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Dendritic Cell Differentiation Signals Induce Anti-Inflammatory Properties in Human Adult Microglia

Caroline Lambert, *† Julie Desbarats,* Nathalie Arbour, ‡ Jeffery A. Hall, § Andre Olivier, † Amit Bar-Or, † and Jack P. Antel2†

Microglia are resident cells of the CNS that belong to the myeloid cell lineage. In experimental models of neuroinflammation, they have limited capacity to function as APCs when compared with dendritic cells (DCs). Human peripheral blood monocytes have the plasticity to differentiate into mature DCs when exposed to GM-CSF and IL-4 followed by LPS. In this study we addressed the potential of human microglia to acquire phenotypic and functional properties of mature DCs under similar inducing conditions. Treated adult and fetal microglia became CD14low and acquired limited expression of CD209 (DC-SIGN); they remained CD1a− and CD83−, and decreased MHCII expression, suggesting that they had not achieved a complete DC phenotype. The monocyte-derived DCs efficiently promoted CD4 T cell proliferation in an allogeneic MLR, whereas differentiated adult microglia had a decreased ability to stimulate CD4 T cell proliferation compared with their untreated counterparts. Differentiated fetal microglia did support CD4 T cell proliferation, whereas untreated cells could not. Fetal and adult microglia produced significant amounts of IL-10 following differentiation but no detectable IL-12 p70, in contrast to differentiated monocytes that produced IL-12 p70. Our data indicate that neither adult nor fetal microglia acquired the full characteristic phenotype of mature stimulatory DCs when treated with DC-inducing cytokines in vitro. Moreover, such treatment, especially of adult microglia, induces functional responses that could promote an anti-inflammatory environment in the CNS.

Although regarded as an immunoprivileged organ, the human CNS can be selectively targeted by immune responses leading to either uniphasic or recurrent disorders as exemplified, respectively, by acute disseminated encephalomyelitis and multiple sclerosis (MS). Experiments performed in animal models of these disorders, particularly in experimental autoimmune encephalomyelitis (EAE), have demonstrated the essential roles of local competent APCs within the CNS for the initiation, persistence, and recurrence phases of these diseases (1). Recent studies in mice with EAE have shown that blood-derived myeloid dendritic cells (DCs), detectable mainly in CNS perivascular areas, play a key role in the progression of EAE, whereas the parenchymal microglial cells have limited and insufficient APC capacity to activate T cells, especially naïve cells (2).

Microglia are derived from myeloid progenitor cells that begin to populate the CNS during fetal development (3). They are usually regarded as long-lived cells (4); however, experiments involving radiation-generated chimeric mice suggest that the rate of repopulation of parenchymal microglia with blood-derived monocytes is greater than previously appreciated (5). Such repopulation may be most apparent following disruption of the blood-brain barrier (6). Previous studies have established that circulating human monocytes retain the capacity to acquire the phenotypic and functional properties of immature or mature DCs in response to defined signals (7, 8). GM-CSF and IL-4, both of which can be produced within the CNS especially under inflammatory conditions (9–11), induce in vitro monocyte differentiation into DCs. LPS, a component of Gram-negative bacteria and a ligand for TLR4, expressed on human monocytes and microglia (12, 13), can provide the additional signal required for full DC maturation of monocytes. Whether microglia, which arise from the same lineage as monocytes, can also acquire DC properties is not completely resolved.

The potential for resident microglia to differentiate into mature DCs may be species-dependent and/or developmentally regulated. Rodent microglia can acquire DC markers and increased APC function in response to GM-CSF alone or in combination with either Th1 cells or other cytokines (14–17). In contrast, Zuiderwijk-Sick et al. concluded that exposure of nonhuman primate microglia to GM-CSF did not change their APC function (18). Previous studies indicate that adult human microglia have distinct properties from those of other species: in situ, human microglia express detectable levels of MHCI in normal CNS specimens (19), whereas mouse microglia do not express detectable MHCI under basal conditions (20). This difference in MHCI expression could reflect constitutive properties of human microglia or repeated exposure of humans to either systemic or CNS environmental stimuli. In vitro, microglia derived from adult human

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CNS survive for weeks under basal culture conditions, in contrast to findings in both rodents and nonhuman primates (18, 21).

We have previously demonstrated that treatment of human adult microglia with Th1-derived supernatant enhances their APC functions, leading to efficient proliferation and IFN-γ production by alloantigen-stimulated naïve (CD45RA⁺) T cells (22). The aim of our present study was to determine whether human microglia can acquire professional APC properties when exposed to the same stimuli inducing DC properties in human blood monocytes. We included human fetal microglia in this study, postulating that such cells would represent a more uncommitted cell population and show increased plasticity to DC-inducing signals in comparisons to their adult counterparts. Our results show that adult and fetal human microglia only acquired a limited subset of DC phenotypic properties upon exposure to the GM-CSF + IL-4 followed by LPS. Whereas this treatment induced blood monocytes to differentiate into competent APCs secreting proinflammatory cytokines, human adult microglia down-regulated MHCII expression and favored the production of IL-10, potentially promoting an anti-inflammatory environment in the CNS.

Materials and Methods

Monocyte and microglia cell culture

Human fetal CNS tissue (cerebral hemispheres) was obtained from the Human Fetal Tissue Repository (Albert Einstein College of Medicine, Bronx, NY), following approved institutional and Canadian Institutes for Health Research (CIHR) guidelines. Cells were isolated as previously described (23). Briefly, fetal brain tissue (gestational age 16–19 wk) was first minced and treated with DNase/trypsin (both from Invitrogen). The suspension was passed through a nylon mesh and plated at 5–7 million cells/ml in high-glucose DMEM supplemented with 5% fetal FBS, penicillin/streptomycin, and glutamine (all from Invitrogen). After 10–14 days in culture, floating microglia were harvested and plated at the desired cell density.

Adult microglia were obtained from normal appearing white matter of temporal lobe brain tissue from patients undergoing surgery for non-tumor-related intractable epilepsy. All studies were approved by our institutional review board. Brain tissue was processed as described (24). Briefly, tissue was obtained in pieces <1 mm³ and treated with DNase/trypsin for 30 min at 37°C. Following sieving through a nylon mesh, cell suspension was separated on a 30% Percoll gradient (GE Healthcare) at 400 × g for 30 min. Glial cells (mostly oligodendrocytes and microglia) were collected from underneath the myelin layer, washed, and then plated in tissue culture-treated vessels. Floating oligodendrocytes were washed away on the subsequent 2 days and the remaining adherent microglia were collected with trypsin with 2 mM EDTA. Cells were grown in MEM containing 5% FBS, penicillin/streptomycin, and glutamine (all from Invitrogen). After 10–14 days in culture, floating microglia were harvested and plated at the desired cell density.

To obtain monocytes, PBMCs collected from venous blood of healthy volunteers were separated on a Ficoll density gradient (GE Healthcare). CD14⁺ monocytes were positively selected to >95% purity by MACS using anti-CD14 beads (Miltenyi Biotec) and then seeded at 1 × 10⁶ cells/well in 24-well plates in RPMI 1640 with 10% FBS, penicillin/streptomycin, and glutamine. Cells were grown in MEM containing 5% FBS, penicillin/streptomycin, and glutamine. The proportion of microglia in the culture was determined using CD11c staining by flow cytometry; purity was always ≥85% and was routinely ≥90%.

To obtain monocytes, PBMCs collected from venous blood of healthy volunteers were separated on a Ficoll density gradient (GE Healthcare). CD14⁺ monocytes were positively selected to >95% purity by MACS using anti-CD14 beads (Miltenyi Biotec) and then seeded at 1 × 10⁶ cells/well in 24-well plates in RPMI 1640 with 10% FBS, penicillin/streptomycin, and glutamine (Invitrogen).

In attempts to induce differentiation of microglia or monocytes toward a DC phenotype, cells were treated with human recombinant GM-CSF (50 ng/ml, PeproTech) and IL-4 (20 ng/ml, PeproTech) for 6 days. LPS (serotype O127:B8, 100 ng/ml, Sigma-Aldrich) was added on day 6 for 24 h to induce DC maturation. Phenotypic characterization and functional assays (supernatant collection for cytokine production, MLR, phagocytosis, OVA uptake) were performed on day 7.

Phenotypic studies

Cultured microglia were harvested by vigorous pipetting and scraping in 2 mM EDTA in PBS. Following overnight blocking of Fc receptors with 3 μg/ml normal mouse IgG (Caltag Laboratories) and 10% human serum, cells were stained with specific or isotype-matched Abs directly conjugated with FITC, PE, PerCP or allophycocyanin fluorochromes. Abs to MHCII (HLA-DR, -DQ, -DP), MHCI (HLA-A, -B, -C), CD1a, CD11c, CD14, CD40, CD80, CD83, CD86, CD209/DC-SIGN, and isotype controls were all from BD Biosciences, except for Ab to CD68 (Dako). Cells were fixed with 1% formaldehyde and analyzed with a FACSCalibur flow cytometer (BD Biosciences) and FlowJo software (Tree Star). In all experiments, microglia from each treatment group were stained with control isotype-matched as well as specific Abs. In total, tissues from 13 different adult and 16 fetal specimens were used in this study to determine the phenotype of microglia.

Allogeneic MLR

CD4 T cells were positively selected from PBMCs using anti-CD4 beads (Miltenyi Biotec) and then labeled with CFSE (Invitrogen) as previously published (25). MLR reactions were performed by adding CFSE-labeled CD4 T cells to wells containing microglia at a ratio of 4:1, 8:1, or 16:1 of original numbers of microglia plated before treatment. Six days later, cells were collected and stained with anti-CD11c and anti-CD3 Abs to discriminate microglia from T cells by flow cytometry. CFSE dilution (proliferation of T cells) was determined in the CD3⁺ population and expressed as percentage of T cells that had proliferated.

Phagocytosis assay

Phagocytosis was assessed through the uptake of Escherichia coli particles labeled with a low pH-sensitive dye (pHrodo E. coli bioparticles, Invitrogen). Before adding the bacterial particles, half of the microglia medium was replaced with HBSS containing 20 mM HEPES (to maintain pH 7.4) and 10% human serum (to enhance opsonization), as suggested by the manufacturer. E. coli particles were added to the microglia culture for 3–4 h at 37°C. Cells were then harvested and washed twice in PBS containing 1% FBS. Analysis of fluorescence from ingested E. coli was monitored by flow cytometry (FL2 channel). Control phagocytosis assays were performed in parallel at 4°C for each treatment group to correct for nonspecific fluorescence.

OVA uptake and processing assay

To assess uptake and processing of soluble Ag by microglia and monocytes, a DQ-OVA (Invitrogen)-based assay was used. In this assay, the fluorescence of OVA labeled with BODIPY FL dye (DQ-OVA) is self-quenched until the OVA is taken up via the mannose receptor and degraded. DQ-OVA uptake was measured as described (26). DQ-OVA was added at 40 μg/ml to cells in phenol red-free growth medium and incubated at 37°C for periods from 30 to 60 min. Cells were then plated on ice, collected, and washed twice with PBS/1% FCS. Mean fluorescence intensity (MFI) values obtained in the FL1 channel were corrected to MFI obtained with cells incubated at 4°C with DQ-OVA in parallel experiment for 1 h.
Cytokine production

Cytokine levels were measured in supernatants collected from fetal and adult microglial cultures treated with GM-CSF and IL-4 with or without LPS. IL-1β, IL-6, IL-10, IL-12 p70, TNF-α (all from BD biosciences), and IL-23 (p19/p40 Ready-SET-Go!, eBioscience) were measured in duplicate using ELISA kits according to the manufacturers’ instructions.

Statistics

Statistical analyses were performed using Prism 3.0 (GraphPad Software). Comparisons for the same cell type under different treatment conditions across different experiments were performed using the Student two-tailed paired t test. Comparisons between cell types under the same treatment conditions were made using the Mann-Whitney U test.

Results

Morphological features of adult and fetal microglia treated with GM-CSF + IL-4 and LPS

Differentiation in the presence of GM-CSF and IL-4 for 6 days followed by LPS maturation for 24 h (designated as “GM-CSF + IL-4 then LPS”) resulted in visible morphological changes in both monocytes and microglia. Only cytokine-treated monocytes survived in large numbers over the course of 1 wk and they remained weakly adherent to the tissue culture vessel. Differentiated monocytes became significantly larger, formed large aggregates, and extended veiled processes (Fig. 1A). In contrast to monocytes, both
untreated and treated adult and fetal microglia survived over 1 wk in culture and adhered strongly to the tissue culture plate. Untreated adult microglia initially displayed a bipolar or multipolar appearance with long wide processes (Fig. 1C). Following treatment with GM-CSF and IL-4, a large proportion of the adult microglia flattened and displayed multiple thin processes (Fig. 1B). Fetal microglia evolved from an initial ameboid into a ramified morphology (Fig. 1C) (26). Adult and fetal microglia both showed more apparent intracellular vesicles following treatment with GM-CSF and IL-4, which further increased upon subsequent stimulation with LPS. We had previously shown that adult microglia in situ and in vitro were CD68+ and CD11c−, but were negative for myeloperoxidase (27). Differentiated microglia remained CD68+ and CD11c− and differentiation was not accompanied by an induction of myeloperoxidase activity (myeloperoxidase leukocyte kit, Sigma-Aldrich, data not shown).

Expression of DC markers on differentiated microglia

We compared expression of surface markers characteristic of DCs on untreated vs treated monocytes and microglia. As expected, ex vivo monocytes expressed high levels of the LPS-binding protein CD14, which was considerably decreased following GM-CSF + IL-4 then LPS treatment (Fig. 2, A and C) (28). CD14 was expressed on the surface of both adult and fetal untreated microglia, albeit at lower levels than on monocytes. Like the monocytes, adult and fetal microglia down-regulated CD14 surface expression in response to treatment (Fig. 2, A and C). Expression of CD209, which characterizes both mature and immature DCs (29), was very low on untreated monocytes and was highly induced in differentiated monocytes (Fig. 2, B and D). Neither adult nor fetal untreated microglia expressed detectable baseline levels of CD209. There was a small increase in expression upon treatment but CD209 levels remained ~100-fold lower than those achieved by monocytes. Treated monocytes also displayed higher CD1a expression (Table I), involved in presenting foreign as well as potentially self-glycolipids to T cells (30). Neither adult nor fetal microglia expressed detectable levels of CD1a before or following differentiation. Monocyte-derived DCs robustly up-regulated the mature DC marker CD83 following stimulation with LPS (Table I). CD83 was undetectable on microglia before treatment. CD83 remained undetectable in treated adult microglia and at the limit of detection in treated fetal microglia. These data show that microglia undergo only a limited phenotypic change in response to treatment with GM-CSF + IL-4 then LPS in comparison to monocytes, which achieve a mature DC phenotype under similar conditions.

MHC and costimulatory molecule expression

As anticipated, monocytes expressed MHCII and MHCI at baseline and strongly increased expression of both in response to GM-CSF + IL-4 then LPS (Fig. 3A–C). MHCI expression was lower on microglia than on monocytes under basal conditions but was up-regulated in response to differentiation signals (Fig. 3B). Baseline MHCII expression levels were consistently high on adult microglia but variable in fetal microglia. In contrast to monocytes, there was a decrease of surface MHCII levels in both adult and fetal microglia treated with GM-CSF and IL-4 without LPS (Fig. 3, D and E). The decrease was most apparent in microglial preparations expressing higher MHCII baseline levels. MHCII tended to increase upon addition of LPS but remained lower than baseline. Adult and fetal microglia only modestly increased levels of the costimulatory molecules CD80 (B7-1), CD86 (B7-2), and CD40 (Fig. 3F–H) when treated with GM-CSF + IL-4 then LPS in contrast to the marked increase observed with monocytes.

Stimulation of allogeneic CD4 T cell proliferation

To characterize whether changes in surface levels of MHC and costimulatory molecules affected the Ag presentation capacity of microglia, we assessed their ability to trigger CD4 T cell proliferation in an allogeneic MLR. As expected, monocyte-derived DCs potently triggered CD4 T cell proliferation compared with untreated monocytes (Fig. 4, A and D) (31). As we previously reported (25), adult human microglia supported significant CD4 T cell proliferation under basal conditions (Fig. 4, B and E) although there was considerable variability in the levels of response between microglia from different individuals (inducing 3–30% of CD4 T cells to proliferate). Differentiation of adult microglia with GM-CSF + IL-4 then LPS resulted in significantly decreased T cell proliferation by at least 40% in all cases (fold decrease, paired t test p < 0.05, n = 4). In comparison, untreated fetal microglia (Fig. 4, C and F) induced only a low percentage of CD4 T cells to proliferate (2–7%), a proportion comparable to ex vivo monocytes. Treatment of fetal microglia with GM-CSF + IL-4 then LPS augmented their capacity to induce CD4 T cell proliferation by 4–20-fold (p < 0.01, n = 4), reaching similar levels to treated monocytes.

Particulate Ag uptake and soluble Ag processing

We performed phagocytosis assays to address whether the noted reduction in APC function of adult microglia would have reflected that they were differentiating into macrophages with increased phagocytic capacity. Phagocytic capacity was tested using E. coli particles labeled with a pH-sensitive fluorochrome (pHrodo E. coli). Phagocytic function was greatly reduced in monocyte-derived DCs as compared with their ex vivo monocyctic counterparts (Fig. 5A). Adult microglia (Fig. 5B) also decreased their phagocytic index in response to differentiation. Fetal microglia treated with GM-CSF + IL-4 then LPS (Fig. 5C) showed a relatively stable phagocytic index with a slight trend toward an increase.

Table I. Expression of cell surface markers in differentiated monocytes vs adult and fetal microglia

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<th>Adult Microglia</th>
<th>Fetal Microglia</th>
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<tr>
<td></td>
<td>Untreated</td>
<td>GM-CSF + IL-4 then LPS</td>
<td>Untreated</td>
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<tr>
<td>CD1a</td>
<td>1 ± 2 (3)a</td>
<td>357 ± 213 (3)</td>
<td>2 ± 2 (3)</td>
</tr>
<tr>
<td>CD83</td>
<td>0 ± 2 (3)</td>
<td>60 ± 6 (3)</td>
<td>1 ± 1 (3)</td>
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<td>MHCI</td>
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<td>CD86</td>
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<td>784 ± 384 (2)</td>
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<tr>
<td>CD40</td>
<td>6 ± 2 (3)</td>
<td>333 ± 179 (3)</td>
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a ΔMFI ± SEM (no. of individuals).

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FIGURE 3. Modulation of MHC and costimulatory molecule expression by microglia and monocytes following differentiation with GM-CSF + IL-4 then LPS. Flow cytometric profiles of monocytes and adult or fetal microglia for MHC I (A) and MHC II (B) under basal conditions (untreated) or after DC-inducing treatment (GM-CSF + IL-4 then LPS). C–E, Actual changes in MFI (ΔMFI) for MHC II in monocytes (C, n = 2), adult microglia (D, n = 3), and fetal microglia (E, n = 5) for individual donors. Representative surface expression profiles for CD80 (F), CD86 (G), and CD40 (H) by flow cytometry are shown.
Thus, the differentiation of microglia did not result in a substantial increase of *E. coli* uptake.

We also assessed uptake and processing of OVA by monocytes and microglia with DQ-OVA. As expected, monocyte-derived immature DCs endocytosed and processed OVA more efficiently than did monocytes; LPS addition resulted in a reduction of this activity (Fig. 5D) (32). Adult and fetal microglia had a measurable processing activity under basal conditions as well as with GM-CSF and IL-4, albeit significantly lower than processing performed by monocytes or immature DCs (Fig. 5, E and F). In the presence of LPS, adult and fetal microglia also displayed low OVA uptake and processing capacity; values were similar to those of LPS-stimulated monocyte-derived DCs.

**Cytokine production by monocytes and microglia**

We then measured the production of cytokines that may influence subsequent T cell responses in supernatants of differentiated monocytes and microglia. These included a Th1-polarizing cytokine IL-12 (heterodimeric form p70, Fig. 6A), IL-10, involved in generation of Th2 and regulatory T cells (Fig. 6B), and IL-23 (Fig. 6C), involved in enhancing the Th17 phenotype (33). Whereas monocyte-derived DCs matured with LPS produced IL-12 p70 (846 ± 323 pg/ml), differentiation did not result in detectable production of IL-12 p70 by microglia (*p* < 0.05), although we previously showed that these cells can secrete this cytokine in response to other stimuli (25). Microglia treated with GM-CSF + IL-4 (without LPS) made substantial amounts of IL-10 (*p* < 0.05), whereas similarly treated monocytes did not increase their IL-10 secretion. Both microglia and monocytes produced enhanced levels of IL-10 following LPS maturation. With regard to IL-23, low levels were observed in monocyte-derived DCs and adult microglia. IL-23 levels were more robustly induced in differentiated fetal microglia compared with other cell types (*p* < 0.05). The acute phase cytokine IL-6 (Fig. 6D) and the early proinflammatory cytokine TNF-α (Fig. 6E) were also measured. IL-6 was induced in all cells tested when stimulated with LPS. TNF-α was also induced upon LPS addition in all cells tested, although the ratio of TNF-α production to IL-10 production was markedly lower in fetal and adult microglia compared with monocytes (*p* < 0.01, Fig. 6, B and E).
further observed that IL-1β was produced at very low levels in monocytes treated with GM-CSF + IL-4 then LPS (26 ± 13 pg/ml) and was below detection threshold in microglia (data not shown). These data show that monocytes adopt a proinflammatory cytokine profile whereas the adult microglia demonstrate an antiinflammatory bias in cytokine production.

FIGURE 5. Particulate Ag uptake and soluble Ag processing by monocytes and microglia. Phagocytosis of pHrodo E. coli is shown in representative flow cytometry profiles of monocytes (A, n = 2 donors), adult (B, n = 2 donors), and fetal (C, n = 3 donors) microglia left untreated or treated with GM-CSF + IL-4 with and without LPS. Shaded curves represent background fluorescence of pHrodo E. coli when added to cells at 4°C. DQ-OVA uptake and processing over time by monocytes (D), adult microglia (E), and fetal microglia (F) in an individual experiment are shown. Experiment was performed as detailed in Materials and Methods.
**Discussion**

In this study, we have examined the response of human microglia to conditions that induce mature DC properties in blood monocytes, namely GM-CSF + IL-4 or with GM-CSF + IL-4 then LPS. In the CNS, GM-CSF can be locally produced by astrocytes and infiltrating T cells (9, 34). Microglia/macrophages have also been reported to be a source of IL-4 (10). The survival of human microglia under basal culture conditions, in contrast to other species, allows us to directly compare the properties of these cells with or without treatment. Our results show that human microglia, unlike monocytes, acquired only partial expression of DC phenotypic properties upon treatment with GM-CSF + IL-4 then LPS. Treated microglia down-regulated MHCII and displayed a less significant up-regulation of costimulatory molecules compared with monocytes. After treatment, adult microglia were less able to support proliferation of allogeneic CD4 T cells in a MLR. Furthermore, treatment of microglia with GM-CSF and IL-4 with or without LPS increased their production of IL-10 but not IL-12 p70. Fetal microglia induced IL-23 production in response to GM-CSF + IL-4 then LPS, whereas monocytes and adult microglia produced low levels. Overall, these data suggest that human microglia have a limited flexibility to develop a stimulatory DC-like program and that conditions that promote such a response in monocytes promote an antiinflammatory response in adult microglia.

Our initial morphological evaluation of the adult and fetal microglia indicated that both cell types responded to GM-CSF and IL-4 treatment. Microglia were more adherent than monocytes treated under the same conditions. With treatment, we observed an increased proportion of the adult microglia with numerous vesicles suggesting a macrophage-like rather than a DC-like transition. Untreated and differentiated adult microglia also showed reactivity for neutral lipids as shown by oil red O staining, consistent with a previous report (data not shown and Ref. 35). This likely is the result of uptake of myelin debris present in the adult CNS glial cell cultures. The detectable albeit limited up-regulation of CD209 on the treated microglia in the absence of other DC markers could also reflect a macrophage-like differentiation since CD209 is also expressed in some macrophage subsets (36). However, we also observed that microglia down-regulated CD14 in response to differentiation, similar to monocyte-derived DCs. Our result is in line with our previous observation that IL-4 treatment alone down-regulated CD14 in response to differentiation, similar to monocyte-derived DCs. Our result is in line with our previous observation that IL-4 treatment alone down-regulated CD14 mRNA expression by human adult microglia (37).

Nonhuman primate microglia are reported to express significant levels of CD14 after treatment with GM-CSF alone (18). We also noted a decreased phagocytic activity in treated adult microglia, which also does not support a macrophage-like differentiation. The fetal microglia appeared to have already engulfed a significant amount of debris before differentiation treatment (Fig. 1); therefore, their relatively low and stable uptake of E. coli may have reflected a certain level of saturation. The vesicles in the fetal microglia only rarely showed reactivity with oil red O staining, consistent with previous reports (38, 39). Although we did not specifically identify the constituents within the fetal microglia,
they may be generated from extensive removal of neural cells undergoing programmed cell death during neurodevelopment as it was suggested in rodent developing CNS (40).

Microglia treated with the combination of GM-CSF and IL-4 then LPS showed significant changes in their APC phenotype and function. After treatment, fetal and adult microglia, unlike monocytes, down-regulated surface MHCII expression. MHCII down-regulation is in line with the reports of others who found that IL-4 down-regulated IFN-γ-induced MHCII expression on neonatal murine microglia (41) and that GM-CSF alone down-regulated MHCII on human fetal microglia (42). We have previously observed MHCII down-regulation on fetal but not adult microglia in response to ligands for TLR2 and TLR4 (25). Changes from baseline expression of costimulatory molecules were very limited on microglia in contrast to those of monocytes. The lack of induction of CD1a may also be relevant in protecting the brain from immune attack mediated by the presentation of self-glycolipids, especially in the context of elevated lipid content in the CNS.

We had previously shown that both immediately ex vivo and cultured adult microglia could drive proliferation of CD4 T cells in an MLR (43). Our MLR results showed that T cell proliferation was reduced in the presence of adult microglia treated with GM-CSF and IL-4 with or without LPS. Zuidervijk-Sick et al. concluded that adult nonhuman primate microglia did not change their APC capacity in response to GM-CSF (18). The baseline ability of human fetal microglia to promote CD4 T cell proliferation was poor, raising the issue of them being more closely related to immature DCs and/or having their function down-regulated by the materials they have ingested (44). However, APC function of the fetal microglia was significantly enhanced by the GM-CSF + IL-4 then LPS treatment. GM-CSF is also reported to enhance neonatal murine microglia APC functions (45). Therefore, human fetal microglia appear to retain some component of plasticity, possibly enhancing their stimulatory immune function when in the presence of T cells (14).

Our results with respect to production of cytokines by microglia in response to DC-inducing conditions again raises the issue of microglia being programmed to favor inhibition of immune responses. We previously showed that although ligands to TLR 2–4 did not change the capacity of human adult microglia to induce allogetic proliferation of T cells (25), they modified production of polarizing Th1 cytokines. Our previous studies have shown that adult microglia can produce IL-12 p70 under specific conditions, including CD40 ligation and TLR3 stimulation with poly(I:C) (13, 46). Treatment of monocytes with GM-CSF + IL-4 then LPS induced IL-12 p70 secretion. However, the fetal and adult microglia treated with GM-CSF + IL-4 then LPS did not produce IL-12 p70 while making increased amounts of IL-10 compared with baseline. All the myeloid cell tested produced IL-6 and TNF-α following LPS treatment, but the ratio of IL-10 to TNF-α production was higher in microglia than in monocytes. We have not yet determined the functional implications of IL-23 induction in differentiated fetal microglia.

The adult microglia we have used were derived from patients undergoing surgery for intractable epilepsy. The tissue was remote from the seizure focus and, in most cases, histological examination showed no definable pathology (47). We can only speculate that these individuals may have a greater chance of having a disrupted blood-brain barrier than absolutely normal individuals. The chi-meric animal studies mentioned in the introduction would predict that we would find a heterogeneous myeloid population due to increased repopulation by blood-borne cells. However, we saw no evidence for small subsets of cells with distinct phenotypes or responses to DC-inducing stimuli by flow cytometry.

The relative contributions of myeloid cells to regulation of the immune response in the human CNS needs to be further examined. Monocytes acquire DC properties after transgressing an experimental blood-brain barrier even in the absence of exogenous GM-CSF or IL-4 (7), indicating their potential contribution to the DC population found in the perivascular region in the CNS (48). Our study has focused on properties of microglia under basal conditions and in response to conditions that induce DC properties in monocytes. The in situ based studies by Boven et al. suggest that the antiinflammatory bias of microglia identified in our study also applies in active MS lesions. Boven et al. found that lipid-laden cells termed “foamy” macrophages expressed antiinflammatory (IL-4, IL-10) rather than proinflammatory cytokines (IL-12) within the lesions, in contrast to “nonfoamy” macrophages found in nonlesion areas (49). Li et al., however, detected IL-23 in microglia/macrophages including foamy cells in active MS lesions, as well as in DCs in perivascular cuffs (50). The survival of human microglia in vitro provides the opportunity for ongoing studies to define the stimuli that activate anti- vs proinflammatory responses in these cells.

Acknowledgments

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Disclosures

The authors have no financial conflicts of interest.

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