

abstract

Peripheral nerve damage is a major complication of leprosy and often occurs during leprosy reactions, leading to permanent loss of nerve function. Peripheral nerve fibers are surrounded by Schwann cells, which are known to be predilection sites of *M. leprae*. Immune mediated destruction of infected Schwann cells may thus play a key role in the pathogenesis of leprosy nerve damage. We recently established a novel model to study human Schwann cell/T cell interactions *in vitro* and showed that type-1 T cells can efficiently kill Schwann cells in an *M. leprae* dependent fashion. Besides being facultative antigen presenting cells (APC), Schwann cells may also produce a variety of immunomodulatory cytokines that may influence local immune reactivity. Here we have investigated the production of pro- and anti-inflammatory cytokines by human Schwann cells and examined their influence on *Mycobacterium leprae* induced T cell activation. An unexpected observation was that human Schwann cells are able to produce IL-4 and IL-10, as determined by ELISPOT. To the best of our knowledge, this is the first demonstration that human IL-4 can be produced by non-hematopoietic cells. Both IL-4 and IL-10 were inducible by *M. leprae* only, but not by *Escherichia coli* derived LPS. Importantly, IL-4 neutralizing antibodies augmented Th1 cell proliferation when added to Schwann cells, but not when macrophages were applied as APC, thus demonstrating that production of endogenous IL-4 by *M. leprae* exposed Schwann cells can inhibit *M. leprae* specific T cell activation.

M. leprae as well as LPS were found to induce the production of IL-6, IL-8, and PGE₂ by Schwann cells, whereas TGF- β was produced constitutively. Production of RANTES was only induced by LPS but not *M. leprae*. No expression of IL-1 β , IL-12, IL-18, TNF- α or IFN- γ could be detected in human Schwann cells. In line with these observations, neutralizing antibodies to IL-12 or IL-18 did not inhibit T cell proliferation induced by *M. leprae* pulsed Schwann cells, whereas these antibodies strongly inhibited T cell responsiveness to *M. leprae* presented by macrophages, indicating that Schwann cells fail to produce type-1 stimulatory cytokines. Our results demonstrate that human Schwann cells predominantly produce anti-inflammatory type-2 like factors, and fail to produce detectable levels of major type-1 cytokines. Thus, Schwann cells may act as type-2 antigen presenting cells. In this fashion Schwann cells likely create a peripheral nerve-tissue specific, immune privileged environment that skews cellular immune responses towards a Th2 type, rather than a potentially noxious, inflammatory Th1 dependent reaction.

introduction

Nerve damage is one of the most prominent and feared features of leprosy. Neuritis occurs in patients across the entire leprosy spectrum, but particularly during reversal reactions. Reversal reactions are observed in approximately 25% of all leprosy patients and represent a temporal upgrade of cellular immunity (Modlin *et al.* 1983). T cells in the granulomas are predominantly CD4⁺, whereas CD8⁺ T cells are mostly present in the surrounding mantle area (Modlin *et al.* 1983; Narayanan *et al.* 1984; Cooper *et al.* 1989). Strong increases of type-1 cytokines have been reported during reversal reactions (Cooper *et al.* 1989; Yamamura *et al.* 1991; Yamamura *et al.* 1992). T cell clones isolated from skin and nerve biopsies of patients with reversal reactions are predominantly of Th1-type (Verhagen *et al.* 1998; Verhagen *et al.* 1997; Spierings *et al.* Unpublished). Moreover, the microanatomical location of serine esterase positive cells within tuberculoid granulomas and reversal reactions overlaps with that of the CD4⁺ CD45RO⁺ subpopulation (Cooper *et al.* 1989). In line with these observations, *Mycobacterium leprae* reactive CD4⁺ cytotoxic T cell clones have been shown to produce serine esterase and to be highly cytotoxic (Mutis *et al.* 1993a). Thus, Th1 like cytotoxic T cells are believed to

play a major role in the immunopathology of leprous neuritis.

Human Schwann cells are predilection sites for *M. leprae* (Boddingius 1974). The neurotropism of *M. leprae* has been attributed to specific binding of the bacillus to the α 2-chain of laminin-2, a molecule that is almost exclusively expressed in the lamina basalis of Schwann cells (Rambukkana *et al.* 1997; Rambukkana *et al.* 1998). *M. leprae* infected Schwann cells can be targets for recognition by cytotoxic CD8⁺ and CD4⁺ T cells in mice (Steinhoff and Kaufmann 1988) and humans (Spierings *et al.* chapter 2a). Nothing is known, however, about the potential immunomodulatory role of human Schwann cells during infection and antigen presentation to T cells. Schwann cells may play an active role in modulating local T cell mediated inflammatory responses via the production of inhibitory or enhancing cytokines. Studies in mice and rats have revealed that rodent Schwann cells are able to produce interleukin(IL)-1 β (Bergsteinsdottir *et al.* 1991), IL-6 (Bolin *et al.* 1995), Transforming Growth Factor- β (TGF- β) (Skoff *et al.* 1998), and Prostaglandin E₂ (PGE₂) (Constable *et al.* 1994). Furthermore, studies on human biopsy material suggested the production of IL-12 (Turka *et al.* 1995). These cytokines may modulate immune responses under pathological and non-pathological conditions.

Here we have studied the role of human Schwann cells in an *in vitro* model, developed to define immunopathogenic mechanisms of leprosy nerve damage. The capacity of Schwann cells to produce chemokines and pro- and anti-inflammatory cytokines was investigated, as well as the role of these cytokines in Schwann cell/T cell interaction.

materials and methods

reagents

M. leprae sonicate was provided through the WHO/IMMLEP programme by Dr. P. J. Brennan (Colorado State University, Fort Collins, CO). LPS of *E. coli* was purchased from Sigma (Sigma Aldrich, St. Louis, MO). Anti IL-12 and anti IL-18 antibodies were gifts of Dr. G. Trinchieri (Wistar Institute, Philadelphia, PA) and Dr. M. Kurimoto (Fujisaki Institute, Okayama, Japan) respectively. Recombinant IL-12 and IL-18 were purchased from R&D (R&D Systems, Minneapolis, MN). IL-4 antibody MP4-25D2 and anti IL-10 antibody JES3-9D7 were purchased from Pharmingen (Pharmingen, San Diego, CA) and Genzyme (Genzyme Diagnostics, Cambridge, MA) respectively.

Schwann cell cultures

Human Schwann cells were isolated from sural nerve biopsies and propagated as described before (van den Berg *et al.* 1995). Briefly, the sural nerve specimens were cut into small pieces and incubated in 85% Iscove's modified DMEM (IMDM) (GIBCO BRL, Grand Island, NY), 10% lymphokine activated killer (LAK) cell supernatant (Lamers *et al.* 1992), 5% fetal calf serum (FCS), 100 U/ml penicillin, 100 μ g/ml streptomycin, and 0.25 μ g/ml phytohemagglutinin (PHA) (Murex Diagnostics, Dartford, UK). The nerve fragments were incubated in humidified 5% CO₂ at 37°C. After 10 days the non-adherent cells were removed and the adherent cells were propagated in medium consisting of 85% IMDM with 0.6% glucose (Sigma), 10% FCS, and 5% LAK cell supernatant.

cytokine induction

Human Schwann cells were harvested using trypsin-EDTA, and seeded at 2000 cells per well into 96 wells flat bottom plates (Greiner GmbH, Solingen, Germany). Cells were allowed to adhere for 24 hours at 37°C, 5% CO₂. After replacement of medium with IMDM, 10% FCS, cells were stimulated with LPS (100 ng/ml) or *M. leprae* sonicate (25 μ g/

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ml) and incubated for 48 hours. Supernatants were collected and stored at -20°C until analysis.

ELISA

Production of cytokines by cultured human Schwann cells was measured in the culture supernatant by ELISA. The detection level of each cytokine detection assay is given in brackets. For IL-4 (1 pg/ml), IL-6 (2 pg/ml), IL-8 (10 pg/ml), and IL-10 (5 pg/ml) measurement a sandwich ELISA was used (Pelikine, CLB, Amsterdam, The Netherlands). IFN- γ (100 pg/ml) (Boonstra *et al.* 1997), TGF- β (1 pg/ml), TNF- α , (100 pg/ml) MCP-1 (400 pg/ml) (van den Berg *et al.* 1998) and RANTES (50 pg/ml) (Deckers *et al.* 1998) sandwich ELISA were performed as described before. IL-12 (20 pg/ml), IL-1 β (5 pg/ml), and PGE₂ (30 pg/ml) production was measured using Quantikine ELISA kits (R&D Systems). IL-18 ELISA (30 pg) was kindly provided by Dr. M. Kurimoto (Fujisaki Institute, Okayama, Japan).

ELISPOT assay

IL-4 and IL-10 secreting cells were detected and quantified by ELISPOT assay using human IL-4 and IL-10 ELISPOT systems (U-CyTech BV, Utrecht, The Netherlands), as described before (van der Meide *et al.* 1995) with minor modifications. After coating overnight with IL-4 or IL-10 specific monoclonal antibodies at 4°C, Schwann cells were added at a density of 500 cells/well, together with either medium, LPS (100ng/ml), or *M. leprae* sonicate (25 μ g/ml) in triplicate. Schwann cells were removed 48 hours later by addition of ice water for 30 minutes. Subsequently, the wells were washed five times with PBS/0.01% tween. Visualization of IL-4 or IL-10 producing cells was performed as recommended by the manufacturer. The spots were examined under low magnification (7x). The number of cytokine producing cells was expressed per 1000 Schwann cells.

RT-PCR

Total RNA was isolated from trypsinized Schwann cell cultures stimulated with LPS or *M. leprae* using the High Pure RNA isolation kit following the instructions of the manufacturer (Boehringer Mannheim GmbH, Mannheim, Germany). First strand cDNA was generated from oligo(dT) primed RNA by incubation at 42°C for 60 minutes in 20 μ l RT mix containing 75 mM KCl, 50 mM Tris-HCl (pH 8.3), 3 mM MgCl₂, 0.4 mM of each dNTP, 10 units AMV reverse transcriptase, and 25 units RNasin (Promega, Madison, WI).

All PCR reactions contained 20 pmol of each primer in 50 μ l of a reaction mixture containing 10mM Tris/HCl (pH 8.4) buffer, 50 mM KCl, 4mM MgCl₂, 0.5 mM dNTPs and 1 unit Taq polymerase (Perkin Elmer Roche Molecular Systems Inc., Branchburg, NJ). Primers used are listed in table 1.

Amplification started with a denaturation step of 5 min at 95°C. Cycling conditions were 95°C for 30 s, 50°C for 30 s, and 72°C for 90 s for 35 cycles, followed by an extension of the last step with 5 min. Following amplification, 10 μ l of each PCR product was applied to a 2% agarose gel, visualized under UV after ethidium bromide staining and photographed.

antigen presentation assay

Schwann cells were collected and seeded 2000/well as described above. After 24 hours, 25 μ g/ml *M. leprae* sonicate was added to the cells in IMDM, 10% human serum. After pulsing with antigens for 40 hours, cells were washed three times with IMDM and subsequently co-cultured for 88 hours with 10⁴ T cells. T cell clone R1E4 recognizing HSP70 of *M. leprae* has been described before (Janson *et al.* 1991). Sixteen hours before

termination of the assay, 50 μ l supernatant was collected for IFN- γ measurement. Subsequently, 10 μ Ci 3 H-Thymidine per well was added. The incorporation of 3 H-Thymidine was assayed by liquid scintillation counting. Anti-IL-12 (1 μ g/ml), anti-IL-18 (1 μ g/ml), anti-IL-4 (20 μ g/ml, 5.0 μ g/ml, and 1.25 μ g/ml), rIL-12 (2.5 μ g/ml) and rIL-18 (100 ng/ml) were added to the cultures for determination of the production of these cytokines.

results

cytokine ELISA

Cytokine production by human Schwann cells was determined after stimulation with LPS or *M. leprae*. No IFN- γ , TNF- α , IL-12, IL-18 or IL-1 β production could be detected by ELISA (figure 1). PGE₂ (figure 2c), TGF- β (figure 2d), IL-6 (figure 3a) and MCP-1 (figure 3d) were produced constitutively in the absence of stimuli, while low levels of RANTES (figure 3c) and IL-8 (figure 3b) were detectable prior to stimulation. Stimulation with *M. leprae* enhanced IL-6, IL-8, and PGE₂ production but had no effect on the production of RANTES, MCP-1, and TGF- β . Like *M. leprae*, stimulation with LPS raised production of IL-6, IL-8, and PGE₂. In contrast to *M. leprae*, LPS induced the production of RANTES and MCP-1 (figure 3c-d).

detection of IL-4 and IL-10 production by ELISPOT assay

To determine the production of IL-4 and IL-10 a more sensitive technique was used: different human Schwann cell cultures were tested for IL-4 and IL-10 production after stimulation with LPS or *M. leprae* in ELISPOT. As shown in figure 2a-b and figure 4, *M. leprae* was able to induce IL-4 and IL-10 production in a significant number of Schwann cells. In contrast, LPS failed to induce significant production of IL-4 or IL-10 by Schwann cells. All Schwann cell cultures showed similar numbers of IL-4 producing cells. IL-4 staining was very intense, suggesting that positive cells produced relatively high amounts of IL-4. The intensity of IL-10 spots was weaker than in the case of IL-4 (figure 4), but the number

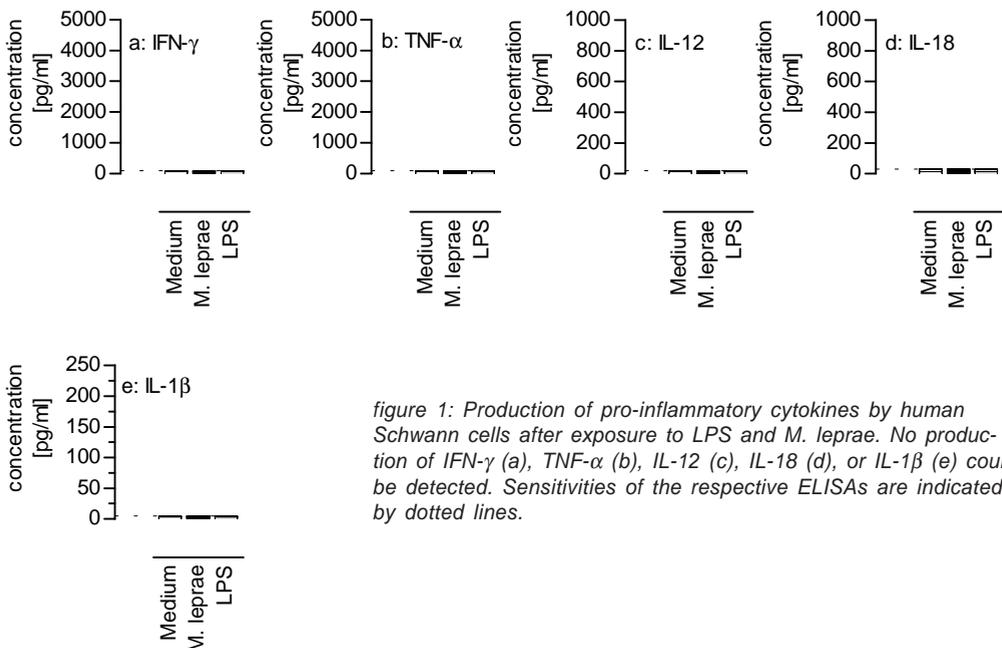


figure 1: Production of pro-inflammatory cytokines by human Schwann cells after exposure to LPS and *M. leprae*. No production of IFN- γ (a), TNF- α (b), IL-12 (c), IL-18 (d), or IL-1 β (e) could be detected. Sensitivities of the respective ELISAs are indicated by dotted lines.

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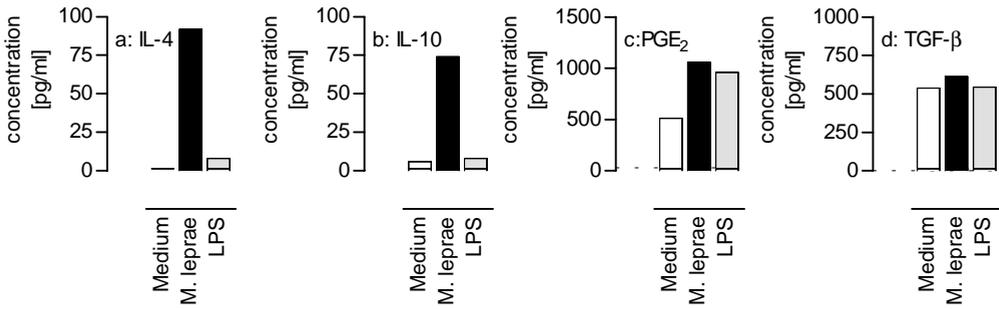


figure 2: Production of type 2 factors by human Schwann cells after exposure to LPS and *M. leprae* as tested in ELISPOT (IL-4 and IL-10) and ELISA (PGE₂ and TGF-β). *M. leprae* but not LPS was found to induce IL-4 (a) and IL-10 (b) production by human Schwann cells. Increased levels of PGE₂ can be found after stimulation with both *M. leprae* and LPS (c). TGF-β is constitutively produced and the production remains unchanged after addition of *M. leprae* or LPS (d). Sensitivities of the PGE₂ and TGF-β ELISAs are indicated by dotted lines.

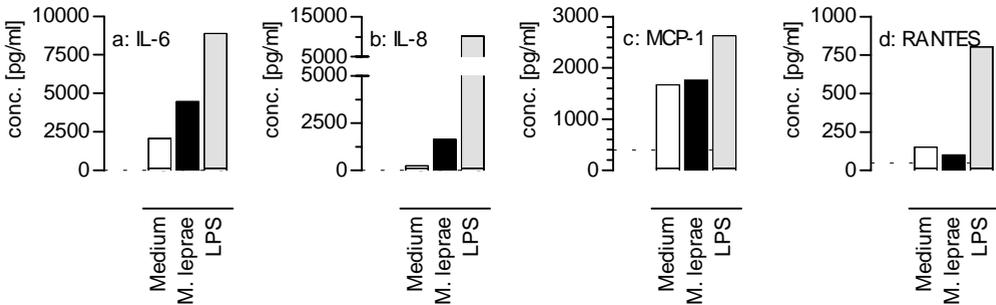


figure 3: Production of chemoattractants by human Schwann cells after exposure to LPS and *M. leprae*. Both *M. leprae* and LPS induce IL-6 (a) and IL-8 (b) production by human Schwann cells. Increased levels of RANTES were found after stimulation with LPS, but not *M. leprae* (c). MCP-1 is constitutively produced and can only be enhanced by LPS (d). Sensitivities of the respective ELISAs are indicated by dotted lines.

of positive spots was of the same magnitude (figure 2). Induction IL-10 by *M. leprae*, but not by LPS was confirmed by RT-PCR (figure 5).

significance of IL-4 in repressing T cell proliferation

IL-4 is a type-2 cytokines that inhibits Th1 development and function. In order to investigate whether IL-4 produced by Schwann cells is able to modulate Th1 responses, IL-4 neutralizing antibodies and control antibodies were used. Proliferation of a CD4⁺ Th1 type T cell clone which does not produce IL-4 itself, increased threefold in the presence of anti IL-4 but not in case of control antibodies (figure 6a). Enhancement of T cell proliferation was dependent on the dose of IL-4 antibodies added. In contrast, addition of IL-4 antibodies did not enhance T cell proliferation when HLA-DR matched PBMC derived monocytes were used as APC, ruling out that IL-4 antibodies are mitogenic and confirming that macrophages do not produce IL-4 in the presence of *M. leprae*. These results strongly suggest that IL-4 production is specifically induced by *M. leprae* in human Schwann cells and has a direct inhibitory effect on Th1 activation.

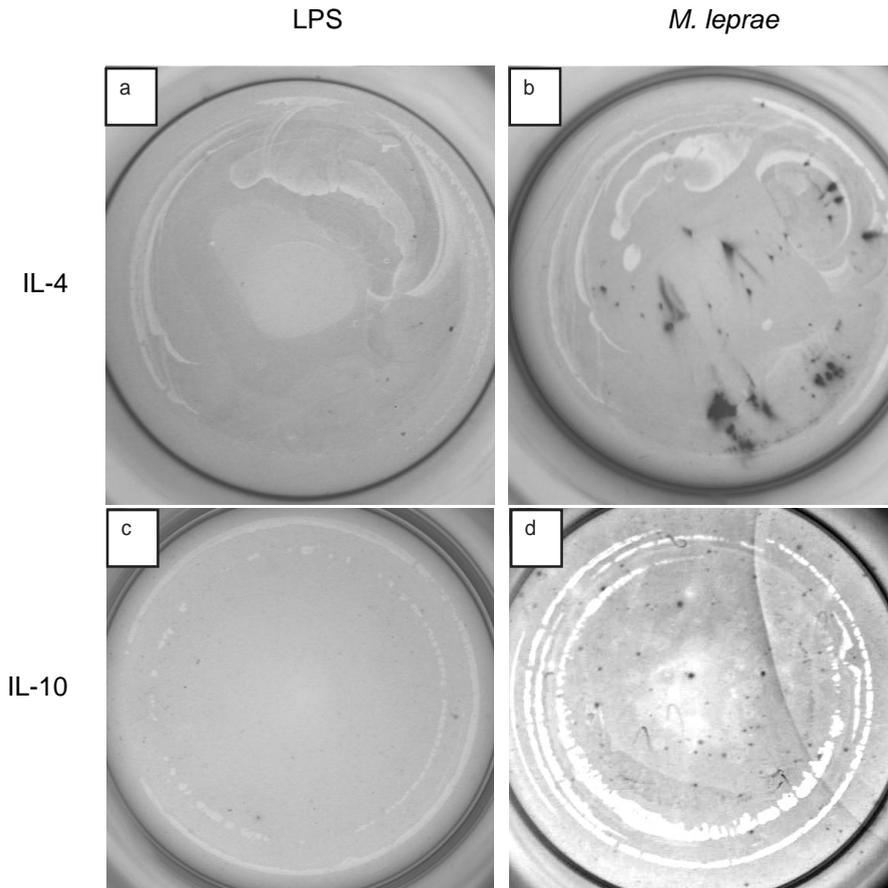


figure 4: ELISPOT analysis of IL-4 and IL-10 producing human Schwann cells, after exposure to LPS (a and c) and *M. leprae* (b and d). Only few small spots could be observed for IL-4 in case of LPS (a). Both the number and the size of the spots increased dramatically when Schwann cells were exposed to *M. leprae* (b). Similar results were obtained for IL-10 (c and d).

IL-12 and IL-18 are not produced by human Schwann cell in vitro

To exclude the production of low amounts of the Th1 cytokines IL-12 and IL-18 and to further document the type-2 cytokine phenotype of human Schwann cells, a functional assay was performed using neutralizing antibodies to IL-12 and IL-18. Addition of anti-IL-12 and anti-IL-18 antibodies had no effect on T cell proliferation induced by Schwann cells (figure 7a), confirming that human Schwann cells are unable to produce IL-12 or IL-18 *in vitro* after exposure to *M. leprae*. In contrast, anti-IL-12 and anti-IL-18 antibodies strongly inhibited T cell proliferation when using monocytes as antigen presenting cells, showing that these antibodies are able to inhibit regular Th1 responses (figure 7b). Addition of the rIL-12 or IL-18 to T cell/Schwann cell co-cultures dramatically increased T cell responses to *M. leprae* (figure 7c-d), demonstrating that IL-12 and IL-18 are limiting factors in Schwann cell mediated antigen presentation.

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mRNA	primer
IL-10 sense	5'-AAATTTGGTTCTAGGCCGGG-3'
IL-10 antisense	5'-GAGTACAGGGGCATGATATC-3'
β 2-m sense	5'-CAGCAGAGAATGGAAAGTC-3'
β 2-m antisense	5'-GATCGCTGCTTACATGTCTCG-3'

table 1: PCR primers used for the detection of IL-10 mRNA. β 2-microglobulin primers were used as control.

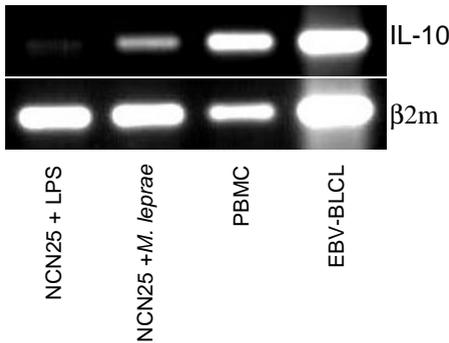


figure 5: RT-PCR analysis of IL-10 mRNA expression by human Schwann cells after exposure to LPS (lane 1) and *M. leprae* (lane 2). IL-10 mRNA was hardly detectable after a 48 hours period LPS exposure, while *M. leprae* induced detectable levels of IL-10 mRNA. Levels of β 2-microglobulin mRNA were similar under both conditions. PBMC and Epstein-Bar Virus transformed B cells were used as positive controls.

discussion

This study documents the cytokine-profile and -function of *in vitro* cultured human Schwann cells after exposure to *M. leprae*. Schwann cells appeared to produce high levels of IL-6 and IL-8, which are chemoattractants, and IL-4, IL-10, TGF- β and PGE₂, factors involved in regulating helper T cell responses. Production of IL-12 and IL-18, which are involved in augmenting Th1 cellular immunity, could not be detected. Human Schwann cells are likely to play a crucial role in the immunopathogenesis of nerve damage in leprosy. Presentation of *M. leprae* antigens to cytotoxic CD4⁺ or CD8⁺ T cells can result in Schwann cell killing in mice (Steinhoff and Kaufmann 1988) and in humans (Spierings *et al.* chapter 2a). The release of particular cytokines by Schwann cells may have important consequences for the outcome of local immune responses.

Our results indicate that Schwann cells may directly regulate *M. leprae* T cell responses in leprosy lesions. For the first time, we here demonstrate production of human IL-4 by non-hematopoietic cells. Production of IL-4, as well as IL-10, was induced only after exposure of Schwann cells to *M. leprae*, but not to LPS. Moreover, neutralizing IL-4 antibodies strongly enhanced T cell proliferation of a Th1 clone recognizing *M. leprae* antigens presented by human Schwann cells. No such effect was observed when monocytes were used as APC, strongly suggesting that IL-4 production is specifically induced by *M. leprae* in human Schwann cells but not monocytes, and has a direct regulatory role in Th1 responsiveness. IL-4 and IL-10 are known to suppress Th1 responses (Salgame *et al.* 1991; Sieling *et al.* 1993; Moore *et al.* 1993). IL-10 suppresses macrophage functions on various levels, including MHC class II expression (de Waal Malefyt *et al.* 1991; Koppelman *et al.* 1997) and pro-inflammatory cytokine production (Gazzinelli *et al.* 1996; Neyer *et al.* 1997), and has been suggested to be responsible for the latent phase during mycobacterial infection (Murray 1999). Moreover, IL-10 drives the generation of a recently described CD4⁺ T cell subset, designated regulatory T cells 1 (Tr1), which suppresses antigen-specific immune responses and actively down-regulates a pathological immune response *in vivo* (Groux *et al.* 1997). In this way, *M. leprae* infected Schwann cells may contribute directly to local suppression of Th1 dependent DTH-like cellular immune reactions that may have detrimental consequences for peripheral

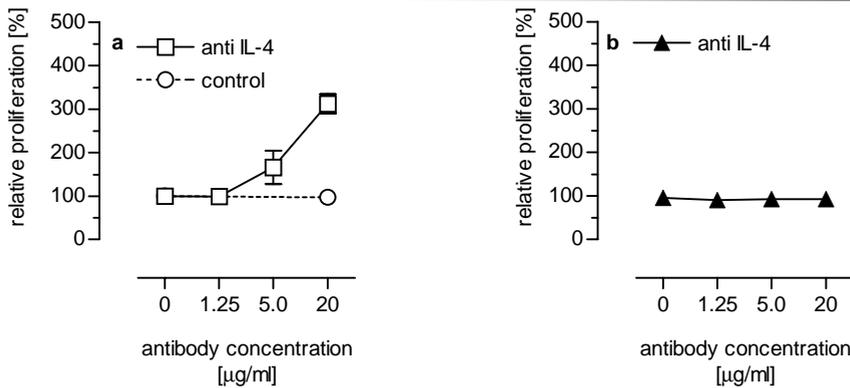


figure 6: Enhancement of *M. leprae* specific Schwann cell induced T cell proliferation of Th1 clone R1E4 in the presence of neutralizing IL-4 antibodies (a). Antibodies recognizing HLA-DP did not enhance T cell proliferation (open triangles), showing the specific effect of IL-4 antibodies. No enhancement was observed when macrophage were used as APC, excluding a mitogenic effect of IL-4 antibodies (b). T cell proliferation in response to *M. leprae* in the absence of antibodies were 21000 cpm and 11000 cpm for Schwann cells and macrophages respectively, while medium values were 475 cpm and 113 cpm respectively.

nerve tissue. Human Schwann cells constitutively produce TGF- β . TGF- β attenuates the IL-12 responsiveness of Th1 cells via down-regulation of IL-12R β 1 and IL-12R β 2 expression (Gorham *et al.* 1998; Bright and Sriram 1998; Zhang *et al.* 1999), or via inhibition of IL-12 production (Skeen *et al.* 1996). In both cases IFN- γ production is reduced. Furthermore, TGF- β reduces MHC expression (Geiser *et al.* 1993) and suppresses iNOS and NO production (Vodovotz *et al.* 1996). Thus, like IL-4 and IL-10, TGF- β drives immune responses away from a likely detrimental Th1 pathway, such that DTH is suppressed. Similarly, the local release of TGF- β and IL-10 has been proposed to be responsible for the immune privileged status of the eye (D'Orazio and Niederkorn 1998) and may be of importance in reducing Th1 autoreactivity in the central nervous system (Schluesener 1990; Bai *et al.* 1997; Cua *et al.* 1999). Our *in vitro* data using neutralizing IL-4 antibodies strongly suggest that endogenous production of IL-4 by Schwann cells has a similar effects on Th1 responses in leprosy. The inability of Schwann cells to produce IL-12 and IL-18 strengthens the concept that Schwann cells are unable to promote Th1 responses, thus maintaining a Th1 immune privileged environment. Killing of *M. leprae* in the nerves by MDT possibly reduces the production of type 2 cytokines by Schwann cells, thus giving room for detrimental Th1 responses, resulting in enhanced predisposition to reactional episodes as indeed often observed during MDT.

Aside to IL-4 and IL-10, human Schwann cells also produce IL-6, but fail to secrete IL-12 and TNF- α . Thus, the cytokine profile of human Schwann cells strongly resembles that of type-2 dendritic cells (reviewed in (Kalinski *et al.* 1999)). Type-2 dendritic cells prime and polarize T cell responses towards a Th2 phenotype (Kalinski *et al.* 1997). PGE₂ has been found to be a key factor in inducing such type-2 dendritic cells. The secretion of PGE₂ by Schwann cells therefore may also be involved in the induction of a type-2 antigen presenting cell.

IL-6 has been reported to play a crucial role in cellular defense against mycobacteria (Appelberg 1994; Ladel *et al.* 1997) and other parasites (Kopf *et al.* 1994; Dalrymple *et al.* 1995). It is produced in an early stage of mycobacterial infection and inhibits bacterial growth in macrophages (Flesch and Kaufmann 1990; Orme *et al.* 1993). As shown in IL-6 *-/-* mice, IL-6 also attracts macrophages and T cells to the nervous system (Eugster *et al.* 1998) and might thus play a role in initiating the inflammation in

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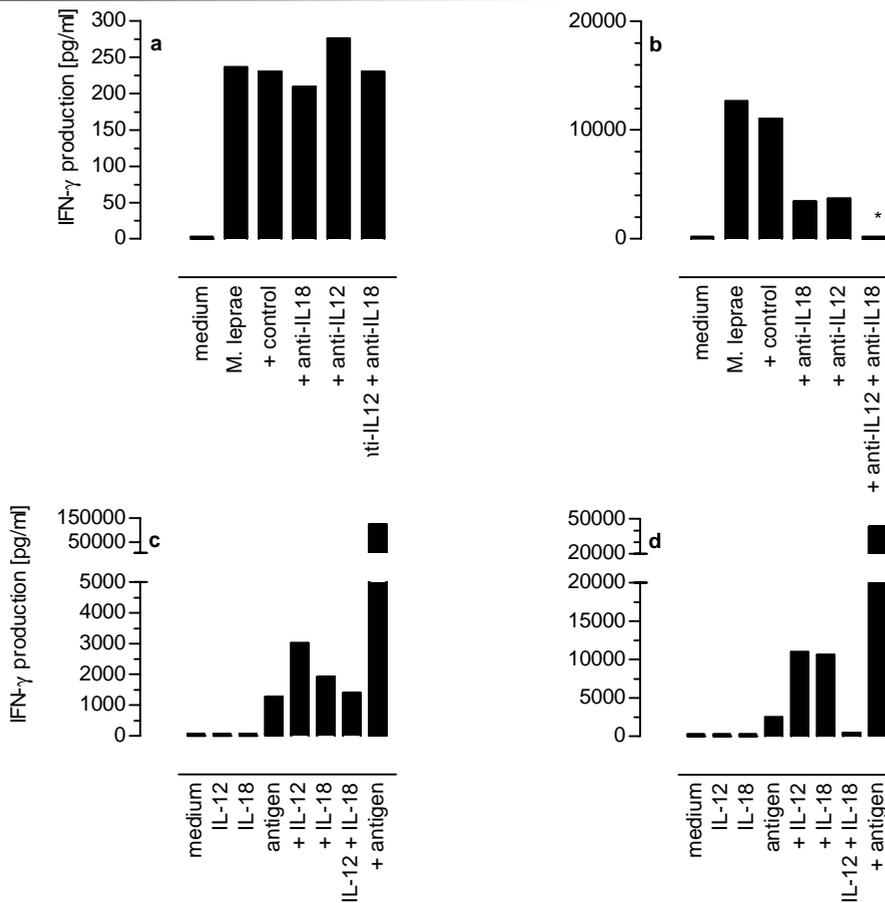


figure 7: Lack of Schwann cell produced IL-12 and IL-18. (a) IL-12 and IL-18 neutralizing antibodies were unable to inhibit Schwann cell mediated antigen presentation, but strongly inhibit antigen presentation by monocytes as controls (b, * = not determined). (c) Addition of rIL-12, rIL-18 or the combination thereof two increases T cell responses in a similar way as with monocytes (d), showing that IL-12 and IL-18 are limiting factors in Schwann cell mediated antigen presentation.

neural tissue. Monocytes and CD4⁺ CD45RO⁺ T cells are central components of reversal reaction granulomata (Cooper *et al.* 1989). IL-8 is able to recruit these cells and initiate local immune responses (Wilkinson and Newman 1992). The raise in chemokine production after infection of Schwann cells with *M. leprae*, may be an important event in the initiation of inflammatory responses in neural tissue.

The pronounced type-2 cytokine profile of human Schwann cells raises the question why Th1 and not Th2 cells are abundantly present in leprosy neuritis lesions. Even though exposure of Schwann cells to *M. leprae* induces IL-4 and IL-10, *M. leprae* infection of local monocytes and dendritic cells is likely to yield strong IL-12 and IL-18 production, resulting in strong enhancement of Th1 immune responses. Under such circumstances, the production of IL-4 and IL-10 by Schwann cells may be too low to influence this process significantly. It will be of interest to see whether host dependent differences in the production of IL-4 and IL-10 by Schwann cells in response to infection are related to inter-individual differences in susceptibility to nerve damage during leprosy reactions.

In summary, our findings indicate that human Schwann cells are able to up-regulate the production of various cytokines upon exposure to *M. leprae*. Some of the detected cytokines are chemokines that recruit T cells and macrophages. *M. leprae* failed to induce

Schwann cell production of the key type-1 cytokines IL-12 and IL-18, but instead enhanced production of IL-4, IL-10 and PGE₂, products which inhibit Th1 development. TGF- β was produced constitutively. We therefore hypothesize that Schwann cells represent type-2 APC, and that infection with *M. leprae* further enhances this type-2 phenotype. By such a mechanism, Schwann cells may be actively involved in the down-regulation of local DTH like cell mediated immune activation, which is a pronounced feature of leprosy pathology.

