INTERNATIONAL JOURNAL OF LEPROSY

Volume 69, Number 2 (Suppl.)
Printed in the U.S.A.
(ISSN 0148-916X)

IMMUNOPATHOLOGY OF LEPROSY; A STATE OF THE ART

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Leprosy, caused by *Mycobacterium leprae,* is a chronic infectious disease inflicting human beings since the ancient times. The disease is manifested in different forms in a spectrum with two polar forms, tuberculoid (TT) and lepromatous (LL) leprosy. In this disease the clinical and pathological manifestations are determined by the host immunity to the invasion of *M. leprae.*

Entry of the organism. Although the exact route of entry of *M. leprae* is not known, from the early appearance of skin lesions it is opined that *M. leprae* infection spreads through skin-to-skin contact. However, experiments in experimental mice have indicated that *M. leprae* are able to enter through the respiratory route ([37]), the abraded skin ([2, 12]) and the mucous membrane ([8]). However, these experimental models fail to simulate the immunopathology of early events of leprosy. Therefore, the present state of the art on the immunopathology would be confined to the findings of established lesions of human leprosy.

Characteristics of T lymphocytes. Extensive studies of T-lymphocyte populations have been performed by researchers using monoclonal antibodies for immunophenotyping of cells in the lesions. van Voohris, *et al.* ([45]) noted the presence of high proportions of T-helper cells ($CD8^+$ T cells; Th cells) as compared to T-suppressor cells (CD8+ T cells; Th cells) in the lesions of leprosy. They noted a CD4/CD8 ratio of 5.6:1 and 1:1.8 in tuberculoid and lepromatous lesions, respectively. On the contrary, Modlin, *et al.* ([18]) and Narayanan, *et al.* ([33]) could not account for the presence of such large numbers of T cells in granulomas of lepromatous leprosy ([22, 25]). Most of the studies carried out on this line ([10, 17, 18, 19, 31, 33, 34]) have shown that in tuberculoid leprosy CD4+ cells were in a majority in these granulomas with an average ratio of 1.9:1; whereas in lepromatous granulomas CD8+ population outnumbered with CD4/CD8 ratio of 0.6:1 ([10, 11, 17–19, 22, 25, 31, 33, 34]). It has been further pointed out that the CD4/CD8 ratios in the lesions are independent of those found in the blood of patients, indicating a selective migration, proliferation and homing of these cells in the granuloma ([24]).

It has been recently pointed out by Modlin, *et al.* ([27]) that although the CD4/CD8 ratio of 2:1 in blood and lesion is not altered in tuberculoid leprosy, when studies were conducted on the memory and naive T-cell ratios it was noted that the T-memory (CD4+ CD29+) and T-naïve (CD4+CD45+) ratio is 1:1 in blood and 14:1 in lesions. On the contrary, in lepromatous lesions half of the CD4+ cells were noted to belong to the naïve subset. In addition, it was noted that CD8+ CD28+ (T cytotoxic) cells were more prevalent in tuberculoid lesions than in lepromatous lesions ([27]).

***In-situ* environment in epidermis and granuloma.** The pattern of distribution and localization of various immune cells in the granuloma and the epidermal layer of skin help in the understanding of the immunological microenvironment. In tuberculoid leprosy the CD4+ T cells remain scattered inside the granuloma along with macrophages, while CD8+ T cells remain in the mantle of the granuloma in the form of a ring ([11, 17, 31, 48]). It has been established that the above pattern of cell arrangement is an indicator for an *in-situ* environment of an immune granuloma. The presence of CD4+ cells (memory cells) and macrophages in the granuloma points toward an active immune interaction with activation and maturation of immune cells, leading ultimately to destruction of *M. leprae.* On the contrary finding of CD8+ cells, macrophages and CD4+ cells inside the granuloma indicate

generation of a suppressive function by CD8+ cells in lepromatus leprosy. In addition to the above, the finding of more numbers of CD1+ Langerhan's cells in the epidermis and in the periphery of tuberculoid granulomas than in lepromatous lesions suggests an environment of effective immunity in tuberculoid leprosy ([3, 9, 11, 20, 21, 32]).

Functional status of T cells. Functional studies on the frequency analysis on the percent prevalence of an *M. leprae* reactive T-cell population indicated that antigen-reactive T-cell content in a tuberculoid granuloma is about 100 times more than that in the blood. Moreover, these antigen-reactive T cells (majority of CD4+ cells) could be expanded very easily by interleukin-2 (IL-2); whereas such cells isolated from lepromatous lesions remained unresponsive to *M. leprae,* indicating that CD4+ cells obtained from tuberculoid lesions were in a state of activation ([27]).

It is well established that the suppression of cell-mediated immunity (CMI) in leprosy is specific to *M. leprae.* The explanation for such an anergy was provided from the induction of generation of Ts cells in lepromatous leprosy. Further, the above hypothesis received support from the finding of the induction of suppression of Th cell function by some identified defined epitopes of *M. leprae* ([13–15, 34]). Further experiments with CD8+ cell clones, lines, generated from cells obtained from lepromatous lesions, suppressing the mitogen- and antigen-induced proliferation of peripheral blood and CD4+ clones in the presence of *M. leprae* only supported the above finding ([25]). Later, the suppressor activity of CD8+ cells was found to be restricted by antigens presented by MHC class II (HLA DQ determinants) ([24, 39]). It was also noted that the receptors of these CD8+ cells were mostly of $\alpha\beta$ heterodimer ([26]). It was further shown that β-chain receptors were mainly involved in inducing the suppression ([39]). Majority of the CD8+ cells of lepromatous lesions also expressed β chain ([26]). Later Salgame, *et al.* ([39]) observed that these CD8+ cells secrete more IL-4 which might be responsible for downregulating macrophage activation by gamma interferon (IFN-γ).

Th1/Th2 functions of lesional cells. Spectral manifestations in human leprosy have provided the immunopathologists great scope in investigating the cytokine secretion profile to unravel a variety of immunological responses to the intracellular parasite, *M. leprae.* It has been clearly demonstrated that the majority of *M. leprae*-responsive T cells generated from the peripheral blood or skin lesions of polar tuberculoid (TT) patients produce IFN-γ, IL-2 and tumor necrosis factor-alpha (TNF-α) but little or no IL-4, IL-5 and IL-6 on *in vitro* stimulation with *M. leprae* ([6, 30, 38]). Further, using the immunoperoxidase technique, IL-2, IFN-γ and TNF-α positive cells have been shown to be present in higher numbers in tuberculoid lesions than in lepromatous lesions ([1, 24, 47]). Using *in-situ* hybridization and mRNA-based polymerase chain reaction (PCR), further proof for the presence of more numbers of IFN-γ, IL-2, TNF-α, LT and GM-CSF-producing T cells in tuberculoid leprosy has been noted. Contrary to the above, these interleukins were found to be at lower levels in lepromatous lesions. However, higher levels of mRNA for IL-4, IL-5, and IL-10 were present in lepromatous lesions than in tuberculoid lesions ([4, 49, 50]). These differential responses of cytokine profiles in leprosy are almost similar to that described in the Th1/Th2 dichotomy which has been described in the mouse ([29]). The Th1-type of response is marked by more IFN-γ and IL-2 production by T cells which activate macrophages and help in killing intracellular pathogens. Conversely, a Th2 response is noted by the production of IL-4, IL-5 and IL-10 which help in antibody production and consequently dampen the CMI induced by the Th1 response. This adequately explains the situation in tuberculoid leprosy in which IFN-γ and IL-2 production induces CMI. On the other hand, in lepromatous leprosy the Th2 response is expressed by production of high levels of antibody, leading to unresponsiveness to *M. leprae.* Further functional analysis of T-cell clones from leprosy lesions revealed that CD4+ clones from tuberculoid lesions liberated mainly IFN-γ and CD8+ clones from lepromatous lesions produced IL-4 ([38]). It was subsequently noted that this preferential dichotomy of the Th1- and Th2-type responses according to the types of leprosy at the level of T-cell clones was not absolute. It was noted that some CD8+ clones produced high levels of IFN-γ in addition to

IL-4. It was also observed that IL-4 and IFN-γ coproducing clones shifted to a Th2-like pattern; whereas non-IL-4-producing clones secreted very high levels of IFN-γ on prolonged culture ([30]).

In addition, when T-cell clones obtained from borderline lepromatous patients were analyzed, it was visualized that the Th1-type of cells produced IFN-γ and TNF-α with minimal IL-6 production; whereas Th2-type T cells produced IL-4, IL-5, IL-13, IL-10 and Th0 cells produced both the types of cytokines ([46]). Simultaneous measurement of memory T cell 1 (MT1, $CD45RA^-$, $CD62L^-$, $CD11a^{bright}$; IFN-γ biased) and memory T cell 2 (TMT2, $CD45RA^-$, CD621+, $CD11a^{dim}$; biased) of the peripheral blood of leprosy patients showed that the ratios of MT1/MT2 differed significantly in patients with tuberculoid and lepromatous leprosy. The above observation strongly indicates that the cells become polarized for one type or the other in leprosy ([16]).

Status of γδ and γβ T cells. It has been established from experiments in mice that the initial immune response induced by several pathogens is evoked by γδ T cells followed by induction and expansion of these cells during immune interaction ([7]). It was also observed that mycobacterial exposure induces γδ T cell expansion of neonatal thymocytes. The finding of γδ T cells in the skin lesions of leprosy ([28]), leishmaniasis ([28]) and tuberculosis ([5, 35]) points toward their role in human disease. Modlin, *et al.* ([28]) noted that 25% to 35% of CD3+ cells of both lepromin skin reaction and reversal reaction are γδ cells which might play an important role in the delayed-type hypersensitivity (DTH) reaction. The finding of proliferation of γδ T-cell lines in expanded culture generated from a skin lesion and peripheral blood in response to *M. leprae* suggests their role along with cytokines in granuloma formation. Uyemura, *et al.* ([44]) further analyzed these γδ T cells using monoclonal antibodies against γδ polypeptide chains, and noted that in skin lesions, TCR of γδ cells in majority express Vδ1 receptor, indicating homing and expansion of Vδ1+ cells in mycobacteria-infested skin lesions. It is known that αβ T cells have many variable regions encoding αβ T-cell receptors. An analysis of leprosy lesions using PCR techniques noted an overexpression of γβ genes in lesions when compared with the peripheral T cells of the same patient. This may indicate that the TCR β population of T cells is important in inducing DTH skin reaction evoked by *M. leprae.*

Status of reaction states. Reactional states are acute exacerbations occurring in the spectral manifestations with reactivation of old lesions and the appearance of new lesions. Reversal reaction (type 1 reaction) is due to a heightened expression of CMI to *M. leprae* antigens, and erythema nodosum leprosum (ENL) (type 2 reaction) is due to the precipitation of immune complexes in tissues along with a transient overexpression of CMI. In both types of reactions heightened T-cell response along with migration of T-memory cells have been noted ([4, 18, 22, 24, 33]). Unlike in the ENL, phenotypic analysis showed that lesions in reversal reactions are predominantly infiltrated by CD8+ T cells, and the majority of these T cells expressed γδ receptors ([28]).

Th1/Th2 phenotypes in lesions. Using mRNA-based *in-situ* hybridization, Cooper, *et al.* ([4]) noted that a higher percentage of cells expressed IFN-γ in reversal reactions than in ENL reactions. They also noted higher signals for human serine esterase (a cytotoxic T-cell marker) in the lesion of reversal reactions than in ENL reactions. While studying the patients with reversal reactions, Verhagen, *et al.* ([46]) noted that regardless of the clinical status of these patients the major subset was TH0 cells which secrete both Th1 and Th2 types of cytokines. These TH0 cells, which produce both IFN-γ and IL-4, shifted to the production of the Th1-type of cytokines only during reactional episodes, which leads to upregulation of CMI resulting in tissue damage. Recently, using reverse transcription PCR (RT-PCR) in peripheral blood mononuclear cells and skin lesions, a higher percentage of patients with reversal reaction showed a Th1 response as compared to ENL patients. Many of the reactional subjects exhibited IFN-γ and IL-4, indicating a preponderance of TH0 cells. The status of the regulatory cytokine IL-12 was observed at a higher percentage in patients with reversal reactions compared to with ENL ([43]).

Conclusion. The above studies helped in understanding many aspects of the host-

parasite relationship in leprosy. However, the mechanism of early events of cellular interactions remain largely unknown. Recently our group ([42]), using organotypic culture of skin lesions, has demonstrated a local antibody response in tuberculoid leprosy in which an environment of Th1 immunity is already being expressed. This finding clearly demonstrated that there is still a great need for research to decipher the cellular interactions and the mechanism of cross talk of effective immune cells in leprosy, especially in a situation of early leprosy.

Acknowledgments. Acknowledgment is due to the help extended by LEPRA, U.K. for carrying out some of the studies which have been mentioned in the text. The secretarial assistance of Shri Anil Chopra is highly appreciated.

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