The significance of Hansen disease, or leprosy, is underscored by the fact that detection of this disease has remained stable over the past 10 yr, even though disease prevalence is reduced. Due to the long incubation time of the organism, health experts predict that leprosy will be with us for decades to come. Despite the fact that Mycobacterium leprae, the causative agent of leprosy, cannot be cultured in the laboratory, researchers are using innovative and imaginative techniques to discern the interactions of M. leprae with host cells both in vitro and in vivo to identify the host and bacterial factors integral to establishment of disease. The studies described in this review present a new and evolving picture of the many interactions between M. leprae and the host. Specific attention will be given to interactions of M. leprae bacilli with host Schwann cells, macrophages, dendritic cells and endothelial cells. The findings described also have implications for the prevention and treatment of leprosy.

Key words Interactions - leprosy - macrophages - Mycobacterium leprae - Schwann cells

The global significance of Hansen disease

In 1997, the World Health Organization (WHO) estimated that 2 million people worldwide were infected with Mycobacterium leprae, the causative agent of Hansen disease or leprosy. Prevalence of leprosy has diminished in recent years, down to approximately 460,000 infections globally, in part because of the success of multiple drug therapy (MDT) to treat infected patients. This has led to a perception that leprosy is no longer a significant health problem. The case detection rate, however, has remained constant. The sustained incidence of the disease is due, in part, to an inability of health professionals to reach isolated areas endemic for the disease. There is also little known about the route of transmission of leprosy. Because of delays in diagnosis and treatment, especially in rural areas, millions of people are permanently disabled by current or past infections. Further, no vaccine exists for the prevention of leprosy. In addition, and related to vaccine development, there are huge gaps in our knowledge of the cell biology associated with M. leprae infection.
The elimination of Hansen disease

In 1981, the WHO recommended MDT to address the emergence of drug resistant strains and to promote compliance and cost effectiveness. In 1991, the World Health Assembly set a year 2000 target for the elimination of leprosy as a public health problem, and defined elimination as less than 1 case per 10,000 population. It should be noted that different presentations of leprosy, e.g. paucibacillary (PB) or multibacillary (MB), require different treatment regimens, and the guidelines for current MDT are available. Since the inception of the elimination plan, the worldwide prevalence of leprosy has decreased dramatically. However, the disease detection rate has remained almost constant over the past 10 yr, with a high rate (17%) of infection in children. Because of these statistics and the potentially long (up to 20 yr) incubation period of leprosy, there has been speculation that the priority of elimination should be reduced in favour of a long-term plan to deal with the chronic and global burden of leprosy. Though millions have benefited from the implementation of MDT treatment to pursue the goal of elimination, lacunae remain in knowledge regarding the basic biology of the disease. This, appropriately, is being slowly addressed as scientists worldwide address the interaction of these organisms with the host.

Mycobacterium leprae research challenges

The causative agent of leprosy, \textit{M. leprae}, has a unique host cell tropism in that it preferentially infects and grows within Schwann cells surrounding the axons of nerve cells. This tropism is believed to contribute to the pathology of leprosy and the resulting injury to patients with this disease. Perhaps, the greatest challenge to investigators is the fact that \textit{M. leprae} cannot be cultured by normal laboratory methods. As a result, no genetic systems for the study of \textit{M. leprae} currently exist. On a more positive note, the genome of \textit{M. leprae} has been sequenced and is readily available for comparative genomics with regard to phenotypic traits of different mycobacterial pathogens. Interestingly, when the genome of \textit{M. leprae} (3.27Mb) is compared to the \textit{Mycobacterium tuberculosis} (the causative agent of tuberculosis or TB) genome (4.41Mb), the \textit{M. leprae} bacterium appears to have undergone “reductive evolution.” There are high levels of inactivated or pseudogenes, and the level of gene duplication is approximately 34 per cent. Only about half of the genome contains functional genes. This gene deletion and decay could, in part, explain the specific niches and host tropism of the organism as well as the inability of researchers to culture the organism in the laboratory.

What has been lacking until recent years is a definition of the molecular basis of \textit{M. leprae} association with the host cell, as well as information relating to the phenotype of the organism in the intracellular milieu. The tropism of \textit{M. leprae} for Schwann cells plus the difficulty in obtaining live \textit{M. leprae} doubles the difficulty in studying bacilli-host interactions. First, it is difficult to obtain primary mammalian Schwann cells. The cells can be harvested from laboratory animals, e.g., the sciatic nerves of rats, or can be isolated from humans. These cells can also be immortalized. Schwannoma cells, in particular the ST 8814 Schwannoma cell line, have also been used to model the primary cell system. A disadvantage of the ST 8814 cell line is that it is, to be sure, a model system. In addition, the immortalized ST 8814 cells may be prone to genetic lesions and rearrangements that would not be seen in primary cells. Regardless, there are distinct advantages in using this cell line, including ease of maintenance and the speed at which relevant data can be obtained.

Secondly, the inability of researchers to grow \textit{M. leprae} in laboratory media adds an additional level of difficulty to the study of this organism. Irradiated organisms are available, but are limited in utility as they are a model, or reflection of, the
interaction of live organisms with the host. The bacteria can be grown to relatively high concentrations in nine-banded armadillo tissue or in the footpads of nude mice, with the latter system appearing to provide organisms at significantly higher viability levels\textsuperscript{20}. These techniques have provided highly viable organisms for the \textit{M. leprae} researchers, but the organisms can only be utilized in the short term. In addition, no animal models exist for the human disease, and neither the mouse nor the armadillo experience a course of infection that sufficiently parallels the human clinical features of leprosy. Regardless of these problems, a small international cadre of \textit{M. leprae} researchers has identified many characteristics of host-pathogen interactions relevant to leprosy that are, in part, described herein. It should be noted that the immunological traits and consequences of \textit{M. leprae} infection are also being described at a commendable rate. Except for specific examples of the influence of host cell-pathogen interactions on the immunology of leprosy disease, this review will be limited to specific host cell-\textit{M. leprae} interactions.

**Interactions of \textit{M. leprae} with the Schwann cell**

Building on histological and clinical studies in the 1950s that indicated that \textit{M. leprae} primarily invade Schwann cells in peripheral nerves\textsuperscript{21}, it was further postulated that nerve damage in leprosy, and the resultant deformities and disabilities related to the disease, resulted from \textit{M. leprae} invasion of human Schwann cells\textsuperscript{22-24}. The molecular basis of this interactions between \textit{M. leprae} and the human Schwann cell has been almost completely unknown until just recently. The late 1990’s saw an intense level of research on, and interest in, the molecular basis of Schwann cell-\textit{M. leprae} interactions. These studies continue to give more and more insight into the basic biology of leprosy and \textit{M. leprae} interactions with the host cell.

The Schwann cell is part of a Schwann cell-axon complex or unit which may or may not be myelinated\textsuperscript{25}. This Schwann cell-axon unit is surrounded by a layer of basal lamina\textsuperscript{26}. This phenotype is specific to the Schwann cell, leading Rambukkana \textit{et al} to postulate specific interactions of \textit{M. leprae} with basal lamina of the Schwann cell-axon unit as a first step to infection of Schwann cells\textsuperscript{27}. This group in fact, showed that a host molecule, laminin-2, was the initial target for \textit{M. leprae} seeking the Schwann-cell niche. The laminin-2 molecule consists of $\alpha_2$, $\beta_1$ and $\gamma_1$ chains, and this study further demonstrated that the globular (G) domain of the laminin $\alpha_2$ chain was the specific subunit with which \textit{M. leprae} interacted\textsuperscript{27}. Consistent with this postulated mechanism of entry, the tissue distribution of the laminin $\alpha_2$ chain is limited to Schwann cells, striated muscle and the placenta\textsuperscript{28}. These tissues correspond to natural sites of \textit{M. leprae} infection in the human.

The importance of laminin-2 to \textit{M. leprae} invasion is based on work from several studies. For example, laminin-2 is anchored to Schwann cells via the laminin receptor\textsuperscript{29}, but laminin also interacts with $\alpha$-Dystroglycan ($\alpha$-DG), another receptor on the Schwann cell-axon unit\textsuperscript{30}. A most exciting extension of the study described above\textsuperscript{27} was published by Rambukkana \textit{et al} in 1998 in which the specific receptor for \textit{M. leprae} on Schwann cells was determined to be the $\alpha$-DG receptor\textsuperscript{16}. This study further demonstrated that the G domain of the laminin $\alpha_2$ chain ($\alpha 2L G$) was required for binding to, and subsequent infection of, Schwann cells. It should be noted that other Schwann cell receptors have been implicated as possible routes of entry for the uptake of the \textit{M. leprae} bacterium into the Schwann cell\textsuperscript{31}. It is also interesting to note that if there is a deficiency in the interaction between laminin-2 and $\alpha$-DG, certain types of muscular dystrophy and other neuropathies can result\textsuperscript{32,33}.

**\textit{M. leprae} adhesins**

The questions that naturally follow from the studies described above have to do with which specific bacterial factors contribute to the observed
Schwann cell tropism. One way to narrow down which bacterial factors are involved in this Schwann cell-\textit{M. leprae} interaction is to compare the cell wall of \textit{M. leprae} with that of \textit{M. tuberculosis} and other mycobacteria and determine those molecules unique to the species \textit{M. leprae}. One such molecule, phenolic glycolipid-1, (PGL-1) is unique to \textit{M. leprae} and has been shown to specifically bind to the laminin \(\alpha_2\) chain \textit{in vitro}\textsuperscript{34}. Specifically, the terminal triglyceride of this molecule has been shown to bind to laminin-2. There is further evidence that this bacterial cell wall component may induce demyelination of nerve cells\textsuperscript{35}. There are two potentially devastating outcomes for the demyelination observed with infection by \textit{M. leprae} and attributed to PGL-1. First, non-myelinated Schwann cells are more susceptible to invasion by \textit{M. leprae}\textsuperscript{16,35}. The organism can infect, demyelinate yet more Schwann cells, and this can lead to the second effect, axonal damage\textsuperscript{36}. This potential cascade effect of \textit{M. leprae} invasion, demyelination, growth within the host cell and release, leading to more invasion, \textit{etc.} is a fascinating theory that could explain, in part, the early events leading to the devastating progression of leprosy disease. Finally, it should be noted that high levels of PGL-1 can be found in the tissue of leprosy patients\textsuperscript{37}.

In addition to the interaction of \textit{M. leprae} PGL-1 with host laminin-2, another specific bacterial adhesin, LBP21, potentiates the interaction of this bacterium with the Schwann cell. The LBP21 protein, coded for by \textit{M. leprae} gene ML1683, also specifically binds laminin-2 on peripheral nerves\textsuperscript{16}. This protein was identified by two groups almost simultaneously. The Shimoji \textit{et al} study\textsuperscript{38} used \(\alpha_2\) laminins as a probe to identify ML1683 in cell wall fractions by Western blot, and the protein was determined by N-terminal amino acid sequencing. This study showed that when fluorescent polystyrene beads were coated with recombinant LBP21, they avidly bound to primary rat Schwann cells as compared to bovine serum albumin (BSA)-coated beads. These experiments indicated a specific role for LBP21 as an adhesin in interactions of \textit{M. leprae} with Schwann cells.

Using a similar Western blot strategy, Marques \textit{et al} isolated a protein that bound to \(\alpha_2\) laminin and analyzed peptide fragments of this protein by mass spectrometry\textsuperscript{39}. The later group designated this protein Hlp for 21 kDa histone-like protein. The Hlp protein is identical to the LBP21 protein. In the Marques study, the gene encoding the Hlp was cloned and expressed. It was determined that the addition of exogenous Hlp enhanced the attachment of mycobacteria to an ST 8814 Schwannoma cell line\textsuperscript{39}. This group further reported that cationic proteins, \textit{e.g.}, host-derived histones, were also able to enhance binding of mycobacteria. In an interesting experiment, the mycobacterial species \textit{M. smegmatis}, which is able to bind ST8814 cells, was compared to an \textit{M. smegmatis} Hlp mutant in a Schwannoma cell binding assay\textsuperscript{39}. Even though the mutant did not produce Hlp, there was no reduction in binding of the mutant to the ST8814 cells as compared to the wild type. These data indicate that other adhesins, or perhaps even non-specific host or other bacterial cationic proteins, may be involved in the binding of \textit{M. leprae} to laminin-2. In additional studies this group, demonstrated that other mycobacterial species are able to bind to \(\alpha_2\)-laminins, and that alanine/lysine rich residues in Hlp and eukaryotic histones may be involved in laminin binding\textsuperscript{40, 41}.

Taken together, these studies indicate that there is more than one adhesin responsible for the initial attachment of \textit{M. leprae} to the Schwann cell. It is clear, however, that \(\alpha_2\)-laminin is an important component of the basal lamina with respect to \textit{M. leprae} attachment to, and invasion of, Schwann cells. Though some work remains to be done regarding the specific host-bacterium interactions when \textit{M. leprae} binds to Schwann cells, basic research thus far indicates that the \textit{M. leprae}-specific PGL-1 molecules, in addition to the Hlp/LBP21 putative adhesin, are important bacterial factors in attachment events. It follows that these
factors can be potential targets for the treatment or prevention of Hansen disease.

**M. leprae uptake and trafficking in the Schwann cell**

Though much has recently been discovered about the factors required for binding of *M. leprae* to the host cell, the intracellular compartment of the organism is virtually uncharacterized. In a study by Alves *et al* the ST 8814 Schwannoma cell line was shown to readily phagocytize both viable, nude-mouse derived *M. leprae* and irradiated organisms. This phagocytic event was shown to be dependant upon host cell kinases, specifically protein tyrosine kinase, calcium-dependent protein kinase and phosphatidylinositol 3-kinase in a series of assays where specific kinase inhibitors were shown to limit uptake of *M. leprae*. These results are not surprising, as phagocytosis is an actin-mediated process and these kinases have been implicated in the actin-mediated phagocytosis of other bacteria. It should be noted that cAMP-dependent protein kinase inhibitors did not affect phagocytosis of *M. leprae* by the ST 8814 cell line. Once *M. leprae* is internalized by the Schwann cell, the bacilli must survive and perpetuate to cause disease. There have been several recent studies that hint at the mechanisms *M. leprae* utilizes to persist in the host cells and cause, in part, the unique pathology seen in leprosy.

In general terms, after organisms have been internalized by host cells, there is a series of events that normally progress to result in the death of invading organisms. In normal endocytic processing, host cells phagocytose particles (including microorganisms), and the resulting phagosomes are transported through a series of events along the phagosomal and endocytic pathway. After exhibiting early, and then late, endosomal characteristics as determined by membrane markers, the phagosome will fuse with lysosomes. These compartments, designated phagolysosomes, are highly acidified and contain degradative enzymes from the lysosomal compartment. This fusion event leads to the degradation of the phagolysosomal contents. Much work has been done to characterize the intracellular compartments of other mycobacterial species, including our work on the intracellular phenotype of *M. marinum*. Phagosomes of other pathogenic mycobacteria, including *M. tuberculosis*, *M. bovis*, and *M. avium* have been shown to contain early endosomal, but not late endosomal or lysosomal markers. In other words, they are arrested for phagosomal development in the early endosomal state. Most importantly, this enables these mycobacterial species to survive the normally hostile intracellular environment by avoiding fusion with the lysosome.

Using the acidotrophic probe Lysotracker™ to label lysosomal compartments, we were able to demonstrate that live *M. leprae* did not reside in acidified compartments at early (up to 48 h) time points in the ST 8814 cell line. Heat killed organisms tested in parallel were, however, trafficked to the lysosomal compartments, even at the earliest (4 h) time point. These data suggest that trafficking of *M. leprae* through the Schwann cell endocytic pathway may be similar to other mycobacteria studied. It should be noted that similar results were obtained in the RAW 264.7 macrophage cell line. Further, the fact that heat killed organisms were not able to evade the host cell endocytic pathway in either host cell model (Schwann cell or macrophage) indicates an active process by which these organisms perpetuate in the host cell. More experiments, however, are required to determine the cellular mechanisms by which phagocytosis of *M. leprae* occurs and to fully characterize the *M. leprae*-containing phagosome.

**Recently elucidated effects of *M. leprae* infection of Schwann cells**

Several studies indicated that a result of *M. leprae* infection of Schwann cells was an increase in Schwann cell proliferation. One signaling pathway involving extracellular signal-
regulated kinases 1 and 2 (Erk1, Erk2) has been shown to play a significant role in cell proliferation\textsuperscript{48,49}. To determine whether this proliferation was a direct result of bacterial insult, long-term (30 day) infections of primary Schwann cells with live \textit{M. leprae} derived from mouse footpads were carried out. The investigators then examined the proliferation of the infected Schwann cells and determined that higher levels of mitosis were occurring at 30 days post-infection and that total numbers of Schwann cells increased as compared to uninfected controls\textsuperscript{50}. Affimetrix chips containing human cell cycle gene substrates were probed with DNA from \textit{M. leprae}-infected Schwann cells. Cyclin D1 and p21, two key G1 phase cell cycle regulators, were found to be upregulated at day 30 post-infection. As several signaling pathways could contribute to this G1-phase cycle induction, a series of elegant inhibition experiments and \textit{in vitro} kinase assays were performed that indicated that the Erk1/2 signaling cascade was activated by intracellular \textit{M. leprae}\textsuperscript{50}. One implication of this study is that the organisms could be increasing non-myelinated Schwann cell proliferation in order to maintain the niche in which they reside. This study\textsuperscript{50} also elucidated an interesting alternative signaling mechanism for cell proliferation. It will be interesting to follow subsequent experiments that will address specific bacterial factors that mediate the host cell proliferation observed and determinations of whether viable organisms are required for this effect.

There has been speculation that other effects of Schwann cell infection by \textit{M. leprae} can lead directly to the Schwann cell presentation of \textit{M. leprae} antigens to T-cells and result in the production of immune modulators such as TNF-\textit{\alpha}\textsuperscript{51}. A recent publication by Pereira \textit{et al}\textsuperscript{52} that also investigated the effect of \textit{M. leprae} infection on specific signaling molecules indicated that NF-kB-dependent transcription repression in ST 8814 Schwannoma cells was a response to infection with irradiated \textit{M. leprae}. Other pathogenic microorganisms have been shown to influence NF-kB activation in order to modulate the innate immune response\textsuperscript{53}. The regulation of NF-kB can also be affected by TNF-\textit{\alpha}. The Pereira study\textsuperscript{52} indicates that \textit{M. leprae} can modulate the immune response of the host via specific signaling pathways. These pathways are, interestingly, also subject to the influence of the TNF-\textit{\alpha} inhibitor, thalidomide\textsuperscript{54}. Thalidomide is used for the treatment of some forms of leprosy in which inflammation and subsequent tissue damage are a factor in the progression of the disease\textsuperscript{55,56}.

**Interactions of macrophages with \textit{M. leprae}**

Krahenbuhl and Adams\textsuperscript{57} put forth an excellent review of the basic biology of macrophage interactions with \textit{M. leprae} in 1994. More recently, however, these investigators, in addition to other researchers, have elucidated interesting facets of the macrophage-\textit{M. leprae} interplay. In a patient infected with \textit{M. leprae}, the organisms can be found in a variety of tissues and cell types, but macrophages can internalize and/or contain many bacilli, especially in bacteraemic infections\textsuperscript{58}. Macrophages in specific tissue and infection sites can play an important role in the pathogenesis of leprosy for two reasons. First, whole \textit{M. leprae} and/or cell wall components can stimulate macrophages to release cytokines, including TNF-\textit{\alpha}, \textit{in vitro}\textsuperscript{59}. This indicates a direct role of the macrophage in the immune response to infection. Secondly, macrophages, like dendritic cells are antigen-presenting cells and will bridge innate and acquired immunity by evoking T-cell and B-cell responses.

\textit{M. leprae} has recently been shown to be readily phagocytosed by RAW 264.7 cells and to evade phagosome-lysosome fusion, at least up to 48 h\textsuperscript{59}. In experiments similar to the kinase inhibitor assays described in this study for the uptake of \textit{M. leprae} by Schwann cells, a study by Lima \textit{et al}\textsuperscript{60} indicated that the uptake of \textit{M. leprae} by the monocytic cell line THP-1 was also kinase dependant. In addition, a study by Charlab \textit{et al}\textsuperscript{61} has shown that human mononuclear cells preexposed to \textit{M. leprae} can be stimulated to produce TNF-\textit{\alpha} after the addition of PGL-1.
Recent studies also define a role for the activation of macrophages in the control of leprosy infection. In an imaginative in vitro model system, which utilizes macrophages isolated from the granulomas in the footpads of M. leprae-infected mice, Hagge et al. established a co-culture system of the granuloma macrophages containing viable M. leprae with either activated or normal effector macrophages. This study then assayed the viability of M. leprae that had been recovered from the respective co-culture systems. It was determined that the M. leprae recovered from the granuloma macrophages co-cultured with normal macrophages had significantly more metabolic activity, as measured by radiorespirometry. These data indicated that those organisms in a background of non-activated macrophages were more viable, i.e., activation of the macrophages is an integral part of the immune response to leprosy infection. Most interestingly, the normal effector macrophages were able to acquire M. leprae from the macrophages of granuloma origin and subsequently augmented the metabolism of resident M. leprae bacilli. This leads to intriguing speculation that this, or some modification of, the co-culture system could lead to the maintenance of viable M. leprae cultures over time.

In a recent study, activated and normal mouse peritoneal macrophages infected with M. leprae were treated with thalidomide to determine whether the viability of intracellular organisms could be affected by this drug. Thalidomide did not exhibit any antimicrobial activity against intracellular organisms in either normal or activated (with endotoxin and IFN-α) macrophages. Further, these investigators suggest that thalidomide does not, therefore, inhibit the release of M. leprae antigens that have been previously shown to exhibit an immunostimulatory effect. The use of thalidomide to modulate the immune response and treat some forms of leprosy is, therefore, most likely directly related to an interaction of the drug with host-specific modulators such as TNF-α.

Interaction of M. leprae with dendritic cells

Recent work has focused on the interactions of M. leprae with another antigen presenting cell, the dendritic cell. Sieling et al. demonstrated that dendritic cells (DCs) were present in tuberculoid lesions of leprosy patients. Soon after, Yamauchi et al. found that T-cells in a similar patient’s tuberculoid lesions expressed CD40 ligand, which is involved in the differentiation and activation of DCs. The DC is thought to be one of the most effective antigen presenting cells, as it can stimulate both CD4+ and CD8+ T cells in the mounting of an effective protective immune response to infection. An actual in vitro study of infection of DCs with footpad-derived M. leprae was published recently that indicated that monocyte-derived DCs were able to actively phagocytose M. leprae. This study further showed that DCs were able to effectively present M. leprae specific antigens, including PGL-1. However, in experiments assaying T-cell activation by DCs, there was less T-cell stimulation with DCs infected with M. leprae than with M. bovis BCG or M. avium. These results indicated a higher level of resistance to DC mediated T-cell immunity. Interestingly, a recent report by Hunger et al. have determined that Langerhans cells, a subset of DCs that initiate the immune response in the skin, are more efficient at M. leprae antigen presentation than monocyte-derived DCs.

In other studies, Makino and colleagues have shown that an isolated and purified M. leprae protein, designated MMP-II (major membrane II) was highly immunogenic. The MMP-II molecule stimulated DCs directly and, when used to pulse monocyte-derived DCs, would result in high levels of T-cell activation. These studies are particularly noteworthy in that this group has identified a novel, immunostimulatory M. leprae protein that could have relevance in the development of vaccines or treatments against leprosy. Continuing the study of the ability of M. leprae to persist in and contribute
to the immunologic functioning of both macrophages and DCs will provide additional information as to the host-pathogen interface that results in disease pathology in the context of the immune response, or lack thereof.

**M. leprae and endothelial cells – a potential delivery system**

An interesting observation that has been made since the establishment of histological techniques for the study of lesions from individuals infected with *M. leprae* has been the frequency with which bacilli are found in endothelial cells lining the blood vessels and lymphatics. Many studies have described the presence of *M. leprae* in the endothelium of the skin, nervous tissue and nasal mucosa. These early studies indicate that the endothelial cell may be a site of *M. leprae* persistence and replication. In 1999, Scollard et al. used an armadillo model of *M. leprae* infection to determine the extent to which the bacilli could be found in endothelial cells. In this seminal study, histopathological evidence suggested that the endothelial cells in the epineurial and perineurial blood vessels could be a reservoir for actively replicating *M. leprae* that would subsequently infect the Schwann cells in adjacent tissue. The implications are that some mechanism of *M. leprae* attachment to endothelial cells could be required for the establishment of infection and that *M. leprae* can reach the peripheral nerve tissue through the bloodstream. This hypothesis was counter to the long-held belief that leprosy infection was a direct result of injury and subsequent Schwann cell exposure to viable bacteria from another exogenous reservoir.

A more detailed study of *M. leprae* association with endothelial cells in vitro followed, wherein monolayers of human umbilical vein endothelial cells (HUVEC) were infected with *M. leprae*. Though the kinetics of uptake of *M. leprae* by the HUVEC was much less than those seen with macrophages, the association appeared to be specific and internalization of the bacilli was observed with both confocal and electron microscopy. Any specific endothelial cell receptors or bacterial ligands responsible for this association and uptake remain to be elucidated. An interesting review of the possible implications of this work is available, but the question as to the contribution of endothelial cell infection to the pathogenesis of leprosy is rather open-ended. A larger question remains in regard to whether endothelial cells or some other cell type can “deliver” viable bacilli from potential exposure sites (e.g. nasal mucosa) to Schwann cells in the extremities and whether tissue damage is integral to the establishment of disease.

The question of how *M. leprae* can be disseminated systemically in the leprosy patient is nicely addressed by Pessolani et al. As the major site of bacterial excretion is the nasal mucosa, a fascinating possibility outlined in this report is that *M. leprae* in nasal discharges from leprosy patients can be transmitted to others via an airborne route. If the bacilli enter the lung, the elucidation of bacterial adhesins that initiate cell contact and infection will be very important. Much remains to be done to determine how, if the organisms are taken up by the respiratory mucosa, the bacilli reach the nerves and skin.

**Conclusions**

It is not difficult to develop an appreciation for the various host cells with which *M. leprae* interact in the pathogenesis of leprosy. The difficulties in working with *M. leprae* in the laboratory are not trivial, but despite this, there has been an explosion of studies on leprosy host-pathogen interactions and other related information in the past few years. This information, now available to researchers and clinicians, will direct the basic science, prevention, and treatment of leprosy in coming decades. Concomitant with studies elucidating the immunologic consequences of, and effects on, leprosy infection, are the recent advances in mycobacterial genomics, eukaryotic cell biology,
new microscopic and histological techniques, and the introduction and/or improvement of \textit{in vitro} and \textit{in vivo} models. These advances, in combination with some of the findings described herein, give the leprosy researcher a variety of exciting investigational roads from which to choose.

Some of the most intriguing questions that still remain have to do with the actual reservoirs of \textit{M. leprae} in the infected individual. Though the organism is thought to reside primarily in Schwann cells and macrophages, many other cell types, including DCs and endothelial cells, appear to harbour the organism. Specific Schwann cell receptors have been elucidated, as have some of the bacterial factors responsible for colonization of the Schwann cells. This begs the question as to whether these bacterial adhesins, \textit{e.g.} PGL-1 and LBP21, are acting as specific adhesins in binding to other cell types.

A further question has to do with the intracellular survival of \textit{M. leprae} in Schwann cells, macrophages, and other host cells. Though there is some evidence that supports the evasion of normal endocytic processing by intracellular \textit{M. leprae}, it remains to be seen whether the intracellular niche of this organism is similar to that of other pathogenic mycobacteria. In addition, it appears that \textit{M. leprae} activates proliferation pathways in the Schwann cell host. This observation is significant for two reasons. First, the organism may be inducing the perpetuation of its own intracellular niche. Secondly, it is intriguing to speculate that specific \textit{M. leprae} factors, without infecting organisms, of course, can be a tool for the induction of nerve regeneration and myelination in the medical setting.

The elucidation of specific bacterial factors important for the organism to establish disease may also lead to candidates for vaccine preparations or targets for specific antimicrobials. Though the volume of work on \textit{M. leprae} interactions with host cells appears to be growing rapidly, many details remain to be resolved. The application of new information regarding the basic biology of \textit{M. leprae} survival and dissemination in the host is the best hope for the true eradication of leprosy.

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