveitis, decreased levels of anterior chamber IL-10 have been identified in patients, whereas increased levels of IL-10 are found both in retinal cells and infiltrating T cells in retinal antigen tolerized animals during EAU. Similarly, within the CNS a modulatory role of IL-10 has been shown in recent evidence that indicates pivotal involvement in the rapid clinical remission of EAE. Furthermore, as observed with our present data supporting an inhibitory action of IL-10, CNS MG-derived IL-10 also down-regulates costimulatory antigen expression. Within the CNS, PVCs turn over, although it remains unconfirmed whether MG behave similarly, despite observations that CNS MG proliferate and migrate within the tissue in response to antigen-specific stimulation.14

During CNS inflammation, however, MG are susceptible to Fas-independent apoptosis, which may in turn regulate the number of MG in the CNS after MG activation and proliferation. Furthermore, reports have shown that TNFα and IFNγ render MG sensitive to FasL-induced apoptosis. Although to date we have found no evidence for increased apoptosis in our experiments, MG apoptosis or death may account for our observed IL-10 production, akin to apoptosis-mediated IL-10 production within the anterior chamber.

We subsequently used a retinal explant model to maintain architecture and morphology, to investigate migratory behavior of retinal MG. Without stimulation, MG rapidly left the retinal explant, and most become adherent and upregulated CD86 and CD40 expression. Phagocytosis is well documented, and we showed the functional presence of ManR on human retinal MG. Phagocytic ability was lost during culture. Such behavior is comparable with that of tissue DCs, which on leaving tissue lose phagocytic ability and increase cell surface antigen expression and antigen-presenting function. Similarly, Langerhans' cells spontaneously cease uptake of dextran-FITC during culture. One hypothesis therefore is that MG may simply remove the threat of a potentially damaging response by removing antigen. MG are resident within the CNS, and no reports have shown that MG emigrate from CNS or retina, although they migrate within tissue. Contrary to DC behavior, the current data show that the migration of MG from a tissue explant was inhibited after IFNγ-LPS stimulation, associated with the increased production of IL-10 that was detected by ELISA in the supernatant. An effect such as that observed in our
initial experiments was also inhibited by a neutralizing anti-
IL-10 mAb. In addition to the observed downregulation of
costimulatory molecules, one further inference is that preven-
tion of migration prevents the presentation of antigen system-
ically and further supports MG’s acting to regulate inflamma-
atory responses in the retina.

There are two apparent paradoxes. First, observance of
retinal inflammation in vivo (e.g., EAU) shows that MG upregu-
late MHC class II expression, and more particularly, during
inflammation, CNS MG migrate within tissue.4,14,46 Yet, in the
current experiments, IFNγ-LPS stimulation resulted in an IL-
10-dependent suppression of migration. Secondly, although
resting MG expressed appropriate molecules inferring APC
capacity, when stimulated, such antigen expression was lost,
which is contrary to some observations that CNS MG when
cultured long term are able to present antigen and stimulate
T-cell responses.47 More recently, cultured MG have been
shown to secrete transforming growth factor-β2,48 while
inducing an allospecific Th2 T-cell response when injected sub-
cutaneously into naïve recipients. Data thus far provide evi-
dence that MG, when conditioned (cultured), are capable of
acting as APCs or releasing proinflammatory cytokines, for
example when isolated from degenerative conditions.49

The role of this cytokine release even under such condi-
tions remains undefined. For example, MG-secreted NO and
TNFα may serve to reduce cellular proliferation and cell mi-
gration and induce T-cell apoptosis, thus regulating tissue
responses. However, despite such paradoxes and although this
series of experiments has not functionally investigated antigen-
expressing capacity nor the effects of cognate interaction with
T cells, the results indicate that activation of MG resulted in
a phenotypic and behavioral change concomitant with IL-10
production and served to modulate further tissue inflamma-
tion. Even if an initial infiltrate of antigen-specific T cells are
presented antigen by MG, previous functional data have shown
such interactions result in T cell anergy and apoptosis.50 Our
data support the notion that the subsequent increase in proin-
flammatory mediators (e.g., IFNγ) during an autoimmune re-
sponse reduces MG capacity to migrate and activate T cells by
downregulation of coaccessory molecule expression.

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