Leprosy: The Immunologist and the Patient

The substantially improved supplies of *Mycobacterium leprae,* the revolution occurring in basic immunology, and the onslaught of molecular biology will all combine to make leprosy one of the most researched infectious diseases. While an extremely impressive body of information has accumulated over the past few years, it is unclear how this knowledge can be integrated to explain clinico-pathological observations in leprosy. The aim of this editorial is to present in a nutshell the current knowledge on the immunology of leprosy in a way that is understandable for those who deal directly with the patients while attempting to retain the interests of immunologists who (understandably) might not have full comprehension of leprosy as seen clinically. We aim for the placement of the patient at the center of the discussion, because we believe that unless leprosy as a disease is viewed *in toto* in terms of its evolution in the patient being studied, grave misconclusions can easily be made. Furthermore, it is extremely important to view all of the experimental data in the context of the real situation, namely the disease process as it occurs in the patient.

The elusive *M. leprae* has not been successfully cultured in cell-free media, but important information with regard to the physico-chemical makeup of the bacilli has already been obtained. The cell wall, for example, has been well studied, and it is clear that this part of the bacillus is a complex structure which presents a formidable task to host cells. For many years, analysis of components of *M. leprae* failed to reveal *M. leprae*-specific determinants.[1–4] The availability of antibodies able to recognize definable units, i.e., monoclonal antibodies, has, however, helped to identify some components which are specific to *M. leprae.* Characterization of these epitopes is important for our understanding of the bacillus and its interaction with the host. As a by-product, important seroepidemiological studies have been conducted using, for example, phenolic glycolipid-I (PGL-I) which is specific to *M. leprae.*[5, 6] Monitoring the synthesis of this molecule may be useful in studies aimed at testing new drugs, drug resistance, and in elucidating mechanisms involved in intracellular killing of the bacilli. Perhaps the most fundamental observation related to PGL-I is that of Mehra, *et al.*[7] These authors found that PGL-I was a potent inducer of suppressor lymphocytes in lepromatous leprosy (LL) patients. The subject of suppressor lymphocytes will be dealt with later, but is mentioned here just to highlight the need for understanding the complexity of *M. leprae* per se.

M. leprae is classically described as being nontoxic; lepromatous leprosy (LL) patients being able to harbor as much as 10^{10} bacilli per gram of tissue without any known clinical side effects.[8] In *in vitro* systems, *M. leprae* does not cause lysis of macrophages, Schwann cells, or other cells. It has, therefore, been accepted that the host immune response is responsible for the pathological consequences of leprosy.[8] The role of *M. leprae* itself in directly influencing the immune response has received rather little attention. Kaplan and others have shown that

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M. leprae can suppress *in vitro* lymphocyte stimulation.[9-11] Since this effect is seen in both healthy unexposed and healthy exposed individuals as well as leprosy patients regardless of their disease classification, the suppressive effect must be due to *M. leprae* itself. The clinical implication of these findings might be the relatively reduced capacity of untreated LL patients to mount strong delayed-type hypersensitivity (DTH) reactions. Furthermore, some investigators have recently suggested that *M. leprae* might also be able to regulate the tempo of the cell-mediated immune (CMI) response by interfering with the expression of major histocompatibility complex (MHC) class II molecules.[12] MHC class II molecules serve to restrict antigen-induced T-lymphocyte stimulation since these lymphocytes will not respond to a nominal antigen unless such antigen is presented by a cell (antigen-presenting cell) which expresses compatible MHC class II molecules on its surface. Kaye, *et al.*[13] have shown that when macrophages are infected with *M. microti* the amount of detectable MHC class II surface molecules are reduced. This phenomenon is dose dependent and is maximally expressed when cells are infected with live bacilli. At the leprosy tissue level, there is conflicting data with regard to expression of MHC class II molecules on the inflammatory cells, and although it is difficult to explain the discrepancies, it would be helpful to have more information with regard to the disease evolution, the bacterial load, and some idea with regard to viability of the bacilli in the lesions.

To date, intracellular mechanisms involved in killing *M. leprae* have not been clarified. Currently, it is thought that such intracellular killing mechanisms involve the generation of hydrogen peroxide (H_2O_2) and toxic oxygen radicals, since H_2O_2 has been reported to be mycobactericidal. Evidence to link the uninterrupted proliferation of *M. leprae* and macrophages' inability to generate H_2O_2 has recently been sought. Sharp and Banerjee[14, 15] showed that monocytes from LL patients were capable of producing normal amounts of H_2O_2 and, therefore, found no correlation between H_2O_2 and proliferation of *M. leprae*. Nathan, *et al.*,[16] on the other hand, found that LL monocytes produced less than half the normal amounts of H_2O_2. The discrepancy between these studies has been thought to be due to technical differences but further work to clarify the issue is needed. Since the cell that is eventually responsible for the killing of intracellular bacilli is the infected macrophage, it is important to assess whether a heavily infected macrophage can, in fact, be activated to kill the bacilli. In this context it is worthwhile noting the recent observations,[13, 17] suggesting that macrophages harboring large numbers of bacilli appear to show an abnormal response to interferon-gamma (IFN-γ). IFN-γ is currently, perhaps, the single well characterized macrophage-activating substance released by activated T lymphocytes. It is important to

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confirm and extend these observations, especially because of the contemplated use of IFN-γ as an adjunct therapy for the treatment of leprosy.[18] Finally, it is important to keep our minds open to the possibility that other effector mechanisms might be involved in the elimination of *M. leprae*. Both Kaufmann, *et al.*[19] and Mustafa, *et al.*[20] have recently shown that clones of T lymphocytes responding to *M. leprae* can lyse antigen-presenting cells in an antigen- and MHC-restricted manner. In light of the well-known pathology of human leprosy, however, it is difficult to assign any clinical significance to these findings. The results should serve to generate more interest in assessing the role of cytotoxic T lymphocytes in the elimination of *M. leprae*-infected cells. The role of anti-*M. leprae* antibodies in the pathogenesis of leprosy is not clearly understood but has been relegated to a minor, if any, significance. Recent data have, however, shown that by using specific monoclonal antibodies it is possible to target cytotoxic T cells[21] and, thus, it appears relevant to re-open the issue of antibody response in leprosy patients, especially with regard to their specificity.

The pathogenesis of lepromatous leprosy. The study of the immunology of leprosy is intimately related to the study of anergy seen in lepromatous leprosy. This anergy is manifested by a failure to form a mature epithelioid cell granuloma in response to *M. leprae* and the absence of *in vitro* T-lymphocyte proliferaton to *M. leprae* antigens. It has become evident that in localized diseases or localized DTH[22–25] the relevance of studies of circulating peripheral blood mononuclear cells (PBMNC), insofar as the pathogenesis of the localized lesion is concerned, is questionable. As such, emphasis has recently been placed on studying the immune response as it takes place in the lesion itself. This approach has received considerable boosting by the availability of monoclonal antibodies recognizing all types of antigens on the cells in the lesion. It must be understood that this method allows us only to characterize cells at a given point in time, depending on the expression of certain molecules (phenotypes), while the functional significance of these cells with regard to the disease process can only be inferred.

Despite these limitations, most such studies show that compared to tuberculoid (TT) leprosy lesions, LL lesions show a marked paucity of cells expressing CD4 molecules.[26–33] Critical analysis of the avail-

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able data shows that, despite the often-repeated statement that LL lesions show an abundance of CD8+ cells, there is in fact no significant difference between LL and TT lesions with regard to the numbers of CD8+ cells. The CD4 molecule is expressed by T lymphocytes that generally but not exclusively function as helper/inducer cells, helping B cells produce antibodies, inducing DTH reactions, or inducing other cells to carry out their functions, including inducing suppressor T cells. The CD8 molecule is expressed by a subset of T lymphocytes that is functionally associated with suppressor or cytotoxic cells.

The presence of certain molecules on T lymphocytes can be used to imply a state of activation. Such molecules include the receptor for interleukin-2 (IL-2 receptor or Tac) and transferrin receptor. IL-2 is produced by activated T lymphocytes, and this substance has been shown to be absolutely necessary for the continued proliferation of T cells. It appears that there is a paucity of IL-2-containing cells in LL lesions.[7, 26–29, 34, 35] There is, however, a controversy with regard to cells expressing the Tac molecule. Modlin, *et al.*[29] reported no difference between LL and TT granulomas, while Nilsen, *et al.*[36] have reported that there are significantly fewer Tac+ cells in LL lesions. The paucity of IL-2-producing cells in the presence of large numbers of Tac+ cells would imply a disturbance of IL-2 production, while the paucity of both IL-2-producing and Tac+ cells could suggest a disturbance at the level of the T-cell activation process or even be a reflection of a paucity of antigen-specific T cells. Because of these reasons and because of the implications of these findings in terms of our understanding the pathogenesis of lepromatous leprosy, it is important to pursue these studies using antibodies that can delineate various phases of T-cell activation.

To our knowledge, the paucity of CD4+ T cells in LL lesions has never been clearly explained. While the migration of T cells into lesions is not fully understood, it is generally thought that such movement depends on adequate antigen recognition by and response of antigen-specific T cells. Activated T cells, however, appear to be able to cross "barriers" which otherwise restrict penetration by normal cells. Activated T cells, for example, can traverse the blood-brain barrier in an antigen-independent manner.[18, 37] Certainly in the LL lesion, antigen concentration is not a limiting factor and, indeed, the large amounts of *M. leprae* antigen may down-regulate the local immune response. Furthermore, since LL patients are capable of mounting a cutaneous DTH reaction to purified protein derivative (PPD) of *M. tuberculosis,* it appears that the mere presence of the lepromatous granuloma does not restrict the movement of the T cell to cutaneous sites. The reduced numbers of CD4+ cells in LL lesions could be secondary to a suppressive phenomenon or could be due to a primary lack of adequate *M. leprae*-specific CD4+ cells. Suppressor cells could operate at the level of T-cell proliferation by influencing such events as IL-2 receptor expression and the production of IL-2.[18, 38, 39] The role of suppressor cells in

derived from lepromatous leprosy lesions. Nature **322** (1986) 459–461.

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the pathogenesis of human infectious diseases is, however, not clearly understood. Current experimental data, suggest, but do not prove, that such suppressor cells are important in determining the outcome of clinical leprosy. In a normally controlled immune response, the generation of suppressor cells is to be construed as a healthy physiological feedback control mechanism.[40] The question, therefore, is not whether there are or there are not suppressor cells in leprosy but rather what is the clinical significance of such cells.

The significance of the observation of leprosy-related suppressor cells by Bloom, *et al.*[41, 42] lies in their demonstration that a bacterial antigen could induce antigen-specific suppressor cells in a certain group of individuals. The implications of these studies are far reaching, especially because they impinge directly on our efforts of developing antileprosy vaccines.[41] It is worthwhile, therefore, to briefly examine the data on suppressor mechanisms as related to leprosy. Extensive reviews on this subject are found elsewhere.[41, 42] While not denying that several cell types might act as suppressor cells, most information is based on suppressor T cells. Several pieces of data have been used to imply that suppressor T cells must be operative in lepromatous leprosy. In the first place, the observation that LL patients appear to respond normally to mycobacterial antigens other than those of *M. leprae*[8, 43] has been taken to imply that the response to *M. leprae* in these patients is specifically suppressed. Secondly, the presence of large amounts of anti-*M. leprae* antibodies in LL patients has been used to suggest that these patients must have T cells that respond to *M. leprae* and help B cells produce the antibodies.[35] Thirdly, the observation that, at least in some LL patients, exogenous supplies of IL-2 could restore *M. leprae* responsiveness[44] indicated that in these patients IL-2 production was being down-regulated, most probably by suppressor cells.[42] Finally, it has recently been pointed out that manipulation of the immune system to achieve functional tolerance (anergy) is almost invariably accompanied by the appearance of suppressor T cells. Close examination of these data, however, suggests that the role of suppressor cells in the pathogenesis of LL is at best poorly understood. Recent data show that the presumed *M. leprae*-specific anergy seen in LL patients is after all not all that specific to *M. leprae*,[45–47] and it appears that these patients show reduced responsiveness to several mycobacterial antigens.

At the clonal level, Ottenhoff, *et al.*[45] could generate *M. leprae*-responding CD4+ T-cell clones only from a borderline lepromatous (BL) leprosy patient who had *a priori in vitro* lymphoproliferative response to *M. leprae*. There is no data showing that *M. leprae*-responding CD4+ clones could be established from a polar lepromatous leprosy (LLp) patient. At the tissue level, Modlin *et al.*,[30, 31] while being able to establish *M. leprae*-specific CD8+ suppressor T cells from LL lesions, have not been able to demon-

reconstituted by interleukin-2. J. Immunol. **135** (1985) 1443–1449.

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strate *M. leprae*-responding CD4+ cells from these lesions.

From the original data of Bloom and Mehra[42] it is clear that suppressor T cells could be demonstrated in the peripheral blood of patients with BT, BL, and LL lesions. It is obvious, however, that BT patients are markedly different clinically from LL patients. While it is generally stated that LL patients appear to make large quantities of anti-*M. leprae* antibodies, it is interesting to note that the specificity of these antibodies appear to be directed toward only a few *M. leprae* components. Most of these components are crossreactive among mycobacteria, although some have *M. leprae*-specific determinants. LL patients respond to *M. leprae* PGL-I by producing large amounts of antibodies to this antigen, and it has been suggested that PGL-I is responsible for the generation of at least some of the *M. leprae*-specific suppressor T cells.[7, 48] Interestingly, the generation of such cells could be abrogated by monoclonal antibodies to the terminal sugars of PGL-I, suggesting that these cells recognize the same epitopes the antibodies recognize.[7] One, therefore, wonders why such antibodies did not block the generation of the suppressor cells *in vivo* or why the suppressor cells do not suppress anti-PGL-I production. In all fairness, however, it is important to note that the communication between T cells themselves and between T cells and B cells is very complicated, and it is unclear whether helper T cells involved in antibody production are exactly the same cells involved in DTH responses.

Finally, data from studies of exogenously added IL-2 on the *in vitro* lymphocyte responses of LL patients to *M. leprae* are at best contoversial.[49] It is difficult to reconcile these data primarily because of insufficient information with regard to disease characteristics at the time of study. The major lesson from these studies appears to be that a simple lack of IL-2 production cannot be the primary cause of anergy in LL patients, which then implies that if suppressor T cells are at all involved in the pathogenesis of LL, such cells would not seem to function by suppressing IL-2 production. Recently, it has been pointed out that lymphocytes from some LL patients appear not to produce IFN-γ in response to *M. leprae*.[50] In the LL lesion, lack of IFN-γ appears to be reflected by the absence of MHC class II molecules on keratinocytes.[51] It is now clear that both CD4+ and CD8+ T cells can produce IFN-γ and, thus, it is uncertain why the activated CD8+ T cells found in LL lesions do not produce IFN-γ. During erythema nodosum leprosum (ENL) reactions, keratinocytes express MHC class II molecules, perhaps reflecting an increased production (locally) of IFN-γ. It is not known whether this increased IFN-γ production reflects an increased local DTH response to *M. leprae* or whether this could be a result of T cells recruited into the lesion in response to other antigens.

While assessing the possible role of suppressor T cells in the pathogenesis of LL, it is equally important to ask ourselves: "What would these suppressor cells suppress?" To answer this question, we must first and unequivocally demonstrate in the first place that LL patients can, in fact, show a T-cell response to *M. leprae,* and to date no data has achieved this. The absence of *M. leprae* responsive CD4+ T-cell clones from LL lesions makes one wonder what the CD8+ suppressor T cells in these lesions would suppress, for they cannot suppress a nonexistent CD4-mediated response. At the moment, with regard to LL, we have accumulated a large amount of negative data, such as paucity of CD4+ cells in the lesions, lack of IL-2-producing cells in both the lesions and peripheral blood, lack of IFN-γ

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producing cells in the peripheral blood, and lack of *M. leprae*-responding CD4+ T-cell clones from the lesions. What we yearn to know is what could account for all of these in the first place.

One possibility that could account for many of the immunological perturbations seen in lepromatous leprosy involves some sort of genetic lesion. This is a mechanism that has been proposed for as long as there has been leprosy. It is not a very popular theory, for it has immense social implications. Furthermore, this theory does not augur well with our current efforts at developing an *M. leprae*-specific vaccine. Recent developments in the analysis of tolerance suggest that clonal deletion may actually be a real phenomenon. It should thus be possible to address the question of whether clonal deletion could underlie the specific anergy seen in LL patients. The possibility that individuals who develop LL might have a self antigen that is sufficiently mimicked by *M. leprae,* and thus tolerance (natural) to the self antigen would also result in tolerance to *M. leprae,* has not been critically examined. Current studies of the genetic control of immune suppression offer a meeting point between those favoring acquired suppressor mechanisms and those in favor of a genetic lesion. It is possible that certain genes control the generation of suppressor cells and such suppressor cells could then influence the outcome of the disease, but it is also possible that the suppressor systems currently available only address an epiphenomenon. It will be extremely surprising if the anergy in LL patients turns out to be mediated by one mechanism. Deletion, suppressing, network interactions, as well as mechanisms not yet understood are bound to be involved—and this, again, argues very strongly for the study of leprosy *in toto.* Understanding the pathogenesis of LL as it occurs in a real patient is extremely important for vaccine development. Taking a cue from the studies of Modlin, *et al.*,[27] who showed that about 50% of all CD4+ T-cell clones from tuberculoid lesions respond to cross-reactive *M. leprae* antigens, one wonders whether one really needs an *M. leprae*-specific vaccine to induce protective immunity against leprosy. Furthermore, if, for one reason or another, certain individuals are unable to develop CD4+ T cells that respond to *M. leprae,* one has to ask oneself whether a *M. leprae*-specific vaccine is the ideal vaccine for such individuals.

Lessons from reactional episodes. To the clinician and the patient, these episodes are important in that they are tissue damaging and thus are a primary cause of morbidity. To the immunologist, they offer a natural disease-related setting to address the question of immunoregulation. It has been proposed and accepted, albeit without much supportive data, that reversal reactions are due to sudden increases in DTH to *M. leprae* antigens. It is not clear what triggers this sudden increase in DTH and whether this DTH is directed at *M. leprae*-specific antigens or not. The all too common explanation that reversal reactions are precipitated by a sudden release of *M. leprae* antigen following chemotherapy appears too simplistic. Clinical evauation of patients shows that BT patients develop one or two clinically obvious reversal reactions, while immunohistological evaluation reveals that bacillary antigens can be detected in the tissue for a considerable period of time.[52] Furthermore, it has recently been shown that histologically detectable microreactions are far more common than has hitherto been thought.[53] Thus, the increased *in vitro* lymphoproliferative responses during reversal reactions appear to only detect the tip of the iceberg and correlate only with the clinically obvious reversal reaction. The most clinically important tissue damaged in leprosy is the peripheral nerve. Since such studies of the immunopathology of leprosy are not complete until we have a fair knowledge of the immunology of the peripheral nerve, it is heartening that studies of neuroimmunology in relation to leprosy neuropathy have started.

In the pre-dapsone era, erythema nodosum leprosum (ENL) was the most common cause of death (by suicide) in some lepro-

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saria. While we have better drugs to manage ENL today, we still do not know how this reaction is precipitated. Many theories have been proposed but none appears to be complete. ENL per se offers an excellent opportunity to study immunoregulation in a state of anergy.

Conclusion. The late 1960s and early 1970s were years of extreme excitement in both experimental and clinical analysis of leprosy immunology. The Ridley-Jopling spectrum received considerable boosting from these studies and the association of delayed-type hypersensitivity and reversal reactions became apparent. The following years marked a phase of consolidation and extension of these findings. It was shown that various *M. leprae* antigens might be involved in the pathogenesis of different tissue-damaging reactions. It was at this time shown that *M. leprae* had a substantial number of antigenic molecules, a majority of which crossreacted with other mycobacteria, and lepromatous leprosy patients produced antibodies to most of these molecules. Modern techniques were used to approach the age-old question of antigen-specific anergy in LL patients. The results of this approach, while not simple or easy to interpret, have generated fascinating discussions which have implications on our understanding of the immunobiology of anergy and tolerance. The availability of monoclonal antibodies together with molecular biology techniques should make it possible to analyze specific epitopes and their significance in the pathogenesis of the disease. The recent application of T-cell cloning techniques to the study of leprosy should be commended. It must be cautioned, however, that *M. leprae* has proved to be a very successful bacterium and a shot-gun approach may not answer all of our questions. Developments in immunology have provided us with an armamentarium of techniques with which to explore the immune response. Application of these techniques has, so far, appeared to have led to diverse results in leprosy immunology, strongly suggesting a need for developing more refined techniques and coherent approaches to probe the immune response in leprosy patients. Whereas there might be a tendency to neglect well-established clinical and immunological techniques in favor of modern ones, we would like to state very strongly that such a decision may not be very wise. Our studies, together with those of others, of clinically well-defined lesions have yielded information that would otherwise not have been obtained.

Finally, it is extremely important to remember that in leprosy the best model so far is the patient. One must view the frequently stated statement that "patients were classified according to the Ridley-Jopling criteria" with extreme caution since the Ridley-Jopling spectrum is dynamic, taking into consideration the clinico-pathological evolution of the disease. Apart, perhaps, from the pure tuberculoid and pure LL (both of which are rare), the spectrum is not static. Basing conclusions and drawing implications, therefore, from data solely relying on a time-point histologic diagnosis of a lesion can prove dangerous. The habit of lumping together BT and TT or borderline lepromatous, subpolar lepromatous and lepromatous leprosy, as if all these groups evolved in exactly the same way, is an oversimplification, and this all too common habit should be re-examined. We believe that if the patient being studied is taken as a whole (and not just as a biopsy report and results of some milliliters of blood), interpretation of currently available data would be made much easier and the results, which at times seem contradictory, could perhaps be reconciled. We admit that many questions need to be answered and several of the currently proposed theories should be reevaluated, but we also feel that the time has arrived to remember the patients, their lesions, and the lessons they are trying to teach us. We do not wish to leave the impression that the immunology of leprosy is merely of academic interest. We believe that advances in our understanding of the immunopathology of leprosy, as are advances in chemotherapy, are terribly important in the eventual control or eradication of this age-old disease. As immunologists, however, we must learn to accept that animal or *in vitro* model systems are no substitute for well-conducted clinical studies.

What we want to ask is: "Now that we have many more tools to dissect the immune system, should we not pause and as-

sess where the patients and their lesions fit in all the data we generate?"

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