State of the Art

The Host Immune Response to Tuberculosis

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SCOPE OF THE PROBLEM

Mycobacterium tuberculosis is one of the most ubiquitous pathogens in the world: estimates are that roughly one third of the world's population is infected with the bacillus, and it is responsible for 8 to 12 million cases of active tuberculosis each year, and 3 million deaths (1). There is compelling clinical evidence that, in addition to the innate virulence of the tubercle bacillus itself, the host response to *M. tuberculosis* plays a major role in determining the clinical manifestations and ultimate outcome of persons who encounter this pathogen. For example, the vast majority of persons infected with the bacillus will never develop any clinical illness. Some will develop active disease in the context of some impairment of their immune system such as that caused by infection with HIV, malnutrition, or advanced malignancy (2), although most cases of active disease occur in persons with no obvious defect in host immunity. In addition, the natural history of active tuberculosis in the preantibiotic era was not uniformly grim (3–5). A substantial proportion of patients with active disease eventually recovered without specific therapy. Even today, a small subset of patients with multidrug-resistant tuberculosis for which little effective chemotherapy is available will have apparent clinical recovery (6, 7). Furthermore, both innate resistance and acquired immunity against tuberculosis seem to exist. The widely used BCG vaccine has at least 50% efficacy in preventing some forms of tuberculosis, and some tuberculin skin-test-positive persons seem protected against developing active tuberculosis despite repeated high level exposure to active cases (8, 9). Reinfection with *M. tuberculosis*, which with the use of restriction fragment length polymorphism analysis has been recently demonstrated to occur on occasion in patients with advanced HIV infection, is apparently a rare event in patients with intact immunity (10–13).

Overall then, a substantial amount of clinical experience indicates that host immunity plays an important role in the host-pathogen interaction occurring in persons exposed to *M. tuberculosis.* Understanding the components of this host response at a basic level is likely to lead to a better understanding of the pathogenesis of tuberculosis in humans and to result in better and novel approaches to prevention and therapy of this disease, which, among adults, remains the leading single cause of death due to infection in the world. In the following review, we will discuss control of initial infection with *M. tuberculosis* by alveolar macrophages, cellular and cytokine responses in the lung during active disease, and the implications of both of these components for understanding the various clinical manifestations of the disease. In this review, we will preferentially review data from human studies or from *in vitro* studies involving human cells or cell lines, though certainly animal models have been extraordinarily useful in understanding the pathogenesis of tuberculosis when human studies are unavailable. Animal and human data will be contrasted, as this comparison is most useful to demonstrate the limitations inherent to models of tuberculosis, despite their critical role in developing and testing hypotheses about host immunity.

INITIAL INFECTION WITH *M. TUBERCULOSIS*

The route of entry of the tubercle bacillus into the body is via the respiratory tract through the inhalation of respiratory droplet nuclei, which are small enough in size (1 to 2 μm or less) to allow passage into the lower respiratory tract (14, 15). Droplets of a larger size are efficiently excluded from the lower respiratory tract by the physical barriers of the nasopharynx and upper respiratory tract. The respiratory bronchial epithelium is remarkably resistant to infection by *M. tuberculosis.* (But virulent mycobacteria are cytotoxic for alveolar type II cells) (16). Although direct evidence of antimycobacterial action is lacking, the bronchial epithelium can produce antimicrobial peptides with a wide spectrum of activity (17).

Once organisms have made their way into the lung, they have four potential fates (18). The initial host response can be completely effective and kill all bacilli, such that the patient has no chance of developing tuberculosis at any time in the future; the organisms can begin to multiply and grow immediately after infection, causing clinical disease known as primary tuberculosis; bacilli may become dormant and never cause disease at all, such that the patient has what is referred to as latent infection, manifest only by a positive tuberculin skin test; or the latent organisms can eventually begin to grow, with resultant clinical disease, known as reactivation tuberculosis. In otherwise healthy hosts with latent infection, a study by Israel and colleagues (19) in nurses indicated that there is a 5 to 10%

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chance over a lifetime of developing active disease (19). A later study of reactivation of inactive tuberculosis in the placebo arm of a Veterans Administration isoniazid preventive therapy trial indicated a reactivation rate as low as 1% over a 7-yr period (20). In severely compromised hosts such as patients with HIV infection, there is perhaps a 7% chance of developing tuberculosis each year after the establishment of latent infection, so that the importance of the host response is readily apparent (21).

Binding of *M. tuberculosis* to Monocytes and Macrophages

The initial defense against infection with *M. tuberculosis*, once it reaches the lower respiratory tract, is the alveolar macrophage, and an overview of the interactions between macrophages and mycobacteria is given in Table 1. This cell is capable of inhibiting growth of the bacillus through phagocytosis, and, as will be discussed later, of participating in a broader context of cellular immunity through the process of antigen presentation and recruitment of T-lymphocytes (22). Although other antigen-presenting cells such as dendritic cells are present in large numbers in the airways, their exact role in host defense against tuberculosis has not been well established to date, and most of the work reviewed below involves monocytes and macrophages (23). Processes involved in phagocytosis include binding of the bacterium to the host cell, internalization, and finally growth inhibition or killing. As a general phenomenon, phagocytosis usually begins with the phagocytic cell engulfing the invading microbe by engulfing it in a membrane-bound tight vacuole, which is created when pseudopods surround the bacterium and fuse distally (24). Substantial experimental evidence exists that in the *M. tuberculosis*/mononuclear phagocyte interaction, the creation of this vacuole, or phagosome, is accompanied by binding of the organisms to the phagocyte through complement receptors CR1, CR3, and CR4, as well as mannose receptors (MR) and other cell surface receptor molecules (25). The addition of non-immune serum to monocyte-derived macrophages enhances binding of organisms to phagocytic cells, indicating the important role of complement in this system (26). In addition, Schlesinger (27) has shown that there may in fact be differences between specific binding mechanisms for virulent and relatively avirulent strains of mycobacteria, as blocking complement receptors with monoclonal antibodies inhibits phagocytosis of *M. tuberculosis* strain H37Ra (an avirulent strain) and the Erdman and H37Rv strains (virulent strains), but downregulation of mannose receptors is associated with decreased binding of only the virulent strains. The interaction between mannose receptors on phagocytic cells and mycobacteria seems to be mediated through the mycobacterial surface glycoprotein lipoarabinomannan (LAM), which is present on the cell wall of the mycobacteria, including virulent strains of *M. tuberculosis*, and is capped by a mannose residue (28). Fc receptors and the β-glucan receptor do not seem to be of major importance in mediating binding of *M. tuberculosis* to mononuclear phagocytes or to alveolar macrophages (26, 29).

TABLE 1

MACROPHAGE-MYCOBACTERIUM INTERACTIONS IN THE HOST RESPONSE AGAINST TUBERCULOSIS

I.	Surface binding of *M. tuberculosis* to the macrophage
	Complement receptors CR1, CR3, CR4
	Mannose receptors
	Surfactant protein receptors
	CD14
	Scavenger receptors
II.	Phagosome-lysosome fusion
III.	Mycobacterial growth inhibition and/or killing
	Production of reactive nitrogen species
	Production of reactive oxygen species
	Apoptosis
IV.	Recruitment of accessory immune cells and development of a local inflammatory response
	Elaboration of cytokines, e.g., TNF-α
	Elaboration of chemokines, e.g., IL-8
	Antigen presentation

The expression of complement and mannose receptors by macrophages seems subject to the influence of a variety of mediators, including PGE_2 and the cytokines IFN-γ and IL-4. PGE_2 and IL-4, a Th2-type cytokine, upregulate CR and MR receptor expression in general, and IFN-γ has been demonstrated to decrease receptor expression and function, with resulting diminished ability of mycobacteria to adhere to macrophages (30, 31). This apparently negative effect of IFN-γ stands in contrast to the demonstrated ability of that cytokine to activate macrophages and increase intracellular growth inhibition (and perhaps actual killing) of mycobacteria.

In addition to complement and mannose receptors, accumulating evidence exists for an important role for surfactant protein receptors in mediating bacterial binding (32, 33). Using cell monolayers composed of human monocyte-derived macrophages, addition of surfactant protein-A (SP-A) obtained from patients with alveolar proteinosis (as well as recombinant rat SP-A) causes enhanced adherence of *M. tuberculosis*; this binding was not diminished after washing away the SP-A. The enhancement of binding seems to be dependent on the carbohydrate moieties of the SP-A molecule, as SP-A protein lacking carbohydrate fails to enhance binding to macrophages.

A role for CD14 has been demonstrated for attachment of mycobacteria to microglia, which are the resident phagocytic cells in the brain, and this ligand may be important for binding of *M. tuberculosis* to alveolar macrophages as well (34). Recent work has shown that antibodies that bind to CD14 or soluble CD14 inhibit infection of microglial cells with a virulent laboratory strain, H37Rv. In addition, it had previously been shown that LAM-stimulated release of TNF-α and IL-1β from the mononuclear phagocytic cell line THP-1 can be inhibited by the addition of anti-CD14 monoclonal antibodies added to the cell line-mycobacterial system (35).

Finally, in addition to the receptors described above, it is likely that recently described scavenger receptors may also play a role in mediating binding of mycobacteria to phagocytic cells (36). These scavenger receptors are located on the macrophage surface and have affinity for a wide variety of ligands, including low density lipoproteins, polyribonucleotides, polysaccharides (including dextran sulfate), anionic phospholipids, and other molecules, including asbestos particles and bacterial endotoxin. When complement receptors C1, C3, and C4, as well as the mannose receptor, are blocked using monoclonal antibodies, overall phagocytosis by human monocyte-derived macrophages is reduced by only about 50 to 60%. However, when competitive ligands (fucoidin and dextran sulfate), which bind scavenger receptors, are added to a system in which C1, C3, C4, and mannose receptors are blocked, phagocytosis is further diminshed, in a dose-dependent manner, to achieve almost complete inhibition of incorporation of the bacillus. Even when complement and mannose receptors are left unblocked, fucoidin and dextran sulfate are able to inhibit nearly 80 to 90% of phagocytosis by themselves.

Fate of *M. tuberculosis* after Phagocytosis: Direct Growth Inhibition by Macrophages

After pathogenic bacteria are engulfed into phagosomes, they are subject to killing via a variety of mechanisms, including

phagosome-lysosome fusion, generation of reactive oxygen intermediates, and generation of reactive nitrogen intermediates, particularly nitric oxide. Understanding of these initial macrophage defenses may lead to important insights into the development of clinically latent infection, as evasion of these macrophage defenses is likely a key step in establishing a focus of infection that may cause active disease later.

Phagosome-lysosome fusion has been extensively studied (and well reviewed by several investigators) with regard to mycobacteria, but the exact role of this cellular process in host defense against *M. tuberculosis* remains somewhat unclear (37–39). Studies by Gordon and D'Arcy Hart (40) demonstrated that mycobacteria are capable of producing ammonia, which could both inhibit phagosome-lysosome fusion and, by alkalinizing the intralysosomal contents, diminishing the potency of the fusion complex. Similarly, sulfatides (derivatives of trehalose 2-sulfate, a glycolipid produced by *M. tuberculosis*) had been previously shown by Goren and colleagues (41, 42) to also inhibit phagosome-lysosome fusion. However, the exact role of these potential "escape" mechanisms in the pathogenesis of human disease is uncertain. Sulfatides, for example, are produced by many mycobacterial species, including some that are nonpathogenic. Similarly, recent evidence that *M. avium* (as well, perhaps, as *M. tuberculosis*), by excluding the proton-ATPase from the phagosome, can prevent acidification of the phagolysosome must be tempered by older work indicating that *in vitro* growth of *M. tuberculosis* (including some guinea pig virulent strains) at an acid pH of 4.5 differed little if at all from growth at pH 7.0 (43, 44). Virulent mycobacteria were able to escape from fused phagosomes into vacuoles with membranes tightly opposed to the mycobacteria, and multiply (45, 46). The arrest of mycobacterial phagosome maturation has been shown to occur at rab-7, a small ras-like GTP-binding protein specific for late endosomes that does not accumulate on the vesicle membrane. In contrast, rab-7 can be identified within hours on vesicle membranes containing latex beads (47).

Once inside the macrophage, there is evidence that *M. tuberculosis* can be killed by several different mechanisms through a host of complicated interactions, mediated by cytokines, between lymphocytes and phagocytes (Figure 1). The macrophage itself is capable of producing both reactive oxygen species and reactive nitrogen species, though most recent evidence suggests the latter is more important than the former in human mycobacterial defense (43, 48–51), though this is still a matter of controversy, and differences between murine and human studies abound. For example, MacMicking and colleagues (52) have recently shown that in genetically altered mice that lack the ability to produce inducible nitric oxide synthase (iNOS$^{-/-}$ knockout mice), *M. tuberculosis* replicates much faster than in wild-type animals, implying a significant role for nitric oxide in mycobacterial host defense, and some evidence for upregulation of iNOS in alveolar macrophages from humans with tuberculosis has also been established (53). On the other hand, Kuo and colleagues (54), using alveolar macrophages obtained from patients with pulmonary tuberculosis, demonstrated a higher capacity for generation of reactive oxygen species (particularly H_2O_2) compared with cells obtained from normal control subjects. The ability of mycobacteria to evade killing by either reactive oxygen or nitrogen species may be a crucial step in the establishment of the latent state of infection. Although latency has not been well studied to date and few clues are available as to its development, one might speculate that mycobacteria may in some way be able to suppress killing through several mechanisms. Perhaps the bacteria produce substances that inactivate reactive oxygen species

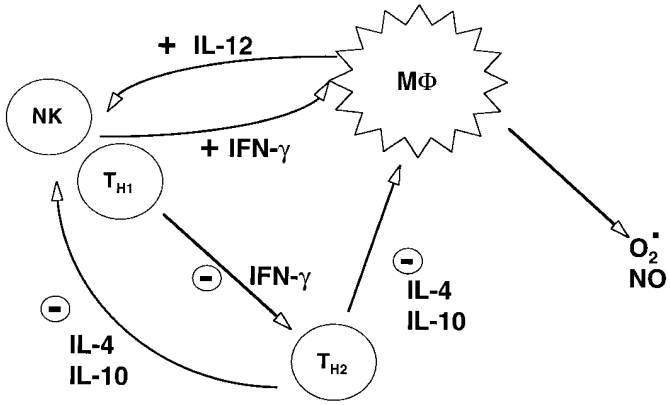

Figure 1. Overview of macrophage-lymphocyte interactions in tuberculosis. Type 1 CD4+ T-lymphocytes (Th1) and natural killer T-lymphocytes (NK cells) secrete interferon gamma, which activates alveolar macrophages to produce a variety of substances, including reactive oxygen and nitrogen species, which are involved in growth inhibition and killing of mycobacteria. Macrophages can also secrete interleukin-12 (IL-12) in a positive feedback loop to amplify this pathway. Although interleukin-4 and -10 can inhibit macrophage function, there is no convincing evidence that these cytokines are present in great amounts in the lungs of patients with tuberculosis, perhaps because of interferon-mediated suppression of Th2 (type 2 CD4+ T-lymphocytes) cell function.

(*M. tuberculosis* is known to produce catalase for example), or perhaps they elaborate substances that can downregulate transcription of genes such as iNOS and thereby evade initial attempts at host defense. This may be followed by the mycobacteria shutting down their own metabolism or cell cycle in a manner not yet understood (55).

Other immune cells offer substantial help to the macrophage in controlling growth of mycobacteria. T-lymphocytes can be recruited to the macrophage and further stimulate it to inhibit growth of or kill mycobacteria (56). Cytotoxic T-lymphocytes can ingest macrophages that have engulfed mycobacteria (57). They can also secrete small proteins such as TIA-1, a cytoplasmic molecule that has been shown to be associated with apoptosis (58). In this section of the review, direct macrophage killing or growth inhibition will be discussed, whereas the role of T-lymphocytes will be discussed later in the context of the cellular profile found in the lungs of patients with active tuberculosis.

The interaction of macrophages with other effector cells occurs in a milieu of both cytokines and chemokines. These molecules serve both to attract other inflammatory effector cells such as lymphocytes and to activate them. An important chemokine in the mycobacterial host-pathogen interaction appears to be interleukin-8 (IL-8), a member of the CXC family of chemokines. (Of interest, the CXC family also includes a number of IFN-γ-stimulated proteins, including the interferon-inducible proteins IP-10 and MIG, a monocyte chemotactic factor. The CXC chemokines include many proteins that recruit granulocytes and lymphocytes to areas of infection and inflammation.) IL-8 has among other functions the ability to recruit neutrophils, T-lymphocytes, and basophils in response to a variety of stimuli (59). It is released primarily by monocytes/macrophages, but it can also be expressed by fibroblasts, keratinocytes, and lymphocytes (60).

Our group has recently shown elevated levels of IL-8 in BAL fluid from patients with pulmonary tuberculosis as well as in supernatants of alveolar macrophages taken from those

patients and maintained in culture for 24 h (58). IL-8 gene expression was also increased in the macrophages as compared with those in normal control subjects. In a series of *in vitro* experiments, it was also demonstrated that intact *M. tuberculosis* or its cell wall component LAM, but not deacylated LAM, could stimulate IL-8 release from macrophages (61). This stimulated release could be blocked substantially by neutralizing antibodies to TNF-α and/or IL-1α or β. These two cytokines are likely to play an important role in tuberculosis: cells recovered by BAL from radiographically involved areas of pulmonary tuberculosis release elevated levels of TNF-α, IL-1β (and IL-6), compared with cells recovered from radiographically normal areas or from normal control subjects (62). As these latter cytokines are also produced in abundance by macrophages and monoctyes, there seems to be an autocrine regulatory loop in operation during the earliest stages of mycobacterial infection in humans. Interestingly, in the murine model of tuberculosis, Orme and his group (63) have shown that although several laboratory strains of *M. tuberculosis* can stimulate production of chemokines (including macrophage inflammatory proteins and interferon-inducible proteins), TNF secretion was stimulated only by certain strains of mycobacteria. In addition, the murine model of pulmonary tuberculosis does not have the intense neutrophil inflammation observed in patients with tuberculosis. These studies show the difficulties in extrapolating animal and *in vitro* data to the human situation, but they also demonstrate the necessity of animal experiments to tease apart the complex strands of mycobacterial host defense in model systems, which can then be studied in humans.

The role of TNF in host defense against tuberculosis is complex. *In vitro,* TNF-α release can be stimulated from monocytes and macrophages by mycobacteria (and their secreted proteins); this cytokine has actually been reported to promote growth of virulent mycobacteria (64). Early on, Kindler and colleagues (65) demonstrated a role for TNF-α in inducing granuloma formation in a murine model of BCG infection. They demonstrated intense immunostaining for TNF in BCG-induced, well-formed granulomas, but mice treated with anti-TNF antibodies failed to make granulomas and died because of disseminated BCG infection. In humans with tuberculosis, thalidomide (a TNF inhibitor) reduces expression of monocyte TNF-α mRNA *in vivo,* increases plasma IFN-γ levels, and promotes weight gain (66).

Murine knockout models of the 55 kD TNF receptor have revealed the importance of TNF-α for survival against *M. tuberculosis* infection, probably through induction of reactive nitrogen species, and they have also demonstrated that TNF-α was not the crucial factor for the development of caseation necrosis (67).

Additional evidence of a role for chemokines in general and for IL-8 in particular in immunity in human tuberculosis comes from Friedland and colleagues (68, 69) who studied a group of mainly HIV-positive patients. Both plasma IL-8 and secretion of IL-8 after *ex vivo* stimulation of peripheral blood leukocytes with lipopolysaccharide remained elevated throughout therapy for tuberculosis. Other investigators (70) had previously shown that IL-8 was also present at other sites of disease such as the pleural space in patients with tuberculous pleurisy. The complicated nature of chemokine secretion is underscored by another study suggesting that persistently high levels of IL-8 were found in patients who succumbed to their disease (69).

Other chemokines that have been implicated in the host response to tuberculosis include monocyte chemoattractant protein-1 (MCP-1) and regulated on activation normal T-cell expressed and secreted (RANTES), which both decrease in the convalescent phase of treatment, as opposed to IL-8 (71).

The ability of the macrophage to inhibit the growth of *M. tuberculosis* seems to depend on the state of activation of the effector cell, and macrophage activation is an intensively studied phenomenon (72). With regard to tuberculosis, great attention has been focused on the role of the cytokines interferon gamma (IFN-γ) and transforming growth factor beta (TGF-β), in terms of their ability to activate and deactivate the macrophage's ability to inhibit mycobacterial growth. A major role for IFN-γ in mycobacterial host defense has been suggested by a variety of *in vitro* and animal experiments. Nagasawa and colleagues (73) added rhIFN-γ to a culture of human alveolar macrophages and found that glucose consumption and cytotoxicity against HeLa cells was markedly increased. On the other hand, Douvas and colleagues (74) found that in response to lymphokine supplementation, intracellular mycobacterial replication actually increased. Rook and colleagues (75), however, found that there was no effect on intracellular growth in the presence of additional r-IFN-γ. Denis (76) found that the addition of rhIFN-γ to a pool of human monocytes endowed them with no tuberculostatic activity; however, if calcitriol was added to the lymphokine preparation, there was total stasis of growth of mycobacteria, though the mechanism by which this interaction between lymphokine and vitamin occurs is unclear. Using human bone-marrow-derived macrophages, Flesch and Kaufmann (77) found that rhIFN-γ significantly augmented killing of *M. tuberculosis* strain Erdman, but this effect was not seen in *M. tuberculosis* strain Middleburg. Rose and colleagues (78) studied the effect of rhIFN-γ on growth of *Mycobacterium avium* complex in human alveolar macrophages, and found that as a lone agent it had no mycobacteriostatic effect, but significant killing was achieved when M-CSF was added to the interferon-macrophage preparation.

Genetically altered mice that lack IFN or its receptor are extraordinarily susceptible to infection with *M. bovis*, though the mechanism of this susceptibility is not precisely determined (79–81). Vilcek and colleagues (82) found IFN-γ release from peripheral blood mononuclear cells to be depressed after lectin or PPD stimulation in patients with active tuberculosis. A more direct role for this cytokine in tuberculosis in humans has been elucidated recently. Jaffe and colleagues (83) demonstrated that aerosol IFN-γ administered to normal human subjects is capable of activating alveolar macrophages, and Holland and colleagues (84) have used systemically administered IFN-γ to treat a group of patients with systemic infections caused by *M. avium* complex and other non-tuberculous mycobacteria, and beneficial effects were seen. We have administered IFN-γ to several patients with multidrug-resistant tuberculosis who were previously failing therapy (as evidenced by persistently positive smears and cultures despite documented adherence to the best possible antibiotic regimen) and have demonstrated improvements in several clinical parameters: patients became sputum-smear-negative, they gained weight, cavitary lesions seen on chest CT scans improved, and the time it took to isolate *M. tuberculosis* from sputum increased, a finding suggestive of a decreasing bacterial burden (85). Additionally, a cohort of patients has recently been described in which a genetic defect in IFN-γ receptor function is present, leading to infections with usually nonpathogenic mycobacteria (86).

Taken together, these experiments do indeed suggest an important role for IFN-γ in host defense, and it is certainly possible that this cytokine is acting primarily as a macrophage activator. The disparity between the *in vitro* effects of IFN-γ and the effects observed when that cytokine is given as a therapeutic agent demonstrate the complexity of the immune net-

works involved in tuberculosis host defense. It is also possible that some of the effect of IFN-γ is due to effects other than direct augmentation of the inhibitory effect of the phagocyte. IFN-γ might also improve or augment antigen presentation, leading to recruitment of CD4+ T-lymphocytes and/or cytotoxic T-lymphocytes, which might participate in mycobacterial killing.

A macrophage inactivator important in human host defense may be TGF-β. This cytokine is widely distributed and produced mainly by monocytes and macrophages (87). Although it has some proinflammatory effects such as enhancement of monocyte chemotaxis and augmented expression of Fc receptors, TGF-β also has important anti-inflammatory effects, including deactivation of macrophage production of reactive oxygen and nitrogen intermediates, inhibition of T-cell proliferation, interference with natural killer and cytotoxic T-lymphocyte function, and downregulation of INF-γ, TNF-α, and IL-1 release (88). Toosi and Hirsch and their colleagues (89–93), in a series of experiments, have elucidated a role for TGF-β in growth inhibition of *M. tuberculosis* by macrophages. When TGF-β is added to cocultures of mononuclear phagocytes and *M. tuberculosis*, both phagocytosis and growth inhibition were inhibited in a dose-dependent manner. TGF-β also blocked the effect of TNF-α on growth inhibition. A role for this cytokine in the pathogenesis of human tuberculosis *in vivo* is further suggested by the finding that tuberculin (PPD) induces TGF-β production by monocytes from healthy subjects, and the demonstration that TGF-β production is increased in peripheral blood mononuclear cells and lung granulomas from patients with pulmonary tuberculosis. Production of interferon gamma after stimulation with PPD by circulating T-lymphocytes taken from patients with tuberculosis increases in the presence of natural inhibitors of TGF-β (91). It seems plausible, therefore, that at least part of the ability of macrophages to inhibit mycobacterial growth may depend on the relative influence of the cytokines IFN-γ and TGF-β in any given focus of infection.

Another candidate macrophage inactivating cytokine in tuberculosis is IL-10 (94–96). Anti-IL-10 antibodies enhance T-cell proliferative responses *in vitro*; interestingly, in patients coinfected with tuberculosis and HIV, expression by peripheral blood mononuclear cells of IFN-γ, IL-2, and IL-4 is suppressed, but IL-10 levels do not differ from patients with HIV infection.

Once a macrophage has been activated to inhibit growth of mycobacteria, a variety of cellular mechanisms are available to accomplish this effector function (97). It has long been known that reactive oxygen species such as superoxide anion and hydrogen peroxide are important components of host defense against a variety of microorganisms, and early experiments by Walker and Lowrie (48) in murine macrophages demonstrated a possible role for reactive oxygen intermediates (ROI) in host defense against mycobacteria (using the species *M. microti*, a member of the *M. tuberculosis* complex), but a large body of work subsequent to this strongly suggests that ROI have a limited, if any, role to play in host defense. Flesch and Kaufmann (98), for example, infected murine bone marrow-derived macrophages with *M. bovis* and determined the ability of these macrophages to inhibit mycobacterial growth in the presence and absence of scavengers of toxic oxygen species. In cell-free conditions, hydrogen peroxide, but not superoxide anion or hydroxyl radical, inhibited the growth of mycobacteria. However, the addition of superoxide dismutase or catalase to macrophages infected with *M. bovis* and stimulated with interferon-γ had no effect on mycobacterial growth within macrophages: growth inhibition of *M. bovis* was not reversed to any significant degree. Protection against ROI by mycobacteria may be achieved by a variety of bacterial components or products, including the detoxifying effects of LAM on toxic oxygen species, sulfatides (which can suppress production of toxic oxygen species in *in vitro* systems), and possibly a substance known as PGL-1 (an oligoglycosylphenolic phthiocerol diester), though the latter may not be present in most strains of *M. tuberculosis* (41). The most abundant protein secreted by *M. tuberculosis* in short-term culture is the 23-kD antigen superoxide dismutase; catalase is secreted as well (99).

Chan and colleagues (50) demonstrated that murine macrophages stimulated by either interferon gamma and either LPS or TNF-α are capable of inhibiting growth of *M. tuberculosis*, and this inhibition is independent of the macrophage's capacity to generate reactive oxygen intermediates, as the inhibition could be achieved using the macrophage cell line D9, which is ROI-deficient. This work further supports the idea that ROI have little role in defense against mycobacteria. These investigators also showed that the antimycobacterial activity of macrophages seemed to correlate with induction of L-arginine-dependent production of toxic nitrogen species, including NO (nitric oxide), NO_2, and HNO_2. More recently, Nicholson and colleagues (53) have examined freshly obtained (by bronchoalveolar lavage) human alveolar macrophages from patients with tuberculosis and have demonstrated that on average, 65% of macrophages from every patient studied reacted with a specific human antibody against inducible nitric oxide synthase (iNOS, or NOS2), whereas only 10% of macrophages from normal subjects stained positively. In addition, BAL samples also contained iNOS mRNA. A knockout mouse model containing a deleted IRF-1 (interferon regulatory factor) gene is unable to clear inoculation with BCG, and succumbs to overwhelming infection (100). Macrophages from these mice are incapable of releasing NO, and IRF-1 has been subsequently shown to be a transcription enhancer of the iNOS gene. These data, taken together with the above and those from other studies, indicate a potentially major role for RNI in mycobacterial defense.

Another potential mechanism involved in macrophage defense against *M. tuberculosis* is apoptosis, or programmed cell death. Placido and colleagues (101) found that using the virulent strain H37Rv, apoptosis was induced in a dose-dependent fashion in BAL cells recovered from patients with tuberculosis, particularly in macrophages from HIV-infected patients. Recently, Klingler and colleagues (102) have demonstrated that apoptosis associated with tuberculosis is mediated through a downregulation of bcl-2, an inhibitor of programmed cell death. Heat-killed H37Ra or *M. bovis* BCG both caused a decrease in bcl-2 gene expression, but no corresponding changes in bax expression, a genetic signal for increased apoptosis. Molloy and coworkers (103) have shown that apoptosis of macrophages results in reduced viability of mycobacteria contained within.

A fascinating aspect of host defense possibly related to the production of reactive nitrogen species involves a protein known as Nramp (natural resistance associated macrophage protein) (104). This protein is apparently crucial to transporting nitrite from intracellular compartments such as the cytosol to more acidic environments such as the phagolysosome, where it can be converted to NO. Defects in Nramp production or function might therefore be expected to lead to defective production of nitric oxide and increased susceptibility to intracellular pathogens such as mycobacteria. Investigations with animal models of infection with intracellular pathogens support this hypothesis. A strain of BALB/c mice known as

Ity/Lsh/Bcg is extraordinarily susceptible to infection with *Mycobacterium bovis*, *Leishmania donovani*, and *Salmonella typhimurium*, and work carried out in the laboratories of Skamene and colleagues (105, 106) and Blackwell and colleagues (107) has demonstrated that this susceptibility can be mapped to defects in the *Nramp1* gene (originally called the *Bcg* gene) (105–107). However, the relevance of Nramp to tuberculosis, even in the animal model, is controversial. Medina and North (108) have recently shown that though *Nramp1* may indeed control resistance to *M. bovis* in mice, resistance to infection with *M. tuberculosis* may be unrelated to mutations at this locus (108, 109). Mice with the mutant (*M. bovis*-susceptible) phenotype were no different in their susceptibility to *M. tuberculosis* infection than were mice with the wild-type or resistant phenotype. In addition, demonstration of mutations in macrophages of patients either with or without tuberculosis has been lacking.

Potentially, Nramp could explain part of the different susceptibility to tuberculosis infection noted in certain human populations such as Eskimos and African Americans (110, 111). The human homologue of murine *Nramp* has been cloned and has been shown to be expressed in macrophages, making it potentially important in host defense against tuberculosis (112, 113). Furthermore, regulatory elements known to be important to macrophage activation such as IFN-γ can plausibly work through Nramp, as the *Nramp1* gene contains interferon response elements as well as IFN-stimulated response elements and IFN-γ-activating sequences (112). However, evidence confirming the importance of Nramp in defense against human tuberculosis is lacking. Newport and colleagues (114) studied a group of children with susceptibility to mycobacterial infection and found no evidence of *Nramp1* mutations as the cause of this susceptibility. Despite the present uncertainty concerning the role of proteins such as Nramp in host defense against pathogenic mycobacteria in humans, it is likely that future investigation will elucidate several factors involved in innate resistance against infection.

GRANULOMA FORMATION

Studies of guinea pigs injected intradermally with BCG have traced the evolution of granuloma formation experimentally (115). Neutrophils migrate early on the site of inoculation, followed by monocytes, which can be observed to differentiate into macrophages within 2 to 3 d. By 5 to 7 d, large granulomas composed of mature macrophages and immature epithelioid cells are seen. By 9 d, the epithelioid cells had matured and Langerhan's giant cells were noted.

In mature granulomas in humans, immunostaining for dendritic cells demonstrates numerous S100+, CD1a+ cells interspersed among epithelioid cells and displaying long filopodia (116). CD4+ T cells are prominent in the lymphocytic layer surrounding the granuloma and CD8+ T cells are also noted (58). Apoptosis is prominent in the epithelioid cells as demonstrated by condensed chromatin viewed by light microscopy or with the TUNEL technique (117). Proliferation of mycobacteria *in situ* occurs in both the lymphocyte- and macrophage-derived cells in the granuloma (118). Heterotypic and homotypic cell adhesion in the developing granuloma is mediated at least in part by the intercellular adhesion molecule ICAM-1, a surface molecule that is upregulated by *M. tuberculosis* or LAM (119). This upregulation is mediated by increased gene expression directly by mycobacteria and can be amplified by the cytokines TNF-α, IL-6, and INF-γ. The latter two cytokines interact by stimulating an IL-6/IFN-γ palindromic response element on the ICAM-1 gene promoter (120). The differentiated macrophage-epithelioid cells produce extracellular matrix proteins (i.e., osteopontin, fibronectin), which provide a cellular anchor through integrin molecules (121).

Mycobacteria are also capable of inducing caseation necrosis in the center of a granuloma. Although TNF-α may contribute to this process, caseation necrosis occurs in animal models lacking the 55-kD TNF receptor. Recently, our group demonstrated that LAM could upregulate interstitial collagenase gene expression in peripheral blood monocytes; in addition, we showed that the 92-kD gelatinase (matrix metalloproteinase-9) gene was also induced. These two proteins of the extracellular matrix can digest collagens I, III, and IV as well as other matrix proteins. The MMP-9 gene was strikingly upregulated in BAL cells recovered from two patients with cavitary tuberculosis (122).

ROLE OF T-LYMPHOCYTES IN HOST DEFENSE AGAINST MYCOBACTERIA

The above discussion has focused on how phagocytic cells, mainly macrophages, inhibit growth of mycobacteria. The next section of the review will discuss the role of lymphocytes as important coeffectors in mycobacterial host defense.

Overview of T-cell Function

Although there is a role for many types of T-lymphocytes (including α/β CD4+ and CD8+ cells, cytotoxic T-lymphocytes, and γ/δ T-lymphocytes) in host defense against *M. tuberculosis*, undoubtedly the major effector cell in cell-mediated immunity in tuberculosis is the CD4+ T-lymphocyte (123). CD4+ T-cells express the α/β T-cell receptor, and they are involved in recognition of antigens that have been processed in phagosomes and presented as small peptide fragments in the context of MHC class II molecules on the surface of antigen-presenting cells such as monocytes, macrophages, or dendritic cells. CD8+ T-cells, on the other hand, recognize antigens that have been processed in the cytosol and that are presented in the context of MHC Class I molecules on the cell surface. In general, CD4+ cells help to amplify the host immune response by activating effector cells and recruiting additional immune cells to the site of disease, whereas CD8+ cells are more likely to be directly cytotoxic to target cells. Mycobacterial cell wall products, especially the cell wall component LAM, are chemotactic for T-cells to sites of infection (124). BAL studies demonstrate enrichment of CD4+ T-cells at sites of disease, and this response is diminished in HIV-infected patients (58). Although blood monocytes sequester *M. tuberculosis* from CD4+ T-cells *in vitro*, there is no evidence that this occurs in the lungs in patients, underscoring the importance of comparing *in vitro* to *in vivo* investigation (125).

In recent years, a paradigm for thinking about the functions of CD4+ T-cells and their relationship to the manifestations of disease has developed (Figure 2). This paradigm, developed initially in the murine model, but which has now accumulated a substantial amount of support in a variety of human disease, holds that CD4+ helper T-cells can be separated into at least two phenotypic classes, Th1 and Th2 (126–128). These cells derive from so-called Th0 or null cells, and their differentiation from these precursor cells may be under the control of cytokines such as interleukin-12 (IL-12). Phenotypically, Th1 cells are characterized mainly by their ability to produce the cytokines IFN-γ and IL-2, whereas Th2 cells produce cytokines such as IL-4, IL-5, and IL-10. Th1-type cytokines are those that activate other inflammatory and phagocytic cells capable of inhibiting the growth of pathogenic bacteria; for example, although Th2 cells are involved in the production of IgE and recruitment of eosinophils. Cytokines such as IL-3,

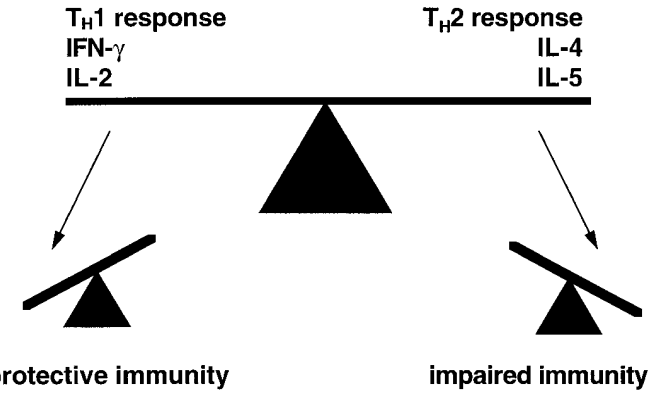

Figure 2. Helper T-cells (CD4+ T-lymphocytes) can be separated into two phenotypes depending on the cytokines that they secrete. CD4+ cells with the Th1 phenotype secrete interferon gamma, a cytokine that is capable of activating macrophages and monocytes. CD4+ cells with the Th2 phenotype secrete interleukin-4 and interleukin-5, cytokines that are involved in recruitment of eosinphils and production of IgE. Although both responses are inflammatory reactions, Th1 reactions typically characterize protective immunity (e.g., organ transplant rejection), and Th2 reactions often represent impaired immunity (e.g., lepromatous leprosy).

lymphotoxin, and granulocyte-macrophage-colony stimulating factor (GM-CSF) are secreted by both phenotypic classes of CD4+ cells.

The relationship of Th phenotype to disease manifestations in humans was demonstrated in studies published by Modlin and colleagues (129–131) in which skin biopsy lesions from patients with leprosy were examined to determine the state of expression of a variety of cytokine genes. The clinical poles of leprosy are represented by lepromatous (susceptible) lesions and tuberculoid (resistant) lesions. Lepromatous leprosy lesions are characterized clinically by extensive cutaneous involvement with poorly defined lesions that infiltrate the dermis diffusely. Importantly, bacilli (*Mycobacterium leprae*) are easily demonstrated in normal skin by staining. In contrast, tuberculoid lesions are sharply demarcated, and are single or few in number on the skin. Histologically, noncaseating granulomas are present in tuberculoid lesions, and bacilli are rare or absent. By using the reverse transcriptase polymerase chain reaction (RT-PCR) to evaluate cytokine gene expression, Modlin and colleagues determined that lepromatous leprosy lesions (those that are less able to control bacterial growth) contained cells expressing the genes from IL-4, IL-5, and IL-10, whereas resistant, tuberculoid lesions contained cells expressing the cytokine genes IFN-γ and IL-2. Thus, the pattern of cytokine gene expression by helper T-cells seems to be associated with different manifestations of disease in humans, and the Th1/Th2 paradigm seems tenable in human disease. Asthma, Crohn's colitis, and organ transplantation are further examples of disease states with clinical manifestations that seem at least in part to be related to the Th phenotype present at the site of disease (132–134).

CD4+ Cell Phenotypes in Tuberculosis

A variety of studies have attempted to characterize the T-lymphocyte responses associated with tuberculosis, though few have studied lymphocytes produced in the lung itself in patients with pulmonary tuberculosis. Surcel and colleagues (135) studied proliferative responses and cytokine production in peripheral blood mononuclear cells taken from patients with tuberculosis and from normal control subjects, in response to stimulation with mycobacterial antigens *in vitro*. They found that patients with tuberculosis had increased proliferation of cells secreting IL-4 but not IFN-γ in response to stimulation and in comparison with cells from healthy subjects. Sanchez and coworkers (136) studied 45 patients with pulmonary tuberculosis and 16 tuberculin skin-test-positive control subjects and found that patients had less IFN-γ than did the control subjects, and more IL-4 production. They concluded that patients with tuberculosis had a Th2-type response in their peripheral blood, whereas tuberculin positive patients had a Th1-type response. These findings were in agreement with the study of Surcel and colleagues cited previously.

More recent work examining cellular response at the actual sites of disease indicates that there may in fact be a compartmentalization of the cellular immune in patients with active tuberculosis, and that IL-12 production may be an important regulator of T-cell phenotypes in tuberculosis. Zhang and colleagues (137) studied cytokine production in pleural fluid from patients with tuberculous pleurisy and found high levels of IL-12 after stimulation of pleural fluid cells with *M. tuberculosis*. As IL-12 is known to induce a Th1-type response in undifferentiated CD4+ cells, these investigators took their findings to suggest a Th1 response at the actual site of disease. In a more recent report, the same investigators examined cytokine production in lymph nodes of patients with and without HIV infection, and compared the results with those found in healthy control subjects (138). Patients showed evidence of high IFN-γ production and no IL-4 secretion by the lymphocytes in the nodes. Both IL-12 and IL-10 were being produced by macrophages within the lymph nodes, but not by lymphocytes themselves. These results were taken to show that there was no enhancement of Th2 responses at the site of disease in human tuberculosis. Th1 and Th2 responses may be controlled by the β-receptor subunit of IL-12. IL-4 downregulates this receptor's expression, leading to a loss of IL-12 responsiveness in CD4+ T-cells. In contrast, IFN-γ enhances IL-12R β2 expression and prevents premature commitment to the Th2 phenotype (139). Of importance in understanding the role of these cells in inflammation, Th1 cells selectively bind to P- and E-selectin in inflamed tissues, whereas Th2 cells do not (140).

Further elucidation of the role of IL-12 as a regulator of the T-cell phenotype response comes from several recent additional studies. Comparing patients with multidrug-resistant tuberculosis (MDR-TB) to tuberculin-negative and tuberculin-positive control subjects, McDyer and colleagues (141) found that stimulated (with *M. tuberculosis*, PPD, or mitogens) peripheral blood mononuclear cells from MDR-TB patients had less proliferation and secretion of IL-2 and IFN-γ than did cells taken from healthy PPD-positive or negative control subjects (141). Interestingly, IFN-γ production could be restored if PBMC were supplemented with IL-12 prior to stimulation, and antibodies to IL-12 caused a further decrease in IFN-γ upon stimulation. Taha and coworkers (142) demonstrated that in patients with drug-susceptible, active tuberculosis both IFN-γ and IL-12 producing BAL cells were abundant as compared with BAL cells recovered from patients with inactive tuberculosis (142). The trigger for IL-12 release appears to be phagocytosis of *M. tuberculosis* by macrophages, as has been shown by several investigators. Of note, release of IL-12 appears to be an early and perhaps somewhat nonspecific response to phagocytosis (143). Ladel and colleagues (144) showed that IL-12 was released by macrophages *in vitro* after infection with *M. tuberculosis* or phagocytosis of latex beads, but TNF and IL-12 were released together only after infection with the mycobacteria.

Evidence regarding Th phenotypes present in the lungs themselves in patients with pulmonary tuberculosis has also recently started to emerge. Robinson and colleagues (145) used *in situ* hybridization to detect cytokine gene expression in bronchoalveolar lavage cells from nine patients with active pulmonary tuberculosis, compared them with healthy control subjects, and found that increased levels of IFN-γ mRNA could be detected and were localized mainly to T-lymphocytes (80% of cells expressing IFN-γ mRNA were T-lymphocytes, the remainder were alveolar macrophages). Actual protein levels were not measured in this study. Additionally, there were no differences detected in expression of IL-2, IL-4, or IL-5 genes. Schwander and colleagues (146) studied a similar group of patients and found that the majority of lymphocytes in the lungs of patients with tuberculosis were T-cells displaying the α/β receptor and were activated, as evidenced by CD69 and HLA-DR expression. In HIV-infected patients, the absolute number and immune activation state of CD4+ lymphocytes may be reduced, as has been shown by Law and colleagues (58). These studies provide some evidence that the local cellular immune response in pulmonary tuberculosis is made up at least in part of Th1-type CD4+ lymphocytes. In addition, two very recent reports suggest that in humans with tuberculosis, the strength of the Th1-type immune response relates directly to the clinical manifestations of the disease. Sodhi and colleagues (147) have demonstrated that low levels of circulating interferon-gamma in peripheral blood are associated with severe clinical tuberculosis (radiographically far-advanced disease). More directly, our group (148) has shown that patients with clinically and radiographically limited tuberculosis (negative sputum smears, no cavitation on chest radiographs) have an alveolar lymphocytosis in infected regions of the lung; these lymphocytes produce high levels of interferon-gamma. In patients with far-advanced or cavitary disease, no Th1-type lymphocytosis is present.

In addition to activation of macrophages by secreted cytokines, there is recent evidence that lymphocytes can kill mycobacteria directly through cytotoxic T-lymphocyte (CTL) activity (57, 149). Lymphocytes taken from the lungs of normal volunteers had the ability to lyse mycobacterial antigen-pulsed alveolar macrophages and peripheral monocytes. The ability to lyse monocytes exceeded the ability to lyse macrophages. CTL was activated with PPD primarily through MHC Class II, whereas when lymphocytes were expanded with both PPD and IL-2, CTL was both Class I and Class II expanded. Although the general assumption has been that CD4+ T-lymphocytes are by far the most important components of cell-mediated immunity in tuberculosis, this study also demonstrated significant ability of CD8+ T-lymphocytes to carry out CTL as well. These experiments establish a direct role for CTL in host defense against tuberculosis that complements their role as stimulators of macrophage function (149).

CD8+ Cell Phenotypes in Tuberculosis

CD8+ T-lymphocytes (suppressor T-cells) recognize peptide fragments of antigens processed in the cytosol and presented in the context of MHC Class I molecules, which are found on the surface of most nucleated cells. These cells participate directly in lysis of infected cells and induction of apoptosis of these target cells. Flynn and colleagues (151, 152) demonstrated the importance of this line of cellular immunity in β2-microglobulin knockout mice that were unable to express MHC Class I molecules and were highly susceptible to infection with mycobacteria. In an earlier study, Rossi and colleagues (152) demonstrated that pleural fluid lymphocytes from patients with anergic tuberculous pleurisy proliferated in response to PPD, whereas circulating lymphocytes did not. The proliferating T-cells in the pleural space contained both CD4+ and CD8+ populations, suggesting that the anergic phase of tuberculous pleurisy may be associated with a sequestration of both CD4+ and CD8+ cells in the pleural space. Similar findings were later reported by Gambon-Deza and colleagues (153). Bose and colleagues (154) reported that peripheral blood CD4/CD8 ratios were depressed in patients with newly diagnosed disease or in chronically nonresponding patients, but ratios normalized if and when patients responded to therapy.

Since those observations, several groups have attempted to characterize the role of CD8+ cells in the blood and lung themselves in patients with pulmonary tuberculosis, and the data obtained have been conflicting at times. Faith and coworkers (155) and Nowakowski and colleagues (156) each found a decreased CD4/CD8 ratio in BAL cells from patients with pulmonary tuberculosis, whereas the ratio on peripheral blood cells was not nearly so depressed. This reduced ratio was felt to be due to depletion of CD4+ cells rather than to proliferation of CD8+ cells. Conversely, Hoheisel and colleagues (157) compared lung lavage cells from 40 patients with tuberculosis with normal control subjects and found that patients with tuberculosis had normal CD4/CD8 ratios. However, the patients in this study were not separated by clinical or radiographic characteristics. Yu and colleagues (158) analyzed CD4 and CD8 populations from patients with rapid, slow, or intermediate regression of disease while receiving therapy and found that slow regression was associated with an increase in CD8+ cells in the BAL. Taha and colleagues (142) found increased CD8+ T-cells in the BAL of patients with active tuberculosis (but not in those with inactive tuberculosis), along with striking increases in the number of BAL cells expressing IFN-γ and IL-12 mRNA. CD8+ T-cells have been recently shown to be able to lyse *Mycobacterium*-infected macrophages by a Fas-independent, granule-dependent mechanism that results in the killing of the bacteria (57). These T-cells were CD1-restricted and had the ability to recognize *M. tuberculosis* lipid and lipoglycan antigens; they kill by exocytosis of the perforin granzyme A.

The above studies point to a potential role for CD8+ T-cells in the immune response to tuberculosis, though the data are not consistent from study to study. The exact function of CD8+ cells in tuberculosis is also unclear. As noted above, CD8+ cells may be involved in cell lysis and apoptosis, but evidence also exists that they are capable of secreting cytokines such as IFN-γ and IL-4, and thus may play a role in regulating the balance of Th1 and Th2 cells in the lungs of patients with pulmonary tuberculosis. Exact elucidation of their role will require futher investigation.

γ/δ T-cells in Tuberculosis

The role of γ/δ T-cells in the host response in tuberculosis has been incompletely worked out. These cells are large granular lymphocytes that can develop a dendritic morphology in lymphoid tissues; they comprise less than 10% of circulating T-lymphocytes (159). They display the TCR-1 receptor, in contrast to α/β T-cells (which comprise most of the CD4+ and CD8+ T-cells), which display the TCR-2 receptor, though some γ/δ cells may be CD8+. In general, γ/δ T-cells are felt to be non-MHC-restricted and they function largely as cytotoxic T-cells. The CD1 molecule has been shown to present *M. tuberculosis* antigens by CD4 and CD8 T-cells, which express either the TCR-1 or TCR-2 receptor (160).

A substantial amount of animal data suggests that γ/δ cells play a significant role in the host response to tuberculosis in

mice and in other species. For example, although mice with severe combined immunodeficiency do not form granulomas and rapidly succumb after BCG infection, they can survive inoculation if they are engrafted with co-isogenic lymph node cells depleted of CD4+ and CD8+ T-cells (161, 162). Presumably, γ/δ T-cells are responsible for this response. In immunocompetent mice, γ/δ T-cells increase by at least one log in lymph nodes draining primary *M. tuberculosis*-inoculated sites.

More recently, data suggesting a role in human disease have been published as well (163, 164). *M. tuberculosis*-reactive γ/δ T-cells can be found in the peripheral blood of tuberculin-positive healthy subjects, and these cells are cytotoxic for monocytes pulsed with mycobacterial antigens, and these secrete cytokines that may be involved in granuloma formation (165). Barnes and colleagues (166) demonstrated that γ/δ cells were relatively more common (25 to 30% of the total) as a percentage of T-lymphocytes in peripheral blood from patients with what the investigators referred to as protective or resistant immunity (patients tuberculin-skin-test positive or with tuberculous pleurisy) as compared with peripheral blood mononuclear cells from patients with ineffective immunity (advanced pulmonary tuberculosis or miliary disease), where they represented 2 to 9% of the total (166). Similar findings were reported by Sanchez and coworkers (136). Ueta and colleagues (167) studied healthy contacts and compared them with persons who had not had contact with patients with tuberculosis. They found that tuberculin-positive persons in frequent contact with active cases had a greater percentage of γ/δ cells in their peripheral blood than did those without constant contact with active cases. Patients with active tuberculosis also had no increase in γ/δ cells as a percentage of total circulating T-lymphocytes. This confirmed earlier work by Tazi and coworkers (168) and others showing no increase in circulating γ/δ cells among a group of patients with active tuberculosis as compared with normal tuberculin-negative control subjects.

Taken together, these studies, as well as others previously done in animals, indicate that γ/δ cells may indeed play a role in early immune responses against tuberculosis and may in fact be an important part of the establishment of protective immunity in those patients with latent infection (169).

CONCLUSION

The human host response to tuberculosis is a complex reaction to infection with a vigorous pathogen. The intricate interaction of the various components of the cellular immune system occurs in a fluid environment containing a wide variety of chemokines and cytokines, and it is likely that the precise balance of these various factors has a large impact on the body's ability to successfully contain infection. In only the last few years have *in vitro* and animal studies begun to be complemented by human studies using material obtained by bronchoalveolar lavage from patients with tuberculosis and from control subjects. This approach, combined with constant reevaluation and reappraisal of model systems of host immunity, should lead to substantial advances in approaches to treatment by immunomodulation and prevention of tuberculosis with more effective vaccines in the coming years.

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