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EDITORIAL

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Cell-Mediated Immunity in Leprosy; An Update

A large body of evidence suggests that in lepromatous leprosy (LL) there is a selective unresponsiveness (anergy) of the T-cell response to *Mycobacterium leprae* antigens and, therefore, the host is unable to mount an adequate cell-mediated immunity (CMI) which could protect the host from the infection. In this form of the disease macrophages, primarily in the peripheral nerves, skin and mucous membranes, get heavily infiltrated with the bacilli.

The disease covers wide intermediary forms of clinical manifestations with two polar types, tuberculoid and lepromatous. An accepted classification based on clinical, histological and immunological parameters which is universally followed has been described elsewhere.[1] The present review will deal mainly with the various types of host cells and their products (effector molecules) which govern CMI and will try to point out the basic immunological defects that may be seen in leprosy.

Cellular Interactions in CMI

The phagocytic cells (macrophages, Langerhans' cells, dendritic cells) are known to engulf and process the invading bacilli and their soluble products. Also designated as antigen-presenting cells (APC), they are capable of presentation of the processed antigens to the T cells through their receptors.[2] It has been well documented that at the initial stages of the CMI response T cells cluster around the surface of the APCs before transforming into blast cells. Thereafter, production of interleukin-2 (IL-2) along with the expression of IL-2 receptors are necessary for the replication of these antigen-specific lymphocytes for clonal expansion.[3] While such clonal expansion goes on, the cellular interaction further liberates a variety of other interleukins (IL-1, IL-3, IL-4 to IL-8) and lymphokines [granulocyte monocyte colony stimulating factors, interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α)] which influence the morphological and functional behavior of various CMI-inducing cells. In general, all of these cells—consisting of activated mononuclear phagocytes, cytotoxic T cells, natural killer (NK) cells and lymphokine ac-

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tivated killer (LAK) cells—create the environment of CMI. Effector molecules such as IFN-γ and TNF-α are known to activate macrophages so that they have an enhanced production of toxic reactive oxygen intermediates (ROI), superoxide anions,[4] and also reactive nitrogen intermediates (RNI)[5] in order to more effectively kill both intracellular and extracellular microorganisms. Further, IL-2 and IL-6 influence T, NK and LAK cells to become cytolytic by increasing their perforin (pore forming protein) content and leukolexin.[6] These interleukins are also known to modify the functions of other cells, such as endothelial cells, keratinocytes, and Langerhans' cells. All of these factors collectively influence the cellular functions *in situ* and also further recruit appropriate cells from the circulation, ultimately leading to the formation of an immune granuloma for the destruction of the invading microorganism.

CMI in Leprosy

Generalized defect

Over two decades ago, researchers often reported on a generalized defect in CMI responses in lepromatous leprosy. A majority of lepromatous leprosy patients from different geographic origins and belonging to different ethnic groups have been shown to have lowered responses to intradermal skin reactions to a variety of reagents,[7-18] allergic skin sensitization to a hapten,[7, 10, 14-17, 19] and homograft rejection.[20] In addition to the above, a lowered response to antigen-[21-25] or mitogen-[7, 9, 22-34] induced lymphocyte transformation, reductions in the number of T lymphocytes in peripheral blood,[33-38] and lowered production of lymphokine[9, 16] have been noted. However, from a few of the above studies it also can be noted that within a group showing an overall generalized depression in CMI many of the in-

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dividuals actually did not exhibit any depression in CMI at all.[16, 19] Further, in many studies the authors failed to observe any depression in general CMI in the entire lepromatous leprosy population, although these patients remained unresponsive to *M. leprae* antigen.[9, 10, 31, 37] In those lepromatous patients who did show some degree of depression in generalized CMI, their generalized CMI improved considerably after antileprosy treatment,[15, 30] but they all still showed unresponsiveness to the lepromin reaction.[38, 39] In a recent study, while conducting skin reactions against new tuberculin and leprosin in untreated lepromatous leprosy cases, all showed a very strong tuberculin reaction, equivalent to those of controls, while being negative to lepromin.[40] This indicates that the state of unresponsiveness in lepromatous leprosy cases is specific to *M. leprae*. They are able to respond to other mycobacterial antigens but are not able to respond to *M. leprae*.

The mechanism for the generalized depression in CMI, if any, in lepromatous leprosy is largely unknown. Recently, Muthukkaruppan, *et al.*,[41, 42] using a pan T-cell marker (OKT3), found normal T-cell levels in the peripheral blood of lepromatous leprosy patients. This observation along with the recent observation of normal CD4/CD8 ratios in the peripheral blood of lepromatous leprosy patients[43] strengthens the view that generalized CMI remains almost unimpaired in lepromatous leprosy. However, reports of the association of other diseases with leprosy are very difficult to interpret. The high incidences of tuberculosis,[44] basal cell carcinoma,[45] and lymphoma[46] among

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lepromatous leprosy patients are very rare and may be related to some local environmental factors. Generally, whenever there is a significant generalized depression in CMI an opportunistic infection occurs, such as is being observed in HIV-1- and HIV-2-infected individuals.[47–49]

Specific defect

The immunological basis for understanding the disease led to the establishment of a polar concept[1] in leprosy. Further, the finer classification of the disease spectrum also has taken into account the CMI response of the host to *M. leprae*.[50]

CMI in leprosy always has been determined using either whole or soluble antigens of *M. leprae in vivo* or *in vitro*.

***In vivo* use of *M. leprae*.** *M. leprae* suspensions designated as lepromin[51] or soluble *M. leprae* antigen[52, 53] have been used to determine the CMI status of patients classified by the Ridley-Jopling scale.[1]

The lepromin reaction is an immunological skin test for leprosy first reported by Mitsuda.[54] This preparation—termed Mitsuda antigen—now a whole bacillary suspension (40 million/ml) in normal saline, when injected intradermally, evokes a weak 24–48-hour skin reaction and a strong 3–6-week skin reaction in sensitized individuals. Later, Dharmendra[55] reported on a lepromin preparation consisting of a defatted bacillary suspension which recently has been standardized.[56] This antigen evokes both the 24–48-hour and 3–4-week skin reactions equally well. More recently, after the successful purification of *M. leprae* from infected armadillo tissue,[57] it was possible to sonicate a large number of bacilli, and the cell-free extract of *M. leprae* could be standardized by its protein content (10 μg/ml).[52] This soluble antigen evokes only a 24–48-hour skin reaction.

Although a lepromin test has no diagnostic potential,[51] it has considerable prognostic value[1] and provides confirmatory evidence for classification of the disease. In most of the TT/BT cases the test is strongly positive, while in BL/LL cases it is negative. The negative reaction in BL/LL leprosy tends to become positive after a reversal reaction. On the other hand, the positive reaction in BT leprosy tends to become negative before the occurrence of a downgrading reaction. However, such changes do not take place in polar TT or polar LL cases.

It has been formally accepted that the 24–48-hour skin reaction is an expression of preexisting delayed-type hypersensitivity (DTH) to the protein antigen(s) of *M. leprae*. In fact, two kinds of soluble proteins were isolated from leprosy nodules by Abe.[58] However, these proteins did not induce a Mitsuda reaction. For the generation of a 3–4-week skin reaction, the presence of whole, intact bacilli in the skin-test antigen is essential.[59]

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Use of *M. leprae* antigen *in vitro*. As early as 1973, Godal and Negassi,[60] while studying the lymphoproliferative response to *M. leprae* antigen in different categories of people, noted that individuals who were not in contact with the disease generally remained unresponsive. On the other hand, 80% of the people who were exposed to leprosy for more than 1 year responded to the antigen. This showed the sensitization of normal individuals who were subclinically infected due to their contact with patients. Myrvang, *et al.*[61] studied the lymphoblastic response of patients along the Ridley-Jopling scale,[1] and noted a gradual fall in the lymphoproliferative response from TT through LL. Various *in vivo* and *in vitro* studies carried out earlier showed that lepromatous patients exhibit a long-lasting anergy to *M. leprae* antigens. This has been extensively reviewed elsewhere.[62, 63]

Scientists have been in doubt in deciding if the lymphoproliferative response to a specific antigen is an *in vivo* correlate of CMI or, rather, whether it may be measuring hypersensitivity which is not a measure of resistance at all.[64, 65] Experimental proof[66] in mice is available. A strain of mice susceptible to *M. lepraemurium* were challenged with different doses of the pathogen and infection (at a certain dose) together with a strong skin DTH reaction against the same pathogen was shown. Moreover, human lymphocyte responses to antigen have been found to vary on several occasions due to the presence of suppressive factors in the plasma of leprosy mothers[67] and dapsone in dapsone-treated patients.[68] Later on it also was pointed out that variability in the lymphoproliferation and leukocyte migration inhibition could be due to the heterogeneous nature of the antigens used in these assays.[64, 69, 70]

A significant number of reactional tuberculoid (BT) patients (type 1 reaction) show lowered *M. leprae* antigen-induced lymphoproliferative response, suppressor cell generation, and lymphokine production.[71] On the other hand, the lymphocyte responses show much improvement in type 1 reaction in BB and BL cases.[70] Similarly, LL patients with erythema nodosum leprosum (ENL; type 2 reaction) generally show heightened T-cell reactivity to *M. leprae* antigen.[38, 72]

Status of T-Cell Subsets and *in Situ* Profile of Accessory Cells and Cytokines

In peripheral blood

Bach, *et al.*[39] and Wallach, *et al.*,[73] while studying the pattern of distribution of T-cell subsets, noted mostly a reduction in the ra-

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tio of helper/inducer (OKT4+ or CD4+) and suppressor/cytotoxic (OKT8+ or CD8+) cells in bacilliferous leprosy patients. On the other hand, the bacillary-negative LL patients showed CD4+ and CD8+ cell numbers equivalent to those of controls. An increase in the CD4+/CD8+ ratio was also noted in ENL patients, with the CD4+/CD8+ ratio returning to normal after subsidence of the reaction. In 1982, Mshana, *et al.*[43] saw no change in the percentage of pan T (OKT3+ or CD3+) cells in tuberculoid, untreated LL, ENL patients, and controls. Similar to the above, however, there was a reduced percentage of OKT4+ cells and an increased percentage of OKT8+ cells, resulting in a reduced OKT4+/OKT8+ ratio in untreated LL. During ENL reactions the helper/suppressor ratio increased due to an increase in the helper and a corresponding decrease in the suppressor cell percentages. However, in the same year Van Voorhis, *et al.*[74] reported an unaltered helper/suppressor cell population in all of the disease types.

All these above studies gave an account of the percent population of the T-cell subsets rather than their absolute values in blood. While enumerating the absolute values of these subsets in a mixture of treated and untreated lepromatous patients, Bullock, *et al.*[75] noted a reduction in the total number of T cells and their helper and suppressor subsets. However, like previous workers, they also failed to record any change in the helper-suppressor ratio. Rea, *et al.*[76] using the same yardsticks observed a significant cytopenia of pan T, helper, and suppressor cells when these values were compared to those of normal individuals. However, when the results were expressed as a percentage of total lymphocytes and again compared, the differences in the groups were abolished. Even when the values of the above parameters of ENL patients were compared with their control values, no significant differences were observed.

From the above-mentioned studies, it becomes clear that when these researchers noted a reduction in CD4+ cells along with an increase in CD8+ cells, it simply indicated a state of immunosuppression.

On the other hand, workers who enumerated the absolute number of these cells could find pan-T-cell cytopenia but failed to find any abnormalities in the CD4+/CD8+ ratio. Considering that lepromatous patients are not prone to get other infections which are known to affect immunosuppressed individuals,[77-82] it is more rational to assume that in lepromatous leprosy there is no biologically significant alteration in the CD4+/CD8+ ratio in the peripheral blood.

In lesions

The *in situ* distribution of T-cell subsets with respect to their proportion and pattern of distribution in the tissues has been widely studied[74, 83-86] in the granuloma itself. Van

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Voorhis, *et al.*[74] noted large numbers of T cells in leprosy lesions and found the helper/suppressor ratio to be 5.6:1 in the tuberculoid granuloma; whereas in the lepromatous lesion they noted this ratio to be 1:1.8. In addition, they observed clusters of OKT4+ cells distributed in the form of rings in the tuberculoid granuloma. OKT6+ cells, on the other hand, remained scattered in both tuberculoid and lepromatous granuloma. In contrast to the above, Narayanan, *et al.*[87] and Modlin, *et al.*[86] could not account for such large numbers of T cells in the granuloma, and the helper/suppressor ratio ranged from 1.2 to 5.0 in tuberculoid and from 0.2 to 1 in lepromatous lesions.[87] Moreover, Narayanan, *et al.*,[87] unlike Van Voorhies, *et al.*,[74] found that in tuberculoid granulomas (concentric variety) the OKT8+ cells were scattered in a "ring-like" fashion among the lymphocytes. Conversely, OKT4+ cells showed a scattered distribution, either singly or in clusters, within the lymphocyte cuff as well as in close association with epithelioid cell aggregates. Similarly, Modlin, *et al.*[85] found lymphocytes expressing CD8+ cells predominantly in the mantle, while CD4+ cells were seen in large numbers within the epithelioid cell aggregates. This type of histological distribution of T-cell subsets also has been noted in the tuberculoid granuloma of sarcoidosis,[85] tuberculosis,[85] and DTH skin reactions.[88–91] In addition, all of these studies showed unequivocally that all lymphocytes and macrophages of leprosy granulomas are Ia positive.

Recently, Narayanan, *et al.* isolated the granuloma-infiltrated cells *in vitro* and studied their characteristics[92–94] and functions.[95] The OKT4+/OKT8+ ratios were found to be similar to those described above in *in situ* situations. Functionally, the immune cells obtained from tuberculoid granulomas exhibited a high incorporation of ^{3}H-thymidine and ^{14}C-leucine. On the other hand, cells from lepromatous granulomas showed poor division without any impairment in their protein synthesis. A similar study[96] was carried out to compare the characteristics of infiltrates in the skin and nerve granulomas of tuberculoid and lepromatous cases. Using phenotypic markers, CD4+ and CD8+ cells of nerve showed similar distributions and proportions as noted with those of skin granulomas. Moreover, in tuberculoid granulomas a higher proportion of lymphocytes of both skin and nerve were activated T cells as compared to those in the lepromatous granulomas. In both types, the granulomas were populated with macrophages which expressed HLA-DR (Ia) antigen. With regard to the helper/suppressor ratio in lesions of both type 1 and type 2 reactional cases, it was noted that the ratio was more than 2 in lepromatous granulo-

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mas due to the increase in the number of OKT4+ cells in the cells of the lesions.[87] It was shown further that in type 1 reaction in BT leprosy, there is a decrease in the mean value of the helper/suppressor ratio when compared to that of nonreactional, untreated leprosy.

Accessory cells (Langerhans' cells, keratinocytes) other than macrophages are known to play a pivotal role in the presentation of antigens to T cells, and they also have been shown to be associated with allergic contact sensitivity and hypersensitivity reactions.[97-100] Using phenotypic markers for T6 and Ia-like antigens, Langerhans' cells have been identified in leprosy lesions. While adequate numbers of these cells were noted in polar tuberculoid leprosy, the cells were virtually absent in polar lepromatous leprosy. However, Ia-like antigens also were found to be associated with macrophages in these lesions.[101] Recent observations[102, 103] revealed that during type 1 and type 2 reactions there is a significant increase in Langerhans' cells in these lesions. In addition, Ia also was seen in all keratinocytes in type 1 reaction; whereas in ENL patients a patchy distribution of these Ia-positive keratinocytes was noticed. All of these events are indicative of a temporary T-cell reactivity in the lesions during manifestation of a reactional phase. Further, Cooper, *et al.*,[104] using mRNA probes for IFN-γ in *in situ* hybridization in reversal reaction biopsy specimens, have elegantly shown that there was a 10-fold rise in IFN-γ-containing cells as compared to those observed in lepromatous patients who were not in reaction. When a probe for the human gene esterase (huHF), a marker for cytotoxic T cells, was used, it was noted that expression of huHF serine esterase was four times more in the reversal reaction and tuberculoid lesions than in lepromatous lesions. Their study further confirmed a selective increase of CD4+ and CD8+ cells, indicating a rise in the DTH response which may help in killing bacilli but which may result in tissue damage. On the other hand, finding a reduction in the expression of IFN-γ and human serine esterase in an atmosphere of a CD4+ rise and transient fall in CD8+ cells suggested a partial or transient boost in the CMI which may be sufficient for antibody production without any effect on bacillary clearance.

Very recently the functional parameter for cytokine production for the locally proliferated immune cells has been worked out very extensively in the tuberculoid and lepromatous groups. It was noted that while mRNAs encoding for IL-2 and IFN-γ were most evident in the tuberculoid granulomas, in lepromatous granulomas mRNAs for IL-4, IL-5 and IL-10 were noted predominantly.[105] Although definite cytokine profiles have been found to be associated with resistant and susceptible types of leprosy, no definite conclusion could be made toward the role of these lymphokines in the etiopathology of such lesions in leprosy.

Understanding the Mechanism of Unresponsiveness

Macrophage/Antigen presenting cell

It is known that *M. leprae* are obligatory parasites that reside inside the macrophages/Schwann cells and produce a gran-

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uloma in the host. The earliest claim[106] that macrophages from lepromatous patients are incapable of killing *M. leprae in vitro* was contradicted by others.[107, 108]

While studying the capability of *M. leprae* antigen presentation by the macrophages of lepromatous leprosy patients, Hirschberg[109] pointed out that, due to the defect in the macrophage presentation of antigen, lepromatous leprosy patients are unable to respond to *M. leprae.* Nath, *et al.*[110] further confirmed this observation in HLA-D-matched, peripheral blood leukocyte coculture experiments. Macrophages from lepromatous patients inhibited the lymphoproliferation of the *M. leprae*-responding individuals. They further showed that lymphocytes of lepromatous patients are capable of undergoing proliferation when macrophages from tuberculoid patients are added to the culture, thereby indicating that antigen-reactive T cells are present in the peripheral blood of lepromatous leprosy patients. Conversely, a study[111] in HLA-D-identical siblings in such situations completely negated the role of the monocyte population as mentioned above. Rather, a T-cell defect accounted for the unresponsiveness. However, more data have accumulated from Nath's laboratory indicating that the adherent cells in the peripheral blood of lepromatous individuals having macrophage characteristics induce a suppressive effect on the antigen-induced lymphoproliferation in HLA-D-identical tuberculoid patients and healthy contacts. Further, it was concluded that the suppression induced by these macrophages is due to some soluble factors liberated by these cells.[112] In support of the above view, Salgame, *et al.*[113] established that lysates of lepromatous macrophages inhibit protein synthesis of normal macrophages in addition to the inhibition of lymphoproliferation.[114] Recently, the soluble factor was characterized and was found to be heat-stable, indomethacin resistant, and of more than 25 kDa.[115]

It has been shown that macrophages, after the phagocytosis of live *M. leprae,* down-regulate their own Fc receptor expression, and biochemical and other functions.[116] In addition, macrophages of lepromatous patients have been shown to have a selective depression in ^{3}H-leucine uptake and exhibit a reduction of Fc receptors only when exposed to *M. leprae.*[117] Mahadevan and Antia[118] proposed from their experiments that, after the phagocytosis of *M. leprae,*

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macrophages lose their capacity to process antigen thereby leading to a CMI defect. At the same time, it has been noted that after the phagocytosis of *M. leprae* Schwann cells lose their capacity to synthesize DNA and are unable to associate themselves with axons, thereby including a defect in C-fiber-function. Recently Mahadevan's group[119] noted that macrophages from the peripheral blood of both paucibacillary and multibacillary leprosy, but not from healthy individuals, are inefficient in killing phagocytosed *M. leprae* due to their inability to produce superoxide (O_2^-) and hydroxyl radicals (OH·).

Immune responses are controlled partially by soluble factors liberated by the lymphoreticular system. IL-1, liberated by macrophages, acts upon T cells in the early G1 phase of the cell cycle, and prepares the T cells to respond to subsequent signals. An initial report by Horwitz, *et al.*[120] of lowered production of cytokine by macrophages of lepromatous patients has been further confirmed and characterized by Watson, *et al.*[121] while studying the IL-1 production in lipopolysaccharide-stimulated monocytes. They noted that 38.5% of BL/LL patients failed to produce IL-1, while TT/BT patients were able to produce either IL-1 in the normal range spontaneously or upon stimulation. A similar report[122] also was established in a *M. leprae*-stimulated situation.

Tumor necrosis factor-alpha (TNF-α) is known as a secretory product of macrophages[123] and activated mononuclear cells of the peripheral blood.[124, 125] Higher levels of TNF-α have been associated with malaria and kala azar.[126] Using a bioassay, higher levels of TNF in serum[127] and higher TNF production by antigen-induced mononuclear cells[128] have been noted in tuberculoid patients compared to lepromatous patients. There is, however, a report of a higher level of TNF-α in the sera of lepromatous patients[129] using an ELISA. This might be due to the presence of inhibitors in the sera of such patients. Such inhibitors might have resulted in relatively low levels of TNF by bioassay but relatively high values in an ELISA. The presence of such inhibitors has been reported in tuberculosis and sarcoidosis.[130] Moreover, the presence of a higher number of TNF-containing cells[131] and a higher concentration of TNF mRNA by *in situ* hybridization[132] and by PCR amplification[105] in tuberculoid skin lesions than in lepromatous lesions have al-

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ready been reported. A protective role of TNF can be explained by the finding of its inhibitory role in mycobacterial multiplication in murine and human macrophages.[133] Moreover, recently TNF has been shown to enhance the production of nitric oxide in mouse macrophages[134] which, in turn, have been shown to kill *M. leprae*.[134] Further, the finding of high levels of TNF[128] in active ENL patients could explain the clinical manifestations of fever and nerve damage which have been noted by TNF inoculation in mice[135] and by *in vitro* experiments,[136, 137] respectively.

T cells

The understanding of a suppressor function of a subpopulation of T cells in mice which regulates the immune response prompted researchers to work on normal and diseased states of human beings. Investigators engaged in leprosy research also generated data on their observations of T-suppressor (OKT8+) cells in leprosy. A preliminary study by Bjune,[138] which was carried out in an Ethiopian population, indicated that *M. leprae* antigens generally suppressed the *in vitro* phytohemagglutinin (PHA)-induced lymphoproliferation in leprosy patients and their household contacts. Working with concanavalin A (ConA)-induced lymphoproliferation in leprosy patients and healthy individuals, Mehra, *et al.*[139] noted that Dharmendra antigen suppressed the ConA-induced proliferation selectively in a majority of lepromatous and borderline patients but not in tuberculoid leprosy patients and healthy individuals. This group further pointed out that the suppression was induced due to the generation of the classical T8+[140] and TH2+[141] phenotype markers-bearing suppressor cells. Although the above work sufficiently indicated that suppressor-T cells were responsible for the suppression of CMI in lepromatous leprosy, Stoner, *et al.*[142] could not establish such a role. Rather, they noted a lack of these cells in most of their Ethiopian patients. Further, from their observation on subclinically infected healthy individuals they established that there is an association of suppressor-cell activity with resistance to *M. leprae* infection.[143] To prove the role of suppressor cells in leprosy, Nath, *et al.*[144] have done extensive work in untreated patients from both hyperendemic and low-endemic areas in India. It was noted in a 4-day culture that ConA-induced suppressor cells selectively suppressed the autologous mitogenic responses of tuberculoid patients. Further, this ConA-stimulated lymphoproliferation was suppressed by the addition of *M. leprae* antigen in a majority of the tuberculoid patients but not in all of the lep-

Bloom, B. R. and Modlin, R. L. Expression of adhesian molecules in leprosy lesions. Infect. Immun. **59** (1991) 4154–4160.

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romatous patients. On the contrary, many of the lepromatous patients showed an enhancement in the lymphoproliferative response. However, when the cultures were continued for 6 days the differences in suppression in the various groups were abolished and the suppressive effect was noted uniformly in all subjects,[145] as noted earlier by Bjune.[138] Earlier studies, when T cells bearing Fc receptors for IgG were considered to be a suppressor-T-cell subset, also indicated the presence of normal levels of these cells in tuberculoid patients[146] with a reduction in their number in lepromatous patients.[147]

The above studies on suppressor-cell activity are very divergent in their views. The suppressor-cell activity in the tuberculoid type of leprosy gains support from only one observation wherein the suppressor-cell generation was mostly associated with a strong CMI response[148] which might possibly play a role in the suppression of unwanted antibody production.[149] On the other hand, with the understanding of murine TH1/TH2 subsets and their biological function,[150, 151] more and more evidence is being accumulated to understand how TH1 lymphocyte proliferation along with IFN-γ secretion are associated with the acute stage of the disease whereas TH2 lymphocyte proliferation with an increase in IL-4 and IL-10 is associated with chronic disease.[152–154] Recently, Salgame, *et al.*[155] elegantly categorized these functional subsets of T cells in leprosy. They established that CD4+ cells cloned from tuberculoid patients produced IFN-γ whereas those obtained from lepromatous patients produced more IL-4. In addition CD8+ T-suppressor clones producing IL-4 isolated from lepromatous patients were found to be essential for the suppression of *in vitro* responses to antigen in these patients. However, more recently the role of contrasuppressor (Cs) cells for antagonizing the suppressor function in leprosy has been reported.[156] Cs cells are known to interact with CD4+ cells and render them unresponsive to the signals of CD8+ cells.[157] A Cs-like functional activity has been noted also in the CD8+ cells in leprosy.[156]

T-cell unresponsiveness to *M. leprae* stimulation in lepromatous leprosy has also been thought to be due to the lack of lymphokine production by the immune cells.[120, 158–161] It has been noted by several

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workers[162–164] that T cells in lepromatous leprosy patients are incapable of specific antigen-stimulated proliferation due to a deficiency of IL-2. However, in spite of an exogenous supply of IL-2 in about one third of the patients, T-cell unresponsiveness could not be corrected.[162] All of these studies also indicated that in the majority of lepromatous cases there is no clonal deletion of *M. leprae*-specific T cells, which was the view taken earlier by Godal, *et al.*[24] This unresponsiveness may be due either to a lack of the required number of *M. leprae*-reactive T cells being circulated[160] or to the presence of monocytes which are liberating suppressive factors[165] as has been mentioned earlier. In contrast to the above findings, Mohagheghpour, *et al.*[166] could not find any IL-2 deficiency in lepromatous patients, and they explained that the unresponsiveness was due to the lack of an IL-2 receptor on the T cells. An interesting observation[167] has recently been made in which CD4+ cells of lepromatous leprosy patients were found to respond to *M. leprae* stimulation after culturing for 48 hours in medium alone. Further, they noted that the recovery of this T-cell reactivity was blocked by the presence of *M. leprae* in the preculture medium. These authors reasoned that the unresponsiveness was due to the persistence of antigen which renders the antigen-responsive T cells unresponsive.

Downregulation of the T-cell response, especially by *M. leprae* modulating the CD2 (E or SRBC receptor), recently has been postulated by Muthukkaruppan, *et al.*[41, 42] Using OKT11 and OKT3 monoclonal antibodies, they showed that bacilliferous lepromatous leprosy patients, while exhibiting low levels of CD2+ cells, show normal levels of CD3+ cells. Further, when *M. leprae* (Dharmendra lepromin only) were exposed to the suspension of peripheral blood T lymphocytes obtained from normal healthy individuals, CD2+ cells were found to become reduced in number, keeping the CD3+ cell number intact. These observations indicated that *M. leprae* antigens, by modulating the E-receptor of T cells, might be inducing the suppression. Contrary to the above, Wong, *et al.*,[168] while looking for the expression of CD2+ and CD3+ receptors on lymphocytes in lepromatous skin lesions and peripheral blood, found that virtually all of the CD3+ cells expressed CD2 in both situations.

Other factors

Serum/Plasma. From time to time the presence of some unknown factors in the blood plasma/serum of leprosy patients which are capable of inhibiting the *in vitro* growth of autologous lymphocytes have been reported.[28, 30, 169–172] Bullock and Fasal[169]

Kushner, P., Granelli-Piperno, A., Vieira, L., Gould, V. C., Levis, W., Steinman, R., Yip, Y. K. and Cohn, Z. A. Defective gamma interferon production in leprosy. Reversal with antigen and interleukin 2. J. Exp. Med. **158** (1983) 2165–2170.

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and Nelson, and others[28, 170, 173] described that some lepromatous leprosy sera are lymphocytotoxic, and these sera also suppress the lymphoproliferation induced by PHA. Later Potts, *et al.*[171] and Kerr, *et al.*[172] showed that the inhibition in lymphoproliferation is due to a decrease in the number of cells responding to mitogen. On the other hand, Kerr, *et al.*[172] recently have shown that the putative inhibitory factor(s) is (are) not cytotoxic. The suppression in the proliferative response may be due to an inherent cellular defect or due to the presence of factors in the serum which inhibit lymphocyte activation. They further characterized the inhibitory factor as being resistant to heating to 60°C for 30 minutes but which becomes denatured at 100°C.[172] A very interesting report[174] indicating that lepromatous leprosy sera causes some chromosomal aberrations of normal lymphocytes in culture, leading to a lowering of the mitotic index and thus inhibiting lymphoproliferation, is also available.

Although the presence of such serum/plasma factors have been reported by different workers in leprosy patients, these factors still need further chemical and structural characterization to substantiate the above findings.

Mycobacterial components. In addition to the isolation of T-cell clones having suppressor functions from lepromatous lesions,[175] *M. leprae* soluble products also have been shown to suppress lymphoproliferation to the antigen in not only lepromatous but also tuberculoid patients.[176] It could also be possible that a nonspecific suppressive function by a microbial fraction may be responsible for the suppression as noted by Molloy, *et al.*[177] Holzer, *et al.*[178] noted that *M. leprae* as a whole fail to stimulate human blood monocytes, neutrophils and murine peritoneal macrophages to generate superoxide anions (respiratory burst). Vachula, *et al.*[179] pointed out the role played by phenolic glycolipid-I (PGL-I) in blocking the respiratory burst of human macrophages. Recently, it also has been hypothesized by Parkash and Sengupta[180] that the failure of the oxidative burst exhibited by macrophages which have phagocytosed *M. leprae* is most probably due to the complement-mediated entry of *M. leprae* into the monocytes. Such a view has already been favored by workers studying various particulate materials including *Leishmania major* as a pathogen.[181–184] With regard to the PGL-I-

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induced suppressive function by macrophages, further evidence has accumulated from the work of Neill and Klebanoff,[186] who showed that both purified PGL-I and its deacylated form abolished the antimicrobial effect by blocking the xanthine oxidase system which is essential for the release of OH·. A blocking effect on the myeloperoxidase-H_2O_2 halide system also was observed using this lipid.

Another cell-wall-associated lipid of mycobacteria, lipoarabinomannan (LAM), which has been shown to inhibit antigen responsiveness of human peripheral blood leukocytes[186] and antigen-induced proliferation of CD4+ T-cell clones,[187] also has been found to block the activation of macrophages without any change in their capability of phagocytosis.[188] The same group of workers have demonstrated further that LAM is able to block the activation of mouse macrophages induced by IFN-γ.[189]

M. leprae protein components and the 120-kDa protein antigen of *M. leprae* have been shown by Sengupta, *et al.*[40] to suppress the tuberculin-induced DTH response *in vivo* in leprosy patients. However, such a suppression could not be reproduced by Fine, *et al.*[190] This discrepancy may be due to the differences in the types of leprosy patients used and differences in the genetics of the ethnic groups used in these studies.

All of the above studies proved beyond doubt that there are several *M. leprae* components which are suppressive for cell-immune functions and these components may be playing major roles in the pathogenesis of leprosy.

Antibodies/Immune complexes. It is known that the presence of *M. leprae* can lead to host immune responses by the production of antibodies and by the development of CMI against the pathogen. It also is known that there is an inverse relationship between CMI and *M. leprae* antibody levels in leprosy patients.[1, 165] It might be expected that if the protective antigens would be capable of eliciting both CMI and antibody in the host, the antibodies would be able to mask the *M. leprae* antigens expressed on the membranes of antigen-presenting cells and this could result in the lowering of CMI.[191] Further, preliminary evidence suggesting that circulating immune complexes from leprosy patients could suppress the *M. leprae*-induced lymphocyte proliferation has been provided by Tyagi, *et al.*[192]

Antigenic mimicry

Since biblical times *M. leprae* have infected human beings. It is quite possible that the organism during these many years might have adapted within the human host in such a way that it generally does not evoke a strong host immune response. The unresponsiveness of the host could be possible if *M. leprae* "share" or "mimic" some of their antigens with the host tissues. Such a possibility is not far fetched when it has been reported that human tissues possess proteins which are very similar to a mycobacterial 65-kDa heat-shock protein on

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the basis of their amino-acid sequences.[193, 194] In addition, very recently Naafs, *et al.*[195] have shown that eight monoclonal antibodies against *M. leprae* determinants (12 kDa, 18 kDa and 65 kDa) reacted with dermal determinants. If these similarities are well founded, then these *M. leprae* antigens would be recognized as self and, due to the presence of some minor dissimilarities in some sequences from the host proteins, they could evoke an autoimmune reaction. Already established evidence for this hypothesis is present in other diseases such as Group A streptococcal mycocarditis,[196] rheumatoid arthritis in *M. tuberculosis* infection,[197] myasthenia gravis,[198] and neuropathy/cardiomyopathy in Chagas' disease.[198, 199]

This review on cell-mediated immunity in leprosy is an effort to cover various aspects of studies concerning cellular immunology which have been carried out in order to understand the disease process. It is known that there are genes for immune responses as well as for immune suppression.[200] Whether a genetic mechanism is also playing a role in modulating the host immune response has not been fully crystallized. However, a significant association of HLA-DR2[201–206] and HLA-DR3[204] molecules with various types of leprosy has been claimed. A detailed review on the genetic correlation with the disease and the role played by HLA-DR antigens in modulating the immune response to *M. leprae* antigens requires a separate review.

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