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EDITORIALS

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The Macrophage in Leprosy: A Review on the Current Status

The immunological response to mycobacterial infections is predominantly cell mediated although it is now realized that humoral factors are also involved.[1] In the study of the cell-mediated immune (CMI) response in leprosy, attention has hitherto been chiefly on the lymphocyte and the macrophage. However, recent studies reveal that several other cells, such as the Langerhans' cells, dendritic cells and B cells, may also play a role, especially in the presentation of mycobacterial antigens.

The reason for the major attention hitherto given to the macrophage and the lymphocyte is because of their predominance in the histology of lesions in leprosy. In the lepromatous form of the disease, the predominant cell is the incompetent macrophage loaded with *Mycobacterium leprae* with the virtual absence of lymphocytes, while the tuberculoid lesion is in the nature of a granulomatous reaction with a core of competent macrophages known as epithelioid cells containing few if any acid-fast bacilli (AFB) and surrounded by a large cuff of lymphocytes.

The earliest studies of Barbieri and Correa[2] followed by that of Beiguelman[3] were related to the *in vitro* study of the macrophages in this disease. They observed that blood-derived macrophages isolated from cases of lepromatous leprosy were unable to cause the *in vitro* lysis of *M. leprae,* while macrophages from tuberculoid patients lysed the bacilli. However, there was no concerted follow-up of these studies. This was partly due to the inability to reproduce the above results by their contemporaries[4, 5] together with the emergence of the importance of T lymphocytes in basic immunology. While literature on the role of T cells in leprosy continued to mount in proportion to the increase in knowledge on lymphocytes, and later its subsets, in general im-

[1] Ferluga, J., Colizzi, V., Ferrante, A., Colston, M. J. and Holborow, E. J. Hypothesis: possible idiotypic suppression of cell mediated immunity in lepromatous leprosy. Lepr. Rev. **55** (1984) 221–227.

[2] Barbieri, T. A. and Correa, W. M. Human macrophage culture; the leprosy prognostic test (LPT). Int. J. Lepr. **35** (1967) 377–381.

[3] Beiguelman, B. Fate of *Mycobacterium leprae* in macrophages. (Letter) Int. J. Lepr. **39** (1971) 896.

[4] Godal, T. and Rees, R. J. W. Fate of *M. leprae* in macrophages of patients with lepromatous or tuberculoid leprosy. Int. J. Lepr. **38** (1970) 439–442.

[5] Talwar, G. P., Krishnan, A. D. and Gupta, P. D. Quantitative evaluation of the process of intracellular infection *in vitro*: incorporation of ^{3}H thymidine into DNA by *M. leprae* in cultivated blood monocytes. Infect. Immun. **9** (1974) 187–191.

munology, studies on the macrophage remained relatively neglected.

In 1978, the importance of the macrophage in the pathogenesis of leprosy was once again highlighted. Bullock, *et al.*[6] reported that splenic cells with macrophage-like characteristics mediated suppression of the immune response to sheep erythrocytes in mice during the 5th–10th week after *M. lepraemurium* infection. Hirschberg[7] used the earlier consistent observation that peripheral-blood lymphocytes from patients suffering from lepromatous leprosy did not normally react *in vitro* to *M. leprae* antigens and showed that T cells from nonresponding patients in combination with macrophages from responding patients or healthy contacts did respond to *M. leprae*. Conversely, T cells from responding patients or healthy contacts in combination with macrophages from nonresponding patients failed to respond. It was concluded, therefore, that the lack of response normally observed in *in vitro* tests using cells from lepromatous leprosy patients was seemingly due to a failure of their macrophages to present *M. leprae* antigens in an immunogenic form. This was corroborated by the later experiments of Nath, *et al.*[8] using HLA-D-matched responders and nonresponders within sibships.

These studies, however, were not extended to obtain an in-depth understanding of the role of macrophages in leprosy. During this period, our group initiated experiments on the role of the macrophage in leprosy in three major areas: a) interaction of *M. leprae* with the macrophage;[9–12] b) macrophage suppressor factors;[13–15] and c) whether the macrophage defect pre-exists before infection.[16]

With the current interest in antigen presentation, the macrophage has gained prominence in general immunology as well as in leprosy. Therefore, we feel that this is an opportune time for this review. This emphasis does not diminish the importance of suppressor-T cells in the pathogenesis of leprosy; their role has been described and discussed exhaustively in several other reviews.[17, 18] However, there may be more than one defect in the chain of events, either in a single-cell type or in a number of varying cell types, and the point at which the defect occurs may also vary across the spectrum of this disease and even among individuals.

Intracellular survival

Abnormal entry. Avoidance of phagocytosis, and even optimal phagocytosis, is an important virulence factor of many intracellular pathogens. It has been stated that

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[6] Bullock, W., Carlson, E. and Gershon, R. The evolution of immunosuppressive cell populations in experimental mycobacterial infections. J. Immunol. **120** (1978) 1709–1716.

[7] Hirschberg, H. The role of macrophages in the lymphoproliferative response to *M. leprae in vitro*. Clin. Exp. Immunol. **34** (1978) 46–51.

[8] Nath, I., van Rood, J., Mehra, N. K. and Vaidya, M. C. Natural suppressor cells in human leprosy: the role of HLA-D-identical peripheral lymphocytes and macrophages in the *in vitro* modulation of lymphoproliferative responses. Clin. Exp. Immunol. **42** (1980) 203–210.

[9] Birdi, T. J., Salgame, P. R. and Antia, N. H. The role of macrophages in leprosy as studied by protein synthesis of macrophages from resistant and susceptible hosts—a mouse & human study. Lepr. India **51** (1979) 23–42.

[10] Birdi, T. J., Mistry, N. F., Mahadevan, P. R. and Antia, N. H. Alterations in the membrane of macrophages from leprosy patients. Infect. Immun. **41** (1983) 121–127.

[11] Birdi, T. J., Mistry, N. F., Mahadevan, P. R. and Antia, N. H. Antigen specific macrophage-lymphocyte interaction in lepromatous leprosy. J. Clin. Lab. Immunol. **13** (1984) 189–194.

[12] Mistry, N. F., Birdi, T. J. and Antia, N. H. *M. leprae* phagocytosis and its association with membrane changes in macrophages from leprosy patients. Parasite Immunol. **8** (1986) 129–138.

[13] Salgame, P. R., Mahadevan, P. R. and Antia, N. H. Mechanism of immunosuppression in leprosy: presence of suppressor factor(s) from macrophages of lepromatous patients. Infect. Immun. **40** (1983) 1119–1126.

[14] Salgame, P. R., Birdi, T. J., Lad, S. J., Mahadevan, P. R. and Antia, N. H. Mechanism of immunosuppression in leprosy—macrophage membrane alterations. J. Clin. Lab. Immunol. **14** (1984) 145–149.

[15] Birdi, T. J., Salgame, P. R., Mahadevan, P. R. and Antia, N. H. An indomethacin sensitive suppressor factor released by macrophages of leprosy patients. J. Biosci. **6** (1984) 125–134.

[16] Mistry, N. F., Birdi, T. J., Mahadevan, P. R. and Antia, N. H. *M. leprae* induced alterations in macrophage Fc receptor expression and monocyte-lymphocyte interaction in familial contacts of leprosy patients. Scand. J. Immunol. **22** (1985) 415–423.

[17] Gill, H. K. and Godal, T. Deficiency of cell mediated immunity in leprosy. Prog. Allergy **37** (1986) 377–390.

[18] Kaplan, G. and Cohn, Z. A. The immunology of leprosy. Int. Rev. Exp. Pathol. **28** (1986) 45–78.

the capacity of macrophages to ingest *M. leprae* is normal.[19] However, studies carried out *in vitro* on the number of bacteria phagocytosed per cell indicated that the largest proportion of macrophages with the highest bacterial load belonged to the lepromatous group.[6] Regulation of phagocytosis was studied by observing the ingestion of *M. leprae* after pulsing the macrophages with a reticuloendothelial system (RES) blocker, carbonyl iron. Phagocytosis of carbonyl iron resulted in decreased *M. leprae* uptake by normal and tuberculoid macrophages. However, in lepromatous cells the uptake of *M. leprae* after carbonyl iron was comparable to untreated control values. These results suggested that lepromatous leprosy macrophages are refractory to normal regulatory mechanisms, resulting in excess phagocytosis.[6] It can be speculated, therefore, that this excessive phagocytosis would result in the neutralization of bactericidal mechanisms such as reactive oxygen intermediates and lysosomal enzymes, leaving a proportion of the bacilli free to exert their virulence. Watson, *et al.*[20] noted a depressed immune response to sheep erythrocytes at an early stage of *M. lepraemurium* infection. The immunosuppression was caused more by macrophages than by lymphocytes. One of the explanations for this observation was that overloading macrophages with mycobacteria could interfere with their ability to ingest, process, and present other antigens.

Invasiveness of the organism into the host cell is another factor noted by Chang[21] in leishmaniasis. Direct penetration through the plasma membrane of phagocytes has also been postulated as a means of entry for protozoa and rickettsia.[22] A similar mode of entry may be operative for *M. leprae*. Cytochalisin B was able to completely block phagocytosis of autoclaved *M. leprae* although a proportion of viable bacilli still entered.[12] This mode of entry would explain the observation that some of the bacteria lie free in the cytoplasm. This entry mechanism may also be important in the parasitization of cells not normally phagocytic, such as Schwann cells and Langerhans' cells.

Phagolysosome. Once intracellular, *M. leprae* may prevent its own destruction either by interfering with the activity of lysosomal enzymes or by preventing phagosome-lysosome fusion. The fusion of a macrophage lysosome with a phagosome is thought to deliver the entire lysosomal contents uniformly into the phagosome.

Decreased levels of lysosomal enzymes would result in the inavailability of sufficient enzymes to degrade *M. leprae*. Avila and Convit[23] and Miyama and Saito[24] reported normal enzyme levels in peripheral-blood-derived macrophages of lepromatous and tuberculoid patients; while studies by Marolia and Mahadevan[25] showed a decrease in the enzyme levels of lepromatous patients compared to tuberculoid patients and normal individuals. These apparently contradictory results may be due to a number of influencing factors, such as heterogenous patient population, variation in treatment, and the state of activation of the patients' macrophages.

Wang and Goren[26] indicated that individual lysosomal enzymes can be selectively and sequentially transferred to phagosomes. Thus, even normal lysosomal enzyme levels may not necessarily result in sufficient enzymes reaching the *M. leprae* contained in the phagosomes.

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[19] Rojas-Espinosa, O. Phagocytosis in leprosy. II. Production of superoxide by circulating blood leukocytes from lepromatous patients. Int. J. Lepr. **46** (1978) 337–341.

[20] Watson, S., Slijivic, V. and Brown, I. Defect of macrophage function in the antibody response to sheep erythrocytes in systemic *M. lepraemurium* infection. Nature **256** (1975) 206–208.

[21] Chang, K. P. *L. donovani* promastigote-macrophage surface interaction *in vitro*. Exp. Parasitol. **48** (1979) 175–189.

[22] Jones, T., Yeh, S. and Hirsch, J. The interaction between *T. gondii* and mammalian cells. J. Exp. Med. **136** (1972) 1173–1194.

[23] Avila, J. L. and Convit, J. Studies on cellular immunity in leprosy; lysosomal enzymes. Int. J. Lepr. **38** (1970) 359–364.

[24] Miyata, T. and Saito, N. Enzymatic activities in the serum of leprosy patients. I) On phosphatase. Lepro **32** (1963) 192–198.

[25] Marolia, J. and Mahadevan, P. R. Hydrolytic enzymes in macrophages from leprosy patients in presence of *Mycobacterium leprae*. Indian J. Lepr. **56** (1984) 776–783.

[26] Wang, Y. and Goren, M. B. Differential and sequential delivery of fluorescent lysosomal probes into phagosomes in mouse peritoneal macrophages. J. Cell Biol. **104** (1987) 1749–1754.

Of greater importance than the intracellular bacterial load is the intracellular location of the bacilli. *M. lepraemurium* multiplies within phagolysosomes, and *M. tuberculosis* prevents phagolysosomal fusion.[27, 28] Job and Verghese[29] and Levy, *et al.*[30] have reported that *M. leprae* appear to multiply within the cytoplasmic matrix outside the phagolysosomes. This may be the result of the facilitative entry mentioned earlier. Recent studies by Sibley, *et al.*[31] reported that in resident peritoneal macrophages from Swiss Webster mice a majority of the phagosomes containing freshly isolated, viable *M. leprae* resisted fusion with lysosomes but that phagosomes containing irradiated *M. leprae* underwent fusion with lysosomes. D'Arcy Hart, *et al.*[28] suggested that the inhibition of phagosome-lysosome fusion after phagocytosis of some viable mycobacteria, such as *M. microti,* might be due to the inhibition of movement of the lysosomes.

Oxidative burst. Various workers have detected a respiratory burst in lepromatous macrophages. However, since *M. leprae* contains superoxide dismutase it is likely that it is able to protect itself from the effects of superoxide radicals.[32] The phenolic glycolipid-I (PGL-I) of *M. leprae* has been shown to scavenge reactive oxygen intermediates, and this may serve to prevent the bactericidal action of these oxygen radicals.[33, 34]

Holtzer, *et al.*[35] reported that *M. leprae* may be phagocytosed by macrophages without triggering an oxidative burst.[36] Similar implicate a role for C3b, since phagocytic cells can ingest C3b-coated particles without triggering an oxidative burst.[36] Similar observations have been made with *Toxoplasma gondii.*[37] Marolia and Mahadevan[38] reported that the production of superoxide in response to *M. leprae* infection is reduced in macrophages from leprosy patients but not in macrophages from normal individuals. This deficiency could be overcome in cells from tuberculoid patients in the presence of lymphokines. However, unlike the observations of Holtzer, *et al.*,[35] they state that *M. leprae* can induce a respiratory burst in macrophages from normal individuals.

A study by Ding and Nathan[39] indicates that the release of small quantities of lipopolysaccharide may be a means by which microorganisms interfere with the immunologically mediated enhancement of the respiratory burst and thereby ensure their survival. Such a sequence of events, if applicable to *M. leprae,* could also partly ex-

[27] D'Arcy Hart, P., Armstrong, J. A., Brown, C. A. and Draper, P. Ultrastructural study of the behavior of macrophages toward parasitic mycobacteria. Infect. Immun. **5** (1972) 803–807.

[28] D'Arcy Hart, P., Young, M. R., Gordon, A. H. and Sullivan, K. H. Inhibition of phagosome-lysosome fusion in macrophages by certain mycobacteria can be explained by inhibition of lysosomal movements observed after phagocytosis. J. Exp. Med. **166** (1987) 933–946.

[29] Job, C. K. and Verghese, R. Schwann cell changes in lepromatous leprosy—an electron microscopic study. Indian J. Med. Res. **63** (1975) 897–901.

[30] Levy, L., Ng, H., Evans, M. J. and Krahenbuhl, J. L. Susceptibility of thymectomized and irradiated mice to challenge with several organisms and the effect of dapsone on infection with *Mycobacterium leprae.* Infect. Immun. **11** (1975) 1122–1132.

[31] Sibley, D., Franzblau, S. G. and Krahenbuhl, J. L. Intracellular fate of *M. leprae* in normal and activated mouse macrophages. Infect. Immun. **55** (1987) 680–685.

[32] Wheeler, P. R. and Gregory, D. Superoxide dismutase activity and catalase in *M. leprae* purified from armadillo liver. J. Gen. Microbiol. **121** (1980) 457–464.

[33] Neill, M. A. and Klebanoff, S. J. The effect of PGL-I from *M. leprae* on the anti-microbial activity of human macrophages. J. Exp. Med. **167** (1988) 30–42.

[34] Holzer, T. J., Arnold, J. J., Vachula, M. and Andersen, B. R. PGL-I of *M. leprae* induces altered monocyte oxidative responses *in vitro.* Int. J. Lepr. **55** (1987) 784–785.

[35] Holzer, T. J., Nelson, K. E., Schauf, V., Crispen, R. G. and Andersen, B. R. *M. leprae* fails to stimulate phagocytic cell superoxide anion generation. Infect. Immun. **51** (1986) 514–520.

[36] Wright, S. D. and Silverstein, S. C. Receptors for C3b and C3bi promote phagocytosis but not release of toxic oxygen from human phagocytes. J. Exp. Med. **158** (1983) 2016–2023.

[37] Wilson, C. B., Tsai, V. and Remington, J. S. Failure to trigger the oxidative metabolic burst by normal macrophages: possible mechanism for survival of intracellular pathogens. J. Exp. Med. **151** (1980) 328–346.

[38] Marolia, J. and Mahadevan, P. R. Superoxide production from macrophages of leprosy patients after stimulation with *M. leprae.* J. Biosci. **12** (1987) 273–279.

[39] Ding, A. H. and Nathan, C. F. Trace levels of bacterial lipopolysaccharide prevent interferon-γ or tumor necrosis factor from enhancing mouse peritoneal macrophage respiratory burst capacity. J. Immunol. **139** (1987) 1971–1977.

plain the differences in the immune response to live and dead bacteria which are discussed in subsequent sections.

Other bactericidal mechanisms. Other bactericidal mechanisms responsible for the killing of *M. leprae in vivo* have also been implicated. Kaufmann, *et al.*[40] suggested that antigen-specific T cells are cytotoxic to macrophages presenting bacterial antigens. This would result in the bacteria being released from an ineffective host cell into the extracellular environment. They may then be rephagocytosed and killed by more activated macrophages, neutrophils, or come in contact with natural-killer (NK) cells, etc. A similar mechanism has been described in tuberculosis.[41]

Another mechanism shared by tuberculosis, cutaneous leishmaniasis, and leprosy is the phenomenon of caseous necrosis which appears histologically to originate from excessive macrophage fusion and a critical level of antigen and antibody.[42, 43] Such a feature is often noted in cutaneous nerves and major nerve trunks of not only tuberculoid cases but also borderline leprosy patients. Its hallmark feature is the preponderance of plasma cells around the caseous mass. Integral bacilli are never visible in or around the caseous mass. However, staining with anti-BCG reveals foci of subcellular mycobacterial antigens.[44]

Response of macrophage to activating signals and the ability to kill *M. leprae*

The ultimate effector for the destruction of pathogenic mycobacteria is the activated macrophage in which the pathogen resides. The questions which arise are: a) can the parasitized macrophage respond to activating signals, and b) if activated, is it able to kill the mycobacterium? Due to the inability to directly measure *M. leprae* viability, various studies have measured the killing of other intracellular parasites as a reflection of the cells' ability to kill *M. leprae*.[45, 46] *T. gondii* and *Listeria monocytogenes* have been most commonly used. The killing of *L. monocytogenes* by macrophages is easily achieved,[47] and this assay may not reflect the ability of a cell to kill *M. leprae*. van Dissel, *et al.*[48] showed that immunologically activated macrophages can kill *L. monocytogenes* and *T. gondii* but not *Salmonella typhimurium*.

Sibley and Krahenbuhl[49] have suggested that at least the tissue macrophages of *M. leprae*-infected nude mice are unable to respond to gamma interferon (γ-IFN) as measured by their ability to kill *T. gondii*. In contrast to tissue macrophages, Sibley, *et al.*[31] observed that activation of peritoneal macrophages of Swiss Webster mice with γ-IFN led to increased phagolysosome fusion, resulting in *M. leprae* fragmentation as seen by electron microscopy. These divergent observations may also be due to variations in macrophage subpopulations.

There may be differences in the responses of tissue macrophages from mice compared to those from lepromatous leprosy patients as seen from the observations of Nathan, *et al.*[50] They reported that local inoculation of

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[40] Kaufmann, S. H. E., Chiplunkar, S., Flesch, I. and de Libero, G. Possible role of helper and cytolytic T cells in mycobacterial infections. Lepr. Rev. **57** Suppl. 2 (1986) 101–111.

[41] Podleski, W. K. and Podleski, U. G. Circulating cytotoxic lymphocytes in human tuberculosis. Am. Rev. Respir. Dis. **108** (1973) 791–798.

[42] Spector, W. G., Marianayagam, Y. and Ridley, M. J. The role of antibody in primary and reinfection BCG granulomas of rat skin. J. Pathol. **136** (1982) 41–57.

[43] Ridley, M. J., Marianayagam, Y. and Spector, W. G. Experimental granulomas induced by mycobacterial immune complexes in rats. J. Pathol. **136** (1982) 59–72.

[44] Antia, N. H. and Mistry, N. F. Plasma cells in caseous necrosis of nerves in leprosy. Lepr. Rev. **56** (1985) 331–335.

[45] Mitsuyama, M., Nomoto, K. and Takeya, K. Direct correlation between delayed foot pad reaction and resistance to local bacterial infection. Infect. Immun. **36** (1982) 72–79.

[46] Horowitz, M. A., Levis, W. R. and Cohn, Z. A. Defective production of monocyte activating cytokines in lepromatous leprosy. J. Exp. Med. **159** (1984) 666–678.

[47] Miyata, M., Mitsuyama, M., Ogata, N., Nomoto, K. and Takeya, K. Two steps in the generation of acquired cellular resistance against *Listeria monocytogenes*: accumulation and activation of macrophages. Immunology **47** (1982) 247–253.

[48] van Dissel, J. T., Stikkelbroeck, J. J. M., van den Barselaar, M. T., Sluiter, W., Leijh, P. C. J. and van Furth, R. Divergent changes in antimicrobial activity after immunologic activation of mouse peritoneal macrophages. J. Immunol. **139** (1987) 1665–1672.

[49] Sibley, L. D. and Krahenbuhl, J. L. *M. leprae* burdened macrophages are refractory to activation by gamma interferon. Infect. Immun. **55** (1987) 446–450.

[50] Nathan, C. F., Kaplan, G., Levis, W. R., Nusrat, A., Witmer, M. D., Sherwin, S. A., Job, C. K., Ho-

γ-IFN into lesions resulted in the activation of macrophages accompanied by a reduction in the bacteriological load. Differences in macrophage populations have also been highlighted by Rook, *et al.*[51] They reported the inhibition of *M. tuberculosis* by γ-IFN-activated mouse peritoneal macrophages but not by human peripheral blood monocytes.

The histopathological studies of Ridley[52] have shown that newly recruited macrophages are the preferential host cells of *M. leprae.* Therefore, the cells which accumulate in response to *M. leprae,* instead of containing the infection, are parasitized, allowing bacillary multiplication. In the same patient bacteria-laden macrophages can be seen to lie alongside uninfected macrophages of sarcoidosis. However, at a later phase of infection *M. leprae* parasitize both inflammatory and tissue macrophages.

Alteration in macrophage metabolism

Whatever the final mode for the intracellular survival of *M. leprae,* macrophage metabolism is affected, as indicated by impaired protein synthesis[9] and the macrophage's inability to respond to activation.

Membrane alterations. Alterations in macrophage metabolism are also reflected in the down-regulation of three membrane markers, namely, the Fc receptor, ConA receptor, and HLA-DR antigen expression.[9–11, 53] Only viable *M. leprae* induce these alterations in the lepromatous macrophage, suggesting that products secreted by viable *M. leprae* may be partly responsible for these alterations rather than structural components of *M. leprae*[54, 55] because the latter would be common to both dead and live bacilli.

These macrophage membrane perturbations have a number of functional implications. T cells see antigens on the surface of antigen-presenting cells (APCs) major in the context of histocompatibility complex (MHC) Class II antigens. The down-regulation of Class II antigens on the macrophage membrane could interfere with its antigen-presenting function. *Leishmania donovani* has also been reported to suppress macrophage expression of MHC gene products.[56]

Down-regulation of the Fc receptor (FcR)[4] could prevent the opsonization of *M. leprae* from having any effect on its uptake and subsequent handling by the macrophage. This was supported by the observation that *M. leprae* opsonization does not enhance phagolysosome formation.[31] Opsonization of *M. lepraemurium,* on the other hand, results in its killing by macrophages.[57] A correlation between susceptibility/resistance and the ability of FcR+/FcR− macrophages to handle *M. leprae* has been attempted by Ohkawa, *et al.*[58] The immunohistological studies on lepromatous skin lesions by Ridley, *et al.*[59] also reported Fc receptor alterations in macrophage-like cells.

Macrophage suppressor activity. For a small proportion of viable bacilli to exert a profound immunosuppressive effect, it is

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rowitz, C. R., Steinman, R. M. and Cohn, Z. A. Local and systemic effects of intradermal recombinant interferon-γ in patients with lepromatous leprosy. N. Engl. J. Med. **315** (1986) 6–15.

[51] Rook, G. A. W., Steele, J., Ainsworth, M. and Champion, B. R. Activation of macrophages to inhibit proliferation of *M. tuberculosis*: comparison of the effects of recombinant gamma interferon on human monocytes and murine peritoneal macrophages. Immunology **59** (1986) 333–338.

[52] Ridley, M. The parasitization of macrophages by *M. leprae.* The Star **46** (1987) 1–5.

[53] Salgame, P. R., Mahadevan, P. R. and Antia, N. H. Study of concanavalin A receptors on macrophages in leprosy. IRCS Med. Sci. **11** (1983) 991–992.

[54] Wadee, A. A., Sher, R. and Rabson, A. R. Production of a suppressor factor by human adherent cells treated with mycobacteria. J. Immunol. **125** (1980) 1380–1386.

[55] Mehra, V., Brennan, P. J., Rada, E., Convit, J. and Bloom, B. R. Lymphocyte suppression in leprosy is induced by unique *M. leprae* glycolipid. Nature **308** (1984) 194–196.

[56] Reiner, N. E., Ng, W. and McMaster, W. R. Parasite-accessory cell interactions in murine leishmaniasis. II. *Leishmania donovani* suppresses macrophage expression of class I and class II major histocompatibility complex gene products. J. Immunol. **138** (1987) 1926–1932.

[57] Brett, S. J. and Butler, R. Interactions of *M. lepraemurium* with resident peritoneal macrophages: phagocytosis and stimulation of the oxidative burst. Clin. Exp. Immunol. **71** (1988) 32–38.

[58] Ohkawa, S., Martin, L. N. and Gormus, B. J. Lepromin induced lymphoproliferative response of experimental leprosy monkeys: regulatory role of monocyte and lymphocyte subsets. J. Immunol. **138** (1987) 3943–3948.

[59] Ridley, M. J., Ridley, D. S. and Turk, J. L. Surface markers on lymphocytes and cells of the mononuclear phagocyte series in skin sections in leprosy. J. Pathol. **125** (1978) 91–98.

logical that these effects may have amplification pathways. Macrophages have been implicated as suppressor cells by a number of workers. Rook[60] suggested that this may result if macrophages are overloaded with mycobacterial antigen. Klimpel and Henney[61] stressed the necessity for bacterial viability. In their studies immunosuppression by macrophage-like cells was noted in the spleens of mice infected with live BCG but not with heat-killed BCG.

Preston[62, 63] studied the differing patterns of *M. lepraemurium* infection in inbred strains of mice. *In vitro,* macrophages from both resistant (C57BL/6) and susceptible (BALB/c) strains of mice were shown to be equally effective in controlling multiplication of *M. lepraemurium. In vivo,* the macrophage-mediated immunity was suppressed in the susceptible BALB/c strain by the soluble factor(s) present in the serum and the peritoneal fluid of infected mice. Using a diffusion chamber technique, the same worker demonstrated two diffusible factors in infected mice from both strains, one able to activate and the other able to suppress macrophage antimycobacterial activity. In C57BL/6 mice, the macrophage-activating factor was dominant; in BALB/c, the suppressor factor seemed to play the major role.

Birdi, *et al.*[9] showed the secretion of a suppressor factor by "susceptible" macrophages. *In vitro,* in *M. leprae*-infected cultures macrophages which did not contain intracellular *M. leprae* had decreased ^{3}H-leucine incorporation, similar to cells with intracellular bacilli. These findings were extended[13–15, 64] and resulted in the identification of two macrophage-suppressor factors; one an indomethacin-sensitive, secretory factor, the other an intracellular, indomethacin-resistant factor. This indomethacin-resistant factor suppressed macrophage functions and lymphocyte stimulation, and induced suppressor-T cells. Both factors were produced in response to infection with viable, but not dead *M. leprae.* The secretion of an indomethacin-resistant suppressor factor by lepromatous monocytes has also been reported by Nath and her colleagues.[65, 66] In functional assays it is similar to the factor described earlier by Salgame, *et al.*[13, 64] More recently, the studies of Krahenbuhl and his colleagues[67, 68] have also demonstrated that at least two effector mechanisms (i.e., PGE_2 and an indomethacin-resistant factor) are involved in macrophage suppression. The presence of an indomethacin-sensitive as well as an indomethacin-resistant macrophage-suppressor factor has also been reported in *M. lepraemurium* infection.[69] Suppression by an indomethacin-resistant pathway has also been reported by Bahr, *et al.*[70] Macrophage-suppressor cells were also reported by Mehra,

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[60] Rook, G. A. W. The potentiating, mitogenic and inhibitory effect on lymphocytes *in vitro* of macrophages in the lymph nodes of mice overloaded with mycobacterial products. Clin. Exp. Immunol. **21** (1975) 163–172.

[61] Klimpel, G. and Henney, C. BCG induced suppressor cells. I. Demonstration of a macrophage-like suppressor cell that inhibits cytotoxic T-cell generation *in vitro.* J. Immunol. **120** (1978) 563–569.

[62] Preston, P. Serum from infected mice suppresses macrophage mediated immunity in *M. lepraemurium* infection: a model for impaired macrophage immunity in human leprosy. Trans. R. Soc. Trop. Med. Hyg. **73** (1979) 212–215.

[63] Preston, P. Macrophages and protective immunity in *M. lepraemurium* infections in a resistant (C57BL) and a susceptible (BALB/c) mouse strain. Clin. Exp. Immunol. **47** (1982) 243–252.

[64] Salgame, P. R., Birdi, T. J., Mahadevan, P. R. and Antia, N. H. Role of macrophages in defective cell-mediated immunity in lepromatous leprosy I. Factors from macrophages affecting protein synthesis and lymphocyte transformation. Int. J. Lepr. **48** (1980) 172–177.

[65] Sathish, M., Bhutani, L. K., Sharma, A. K. and Nath, I. Monocyte derived soluble factor(s) in patients with lepromatous leprosy. Infect. Immun. **42** (1983) 890–899.

[66] Nath, I., Jayaraman, T., Sathish, M., Bhutani, L. K. and Sharma, A. K. Inhibition of interleukin-2 production by adherent cell factors from lepromatous leprosy patients. Clin. Exp. Immunol. **58** (1984) 531–538.

[67] Krahenbuhl, J. L., Sibley, L. D. and Chae, G. T. Defective macrophage effector function in lepromatous leprosy. Int. J. Lepr. **55** (1987) 782–783.

[68] Sibley, L. D., Ramasesh, N., Franzblau, S. G. and Krahenbuhl, J. L. Functional responses of normal and activated macrophages infected with *M. leprae in vitro.* Int. J. Lepr. **55** (1987) 783–784.

[69] Akiyama, T., Esashika, I. and Yamamura, N. Suppressor cells in experimental murine leprosy. Int. J. Lepr. **50** (1982) 595.

[70] Bahr, G. M., Rook, G. A. W. and Stanford, J. L. Prostaglandin dependent regulation of the *in vitro* proliferative response to mycobacterial antigens of peripheral blood lymphocytes from normal donors and from patients with tuberculosis and leprosy. Clin. Exp. Immunol. **45** (1981) 646–653.

et al.[71] in an *in vitro* system in which lepromin induced suppression of the mitogenic response of peripheral blood mononuclear cells to ConA from lepromatous and borderline leprosy patients but not from tuberculoid leprosy patients.

Role of lipids in macrophage function. A general review on the role of lipids in the immune response was published by Gurr.[72] Since mycobacteria are rich in lipids and reside intracellularly in macrophages, there is a possibility that these lipids can alter macrophage metabolism. As mentioned earlier, PGL-I may scavenge hydroxyl ions and thus contribute to the survival of *M. leprae.*[33, 34] An association between resistance to H_2O_2 and the lipid content of the bacillus is also seen in *M. tuberculosis.*[73]

Lipids can also be involved in the adherence of the pathogen to the host, and thus modulate the selective entry of the organism into its preferred host cell. An example of this is seen in *Candida* infections.[74] *M. leprae* adherence, at least to Schwann cells, is mediated partially by PGL-I.[75]

Foamy macrophages, characteristic of lepromatous lesions, are an indicator that lipid metabolism may be affected in *M. leprae*-parasitized macrophages. Kurup and Mahadevan[76] demonstrated the accumulation of cholesterol esters in susceptible macrophages on *in vitro* infection with viable *M. leprae.* Similar observations have been reported by Kondo and Kanai[77] following *M. tuberculosis* infection.

A marked increase in phospholipids and triglycerides in lepromatous skin lesions has been shown by Khandke, *et al.*[78] and Kumar, *et al.*[79] Triglycerides and phospholipids have the ability to induce macrophage proliferation.[80] This might provide an explanation for the observations of Mor, *et al.*[81] Using the *M. marinum* infection in mice as a model, they suggested that the increase in the number of macrophages in lepromatous lesions is partly the result of the division of local macrophages rather than an influx of inflammatory cells only.

Another group of host lipids reportedly increased during *M. leprae* infection are the gangliosides, especially GM1 and GM3.[82] A number of immunological functions of gangliosides have been reported. Welte, *et al.*[83] report that the GD3 ganglioside is involved in the proliferation of a subpopulation of lymphocytes. However, the studies of Offner, *et al.*,[84] stating that GM1 induces selective modulation of CD4 from T-helper cells, is of greater interest to this review, especially since recent reports state that CD4 antigen is also present on macrophages.[85] It

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[71] Mehra, V., Mason, L. H., Rothman, W., Reinherz, E., Schlossman, S. F. and Bloom, B. R. Delineation of a human T-cell subset responsible for lepromin induced suppression in leprosy patients. J. Immunol. **125** (1980) 1183–1188.

[72] Gurr, M. I. The role of lipids in the regulation of the immune system. Prog. Lipid Res. **22** (1983) 257–287.

[73] Goren, M. B., Grange, J. M., Aber, V. R., Allen, B. W. and Mitchison, D. A. Role of lipid content and hydrogen peroxide susceptibility in determining the guinea-pig virulence of *M. tuberculosis.* Br. J. Exp. Pathol. **63** (1982) 693–700.

[74] Ghannoum, M. A., Burns, G. R., Elteen, K. A. and Radwan, S. S. Experimental evidence for the role of lipids in adherence of *Candida* spp. to human buccal epithelial cells. Infect. Immun. **54** (1986) 189–193.

[75] Choudhary, *et al.*, unpublished data.

[76] Kurup, I. G. and Mahadevan, P. R. Cholesterol metabolism of macrophages in relation to the presence of *M. leprae.* J. Biosci. **4** (1982) 307–316.

[77] Kondo, E. and Kanai, K. Accumulation of cholesterol esters in macrophages incubated with mycobacteria *in vitro.* Jpn. J. Med. Sci. Biol. **29** (1976) 123–137.

[78] Khandke, L., Penumarti, N. and Mahadevan, P. R. Phosphatidyl-inositol mannosides in lepromatous leprosy nodules. Indian J. Med. Res. **80** (1984) 259–263.

[79] Kumar, B., Jamumdar, S., Dahiya, R., Kaur, S. and Ganguly, N. K. Lipid composition of human leprous tissue. Int. J. Lepr. **55** (1987) 162–164.

[80] Yui, S. and Yamazaki, M. Relationship of ability of phospholipids to stimulate growth and bind to macrophages. J. Leuk. Biol. **41** (1987) 392–399.

[81] Mor, N., Lutsky, I. and Levy, L. Role of popliteal lymph node in infection with *M. marinum* of the hind foot-pad of the mouse and source of the cells that characterize the process. Scand. J. Immunol. **15** (1982) 429–438.

[82] Harris, E. B., Li, Y. T. and Li, S. Ganglioside patterns in normal and lepromatous armadillo tissues. Int. J. Lepr. **54** (1986) 289–293.

[83] Welte, K., Miller, G., Chapman, P. B., Yuasa, H., Natoli, E., Kunicka, J. E., Cordon-Cardo, C., Buhrer, C., Old, L. J. and Houghton, A. N. Stimulation of T-lymphocyte proliferation by monoclonal antibodies against GD3 ganglioside. J. Immunol. **139** (1987) 1763–1771.

[84] Offner, H., Thieme, T. and Vandenbark, A. A. Gangliosides induce selective modulation of CD4 from helper T lymphocytes. J. Immunol. **139** (1987) 3295–3305.

[85] Perry, V. H. and Gordon, S. Modulation of CD4 antigen on macrophages and microglia in rat brain. J. Exp. Med. **166** (1987) 1138–1143.

may be speculated that GM1 could affect both macrophage and lymphocyte membranes, causing a decrease in CD4 antigen expression.

Genetic influences

The concept of a genetic influence on the ability of the macrophage to kill *M. leprae* was first stated by Beiguelman.[86] However, because these observations were not reproduced,[87] this approach was shelved for over a decade. Interest in the genetic control of innate resistance to mycobacterial infections was revived with the observation that a single gene on murine chromosome 1 controls innate resistance to *L. donovani, S. typhimurium,* BCG, and *M. lepraemurium* infections.[88]

While the background genes play a major role in determining resistance to *M. lepraemurium,* genes within the H-2 complex only have a modifying influence.[89] The two aspects of resistance, natural resistance and acquired resistance, have to be considered separately. C3H mice, which are naturally resistant to BCG and *M. lepraemurium,* do not acquire protective immunity when infected with *M. lepraemurium* subcutaneously.[90] Thus, natural resistance and immune resistance do not operate in the same way, are not elicited by the same route of infection, and probably do not have the same effector cells.

On the basis of these experimental mouse models, it is possible that innate resistance to *M. leprae* may also be controlled by non-HLA-linked gene system(s). Susceptibility to leprosy per se does not appear to be HLA linked, but the type of disease developed by susceptible individuals is influenced by their HLA haplotype. Significant associations were found between DR3 and DQw1 haplotypes and tuberculoid and lepromatous leprosy, respectively.[91] In an *in-vitro* study using a monoclonal antibody directed against DQw1, Kikuchi, *et al.*[92] were able to abolish *M. leprae*-mediated immune suppression. However, using a suppressor-T cell line from a borderline lepromatous patient, Ottenhoff and de Vries[91] have been unable to convincingly demonstrate any influence of a DQw1 gene product.

The high *M. leprae* T-cell responsiveness associated with tuberculoid leprosy and, hence, DR3 could be demonstrated *in vitro* in cells from normal healthy individuals. However, cells from tuberculoid patients surprisingly displayed a DR3-associated low responsiveness.[91] The authors postulate that this DR3-associated low responsiveness is a consequence of the initial high response which results in tissue damage and triggers a suppressive signal.

In leprosy, partly because of the difficulties involved in measuring *M. leprae* viability, studies on the innate resistance to *M. leprae* infection have not been carried out. Secondly, the type of leprosy is believed to correlate with the ability of T cells to respond to *M. leprae* and, hence, greater emphasis has been given to the genetics of acquired immunity.

To determine whether macrophage *in vitro* responses to viable *M. leprae* (i.e., down-regulation of Fc receptors; negative antigen-specific macrophage-lymphocyte interaction) were of primary importance for the development of disease or a consequence of infection, familial contacts were studied.[10] A Mendelian form of inheritance of these two macrophage parameters was seen. The result also stressed the independence of these two macrophage parameters from each other and also from factors such as age, sex, age at onset of exposure and, to some extent, the duration of exposure, implying that the macrophage defect could be an innate one.[10] If so, this could answer certain questions

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[86] Beiguelman, B. Lepromin reaction: genetic studies including twin pair analysis. Acta Leprol. (Genève) **44** (1971) 5–65.

[87] Godal, T., Rees, R. J. W. and Lamvik, J. Lymphocyte mediated modification of blood derived macrophage function *in vitro*; inhibition of growth of intracellular mycobacteria with lymphokines. Clin. Exp. Immunol. **8** (1971) 625–663.

[88] Brown, I., Glynn, A. and Plant, J. Inbred mouse strain resistance to *M. lepraemurium* follows the *Ity/Lsh* pattern. Immunology **47** (1982) 149–156.

[89] Curtis, J., Abu, H. and Turk, J. H-2 linkage control of resistance to subcutaneous infection with *M. lepraemurium.* Infect. Immun. **38** (1982) 434–439.

[90] Lagrange, P. H. and Hurtrel, B. Local immune response to *M. lepraemurium* in C3H and C57BL/6 mice. Clin. Exp. Immunol. **38** (1979) 461–474.

[91] Ottenhoff, T. H. M. and de Vries, R. R. P. HLA class II immune response and suppression genes in leprosy. Int. J. Lepr. **55** (1987) 521–534.

[92] Kikuchi, I., Ozawa, T., Hirayama, K. and Sasazuki, T. An HLA-linked gene controls susceptibility to lepromatous leprosy through T cell regulation. Lepr. Rev. **52** Suppl. 2 (1986) 139–142.

raised about the specificity of the defective macrophage response to *M. leprae*. It is conceivable that genes (external to the MHC and hitherto unknown) may operate in individuals susceptible to leprosy to govern some unique interaction between the pathogen and the host macrophage just as the *Lsh/Bcg/Ity* gene governs the interaction of macrophages with leishmania, *M. lepraemurium*, BCG, and salmonella. If they exist, the genes responsible for the defective macrophage response in lepromatous leprosy could regulate responses to other pathogens whose clinical status may not be obviously linked to leprosy.

Antigen presentation and lymphocyte stimulation

Live vs killed organisms. The key question in the understanding of the disease is not whether the host can or cannot respond to antigens of killed *M. leprae* but can the patient cope with viable organisms and, if so, with how many. Most immunological studies for reasons of convenience have used antigens of heat-killed/irradiated bacteria or sonicated bacterial preparations. Some workers however have addressed themselves to this question.

Animal experiments prove that major differences between C3H and the C57BL mice lie in the differing capacities of these strains to respond to live *M. lepraemurium*; whereas their immune responses to dead bacteria are quite similar.[93] Therefore, whether antigens from live *M. lepraemurium* are presented remains uncertain. However, evidence exists that T cells mediating immunity to antigens of live organisms are restricted by Class I antigens on the APC, while structural antigens are restricted by Class II antigens.[94]

Resistance to most intracellular bacteria, such as *M. tuberculosis, M. bovis,* salmonella, brucella and listeria, can only be achieved by immunization with viable organisms. This may also apply to mycobacterial infections, as highlighted by the observation that many human T-cell clones that recognize sonicated mycobacteria fail to recognize live mycobacteria.[95] If one extrapolates from the *M. lepraemurium* story it is possible that the antigens necessary for the induction of a protective immune response are produced by the bacteria during a period of active growth.

Secreted antigens are likely candidates for induction of protective immunity. Leprosy patients have an antibody which binds to a secretory protein of BCG and *M. tuberculosis* which is not detectable in sonicated preparations of *M. leprae*.[96]

A feature that differentiates between live and dead *M. leprae* in concordance with the clinical spectrum of leprosy is the macrophage expression of membrane markers such as Fc receptor and HLA-DR antigens.[10, 11] The identification of this "immunological lesion" may prove useful in determining a central mechanism as to how viable organisms subvert the immune response.

Interleukin-1 (IL-1) production. The lack of responsiveness to *M. leprae* could be due to impaired IL-1 production by monocyte APCs from patients. This was investigated by Watson, *et al.*[97] A significant proportion (40%) of lepromatous patients failed to produce IL-1 in response to lipopolysaccharide (LPS) stimulation. Cells from all of the tuberculoid patients and normal individuals tested did so, even spontaneously. Salgame and Antia[98] also found a lack of IL-1 secretion by macrophages of lepromatous pa-

[93] Lovik, M. and Closs, O. Induction of delayed type hypersensitivity against ultrasonicated *M. lepraemurium* bacilli without simultaneous local reactivity against live bacilli or protective immunity. Clin. Exp. Immunol. **53** (1983) 319–327.

[94] Orme, I. M. and Collins, F. M. Adoptive protection of the *M. tuberculosis* infected lung; dissociation between cells that passively transfer protective immunity and those that transfer DTH to tuberculin. Cell. Immunol. **84** (1984) 113–120.

[95] Rook, G. A. W., Steele, J., Barnass, S., Mace, J. and Stanford, J. L. Responsiveness to live *M. tuberculosis* and common antigens of sonicate stimulated T cell lines from normal donors. Clin. Exp. Immunol. **63** (1986) 105–110.

[96] Wiker, H. G., Harboe, M., Nagai, S., Patarroyo, M. E., Ramirez, C. and Cruz, N. MPB59, a widely cross reacting protein of *Mycobacterium bovis* BCG. Int. Arch. Allergy Appl. Immunol. **81** (1986) 307–314.

[97] Watson, S., Bullock, W., Nelson, K., Schauf, R., Gelber, R. and Jacobson, R. Interleukin 1 production by peripheral blood mononuclear cells from leprosy patients. Infect. Immun. **45** (1984) 787–789.

[98] Salgame, P. R. and Antia, N. H. *Mechanism of immunosuppression in lepromatous leprosy: role of macrophage suppressor factors.* Ph.D. thesis, University of Bombay, 1984.

tients in the presence of *M. leprae* as an IL-1 inducer.

The inability to produce IL-1 also appears to be a common feature of other intracellular parasites, such as leishmania.[99] It has been suggested that a tolerogenic signal may result from T-cell recognition of a nondegraded antigen in the absence of an IL-1 signal.[100]

HLA-DR expression. Alterations in the levels of Ia expression directly affect the ability of APCs to interact with antigen-reactive T cells. Therefore, the down-regulation of HLA-DR antigens of lepromatous macrophages on *in vitro* infection with viable *M. leprae* could thwart the induction of antimycobacterial immunity.[11] Similar observations have been reported by Poulter, *et al.*[101] in Langerhans' cells. *M. leprae* infection may not be unique in this respect since it has been shown that other pathogens, such as *M. tuberculosis*[102] and *L. donovani*,[56] have similar effects.

While decreased HLA-DR expression could account in part for defective immunity, it does not explain the negative lymphoproliferative response which has been extensively documented nor the lack of antigen-specific macrophage-lymphocyte interaction.[11]

Antigen presentation. Substantial evidence has accumulated to support the "determinant selection hypothesis" of antigen presentation. This hypothesis states that the antigenic determinants are selected by Ia molecules which interact specifically with unique sequences of the antigen and bring about their presentation.[103] In macrophages from susceptible individuals, the Ia molecule may not be able to combine with the relevant antigenic determinant necessary for protection.

Alternatively, the two antigens to which immune responsiveness is regulated by the same immune response genes may compete for binding to the Ia molecule and, hence, for presentation by the macrophage. In tuberculoid leprosy, it may be postulated that the APC may select from a cocktail of *M. leprae* antigen determinants that induce delayed-type hypersensitivitiy (DTH) rather than protection.

Various groups have demonstrated the presence of *M. leprae*-reactive lymphocytes in the circulation of lepromatous patients,[104–107] thus arguing against a total defect in the afferent limb of the immune system.

Resting lymphocytes from lepromatous patients in simple medium before pulsing with antigen reportedly restores their lymphoproliferative ability,[104] but the system used is open to various interpretations. For example, in the resting phase T-4 cells may become refractory to the action of suppressive monocytes, an event that is not likely to occur *in vivo*. It may be that once cells are initiated into an activation pathway they may escape the purview of suppressive APCs in the same way that activation mechanisms are ineffective after suppression has been

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[99] Reiner, N.E. Parsite accessory cell interactions in murine leishmaniasis. I. Evasion and stimulus-dependent suppression of the macrophage interleukin-1 response by *Leishmania donovani*. J. Immunol. **138** (1987) 1919–1925.

[100] Levich, J. D., Signorella, A. P., Wittenberg, G. and Weigle, W. O. Macrophage handling of a tolerogen and the role of IL-1 in tolerance induction in a helper T cell clone *in vitro*. J. Immunol. **138** (1987) 3675–3679.

[101] Poulter, L. W., Collings, L. A., Tung, K. S. and Waters, M. F. R. Parasitism of antigen presenting cells in hyperbacillary leprosy. Clin. Exp. Immunol. **55** (1984) 611–617.

[102] Tweardy, D. J., Schacter, B. Z. and Ellner, J. J. Association of altered dynamics of monocyte surface expression of HLA-DR with immunosuppression in tuberculosis. J. Infect. Dis. **149** (1984) 31–37.

[103] Werdelin, O. A hypothesis for the activity of immune response genes in the processing and presentation of antigens by macrophages. Scand. J. Immunol. **24** (1986) 625–636.

[104] Mohagheghpour, N., Gelber, R. R. and Engleman, E. G. T-cell defect in lepromatous leprosy is reversible *in vitro* in the absence of exogenous growth factors. J. Immunol. **138** (1987) 570–574.

[105] Haregewoin, A., Godal, T., Mustafa, A. S., Belehu, A. and Yemaneberhan, T. T cell conditioned media reverse T cell unresponsiveness in lepromatous leprosy. Nature **303** (1983) 342–344.

[106] Kaplan, G., Weinstein, D. E., Steinman, R. M., Levis, W. R., Elvers, U., Patarroyo, M. E. and Cohn, Z. A. An analysis of *in vitro* T cell responsiveness in lepromatous leprosy. J. Exp. Med. **162** (1985) 917–929.

[107] Ottenhoff, T. M. H., Elferink, D. G. and de Vries, R. R. P. Unresponsiveness to *M. leprae* in lepromatous leprosy *in vitro*: reversible or not? Int. J. Lepr. **52** (1984) 419–422.

initiated.[108] The results obtained on resting normal *M. leprae* responders, as in the above study,[104] stress our contention. Moreover, in the same study the suppressive function of the monocyte could have been impaired due to the irradiation step incorporated into the protocol.

Another piece of evidence in support of a lymphocyte defect is that lepromatous patients have peripheral blood mononuclear cells which respond to *M. leprae in vitro* in the presence of IL-2. However, this is seen only in a fraction of the patients.[107] This may be due to an expansion of a few clones crossreactive with environmental mycobacteria.

An alternative hypothesis is that the primary defect in the immune response in lepromatous leprosy is that lymphocytes cannot be stimulated and do not secrete lymphokines. Its support comes from the experiments in which γ-IFN was injected into treated lepromatous patients and a marginal reduction in the bacterial index was noted.[50] Nevertheless, since the patients had already undergone chemotherapy, most of the bacilli would already have been rendered nonviable. Thus, γ-IFN may have assisted in the clearance of dead bacilli rather than the killing of viable ones.

In light of the above arguments, two points need emphasis: a) the lack of lymphokine production could be directly linked to aberrant macrophage function, and b) lymphoproliferative responses are not a true reflection of restored immunity. On the basis of these experiments,[50, 107] the conclusion that the lepromatous macrophage is normal in its function of antigen presentation needs reappraisal.

Interplay between macrophages and other APCs

Differences in antigens as seen by T cells may not be due to differences in the antigens per se but due to differences in the handling of the bacterium by the individual cell types in various tissues. Lovik, *et al.*[109] observed that the lymph node and spleen of *M. lepraemurium*-infected mice showed greater differences in bacillary numbers between C3H and C57BL/6 mice than those differences found in the foot pad.

In leprosy, the lymph node enables the study of an interplay and/or interdependence of various APCs, such as macrophages, dendritic cells, and B cells. The studies of Barros, *et al.*,[110] using antisera against BCG, have demonstrated that mycobacterial antigens are present in the foamy macrophages and B cells within lymph nodes of bacteriologically positive lepromatous patients and in small cell clusters suggestive of dendritic cells in tuberculoid and treated, bacteriologically negative, lepromatous patients. Various studies have reported the ability of dendritic cells and B cells to function as effective accessory cells for antimycobacterial responses.[111, 112]

Desai, *et al.*[113] showed an *M. leprae*-specific lymphoproliferative response of mononuclear cells isolated from the lymph nodes of lepromatous leprosy patients, and suggested that lepromatous patients are able to respond to viable *M. leprae* despite an apparent anergy in their peripheral blood. Since in this study the entire mononuclear cell population was used in the lymphoproliferative assay, one would expect the *M. leprae*-antigen-laden macrophage to suppress the proliferative response. Instead, a significant stimulation was observed. Two explanations can be offered for this observation: a) APCs other than macrophages are not sensitive to the macrophage suppressor factors, and b) the tissue macrophage present in the

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[108] Gromo, G., Inverardi, L., Geller, R. L., Alter, B. J. and Bach, F. H. The stepwise activation of cytotoxic T lymphocytes. Immunol. Today **8** (1987) 259–261.

[109] Lovik, M., Collins, F. M. and Closs, O. Inbred C3H mouse substrain differences demonstrated in experimental murine leprosy. Immunogenetics **16** (1982) 607–611.

[110] Barros, U., Ladiwalla, U., Birdi, T. J. and Antia, N. H. Localization and retention of mycobacterial antigens in lymph nodes of leprosy patients. Br. J. Exp. Pathol. **68** (1987) 733–742.

[111] Nath, I. Reversal of T cell unresponsiveness in lepromatous leprosy. Lepr. Rev. **57** Suppl. 2 (1986) 207–212.

[112] Elferink, B. G., Ottenhoff, T. H. M. and de Vries, R. R. P. Epstein-Barr virus-transformed B cell lines present *M. leprae* antigens to T cells. Scand. J. Immunol. **22** (1985) 585–589.

[113] Desai, S., Birdi, T. J. and Antia, N. H. Presence of *M. leprae* specific lymphocytes in lymph nodes of lepromatous leprosy patients. Scand. J. Immunol. **28** (1988) 211–216.

lymph node compartment may be exhibiting a different APC function as compared to circulating monocytes and may not be suppressive in function. The lymph node then may be a tissue where suppressor-cell influences may be minimized.

Role of macrophage and other APCs in granuloma formation

Intact *M. leprae* or its antigens have been detected in Langerhans' cells, endothelial cells, dendritic cells, plasmacytoid-like cells and, most extensively of all, in Schwann cells. These cells are nonprofessional phagocytes, and the interaction of *M. leprae* with these cells may differ from its interaction with the macrophage. Moreover, parasitization of some of these cell types by *M. leprae,* i.e., endothelial cells and Schwann cells, may result in the antigens traveling directly to the spleen and bypassing the regional lymph node without stimulating cell-mediated immunity.[114]

Skin. In lepromatous leprosy the granuloma is characterized by: a) the absence of neutrophils (PMN), which may be the result of a serum inhibitory factor-induced decrease in their chemotactic ability,[115] and b) the presence of large numbers of histiocytes which have not been activated into epithelioid cells. The absence of epithelioid cells in a lepromatous granuloma does not necessarily mean a complete lack of macrophage activation. The studies by Flad, *et al.*[116] have demonstrated an increase in the percentage of macrophages stained with the monoclonal antibody, Mac 675 (which recognizes an 80-kDa protein on activated macrophages), in lepromatous skin lesions compared with tuberculoid or borderline lepromatous lesions. This type of activation in lepromatous lesions does not seem to serve any protective function and could, in fact, have a deleterious effect.

This alternative sort of stimulation is associated with a high-turnover as opposed to a low-turnover granuloma.[117] The regulation of this turnover rate lies in the nature of the inducing agent. In the high-turnover granulomas, on the basis of histology, Ridley[52] suggests that bacterial multiplication stimulates an influx of macrophages. Alternatively, Mor, *et al.*,[81] using *M. marinum* infection in mice as the model, suggest that the origin of the macrophages within the lesion are the tissue macrophages themselves which have been stimulated into proliferating. This is supported by the studies of Schuller-Levis, *et al.*[118] who demonstrated defective monocyte chemotaxis in lepromatous leprosy patients.

In skin lesions, besides macrophages, Langerhans' cells and interdigitating cells also function as APCs. Mathur, *et al.*[119] have reported a decrease in the numbers of Langerhans' cells in skin lesions of lepromatous patients. Poulter, *et al.*[101] showed that these cells in lepromatous lesions contained intracellular bacilli. This suggests that *M. leprae* can actively enter a cell (discussed in greater detail earlier) and may affect its functional capacity as an APC. In support of this hypothesis, the expression of HLA-DR antigens by Langerhans' cells is reduced in lepromatous infiltrates associated with large numbers of bacilli.[120]

In tuberculoid skin granulomas, the number of Langerhans' cells are increased in the epidermis and the dermis, and they are also strongly HLA-DR positive.[120] Besides Langerhans' cells, the keratinocytes in tuberculoid lesions are also activated, as characterized by the strong HLA-DR and IP-10

[114] Stoner, G. L. Importance of the neural predilection of *M. leprae* in leprosy. Lancet **2** (1979) 994–996.

[115] Wahba, A., Cohen, H. and Sheskin J. Neutrophil chemotactic responses in lepromatous leprosy: an *in vitro* study of 52 patients. Clin. Immunol. Immunopathol. **17** (1980) 556–561.

[116] Flad, H. D., Peters, B., Arndt, R., von Ballestrem, W. G. and Alvarenga, A. E. Cutaneous infiltrates in leprosy: relationship between bacterial content and phenotypes of mononuclear cells. In: Proceedings of the IV European Leprosy Symposium on Leprosy Research. Quad. Coop. Sanit. **7** (1988) 117–124.

[117] Ridley, D. S. Macrophage stimulation and activity in lepromatous leprosy. Lepr. Rev. **51** (1980) 111–116.

[118] Schuller-Levis, G., Harris, D., Cutler, E., Meeker, H. C., Haubenstock, H. and Levis, W. R. Defective monocyte chemotaxis in active lepromatous leprosy. Int. J. Lepr. **55** (1987) 267–272.

[119] Mathur, N. K., Mangal, H. N., Mathur, D., Bedwal, R. S. and Mathur, R. S. Langerhans' cells and leprosy. Lepr. India **55** (1983) 22–28.

[120] Collings, L. A., Waters, M. F. R. and Poulter, L. W. The involvement of dendritic cells in the cutaneous lesions associated with tuberculoid and lepromatous leprosy. Clin. Exp. Immunol. **62** (1985) 458–467.

positivity as a result of activating factors (γ-IFN) produced within the granuloma.[121] This increase in Class II antigens may result in augmentation of the immune response. The prolonged activation of keratinocytes by γ-IFN may also result in the inhibition of cell multiplication required in wound healing should a trauma occur.[122]

Peripheral nerves. There is considerable evidence that *M. leprae* have a predilection for Schwann cells.[123] There are a number of indications that the normal metabolic functions of the Schwann cell are altered after parasitization by *M. leprae. In vivo,* this is evident from the multiple axonal myelineation[124] and in the decreased Schwann-cell proliferation in response to crushed nerve injury[125] seen in the nerves of both leprosy patients and mice inoculated with *M. leprae.* The ability of *M. leprae* to hamper Schwann-cell proliferation was also observed *in vitro.*[126, 127]

Both B-cell[44, 128] and T-cell responses (unpublished observations) in inflamed tuberculoid nerves are directed to *M. leprae* antigens. While it may be construed that many of the responses are due to the presence of classical APCs in a nerve, it is also possible that under certain conditions Schwann cells could serve as APCs.

Schwann cells can be induced by γ-IFN to express Class II antigens. Nevertheless, while *M. leprae* infection by itself does not induce Class II expression in Schwann cells it allows the cell to respond to γ-IFN.[129, 130] In nerve lesions of tuberculoid patients, an influx of stimulated inflammatory cells could result in the production of γ-IFN in a localized focus. Since *M. leprae* antigens are already present within the Schwann cells,[131] response to the γ-IFN signal could induce Class II expression and antigen presentation and thereby augment the T-cell response further, resulting in the increased DTH responses leading to nerve damage which is characteristic in this part of the spectrum of the disease.

In the absence of γ-IFN, Schwann cells express Class I antigens constitutively which are not modulated by *M. leprae* infection.[127] Infected Schwann cells also express *M. leprae* antigens on their surface, and their expression is not increased with treatment with γ-IFN (unpublished observations). From the studies of Orme and Collins[94] and Jungi, *et al.,*[132] it appears that T cells mediating DTH to dead structural antigens in mycobacterial and listerial infections are restricted by Class II antigens; whereas T cells mediating immunity to metabolic antigens of live organisms are restricted by Class I antigens. This corroborates the recent finding that Class I-restricted cells are protective in nature.[40] If these results can be extended to the nerves and it can be demonstrated

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[121] Kaplan, G., Luster, A. D., Hancock, G. and Cohn, Z. A. The expression of a γ-interferon induced protein (IP-10) in delayed immune responses in human skin. J. Exp. Med. **166** (1987) 1098–1108.

[122] Morhenn, V. B. Keratinocyte proliferation in wound healing and skin disease. Immunol. Today **9** (1988) 104–107.

[123] Antia, N. H. Leprosy: a disease of the Schwann cell. Lepr. India **54** (1982) 599–604.

[124] Shetty, V. P. and Antia, N. H. Multiple axonal myelination in the experimental mouse leprosy model. Int. J. Lepr. **52** (1984) 249–251.

[125] Verghese, G. and Shetty, V. P. *In vivo behaviour of Schwann cells in leprous neuritis.* M.Sc. thesis, University of Bombay, 1984.

[126] Mukherjee, R., Mahadevan, P. R. and Antia, N. H. Organized nerve culture. II. DNA synthesis in Schwann cells in the presence of *M. leprae.* Int. J. Lepr. **48** (1980) 189–192.

[127] Samuel, N. M., Mirsky, R., Grange, J. M. and Jessen, K. R. Expression of MHC class I and class II antigens in human Schwann cell cultures and effects of infection with *M. leprae.* Clin. Exp. Immunol. **68** (1987) 500–509.

[128] Mshana, R. N., Harboe, M., Stoner, G. L., Hughes, R. A. C., Kadlubowski, M. and Belehu, A. Immune responses to bovine neural antigens in leprosy patients. II. Absence of antibodies to an isolated myelin protein. Int. J. Lepr. **57** (1983) 33–40.

[129] Samuel, N. M., Jessen, K. R., Grange, J. M. and Mirsky, R. Gamma interferon, but not *Mycobacterium leprae,* induces major histocompatibility complex class II antigens on cultured rat Schwann cells. J. Neurocytol. **16** (1987) 281–287.

[130] Mshana, R. N., Krahenbuhl, J. L. and Hastings, R. C. Interferon-gamma induces the expression of major histocompatibility complex antigens by *in vitro* cultured murine Schwann cells. Int. J. Lepr. **55** (1987) 781.

[131] Barros, U., Shetty, V. P. and Antia, N. H. Demonstration of *M. leprae* antigens in nerves of tuberculoid leprosy patients. Acta Neuropathol. **73** (1987) 387–392.

[132] Jungi, T. W., Gill, T. J., Kunz, H. W. and Jungi, R. Cellular immunity to *L. monocytogenes* in the rat: different restriction elements are involved in T cell triggering *in vivo* by infective organisms or bacterial antigens. Transplant. Proc. **15** (1983) 1606–1610.

that the antigens are indeed presented by Schwann cells in the context of Class I, a better understanding of the mechanisms operating within the nerve may be obtained.

In the nerves, besides Schwann cells and macrophages, antigen is also present in the infiltrating plasma cells.[131] The relevance of this in pathogenesis is, at present, unknown but could implicate humoral immunity in nerve damage.

Concluding remarks

The inherent difficulties in dissecting the macrophage defect in leprosy stems, in part, from those experienced in basic immunological studies. Macrophage activation and differentiation markers have only recently been classified.[133] The functional significance of these markers, however, is yet to emerge. It is probable that, as in lymphocytes, there exist discrete subpopulations of macrophages differing in functional capacities. Considering macrophages as a homogenous population is likely to yield complacent generalizations which may be inaccurate. In addition, genetic systems that may govern the innate functioning of the cell are only now being detected.[134]

It is also a matter of controversy, particularly in a disease such as leprosy which shows no features of overall immunodepression, whether the macrophage can exhibit specificity in its ability to handle *M. leprae*. While one group advocates the generation of immunosuppression through a group of specific antigens,[55, 135] others propound that the inability encompasses a whole range of crossreactive mycobacterial antigens.[136] There is room, however, for a third point of view: that the specificity may not be at the level of bacterial structural antigens but may be a feature of a distinct metabolic property of the bacilli brought into relief in its interaction with a susceptible target cell. Alternatively, specificity may be bestowed on the macrophage through its interaction with the humoral immune system. This, in our opinion, is a favorable avenue for further investigation.

It is critical at this juncture to design experiments that would be indicative of a protective immune response operating through the macrophage. At present, intracellular killing of bacilli appears to be the most widely studied parameter. An alternative parameter that needs examination is intracellular processing of viable *M. leprae*. This should be studied both a) in normal, nonsusceptible individuals who are able to overcome infection without an overt immune response and b) in lepromatous patients with the maintenance of long-term suppression who may face a constant risk of either reinfection by viable *M. leprae* or reactivation of the disease.

Information on the role of the macrophage is likely to be used in its manipulation for protecting populations at risk. Macrophages can be activated through lymphokines, neurohumoral peptides, or bacteria such as BCG and *Corynebacterium parvum*. What is not known, however, is the sustenance of such activation over a substantial period of time. The results obtained through such experiments will have considerable bearing on current attempts in immunoprophylaxis.

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[133] Hogg, N. Human mononuclar phagocyte molecules and the use of monoclonal antibodies in their detection. Clin. Exp. Immunol. **69** (1987) 687–694.

[134] Rose, M. Linkage disequilibrium in the monocyte alloantigen system. Exp. Clin. Immunogenet. **4** (1987) 227–230.

[135] Kaplan, G., Gandhi, R. R., Weinstein, D. E., Levis, W. R., Patarroyo, M. E., Brennan, P. J. and Cohn, Z. A. *Mycobacterium leprae* antigen-induced suppression of T cell proliferation *in vitro*. J. Immunol. **138** (1987) 3028–2034.

[136] Rook, G. A. W., Barnass, S., Torres, P., Gozalbes, J., Terencio de las Aguas, J. and Stanford, J. Mycobacterium-responsive T cell clones from leprosy patients reveal defects not explicable by defective Il-2 release or *M. leprae*-specific suppressor cells. In: Proceedings of the IV European Leprosy Symposium on Leprosy Research. Quad. Coop. Sanit. **7** (1988) 113–116.