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Microglia share a lineage relationship with bone marrow-derived monocytes/macrophages and dendritic cells, and their inclusion in retinal and brain transplants may function as “passenger leukocytes.” In other solid allografts, passenger leukocytes are the primary sources of immunogenicity, triggering allogeneic rejection. We have conducted a series of in vitro and in vivo studies examining the capacity of microglia cultured from forebrain to activate alloreactive T cells and to induce and elicit allograft rejection. Cultured microglia expressed class II MHC molecules and costimulatory molecules (B7-1, B7-2, and CD40), and they secreted IL-12. Cultured microglia injected s.c. into naive recipients induced alloreactive delayed hypersensitivity and elicited delayed hypersensitivity directed at alloantigens. Cultured microglia differed from conventional APCs by secreting significant amounts of mature TGF-β2, but smaller amounts of IL-12. Moreover, while both cultured microglia and conventional APC stimulated T cell proliferation in vitro, microglia directed the responding T cells toward the Th2 pathway in which IL-4, but not IL-2 and IFN-γ, was secreted. The abilities of microglia to secrete TGF-β2, to stimulate alloreactive Th2 cells, and to induce anterior chamber associated immune deviation when injected into the eye of naive allogeneic mice suggest that they are not typical passenger leukocytes. The unique functional properties of cultured microglia may account for the capacity of neonatal retinal tissue transplanted into the eye to alter the systemic alloimmune response in a manner that delays, but does not prevent, graft rejection. The Journal of Immunology, 1999, 162: 4482–4489.

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potential to become “passenger leukocytes.” In support of this possibility is our observation that NNR allografts that contain class II-expressing microglia acquire recipient-derived T cells whose appearance correlates with evidence of graft break-down and deterioration.

Although our long-term interests are in retinal transplantation, the small size of the neonatal mouse retina precluded our conducting extensive studies with microglia from this source. Therefore, in this communication, we describe the results of experiments in which microglia derived from neonatal mouse forebrain have been examined for their abilities to induce alloimmunity in vivo and in vitro. The results indicate, on the one hand, that microglia have the ability to activate MHC-specific alloreactive T cells, but that, on the other hand, the functional phenotype of the responding T cells suggests that Th2, rather than Th1, cells are preferentially activated.

Materials and Methods

Microglial isolation and purification

Primary mixed glial cultures were established from the neonate forebrains of either BALB/c or C57BL/6 mice (<24 h), based on the previously published procedure (13, 17). Briefly, mouse forebrains were gently dissected free from meninges. The tissue was pressed through a sterile nylon mesh to generate a single cell suspension. Spleen and lymph nodes were obtained from naive C57BL/6 mice (6 – 8 wk) and pressed through nylon mesh to generate a single cell suspension. T cell purification with an EPICS XL flow cytometer (Beckman Coulter, Miami, FL).

B7.1, B7.2, MHC class II (I-Ad), and CD40 (PharMingen) with 1/20 dilution of RPMI 1640 medium, 10% FCS, 10 mM HEPES, 1 mM sodium pyruvate, penicillin (100 U/ml), streptomycin (100 μg/ml), and 0.2 mM 2-ME (Sigma) at 37°C in a 92% air/8% CO₂ humidified atmosphere in DMEM medium containing 10% FCS, 2 mM glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml) (BioWhittaker, Walkersville, MD). The medium was changed after 24 h and again on days 4 and 9. After ~10 days in vitro, microglia were detached from the astrocyte monolayer by rapid and gentle manual shaking of the culture flasks, and the supernatant was collected, centrifuged, and resuspended in the same medium as above to 75-cm² flasks. After 1 h of culture, the medium was replaced to remove nonadherent cells. These cultures generate microglia that are >95% Mac-1 and Grifonia simplicifolia positive (13, 27, 28). The above procedure was repeated 3 days later, and the purity of microglial cells was >99%.

Preparation of peritoneal exudate cells (PEC)

To compare the functional properties of cultured microglia with conventional APCs in induction of alloimmunity, PECs were prepared from normal BALB/c or C57BL/6 mice that had received 2 ml of thioglycolate (Sigma, St. Louis, MO) i.p. 3 days before. PECs were collected from the abdominal cavity, washed, and resuspended in the complete medium containing RPMI 1640 medium, 10% FCS, 10 mM HEPES, 1 mM sodium pyruvate, penicillin (100 U/ml), and 0.2 mM 2-ME (Sigma) at 1 × 10⁷/well in a 96-well plate. After 2 h of culture, the medium was replaced to remove nonadherent cells, and the cells were used for all subsequent experiments. In this culture, >90% of the adherent cells are Mac-1⁺ dendritic cells or macrophages (29, 30).

Analysis of cell immunophenotypes by flow cytometry

To determine whether cultured microglia express similar immunophenotypic characteristics to conventional APCs, cultured microglia and isolated PEC were collected and incubated with CD16/323 Fc block (PharMingen, San Diego, CA) in PBS containing 2% normal mouse serum for 10 min on ice. The cells were then incubated, respectively, with biotinylated, FITC-conjugated and phycoerythrin (PE)-conjugated mAbs against mouse B7.1, B7.2, MHC class II (I-A), and CD40 (PharMingen) with 1/20 dilution for 30 min on ice. FITC-conjugated nonspecific rat IgG2b and PE-conjugated nonspecific rat IgG2a were used as isotype controls. Following staining, the cells were washed, and the fluorescence intensity assessed with an EPICS XL flow cytometer (Beckman Coulter, Miami, FL).

T cell proliferation assay

To examine the allosensitizing capacity of cultured microglia, purified T cells (3 × 10⁵ cells/well) from C57BL/6 or BALB/c mice, as responder cells, were cocultured with irradiated syngeneic or allogeneic stimulator cells (5 × 10⁵ cells/well) in complete medium in round-bottom 96-well plates. These stimulator cells included cultured microglia and PEC derived from either BALB/c or C57BL/6 mice. Cells were cultured for 1–4 days at 37°C in a 95% air/5% CO₂ humidified atmosphere. [₃H]Thymidine (0.5 μCi) was added into the culture 12 h before the measurement. The cells were harvested onto glass filters through an automatic cell harvester (Tomtec, Orange, CT). Radioactivity was assessed by liquid scintillation spectrometer, and the amount expressed as cpm.

Cytokine assays

To analyze the cytokine production by mixed cultures containing T cells and microglia or PEC, supernatants from the cultures described above were collected every 24 h from 1 to 4 days. The concentration of IL-2, IL-4, IL-12 (p40), and IFN-γ was examined by quantitative ELISA, according to the manufacturer’s instructions (PharMingen). Rat mAbs against mouse cytokine IL-2 (JES6-1A12), IL-4 (BVD4-1D11), IL-12 (C15.6), and IFN-γ (R4-6A2) were obtained commercially (PharMingen) and used as coating Abs. Biotinylated rat mAbs to IL-2 (JES6-5H4), IL-4 (BVD6-24G2), IL-12 (C17.8), and IFN-γ (XMG1.2) were used as detecting Abs. The concentration of TGF-β2 in the supernatant of APCs-T cells response was detected using TGF-β2 Emax ImmunoAssay Systems (Promega, Madison, WI).

Induction of delayed hypersensitivity and AC-associated immune deviation (ACAI)

To study the capacity of microglia to sensitize allogeneic recipients in vivo, syngeneic or allogeneic microglia or PEC were injected s.c. (50,000 cells/100 μl HBSS), or into the AC (2 × 10⁵ cells/2 μl HBSS) of naive BALB/c mice (6 – 8 wk). Ten days after s.c. injection, the ear pinnae were challenged with intradermal injections of irradiated splenocytes (1 × 10⁶) from C57BL/6 mice. Mice that first received an AC injection of microglia received an s.c. priming 1 wk later with 10² splenocytes from C57BL/6 mice. One week later, the ear pinnae were challenged with irradiated splenocytes from C57BL/6 mice. Ear swelling responses were measured at 24 and 48 h thereafter with an engineer’s micrometer (Mitutoyo, Paramus, NJ).

Elicitation of delayed hypersensitivity

To detect the capacity of allogeneic microglia to elicit delayed hypersensitivity responses in presensitized mice, naive BALB/c mice (6 – 8 wk) were first sensitized s.c. with 10² splenocytes from C57BL/6 mice. Ten days later, the ear pinnae were challenged with irradiated PECs (2 × 10⁴ cells) from C57BL/6 mice or cultured microglia (2 × 10⁴ cells) derived from either BALB/c or C57BL/6 mice. Ear swelling responses were assessed as described above.

Statistical analysis

Levels of significance for comparison between different samples were determined using Student’s t test distribution. Values of p < 0.05 were considered statistically significant.

Results

Cultured microglia express surface molecules similar to conventional APCs

The dendritic cells and macrophages that function as passenger leukocytes in solid tissue grafts, such as skin, heart, and kidney, express an array of cell surface molecules that are essential to the activation of naive alloreactive T cells. These molecules include class II MHC molecules, which present peptides to CD4⁺ T cells, and costimulatory molecules including B7-1, B7-2, and CD40, which are coreceptors for CD28 and CD40 ligand, respectively. Many microglia in neonatal forebrain are “reactive” and already contain a large store of autologous alloantigen. Therefore, in this communication, we describe the results of many microglia in neonatal forebrain are “reactive” and already contain a large store of autologous alloantigen. Therefore, in this communication, we describe the results of...
microglia that had been cultured for 10 days from neonatal mouse forebrain still expressed class II MHC and costimulatory molecules. For comparison, we examined surface expression of these molecules (within 1 h of harvesting) on cultured microglia and cultured PEC by subjecting the cells to flow cytometric analysis using Abs directed at class II MHC, B7-1, B7-2, and CD40. The results presented in Fig. 1 indicate that ~60% of microglia and similar percentage of PEC stained positively for class II MHC and CD40. Both cell types also stained for B7-1 and B7-2, with more microglia than PEC being positive for B7-2 (73%). We interpret these results to mean that cultured microglia that have been isolated and purified from neonatal brain express a spectrum of MHC and costimulation molecules appropriate to consider them candidates for APCs to alloreactive T cells. It is important to point out that pure microglia used in our experiments were from 10-day mixed glial cultures that were not subcultured for 24 h. This may explain the difference of the microglial expression of MHC class II and CD40 between our study and the previous studies that used pure microglia that sustained an additional 24 h of culture (13).

Cultured microglia induce proliferation among alloreactive T cells in vitro

To begin to assess the potential capacity of microglia to activate alloreactive T cells, cultured C57BL/6 microglia or PEC were used as stimulators in mixed lymphocyte reactions in which spleen and lymph node T cells from BALB/c donors were used as responders. Syngeneic cultures contained BALB/c microglia and BALB/c spleen cells. T cell proliferation was measured at each 24-h interval for a total of 4 days by adding [3H]thymidine 12 h before termination of the culture. The results of a representative experiment are presented in Fig. 2. No significant proliferative response was detected during the first 48 h in any cultures. Thereafter, allogeneic microglia and allogeneic PEC induced significant [3H]thymidine incorporation compared with cultures in which BALB/c microglia were used to stimulate BALB/c T cells. At 72 h, the proliferation induced by allogeneic PEC slightly exceeded that induced by allogeneic microglia, even though this difference was not statistically significant, whereas, at 96 h, microglia were similar to PEC in inducing alloreactive T cell proliferation in vitro. These data indicate that cultured allogeneic microglia were capable of inducing alloreactive T cell proliferation of a magnitude similar to that induced by PEC. These results confirm the earlier report of Cash and Rott (31), who showed that microglia isolated from rat brain can induce significant proliferation among alloreactive CD4+ and CD8+ T cells.

FIGURE 1. Histograms illustrating surface expression of B7-1, B7-2, MHC class II, and CD40 in PECs and cultured microglia. The cells were stained with FITC-conjugated anti-Mac-1, and PE-conjugated anti-B7-1, anti-B7-2, anti-MHC class II, and anti-CD40. FITC-conjugated nonspecific rat IgG2b and PE-conjugated nonspecific rat IgG2a were used as isotype controls and showed no positive labeling with the cells. The intensity of surface phenotypes on the cells was analyzed by an EPICS XL flow cytometer. The x-axis represents FITC fluorescence, and y-axis represents PE fluorescence. Numbers indicate percentages within quadrants.

FIGURE 2. Proliferation response of purified T cells stimulated in vitro by allogeneic or syngeneic PECs and cultured microglia isolated from either BALB/c mice or C57BL/6 mice. T cell proliferation was measured every 24 h for a total of 96 h following stimulation using [3H]thymidine incorporation. [3H]thymidine was added to the cultures 12 h before detection. Each data point represents the mean ± SD of three separate experiments at every 24-h time point. The value of p represents comparisons between cocultures of allogeneic microglia T cells and syngeneic microglia T cells.
Cultured microglia and cultured PEC stimulate alloreactive T cells to secrete different cytokine patterns

Although alloreactive T cells activated in mixed lymphocyte cultures can produce a wide range of cytokines, IL-2, IFN-γ, and TNF-α predominate, while IL-4 and IL-10 are poorly represented. Next, we examined the cytokine secretion profiles of T cells stimulated with allogeneic cultured microglia and cultured PEC. Mixed lymphocyte cultures similar to those described above were established. At 24-h intervals, supernatants were harvested from these cultures and analyzed for content of IL-2, IL-4, and IFN-γ by ELISA. Results from three separate experiments are displayed in Fig. 3. BALB/c T cells stimulated with allogeneic PEC produced significantly higher amounts of IL-2 and IFN-γ than did T cells stimulated with allogeneic microglia. Particularly, microglia-stimulated T cells produced virtually no IFN-γ. T cells activated by both allogeneic microglia and PEC produced similar amounts of IL-4 within 24 h of stimulation, but thereafter, T cells stimulated with microglia secreted significantly more IL-4 than did T cells stimulated with PEC. As anticipated, T cells exposed to syngeneic microglia produced only trivial amounts of these cytokines. Thus, although microglia and PEC similarly stimulate alloreactive T cells to proliferate, the phenotype of the responding T cells is decidedly different. Whereas allogeneic PEC stimulate T cells that secrete predominantly Th1-type cytokines, allogeneic microglia stimulate T cells that appear to be shifted in the direction of Th2-type responses. It has been noted previously that cultured microglia isolated from adult human tissue produce IL-10 following the stimulation with IFN-γ/LPS (32). However, in our hands, purified murine microglia neither secreted IL-10 spontaneously, nor did the supernatants of mixed lymphocyte cultures containing microglia contain this cytokine (data not shown).

Cultured microglia produce IL-12 (p40) similar to PEC in the presence of alloreactive T cells

IL-12 is a costimulatory molecule secreted by APCs that promotes the activation of Th0- and Th1-type T cells. To determine whether this cytokine was produced by microglia, supernatants of mixed lymphocyte cultures containing allogeneic PEC or microglia as stimulators were collected as described above and analyzed by ELISA for IL-12 (p40) content. Syngeneic control cultures contained BALB/c microglia and BALB/c T cells. As revealed by the results presented in Fig. 4, cultured microglia produced small amounts of IL-12 in the presence of syngeneic T cells. When confronted by allogeneic T cells, the cells produced considerably more IL-12 at 24 h and throughout the culture interval. Similarly, PEC exposed to allogeneic T cells also produced IL-12, especially at 72 and 96 h of culture, these cells produced much more IL-12 than did their microglial counterparts. The almost 2-fold greater IL-12 production at 72 and 96 h by PEC compared with microglia correlates with the peak IL-2 and IFN-γ production by responding T cells in cultures containing PEC as stimulators. These findings confirm previous reports that cultured microglia following stimulation produce IL-12 p40 (13, 33).

Cultured microglia secrete TGF-β2 in the presence of T cells

We have recently shown that PEC treated with TGF-β2 before being pulsed with Ag increase their endogenous production of TGF-β and secrete this growth factor predominantly in its active form. Moreover, these TGF-β2-pretreated PEC stimulate Ag-specific T cells to differentiate down a Th2, rather than Th1, pathway in vitro (29, 30). Since microglia arise from an environment rich in TGF-β and are cultured in the presence of astrocytes that also

![FIGURE 3](http://www.jimmunol.org/)

**FIGURE 3.** Secretion of IL-2, IL-4, and IFN-γ by T cells stimulated by allogeneic or syngeneic PECs and cultured microglia isolated from either BALB/c mice or C57BL/6 mice. The culture supernatants were harvested and assayed for IL-2, IL-4, and IFN-γ content by ELISA. Each data point represents the mean ± SD of three separate experiments at every 24-h time point. The value of p represents comparisons between cocultures of allogeneic microglia-T cells and allogeneic PEC-T cells.

![FIGURE 4](http://www.jimmunol.org/)

**FIGURE 4.** Secretion of IL-12 by PECs and cultured microglia from either BALB/c or C57BL/6 when reacted with syngeneic or allogeneic T cells. Culture supernatants were harvested and assayed for IL-12 content by ELISA. Each data point represents the mean ± SD of three separate experiments at every 24-h time point. The value of p represents comparisons between cocultures of allogeneic microglia-T cells and allogeneic PEC-T cells.
Cultured microglia can induce and elicit allospecific delayed hypersensitivity in vivo

Animals that reject solid tissue allografts typically acquire donor-specific delayed hypersensitivity responses. When the alloantigens on the graft are encoded by genes within the MHC, passenger leukocytes within the graft are responsible for inducing this form of immunity. We next wished to determine whether cultured microglia have the capacity to induce delayed hypersensitivity when injected into naive recipient mice. Therefore, suspensions of C57BL/6 or BALB/c microglia were injected (50,000 cells/recipient) s.c. into normal BALB/c mice. Positive control recipients were immunized s.c. with C57BL/6 splenocytes (10 × 10⁶ cells). Ten days later, the ears of these mice were challenged with C57BL/6 PEC, C57BL/6 microglia, or BALB/c microglia (20,000 cells/injection). When the ear swelling responses were assessed (see Fig. 7), both C57BL/6 microglia and PEC proved capable of eliciting delayed hypersensitivity.

Allogeneic cultured microglia also induced delayed hypersensitivity, and of virtually the same intensity.

Having established that microglia are capable of inducing allospecific delayed hypersensitivity, we then examined their capacity to elicit this immune response in presensitized mice. BALB/c mice were immunized s.c. with C57BL/6 splenocytes (10 × 10⁶). Ten days later, the ears of these mice were challenged with C57BL/6 PEC, C57BL/6 microglia, or BALB/c microglia (20,000 cells/injection). When the ear swelling responses were assessed (see Fig. 7), both C57BL/6 microglia and PEC proved capable of eliciting delayed hypersensitivity.
Cultured microglia can induce alloantigen-specific ACAID

Our eventual goal is to graft neuronal retinal tissue successfully into the eye, an immune privileged site. We and others have shown that allogeneic APCs from extraocular sites induce immune deviation when injected into the AC of the eye (35). Therefore, we wished to know whether allogeneic cultured microglia have a similar capability. Cultured microglia or PEC from C57BL/6 donors were injected (20,000 cells/2 μl) into the AC of one eye of BALB/c mice. Seven days later, these animals, along with positive (non-eye injected) controls, were immunized by an s.c. injection of BALB/c mice. Seven days later, recipients were sensitized s.c. with C57BL/6 splenocytes (1 × 10^6 cells). Ear swelling was assessed 24 and 48 h after ear challenge by micrometer. Data represent the mean ± SD of ear swelling responses of each group at the time point of optimal reaction (n = 10 in each group).

Discussion

When NNR tissues are implanted into the AC or subretinal space of normal mouse eyes, microglial cells within these grafts become activated. Not only do the cells adopt a morphology that is consistent with the activated state, but they up-regulate the expression of class I MHC molecules and initiate surface expression of class II MHC molecules. Although these changes occur whether the grafts are placed in eyes of syngeneic or allogeneic mice, the expression of class II Ags on donor-derived microglia within grafts placed in allogeneic mice is much more intense than that of syngeneic grafts. Moreover, as the allogeneic NNR grafts deteriorate between days 12 and 35 postimplantation, they become infiltrated with small but significant numbers of recipient T cells, and, during this interval, donor-derived microglia gradually disappear (26). Because of these striking changes among microglia within NNR allografts, we have formulated the hypothesis that retinal microglia can assume the functional properties of passenger leukocytes, similar to cells described in most other solid tissue allografts, and thereby confer enhanced “immunogenicity” upon the graft. The results of the studies reported here provide strong support for this idea.

Cultured microglia derived from forebrain of 1-day-old mice acquire surface molecules that are known to be important in the activation of alloreactive T cells: enhanced expression of class I and de novo expression of class II, B7-1, B7-2, and CD40 (13, 36, and the present study). In addition, neonatal forebrain microglia secrete IL-12, a cytokine known to be important in the activation of naive T cells (13, 17, 33). Moreover, cultured microglia that express these important immunostimulatory molecules also acquire the capacity to activate alloreactive T cells both in vitro and in vitro. Thus, cultured microglia of C57BL/6 forebrain origin stimulated naive BALB/c T cells to proliferate in vitro, and these same microglia induced donor-specific alloimmunity when injected s.c. into naive BALB/c mice. When challenged in the ear pinnae with C57BL/6 cells, BALB/c recipients of cultured C57BL/6 microglia displayed intense ear swelling responses. Due to size constraints of the neonatal mouse retina, we were able to conduct only a few experiments with retina-derived microglia. The results of these experiments were similar to those of experiments with forebrain-derived microglia (data not shown).

In the aggregate, expression of immunostimulatory molecules and the capacity to activate alloreactive T cells in vitro and in vivo strongly suggest that cultured microglia have properties similar to those described for passenger leukocytes. If true, then this would explain why NNR allografts eventually sensitize their recipients to MHC-encoded alloantigens. In the neonatal retina, MHC class I expression is strictly limited to microglia, and no cells express MHC class II molecules. As the retina matures, especially within intraocular NNR grafts, MHC class II appears, but solely on graft-derived microglia (26). The appearance of MHC class II expression on graft microglia heralds the acquisition of donor-specific delayed hypersensitivity and the eventual destruction of the graft.

Rejection of NNR allografts in the AC and subretinal space takes place gradually and is largely accomplished by 35 days postimplantation (23–25). At that time, the only cells expressing donor class II molecules within the graft are the few remaining microglia in the center of disintegrating rosettes (26). Since the mice bearing these intraocular grafts display systemic delayed hypersensitivity to donor alloantigens at this time (24, 25), the possibility exists that the targets of this recipient alloimmune response are the microglia themselves. The results reported here indicate that microglia are fully capable of eliciting delayed hypersensitivity reactions when injected into the pinnae of presensitized mice. Thus, we suspect that the T cells responsible for rejection of intraocular NNR allografts are targeted to the donor-derived microglia within these grafts, and that T cell attack upon the microglia triggers the grafts’ demise.

As mentioned above, NNR allografts are rejected slowly within the AC or subretinal space (24, 25). Moreover, the acquisition of donor-specific delayed hypersensitivity by recipients of these grafts is also delayed. In fact, mice bearing allogeneic NNR grafts in the AC first display donor-specific ACAID at 12 days postimplantation, rather than delayed hypersensitivity. The latter reactivity only emerges by 35 days, which coincides with the time of maximum deterioration of the graft (24, 25). It is of considerable interest, therefore, that cultured allogeneic microglia injected into the AC of the eye induced ACAID. We suspect that small numbers of donor-derived microglia emigrate from NNR grafts into the ocular microenvironment and proceed to promote ACAID.
From several different vantage points, the immunogenic characteristics of cultured microglia are compatible with the hypothesis that these cells can function as passenger leukocytes. However, there are details of our experimental results that give us pause. First, peritoneal exudate cells (conventional APCs) and cultured microglia both expressed B7-1, B7-2, and CD40, but microglia expressed relatively more B7-2 (~15%) and less B7-1 (~20%). Second, whereas both PEC and cultured microglia secreted IL-12, microglia also secreted large and sustained amounts of TGF-β2. Third, whereas conventional APCs induced alloreactive T cells to proliferate and secrete IL-2 and IFN-γ, cultured microglia induced proliferating T cells that secreted predominantly IL-4, but not IL-2 and IFN-γ, and very small amounts of IL-2. These results indicate that cultured microglia and PEC are not functionally identical as stimulators of alloreactive T cells, and that PEC promote responding T cell differentiation down the Th1 pathway (29, 30, 37, and the present study), whereas cultured microglia direct responding T cells toward the Th2 pathway. The T cell-activating properties of cultured microglia strongly resemble the T cell-activating properties of PEC that have been pretreated with TGF-β2. Takeuchi et al. (29, 30) have recently reported that exposure of PEC to TGF-β2 causes the cells to secrete less IL-12, express less CD40, and secrete their own mature TGF-β. Nguyen et al. (38) have also demonstrated similar findings when cultured microglia from a cell line were exposed to TGF-β. Moreover, when TGF-β2-pretreated PEC were pulsed with Ag and used to stimulate naive, Ag-specific T cells, the responding T cells secreted Th2- rather than Th1-type cytokines (29, 30). Since our cultured microglia grow on a substrate of astrocytes, which constitutively secrete small amount of TGF-β, the microglia may already be entrained to secrete their own TGF-β at the time they are added to our mixed lymphocyte cultures, and this may explain why they stimulate alloreactive T cells to produce IL-4, rather than IL-2 and IFN-γ. Activation of Th2 cells has now been described in ACAID experiments conducted in several different laboratories (29, 30). Perhaps the ability of NNR allografts to induce ACAID shortly after they have been implanted into the AC reflects the inherent capacity of graft microglia to activate Th2-type alloreactive T cells. Moreover, since Th2 cells are thought to be protective of allografts, compared with Th1 cells (39, 40), the ability of microglia to activate Th2 cells preferentially may help to explain why NNR allografts in the eye enjoy a prolonged existence before their destruction.

The ability of brain microglia to function as APCs has long been appreciated (3, 9–12, 14). This knowledge is derived, on the one hand, from studies of experimentally induced autoimmune inflammatory diseases of the brain (3, 9–11), and, on the other hand, from studies of brain and retinal transplants into the CNS (25, 41–46). In addition, in vitro studies of brain-derived microglia have revealed their capacity to present nominal Ags to sensitized T cells (12–14). Our results with cultured microglia expand on this knowledge base by revealing that microglia: 1) can directly activate alloreactive naïve T cells, 2) can immunize recipients to donor-derived alloantigens, and 3) can act as targets of alloreactive T cells that mediate delayed hypersensitivity. It is important to acknowledge that some of the results in our study were generated in vitro using neonatal microglia. It is likely that cultured cells may have adjusted their properties during the culture when compared with their normal in vivo counterparts. As suggested previously, cultured microglia can be regarded as a form of reactive microglia similar to those appearing in the immature CNS tissue during development (13, 17, 36). Therefore, it is possible that the allostimulatory capacities of cultured microglia revealed in our studies reflect the immunogenic properties of activated microglia found in NNR grafts, but may not be representative either of normal neonatal microglia, or of mature, resting microglia within NNR grafts. At the moment, technical considerations make it virtually impossible to obtain routinely sufficient microglia directly from the murine neonatal retina to test the cells in repeated and diverse in vitro assays. The challenge to experimentalists is to develop such strategies that will enable unmodified microglia to be evaluated for their immunogenic potential directly. Until then, our findings suggest that activated microglia within neonatal retinal transplants have the potential to function as passenger leukocytes, albeit functionally unusual ones.

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References