Fc Receptor-Positive Cells in Remyelinating Multiple Sclerosis Lesions

Jin Nakahara, MD and Sadakazu Aiso, MD, PhD

Abstract

The capacity for spontaneous remyelination in cases of multiple sclerosis (MS) is limited and lesions are not fully repaired. Recent evidence has shown that oligodendrocyte precursor cells and immature oligodendrocytes (OPC/iOligs) are preserved in MS lesions. Induced differentiation of these cells into myelinating cells may ultimately lead to a novel remyelination therapy. A previous study showed that the γ chain of immunoglobulin Fc receptors (FcRγ), expressed in OPC/iOligs, is essential for their differentiation. Whether FcRγ is expressed in preserved OPC/iOligs within MS lesions, however, remains uncertain. In the present study, we examined 10 autopsy cases of MS for the expression of FcRγ both in remyelinating areas and demyelinated plaques. The expression of FcRγ was confirmed in both OPC/iOligs and microglia in MS lesions. Statistical analysis showed that the density of FcRγ-positive OPC/iOligs was approximately 3 times greater in remyelinating areas compared with demyelinated plaques; the opposite was true for FcRγ-positive microglia. The distribution of FcRγ-negative OPC/iOligs did not differ between the 2 types of lesions. Thus, an increase in FcRγ-positive OPC/iOligs and a decrease in FcRγ-positive microglia, but not in FcRγ-negative OPC/iOligs, are associated with spontaneous remyelination in MS brains, suggesting a possible role for FcRγ in the induction of remyelination.

Key Words: Immunoglobulin Fc receptors, Multiple sclerosis, Oligodendrocyte, Remyelination.

INTRODUCTION

Multiple sclerosis (MS) is the most pervasive demyelinating disease of the central nervous system (CNS), affecting more than one million people worldwide. The disease is characterized by multifocal demyelination that relapses and remits in time and space. Although the exact etiology remains unknown, studies of rodents with experimental autoimmunity encephalomyelitis (EAE) as a model of MS and histopathologic studies of chronic cases of MS have suggested that MS is a T-cell-mediated autoimmune disease (1). A recent autopsy study of patients with MS who died immediately after the onset of symptoms, however, revealed little evidence of T-cell infiltration (2). Additionally, a study of a rodent model of chronic demyelination showed that the induction of acute inflammation induces remyelination (3), indicating that immune reactions are not merely injurious but occur as a part of the repair process. Several studies have shown that immunoglobulin mediates CNS repair, including remyelination (4–6). These studies challenge our classic view of MS pathology and our efforts to control the disease by simply suppressing the immune response.

Another possible approach for the treatment of MS is to enhance remyelination. Unlike congenital dysmyelinating diseases, there is no evidence of white matter abnormalities in patients with MS before the onset of the disease or during childhood, when myelination takes place, indicating that the myelination machinery of the oligodendrocytes, the myelinating cells of the CNS, is largely intact. Although spontaneous remyelination eventually does occur (7), the level is not sufficient to fully restore the neural functions of patients with MS. In contrast to the old view that MS lesions are deprived of oligodendrocytes and that the insufficient remyelination activity is the result of the lack of myelinating cells, recent evidence has shown that oligodendrocyte precursor cells and immature oligodendrocytes (OPC/iOligs) are preserved in the lesions (8, 9). This evidence has prompted researchers to explore a remyelination therapy in which the differentiation of the preserved OPC/iOligs into myelinating cells is stimulated.

The mechanisms underlying the differentiation of OPC/iOligs into myelinating oligodendrocytes, however, are not fully understood, and the key “trigger” molecule of the process, which would be a potent therapeutic target, has remained uncertain (10). In a study using rodents, we have recently revealed that the γ chain of immunoglobulin Fc receptors (FcRγ) may play such a role (11). FcRγ is expressed in rodent OPC/iOligs, and activating this molecule (e.g. by crosslinking immunoglobulin Fc receptors using IgG and antigen), turns on the differentiation cascade that results in the induction of the morphologic differentiation and stimulation of myelin basic protein expression. We therefore hypothesized that FcRγ may also be involved in remyelination in MS.
lesions. If this were the case, we not only hope to develop a remelination therapy, but may also introduce a new avenue to understand the complicated immunologic nature of this disease.

In this study, we show that FcR\(\gamma\) is expressed in preserved OPC/iOligs and microglia in MS lesions. The density of FcR\(\gamma\)-positive OPC/iOligs was substantially greater in remyelinating areas than in demyelinated plaques, whereas that of FcR\(\gamma\)-negative OPC/iOligs did not differ between the 2 types of lesions. Demyelinated plaques contained numerous FcR\(\gamma\)-positive microglia, although the edges of plaques adjacent to remyelinating areas revealed an exceptional increase of FcR\(\gamma\)-positive OPC/iOligs. Thus, an increase in the density of FcR\(\gamma\)-positive OPC/iOligs correlates with remyelination in MS lesions, which suggests the involvement of FcR\(\gamma\) in the remyelination process. Together with our previous findings (11), these results suggest the hypothesis that FcR\(\gamma\) is involved in the differentiation of remyelinating oligodendrocytes in MS brains.

**MATERIALS AND METHODS**

**Brain Specimens**

The brains of 10 deceased patients with MS (average age, 54.9 years; age range, 47–72 years) and 5 control patients without brain disease (average age, 76.2 years; age range, 54–91 years) were investigated in this study. All of the specimens were obtained from the Human Brain and Spinal Fluid Resource Center in Los Angeles, California. Each of the MS specimens obtained contained one demyelinated plaque within it. Autolysis time varied from 11 to 58 hours (average, 21 hours) for patients with MS and from 9 to 19 hours (average, 12 hours) for control patients. Available clinical

<table>
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<tr>
<th>Table 1. Clinical Characteristics of the Patients Analyzed in This Study</th>
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<td>MS-1</td>
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Clinical information regarding the patients was provided by the Human Brain and Spinal Fluid Resource Center. The presence of “remelinating activity” was determined by the relative density of immature oligodendrocytes with cytoplasmic substances (Fig. 1F) within the remyelinating areas. The “lobe” indicates the lobe of the cerebrum where the lesions were analyzed.

*Clinical course information was not available.
†The remyelinating area could not be precisely determined as a result of the presence of active demyelination (this case was omitted from the statistical analysis).

RR, relapse remitting; PP, primary progressive; SP, secondary progressive; MS, multiple sclerosis; CTRL, control; CAD, coronary artery disease; NA, not applicable; ND, not determined.

**Table 2. Primary Antibodies Used in This Study**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Host/Clone</th>
<th>Dilution</th>
<th>Manufacturer</th>
<th>Target (reference)</th>
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<tr>
<td>FcR(\gamma)</td>
<td>Rabbit/polyclonal</td>
<td>1:500</td>
<td>Upstate Biotechnology, Lake Placid, NY</td>
<td>OPC/iOligs involved in remyelination, Microglia ([11] and this study)</td>
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<tr>
<td>Olig1</td>
<td>Mouse/clone 257219</td>
<td>1:50</td>
<td>R&amp;D Systems, Minneapolis, MN</td>
<td>OPC/iOligs committed to remyelination (if positive in the nucleus) [19]</td>
</tr>
<tr>
<td>Olig2</td>
<td>Rabbit/polyclonal</td>
<td>5000</td>
<td>Dr. H. Yokoo, Department of Pathology, Gunma University Graduate School of Medicine, Gunma, Japan</td>
<td></td>
</tr>
<tr>
<td>NG2</td>
<td>Mouse/clone 9.2.27</td>
<td>1:50</td>
<td>BD Biosciences Pharmingen, San Jose, CA</td>
<td>OPC/iOligs [9]</td>
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<tr>
<td>CNPase</td>
<td>Mouse/clone 11-5B</td>
<td>1:100</td>
<td>Sigma Aldrich, Tokyo, Japan</td>
<td>OPC/iOligs, myelin</td>
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<tr>
<td>MHC class II</td>
<td>Mouse/clone CR3/43</td>
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<td>DakoCytomation, Glostrup, Denmark</td>
<td>Microglia</td>
</tr>
<tr>
<td>NF</td>
<td>Mouse/clone SMI-35</td>
<td>1:250</td>
<td>Sternberger Monoclonals, Berkeley, CA</td>
<td>Axon</td>
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FcR\(\gamma\), the common \(\gamma\) chain of immunoglobulin Fc receptors; CNPase, 2, 3-cyclic nucleotide 3-phosphodiesterase; MHC class II, major histocompatibility complex class II; NF, neurofilament.
information about the patients is summarized in Table 1. Frozen specimens were thawed and fixed in 4% paraformaldehyde phosphate-buffer solution (Wako, Osaka, Japan) for 20 hours. Fixed specimens were washed thoroughly in phosphate-buffered saline at pH 7.4 (PBS; Ambion, Tokyo, Japan) and immersed in an PBS solution containing 20% sucrose (Wako) until the specimens sank. The cryoprotected specimens were frozen and embedded in a compound medium (Tissue-Tek O.C.T. Compound; Sakura Finetechical, Tokyo, Japan). Embedded specimens were sliced into 10-μm-thick sections using a cryostat microtome (CM3050; Leica Instruments, Nubloch, Germany). Slices were collected on glass slides with an aminosilan precoat (Matsunami Glass, Osaka, Japan).

Myelin Staining
A Luxol fast blue (LFB; Acros Organics, Fair Lawn, NJ) solution (0.1% LFB in 95% ethanol containing 0.05% acetic acid [Wako]; stained overnight at 60°C) and a nuclear fast red (NFR) counterstain (Vector Laboratories, Burlingame, CA; stained for 5 minutes at room temperature [RT]) were routinely used for myelin staining. 0.05% lithium carbonate (Wako) solution was used for the color differentiation of LFB.

Immunohistochemistry
A precise step-by-step method is available on written request to the authors. Briefly, antigen retrieval was performed by placing the slides (excluding the slides used for NG2 staining) in boiling 0.01 M citrate buffer solution at pH 7.0 (Muto Pure Chemicals, Tokyo, Japan) for 10 minutes. For NG2 staining, 0.3% Triton X-100 (Sigma Aldrich, Tokyo, Japan) was applied for 30 minutes at RT. Quenching of internal peroxidase activity was performed by placing the slides in 3% hydrogen peroxide (Wako) for 10 minutes at RT. This procedure was carried out for the slides stained using the indirect immunoperoxidase method but omitted for those labeled using the immunofluorescence method. After these procedures, the slides were incubated with primary antibodies diluted in 0.5% skim milk (Becton Dickinson, Sparks, MD) in PBS for 3 days at 4°C, and then with secondary antibodies diluted in 0.5% skim milk in PBS overnight at 4°C. For the immunofluorescence method, the specimens were mounted in Vectashield (Vector Laboratories, Burlingame, CA). For the development of color in immunoperoxidase slides, a solution of 0.01% 3,3-diaminobenzidine, tetrahydrochloride (DAB; Dojindo, Kumamoto, Japan), 0.01% hydrogen peroxidase (Wako), and 0.05 M Tris buffer (Research Organics, Cleveland, OH) was used. The specimens were then mounted in Entellan neu (Merck, Darmstadt, Germany). For double-labeling experiments using 2 rabbit antisera (i.e. anti-Olig2 and anti-FcR) in this study, the antibodies that targeted the nuclei (i.e. anti-Olig2) were visualized first. After immunoperoxidase staining for the first set of antibodies, the slides were

**FIGURE 1.** Demyelination and remyelination in multiple sclerosis (MS) brains. Luxol fast blue (LFB) and nuclear fast red staining results of an MS brain ([A]: a 51-year-old woman, MS-5) and a control brain ([B]: a 54-year-old man, Ctrl-5) are shown in the upper panels. The demyelinated plaque (inactive) is devoid of LFB staining, and the remyelinating area appears as a “shadow plaque” at a lower magnification. At a higher magnification, the characteristics of the MS lesions were determined as follows: “demyelinating plaques (active)” were lesions where actively demyelinating macrophages engulfing LFB-positive myelin debris were observed ([C], arrowheads and inset), “demyelinated plaques (inactive)” were lesions in which LFB staining was diminished and demyelinating macrophages were absent ([E]), and “remyelinating areas” were lesions where abnormally thin and short myelin was observed together with immature oligodendrocytes shortly before myelination (cells with abundant LFB-positive myelin products in their cell body; arrowheads and inset in [F]) were observed. ([D]) Normal myelin sheaths and oligodendrocytes observed in a control brain are shown. Scale bars = ([A, B]) 1 mm; ([C-F]) 50 μm.
washed once in running water for 10 minutes and twice in 0.1 M glycine-HCl (Wako) at pH 2.2 for 30 minutes to remove the bound antibodies. The second set of antibodies (i.e. anti-FcRγ) was administered similarly, and a solution of 0.02% 4-chloro-1-naphthol (Wako), 0.01% hydrogen peroxidase, and 0.05 M Tris buffer was used for the development of blue staining. The specimens were then mounted in Aquatex (Merck). Immunofluorescent slides were viewed under a confocal laser-scanning microscope (Digital Eclipse C1; Nikon, Kanagawa, Japan), whereas immunohistochemistry slides were observed under a stereotactic optical microscope (Coolscope; Nikon).

**Antibodies**

The primary antibodies used in this study are listed in Table 2. The secondary antibodies and reagents used in this study included peroxidase-conjugated antirabbit IgG goat antibodies (gAb) (MBL, Nagoya, Japan; diluted 1:100), peroxidase-conjugated antimouse IgG gAb (MBL; diluted 1:100), indocarbocyanine (Cy3)-conjugated antirabbit IgG gAb (Jackson ImmunoResearch Laboratories, West Grove, PA; diluted 1:100), and fluorescein (FITC)-conjugated anti-mouse IgG gAb (Jackson ImmunoResearch Laboratories; diluted 1:100). TO-PRO-3 iodide nuclear stain (Molecular Probes, Eugene, OR; diluted 1:500) was additionally used to show the nuclear localization of Olig1.

**Statistical Analysis**

Two consecutive slices were selected for each case. One slide was then stained with LFB + NFR, whereas the other was doublelabeled for FcRγ and Olig2. Using the LFB + NFR slide, the locations of the demyelinated plaques and remyelinating areas were stereotactically mapped. The corresponding FcRγ/Olig2 doublestained slide was then analyzed. For slides stained with LFB + NFR, demyelinated plaques (inactive) were identified by the complete loss of LFB staining, and remyelinating areas were identified by the existence of abnormally thin or short myelin and immature oligodendrocytes shortly before myelination (cells with relatively large nuclei and cytoplasmic LFB-positive myelin-like substance). An actively demyelinating lesion, as identified by the presence of macrophages phagocytosing LFB-positive particles, was observed in one of the 10 MS cases analyzed. This case was omitted from the statistical analysis as a result of the difficulty in identifying precisely the remyelinating area; thus, a total of 9 MS cases and 5 control cases were included in the statistical analysis. Photographs of immunohistochemistry slides (labeled for Olig2 and FcRγ) were stereotactically taken for the corresponding demyelinated plaques (inactive) and remyelinating areas in accordance with the results from the LFB + NFR staining. The borders between demyelinated plaques and remyelinating areas (approximately 200 μm in width) were avoided, considering the potential overlap of these areas. Areas with obvious postmortem changes were also avoided. Digital photographs representing 0.146 mm² were taken with a CCD camera at a resolution of 1280 × 960 pixels with an original magnification of 200× under a stereotactic optical microscope (Coolscope; Nikon). At least 3 different photographs that were more than 1 mm apart from each other in the X and Y directions were randomly taken for every plaque and remyelinating area. For Olig2-positive cells (OPC/iOligs), only cells with detectable nuclei were included.
in the count. For Olig2-negative cells (microglia), only cells with detectable cytoplasm were included in the count. The number of FcRγ-positive/Olig2-positive cells (FcRγ-positive OPC/iOligs), FcRγ-positive/Olig2-negative cells (FcRγ-positive microglia), and FcRγ-negative/Olig2-positive cells (FcRγ-negative OPC/iOligs) in each photograph were counted. The average density of the cells per millimeter squared was calculated from the results for demyelinated plaques (inactive) and remyelinating areas. The statistical significance of the differences in average density between demyelinated plaques and remyelinating areas was determined by 2-sided paired t-tests, and differences that resulted in p values less than 0.05 were considered significant. Statistical analysis of normal and MS specimens was not applicable as a result of the original biases in the average age and autolysis time, which may influence the results.

RESULTS

Patients and Multiple Sclerosis Lesions

Brain (cerebrum) specimens from 10 patients with MS and 5 subjects without brain disease were included in this study (Table 1). We analyzed each specimen by myelin staining and nuclear counterstaining (LFB + NFR) to identify and categorize the lesions (Fig. 1). A demyelinating plaque (active) was identified by the presence of macrophages phagocytosing LFB-positive granules (Fig. 1C; normal white matter is shown in Fig. 1D). One of the 10 MS specimens analyzed (MS-1 in Table 1) revealed the presence of an active demyelinating plaque. An area was identified as a demyelinated plaque (inactive) or a chronically demyelinated area if myelin staining and macrophages were both absent (Fig. 1E).

Some degree of remyelination occurs spontaneously in MS lesions. An area in which remyelination takes place often appears as a “shadow plaque” as a result of the paucity of LFB staining at a low magnification (Fig. 1A) when compared with the darker staining observed in normal white matter (Fig. 1B). Although whether a “shadow plaque” signifies the presence of remyelination or incomplete demyelination was a matter of debate in the past, it is now considered to be a remyelinating area (7). Remyelinating oligodendrocytes produce thinner and shorter myelin (13), characteristics that can be detected by LFB staining (Fig. 1F). In the present study, we additionally looked for the presence of immature oligodendrocytes with cytoplasmic LFB-positive myelin-like substance as evidence of remyelinating areas. During the differentiation of OPCs into myelinating oligodendrocytes, immature oligodendrocytes initially produce LFB-positive myelin-like substance and store it in their cytoplasm (14). An area was determined to be remyelinating only if cells such as these were visualized within the shadow plaque (Fig. 1F, arrowheads and inset). Remyelinating areas near an actively demyelinating plaque were not easily discerned, and thus the case with a

FIGURE 3. The identity of FcRγ-positive cells in remyelinating multiple sclerosis lesion. In remyelinating areas where numerous FcRγ-positive oligodendrocyte precursor cells and immature oligodendrocytes (OPC/iOligs) were observed, NG2, an OPC marker ([A–C], arrows) and CNPase, an oligodendrocyte marker ([D–F], arrows and inset) were expressed in the cells. Note that NG2 is also expressed in blood vessels. FcRγ-positive remyelinating oligodendrocytes around neurofilament (NF)-positive axons are also observed (arrows in [G–I]). Scale bars = (A–C, G–I) 33 μm; (D–F) 100 μm.
A demyelinating plaque (MS-1 in Table 1) was excluded from further analysis in this study.

**Fcγ-positive Oligodendrocyte Precursor Cells in Multiple Sclerosis Lesions**

For the detection of OPC/iOligs in MS lesions, we used Olig2, a basic helix–loop–helix (bHLH)-type transcription factor (15–17) as a molecular marker. Olig2 is involved in the specification of oligodendrocytes and neurons during development (18). Because the latter are absent from the white matter, Olig2 can be used as a specific marker for OPC/iOligs. Additionally, Olig2 is unique among OPC/iOligs markers in its nuclear expression, which makes it easy to distinguish from the expression of Fcγ at the cell membrane. The specificity of the antibodies used for Olig2 detection in this study has been established and reported previously by others (12).

We performed double-staining immunohistochemical analysis for Olig2 and Fcγ on sections consecutive to the ones used for LFB + NFR analysis. In demyelinated plaques, many Fcγ+/Olig2 cells (Fcγ-positive microglia; arrows in Fig. 2A) were observed, whereas Fcγ+/Olig2+ cells (Fcγ-positive OPC/iOligs) were much less frequently observed. In remyelinating areas, many Fcγ-positive OPC/iOligs were observed (arrows in Fig. 2C), whereas Fcγ-positive microglia were less frequently observed, indicating an inverse relationship between the distributions of Fcγ-positive OPC/iOligs and microglia in demyelinated plaques and remyelinating areas. Normal white matter contained an intermediate number of both Fcγ-positive OPC/iOligs and microglia (Fig. 2B).

**FIGURE 4.** Fcγ-positive microglia. Major histocompatibility complex class II, a marker for microglia, was expressed in virtually all of the Fcγ-positive cells in demyelinated plaques, where numerous Fcγ-positive microglia were observed ([A–C], arrows and inset; also see Fig. 2). Scale bars = (A–C) 100 μm.

**FIGURE 5.** Statistical analysis. The densities of Fcγ-positive oligodendrocyte precursor cells and immature oligodendrocytes (OPC/iOligs), Fcγ-positive microglia, and Fcγ-negative OPC/iOligs were statistically analyzed in both demyelinated plaques (inactive) and remyelinating areas (see “Materials and Methods” for details). Data representing the same specimen are connected with lines. The red lines indicate averages and standard deviations of the 9 multiple sclerosis specimens analyzed. The solid and 2 dotted horizontal gray lines indicate the averages ± the standard deviations of the results from 5 control subjects (133.83 ± 26.44, 253.69 ± 54.71, and 246.58 ± 59.52 cells/mm² for Fcγ-positive OPC/iOligs, Fcγ-positive microglia, and Fcγ-negative OPC/iOligs, respectively). The average density of Fcγ-positive OPC/iOligs was significantly greater in remyelinating areas compared with demyelinated plaques (310.55 ± 127.68 vs 90.69 ± 45.57 cells/mm²; p < 0.001), whereas that of Fcγ-positive microglia was inversely distributed (176.70 ± 82.64 vs 441.40 ± 195.97 cells/mm²; p = 0.003). No statistically significant difference was found between the 2 areas in the density of Fcγ-negative OPC/iOligs (307.02 ± 78.17 vs 334.73 ± 77.42 cells/mm², p = 0.34).
when compared with the 2 types of MS lesions. There was no remarkable difference in the distribution of FcRγ-Olig2+ cells (FcRγ-negative OPC/iOligs; arrowheads in Fig. 2A–C). Interestingly, FcRγ-positive OPC/iOligs occasionally appeared as clusters in remyelinating areas (left bottom corner of Fig. 2C), suggestive of local proliferation and/or accumulation of the cells.

The morphology of FcRγ-positive OPC/iOligs can be classified into at least 3 types. Type 1 cells were the most morphologically immature cells or OPCs (Fig. 2D) with short, thick processes and occasionally appeared in pairs or groups, possibly reflecting cell divisions that took place shortly before fixation. Type 2 cells were more differentiated cells with thinner web-like processes (late OPCs or early immature oligodendrocytes; Fig. 2E). Type 3 cells looked like immature oligodendrocytes shortly before myelination, which extend thin, long processes that are aligned parallel to the direction of the axons (Fig. 2F). FcRγ-positive OPC/iOligs in normal white matter tended to have a more differentiated morphology (type 2 or type 3; Fig. 2B, H), whereas all 3 cell types were observed in remyelinating areas. Rarely, type 1 FcRγ-positive OPC/iOligs were observed in demyelinated plaques. The FcRγ-positive microglia in demyelinated plaques displayed a variety of morphologic forms with small cytoplasmic volumes, a characteristic of resident microglia (Fig. 2A, G).

To further confirm the identity of the FcRγ-positive OPC/iOligs, we carried out several immunohistochemical analyses in remyelinating areas. NG2 has previously been used as an OPC marker to identify OPCs in MS lesions (9). In agreement with this study, we observed FcRγ-NG2 double-positive cells in these areas (Fig. 3A–C). In the areas where many type 3 FcRγ-positive OPC/iOligs were observed, we detected many cells expressing CNPase, a marker for myelin, with processes elongating in the direction axons run (Fig. 3D–F). Occasionally, FcRγ-positive remyelinating oligodendrocytes were observed in these areas (Fig. 3G–I). FcRγ-positive resident microglia were positive for MHC class II (Fig. 4A–C).

**Statistical Analysis**

We have conducted a statistical analysis of the density of FcRγ-positive OPC/iOligs, FcRγ-positive microglia, and FcRγ-negative OPC/iOligs in demyelinated plaques and in remyelinating areas. The results, presented in Figure 5, clearly indicate that the density of FcRγ-positive OPC/iOligs was substantially greater in remyelinating areas compared with demyelinated plaques (310.55 ± 127.68 vs 90.69 ±...
In the present study, we have shown for the first time that FcRγ is expressed in preserved OPC/iOligs in MS brains (Figs. 2 and 3). We have previously reported that in rodents, FcRγ is the key molecule that induces the differentiation of OPC/iOligs into myelinating oligodendrocytes (11). Together with the results from the current study and for the following reasons, we hypothesize that FcRγ is also the key factor for the differentiation of OPC/iOligs into remyelinating oligodendrocytes in MS brains. First, the density of FcRγ-positive OPC/iOligs was greater in remyelinating areas compared with demyelinated plaques (Fig. 5). Second, FcRγ-positive remyelinating oligodendrocytes (Fig. 3G–I) were present in remyelinating areas. Third, the increase in FcRγ-positive OPC/iOligs at the edge of demyelinated plaques, which appears to be the potential remyelinating area, was demonstrated (Fig. 6) and also confirmed by their nuclear expression of Olig1, a transcription factor involved in remyelination (19) (Fig. 7). The limit of this study is the lack of temporal information resulting from the use of autopsied brains. It is therefore important to test this hypothesis in animal models. To our knowledge, however, there are no suitable models available for this purpose at present.

Several previous studies reported the expression of FcRγ and other relevant molecules in MS brains. For example, reactive microglia in MS lesions were shown to be positive for Olig1 and other relevant molecules in MS brains. For example, reactive microglia in MS lesions were shown to be positive for Olig1 and other relevant molecules in MS brains.

**DISCUSSION**

In the present study, we have shown for the first time that FcRγ is expressed in preserved OPC/iOligs in MS brains (Figs. 2 and 3). We have previously reported that in rodents, FcRγ is the key molecule that induces the differentiation of OPC/iOligs into myelinating oligodendrocytes (11). Together with the results from the current study and for the following reasons, we hypothesize that FcRγ is also the key factor for the differentiation of OPC/iOligs into remyelinating oligodendrocytes in MS brains. First, the density of FcRγ-positive OPC/iOligs was greater in remyelinating areas compared with demyelinated plaques (Fig. 5). Second, FcRγ-positive remyelinating oligodendrocytes (Fig. 3G–I) were present in remyelinating areas. Third, the increase in FcRγ-positive OPC/iOligs at the edge of demyelinated plaques, which appears to be the potential remyelinating area, was demonstrated (Fig. 6) and also confirmed by their nuclear expression of Olig1, a transcription factor involved in remyelination (19) (Fig. 7). The limit of this study is the lack of temporal information resulting from the use of autopsied brains. It is therefore important to test this hypothesis in animal models. To our knowledge, however, there are no suitable models available for this purpose at present.

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of FcR increase in the density, as well as in morphologic development, plaque edge, and remyelinating areas, there was a gradual increase in the areas (Fig. 2C). Going from demyelinated plaques, the expression of FcR was upregulated in chronic MS lesions.

In agreement with our study, we have also detected FcR in MHC class II-positive microglia (Fig. 4). The discrepancy between theirs and our study regarding the detection of FcR in OPC/iOligs may come from the availability of appropriate markers such as Olig2 for these cells. A different study used DNA microarray analysis to suggest that the expression of FcR is upregulated in chronic MS plaques when compared with acute lesions (21). Although histopathologic analysis was not performed in this study, the results indicated that because classic immune cells were more abundant in acute lesions, these cells were not responsible for the upregulated FcR expression. For this reason, the mechanism underlying the upregulation of FcR in chronic lesions remains uncertain (22), although FcR has been shown to be critical in the induction of EAE (23). We propose that FcR is involved in the remyelination process in chronic MS lesions.

The exact origin of FcR-positive OPC/iOligs in remyelinating areas and the plaque edge remains unclear. Proliferation of the cells within remyelinating areas may take place, as we have observed a clustering of FcR-positive OPC/iOligs in the areas (Fig. 2C). Going from demyelinated plaques, the plaque edge, and remyelinating areas, there was a gradual increase in the density, as well as in morphologic development, of FcR-positive OPC/iOligs, but an inverse decrease in those properties of FcR-positive microglia (Figs. 5 and 6). Migration of cells between these lesions cannot be assessed in the present study but is unlikely to explain the phenomenon, because we did not observe any cells with migratory morphology such as asymmetric morphology of the nucleus or the cell body in any of the relevant areas. Acquisition of FcR expression in FcR-negative OPC/iOligs observed in all 3 lesions could not be definitively excluded, although it was rather unlikely owing to the lack of difference in the distribution of the cells (Fig. 5). Thus, one can alternatively hypothesize that FcR-positive microglia, most likely those in the plaque edge, may differentiate into OPC/iOligs in response to changes in their environment. The precise identification of microglia remains difficult. Although the definition of microglia has relied on its morphology in the past, the expression of immunologic molecules (including FcR in some cases) has been used more recently to identify these cells, because microglia are considered to be of hematopoietic origin. Previous studies have shown that microglia can produce OPCs (24) or transdifferentiate into oligodendroglial cells (25). The possibility that FcR-positive microglia in demyelinated plaques are preventing OPC/iOligs differentiation and remyelination is unlikely, because inhibiting microglial activity using minocycline impairs remyelination after demyelination events (26). Although a reevaluation of the definition of microglia may be necessary, these reports may support the previously mentioned hypothesis that FcR-positive microglia differentiate into OPC/iOligs under certain conditions. Our proposal is summarized in Figure 8.

We have previously shown that FcR in OPC/iOligs is coupled with Fc receptors for IgG but not for IgE (11). Thus, humoral immune responses, especially those that are IgG-mediated, may contribute to the remyelination process in MS. This proposal is further supported by the notion that certain components of the inflammatory response are beneficial in promoting remyelination after a demyelinating insult (3). Furthermore, several studies by Rodriguez and colleagues have shown that immunoglobulin is involved in central nervous system repair (4–6). No evidence of the promotion of remyelination, however, was found in patients with MS who received intravenous immunoglobulin therapy (IVIg) (27). It must be noted, however, that the intact blood–brain barrier carries neonatal Fc receptors, which transcytose IgG from the brain to the blood (28). Thus, the contribution of IVIg to an increase in the local concentration of IgG within an
MS brain may be limited, especially in chronic cases. Also, an inhibitory IgG-Fc receptor, which can counter FcRγ signaling, has been detected in OPC/iOligs (11). Therefore, a simple increase in the local IgG concentration may not result in a positive effect. Furthermore, our previous study showed that the crosslinking of IgG with a multivalent antigen was required for efficient FcRγ signaling (11). Theoretically, the development of novel medicines that mimic the antigen−IgG complex, but do not activate complement pathways, which could be delivered specifically to MS lesions to selectively stimulate the FcRγ signaling pathway for remyelination purposes, may be beneficial and should be considered in the future.

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