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EDITORIAL

Editorial opinions expressed are those of the writers.

Host-Parasite Interrelationship Between *M. leprae* and Schwann Cells *in Vitro*

The study of the host-parasite interrelationship between *Mycobacterium leprae* the causative agent and the Schwann cell is of special interest in leprosy since there is substantial clinical and histopathological evidence to suggest that in the human body the Schwann cell is the target cell for this mycobacterium.[1] There are indications that the Schwann cell serves not only as the natural host for the growth and multiplication of *M. leprae* but also protects the organism from the body's immune responses as well as from drugs.[2,3] These observations have further led to the speculation that the Schwann cell resident *M. leprae* may possibly be a primary and persistent source of infection for the continuous leakage of bacilli or bacillary antigen into the circulation, hence responsible for the state of persistent infection or relapse seen in some lepromatous cases.[4,5] It is vital, therefore, to understand this host-parasite relationship directly at the cellular and molecular level. Studies carried out in our laboratory over the last 8 years have enabled us to make important observations in this direction.[6-14]

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Nerve tissue culture models. The nerve tissue culture model developed by Murray[15] has been substantially improved by many tissue culturists. As a result, it is now possible to obtain cultured Schwann cells as components of organized nerve cultures or as pure populations.[16–18] In our laboratory, we have developed four culture models which are illustrated in Figure 1. These culture models enabled us to study the direct interaction between *M. leprae* and components of nerve, particularly the Schwann cell.

Basis for affinity to and invasion by *M. leprae* of peripheral nerve. Cells cultured as in Figure 1 were infected with *M. leprae* and the effect, both short-term and long-term, was studied.[6,8] In all these culture systems, *M. leprae* readily parasitized the Schwann cell. They did not infect the neurons or axons. Bacilli were present in 70–80% of the Schwann cells and 20–30% of the neurofibroblasts, but not in the axons or neurons (unpublished observations). In long-term infected cultures, *M. leprae* were found in 95% of the Schwann cells but not in the axons or neurons (unpublished observations). A preferential affinity of *M. leprae* for Schwann cells was also evident when the relative ability of *M. leprae* to parasitize Schwann cells, skin fibroblasts, and muscle cells was compared under similar culture conditions.[19] These *in vitro* observations, therefore, provide direct evidence of susceptibility of Schwann cells and resistance of neurons and axons to direct invasion by *M. leprae*. Hence, these results do not support the speculation that in the peripheral nerves *M. leprae* are initially phagocytosed by regenerating growth cones of neurons and disseminated into the axoplasm[20,21] but, instead, provide evidence for a second hypothesis, that the Schwann cells are the target for invasion and dissemination of *M. leprae* in the peripheral nervous system.[22,23]

Subsequent experiments conducted in our laboratory provided further evidence for the latter hypothesis, i.e., that the ability of *M. leprae* to adhere to and get ingested by the Schwann cells, is the major contributory factor for invasion of the peripheral nerves.[10,13]

Adherence. In both the organized nerve culture and pure Schwann cell culture models, *M. leprae* readily bind to the surface of cultured Schwann cells but not to the neuronal cell body or to axons[13] (and unpublished observations). The adherence of eight other species of known human pathogenic mycobacteria closely related to *M. leprae*, such as *M. kansasii*, *M. tuberculosis*, and *M. lepraemurium*, was poor.[10] It was significantly blocked by the treatment of the host cell surface with lipase, reduced by glucose, and enhanced by trypsin.[13]

Phagocytosis. The adherent organisms were subsequently ingested. It then becomes

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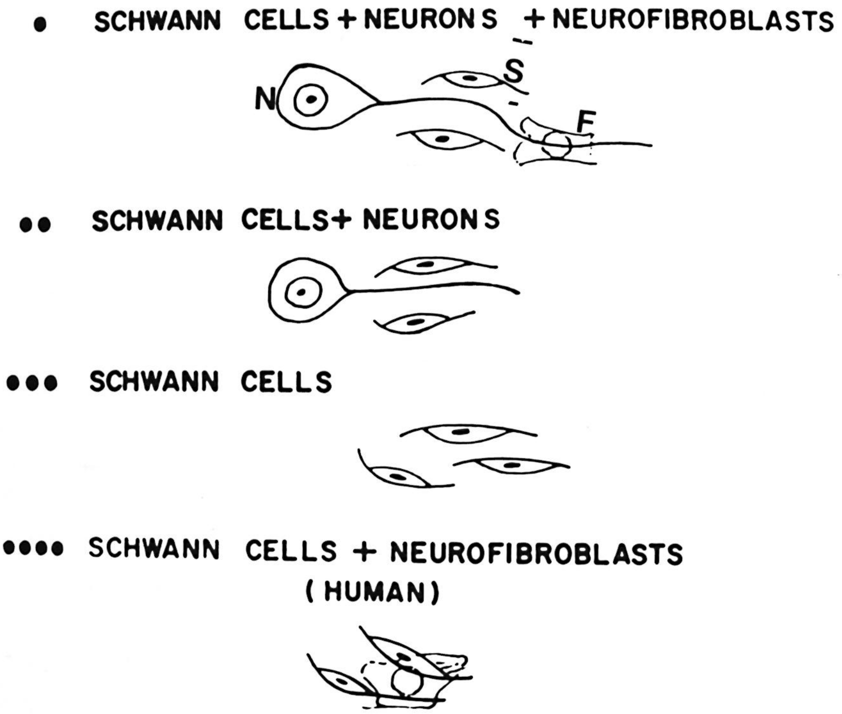

FIG. 1. Schematic representation of four types of nerve tissue culture models used in our studies. Dorsal root ganglion explant cultures contain three main cellular components of peripheral nerve—neurons (N), Schwann cells (S), and neurofibroblasts (F) precursors of perineurial cells[6] (•). Neurofibroblasts are selectively killed by antimitotic drugs, leaving behind a mixture of just Schwann cells and neurons[6] (• •). A monolayer Schwann cell culture of 95% purity can be prepared from sciatic nerves[10] (• • •). Adult human nerve explant cultures give rise primarily to Schwann cells and neurofibroblasts[11] (• • • •).

necessary to examine in detail the mechanism of phagocytosis of *M. leprae* by the Schwann cells. It is important to remember that the Schwann cell is not a professional phagocyte, its main function being myelin synthesis.[24] Therefore, its phagocytic characteristics are quite distinct from that reported for macrophages.[6,25] It is slowly phagocytic, the optimum phagocytosis being attained only 72 hours after inoculation with the bacilli. Phagocytosis of the organism is significantly reduced by heat killing or formal-saline treatment,[6] or drastic purification procedures to remove host tissue contamination (unpublished observations). It is blocked by lipase and enhanced by trypsin (unpublished observations). It is also significantly inhibited by agents that cause metabolic inhibitions or disruption of cytoskeletal elements of the host (unpublished observations) and is also influenced by the state of association of the Schwann cell with axon.[6] Some of these factors may possibly have contributed to the reported un-ingestion or poor ingestion of *M. leprae* in the systems of Fildes[26] and Saito, *et al.*[27] Phagocytosis of other mycobacteria by Schwann cells in our system is poor (unpublished observations).

Possible involvement of surface receptors in the adherence-phagocytosis phenome-

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non. It is regarded in general that adherence of the microorganisms to the host cells is a prerequisite for tissue invasion. The ability of a bacterial pathogen to adhere to mammalian tissue has been shown to equate with pathogenicity.[28] Such an effect has been explained on the basis of existence of specific molecule(s) on the surface of the pathogen that can recognize a receptor on the host cell surface.[29]

There is ample clinical and histopathological evidence to show that *M. leprae* is unique in its predilection for the peripheral nerve, especially the Schwann cell.[30] Our data on adherence and phagocytosis suggest the involvement of surface molecules for recognition. The existence of such a specific receptor for the interaction between *M. leprae* and the Schwann cell is worth pursuing.

It is well established in macrophages that the phagocytosis of mycobacteria, including *M. leprae,* is mediated by the receptors for the Fc portion of immunoglobulin G[31,32] as well as for the C3b portion of complement.[33,34] More recently, lectin receptors have also been implicated.[35,36] There is no experimental demonstration of the existence of any of these receptors in the mouse Schwann cell membrane. Preliminary experiments carried out in our laboratory did not reveal the presence of Fc or C3b receptors in these cells (unpublished observations). The absence of an Fc receptor in the cultured rat Schwann cells has also been reported.[37] Based on our observations, it can be speculated that phagocytosis of *M. leprae* by the Schwann cell is mediated by the other receptor(s), possibly lipid in nature, and not either monosaccharide or a protein residue. Further experiments directed toward isolation and characterization of receptors on Schwann cell membrane may lead to a better understanding of the molecular basis for the ability of *M. leprae* to invade Schwann cells.

Host cell modification. Once ingested, *M. leprae* cause prolonged and persistent infection of the host cells.[8] During the course of long-term infection *in vitro,* there was no evidence of significant host cell lysis or release of bacilli into the extracellular medium. No cytopathic changes in the host cell were evident in our biochemical and functional studies conducted on organized nerve cultures maintained 1–6 weeks post-infection.[8] The light and ultrastructural morphology of these cells was also normal. The incorporation of a general metabolic precursor was also near normal. The rate of protein synthesis and the protein profile remained unaltered.[7] However, once *M. leprae* invaded the Schwann cells, they were rendered incapable of synthesizing DNA or effectively associating or interacting with axons.[6] Studies with human lepromatous nerve cultures have also revealed that *M. leprae* inhibit migratory and proliferative activity of the host Schwann cells.[11]

The properties of attachment, migration, and proliferation of Schwann cells are important for their differentiation and acquisition of specialized functions, such as association, interaction, and myelination of the axons.[38-40] The alteration in these host

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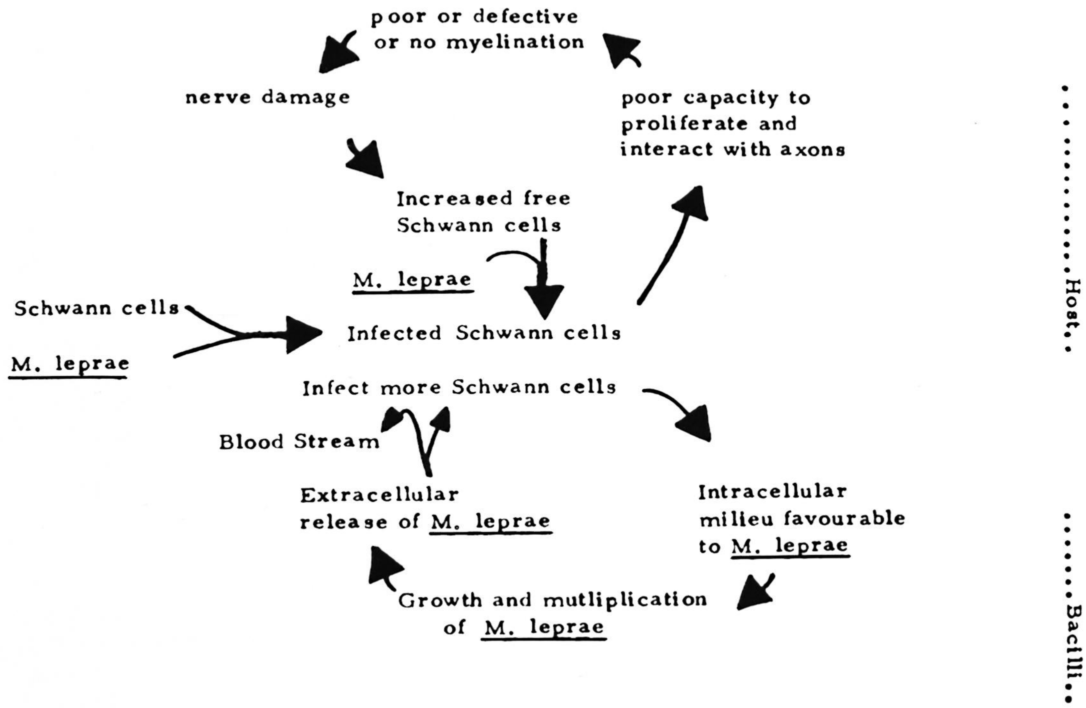

FIG. 2. Possible sequence of events following entry of *M. leprae* into the Schwann cells that leads to the slow and progressive nerve damage in the lepromatous spectrum of leprosy. The presence of *M. leprae* in the cytoplasm renders the host Schwann cell incapable of synthesizing myelin or responding normally to trauma or any other conditions requiring regeneration and remyelination of nerve fibers. The axons surrounded by Schwann cells harboring *M. leprae* are, therefore, vulnerable to repeated trauma and damage. *M. leprae*, on the other hand, finds the host intracellular milieu favorable for its survival and growth. It multiplies. The bacilli grown within the Schwann cells are then either released into the extracellular milieu and transported to the other parts of the body through the blood stream, or they may infect the neighboring Schwann cells and repeat the entire cycle.

cell functions was a direct inhibitory effect of the intracellular organism. There was no evidence of this effect being mediated through the release of any soluble product or toxic factor.[8, 11] Unparasitized Schwann cells in the same culture had normal light and ultrastructural morphology, and good migratory and proliferative activities. The infected cultures were rich in healthy axons and neurons (unpublished observations).

Our studies, therefore, experimentally demonstrate the nontoxic effect of *M. leprae* on the peripheral nerves, and suggest that the infected cells are unable to participate in the regenerative activity of the nerves but continue to survive for a prolonged period of time. This phenomenon may, however, initiate slow but progressive nerve damage and also contribute to the spread of bacilli within the nerve as well as in the systemic circulation. The possible sequence of events is schematically presented in Figure 2. These effects can, to an extent, be reversed by prolonged chemotherapy.[11] Similar observations have also been made in histopathological studies of lepromatous nerves where poor proliferation as well as poor axon association were observed.[41]

The mechanism(s) by which *M. leprae*

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alter the functional status of the parasitized host cell is not clear. Physical occupation of the host cytoplasm by these organisms may alter the content as well as the organization of the cytoskeletal element that controls the proliferative as well as the migratory activity of the cell in general.[42,43]

Intracellular growth. The lack of direct detrimental effects of and induction of alteration in only the specialized functions of the host cell by *M. leprae* provide some clue to the sequence of events which occur during the process of appropriation of peripheral nerves. The semblance of symbiosis between *M. leprae* and cultured nerve cells observed in the early infected cultures may be of ultimate advantage to the pathogen.

There was evidence for the synthesis of capsule and cell wall by *M. leprae* using monoclonal antibody to phenolic glycolipid-I (PGL-I) in an indirect immunofluorescence assay.[8] This was followed by significant multiplication of the organism within the Schwann cell component of the cultures. The active replication of the acid-fast bacilli was evinced by its ability to incorporate radiolabeled thymidine as well as bacteriological counts.[8] Thus, our study demonstrates that the organized nerve culture in which the Schwann cells are physiologically analogous to their *in vivo* counterparts serves as host for the *in vitro* survival and growth of *M. leprae.*

Mycobacteria cultivated within Schwann cells exhibited several properties distinct from those of other leprosy-derived mycobacteria cultured directly in the medium. Their growth was strictly intracellular, and was not observed in conventional bacteriological media. They induced inhibition of host cell proliferation, a feature reported to be specific for *M. leprae.*[7] Growth was inhibited by the antileprosy drugs dapsone (DDS) and rifampin. Besides these characteristics, these organisms exhibited the capacity to produce PGL-I (a component of its capsule) commonly used as a taxonomic marker for *M. leprae.*[44] Therefore, these organisms have been designated as "Schwann-cell-derived PGL+AFB."

Other studies conducted in the Schwannoma cell line (33B) demonstrated that intracellular *M. leprae* readily synthesized lipids. Within 3 weeks of incubation *in vitro,* substantial incorporation of ^{14}C-acetate was evident in all of the lipid components,[9] the most remarkable being incorporation into the PGL-I fraction. The time kinetics of the incorporation and its correlation with bacillary numbers indicated active synthesis of this molecule by *M. leprae.* PGL-I is speculated to be resistant to the action of host bactericidal activity.[44,45] However, its sensitivity to antileprosy drugs indicates that chemotherapy should curb the synthesis and, hence, the deposition of this molecule within the Schwann cells. Whether the lipid synthesis observed in these cells is related to the availability of the precursors or intermediate metabolites of host lipid metabolism remains to be established. Schwann cells have the capacity to synthesize *in vitro* bulk quantities of myelin components and other lipids that could be utilized by the bacillus.[17,46,47]

Relationship to host immune response. The mechanism(s) by which Schwann-cell-resident *M. leprae* evade the host microbicidal action and immune surveillance is not clear. It may use conventional methods, such as avoiding the potentially harmful action of lysosomal hydrolases or subverting the lethal effect of the oxidative burst, or some other, yet unknown mechanism(s). There is as yet no study available relating to the oxidative metabolism of the Schwann cell. New insight would require detailed biochemical investigations into its interaction

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t cells as well as the analysis of its antigenic constitution.

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