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The ontogenesis of Fcγ receptors and complement receptors CR1 in human peripheral nerve*

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Summary. The ontogenesis of Fcγ receptors (FcR) and C3b/C4b receptors (CR1) was studied in peripheral nerves from ten fetuses aged from 20 to 38 weeks using immunohistochemical and functional assays. Monoclonal antibodies (mAbs) against FcR and CR1 stained nerve fibers at 10 weeks of gestation and the staining intensity increased during nerve maturation. FcR and CR1 are probably expressed on Schwann cells and are early markers during the development of peripheral nerves. Functional FcR activity was detected in nerve sections before initiation of myelination, which occurs at approximately 18–19 weeks, whereas functional CR1 activity was found in the sections after myelination. Functional CR1 activity may, therefore, be related to myelin. The ontogenesis of FcR and CR1 was also studied on Schwann cells in culture from three fetuses aged 14, 16 and 19 weeks, using immunofluorescence technique with mAbs. The FcR and CR1 are lost on cultured Schwann cells. This suggests that the receptors are not intrinsic to the cells or that Schwann cells require axonal contact for the expression of FcR and CR1.

Key words: Fetal nerve – Fcγ receptor – C3b/C4b receptor

Fcγ receptors (FcR) and C3b/C4b receptors (CR1) are present on various cells of the immune system, such as phagocytes and lymphoid cells. FcR have also been demonstrated on endothelial cells and on trophoblasts in human placenta [8], in choroid plexus and in arachnoid granulations in the central nervous system [13]. CR1 have been demonstrated on erythrocytes [4], leukocytes [4] and glomerular podocytes [10]. Recently, we have demonstrated both receptors on human Schwann cells [29]. Schwann cells are neural crest-derived cells which accumulate along developing axons [20]. Schwann cells synthesize and assemble a myelin sheath at approximately 18–19 weeks of gestation [18], probably as a result of an axonal signal [3].

There are few studies on the ontogenesis of FcR and CR1. Data presented however, indicate that these receptors are early markers during ontogenesis as FcR are found on human thymocytes at 10 weeks of gestation [5] and CR1 are present on human B lymphocytes at 12 weeks [21] and on glomerular podocytes at 9 weeks of gestation [2]. In the present report, immunohistochemical and functional assays were used to study whether FcR and CR1 are early markers on human Schwann cells. Previously, we have found that functional CR1 activity is present in myelinated, but not in unmyelinated adult nerves [25]. It was, therefore, of particular interest to study the relation between functional CR1 activity and the myelination process during the ontogenesis.

Materials and methods

Tissue sections

Fetal femoral nerves were obtained from eight therapeutic pregnancy interruptions according to current Italian laws. Samples from one 10-week, two 14-week, two 16-week, two 19-week and one 20-week fetus, as determined by crown-rump length [14] were examined. Femoral nerves were also obtained from two fetuses of 28 and 36 weeks of gestation who died of intrauterine asphyxia. Adult femoral nerves were obtained from amputations performed in the Department of Surgery. The nerves were frozen in isopentane precooled with liquid nitrogen and mounted on specimen holders for preparation of tissue sections. Sections (5 μm) were cut on a cryostat and placed on glass coverslips. For immunofluorescence staining, sections were fixed in cold methanol for 1 min and washed in phosphate-buffered saline, pH 7.2 (PBS).

Tissue culture

Human fetal Schwann cells were cultured as previously described [16]. Briefly, nerves were removed aseptically from fetuses of 14-

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weeks, 16-weeks and 19-weeks of gestation, put in ice-cold PBS, cleaned of epineurial connective tissue and transferred in PBS containing 0.37% (w/v) trypsin (2.5% solution, code No. 043-5090-H, Gibco, Grand Island, NY, USA) and 0.03% (w/v) collagenase (800 U/mg, code No. C 0255, Sigma, St. Louis, Mo, USA). After mincing with scalpels, they were incubated in the enzyme solution for 45 min at 37° C. The suspension was pipetted with a Pasteur pipette 20 times, centrifuged (447 *g* for 3 min) at room temperature and the pellet washed 3 times in Minimal Essential Medium (MEM) with Earle's salts (Gibco) containing 20% fetal calf serum (FCS), 600 mg/100 ml glucose, 75 U/ml penicillin, 75 mg/ml streptomycin and 0.025 mg/ml amphotericin B. The cells were resuspended in the medium, counted (50,000 cells/ml) and seeded on glass coverslips which were maintained in 24-multi-well plates.

Schwann cells were also obtained from four adult femoral nerves and cultured as previously described [17]. Briefly, nerves, free of epineurium, were minced with scalpels and transfered in MEM with Earle's salts containing 15% FCS, 25 mM Hepes (code no. 1059432, Boehringer, Mannheim, FRG), 1.25 U/ml dispase (code no. 165859, Boehringer), 0.05% (w/v) collagenase, 0.1% (w/v) hyaluronidase (code no. H 3884, Sigma), 600 mg/100 ml glucose, 75 U/ml penicillin, 75 mg/ml streptomycin and 0.025 mg/ml amphotericin B. The suspension was pipetted with a Pasteur pipette 20 times and incubated overnight at 37° C. The tissue was then centrifuged (447 *g* for 3 min), the medium removed and replaced by MEM with Earle's salts containing 1.25 U/ml dispase and 0.05% (w/v) collagenase. After incubation for 45 min at 37° C, the suspension was centrifuged (447 *g* for 3 min) and the pellet washed 3 times in MEM with Earle's salts containing 15% FCS, 600 mg/100 ml glucose, 75 U/ml penicillin, 75 mg/ml streptomycin and 0.025 mg/ml amphotericin B. The cells were then seeded on glass coverslips which were maintained in 24-multi-well plates.

Sera

A mouse monoclonal antibody (mAb) B1D6 against a 40-kDa placental FcR has been described previously [11, 12]. mAb against CR1 (code No. M710; IgG conc. 133 μg/ml), rabbit antiserum to bovine S-100 protein (code No. Z311; IgG conc. 95 μg/ml), which is a marker for human Schwann cells [15], rabbit antiserum to human myelin basic protein (code No. A623; IgG conc. 131 μg/ml), fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse Ig (code No. F232), FITC-conjugated swine anti-rabbit Ig (code No. P217) and tetramethyl rhodamine isothiocyanate (TRITC)-conjugated swine anti-rabbit Ig (code No. R156) were purchased from Dakopatts, Glostrup, Denmark.

Indicator cells

Indicator cells for demonstration of FcR were sheep erythrocytes (E) sensitized with rabbit IgG antibodies (A) (EA) [8]. Sheep E were also sensitized with A of IgM class (EA) and incubated in zymosan-treated human serum (C) (EAC3b) for demonstration of CR1 [9]. Hemadsorption to nerve sections was performed using the closed chamber technique as described previously [9].

Immunofluorescence technique

Sections were incubated overnight in a moist chamber at 4° C with the mAbs diluted in PBS. After washing in PBS, the sections were incubated for 30 min at room temperature with FITC-conjugated rabbit anti-mouse Ig diluted 1:30 in PBS containing 20% pooled human serum (PHS). The sections were then washed in PBS and incubated with swine anti-rabbit Ig diluted 1:30 in PBS containing 20% PHS. Sections were also incubated overnight in a moist chamber at 4° C with anti-S-100 protein diluted 1:40 in PBS or with anti-myelin basic protein diluted 1:40 in PBS, washed in PBS and then incubated with swine anti-rabbit Ig diluted 1:30 in PBS containing 20% PHS. Controls were prepared by substituting the mAbs, the anti-S-100 protein or the anti-myelin basic protein with PBS, normal mouse serum, normal rabbit serum or supernatant from the myeloma cell line P3 × 63Ag8 which was used for the production of the anti-FcR mAb.

Schwann cells cultured on coverslips were incubated for 30 min at room temperature with undiluted anti-FcR or anti-CR1 mAbs, washed in MEM and incubated for 30 min at room temperature with FITC-conjugated rabbit anti-mouse Ig diluted 1:30 in PBS. In double-staining experiments, Schwann cells were first incubated as described, then fixed in 3% formaldehyde for 5 min at 4° C, washed in MEM and incubated overnight in a moist chamber at 4° C with anti-S-100 protein diluted 1:100 in PBS. The cells were then washed in MEM and incubated for 30 min at room temperature with TRITC-conjugated anti-rabbit Ig diluted 1:80 in PBS. The tissue sections and Schwann cells were mounted in glycerol-PBS and examined in a Zeiss immunofluorescence microscope.

Results

FcR on peripheral nerve fibers

The anti-FcR mAb stained sections of the 10- and 14-week nerves up to a dilution of 1:4 (Fig. 1, Table 1). Sections of the 16-week nerves were stained with the mAb diluted up to 1:32 and sections of the 19-, 20-, 28- and 36-week nerves as well as sections of adult nerves were stained with the mAb diluted up to 1:512. The staining obtained with the anti-S-100 protein, indicated that the FcR were localized to the Schwann cells. Myelin formation was demonstrated at 19 weeks by staining with antibody against myelin basic protein and by the appearance of birefringent rings detected using phase-contrast microscopy (Fig. 1). The unmyelinated areas were stained by the anti-FcR mAb with a stronger intensity than the myelinated areas. In the 28-week nerve most of the nerve fibers were myelinated and the mAb stained equally well the myelinated and the unmyelinated fibers.

EA adhered to sections of adult peripheral nerves as previously described [24]. In addition, EA adhered to sections of 16-week nerves, but not to 14-week nerves (Table 1). The adherence to the 16-week nerves was weak, whereas the adherence to the 19-week nerves was as strong as to adult nerves. Unsensitized E or E sensitized with IgM did not adhere.

CR1 on peripheral nerve fibers

Undiluted anti-CR1 mAb showed weak staining of sections of 10- and 14-week nerves (Fig. 1, Table 1). The staining intensity increased with maturation and the 16-week nerves were stained with the mAb diluted up to 1:4 while the 19-, 20-, 28- and 36-week nerves as well as the adult nerves were stained with the mAb diluted up to 1:8. The staining obtained with the anti-S-100 protein, indicate that the CR1 were localized to the Schwann cells. During myelination at 19 weeks, the mAb stained both the unmyelinated and myelinated areas equally well (Fig. 2).

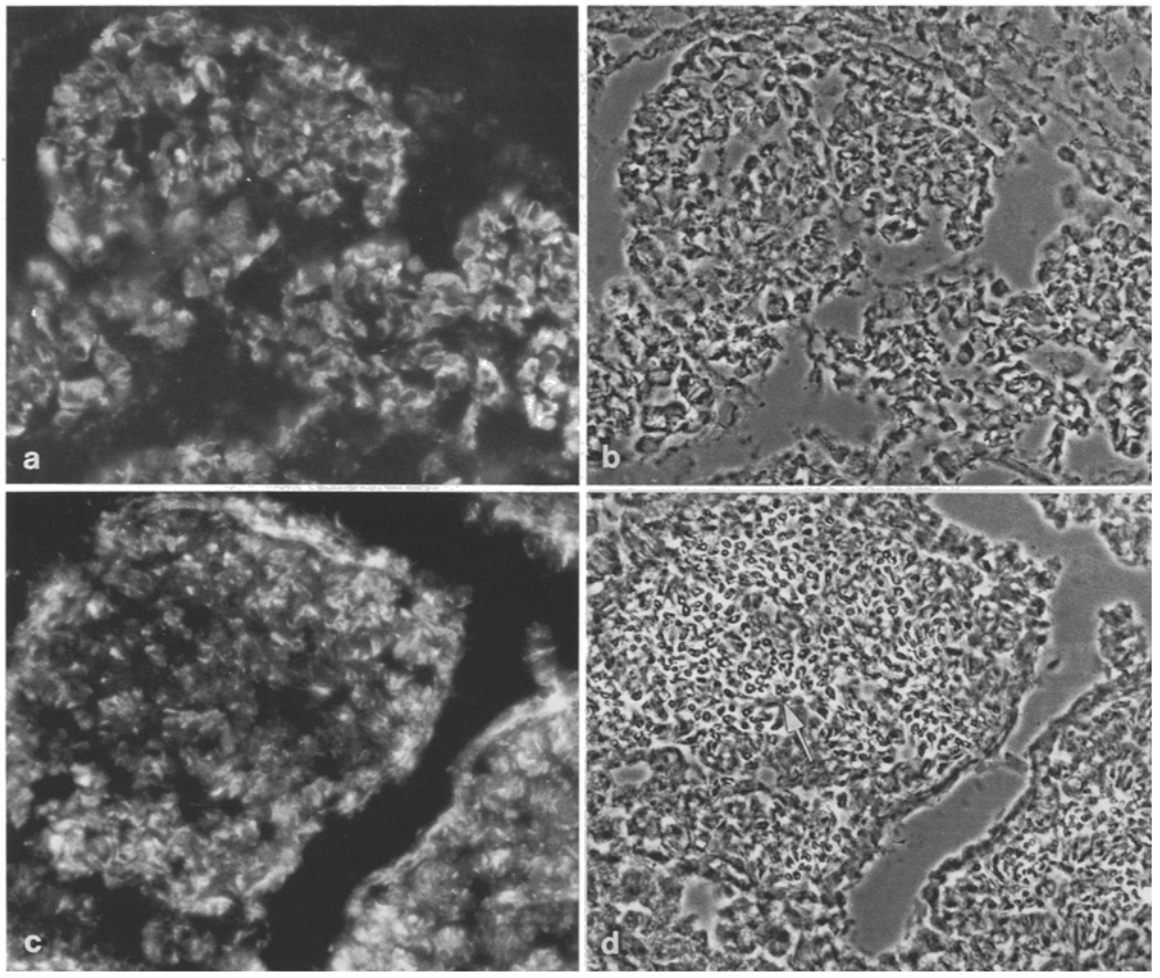

Fig. 1 a – d. Immunofluorescence staining with monoclonal antibody against FcR and phase-contrast microscopy of fetal nerves. The staining was localized to the nerve fibers. The gestational ages of the nerves are 10 weeks (**a, b**) and 19 weeks (**c, d**). The 19-week nerve displays birefringent myelin rings (*arrow*). ×250

Table 1. Expression and functional activity of Fcγ receptors (FcR) and complement receptors CR1 during the ontogenesis of human peripheral nerves. The receptors were demonstrated by immunofluorescence technique using monoclonal antibodies (mAbs) and functionally by hemadsorption of indicator cells coated with IgG (EA) or C3b (EAC3b), respectively. Myelin formation was demonstrated by staining with antibody against myelin basic protein and by the appearance of birefringent rings detected using phase-contrast microscopy

Receptors/ myelin	Fetal nerves (weeks)							Adult nerve
	10	14	16	19	20	28	36	
FcR (mAb)	+	+	+	+	+	+	+	+
FcR (EA)	−	−	+	+	+	+	+	+
CR1 (mAb)	+	+	+	+	+	+	+	+
CR1 (EAC3b)	−	−	−	−	−	+	+	+
Myelin	−	−	−	+	+	+	+	+

EAC3b adhered to adult peripheral nerves as previously described [25]. However, EAC3b did not adhere to the 20-week nerve, whereas the indicator cells showed weak adherence to the 28-week nerve (Table 1).

Schwann cell culture

Schwann cells were identified by morphology (elongated, bipolar or multipolar cells with oval nuclei) and nuclear and cytoplasmic staining with the anti-S-100 protein (Fig. 3). The anti-FcR and anti-CR1 mAbs did not stain Schwann cells in any of the cultures from 14-, 16-, 19-week nerves or from adult nerves, when examined at 3, 7, 21 and 35 days in culture.

Discussion

The expression of FcR and CR1 in human peripheral nerves was demonstrated from the 10th week of gestation by immunohistochemical technique using mAbs. The staining was apparently localized to the Schwann cells. CR1 have previously been detected on kidney podocytes at 9 weeks of gestation [2] and on B cells in liver, bone marrow and spleen at 12 weeks of gestation [21]. Apparently, FcR and CR1 are early markers for Schwann cells during the development of peripheral nerves.

The staining intensity of the mAbs increased during development up to 19 weeks of gestation, indicating that

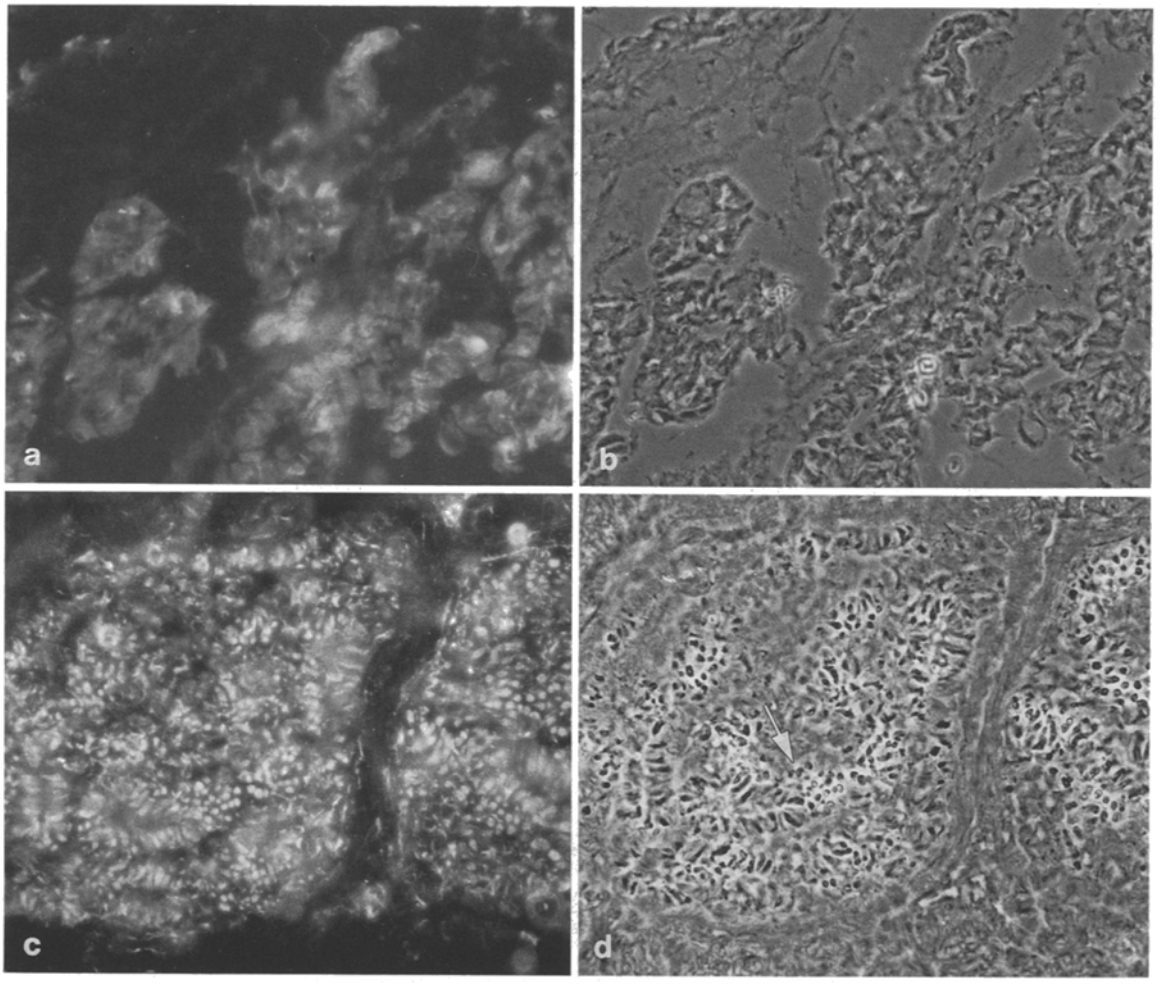

Fig. 2a–d. Immunofluorescence staining with monoclonal antibody against CR1 and phase-contrast microscopy of fetal nerves. The staining was localized to the nerve fibers. The gestational ages of the nerves are 10 weeks (**a, b**) and 19 weeks (**c, d**). The 19-week nerve displays birefringent myelin rings (*arrow*). ×250

Schwann cells increase their number of receptor molecules. An increased number of CR1 molecules has been demonstrated in the development of kidney podocytes [2].

The anti-CR1 mAb stained myelinated and unmyelinated areas equally well during initiation of myelination which occurred at approximately 19 weeks. The anti-FcR mAb however, stained unmyelinated areas stronger than myelinated areas at the initiation of myelination, whereas no difference in the staining intensity was seen at 28 weeks of gestation. The reason for this apparently temporary down-regulation of the FcR expression during initiation of myelination is at present unknown.

The data obtained using hemadsorption assay indicate that the functional activity of FcR and CR1 appears after the demonstration of receptor molecules by mAbs. This is in line with data reported by Appay et al. [2] who found that the CR1 molecules on podocytes were detected at 9 weeks of gestation, whereas functional CR1 activity could only be detected in well-differentiated glomeruli. The late acquisition of functional CR1 activity may be due to the requirement of a minimal density of CR1 molecules on the cells to allow multivalent attachment of EAC3b.

Functional FcR activity appeared just before initiation of the myelination. Previously, functional FcR activity has been demonstrated in both adult myelinated and unmyelinated nerves [24]. The FcR activity is, therefore, not dependent on the presence of myelin. Functional CR1 activity, however, appeared after initiation of the myelination. Furthermore, CR1 activity has been demonstrated only in adult myelinated nerves and not in unmyelinated nerves [25]. The results, therefore, indicate that functional expression of CR1 activity is associated with myelin production. These findings are of particular interest since myelin can activate complement [6], and CR1, both in situ [26] and in soluble form [27], can down-regulate the complement cascade. Accordingly, CR1 may be of significance in preventing the formation of the terminal lytic complex which can cause damage to the cell membrane.

The biological functions of FcR on Schwann cells remain unclear. The FcR may be of significance in the binding of immune complexes, in phagocytosis and in local immunoregulation [23].

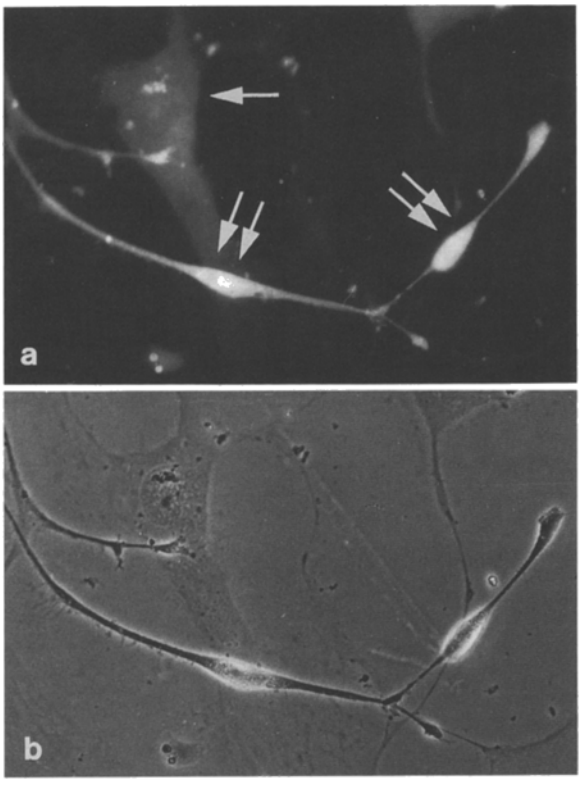

Fig. 3. Immunofluorescence staining (**a**) with polyclonal antibody against S-100 protein and phase-contrast microscopy (**b**) of cultured fetal Schwann cells. Schwann cells from a 19-week nerve after 7 days in culture are positive (*arrows*) and the fibroblasts are negative (*arrow*). ×250

The FcR and CR1 are lost as Schwann cells from both fetal and adult nerves grow in culture. This accords with previous findings showing that CR1 on glomerular epithelial cells are lost in culture [19]. The loss of FcR and CR1 may be due to the possibility that the receptors are not intrinsic to the cells but merely absorbed from FcR and CR1 present in sera [22, 30]. Alternatively, the expression of FcR and CR1 are dependent on a Schwann cell–axon interaction. An intriguing observation is that the expression of myelin protein Po and myelin basic protein in cultured rat Schwann cells are dependent on axonal contact [7].

FcR expressed on human leukocytes have been divided into three distinct classes – FcRI, FcRII and FcRIII – on the basis of ligand affinity and reactivity with class-specific mAbs [1]. Preliminary data obtained in transfection experiments with COS (CV-1, origin of Sv40) cells indicate that the mAb B1D6 probably reacts with a FcR belonging to the FcRII family. The mAb has also been used to purify a 40-kDa FcR with low affinity for IgG from human peripheral nerves further indicating that the isolated receptor belong to the FcRII family [28]. Recent data indicate that human Schwann cells also possess FcRIII (data to be published). Further studies will address the expression of FcRIII during peripheral nerve development.

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