Tuberculosis: Pathogenesis, Protection, and Control
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Chapter 24

Immune Mechanisms of Protection

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Acquired resistance against tuberculosis paradigmatically rests on cell-mediated immunity, with the major factors being mononuclear phagocytes (MP) and T lymphocytes. While the former cells act as the principal effectors, the latter ones serve as the predominant inducers of protection. At the same time, however, MP provide the preferred biotype for the etiologic agent of tuberculosis, *Mycobacterium tuberculosis*, and hence play a dual role in tuberculosis, promoting not only protection against the disease but also survival of the pathogen. Similarly, T cells not only are indispensable for protective immunity but also contribute to pathogenesis. A coordinated cross-talk between MP and T cells, therefore, is essential for optimum protection. Such coordination is best achieved in the granulomatous lesion, which provides the tissue site for defense against tuberculosis. Even in the face of coordinated T-cell–MP interactions, full eradication of the pathogen is frequently not achieved, so that the individual remains infected without developing active disease. Any later imbalance of the immune system will promote microbial reemergence and ultimately result in clinical disease. This chapter focuses on the immune mechanisms involved in protective immunity against tuberculosis, with the awareness that in most cases the immune response activated during infection with *M. tuberculosis* may be remarkably powerful yet insufficient.

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A HISTORICAL NOTE

In his epoch-making description of the etiologic agent of tuberculosis in 1882, R. Koch noted the intracellular location of *M. tuberculosis* within giant cells (end-stage-differentiated MP) in granulomatous lesions (Koch, 1882). In his endeavor to develop an active vaccination protocol for treating tuberculosis, Koch found that after administration of glycerin extracts of *M. tuberculosis* culture supernatants, the lesions of tuberculous guinea pigs became heavily necrotized (Koch, 1890). In these necrotic reactions, many microorganisms died because of nutrient and oxygen deficiencies. Although Koch had already noted that *M. tuberculosis* organisms can be disseminated from such necrotizing lesions to other tissue sites, he underrated the detrimental consequences of this effect, which soon brought therapeutic vaccination with tuberculin to an end. E. Metchnikoff, a contemporary but not a close friend of Koch, was the first to fully realize the importance of MP in antibacterial immunity in general and in defense against tuberculosis in particular (Metchnikoff, 1905). The great success around the turn of the century in transferring protection against toxin-producing bacteria by using antisera from immune animals prompted numerous scientists to attempt passive vaccination against tuberculosis with antisera. Soon, however, it was realized that such antisera failed to transfer protection against tuberculosis. The first success in this direction was obtained in 1909 to 1910 by H. Helmholtz and O. Bail, who independently succeeded in adoptively transferring delayed-type hypersensitivity to tuberculosis with whole blood (containing leukocytes) or spleen homogenate, respectively (Helmholtz, 1909; Bail, 1910). Formal proof for the cellular dependence of delayed-type hypersensitivity to tuberculin was provided by M. Chase in 1945 (Chase, 1945). M. Lurie and E. Suter independently found that macrophages from immune animals expressed tuberculostatic activities, whereas those from normal animals permitted unrestricted bacillary multiplication (Suter, 1953; Lurie, 1964). Although these studies suggested involvement of specific immune mechanisms, the investigators did not contest alternative strategies when they realized that immune serum did not influence tuberculostasis by MP. It was the achievement of G. B. Mackaness to show that activation of antimycobacterial macrophage functions is controlled by lymphocytes (Mackaness and Blanden, 1967). That this activation is afforded by soluble mediators, now termed cytokines, was noted by B. R. Bloom and J. R. David (Bloom and Bennett, 1966; David, 1966).

IN VITRO ACTIVATION OF MACROPHAGE ANTIMYCOBACTERIAL FUNCTIONS

Evidence has long existed that murine macrophages have an antimycobacterial function in tissue culture systems (Lurie, 1942; Suter, 1952; Mackaness, 1969). Earlier work by various laboratories demonstrated that these cells, when activated in vitro by supernatants of immunologically stimulated lymphocytes, had various degrees of antimycobacterial activity (Patterson and Youmans, 1970; Klun and Youmans, 1973a, b; Cahall and Youmans, 1975a, b; Muroaka et al., 1976a, b; Turcotte et al., 1976). Soon, hydrogen peroxide (H_2O_2), one of the reactive oxygen intermediates (ROI) generated by macrophages during the oxidative burst (Sbarra and Karnovsky, 1959; Iyer et al., 1961; Klebanoff, 1980), was identified as the molecule that mediated mycobacteriocidal effects of MP (Walker and Lowrie, 1981). This finding marked the beginning of much debate concerning the significance of ROI in host defense against *M. tuberculosis*. Later, gamma interferon (IFN-γ) was found to be the key endogenous activating agent that triggers the antimycobacterial effects of murine macrophages (Rook et al., 1986; Flesch and Kaufmann, 1987), furnishing a better-defined system (compared to one using crude supernatants obtained from stimulated lymphocytes) in which to examine the antimycobacterial effects of macrophages. Recent remarkable advances made in the cloning, characterization, and production of numerous cytokines by recombinant DNA technology have facilitated similar in vitro experimentation designed to explore the potential of these interesting molecules in host defense against *M. tuberculosis*. Thus, tumor necrosis factor alpha (TNF-α), although ineffective when used alone, synergizes with IFN-γ to induce antimycobacterial effects of murine macrophages in vitro (Flesch and Kaufmann, 1990a). TNF-α also appears to play a critical role in the control of BCG infection in vivo, although its direct effect on the antimycobacterial capacity of macrophages has not been addressed in this model. Nevertheless, when TNF-α-specific monoclonal antibodies were used to probe the significance of this cytokine in defense against mycobacteria, deficient TNF-α resulted in poor granuloma formation and disseminated BCG infection in mice (Kindler et al., 1989). The significance of TNF-α in granuloma formation has been demonstrated in other infectious disease models (Chensue et al., 1989; Amiri et al., 1992). More importantly, preliminary studies suggest that anti-TNF-α antibodies markedly exacerbate disease progression in murine experimental tuberculosis (Flynn et al., personal communication).

Other cytokines have been implicated in macrophage defense against *M. tuberculosis*, although their roles are not as well established as those of IFN-γ and TNF-α. In vitro, interleukin-4 (IL-4) and IL-6 have the ability to induce macrophage antimycobacterial activity (Kaufmann et al., 1989; Flesch and Kaufmann, 1990a, b) by mechanisms presently undefined. Infection of the human myelomonocytic cell line THP-1 with *M. tuberculosis* enhances production of IL-6 (Friedland et al., 1993) compared to that in cells infected with *Toxoplasma gondii*, an intracellular protozoan known to elicit little inflammatory response even in immunocompetent patients. In the murine system, BCG or its subcellular components are capable of inducing production of IL-6 by splenocytes (Huygen et al., 1991). The antimycobacterial effects of IL-4 and IL-6 (Flesch and Kaufmann, 1990a, b) in the in vitro macrophage system are seen only when these cytokines are added to macrophage cultures after, but not before, the establishment of BCG infection. This phenomenon sharply contrasts with the ability of IFN-γ to induce antimycobacterial activity in macrophages, which is markedly blunted if it is given after initiation of infection (Flesch and Kaufmann, 1990a). The mechanism and the significance of this observation are currently obscure, but it illustrates well the complexity of the interaction between macrophages, cytokines, and the organisms as well as the limitations of existing in vitro systems in dissecting the likely complex cytokine network involved during tuberculous infection. Thus, it is known that THP-1 cells produce IL-8 in response to *M. tuberculosis* infection in vitro, but the role of this cytokine in host defense in tuberculosis is completely unknown (Friedland et al., 1992, 1993). Nevertheless, it has been postulated that IL-8 plays a role in granuloma formation by virtue of its ability to act as a chemotactic agent for T cells (Larsen et al., 1989; Friedland et al., 1992). IL-1 (Kobayashi et al., 1985; Dunn et al., 1988; Kasahara et al., 1988), IL-2 (Mathew et al., 1990; Cheever et al., 1992), IL-4 (McInnes and Rennick, 1988; Chensue et al., 1992), and IFN-γ (Squires et al., 1989; Chensue et al., 1992) may similarly contribute to resistance against *M. tuberculosis*, since these cytokines have been implicated in granulomatous reactions in various in vitro systems, including a murine schistosomiasis model. Recently, IL-10 (Bermudez and Champsi, 1993) and transforming growth factor beta1 (TGF-β1) (Denis and Ghadirian, 1991; Bermudez, 1993) have been shown to be associated with diminution of macrophage antimycobacterial effect in vitro and with disease exacerbation in mice infected with *M. avium*. In contrast, preliminary studies (Flynn and Bloom, personal communication) indicate that administration of recombinant IL-12, a recently characterized heterodimeric glycoprotein produced by various immune cells including macrophages (D'Andrea et al., 1992; Schoenhaut et al., 1992; Gazzinelli et al., 1993), may confer resistance to tuberculosis in mice. IL-12 has recently been shown to play an important role in resistance to *Leishmania* *major*, *T. gondii*, and *Listeria monocytogenes* (Gazzinelli et al., 1993; Heinzel et al., 1993; Locksley, 1993; Tripp et al., 1993). The events triggered by IL-12 help identify natural killer (NK) cells as a critical cellular component in defense against *M. tuberculosis*. By virtue of their ability to produce IFN-γ in response to IL-12 (Kobayashi et al., 1989; Wolf et al., 1991), NK cells can rapidly activate macrophages to express microbicidal functions during the early "nonimmune" phase of tuberculous infection, before the expansion and differentiation of specific T lymphocytes. As cytokines are being examined in experimental mycobacterial infection, it is becoming clear that these molecules interact dynamically to form a highly coordinated network that is configured by both host- and pathogen-specific factors, which together influence disease outcome and progression.

Compared to the murine system, much less is known about the activation of antimycobacterial activity in human macrophages. While it is clear that IFN-γ has the capability to induce significant antimycobacterial activity in murine macrophages, its role in the human system is unsettled. Thus, reports of the effect of IFN-γ-treated human macrophages on the replication of *M. tuberculosis* ranges from being inhibitory (Rook et al., 1986) to enhancing (Douvas et al., 1985). This inconsistency had cast considerable doubts on the antimycobacterial capability of human mononuclear phagocytes until the demonstration that 1,25-dihydroxy vitamin D_3 [1,25-$(OH)_2D_3$], alone or in combination with IFN-γ and TNF-α, was able to activate macrophages to inhibit and/or kill *M. tuberculosis* in the human system (Crowle et al., 1987; Rook, 1988; Denis, 1991b). Interestingly, IFN-γ stimulates human (Adams and Gacad, 1985; Koeffler et al., 1985; Reichel et al., 1987) but not murine (Rook, 1990) macrophages to produce 1,25-$(OH)_2D_3$, probably via induction of 25$(OH)D_3$-1α-hydroxylase, the enzyme that converts 25$(OH)D_3$ to the biologically more potent dihydroxylated form, which may explain the inability of 1,25$(OH)_2D_3$ to affect antimycobacterial activity in the murine system. This difference in 1,25$(OH)_2D_3$ metabolism between murine and human macrophages should serve as a reminder that species variations exist and a caution against the occasional readiness with which cross-species extrapolations of experimental results are made. The value of existing in vitro and in vivo murine models in understanding tuberculosis must, however, not be understated.

ANTIMYCOBACTERIAL EFFECTOR FUNCTIONS OF MACROPHAGES: HOW DOES *M. TUBERCULOSIS* SURVIVE?

The mononuclear phagocyte constitutes a potent antimicrobial component of cell-mediated immunity. The precise mechanisms by which these cells mediate killing or inhibition of bacterial pathogens are, however, not clearly understood. Nonetheless, in this section, some of the best-characterized antimicrobial effector functions of macrophages—phagosome-lysosome fusion, generation of ROI by the oxidative burst, and production of reactive nitrogen intermediates (RNI) via the L-arginine-dependent cytotoxic pathway—will be discussed in the context of tuberculous infection together with the possible evasion mechanisms employed by the tubercle bacillus to escape killing by activated macrophages (Fig. 1).

Phagosome-Lysosome Fusion

The lysosome is a highly complex organelle containing numerous enzymes within its own limiting membrane that are capable of degrading a whole range of macromolecules (reviewed in de Duve and Wattiaux [1966], Bainton [1981], and Kornfeld [1987]). To provide optimal conditions for the functioning of these degradative enzymes, the intralysosomal milieu is main-

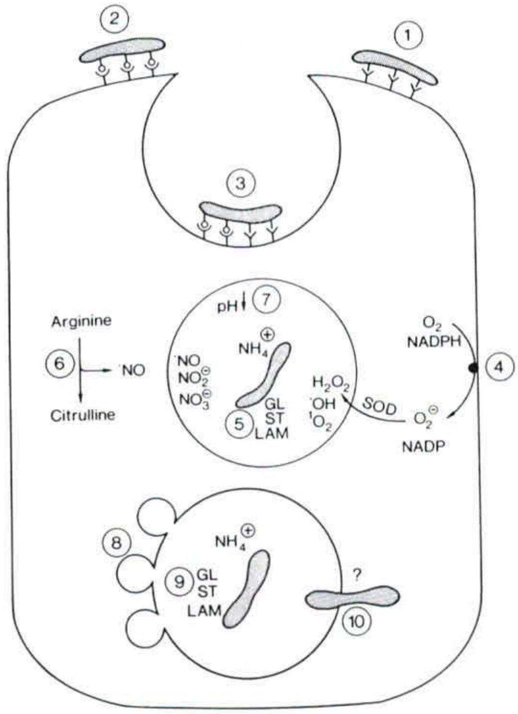

Figure 1. Antituberculous macrophage activities and evasion mechanisms. Accumulating evidence suggests that *M. tuberculosis* enters macrophages via specific binding to cell surface molecules of phagocytes. It has been reported that the tubercle bacillus can bind directly to the mannoase receptor via the cell wall-associated, mannosylated glycolipid LAM (1) or indirectly via complement receptors of the integrin family (CR1, CR3) or Fc receptors (2). Phagocytosis (3), triggered by engaging certain cell surface molecules such as the Fc receptor, stimulates the production of ROI via activation of the oxidative burst (4). Experimental data indicate that *M. tuberculosis* can interfere with the toxic effect of ROI by various mechanisms. First, various mycobacterial compounds including glycolipids (GL), sulfatides (ST), and LAM can downregulate the oxidative cytotoxic mechanism (5; see text for details). Second, uptake via CR1 bypasses activation of the respiratory burst. Cytokine-activated macrophages produce RNI that, at least in the mouse system, mediate potent antimycobacterial activity (6). The acidic condition of the phagolysosomal vacuole can be conducive to the toxic effect of RNI (7). However, NH_4^+ production by *M. tuberculosis* may attenuate the potency of the L-arginine-dependent antimycobacterial mechanism and that of lysosomal enzymes (8), which operate best at an acidic pH. In addition, mycobacterial products such as sulfatides and NH_4^+ may interfere with phagolysosomal fusion (9). Finally, the tubercle bacillus may evade the highly toxic environment by escaping into the cytoplasm via the production of hemolysin (10).

tained at a relatively acidic state (pH ~5) by an ATP-dependent proton pump (Ohkuma and Poole, 1978; Ohkuma et al., 1982). It is generally accepted that certain microorganisms, sequestered within the phagosome upon ingestion by phagocytic cells including macrophages, are subject to degradation by the various lysosomal digestive enzymes transferred into this subcellular compartment as a result of phagolysosomal fusion (Cohn, 1963). This fusion process, a highly regulated event, most likely constitutes a significant antimicrobial mechanism of phagocytes. Examination of the interaction between isotopically labeled bacteria and macrophages, using the generation of acid-soluble radioactive materials as an indicator of degradation, suggests that certain organisms are degraded extensively within 2 h after having been phagocytized (Cohn, 1963). Also, electron microscopic studies indicate that the cell wall of *Bacillus subtilis* is degraded extensively within 30 min after phagocytosis by polymorphonuclear leukocytes (Cohn, 1963). How, then, does *M. tuberculosis* survive the hostile environment of phagolysosomes?

M. tuberculosis has the ability to produce ammonia in abundance (Gordon et al., 1980). This volatile weak base accumulates in *M. tuberculosis* culture filtrates in concentrations of up to 20 mM and is thought to be responsible for the inhibitory effect of culture supernatants of virulent mycobacteria on phagolysosome fusion (Gordon et al., 1980). In addition, ammonium chloride (NH_4Cl) has been shown to affect the saltatory movement of lysosomes (D'Arcy

Hart et al., 1983) and to alkalinize the intralysosomal compartment (D'Arcy Hart et al., 1983). Thus, by virtue of its ability to produce a significant amount of ammonia, the tubercle bacillus can potentially evade the toxic environment within the lysosomal vacuole by (i) inhibiting phagosome-lysosome fusion and (ii) diminishing the potency of the intralysosomal enzymes via alkalinization. This latter attribute of raising intralysosomal pH might also be protective against the RNI cytotoxic mechanism of macrophages (see below).

Another mycobacterial product thought to have the ability to inhibit phagolysosomal fusion is the sulfatides (Goren et al., 1976b), derivatives of multiacylated trehalose 2-sulfate, a lysosomotropic polyanionic glycolipid produced by *M. tuberculosis* (Middlebrook et al., 1959; Goren et al., 1976a). Because of the ability of certain polyanionic compounds to entrap commonly used lysosomal markers employed to study phagolysosome fusion, artifactual "inhibition" of this process can occur and has spawned much controversy (Goren et al., 1987a, b). These entrapment phenomena could be secondary to the formation of gelatinous, sluggishly moving hydrocolloids that physically retain lysosomal markers or to ionic interaction with cationic markers such as acridine orange. Although sulfatides do not form hydrocolloids, the polyanionic nature of these glycolipids poses questions concerning their ability to inhibit phagolysosomal fusion (Goren et al., 1987a, b). Careful reanalysis of the effect of these glycolipids on phagolysosome fusion appears to be warranted. Regardless of the chemical components of the tubercle bacillus that contribute to the inhibition of phagolysosomal fusion, this phenomenon (controversy notwithstanding) has been extensively studied (Armstrong and D'Arcy Hart, 1971, 1975; Goren et al., 1976b; Myrvik et al., 1984; D'Arcy Hart et al., 1987) and is certainly a mechanism by which *M. tuberculosis* could evade cytotoxic effects of macrophages. This issue could perhaps be addressed more rigorously and definitively by direct immunohistochemical labeling of vacuolar membranes enclosing intracellular *M. tuberculosis* with antibodies specific to lysosomal glycoproteins (Joiner et al., 1990) or by using the "trap-resistant" ionic impermeant fluors (lucifer yellow, lissamine rhodamine, and sulforhodamine) as alternative lysosomal markers (Goren et al., 1987a, b). Finally, it is likely that virulent tubercle bacilli, like certain intracellular pathogens, including rickettsiae (Winkler, 1990), listeriae (Bielecki et al., 1990), and shigellae (Sansonetti et al., 1986), evade killing by escaping from phagocytic vacuoles into the cytoplasm (for a review, see Falkow et al. [1992]). Hemolytic activities capable of lysing vacuolar membranes are thought to be the common virulent determinant that enables successful parasitization of the cytoplasm (Falkow et al., 1992). Indeed, the translocation of *M. tuberculosis* from within phagocytic vacuoles into the cytoplasmic compartment has been reported (Myrvik et al., 1984; McDonough et al., 1993). These observations are reinforced by the presence of a hemolytic activity in the tubercle bacillus (King et al., 1993). Also, the cytoplasmic location made possible by this potential evasion mechanism could, in theory, facilitate the routing of mycobacterial components into the major histocompatibility class I (MHC I) pathway of antigen presentation, thus explaining at least in part the importance of MHC I molecules and $CD8^+$ T cells in defense against *M. tuberculosis* (Kaufmann, 1988; Flynn et al., 1992).

The Respiratory Burst

That ROI play a significant role in host defense against microbes is best exemplified by the frequent infectious complication experienced by chronic granulomatous disease patients (reviewed in Forrest et al. [1988]), whose phagocytes cannot mount an oxidative burst (Sbarra and Karnovsky, 1959; Iyer et al., 1961; Klebanoff, 1980). The significance of these toxic oxygen species in defense against *M. tuberculosis*, however, remains controversial. Since the report that H_2O_2 produced by lymphokine-activated murine macrophages kills *M. microti* (Walker and Lowrie, 1981), much effort has been focused on testing the role of the oxygen radical-dependent killing mechanism in defense against *M. tuberculosis*. Such effort, however, provided evidence indicating that oxygen radicals may not be sufficient to inhibit and/or kill *M. tuberculosis* (Flesch and Kaufmann, 1987, 1988; Chan et al., 1992). The validity of these findings has been reinforced by the demonstration of evasion mechanisms employed by the tubercle bacillus to elude the toxic effect of ROI. Of these mechanisms, those that are mediated by mycobacterial components lipoarabinomannan (LAM) and phenolicglycolipid I (PGL-I) are among the best studied and characterized (for reviews, see Brennan [1989] and Brennan et al. [1990]).

LAM, a major cell wall-associated, phosphatidylinositol-anchored complex lipopolysaccharide, is produced by *M. tuberculosis* in large amounts (15 mg/g of bacteria) (Hunter et al., 1986; Hunter and Brennan, 1991). Immunogold staining has demonstrated that LAM exists in a capsular sheath encasing *M. tuberculosis* (Hunter and Brennan, 1991). This strategic location places LAM at the frontline of attacks directed by the various antimicrobial mechanisms of macrophages. It has now been shown that LAM can incapacitate the oxygen radical-dependent antimicrobial mechanism at at least two levels: (i) studies using electron spin resonance spectroscopy and spin-trapping have shown that LAM is an effective ROI scavenger (Chan et al., 1991); and (ii) LAM can downregulate the oxidative burst by inhibiting protein kinase C (Chan et al., 1991), an enzyme that plays an important role in activation of the oxidative burst in phagocytic cells (Gennaro et al., 1985; Pontyremoli et al., 1986; Wilson et al., 1986; Gavioli et al., 1987). In addition, since IFN-γ is a major factor for macrophage activation (Hamilton et al., 1984; Hamilton and Adams, 1987; Fan et al., 1988) and has the ability to enhance ROI production by phagocytic cells, it is possible that LAM, by virtue of its ability to inhibit transcriptional activation of IFN-γ-inducible genes (Chan et al., 1991), is able to block the expression of an as yet unidentified factor(s) inducible by this cytokine that is required for the oxidative burst. These results are in keeping with the findings that mouse peritoneal macrophages treated with LAM or infected with *M. leprae* (a LAM-producing pathogenic mycobacterium) are not responsive to IFN-γ activation as assessed by microbicidal and tumoricidal activities, O_2^- production, and surface Ia antigen expression (Sibley et al., 1988; Sibley and Krahenbuhl, 1988) and may partially explain the inability of IFN-γ-stimulated macrophages from both humans and mice to effectively kill *M. tuberculosis* in vitro (Rook et al., 1986; Flesch and Kaufmann, 1987).

Other mycobacterial components that interfere with the oxygen radical-dependent antimicrobial mechanism of macrophages are PGL-I and the sulfatides. PGL-I is an oligoglycosylphenolic phthiocerol diester with its species-specific trisaccharide moiety glycosidically linked to a phenyl group that in turn is attached to the branched glycolic chain, phthiocerol; two hydroxyl functions of the phthiocerol are esterified by methyl-branched fatty acids (mycocerosates) (Hunter and Brennan, 1981; Hunter et al., 1982). Although universally distributed among *M. leprae*, the expression of PGL-I in the various strains of *M. tuberculosis* is much restricted (Daffe et al., 1987; Brennan, 1989; Brennan et al., 1990). In contrast, the sulfatides, derivatives of multiacylated trehalose 2-sulfate (Middlebrook et al., 1959; Goren et al., 1976a), are widely expressed among different strains of *M. tuberculosis* (Middlebrook et al., 1959; Goren et al., 1974, 1976a). Because of its restricted distribution among tuberculous isolates, the significance of PGL-1 in the pathogenesis of tuberculosis remains to be determined. Nonetheless, both PGL-I and the sulfatides have the capacity to down-regulate ROI production in in vitro macrophage culture systems (Neill and Klebanoff, 1988; Pabst et al., 1988; Vachula et al., 1989; Brozna et al., 1991), and PGL-I directly scavenges oxygen radicals in a cell-free system (Chan et al., 1989). Another mechanism by which *M. tuberculosis* could evade the toxicity of ROI is to avoid binding to macrophage cell surface components, such as Fc receptors, that would provoke an oxidative burst. Instead, the tubercle bacillus parasitizes MP via complement receptors CR1 and CR3, molecules of the integrin family whose interaction with a ligand does not trigger ROI production (Wright and Silverstein, 1983), in resting macrophages (Schlesinger et al., 1990). Thus, as in other parasites (for reviews, see Isberg [1991] and Falkow et al. [1992]), including *Bordetella pertussis* (Relman et al., 1990), *Histoplasma capsulatum* (Bullock and Wright, 1987), *Legionella pneumophila* (Payne and Horwitz, 1987), and *Leishmania* spp. (Mosser and Edelson, 1987; Russell and Wright, 1988; Talamas-Rohana et al., 1990), exploitation of integrin receptors may be a common scheme of invasion among pathogenic mycobacteria.

Although these in vitro data provide substantive evidence to suggest pathogenetic roles of the various mycobacterial glycolipids, their in vivo significance is presently undefined and awaits rigorous genetic analyses. Nonetheless, it is undeniable that *Mycobacterium* spp. are extremely well adapted to the hostile environment of phagocytic cells, their deftness reflected by the alarming morbidity and mortality caused by tuberculosis worldwide (Murray et al., 1990). However, since infection with the tubercle bacillus does not equal disease the host must be equally sophisticated in evolving effective defensive strategies against this formidable invader. It follows, then, that there must exist antimicrobial mechanisms to which the bacillus succumbs.

Reactive Nitrogen Oxides

The L-arginine-dependent cytotoxic pathway of activated macrophages constitutes an important antimicrobial mechanism against intracellular parasites (for reviews, see Nathan and Hibbs [1991], Liew and Cox [1991], and Nathan [1992]). The cytotoxic effect of this pathway is mediated through nitric oxide (NO) and related RNI generated from the substrate L-arginine via the action of the inducible form of the enzyme nitric oxide synthase (iNOS) (Nathan and Hibbs, 1991; Nathan, 1992). Recent studies have demonstrated an association between the antimycobacterial effect of cytokine-activated murine macrophages and the activation of the L-arginine-dependent cytotoxic pathway (Denis, 1991b; Flesch and Kaufmann, 1991; Chan et al., 1992). Thus, the capability of macrophages activated by IFN-γ and *Escherichia coli* lipopolysaccharide or TNF-α to kill and/or inhibit the virulent Erdman strain of *M. tuberculosis* correlates well with RNI production, and nitrogen oxides generated by acidification of nitrite are also mycobactericidal (Chan et al., 1992). Deletion analyses of the 5′ flanking promoter sequence of murine iNOS indicate that IFN-γ alone is insufficient for transcriptional activation of this gene (Xie et al., 1993). The synergistic effect of IFN-γ and TNF-α in inducing macrophage antimycobacterial function via RNI production underscores the importance of these cytokines in defense against *M. tuberculosis*. Indeed, IFN-γ and IFN-γ receptor "knockout" mice that are deficient in mounting an RNI response to infection with the tubercle bacillus experience a globin, a direct connection of iron and infection is made (Eaton et al., 1982). In human diseases, the mortality rate of *Vibrio vulnificus* is markedly increased in patients suffering from iron overload as a result of conditions such as hemochromatosis and alcoholism (Brennt et al., 1991; Bullen et al., 1991). These experimental data thus suggest a possible role of siderophores in bacterial virulence.

Mycobactins, a group of iron-chelating growth factors of mycobacteria, have been considered a possible virulence factor of *M. tuberculosis* (Snow, 1970). These hydroxamate derivatives chelate ferric ions with a stability constant exceeding 10^{30} (Snow, 1970). Thus, mycobactins compete favorably for chelating Fe^{3+} with human ferritin and transferrin, the major iron storage and iron-transporting proteins, respectively. The significance of these mycobacterial iron-binding agents in the pathogenesis of tuberculosis, however, remains to be established. Recently, the L-arginine–NO pathway has been reported to participate in posttranscriptional regulation of the expression of ferritin, transferrin receptor, and 5-aminolevulinate synthase (a rate-limiting enzyme in erythroid heme synthesis) in macrophages (Drapier et al., 1993; Weiss et al., 1993). It is fascinating that the very same pathway that produces potent antimycobacterial activities in macrophages participates also in the regulation of the metabolism of iron, whose availability is essential to the optimum growth of *M. tuberculosis*. Dissecting this likely complex tangle may uncover additional roles for the NO pathway in tuberculous infection and shed light on the significance of iron in the pathogenicity of *M. tuberculosis*.

DOES *M. TUBERCULOSIS* INVADE CELLS OTHER THAN PROFESSIONAL PHAGOCYTES?

There is little doubt that *M. tuberculosis* has the ability to establish infection in and replicate inside of a wide variety of mammalian cells in vitro (Sheppard, 1958). Yet in infected tissues, the tubercle bacillus is to be found only in polymorphonuclear leukocytes and MP (Filley and Rook, 1991). The findings by Filley and Rook that endothelial cells and fibroblasts infected by *M. tuberculosis* exhibit increased sensitivity to the cytolytic effect of TNF have led to the hypothesis that this cytokine contributes significantly to the immunopathology of tuberculosis (Filley and Rook, 1991). The enhanced susceptibility of nonphagocytic cells to TNF upon mycobacterial infection may also partially explain the difficulties encountered in identifying such target cells in vivo. It is also possible that these nonphagocytic cells serve as a reservoir for bacterial multiplication and thus aid in disease dissemination upon lysis by TNF. Research in these areas is just beginning to draw attention and is likely to help provide insight into the pathogenic strategies of *M. tuberculosis*. Finally, unlike the processes of other pathogenic bacteria such as the enteric shigellae and salmonellae and the gram-positive listeriae (for reviews see Isberg [1991] and Falkow et al. [1992]), the processes of adhesion and invasion by which *M. tuberculosis* enters host cells are just beginning to be understood. *M. tuberculosis* gains entry into MP via cell surface molecules, including the integrin family CR1 and CR3 complement receptors (Schlesinger et al., 1990) and the mannose receptor (Schlesinger, 1993). Recently, *M. avium* has been shown to enter macrophages via $\alpha_v\beta_3$, another molecule of the integrin family (Rao et al., 1993). Parasitization of phagocytes via the CR1 and CR3 receptors by various pathogens avoids triggering the oxidative burst (Wright and Silverstein, 1983). Whether the same advantage is gained by engaging the mannose receptor or the $\alpha_v\beta_3$ integrin is presently unclear. Since the cytoplasmic domain of β subunit of integrin is coupled to the cytoskeleton (Albelda and Buck, 1990), it is possible that binding to such cell surface receptors serves to initiate the process of internalization by the host cell (Isberg, 1991). Does the recently described mycobacterial invasin (Arruda et al., 1993) bind also to integrin receptors? Comprehension of these adhesion and invasion events is very important in advancing our understanding of the pathogenicity of *M. tuberculosis*.

CONTRIBUTION OF T CELLS TO ACQUIRED RESISTANCE

T lymphocytes are obligatory mediators of protection. They do not act alone but must interact with other cells of the immune system to achieve optimum resistance. All T-cell populations (CD4 α/β T cells, CD8 α/β T cells, and γ/δ T cells) contribute to protection. The central role of T lymphocytes has been exemplified by experiments showing that *nu/nu* and *scids* mice suffer more severely from experimental *M. tuberculosis* and BCG infections than their control counterparts (Sher et al., 1975; Izzo and North, 1992).

T-Cell Populations

T cells expressing an α/β-T-cell receptor constitute more than 95% of postthymic T cells in peripheral organs and blood. In contrast, γ/δ T cells are a minority at these sites, but they are more prominent in mucosal tissues such as the lung. Formal proof that α/β T cells are crucial for acquired resistance against tuberculosis was provided recently with mutant mice lacking all α/β T cells. In these mice, the gene encoding the T-cell receptor β chain had been deleted by homologous recombination (Mombaerts et al., 1992). Although these α/β-T-cell-deficient mice are relatively resistant to sublethal BCG infection during the first 4 weeks of infection, growth of BCG markedly increases afterwards, and ultimately the α/β-T-cell-deficient mice succumb to BCG infection (Ladel and Kaufmann, unpublished data). α/β T cells car further divided into CD4 T cells, wh recognize antigenic peptides in the cont of MHC class II molecules, and CD8 cells, which respond to peptides present by the MHC class I gene products. Myc bacterium-specific CD4 T lymphocyt have been identified consistently in expe mental and human tuberculosis (Kaufmar and Flesch, 1986; Ottenhoff et al., 198 Barnes et al., 1989). Furthermore, CD T-cell depletion by specific monoclonal an tibodies exacerbates experimental infectio of mice with *M. tuberculosis* and BC (Müller et al., 1987; Pedrazzini et al., 1987). Conversely, adoptive protection against *M. tuberculosis* and BCG largely depends on transfer of selected CD4 T cells (Orme and Collins, 1984; Orme, 1987). Consistent with these findings, mutant mice with a deficiency in the MHC class II gene that are devoid of functionally active CD4 T cells die of BCG (Ladel and Kaufmann, unpublished data) and *M. tuberculosis* (Flynn et al., unpublished observation) infections. In conclusion, these experiments strongly point to an essential role of CD4 T cells in protection against tuberculosis. Consistent with these data, CD4 depletion as a result of human immunodeficiency virus infection frequently results in clinical tuberculosis in AIDS patients.

A substantial role for CD8 T cells in protection against tuberculosis is indicated by several lines of experimental studies. Depletion of CD8 T cells with specific monoclonal antibodies exacerbates *M. tuberculosis* infection in mice, and selected CD8 T cells transfer adoptive protection against tuberculosis (Orme and Collins, 1984; Müller et al., 1987; Orme, 1987; Pedrazzini et al., 1987). These findings have been further substantiated recently by application of mutant mice in which the β2-microglobulin (β2m) gene had been deleted (Flynn et al., 1992). Because β2m is required for MHC class I surface expression, β2m-deficient mutant mice are devoid of functionally active CD8 T cells. These mice die rapidly from *M. tuberculosis* but not from BCG infection. Impressive as these studies are, it should be kept in mind that β2m not only serves to stabilize MHC class I surface expression but may also perform other functions that could influence survival of *M. tuberculosis* in β2m-deficient mice. Furthermore, mycobacterium-specific CD8 T cells have been isolated from *M. tuberculosis*- and BCG-immune mice (DeLibero et al., 1988). In contrast, such mycobacterium-specific CD8 T cells were rarely identified in patients suffering from human tuberculosis (Rees et al., 1988). CD8 T-cell lines derived from *M. tuberculosis*- and BCG-immune mice are MHC class I restricted, thus raising the question of how *M. tuberculosis* and BCG proteins gain access to the MHC class I processing pathway (DeLibero et al., 1988). Although it is generally assumed that *M. tuberculosis* remains in the endosomal compartment, clear evidence for escape of *M. tuberculosis* from phagolysosomes into the cytoplasm has been presented (Leake et al., 1984; McDonough et al., 1993). Microbes residing in the cytoplasm could then produce proteins that contact MHC class I molecules, as has been clearly shown for *Listeria monocytogenes*. Alternatively, it can be assumed that during persistent replication within the phagosome, mycobacterial proteins or peptides are translocated into the cytoplasm, where they contact the MHC class I processing machinery. Recent evidence indicates that MHC class I processing can occur independently of microbial egression into the cytoplasm (Pfeifer et al., 1993).

Besides conventional MHC class I-restricted CD8 T cells, T cells that are apparently MHC class I nonrestricted have been described (DeLibero et al., 1988). Similar T cells have been identified in the listeriosis system, where these T lymphocytes are focused on peptides containing the *N*-formylmethionine (*N*-fMet) sequence presented by nonconventional MHC class Ib molecules (Kaufmann et al., 1988; Kurlander et al., 1992; Pamer et al., 1992). The *N*-fMet sequence probably serves as a secretion signal in prokaryotic cells. In mammals, the *N*-fMet sequence is present only in proteins encoded by the mitochondrial genome (probably of prokaryotic origin). Furthermore, nonconventional MHC class Ib gene products are highly conserved and vary in only few mouse strains. Thus, it appears that a subset of bacterium-specific CD8 T cells is focused on (i) conserved bacterial peptides and (ii) nonpolymorphic presentation elements. If these observations can be generalized to human tuberculosis, important consequences for peptide vaccination against bacteria with few peptides and independent of human lymphocyte antigen polymorphism can be envisaged.

A contribution of γ/δ T cells to protection is suggested by indirect evidence. They have been identified in reversal reactions of leprosy patients and in tuberculous lymphadenitis lesions (Falini et al., 1989; Modlin et al., 1989). No evidence for increased γ/δ T cell numbers, however, has been observed in lymph node granulomas of tuberculosis patients (Tazi et al., 1991). In mice, γ/δ T cells accumulate early at the site of BCG replication, in draining lymph nodes after immunization with complete Freund's adjuvant, and in the lung after aerosol immunization with mycobacterial components (Augustin et al., 1989; Janis et al., 1989; Inoue et al., 1991). Furthermore, the progressive BCG infection in *scid* mice compared to *nu*/*nu* mice and mice depleted of CD4 and CD8 T cells has been taken as evidence for a role of γ/δ T cells (Izzo and North, 1992). Direct proof, however, has to await experiments with mutant mice devoid of γ/δ T cells. The γ/δ T cells from healthy individuals proliferate vigorously in response to mycobacterial components (Kabelitz et al., 1990; Munk et al., 1990). Although preferential γ/δ-T-cell expansion by mycobacteria is caused to a large degree by low-molecular-weight nonproteinaceous components that act in a superantigen-like fashion, γ/δ T cells also appear to be stimulated by *M. tuberculosis* antigens (Munk et al., 1990; Pfeffer et al., 1990). Thus far, the kind of antigens and presentation molecules required for γ/δ-T-cell stimulation remain virtually unknown. Evidence from other systems indicates that the relevant peptides are presented by nonconventional MHC molecules (Pamer et al., 1993). Perhaps the MHC class Ib molecules involved in CD8 T-cell stimulation also participate in γ/δ-T-cell stimulation.

T-Cell Functions

Various in vitro studies of the human and murine systems show that mycobacterium-reactive CD4 T cells are potent IFN-γ producers (Emmrich et al., 1986; Kaufmann and Flesch, 1986). IFN-γ is also produced by murine CD8 T cells with mycobacterial specificity (DeLibero et al., 1988). As described above, this cytokine is the principal mediator of antituberculous resistance. Mycobacterium-reactive CD4 T cells and CD8 T cells also express specific cytolytic activities; i.e., they lyse macrophages primed with mycobacterial antigens or infected with BCG or *M. tuberculosis* (DeLibero et al., 1988; Ottenhoff et al., 1988). It appears that these two functions not only are demonstrable in vitro but also contribute to protection in vivo. Besides the well-characterized α/β T cells, other cells also produce IFN-γ and express cytolytic activities, suggesting their participation in acquisition of resistance. In particular, both NK cells and γ/δ T cells produce IFN-γ and lyse mycobacterium-pulsed target cells (Munk et al., 1990; Bancroft et al., 1991; Follows et al., 1992; Molloy et al., 1993). In addition, polymorphonuclear granulocytes (PNG) produce highly proteolytic enzymes causing tissue liquefaction (Weiss, 1989). At the site of *M. tuberculosis* growth, these cells appear sequentially in the following order: PNG, NK cells, γ/δ T cells, α/β T cells.

Evidence has been presented elsewhere that T-cell lysis of BCG-infected macrophages causes bacterial growth inhibition in vitro (DeLibero et al., 1988). Perhaps target cell lysis promotes discharge of toxic macrophage products that inhibit mycobacterial growth. This in vitro observation may be taken as evidence for a direct protective effect afforded by cytolytic T cells. More importantly, a coordinated interplay between macrophage activation by IFN-γ (probably in conjunction with additional mediators) and target cell lysis appears to be required for optimum protection (Kaufmann, 1988). *M. tuberculosis* is extremely resistant to macrophage killing. The persistence of *M. tuberculosis* in healthy individuals for years without causing disease indicates that the immune system generally fails to sterilely eradicate this pathogen and must rely on mycobacterial containment and growth inhibition. Not only prior to but also after IFN-γ stimulation, macrophages are largely abused as habitat. Lysis of such macrophages promotes bacillary release from a shelter. Provided that the microorganisms are taken up by more efficient phagocytes soon after their liberation, this mechanism should improve host defense against tuberculosis. Such an interplay between lysis and activation of MP would best be controlled in productive granulomas (see below). At the same time, target cell lysis causes tissue damage, affects organ functions, and, in the absence of phagocytosis, promotes microbial dissemination. Lysis of infected MP, therefore, is a double-edged sword that, depending on the general situation, has a beneficial or a detrimental outcome.

T-Cell Antigens

At least two characteristics of *M. tuberculosis* and BCG influence the type of antigens that are recognized by protective T cells. First, the intracellular location (phagosome versus cytosol) dictates processing via the MHC class I or class II pathway. Second, the intracellular viability of the pathogen determines availability of polypeptides for processing (Fig. 2). MHC class I versus MHC class II processing has been discussed above. Because soluble protein antigens are not introduced into the MHC class I pathway, the design of subunit vaccines requires use of appropriate adjuvants or viable carriers capable of targeting both the MHC class I and the MHC class II pathway. As long as MP fail to kill significant numbers of intracellular *M. tuberculosis*, secreted proteins and metabolically produced peptides are the main, if not the sole, source of antigens. Later, when *M. tuberculosis* and *M. bovis* die in the activated macrophage, somatic proteins become a major source of T-cell antigens. The less metabolically active bacteria are, the lower the relative proportion of secreted protein antigens will be. Dormant tubercle bacilli without significant metabolic activity but resisting macrophage killing will be an ineffectual source of any antigen. Both features may be relevant to the low effectiveness of the only vaccine against tuberculosis available, BCG. First, BCG seems to primarily activate CD4 T cells (Pedrazzini et al., 1987). While this seems to be sufficient for protection against BCG, it appears to be insufficient for effective vaccination against tuberculosis. Perhaps the shorter intracellular survival of BCG together with a deficiency in cytolysins restricts access of BCG-derived proteins to the MHC class I pathway. Second, owing to the shorter survival time of BCG, somatic antigens will predominate early after infection. Early recognition of *M. tuberculosis*-infected macrophages, however, primarily depends on T cells that recognize secreted proteins. Thus, the preponderance of CD4 T cells and somatic antigens may explain, at least in part, the insufficient protection against *M. tuberculosis* afforded by BCG vaccination.

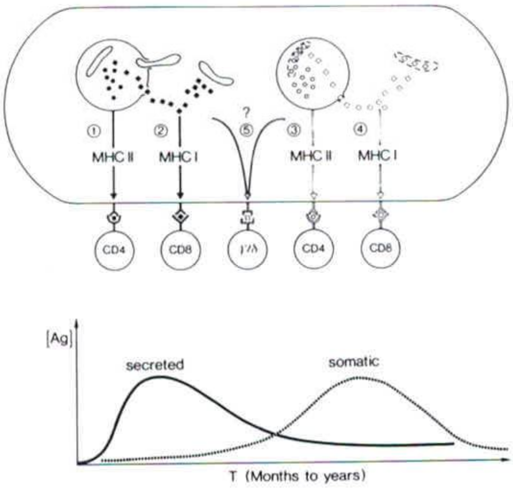

Figure 2. Relationship between intracellular persistence of *M. tuberculosis*, antigen type, and T-cell subset activation. (1) *M. tuberculosis* replicating in the phagosome secretes proteins that are degraded into peptides and then translocated to the cell surface by MHC class II molecules. (2) MHC class I molecules capture *M. tuberculosis* peptides derived from secreted proteins in the cytoplasm. Either the proteins or peptides had been translocated from the endosomal into the cytoplasmic compartment, or they were secreted into the cytoplasm by *M. tuberculosis* after its evasion of the phagosome. Later, *M. tuberculosis* is killed and degraded, thus giving rise to somatic proteins. (3) Peptides derived from *M. tuberculosis* killed in the phagosome contact MHC class II molecules. (4) Peptides from somatic proteins present in the cytoplasm are charged to MHC class I molecules. (5) Neither the source of peptides nor the presentation molecules involved in γ/δ T-cell stimulation are fully understood. This sequence of events leads to a first wave of T cells with specificity for secreted proteins followed by a second wave of T cells with specificity for somatic proteins. Ag, antigen.

THE IN VIVO SITUATION

In tuberculosis, the port of entry as well as the major organ of disease is the lung. After being inhaled, the pathogen is engulfed by alveolar macrophages that appear to be insufficiently equipped for microbial killing. Probably these alveolar macrophages transport the pathogen into the lung parenchyma and into draining lymph nodes, where the microbe replicates. Infected macrophages produce chemokines that cause the extravasation of additional phagocytes (Oppenheim et al., 1991; Friedland et al., 1992). These inflammatory phagocytes (PNG and blood monocytes) secrete significant amounts of proteolytic enzymes, generating an exudative lesion. Activated MP also secrete TNF, which initiates granuloma formation (Kindler et al., 1989). Eventually, T cells activated in draining lymph nodes as well as NK cells are attracted to the site of inflammation. Although NK cells and γ/δ T lymphocytes seem to precede α/β T cells, the former two are soon outnumbered by the last. The α/β T cells and γ/δ T cells interact with MP that present mycobacterial peptides in the context of adequate MHC molecules. They produce IFN-γ, as do NK cells, which in turn activates tuberculostatic macrophage functions. A productive granuloma with a high cellular turnover develops; bacteria are confined in it, and their growth is restrained. Although these granulomas effectively inhibit bacterial replication, they are generally unable to sterilely eradicate the pathogens. In particular, the multinucleated giant cells harbor *M. tuberculosis* and seem to be unable to eradicate their intracellular predators. Lysis of such cells, therefore, may contribute to protection by allowing uptake by more efficient phagocytes. Later, the productive granuloma may become encapsulated by a fibrotic wall, and the center of the granuloma may necrotize. TNF seems to play a notable role in fibrotic encapsulation and central necrosis (Vassalli, 1992). Encapsulation further contributes to microbial containment, and the low partial O_2 pressure (pO_2) in the necrotic center provides unfavorable growth conditions for *M. tuberculosis*. Uncontrolled cell destruction by cytolytic T cells, NK cells, activated MP, and/or PNG may promote granuloma liquefaction and rupture into the bronchoalveolar and vascular systems. The cellular detritus and the elevated pO_2 thus arising provide an excellent medium for *M. tuberculosis* that favors its uncontrolled multiplication. Rupture of the granuloma promotes microbial dissemination through the bronchoalveolar system into the environment and through the vascular system to other tissue sites.

WHY DO WE NEED MORE THAN ONE T-CELL POPULATION FOR PROTECTION?

Given that in vitro CD4 T cells, CD8 T cells, and γ/δ T cells are so highly similar with respect to their functional competence, why do we need several T-cell subsets for optimum protection to occur? At the moment, this question cannot be fully answered. A first advantage of CD8 T cells and γ/δ T cells over CD4 T cells is their restriction by MHC class I molecules, which are expressed on virtually all host cells, while MHC class II expression is restricted to certain host cells such as MP. Although *M. tuberculosis* preferentially resides in MP, a few parenchyma cells, typically in the lung, may become infected. These cells remain unnoticed by CD4 T cells and are identified only by CD8 T cells (and perhaps γ/δ T cells). Second, the three T-cell populations may differ in their activation kinetics, with γ/δ T cells probably arriving first at the site of mycobacterial growth. Thus, γ/δ T cells may perform essential effector functions before α/β T cells do. Although γ/δ T cells may be less effective, their faster kinetics of mobilization and activation may give them some advantage. Third, these T-cell populations may differ in effector functions thus far unclear, e.g., in their capacity to leave the vascular bed or in their responsiveness to inflammatory signals. Fourth, α/β T cells and γ/δ T cells vary remarkably in their tissue distributions. In mucosal tissues, including the lung, as preferred port of entry and site of disease manifestation in tuberculosis, the percentage of γ/δ T cells is markedly higher than in peripheral blood and central lymphoid organs. Finally, regulatory interactions between these T-cell subsets may be required. In support of this last possibility, evidence has been presented that γ/δ T cells control activation of α/β T cells not only in vitro but also in vivo (Kaufmann et al., 1993). Most impressively, in the model of experimental listeriosis of γ/δ T-cell-deficient mutant mice, huge, abscess-like lesions develop that are strikingly different from the granulomatous lesions at the site of listerial implantation in healthy controls (Mombaerts et al., 1993).

GENETIC DETERMINANTS FOR SUSCEPTIBILITY AND RESISTANCE IN TUBERCULOSIS

While there is little formal genetic evidence in humans, data obtained from epidemiological investigations suggest that susceptibility to many infectious diseases, including tuberculosis, is under some genetic control (Motulsky, 1979; Skamene, 1986). The annual death rate from tuberculosis reached 10% when the disease first became prevalent in the Qu'appelle Valley Indian Reservation in Canada, eliminating half the Indian families in the first three generations; yet 40 years later, the annual death rate had dwindled to 0.2%, suggesting selection for host resistance (Goodman and Motulsky, 1979). Clearly, it is conceivable that different genetic strains of the same pathogen cause diseases in different geographical regions, so that with continued passage, as could be in the case of tuberculosis in the Qu'appelle Valley, attenuated virulence and thus in a drastic drop in death rate over time result. While this confounding factor is difficult to rule out, nonetheless, the higher degree of concordance of tuberculosis among monozygotic than dizygotic twins (Comstock, 1978) and the tragic incident of Lubeck in 1927 (Anonymous, 1935), in which infants inadvertently immunized with a single viable virulent *M. tuberculosis* strain displayed marked differences in susceptibility ranging from death to recovery, argue for a genetic basis for resistance to mycobacterial diseases.

In contrast to work with the human system, experimental studies on the genetics of resistance to an enormous variety of infectious agents (salmonellae, leishmaniae, mycobacteria, murine leukemia viruses, rickettsiae, etc.) in inbred strains of mice are abundant (Skamene, 1985). In the case of resistance to *Salmonella typhimurium*, *Leishmania donovani*, and BCG, compelling experimental evidence obtained from backcross linkage analyses (Skamene et al., 1982) suggests that resistance against these three pathogens is under monogenic control. This allele has been designated *Ity*, *Lsh*, and *Bcg* in the resistance models of *S. typhimurium*, *Leishmania donovani*, and BCG, respectively. Through typing for resistance and susceptibility to BCG among recombinant inbred mouse strains together with linkage analyses and detailed dissection of a 30-centimorgan segment on murine chromosome 1, the cloning of the cDNA for the *Bcg* gene, designated *Nramp* (natural-resistance-associated macrophage protein), has recently been achieved (Vidal et al., 1993). Sequence analysis of the *Nramp* cDNA reveals a 1,452-nucleotide open reading frame that encodes a 484-amino-acid protein with structural homology to a eukaryotic nitrate transporter. Analysis of *Nramp* cDNAs from seven Bcg^r and six Bcg^s mouse strains indicates that BCG susceptibility is the result of a G-to-A transition at position 783 associated with a nonconservative substitution of Asp-105 for Gly-105 within a predicted transmembrane domain of *Nramp*. Comparison of amino acid sequences of the murine *Nramp* and a human homolog deduced from a partial cDNA clone reveals 89% homology between the two species. Nucleic acid sequence analysis indicates that Gly-105 of murine *Nramp* is conserved in the human sequence.

While it is known that the *Bcg*ʳ gene confers resistance against mycobacteria by acting early during the nonimmune phase of infection in mice (in contrast to the MHC genes, which appear to be associated with recovery after infection), the precise biochemical and molecular mechanisms of how *Nramp* regulates resistance and susceptibility to infection remain to be defined (reviewed in Skamene [1986]). Experimental evidence strongly suggests that the *Nramp* phenotype is mediated via macrophages. It has been demonstrated that the cell type expressing the *Nramp* phenotype is derived from the bone marrow and is relatively radioresistant. In addition, the phenotypic expression of *Nramp* can be inactivated by chronic exposure of mice to silica, a macrophage poison (Gros et al., 1983). Finally, *Nramp* mRNAs are preferentially expressed in the reticuloendothelial system, particularly in macrophages. The recent finding that RNI generated via the macrophage L-arginine-dependent cytotoxic mechanism is effectively antimycobacterial (Denis, 1991a; Flesch and Kaufmann, 1991; Chan et al., 1992) and the demonstration of marked structural resemblance of *Nramp* protein to a eukaryotic nitrate transporter (Vidal et al., 1993) lend support to the hypothesis that regulation of RNI trafficking in macrophages might be one way by which the resistance phenotype of this gene is expressed. It is thus possible that *Nramp* participates in the L-arginine-dependent antimycobacterial pathway by transporting NO_2^-, a relatively stable and nontoxic nitrogen oxide formed via the oxidation of nitric oxide in the aqueous phase, into the phagolysosomal compartment, whose acidic environment is requisite to and allows the formation of nitrous acid, which dismutates to generate NO (Shank et al., 1962) and other more reactive and perhaps more toxic reactive nitrogen species such as the nitrogen dioxide radical. A corollary of this possibility is that ammonia production by *M. tuberculosis* (Gordon et al., 1980) is a means by which generation of toxic RNI could be intercepted via alkalinization of the phagolysosomal content. The existence of a human homolog of *Nramp*, at least by cDNA analyses (Vidal et al., 1993), together with the presence on human chromosome 2q of a region syntenic to the 30-centimorgan segment on murine chromosome 1 that contains the *Bcg* allele (Schurr et al., 1990) should presage optimism in unraveling the genetic basis for resistance and susceptibility to mycobacterial diseases, at least at the early phase of infection. It is hoped that the elucidation of one aspect of this difficult question will form a firm springboard for understanding other as yet unknown genetic factors, e.g., the MHC molecules (Skamene, 1986), that aid in determining the outcome of mycobacterial infection.

CONCLUDING REMARKS

Around the world, as many as 60 million people suffer from tuberculosis. This high figure may lead to the false conclusion that protective immunity is totally insufficient for control of this disease. The figure, however, is clearly qualified by the even higher number of more than 1.7 billion infected individuals, i.e., one-third of the world population, illustrating that in the vast majority of infected individuals, disease does not develop in the face of an ongoing infection. Hence, protective immunity is extraordinarily inefficient in terminating infection and, at the same time, highly efficacious in preventing disease. Because the relationship between *M. tuberculosis* and host immunity underlying infection is a labile one, any diminution of protective immunity will cause progression into clinical disease.

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