Imbalanced immunity in multiple sclerosis

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Isolation and culture of human post-mortem resident microglia

A pilot study of CD200 and CD200R regulation in the human CNS

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Abstract

Studies of human microglia are exceptional as these cells are difficult to access. It is however, very important to study microglia biology, because they have been implicated in many neurodegenerative conditions. In the present study we show a rapid procedure to isolate, sort and culture microglia with high purity from human post-mortem brain tissue. The techniques that are used are based on density gradients and cell surface expression of CD11b and CD45, as elucidated in cell populations derived from human blood, choroid plexus and corpus callosum white matter. Because in vitro stimulation of monocyte-derived macrophages results in polarized cells with different pro- and anti-inflammatory properties, we tested whether microglia, the resident macrophages of the brain, may polarize as well. We therefore stimulated cultured primary microglia with IL-4, as we recently demonstrated that IL-4 induced expression of the immune inhibitory molecule CD200R and mannose receptor (MR) in human alternatively activated macrophages. Although microglia expressed CD200R, expression was not up-regulated by IL-4. In addition, MR was not detectable in microglia, independent of IL-4 stimulation. This was in contrast to choroid plexus macrophages where IL-4 increased both CD200R and MR expression. Thus, MR may be the first discriminating molecule between human macrophages and microglia. Since CD200 provides an important mechanism to control microglia activation via CD200R, we additionally addressed CD200 expression on a human neuroblastoma cell line. IFN-γ, IL-1β, TNF, IL-4, IL-10 or dexamethasone did not influence CD200 mRNA expression. Conclusively, these data demonstrate that microglia behavior differs from that of other tissue macrophages. Furthermore, the expression of CD200R on human microglia and CD200 on neurons is confirmed but their regulation remains unidentified. Most importantly, the currently described techniques offer many possibilities to study the biology and behavior of human microglia directly ex vivo.

Introduction

Microglia originate from myeloid progenitor cells and are the predominant type of immune cells in the brain. They closely resemble macrophages, and share surface markers like CD11b and functions, such as their phagocytic capacity. Depending on the stimulus, macrophages can turn on so-called classical or alternative activation programs. Alternative macrophage polarization results in a group of divergent cells named M2a, M2b and M2c, that emphasize the different anti-inflammatory properties such as tissue repair and remodeling, scavenging of debris and dampening inflammation by promoting type II immune responses.[22,23,172-174] Whether microglia can polarize is at present unknown, and is an important topic of investigation as there are many controversies about the contribution of activated microglia in many neurodegenerative diseases. For example, it has been proposed that a disproportionate activation of microglia leads to an uncontrolled (inflammatory) reaction in neurodegenerative diseases.[112] On the other hand, several studies suggest a protective role for microglia, implying that also microglia activation may be heterogeneous resulting in different outcomes.[31]

Microglia activation is tightly regulated amongst others by the inhibitory molecule CD200 receptor (CD200R). CD200R is present on all myeloid cells where it has been shown to be a potent immune suppressor by dampening immunological activity via inhibition of MAPK pathways.[121,123,124] Although microglia express only low levels of CD200R,[121,136,183] absence of its ligand CD200, expressed by neurons and oligodendrocytes, is sufficient for spontaneous activation of microglia.[125] It is known that IL-4 induces alternatively activated monocyte-derived macrophages of the M2a subtype, which are involved in anti-inflammatory type II responses. We recently demonstrated that CD200R expression is enhanced by IL-4 in these cells, with even higher specificity than mannose receptor (MR), the currently most common M2a marker, since MR was also induced by GM-CSF (Koning *et al.* submitted). We therefore hypothesized that IL-4 stimulation could also induce alternative activation in microglia, the resident macrophages of the brain, and wondered whether this would also be reflected by increased CD200R expression levels. In order to address this question, we needed to develop a method to isolate and culture human microglia.

The biology of human primary microglia in a pathological setting, such as multiple sclerosis (MS), is a largely unexplored field, primarily because these cells are poorly accessible, and brain tissue from MS patients is scarce. It is important that techniques are developed that allow the studying of primary microglia. The currently most frequent used technique to isolate microglia is based on adhesive prop-

erties and is derived from neonatal rodent brains. There are many disadvantages of this technique, for example the source of the material, the time that is needed to obtain the cells and the purity of the cultures. A more reliable method to obtain pure microglia would be to use fluorescence activated cell sorting (FACS). Pioneers in this field are Sedgwick and colleagues, who showed the characterization and isolation of rodent, and later also human resident microglia using flow cytometric sorting based on expression profiles of CD11b and CD45.[162,184,185] Based on these experiments, we show in the present study a functional procedure to isolate, sort and culture pure resident microglia from post-mortem human brain tissue. In a pilot study, we further show that unlike macrophages, IL-4 does not induce CD200R or MR expression in these cells. Furthermore, since its ligand CD200 is highly expressed on neurons and is essential for controlling microglia activity via CD200R,[111,125] we studied the regulation of CD200 on a human neuroblastoma cell line in parallel.

Methods

Human microglia isolation

All material has been collected from donors from whom a written informed consent for brain autopsy and the use of the tissue and clinical information for research purposes had been obtained by the Netherlands Brain Bank (NBB). Corpus callosum white matter (4 grams) and choroid plexus, provided by the NBB, was obtained at autopsy and stored in Hibernate A medium (Brain Bits LLC, Springfield, IL) at 4°C. Tissue was obtained from 8 male and 6 female donors diagnosed with Alzheimer's disease (4 donors), Pick's disease (2 donors), Lewy Body disease (2 donors), Parkinson's disease (1 donor), epilepsy (1 donor), bipolar disorder (1 donor), progressive supranuclear palsy (1 donor) and without neurological condition (2 donors). The average age of the donors was 78 years (range 50 – 93 years). The average post-mortem delay was 5 hours and 48 minutes (range 4 hours and 10 minutes – 9 hours). Within 3 – 18 hours after autopsy, cells were isolated using a Percoll gradient, as described previously,[162] with some adaptations. In brief, tissue (per 2 grams) was mechanically dissociated using a metal sieve in a total volume of 50 ml GKN/0.2% BSA (8 g/l NaCl, 0.4 g/l KCl, 1.77 g/l $Na_2HPO_4.2H_2O$, 0.69 g/l $NaH_2PO_4.H_2O$, 2 g/l D-(+)-glucose, 2 g/l BSA, pH 7.4). After centrifugation (400 x g, 7 min), the pellet was reconstituted in 5 ml dissociation buffer (4 g/l $MgCl_2$, 2.55 g/l $CaCl_2$, 3.73 g/l KCl, 8.95 g/l NaCl, pH 6-7) and enzymatically digested in 150 U Collagenase Type I (Worthington, Lakewood, NJ) and 200 µg/ml DNase I (Roche Diagnostics, Mannheim, Germany) for 1 h at 37°C. During this incubation, the tissue was mechanically dis-

rupted by passing the mixture through a p1000 pipet tip with decreasing bore size every 5-10 minutes. After washing in GKN/0.2% BSA, the cells were resuspended in 20 ml Percoll (ρ = 1.03), underlain by 10 ml Percoll (ρ = 1.095) and overlain by 5 ml GKN/0.2%BSA and centrifuged at 1200 x g for 30 min with slow acceleration and no break. The myelin layer was removed from the GKN-Percoll 1.03 interface and discarded, after which the cells from the 1.03-1.095 interface were collected. The average number of recovered cells was $3x10^6$ cells/gram tissue.

Fluorescent activated cell sorting

The freshly isolated cells from corpus callosum, choroid plexus or human blood, were incubated with phycoerythyrin (PE)-labeled CD11b (Clone ICRF44, DakoCytomation, Glostrup, Denmark), fluorescein isothiocyanate (FITC)-labeled antibody against human CD45 (Clone HI30, Dako) or with isotype-matched controls in the presence of 10% human pool serum (HPS) for 1 h at 4°C. Antibody dilutions and washing steps were performed in FACS buffer (PBS, 0.2% BSA, 10 mM sodium azide). After washing, the cells were analyzed using a FACSCalibur flow cytometer (BD PharMingen, San Jose, CA) and the data was analyzed using FlowJo software version 8.7.1 (Treestar, Inc. Ashland, OR). To isolate microglia from the CNS, cells were stained with PE-labeled CD11b in the presence of 10% HPS for 1 h at 4°C. Antibody dilutions and washing steps were performed in GKN/0.3%BSA. After washing, the $CD11b^+$ cells were sorted on a FACS Aria cell sorter (BD Biosciences).

Cell culture

Isolated primary cells obtained after sorting were cultured in 96-wells flat bottom culture plates in RPMI (Gibco, Invitrogen, Breda, The Netherlands) with 1% heat-inactivated HPS, and were stimulated with 40 ng/ml human IL-4 (Peprotech, London, UK) for 72 hours. The human neuroblastoma cell line SH-SY5Y (kindly provided by dr. E. Hol, Netherlands Institute for Neuroscience, Amsterdam) was cultured in 24-wells in serum-free Neurobasal medium (Invitrogen, Carlsbad, CA, USA), supplemented with the neuronal growth factor B-27 (Invitrogen). Cells were stimulated with $1x10^4$ nM dexamethasone (Sigma Aldrich, Zwijndrecht, the Netherlands), 50 ng/ml IL-1β, 5 ng/ml IL-4, 20 ng/ml IL-10, 10 ng/ml TNF or 50 ng/ml IFN-γ (Peprotech) for 6 and 24 hours.

RNA isolation

The cells were washed in PBS and lysed in 1 ml (SH-SY5Y cells) or 800 µl (primary cells) TRIZOL (Invitrogen). Respectively 200 or 160 µl chloroform (Sigma) was added after which the lysate was thoroughly mixed and centrifuged at 12,000 rpm, 4°C for

15 minutes to recover the aqueous phase. RNA from SH-SY5Y cell lysates was precipitated with isopropyl alcohol, dissolved in 30 µl RNAse free water and stored at -20°C until further use. For the primary cell lysates, an equal volume of 70% ethanol was added to the collected aqueous phase, and loaded onto an RNeasy Mini Kit column (Qiagen, Hilden, Germany). Further RNA isolation was performed according to the RNeasy protocol (RNeasy Mini Handbook 06/2001). The amount (OD260) and purity (OD260/280 ratio) of the RNA was determined using a nanodrop (ND-1000, NanoDrop Technologies, Rockland, DE, USA).

cDNA synthesis and quantitative PCR

The synthesis of cDNA was performed as described previously.[111] Real-time quantitative PCR (qPCR) was performed with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) with samples containing equal cDNA concentrations of 10 ng initial total RNA per reaction. Analysis was performed according to manufacturer's protocol and the ABI Prism 7300 Sequence Detection System (Applied Biosystems). Expression of CD200 (fw: 5'-CCAGGAAGCCCTCATTGTGA-3', rv: 5'-TCTCGCTGAAGGTGACCATGT-3'), CD200R (fw: 5'-GAGCAATGGCACAGTGACTGTT-3', rv: 5'-GTGGCAGGTCACGGTAGACA-3'), MR (fw: 5'-TGCAGAAGCAAACCAAACCT-3', rv: 5'-CAGGCCTTAAGCCAACGAAACT-3') and HLA-DR (fw: 5'-CCCAGGGAAGACCACCTTT-3', rv: 5'-CACCCTGCAGTCGTAAACGT-3') were normalized to 18S ribosomal RNA. For each primer pair, the primer efficiency (Effpr) was calculated using LinRegPCR software.[145] Fold differences were calculated by $(Effpr)^{-\Delta\Delta CT}$ method.[146]

Results

Isolation and sorting of human primary microglia based on CD11b/CD45 expression

Reliable isolation and culture of human microglia facilitates the study of gene or protein regulation in these cells. Currently used methods to isolate microglia from brain homogenates based on adhesion are time-consuming and may be contaminated with other CNS cell types or blood-derived myeloid cells. A more reliable way of isolating microglia would be to sort these cells based on a cell surface marker, but the lack of a specific microglia marker impedes the discrimination of microglia from CNS associated macrophages or monocyte-derived macrophages. Successful microglia isolation in rodents was established based on the staining for CD11b (myeloid cells) and CD45 (leukocytes) cell surface markers, as macrophages are $CD11b^{+}CD45^{hi}$ while microglia are $CD11b^{+}CD45^{dim}$.[162,184,185] To analyze whether this feature would come to our aid also in the isolation of human primary microglia, we

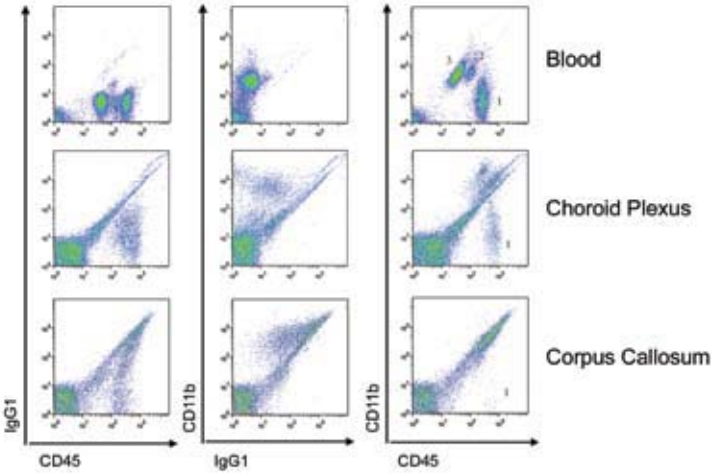

Fig. 1 Flow cytometric analysis of CD11b and CD45 on cells isolated from peripheral blood, choroid plexus and corpus callosum. The left and middle panel show single CD11b$^+$ and CD45$^+$ labeled populations in the three tissues. In choroid plexus and corpus callosum, autofluorescent cell populations are evident. The left panel shows double labeling for CD11b and CD45. In blood, the double labeling shows three populations: lymphocytes (population 1), granulocytes (population 2) and granulocytes (population 3). Population 1 is also present in cells isolated from choroid plexus, but contained very few events when derived from corpus callosum. The CD11b$^+$CD45$^+$ cells from choroid plexus may contain both macrophages and granulocytes. The CD11b$^+$CD45$^+$ population derived from corpus callosum lies underneath the autofluorescent cells. Representative data of 3 (blood and choroid plexus) and 10 (corpus callosum) experiments are shown.

set out to isolate cells from peripheral blood, choroid plexus and corpus callosum. Blood-derived cells showed two CD45$^+$ populations (Fig. 1, left panel) and a single CD11b$^+$ population (Fig. 1, middle panel). These leukocyte populations were separated by double labeling for CD45 and CD11b (Fig. 1, right panel) into lymphocytes (population 1), granulocytes (population 2) and monocytes (population 3). Cell isolates from choroid plexus, a well vascularized site lining the ventricles and highly enriched for macrophages, contained a single CD11b$^+$ and CD45$^+$ population as well as an autofluorescent cell population (Fig. 1). By double labeling for CD11b and CD45, the lymphocyte population was identified (population 1). The CD11b$^+$CD45$^+$ population consisted mostly of monocytes/macrophages, but probably also contained granulocytes. However, the discrimination between these cell types was hampered by interference of autofluorescent events. Similar to choroid plexus, cells derived from corpus callosum contained a large autofluorescent population (Fig. 1). Whereas single staining clearly identified CD45$^+$ and CD11b$^+$ populations, double labeling resulted in only one double positive population, which largely overlaps with the autofluorescent population. This is in contrast to CNS myeloid cells from

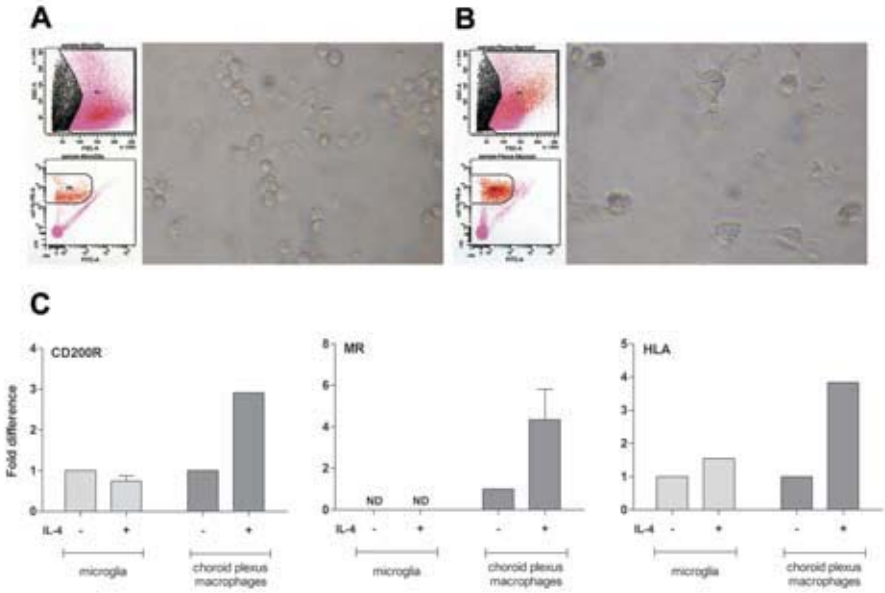

Fig. 2 Morphology of and gene expression in microglia and choroid plexus macrophages sorted based on CD11b expression. (A) Microglia are small cells with little granularity as seen in the forward side scatter analysis (red dots in upper FACS plot). In culture, microglia are small, round cells without ramifications. (B) Choroid plexus macrophages are much larger and more granular as seen in both the forward side scatter analysis (red dots in upper panel) and in culture, where some cells also displayed extensions. (C) After 72 hours of IL-4 stimulation, gene expression of CD200R, MR and HLA-DR was determined and compared to unstimulated cells. Expression of MR was not detectable (ND) in microglia. Results of one or two experiments are shown, and where applicable, mean ± SD are indicated.

rodent brain tissue that comprise of the $CD11b^{+}CD45^{hi}$ and $CD11b^{+}CD45^{dim}$ populations as mentioned before.[162] Moreover, the merger of the double positive population with the autofluorescent events, made it impossible to sort these cells on the basis of differential CD45 staining. In addition, these stainings show hardly any $CD11b^{+}CD45^{hi}$ cells, indicating that nearly all CD11b expressing cells are microglia. Therefore, we chose to sort and culture the $CD11b^{+}$ cells. Also note that there were very few $CD11b^{-}CD45^{+}$ cells (population 1) in corpus callosum, indicating that the fraction of lymphocytes was scarce in this tissue. Since lymphocytes are most likely derived from the vasculature, and are rare in the CNS parenchyma, this suggests that the contamination of blood-derived leukocytes, including monocytes/macrophages, was very low.

IL-4 activation does not induce CD200R expression in human primary microglia

Up to 135,000 $CD11b^{+}$ cells derived from corpus callosum (microglia) and up to 210,000 $CD11b^{+}$ cells from choroid plexus (macrophages) were sorted and cultured

(Fig 2A,B). Both cell types adhered to the culture dish within 60 minutes. Primary microglia in culture displayed a small and round morphology (Fig. 2A), whereas choroid plexus macrophages were much larger, showed several extensions and had a granular appearance (Fig. 2B). We previously showed that IL-4 induced alternative activation of macrophages of the M2a subtype is associated with enhanced CD200R expression (Koning *et al*. submitted). Because microglia originate from myeloid progenitor cells and are commonly called 'the resident macrophages of the brain', we studied this feature in primary microglia and choroid plexus macrophages. Although CD200R mRNA was clearly present in microglia, culturing them for 72 hours in the presence of IL-4 did not alter its expression levels (Fig. 2C). In contrast, IL-4 treatment during this culture period did induce CD200R expression 2.9 times in choroid plexus macrophages isolated from the same donor. Currently the most common marker for M2a cells is the mannose receptor (MR) that is also induced upon IL-4 stimulation in blood-derived macrophages.[172] However, MR mRNA was not detectable in microglia either in the absence or presence of IL-4 (Fig. 2C). In contrast, MR expression in choroid plexus macrophages was present in unstimulated cells, and increased 3.9-fold upon IL-4 stimulation. To confirm whether IL-4 could activate both cell types, HLA-DR expression was determined. HLA-DR was expressed on both cell types with absolute expression levels in microglia resembling that of unstimulated choroid plexus macrophages (data not shown). IL-4 slightly increased HLA-DR expression in microglia but more robustly induced expression in choroid plexus macrophages by 3.8 times (Fig. 2C). Collectively, these data indicate that choroid plexus macrophages resemble blood-derived macrophages, but microglia do not.

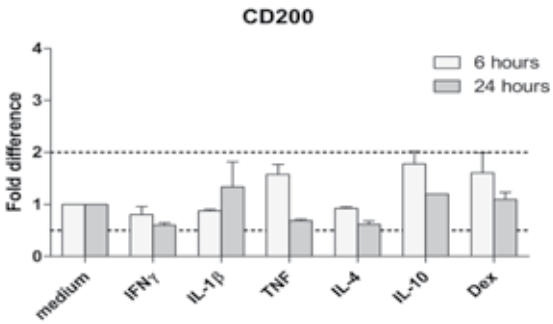

Fig. 3 Regulation of CD200 on neuroblastoma cells. Expression of CD200 mRNA was determined in the neuroblastoma cell line SH-SY5Y upon stimulation with IFN-γ (50 ng/ml), IL-1β (50 ng/ml), TNF (10 ng/ml), IL-4 (5 ng/ml), IL-10 (20 ng/ml) or dexamethasone (Dex, $1x10^4$ nM) for 6 and 24 hours. Dotted lines at 2 and 0.5 correspond to a 2 and -2-fold difference and indicate the sensitivity of the technique, i.e. a difference of at least one cycle threshold. Data represent the mean ± SEM from at least 2 experiments.

CD200 expression is stable on human neuroblastoma cells

To establish whether CD200 expression can be altered, we applied a panel of pro- and anti-inflammatory stimuli to the human neuroblastoma cell line SH-SY5Y. As shown in Figure 3, none of the stimuli convincingly induced or reduced CD200 mRNA expression in these cells, neither after 6 nor after 24 hours. Varying the concentrations of the applied stimuli had no effect either and stimulating the cells for up to 48 hours resulted in reduced viability of the cells (data not shown). These results indicate that CD200 expression on neuroblastoma cells is stable and hardly influenced by cytokines or dexamethasone.

Discussion

In the present pilot study, we show a successful and rapid procedure to isolate and culture human microglia using FACS techniques. Although the isolation procedure of using a density gradient combined with flow cytometry was based on techniques described for rodents by Sedgwick *et al*.[162] and for human biopsy material by Dick *et al*.,[185] the method in the present study using human post-mortem brain tissue from which microglia are sorted and cultured within a few hours after the donor's death is unique and not described elsewhere. This fast procedure allows rapid microglia manipulation in culture as well as gene and protein expression analyses.

Although no specific microglia marker is known, Sedgwick *et al*. used CD11b and CD45 staining intensity to discriminate macrophages that are $CD11b^{+}CD45^{hi}$ from microglia that are $CD11b^{+}CD45^{dim}$.[162] In the present study however, we only detected a single $CD11b^{+}CD45^{+}$ cell population. In the studies of Sedgwick *et al*. and Ford *et al*., a clear $CD11b^{-}CD45^{+}$ lymphocyte population was present in their isolates. As whole rodent brain homogenates were used, these lymphocytes were most likely derived from meninges, subarachnoid space and choroid plexus. Using white matter from corpus callosum that obviously lack these structures, we detected only few lymphocytes, confirming a previously reported observation in human biopsy brain tissue.[185] The contribution of blood-derived $CD11b^{+}CD45^{hi}$ myeloid cells is therefore expected to be low. However, it is still surprising that we could not distinguish between a $CD11b^{+}CD45^{dim}$ and $CD11b^{+}CD45^{hi}$ population in post-mortem corpus callosum as this was previously shown in isolates from human biopsy tissue, although the latter population was remarkably small.[185] It is therefore most likely that blood-derived or perivascular macrophages are not present in our isolates, for example because they actively migrated out of the tissue in the intervening time between isolation and processing of the tissue or they underwent apoptosis.

The most frequently applied technique to obtain microglia, predominantly from neonatal rodent CNS tissue, is based on adhesive properties of microglia after vigorously agitating a mixed cell culture containing amongst others astrocytes, microglia and neurons, for many hours or days with or without GM-CSF or M-CSF.[186-188] The purity of such microglia cultures is assessed on the basis of morphology and immunohistochemical analysis after isolation, and frequently shows some contamination with astrocytes. In addition, as whole rodent brain homogenates contain major blood vessels and frequently also the meninges, these cultures include CNS-associated macrophages or cells derived from the circulation that contaminate these preparations. In contrast, by sorting the cells, the definition and criteria for microglia isolation are determined beforehand. We sorted and cultured resident microglia based on CD11b expression. Although other cells also express CD11b like blood-derived monocytes/macrophages, a subset of dendritic cells, granulocytes and perivascular macrophages, contamination with these cells in our procedure is expected to be negligible for a number of reasons. First, dendritic cells and granuloctyes are hardly present in the brain parenchyma. If these cells would be present in the isolates, they would derive from the circulation. We demonstrated that the fraction of $CD11b^{-}CD45^{+}$ lymphocytes is very small, clearly indicating that the contribution of cells derived from the blood compartment is low. Accordingly, also the number of blood-derived monocytes/macrophages will be insignificant. Second, we and others have previously demonstrated that perivascular macrophages express high levels of MR.[163,183] However, even with the highly sensitive technique of qPCR, MR expression was not detectable in the microglia cultures, indicating that there was no significant contribution of these cells to the cultures. Finally, confirming previous observations,[184] microglia were smaller than choroid plexus macrophages, as seen in the forward versus side scatter profiles of the unpurified cells, as well as consistently seen in culture, corresponding with their morphology in the brain parenchyma. This finding indicates that with respect to the size of the cells, cultured microglia display a homogenous cell population, which is in line with data on mouse microglia.[184]

Besides microglia, choroid plexus cells were also sorted based on CD11b expression. Because a substantial amount of lymphocytes was detectable in the initial isolation, contamination of blood-derived macrophages or granulocytes can not be excluded. However, since the choroid plexus is highly enriched for macrophages, we expect that the majority of the cells are macrophages. Future experiments have to address the purity of this culture. Nevertheless, for the present study, choroid plexus cells were used as control for the microglia culture. In keeping with our previous study (Koning *et al*. submitted), we showed that CD200R and MR are induced

on choroid plexus macrophages following IL-4 stimulation. This suggests an IL-4 induced polarization of these cells and also confirms that the isolation and sorting procedure had no ill effect on IL-4 stimulation of macrophages.

Human microglia biology, especially in MS, is a largely undiscovered field. Because of their controversial association with many neurodegenerative diseases, it is very important to know whether activation of microglia can lead to polarization as seen in other types of macrophages. Under influence of IL-4, choroid plexus macrophages increased CD200R expression, but this was not demonstrated in microglia, although expression of the molecule was evident in these cells. MR is a commonly used marker for alternatively activated macrophages[172] and was indeed up-regulated in choroid plexus macrophages upon IL-4 stimulation, but again, not in microglia. Because MR mRNA levels were undetectable in microglia, MR expression might in addition be useful as a discriminator between macrophages and microglia in man. These data contradict a study on mouse microglia, demonstrating MR expression and up-regulation by IL-4 on these cells.[189,190] However, microglia were obtained 4 to 5 weeks following preparation of the neonatal brains, based on adherence. As stated above, it is possible that these cultures included macrophages derived from blood, perivascular and subarachnoidal spaces, meninges and choroid plexus and astrocytes that are claimed by this group to express MR as well.[189] From our data however, we will further address the usability of MR protein expression in cell sorting as a functional discriminator between human macrophages and microglia.

Although microglia are often referred to as 'macrophages of the brain', the preliminary data from the present study suggest that human microglia do not behave like macrophages in terms of classic or alternative macrophage polarization. On the other hand, HLA-DR expression levels indicated that the cultured microglia were in an activated state. This activation may have caused polarization in either direction. This is an interesting subject for further study. For example, would the activation state of microglia in culture change following soluble CD200 treatment, and would IL-4 treatment then induce polarization and enhance CD200R expression? Future studies will also have to confirm the presence of the receptor for IL-4 on these microglia, as well as its downstream pathway molecules STAT6 and PPARδ, that have been implicated in the induction of alternatively activated macrophages.[191,192]

CD200-CD200R interaction provides an essential inhibitory mechanism to maintain an immune suppressed environment in the brain.[111] Absence of CD200 in mice causes spontaneously activated microglia and results in accelerated onset of experimental autoimmune encephalomyelitis (EAE), the animal model for MS.[125] For such an important system it is peculiar that no regulators of CD200R, other than

IL-4 in monocyte-derived macrophages, have been identified. Also in our hands, none of the different pro- and anti-inflammatory compounds tested convincingly altered CD200 expression on neuroblastoma cells. A recent publication suggests that IL-4 enhanced neuronal CD200 expression.[193] However, the authors used a concentration of 20 µg/ml, 4000 times the concentration used in the present study, and overwhelmingly exceeding physiological concentrations.

In conclusion, the present data suggest that microglia resemble macrophages to a lesser extent than previously assumed. The expression of CD200R on human microglia and CD200 on neurons is confirmed but their regulation remains unidentified. Finally, this study demonstrates a functional technique of isolating, sorting and culturing microglia and provides a powerful tool to further study CD200R regulation in these cells as well as human primary microglia biology in general.