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M. leprae and Macrophage Secretory Products Modulate the Expression of NgCAM on Schwann Cell Surface

TO THE EDITOR:

During embryogenesis and under pathological conditions, Schwann cells (SC) of the peripheral nerve are induced to express cell-surface molecules, such as the neuroglial cell adhesion molecule (NgCAM), which aid in the initial SC-axon associations needed for axon fasciculation ([5]). Infection with *Mycobacterium leprae* of its host cell, the macrophages, renders them defective in a number of functions, including the expression of cell-surface molecules ([1]). The possibility exists that SCs, for which *M. leprae* have a special affinity, also could be rendered defective in the expression of cell-surface molecules and, therefore, contribute to the peripheral nerve pathology in leprosy. The presence of features like aberrant myelination in the sciatic nerve of the murine animal model inoculated with *M. leprae* in the foot pad ([7]) may indicate variation in adhesion molecule expression. Besides this, macrophages which infiltrate the site of a nerve lesion in order to aid SCs in nerve regeneration ([6]), secrete a host of cytokines which have been shown to regulate the expression of cell-adhesion molecules ([4]).

The aim of this study was, therefore, to determine if *M. leprae* infection and macrophage secretory products modulate the expression of NgCAM on the SC surface, comparing the differences in cells derived from two strains of mice, Swiss white (SW) and C57BL/6 mice, in their response to *M. leprae* infection ([2]). (The viable *M. leprae* used in our study was derived from frozen armadillo liver biopsies supplied by Dr. E. Storrs, Florida Institute of Technology, Melbourne, Florida, U.S.A.)

THE TABLE. *Effect of macrophage conditioned medium on expression of NgCAM by Schwann cells.*[a]

	SW mice			C57BL/6 mice		
Schwann cells	–	MOU	MOI	–	MOU	MOI
Uninfected	++	+++	++++	++	+++	+++
ML Infected	+	+++	+	++	+++	++

[a] Intensity of immunofluorescence staining of NgCAM was compared in uninfected Schwann cells and cells infected with viable *M. leprae* (ML) for 6 days. NgCAM expression of Schwann cells was also compared with cells exposed to uninfected (MOU) and 3-day postinfected (MOI) macrophage conditioned medium for 72 hr in both mouse strains. NgCAM expression was assessed qualitatively, and cells showing minimum intensity of fluorescence were graded as +.

Dissociated Schwann cells (DSC) from the sciatic and brachial nerves of 1–2-day-old mice were cultured on coverslips in a 10% feeding medium (FM) essentially by the method of Brockes, *et al.* ([3]). Five-day-old cultures were infected with *M. leprae* (5×10^8/ml) for 24 hr following which the excess bacilli were washed off. Conditioned medium from cultures of peritoneal macrophages was added to uninfected and 3-day postinfected DSC cultures with FM in a ratio of 1:1 for 72 hr. NgCAM expression was determined by indirect immunofluorescence staining as follows: Cells were fixed with 70% ethanol and incubated with a 1:50 dilution of rabbit polyclonal antibody to human brain NgCAM and a 1:200 dilution of FITC-labeled anti-rabbit IgGs. The cells were mounted on PBS-glycerol and observed under a fluorescence microscope.

The NgCAM was expressed by almost all of the SCs, and the few fibroblasts present in the culture were negative. NgCAM was expressed to an equal extent by SCs from both strains of mice. However, following *M. leprae* infection, the expression decreased in intensity in SCs from SW mice. Uninfected SCs showed enhancement in NgCAM expression in the presence of both uninfected and *M. leprae*-infected macrophage conditioned medium. However, NgCAM expression of infected SCs was enhanced only in the presence of uninfected macrophage conditioned medium, especially SW SCs in which the *M. leprae* infection had initially decreased the NgCAM expression (The Table).

This indicates that *in vivo* regeneration attempts in an early leprous nerve could be hampered, in combination with other factors, by aberration in the SC association with the axons due to decrease in NgCAM expression following parasitization with *M. leprae*. But this does not take into consideration the status of axonal NgCAM expression which could be, alternatively, over- or under-expressed in a leprous nerve, thereby further influencing SC-axon associations. In addition, variation in response to infection in the two mouse strains also implicates a role for NgCAM in the divergence observed in the sciatic nerve pathology in foot pad-inoculated mice, with early comparable pathology in SW and C57BL/6 progressing to extensive demyelination only in SW mice ([2]). On the other hand, an influx of macrophages in the leprous nerve would enhance the expression of NgCAM and thus aid SCs in nerve regeneration. The fact that nerve damage still ensues in spite of a high macrophage population in the leprous nerve indicates the involvement of other complex mechanisms.

The infection of macrophages by *M. leprae* results in the downregulation of a number of cell-surface receptors ([1]). Similarly, invasion by *M. leprae* seems to result in membrane perturbation of the SC surface also, as indicated by the decrease in NgCAM expression. NgCAM may be just one of a number of other SC surface molecules to be altered on *M. leprae* invasion, and these changes in conjunction with one another may contribute to the leprous nerve pathology.

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Low Rates of Detection of Mycobacterial Secretory Antigen 85 in Sera of Untreated Leprosy Patients

To the Editor:

Leprosy continues to be one of the major infectious diseases, affecting about 2.4 million people worldwide (estimate for December 1993) ([7]), despite concerted efforts to control this disease. The World Health Assembly call for the elimination of leprosy by 2000 A.D. and the World Health Organization (WHO) recommendation of the use of fixed duration multidrug therapy (WHO/MDT) has made the monitoring of therapy a matter of importance. In this regard, mycobacterial proteins, such as the antigen 85 (Ag85) complex, actively secreted by growing microbacteria ([8, 13]), were used for the assessment of MDT in leprosy.

Sera of leprosy patients were used for the concurrent detection of Ag85 (secretory protein) and Ag82 (cytoplasmic 65-kDa heat-shock protein) by ELISAs. A ratio of the secretory to the cytoplasmic antigen (SCR) indicated secretory efficacy and, hence, viability of *Mycobacterium leprae*. Only 6 of 21 (28.5%) untreated multibacillary (MB) and 11 of 34 (32.3%) untreated paucibacillary (PB) patients showed significant positivity for Ag85, although among the positive cases the SCR was high (> 1) and Ag85 levels between 0.1–10 ng/ml were detected, indicating bacterial viability. Immune complexes, which have been demonstrated in MB patients' sera ([6, 12]) and which could lower the level of free antigen detection, were suspected to be the cause of such low sensitivity. However, immune complex dissociation attempted with MB sera failed to improve sensitivity. Furthermore, no correlation was apparent between the bacterial index (BI) in skin smears and the positivity in ELISA.

The low sensitivity in ELISA could not be attributed to the lack of antigen secretion by intracellular bacilli since the presence of Ag85 in the skin and nerve tissue of untreated leprosy patients was clearly demonstrable by immunocytochemistry. Skin sec-