Th1-Th2 Paradigm: Insights from Leprosy

Robert L. Modlin
Division of Dermatology and Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, California, U.S.A.

The mechanism by which T cells and cytokines regulate immune processes in skin can be investigated by studying patients with leprosy. The disease, caused by the obligate intracellular bacterium *Mycobacterium leprae*, forms a spectrum. At one pole, patients with tuberculoid leprosy are able to restrict the growth of the pathogen and their skin lesions are characterized by a predominance of CD4+ T cells and type 1 cytokines including interleukins 4 and 10. At the opposite pole, patients with lepromatous leprosy are unable to contain the infection and their skin lesions are characterized by a predominance of CD8+ T cells and type 2 cytokines including interleukin 12, which selectively favors expansion of CD4+ T cells producing interferon γ. By understanding the factors that regulate T-cell and cytokine responses in leprosy, it should be possible to devise specific immunologic interventions in diseases of skin. J Invest Dermatol 102:828–832, 1994

Leprosy, or Hansen’s Disease, is a chronic, infectious disease of human beings that primarily affects the skin, mucous membranes, and nerves. The disease is caused by a rod-shaped bacillus, *Mycobacterium leprae*. The leprosy bacillus was identified in 1874 by the Norwegian physician Gerhard Henrik Armauer Hansen (1841–1912).

In both the Old and New Testaments the name leprosy is given to a number of physical conditions unrelated to leprosy. These conditions were considered a punishment from God for sin. The victim was said to be in a state of tsara‘ath, or defilement. This Hebrew term was later translated as lepros, from which came the word leprosy.*

This excerpt, recently published on CD-ROM, suggests that the earliest case reports of leprosy were misdiagnosed. Nevertheless, in the current era, sufferers of leprosy are some of the most stigmatized individuals in society. Although Hansen discovered the leprosy bacillus 120 years ago, the pathogen evades attempts at culture and has recently become resistant to some antibiotic therapy. These are clearly reasons enough to study leprosy. But an equally compelling reason is that leprosy provides an extraordinary window onto immune regulation in humans.

Leprosy is an ideal model because it presents as a spectrum of clinical manifestations that correlate with immune responses to the pathogen. At one end of the spectrum, patients with tuberculoid leprosy typify the resistant response that restricts the growth of the pathogen. The number of lesions is few, although tissue and nerve damage is frequent. At the opposite end of this spectrum, patients with lepromatous leprosy represent extreme susceptibility to *Mycobacterium leprae* infection. In lepromatous leprosy, the skin lesions are numerous and growth of the pathogen is unabated, resulting in many viable *M. leprae* throughout the skin lesions. These clinical presentations correlate with the level of cell-mediated immunity (CMI) against *M. leprae*. The standard measure of CMI to the pathogen is the Mitsuda reaction. Patients are challenged by intradermal injection of *M. leprae* and induration is measured 3 weeks later. The test is positive in tuberculoid patients and negative in lepromatous patients.

It is widely agreed that T cells involved in cell-mediated immunity are pivotal in determining the outcome of infection with *M. leprae*, because skin test and lymphocyte reactivity are positive in tuberculoid patients but negative in lepromatous patients. Yet there is an interesting paradox in that CMI and humoral responses exhibit an inverse relationship. Anti-*M. leprae* antibodies are most elevated in patients with the lepromatous form of the disease, and therefore are not thought to play a role in protection. A goal of our studies was to determine whether the resistance to *M. leprae* infection (associated with CMI) versus the susceptibility to *M. leprae* infection (associated with humoral responses) could be correlated with distinct cytokine patterns.

THE TH1-TH2 PARADigm

A major paradigm shift in how we think about immunoregulation resulted from the analysis of cytokine patterns produced by murine CD4+ T-cell clones [2]. T cells that produce interleukin 2 (IL-2) and interferon γ (IFN-γ), termed Th1 cells, could be envisioned as contributing to CMI, whereas T cells that produce IL-4 and IL-5, termed Th2 cells, augment humoral responses. In murine models of intracellular infection, resistant versus susceptible immune responses appear to be regulated by these two T-cell subpopulations [3–5]. Th1 cells preferentially activate macrophages to kill or inhibit the growth of the pathogen, resulting in mild or self-curing disease. In contrast, Th2 cells facilitate humoral responses and inhibit some cell-mediated immune responses, resulting in progressive infection. Through the investigation of the active lesions of leprosy, we have found that this paradigm may explain the dichotomy of responses to many human pathogens (Fig 2).

CYTOKINE PATTERNS IN LEPROSY LESIONS

Initially, IL-2 was detected in leprosy lesions by monoclonal antibodies and immunoperoxidase techniques [6,7]. An order of magnitude greater number of IL-2-containing cells were present in tuberculoid lesions as compared with lepromatous lesions. By in situ hybridization, it became readily apparent that IFN-γ mRNA was also more strongly expressed in tuberculoid lesions [8]. These early studies indicated that the “Th1” cytokines were most prominent in the form of leprosy that is characterized by CMI to the pathogen.

To more fully probe patterns of cytokines in lesions at the ex-

Reprint requests to: Dr. Robert L. Modlin, Division of Dermatology, UCLA School of Medicine, Los Angeles, CA 90024.

Abbreviations: CMI, cell-mediated immunity; DCL, diffuse cutaneous leishmaniasis; LCL, localized cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis.

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In contrast to the set of cytokine mRNAs present in tuberculoid lesions that might be involved in CMI and inflammation, those found to be increased in lepromatous lesions might be expected to contribute to the immune unresponsiveness and failure of macrophage activation in these individuals. IL-4 was increased in lepromatous lesions compared with tuberculoid lesions. IL-4 may contribute to the elevated anti-M. leprae antibodies in lepromatous patients via its role in differentiation and immunoglobulin class switching of B cells [23] as well as its ability to stimulate Th2 proliferation [24]. IL-4 also has a negative immunoregulatory effect on CMI that could lead to enhanced bacterial growth, because it 1) blocks IL-2-dependent proliferation of human T cells by downregulation of IL-2 receptors [25]; 2) abrogates both the IFN-γ-mediated activation of monocytes and their anti-leishmanial activity [26]; 3) downregulates CD14 expression on monocytes and production of IL-1β and tumor necrosis factor α (TNF-α) [27]; and 4) blocks macrophage nitric oxide generation necessary for killing intracellular pathogens [28].

**CYTOKINE PROFILES OF CD4 AND CD8 T CLONES**

What are the T-cell populations responsible for the production of these distinct cytokine patterns? Earlier immunoperoxidase and immunofluorescence studies had indicated differences in the CD4 : CD8 ratio at the poles of the leprosy spectrum [6,7,29-31]. The data from these studies indicate that in tuberculous leprosy lesions the CD4+ population predominated, with a CD4 : CD8 ratio of 1.9 : 1, whereas in the lepromatous lesions the CD8+ population predominates with a CD4 : CD8 ratio of 0.6 : 1. Furthermore, CD4+ cells in tuberculoid lesions express the T-memory phenotype (CD45R0+) [32]. The majority of CD8+ cells in lepromatous lesions are CD28−, indicating that they are of the T-suppressor phenotype [32]. Of CD4+ cells cultured directly from tuberculoid lesions, one in 60 react to M. leprae antigens [32]. CD8+ cells from lepromatous lesions fail to proliferate to antigen but can be activated by M. leprae to inhibit proliferative responses by CD4+ cells and are termed “T-suppressor cells” [33,34].

To gain insight into the molecular and cellular basis of T-cell-mediated protection and suppression, as well as to correlate the cytokine patterns in lesions with functional subsets of CD4+ and CD8+ T-cells, we examined the profile of cytokines produced by T-cell clones derived from patients across the spectrum of leprosy [35]. The patterns of cytokine secretion of these clones were compared to the cytokine profiles of cells specific for other antigens bearing the same surface phenotype, but differing in function. CD4 clones specific for tetanus toxoid and CD8 major histocompatibility complex class I-restricted T-cytotoxic cells specific for HLA-B27 were studied. The T-cell clones were stimulated via the T-cell receptor-CD3 complex using anti-CD3 monoclonal antibodies, and the cytokines that they released were measured [35].

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**Figure 1.** The spectrum of leprosy.

**Figure 2.** A model for immunoregulation in leprosy.
All of the *M. leprae*-specific CD4+ clones obtained from tuberculooid patients produced IL-2, IFN-γ, and granulocyte/macrophage colony-stimulating factor, but little or no IL-4 or IL-5, similar to the pattern of cytokines characteristic of murine Th1 cells. These clones also lacked helper activity for antibody formation; we therefore designated them as “type 1” CD4+ T cells. The CD4+ tetanus toxoid clones produced low levels of IFN-γ and IL-2, but high levels of IL-4 and IL-5, a pattern similar to that of Th2 cells. These clones were found to have B-cell helper activity and were designated as “type 2” CD4+ T cells. Thus, functionally different CD4+ T cells from strongly antigen-reactive donors can be subtyped into two groups, similar to the murine counterparts, based on cytokine patterns.

Similarly, we were able to define subpopulations of CD8+ T cells based on their cytokine pattern. The majority of the CD8+ alloreactive T-cytotoxic clones tested secreted IFN-γ, but made no detectable IL-4 or IL-5. Of particular interest, the pattern of cytokine production by the CD8+ T-suppressor clones was characterized by high levels of IL-4 and low levels of IFN-γ. Based on patterns of cytokine secretion, particularly of IL-4, the data suggest that the human CD8 population can also be divided into two functional subsets, which we designate as “type 1” and “type 2” CD8+ T cells.

IL-4 facilitated the growth of CD8+ T cells and T cell clones from lepromatous donors, suggesting that it may act as an autocrine growth factor for the CD8+ type 2 subset [36]. The critical role for IL-4 in mediating suppression of CD4+ T-cell responses was shown using neutralizing anti–IL-4 antibodies [35]. We also found that the suppressor activity of CD8+ type 2 cells could be abrogated by the addition of anti–IL-4 antibodies to the cultures.

It is a well-known immunologic generalization that a dichotomy exists between antibodies to a pathogen and cell-mediated immunity. As a case in point, lepromatous leprosy patients, lacking CMI, have significantly higher levels of anti-*M. leprae* antibodies than tuberculooid patients. These data and the results of murine infection models can best be reconciled by appreciating that IL-4 has the capacity not only to enhance antibody formation, but also to depress multiple components of CMI required for protection. However, it should be noted that there may be significant contributions to the outcome of infection by other cytokines. More likely it is the matrix of cytokines that determine the ultimate biologic response at the locus of infection, protective immunity, and/or immunopathology.

**A ROLE FOR IL-10**

Although IL-10 mRNAs were prominent in lepromatous lesions, the T-cell clones from the lesions produced only small amounts of IL-10 protein [35,36]. The presence in lepromatous lesions of IL-10, a cytokine that can down-regulate cytokine production by CD4 T cells in the relative absence of IFN-γ or IL-2, suggests a possible role for this cytokine in the specific immunologic unresponsiveness to *M. leprae* antigens [37,38]. To define more precisely the role of IL-10 in human infection, we studied in vitro responses to *M. leprae* [36]. *M. leprae* triggered IL-10 release from peripheral blood mononuclear cells of patients and healthy donors; the predominant source of the IL-10 was found to be macrophages. Neutralizing anti–IL-10 monoclonal antibodies significantly enhanced *M. leprae*-specific T-cell proliferation and release of TNF-α, granulocyte/macrophage colony-stimulating factor, and IFN-γ. These data indicate that *M. leprae* induces IL-10 production, which inhibits T-cell proliferation and the release of cytokines that have anti-mycobacterial properties.

**REVERSING CYTOKINE PATTERNS**

Leprosy is not a static disease but an extremely dynamic condition in which immune changes alter the clinical manifestations, in the form of “reactional states.” These reactional states provide a window onto the dynamic immune events associated with immunoregulation in human disease. Reversal reactions are generally known to be naturally occurring delayed-type hypersensitivity (DTH) responses to *M. leprae*, associated with clearance of bacilli from lesions and upgrading towards the tuberculoid pole [39–43]. The onset of reversal reactions is associated with the emergence of T-cell responsiveness to *M. leprae*. Reversal reaction lesions are characterized by the influx of CD4+ T cells [44]. We analyzed the dynamic changes in cytokine patterns by studying biopsy specimens from patients before the onset of reversal reaction and during the reaction [10]. As patients with reversal reaction upgrade from the lepromatous pole, we found a concomitant switch from type 2 to type 1 cytokine production locally. These data provide clear evidence that DTH responses involve the influx of T cells secreting type 1 cytokines. Furthermore, because the cytokine pattern changes during a naturally occurring reaction, it should be possible to devise immunotherapeutic tools to artificially reverse the cytokine profile. In fact, cutaneous administration of either IL-2 or IFN-γ to lepromatous patients results in some clearance of bacilli from lesions [19,45], although there is little or no gain in immunologic memory.

**LEARNING FROM LEISHMANIASIS**

Leishmaniasis, like leprosy, is not a single disease entity, but a set of clinical entities each with a differing immunopathogenesis. These different clinical presentations may comprise an immunologic spectrum [46]. Patients with localized cutaneous leishmaniasis (LCL) have few skin lesions in which the growth of the parasite is restricted. In contrast, patients with diffuse cutaneous leishmaniasis (DCL) have widely disseminated lesions and the growth of the parasite appears to be unabated. The Montenegro test, a DTH response to intradermal challenge with *Leishmania* antigen is positive in LCL patients and negative in DCL patients. A minority of LCL patients heal, but subsequently relapse to the mucocutaneous (MCL) form. Patients with MCL may fall somewhere in between the two poles of the LCL-DCL spectrum, having some resistance against disseminated infection, but not being able to fully eliminate the parasite, a situation resulting in chronic destructive lesions.

By PCR we determined that the type 1 cytokine pattern was present in the Montenegro reaction and the self-healing LCL group [47]. The abundance of IL-2, IFN-γ, and lymphotixin is likely to contribute to the resistant state of immunity and elimination of parasites. On the other hand, MCL lesions appear to be characterized by a mixture of type 1 and type 2 cytokines. The greatest difference found in MCL versus LCL lesions was the several orders of magnitude greater amount of IL-4 mRNA in MCL lesions [48]. Although IFN-γ is present, the levels of IL-4 may be sufficient to inhibit macrophage function so that all the parasites are not eliminated and the infection persists. In DCL lesions, the level of IL-4 is greatest, so that the parasite’s growth is unabated. These data indicate the crucial role of IL-4 in determining outcome in cutaneous leishmaniasis. Furthermore, these studies provide evidence that the pathogenesis of human leishmaniasis is associated with distinct cytokine patterns that conform to the Th1-Th2 paradigm.

To gain insight into the functional role of the CD4+ and CD8+ subsets in LCL lesions in contributing to the observed cytokine patterns, we examined the cytokine mRNAs of the sorted populations from lesions [49]. The CD4+ subset was characterized by high levels of IFN-γ and lymphotoxin in MCL vs. low levels of IFN-γ and lymphotoxin but weak expression of IL-4 in DCL lesions. In contrast, the CD8+ subset was characterized by high levels of IL-4 mRNAs with weak expression of IFN-γ and lymphotoxin RNAs, consistent with a type 2 cytokine pattern. Finally, IL-10 mRNA was present in both subsets but more strongly expressed in the CD8+ subset. The outcome of the human immune response to leishmania, in a manner analogous to mycobacteria, may depend on the balance between CD4+ type 1 T cells and CD8+ type 2 T cells.

**FACTORS THAT INFLUENCE THE CYTOKINE PATTERN**

One of the major issues in immunology today is the elucidation of the key factors that determine or bias Th1 versus Th2 responses. In the murine model of leishmaniasis, distinct antigen fractions of *Leishmania*, as well as antigen dose, select the functional T-cell population [50]. Yet more current data in a mouse model indicates
that identical T-cell receptors, hence identical antigens, can give rise to the Th1 versus Th2 patterns [51]. Our studies of human leishmaniasis indicate that the human T-cell populations responsible for the Th1 and Th2 patterns bear distinct T-cell receptors and hence may recognize distinct antigens [49].

In mouse models, the antigen-presenting cell may be a determining factor. Macrophages may preferentially present antigen to Th1 cells, but B-cells present antigen to Th2 cells [52]. It is noteworthy that Langerhans cells, powerful antigen-presenting cells of the skin, can present to both Th1 and Th2 cells, but when ultraviolet irradiated present only to Th2 cells [53]. Cytokine patterns may be specific according to anatomic location: T cells in lymphoid organs draining nonmucosal tissue sites produce IL-2 whereas those draining mucosal sites produce IL-4 [54]. It is also important to consider the major histocompatibility complex genotype that may select particular T-cell populations [55]. In leprosy, the major histocompatibility complex restricting element may be critical: the majority of CD4 type 1 cells are restricted by HLA-DR [56], but the CD8 T-suppressor type 2 cells are restricted by HLA-DQ [57].

Many immunologists consider that "natural immunity" is involved in determining outcome in that cells other than T cells may rapidly produce cytokines that bias the T-cell cytokine response [58]. Infected macrophages usually release IFN-γ and IL-12, which activate natural killer cells to release IFN-γ, with a subsequent bias towards a Th1 response [59]. In vivo, susceptible mice are cured of L. major infection when recombinant IL-2 is administered before or at the time of infection [60,61].

**IL-12: A KEY DETERMINANT?**

We investigated the role of IL-12 in regulating type 1 T-cell responses in human infection by employing leprosy as a model [62]. The local production of IL-12 in leprosy lesions was evaluated using the polymerase chain reaction. IL-12 is a 70-kd heterodimer produced by macrophages. The constitutively produced IL-12 p35 mRNA was present at equivalent levels in both forms of the disease. In contrast, IL-12 p40 mRNA levels were significantly higher in tuberculoid than lepromatous lesions. Therefore, the local expression of IL-12 was greatest in the group of patients characterized by type 1 cytokine responses.

One characteristic of IL-12 is its potent T-cell growth factor activity [63]. We found that T-cell proliferation to M. leprae was dependent on the endogenous production of IL-12 in that we could block proliferation in vitro by the addition of neutralizing IL-12 antibodies. IL-12 has been shown to direct T cells in a primary response towards a type 1 cytokine pattern [64-66]. A striking finding of our analysis of T-cell responses in leprosy was that rIL-12 induced proliferation of CD4+ type 1 cell clones from tuberculoid lesions but not CD8+ type 2 cell clones from lepromatous lesions. All clones proliferated in response to rIL-2. Therefore, a mechanism by which IL-12 may contribute to CMI is via the preferential expansion of differentiated T cells committed to the type 1 cytokine pattern.

Can IL-2 be used as an immunotherapeutic adjuvant to reverse T-cell unresponsiveness and induce type 1 cytokine responses? Towards this goal, we explored whether rIL-12 could potentiate antigen-induced T-cell responses and augment CMI in unresponsive lepromatous patients. We observed an IL-12-dependent enhancement of T-cell responses in lepromatous patients that could be attributed to an increase in CD4+ T cells. Furthermore, IL-12 induced a preferential increase in the capacity of T cells to produce the type 1 cytokine, IFN-γ, versus the type 2 cytokine, IL-4. Perhaps more intriguing was the ability of anti-IL-10 to augment rIL-12 plus M. leprae-stimulated T-cell proliferation in some patients to levels approaching those of tuberculoid patients. It should be noted that T-cell responses and IFN-γ production could not be augmented in all lepromatous patients. Therefore, CMI may be responsible for inducing T-cell responsiveness and type 1 cytokine responses to infectious pathogens. Yet our data and studies of human immunodeficiency virus infection [67] indicate the potential usefulness as IL-12 in the immunotherapy of infectious disease.

Perhaps combination immunotherapy, employing antagonists to "negative" regulatory cytokines, would be a more effective strategy in combating human infectious disease [66,68]. Hopefully, such approaches can help patients with leprosy and other devastating diseases.

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