Review Article

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Immune Response In Tuberculosis: An Overview

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ABSTRACT

Tuberclosis is one of the most infectious and ancient disease. it is caused by bacteria mycobacterium tuberculosis, but very few people have risk of its reoccurrence round 5-10%.one third of the population is infected with this deadly disease and comes next to HIV. When active tuberculosis develops, disease localization, severity and outcome are highly variable. It may develop anywhere in the body but usually presents as pulmonary infection ,ranging from mild infection to chronic, cavitary and severely destructive disease. According to world health organization (WHO),TB is a worldwide pandemic. The different manifestation of infection with M.tuberclosis reflect the balance between the bacillus and host defence system. However one should be were that dissecting innate and acquired host responses are complementary and synergistic.

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INTRODUCTION

One third of the world population is infected with Mycobacterium tuberculosis, but only 5 to 10% of this of this population has a life time risk of developing active tuberculosis, either within 1 or 2 years after infection (primary tuberculosis) or thereafter (postprimary tuberculosis) (Fig.1). When active tuberculosis develops, disease localization, severity, and outcome are highly variable. Miliary tuberculosis, characterized by the hematogenous dissemination of large numbers of mycobacteria throughout the body, is the most serious disease manifestation. On the other end of the clinical spectrum, tuberculosis pleuritis is usually self-limiting. Tuberculosis may develop anywhere in the body, but usuallypresents as pulmonary infection, ranging from mild infiltration to chronic, cavitary, and severely destructive disease.The different manifestation of infection with M. tuberculosis reflect the balance between the bacillus and host defense mechanisms, in which the quality of host defense determines outcome. In this review, emphasis is placed on the natural, innate host defense mechanisms against M.tuberculosis.

However, to enable the reader to place those mechanisms in the context of both innate and acquired defense, a complete picture of M.tuberculosis infection is briefly reviewed first. One should be aware that dissecting innate and acquired host response are complementary and synergistic.

Chronological Event in the Pathogenesis of Tuberculosis

Based on lurie's fundamental studies in rabbits, four stages of pulmonary tuberculosis have been distinguished. The first stage begins with inhalation of tubercle bacilli. Alveolar macrophages ingest the bacilli and often destroy them. At this stage, the destruction of mycobacteria depends on the intrinsic microbicidal capacity of host phagocytes and virulence factors of the ingested mycobacteria. Mycobacteria which escape the initial intracellular destruction will multiply, and this will lead to disruption of the macrophage. When this happens, blood monocytes and other inflammatory cells are attracted to the lung (second stage). These monocytes will differentiate into macrophages which again readily ingest but do not destroy the mycobacteria. In this symbiotic stage, mycobacteria grow log-arithmically , and blood-derived macrophages accumulate, but little tissue damage occurs. Two to three weeks after infection, T-cell immunity develops, with antigen-specific T lymphocytes that arrive, proliferate with in the early lesions or tubercles, and then activate macrophages to kill yje intracellular mycobacteria. Subsequent to this phase the early logarithmic bacillary growth stops (third stage). Central solid necrosis in these primary lesions inhibits extracellular growth of mycobacteria. As a result, infection may become stationary or dormant. Disease may progress, and hematogenous dissemination may take place after primary infection, as well as months or years afterwards (postprimary tuberculosis), under conditions of failing immune surveillance. Liquefiedcaseous foci provide excellent conditions for extracellular growth of M. tuberculosis. Cavity formation may lead to rupture of nearby bronchi, allowing the bacilli to spread through the airways to other parts of the lungs and the outside environment. In summary, after entry in the human lung, M. Tuberculosishas a series of encounters with different host defense mechanisms. The final outcome of infection with M. Tuberculosis depends on the balance between (i) outgrowth and killing of M. Tuberculosis and (ii) the extent off tissue necrosis, fibrosis, and regeneration.

After inhalation of M. Tuberculosis (MTB) droplet nuclei, several scenarios may follow. Mycobacteria may be destroyed by alveolar macrophages, in which case no real infection will take place. Alternatively, M. tuberculosis may not be immediately killed, and so a primary complex consisting of a small infiltrate and a draining lymph node will develop. Small calcifications may be seen on radiographic examination and the PPD skin test, as a marker of an M. tuberculosis-specific T-cell response, becomes positive. Most often, infection is stabilized at this point. In a minority of cases active disease now develops (primary tuberculosis[TB]),either in the lungs or anywhere else after hematogenous dissemination of M.

tuberculosis. Months or years afterwards, usually under condition of failing immune surveillance, latest infection may reactivate (postprimary TB).
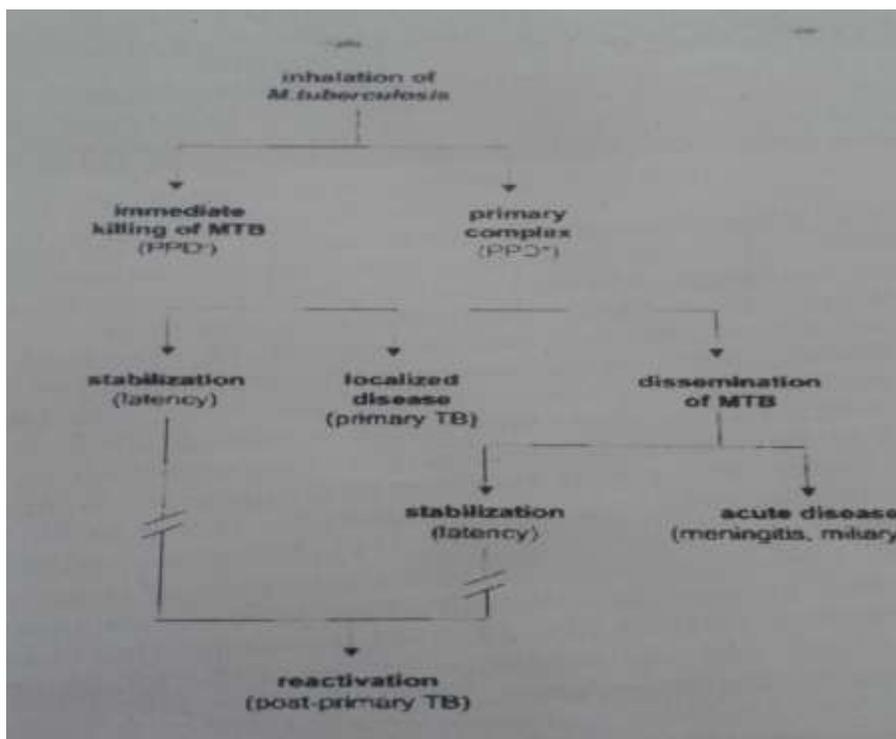

FIG. 1. Chronological events after inhalation of M. tuberculosis.

Protection against Tuberculosis

Acquired T- cell- mediated immunity: Elimination of M. Tuberculosis infection mainly depends on success of the interaction between infected macrophages and T lymphocytes. Primary as well as acquired immunodeficiencies, especially human immunodeficiency virus infection, have dramatically shown the importance of cellular immunity in tuberculosis. CD4_ T cell exert their protective effect by the production of cytokines, primarily gamma interferon (IFN-Γ), after stimulation with mycobacterial antigens. Other T-cell subsets, like CD8_T cell, are likely to contribute as well, by secreting cytokines and lysing infected cells . The T-cell response is mostly antigen specific, and attention has focused on the identification of immunodominats antigens which might be used for the development of effective vaccines. The acquired T-cell response develops in context of the major histocompatibility complex (MHC), and polymorphism of MHC may contribute of differences in diseases susceptibility or outcome. Functional diversity of T lymphocytes may also be relevant. In 1986, it was reported that murine helper T (Th) lymphocytes could be divided into two subsets: Th1 clones were characterized by the production of IFN-γ, and Th2 clones were characterized by the production of interleukin 4 (IL-4).Both subsets develop from naïve T cell, whose differentiation is influenced by the environment:IL-12, produced by activated macrophages and dendritic cells, is the principal Th1- inducing cytokine, while IL-4 promotes induction of Th2 cells. More cytokines and different cellular subsets have been included in this Th1- Th2 concept , which is thought to be relevant in many disease entities. In mycobacterial infection, Th1 –type cytokines seem essential for protective immunity. Indeed, IFN-Γ gene knockout (KO) mice are highly susceptible to M. Tuberculosis, and individuals lacking receptors for IFN-Γ suffer from recurrent, sometime lethal mycobacterial infections. Th2- type cytokines inhibit the in vitro production of IFN-as well the activation

of macrophages, and my therefore weaken host defense We and others have shown an increase in Th2-type cytokines in tuberculosis patients However, this is not a consistent finding, and the relevance of the Th1-Th2 concept in disease susceptibility or presentation remains uncertain.

Evidence for innate immunity
Phagocytic cells play a key role in the initiation and direction of adaptive T-cell immunity by presentation of mycobacterial antigens and expression of costimulatory signals and cytokines. In addition, innate defense mechanisms of phagocytic cells may be important, as highlighted in lurie' s fundamental studies with resistant and susceptible inbred rabbits. Seven days after primary infection through inhalation of tubercle bacilli, the lungs of mycobacterium-susceptible rabbits contained 20-to 30-fold more viable mycobacteria then did the lungs of mycobacterium-resistant rabbits. Obviously, this difference during early infection cannot be attributed to T-cell immunity. More recently this was found that acquired T-cell immunity in vaccinated mice effectively protects them from disseminated tuberculosis but does not prevent the initial pulmonary infection.

In human disease, the same holds true. Acquired T-cell immunity after vaccination with Mycobacterium bovis BCG is more effective against disseminated infection than against pulmonary disease.
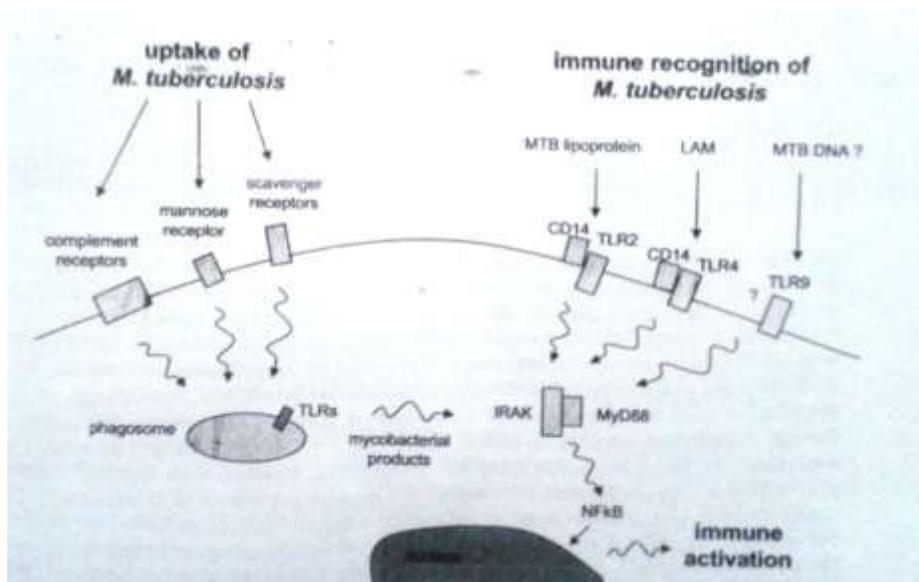
Various receptors have been identified for phagocytosis of M. tuberculosis (MTB) by macrophages and dendritic cells: complement receptors are primarily responsible for uptake of opsonized M. tuberculosis; MRs and scavenger receptors for uptake of nonopsonized M. tuberculosis. TLRs play a central role in immune recognition of M. tuberculosis. In the context of CD14, TLR2 binds lipoarabinomannan, a heterodimer of TLR2 and TLR6 binds a 19-KDa M. tuberculosis lipoarabinomannan, TLR4 binds to an undefined heat-labile cell-associated factor, and (possibly) TLR4 binds to M. tuberculosis DNA. After binding to TLRs, common signalling pathways lead to cell activation and cytokine production. TLRs are expressed not only at the cell surface but also in phagosomes; therefore, immune activation may occur with or without phagocytosis. On the other hand, phagocytosis alone probably does not lead to immune activation without the involvement of TLRs.

Occurred twice as often in black as in white individuals who were equally exposed to active tuberculosis. Apparently, innate host defense mechanisms at this early stage were less efficient in black residents. In accordance with this, macrophages from Afro-Americans demonstrate a relative permissiveness for intracellular growth of virulent mycobacteria. Support for the relevance of T-cell-independent, intrinsic

bactericidal activity of macrophages is also found in genetic studies which have shown associations between tuberculosis and functional gene polymorphism for various macrophage products . There is more evidence, from both clinical and experimental studies, to support the relevance of innate immunity in tuberculosis. In the following paragraphs, the various components and processes that make up the innate host response to M. tuberculosis are discussed in more detail.

PHAGOCYTOSIS OF M.TUBERCULOSIS

Alveolar resident macrophages are the primary cell type involved in the initial uptake of M. tuberculosis. After this first encounter, dendritic cells and monocyte-derived macrophages also take part in the phagocytic process. Endocytosis of M. tuberculosis involves different receptors on the phagocytic cell (Fig.2) which either binds to nonopsonized M. tuberculosis or recognize opsonins on the surface of M. tuberculosis. As an example of the latter mechanism, mycobacteria can invade host macrophages after opsonisation with complement factor C3, which is followed by binding and uptake through complement receptor 1 (CR1), CR3, and CR4 . The relative importance of the various receptors for complement factor C3 is apparent form experiments in vitro, in which in the absence of CR3, phagocytosis of M. tuberculosis by human macrophages and monocytes is reduced by approximately 70 to 80%.For opsonisation with C3, the split product C3b should first be generated by activation of the complement system. M. tuberculosis also utilize part of the classical pathway of complement activation by direct binding to C2a, even in the absence of C4b; in this way the C3b necessary for binding to CR1 is formed This mechanism facilitates mycobacterial uptake in environments low in opsonins, such as the lung. Nevertheless, nonopsonized M. tuberculosis can behind directly to CR3 and CR4. However, the best- characterized receptor for non-opsonin-mediated phagocytosis of M. tuberculosis is the mycobacteria .When uptake by CRs and MR is blocked, macrophages may also internalize M. tuberculosis through the type A scavenger receptor. Fc_ receptors, which facilitate phagocytosis of particles coated with antibodies of the immunoglobulin G class, seem to play little role in tuberculosis.

Enhanced binding of M. tuberculosis to epithelial cell or alveolar macrophages may represent a risk factor for developing clinical tuberculosis. Collection, a structurally related group of proteins that includes surfactant proteins, mannose-binding lectins (MBLs), and C1q, seem to be important in this respect. Surfactant proteins A (Sp-A) facilitates the uptake of M. tuberculosis through binding to either the macrophages, type II pneumocytes, or neutrophils. Interestingly, it has been reported that human immunodeficiency virus infected individuals have increased levels of Sp- A in the lungs, and this results in a threefold-greater attachment of M. tuberculosis to alveolar macrophages. In contrast another surfactant protein, SP-D, has been found to block the uptake of pathogenic strains of M. tuberculosis in tuberculosis in macrophages. It may therefore be hypothesized that the relative concentration of different surfactant proteins correlate with the risk of infection.

Another member of the collection family, the plasma factor MBL, may also be involved in the uptake of mycobacteria by phagocytic cells. MBL recognize carbohydrate configuration on a wide variety of pathogens and induces phagocytosis directly through a yet-undefined receptor or indirectly by activation of the complement system. Genetic polymorphisms of the MBL gene account for significant variability of serum MBL concentration in different population. One study has reported elevated concentrations of MBL in the serum of tuberculosis patients and genetic polymorphisms associated with increased production of MBL have been reported to be a relative disadvantages in mycobacterial infections.

Although M. tuberculosis has a tropism for phagocytic cell, it may also interact with nonprofessional phagocytic cell, such as alveolar epithelial cells This binding may involve fibronectin, a glycoprotein found in plasma and on the outer surface of many cell types .Similar to mycobacterium lepra. M. tuberculosis may bind to epithelial cells since the bacterium produces and secretes the 30-to31k Da

antigen 85 complex, a member of the fibronectin-binding protein family. In addition, a 28-KDa heparin-binding adhesin, produced by M. tuberculosis, will bind to sulfated glycoconjugates on host cells.

Thus, there are multiple mechanisms for the uptake of M. tuberculosis, involving a number of different host cell receptors. Most of these interaction have been demonstrated in vitro, and their relative importance in vivo remains to be shown. Distinct routes of entry of M. tuberculosis may lead to differences in signal transduction, immune activation, and intracellular survival o M. tuberculosis. For example, Fc_ receptormediated phagocytosis is directly linked to an inflammatory response, and binding to CR is not. Survival of M. tuberculosis after binding to CR1 is better than that after binding to CR3 or CR4. Also, phagocytosis of Sp-A-opsonized mycobacteria by alveolar macrophages suppresses reactive nitrogen intermediates, one of the putative effector mechanisms involved in the killing of mycobacteriaLikewise, virulent strains of M. tuberculosis are phagocytosed through MR, while attenuated strains are not, suggesting that this route of entrance is advantages to the mycobacterium. Indeed, phagocytosis through MR does not trigger O2 production and M. tuberculosis exerts an anti-inflammatory signal through MR. In vivo, the possible role of these events in immune evasion byM. tuberculosis remains to be determined. Of interest, strong linkage was found with several markers on chromosome 10p 13, where the MR gene is located, in a recent genome-wide scan of 245 families with leprosy in India.

RECOGNITION OF M.TUBERCULOSIS: ROLE OF TOLL-LIKE RECEPTORS

Beside phagocytosis recognition of M. tuberculosis or mycobacterial product is a crucial step in an effective host response. Immune recognition of the major mycobacterial cell wall component, lipoarabinomannan (LAM), appears to resemble that of gram negative bacterial lipopolysaccharide (LPS). Several circulating factor and receptors are involved. Plasma LPS-binding protein enhances macrophage responses to LPS and LAM by transferring these microbial product to the cell surface receptor CD14(68). Similarly, soluble CD 14 confers responsiveness to both LAM and LPS in CD 14- negative cells. Of interest, concentration of CD14 and LPS-binding protein in serum were elevated in patients with active tuberculosis.

Toll- like receptors (TLRs) are phylogenetically conserved mediators of innate immunity which are essential for microbial recognition on macrophages and dendritic cell. Member of the TLR family are transmembrane proteins containing repeated leucine-rich motifs in their extracellular domains, similar to other pattern-recognizing proteins of the innate system. The cytoplasmic domain of TLR is homologous to the signaling domain of IL-1 receptor (IL-1R) and links to IRAK (IL-1R-associated kinase), a serine kinase that activates transcription factors like NF-B to signal the production of cytokines. To date, at least 10 TLR have been identified; o those TLR2, TLR4, and TLR9 seem responsible for the cellular responses to peptidoglycan and bacterial lipopeptides, endotoxin of gram-negative bacteri and bacterian DNA ,respectively. TLRs are also involved in cellular recognition of mycobacteria (Fig.2). Through TLRs, M. tuberculosis lysate or soluble mycobacterial cell wall-associated lipoproteins induce production of IL-12, a strong proinflammatory cytokine. My D88 (myeloid differentiation proteins 88), a common signalling component that links all TLRs to IRAK, was found to be essential for M. tuberculosis-induced macrophage activation. A mutation of TLR2 specifically inhibited M. tuberculosis-induced tumor necrosis factor alpha (TNF-α) production; this inhibition was incomplete, thereby suggesting that besides TLR2, other TLRs may be involved. In a transfection model using Chinese hamster ovary (CHO) cells (which are relatively deficient in TLR), expression of TLR2 or TLR4 conferred responsiveness to both virulent and attenuated M. tuberculosis. TLR2, and not TLR4, was necessary for signalling of the mycobacterial LPS LAM and a 19-KDa M. tuberculosis lipoprotein, while an undefined heat-labile cell-associated mycobacterial factor was found to be the ligand for TLR4. Interestingly, mycobacterial infection and proinflammatory cytokines increase surface expression of TLR2. Besides TLR2 and TLR4,

other TLRs may be involved in immune recognition of M. tuberculosis: heterodimerization of TLR2 with TLR6 or TLR1 is necessary for signal transduction (31, 167), and TLR9 binds CpG dinucleotides in bacterial DNA.

From several lines of evidence it has become clear that phagocytosis does not lead to immune activation in the absence of functional TLRs (Fig. 2). Even though TLR2 is recruited to phagosomes during phagocytosis, cytokine production was eliminated by the expression of a mutant TLR2, but particle binding and internalization were unaffected. Furthermore, the expression of CD14 and TLRs did not alter uptake of M. tuberculosis in vitro studies. Apparently, TLRs play an important role in innate recognition of mycobacteria, and this also hold for humans. Interestingly, a recent study showed that TLR2 activation directly led to killing of intracellular M. tuberculosis in alveolar macrophages in vitro. It may be anticipated that genetic polymorphism, or perhaps mutations, in the relevant TLR or the downstream signalling proteins will affect the performance of the innate host response to mycobacteria.

CYTOKINE PRODUCTION DRIVEN BY M. TUBERCULOSIS

Proinflammatory cytokines

Recognition of M. Tuberculosis by phagocytic cells lead to cell activation and production of cytokines, which in itself induces further activation and cytokine production in a complex process of regulation and cross -regulation. This cytokine network plays a crucial role in the inflammatory response and the outcome of mycobacterial infections (Fig. 3). Several proinflammatory cytokine are discussed here.

TNF-a.: stimulation of monocytes, macrophages, and dendritic cell with mycobacteria or mycobacterial products induces the production of TNF-α, a prototype proinflammatory cytokine. TNF-α plays a key role in granuloma formation, induces macrophages activation, and has immunoregulatory properties. In mice, TNF-α is also important for containment of latent infection in granulom. In tuberculosis patients, TNF-α production is present at the site of disease. Systemic spillover of TNF-α may account for unwanted inflammatory effects like fever and wasting. Clinical deterioration early in treatment is associated with a rapid selective increase of TNF-α in plasma and quick recovery is associated with a rapid decrease of TNF-α in plasma.To limit the deleterious effect of TNF-α systemic production of TNF-α is downregulated and soluble TNF-α receptors which block TNF-α activity are increased. KO mice which are unable to make TNF or the TNF-α receptor p55 display an increased susceptibility for mycobacteria. In line with this, the use of potent monoclonal anti-TNF-α antibodies in Crohn's disease and rheumatoid arthritis has been associated with increase reactivation of tuberculosis (including miliary and extrapulmonary disease). In human tuberculosis, no TNF-α gene mutations have been found and no positive association has yet been established between gene polymorphism for TNF-α and disease susceptibility.

IL-1 A second proinflammatory cytokine involved in the host response to M. tuberculosis is IL-1. Like TNF-α, IL-1 is mainly produced by monocytes, macrophages, and dendritic cell. In tuberculosis patients, IL-1 is expressed in excess and at the site of disease Studies with mice suggest an important role of IL-1 in tuberculosis: IL-1 and -1 double- KO mice and IL-IR type I-deficient mice (which do not respond to IL-1)display an increased mycobacterial outgrowth and also defective granuloma formation after infection with M. Tuberculosis. In addition, among 90 Hindu tuberculosis patients in London, haplotypes for IL-1 and IL-1R antagonist (IL-1RA) (a naturally occurring antagonist of IL-1) were unevenly distributed: a "proinflammatory haplotype," reflected in an increase ratio of IL-1 production to IL-1Ra production, was significantly more common in tuberculosis pleurisy than in other types of tuberculosis.

Because tuberculosis pleurisy is a usually self-resolving type of primary tuberculosis, one may hypothesize that an increased IL-1_/IL-1Ra ratio protects against a more severe presentation of tuberculosis.

IL-6.IL- 6, which has both pro- and anti-inflammatory properties, is produced early during mycobacterial infection and at the site of infection). IL-6 may be harmful in mycobacterial infections, as it inhibits the production of TNF-α and IL-1 (194) and promotes in vitro growth of Mycobacterium avium. Other reports support a protective role for IL-6: IL-6-deficient mice display increased susceptibility to infection with M. Tuberculosis, which seems related to a deficient production of IFN-early in the infection, before adaptive T-cell immunity has fully developed.

IL- 12. IL-12 is a key player in host defense against M. tuberculosis. IL-12 is produced mainly by phagocytic cells, and phagocytic cell, and phagocytosis of M. tuberculosis seems necessary for its production. IL-12 has a crucial role in the induction of IFN-γ production. In tuberculosis, IL-12has been detected in lung infiltrates, in pleurisy, in granulomas , and in lymphadenitis. The expression of IL-12 receptors is also increased at the site of disease. The protective role of IL-12 can be inferred from the observation that IL-12 KO mice are highly susceptible to mycobacterial infections,. In human suffering from recurrent nontuberculous mycobacterial infections, deleterious genetic mutations in the genes encoding IL-12p40 and IL-12R have been identified .These patients display a reduced capacity to produce IFN-γ. Recently, an IL-12R defect has also been identified in a patient with abdominal tuberculosis. Apparently, IL-12 is a regulatory cytokine which connects the innate and adaptive host response to mycobacteria and which exerts its protective effects mainly through the induction of IFN-γ.

IL-18 AND IL-15. In addition to IL-12, two cytokines are important in the IFN-x_ axis. IL-18, a novel proinflammatory cytokine which shares many feature with IL-1, was initially discovered as an IFN-γ inducing factor, synergistic with IL-12 . It has since been found that IL-18 also stimulates the production of other proinflammatory cytokines, chemokines, and transcription factors.
There is evidence for a protective role of IL-18 during mycobacterial infections: IL-18 KO mice are highly susceptible to BCG and M. tuberculosis, and in mice infected with M. Leprae, resistance is correlated with a higher expression of IL-18. Il-18's major effect in this model seems to be the induction of IFN-γ. Indeed in tuberculosis pleurisy, parallel concentrations of IL-18 and IFN-γ were found. Also, M. tuberculosis-mediated production of IL-18 by peripheral blood mononuclear cells is reduced in tuberculosis patients, and this reduction may be responsible for reduced INF-y production. IL-15 resembles IL-2 in its biologic activities, stimulating T-cell and NK-cell proliferation and activation. Unlike IL-2, however, IL-15 is primarily synthesized by monocytes and macrophages. IL-15 Mrna was found to be expressed more strongly in immunologically resistant tuberculoid leprosy than in unresponsive lepromatous leprosy. As far as we know, no report has been published yet about IL-15 in tuberculosis.

IFN-γ The protective role of IFN-γ in tuberculosis is well established , primarily in the context of antigen-specific T-cell immunity. Mycobacterial antigen-specific IFN-γ production in vitro can be used as a surrogate marker of infection with M. tuberculosis . In principal, naïve (tuberculin skin test-negative) individuals do not show purified protein derivative (PPD)-stimulated IFN-γ production in vitro. However, in both PPD- positive and PPD-negative individuals, M. tuberculosis-infected monocytes stimulate lymphocytes for the in vitro production of INF-y (103). We found that PPD (consisting of mycobacterial proteins) selectively induces IFN-γ production in PDP- positive individuals, while M. tuberculosis sonicate, which contains mycobacterial polyglycans and phospholipids, nonselectively induced IFN-γ production in PPD positive and PPD-negative individuals alike (R. van Crevel et al, unpublished data).

This M. tuberculosis sonicate stimulates production of monocyte-derived cytokines like TNF-α and IL-1. These, as well as IL-12 and IL-18, may act as costimuli for antigen-independent IFN-γ production .
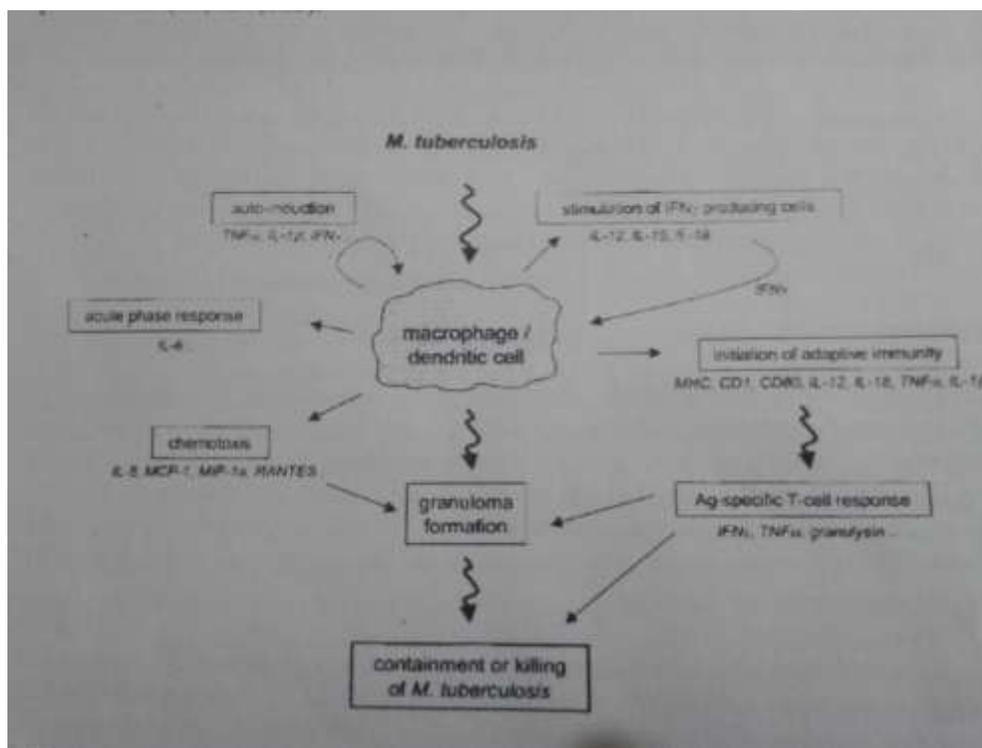

FIG.3. Inflammatory response of phagocytic cell upon activation with M. tuberculosis.

Immune recognition of M. tuberculosis by macrophages and dendritic cell is followed by an inflammatory response with a crucial role for cytokine production. Initial event in this cellular response include nonspecific host defence mechanism, which may lead to early killing or containment of infection. In addition, various cellular products, including cytokines and cell surface markers, are involved in these processes as depicted in the figure (in italics). The anti-inflammatory cytokines (see text ["Anti-Inflammatory Cytokines"]) are not represented in this picture.

Which cell are responsible for this nonspecific production of IFN-γ_? First, before adaptive T-cell immunity has fully developed, NK cells may be the main producers of IFN-γ either in response to IL-12 and IL-18 or directly by exposure to mycobacterial oligodeoxynucleotides. Second, lung macrophages were found to produce IFN-γ in M. tuberculosis- infected mice. This remarkable observation needs confirmation. Third, T cell bearing limited T-cell receptor diversity, including T cells expressing_ T-cell receptors (T cell) and CDI- restricted T cells, may produce IFN-γ_ during early infection. T cell may directly recognize small mycobacterial proteins and nonprotein ligands in the absence of antigen-presenting molecules. In mice, a single priming with M. tuberculosis substantially increases the number of T cell, but not the number of T cells (CD4 and CD8T cells) in draining lymph nodes. In mice infected with M. tuberculosis, T cell accumulate at the site of disease and seem necessary for early containment of mycobacterial infections. Like T cells, CDI- restricted T cells do not react with mycobacterial protein antigens in the context of MHC class I and II molecules.

Instead, these T cells react with mycobacterial lipid and glycolipid antigens bound to CDI on antigen-presenting cells. CDI molecules have close structural resemblance to MHC class I but are relatively nonpolymorphic. In mycobacterial infections, several different T- cells subsets have been found to

interact with CDI, including CD4, CD8 (double-negative) T cells, CD4 or CD8 single-positive T cells, and T cells. CDI-restricted T cells display cytotoxic activity and are able to produce IFN-γ.

Anti-Inflammatory Cytokines
The proinflammatory response which is initiated by M. tuberculosis is antagonized by anti-inflammatory mechanisms. Soluble cytokine receptors (e.g., soluble TNF-α receptors I and II) prevent binding of cytokines to cellular receptors, thereby blocking further signaling. As already mentioned, IL-1_ is counteracted by a specific antagonist, IL-1Ra. In addition, three anti-inflammatory cytokines, IL-4, IL-10, and transforming growth factor beta (TGF), may inhibit the production or the effects of proinflammatory cytokines in tuberculosis.

IL-10. IL-10 is produced by macrophages after phagocytosis of M. tuberculosis and after binding of mycobacterial LAM (49). T lymphocytes, including M. tuberculosis-reactive T cells, are also capable of producing IL-10. In patients with tuberculosis, expression of IL-10 Mrna has been demonstrated in circulating mononuclear cells, at the site of disease in pleural fluid, and in alveolar lavage fluid. Ex vivo production of IL-10 was shown to be upregulated in tuberculosis by some investigators, but this was not found by others IL-10 antagonizes the proinflammatory cytokine response by downregulation of production of IFN-γ, TNF-α, and IL-12. Since the last of these cytokines-as discussed under the previous section heading- are essential for protective immunity in tuberculosis, IL-10 would be expected to interfere with host defence against M. tuberculosis.

Indeed, IL-10 transgenic mice with mycobacterial infection develop a larger bacterial burden. In line with this, IL-10- deficient mice showed a lower bacterial burden early after infection in one report, albeit normal resistance in two other reports. In human tuberculosis, IL-10 production was higher in anergic patients, both before and after successful treatment, suggesting that M. tuberculosis-induced IL-10 production suppresses an effective immune response.

TGF-β .TGF-β also seems to counteract protective immunity in tuberculosis. Mycobacterial products induce production of TGF-β by monocytes and dendritic cells. Interestingly, Lam from virulent mycobacteria selectively induces TGF-β products. Like IL-10, TGF-β is produced in excess during tuberculosis and is expressed at the site of disease . TGF-β suppresses cell-mediated immunity: in T cells, TGF-βinhibits proliferation andIFN-γ production; in macrophages it antagonizes antigen presentation, proinflammatory cytokine production, and cellular activation. In addition, TGF-β may be involved in tissue damage and fibrosis during tuberculosis, as it promotes the production and deposition of macrophage collagenases and collagen matrix. Naturally occurring inhibitors of TGF-β eliminate the suppressive effects of TGF-β on mononuclear cells from tuberculosis patients and in macrophages infected with M. tuberculosis . Within the anti-inflammatory response, TGF-β and IL-10 seem to synergize: TGF-β selectively induces IL-10 production, and both cytokines show synergism in the suppression of IFN-γ production. TGF-β may also interact with IL-4. Paradoxically, in the presence of both cytokines, T cell may be directed towards a protective Th 1-type profile.

IL-4. The deleterious effect of IL-4 in intracellular infection (including tuberculosis) have been ascribed to this cytokine's suppression of IFN-γ production and macrophages activation .In mice infected with M. tuberculosis, progressive disease and reactivation of latent infection are both associated with increased production of IL-4. Similarly, overexpression of IL-4 intensified tissue damage in experimental infection. Conversely, inhibition of IL-4 production did not seem to promote cellular immunity: IL-4 mice displayed normal instead of increased susceptibility to mycobacteria in two studies, suggesting that IL-4 may be a

consequence rather than the cause of tuberculosis development. In contrast, a recent study on IL-4 KO mice showed increased granuloma size and mycobacterial outgrowth after airborne infection.

TGF-β .TGF-β also seems to counteract protective immunity in tuberculosis .mycobacterial products induce production of TGF-β by monocytes and dendritic cells .Interestingly, LAM from virulent mycobacteria selectively induces TGF-β production. Like IL-10,TGF-β is produced in excess during the tuberculosis and is expressed at the site of the disease. The deleterious effects of IL-4 in intracellular infections (including tuberculosis) have been ascribed to this cytokine's suppression of IFN-γ production and macrophage activation . In mice infected with M. tuberculosis, progressive disease and reactivation of latent infection are both associated with increased production of IL-4. Similarly, over expression of IL-4 intensified tissue damage in experimental infection . Conversely, inhibition of TGF-beta also seems to counteract protective immunity in tuberculosis. Mycobacterial products induce production of TGF-β by monocytes and dendritic cells. Interestingly, LAM from virulent mycobacteria selectively induces TGF-β production . Like IL-10, TGF-β is produced in excess during tuberculosis and is expressed at the site of disease. TGF-β suppresses cell-mediated immunity: in T cells, TGF-β inhibits proliferation and IFN-γ production; in macrophages it antagonizes antigen presentation, pro-inflammatory cytokine production, and cellular activation. In addition, TGF-β may be involved in tissue damage and fibrosis during tuberculosis, as it promotes the production and deposition of macrophage collagenases and collagen matrix. Naturally occurring inhibitors of TGF-β eliminate the suppressive effects of TGF-β on mononuclear cells from tuberculosis patients and in macrophages infected with M. tuberculosis . Within the anti-inflammatory response, TGF-β and IL-10 seem to synergize: TGF-β selectively induces IL-10 production, and both cytokines show synergism in the suppression of IFN-γ production. TGF-β may also interact with IL-4. Paradoxically, in the presence of both cytokines, T cells may be directed towards a protective Th1-type profile .

IL-4 production did not seem to promote cellular immunity: IL-4 mice displayed normal instead of increased susceptibility to mycobacteria in two studies, suggesting that IL-4 may be a consequence rather than the cause of tuberculosis development. In contrast, a recent study on IL-4 KO mice showed increased granuloma size and mycobacterial outgrowth after airborne infection. Compared with control mice, production of proinflammatory cytokines was increased in these animals and accompanied by excessive tissue damage. We and others have detected increased production of IL-4 in human tuberculosis patients, especially those with cavitary disease. However, this is not a consistent finding, and it still remains to be determined whether IL-4 causes or merely reflects disease activity in human tuberculosis. Thus, the role of IL-4 in tuberculosis susceptibility is not yet entirely resolved.

Production of soluble cytokine receptors and anti-inflammatory cytokines may help regulate the inflammatory response during tuberculosis. An unrestrained proinflammatory response may lead to excessive tissue damage (as in IL-4 KO mice), while a predominance of anti-inflammatory effects may favor outgrowth of M. tuberculosis. M.tuberculosis may evade protective immune mechanisms of the host by selective induction of anti-inflammatory cytokines. In addition, individuals genetically predisposed to higher production of these cytokines may display increased innate susceptibility to M. tuberculosis. To date, such genetic predisposition has not yet been reported in humans.

Chemokines:

Chemotactic cytokines (chemokines) are largely responsible for recruitment of inflammatory cells to the site of infection. About 40 chemokines and 16 chemokine receptors have now been identified. A number of chemokines have been investigated in tuberculosis. First, several reports have addressed the role of IL-8, which attracts neutrophils, T lymphocytes, and possibly monocytes. Upon phagocytosis of M.

tuberculosis or stimulation with LAM, macrophages produce IL-8 . This production is substantially blocked by neutralization of TNF-alpha and IL-1_, indicating that IL-8 production is largely under the control of these cytokines.

Pulmonary epithelial cells also produce IL-8 in response to M. tuberculosis. In tuberculosis patients, IL-8 has been found in broncho alveolar lavage fluid and serum for months. This finding is puzzling, because first of all it is unclear what drives such prolonged production and second IL-8 is a potent neutrophil attractant and a neutrophilic response is not prominent in established tuberculosis.

A second major chemokine is monocyte chemoattractant protein 1 (MCP-1), which is produced by and acts on monocytes and macrophages. M. tuberculosis preferentially induces production of MCP-1 by monocytes . In murine models, deficiency of MCP-1 inhibited granuloma formation . Also, C-C chemokine receptor 2-deficient mice, which fail to respond to MCP-1, display reduced granuloma formation and suppressed Th1-type cytokine production and die early after infection with M. tuberculosis .In alveolar lavage fluid, serum ,and pleural fluid from tuberculosis patients, concentrations of MCP-1 were found to be elevated.

A third chemokine is RANTES, which is produced by a wide variety of cells and which shows promiscuous binding to multiple chemokin receptors. In murine models, expression of RANTES was associated with development of M. bovis induced pulmanory granulomas. In human patients, RANTES has been detected in alveolar lavage fluid.

Apart from IL-8, MCP-1, and RANTES, other chemokines may be involved in cell trafficking in tuberculosis. Inhibition of chemokines production may lead to an insufficient local tissue response. However, due to the redundancy of the chemokine system, the contribution of individual chemokines is difficult to evaluate. As far as we are patients with mycobacterial infectious diseases.

EFFECTOR MECHANISMS FOR KILLING OF M. TUBERCULOSIS

Macrophages are the main effector cells involved in killing of M. tuberculosis. To become active against mycrobacteria, macrophages need to be activated. In vitro models of macrophage activation for the killing of M. tuberculosis seem rather artificial, and therefore the exact conditions for optimal activation remain unknown. However, it is clear that lymphocyte products, mainly IFN-γ, and proinflammatory cytokines like TNF-beta are important. In addition, vitamin D seems involved in macrophage activation.

The active metabolite of vitamin D, I,25-dihydroxyvitamin D, helps macrophages suppress growth of M. tuberculosis .Concentrations of vitamin D in serum have been reported to be lower in tuberculosis patients in some populations but not in others. A recent study among Gujarati Hindus, a mainly vegetarian immigrant population in London, showed that vitamin D deficiency was a risk factor for tuberculosis. When considered in combination with vitamin D deficiency, three polymorphisms of the vitamin D receptor were also associated with disease susceptibility in this population. For another variant of the vitamin D receptor (tt genotype), 6% of tuberculosis patients in The Gambia proved homozygous compared with 12% of control subjects suggesting that this polymorphism protects against active tuberculosis.

It should be noted, however, that no functional changes which might affect macrophage activation have yet been described for any of the vitamin D receptor polymorphisms associated with disease. Putative mechanisms involved in killing of M. tuberculosis within the phago lysosomes of activated macrophages include the production of reactive oxygen intermediates (ROI) or reactive nitrogen intermediates (RNI). The study of these mechanisms has been hampered by differences between mice (the most important animal model used for mycobacterial infections) and humans. However, when we restrict ourselves to data derived from human cells or patients, controversy remains.

In vitro, mycobacteria seem resistant to killing by ROI such as superoxide and hydrogen peroxide. A possible explanation lies in the fact that several mycobacterial products including sulfatides and LAM, are

able to scavange ROI In vivo, it was found that p47phox_/_mice, which lack a functional p47 unit of NADPH-oxidase needed for superoxide production, suffer from increased early soutgrowth of mycobacteria in experimental infection (46). Therefore, this supports a role for ROI in the killing of M. tuberculosis. On the other hand, patients with chronic granulomatous disease, who have defective production of ROI, do not seem to display increased susceptibility to tuberculosis.

The role of RNI in tuberculosis also remains a matter of debate. In vitro human alveolar macrophages infected with M. bovis.BCG display increased inducible nitric oxide synthase (iNOS) mRNA and inhibition of iNOS is followed by increased bacterial outgrowth. In tuberculosis patients, alveolar macrophages show increased production of iNOS as well However, whether iNOS gene expression leads to in vivo NO production remains uncertain, as in humans posttranslational modification of iNOS may be necessary for functional activity Therefore, the exact contribution of RNI in human tuberculosis remains to be elucidated.

Sustained intracellular growth of M. tuberculosis may depend on its ability to avoid destruction by lysosomal enzymes, ROI, and RNI. When phagocytosed by macrophages bacteria typically enter specialized phagosomes that undergo progressive acidification followed by fusion with lysosomes. However, M. tuberculosis delays or inhibits fusion of phagosomes and lysosomes. In addition, M tuberculosis prevents phagosomal maturation and acidification of phagosomes, thereby blocking the digestive activity of acidic hydrolases .Nramp1, which codes for natural-resistance-associated macrophase protein (Nramp), is an interesting gene involved in macrophage activation and mycobacterial killing .

The protein is an integral membrane protein which belongs to a family of metal ion transporters. These metal ions, particularly Fe2_, are involved in macrophage activation and generation of toxic antimicrobial radicals. Following phagocytosis, Nramp1 becomes part of the phagosome. Nramp1 mutant mice display reduced phagosomal maturation and acidification. Surprisingly, mycobacterial outgrowth is unaffected in these animals .In humans, functional polymorphism in the promoter region of Nramp1, associated with reduced gene expression, was found to be associated with susceptibility to tuberculosis in studies from West Africa .Thus, genetic variation in Nramp1 may affect the outcome of infection with M. tuberculosis. However, to prove the significance of this gene in human tuberculosis further epidemiological and mechanistic studies are needed.

Apoptosis may constitute another effector mechanism for the infected host to limit outgrowth of M. tuberculosis . Apoptosis of phagocytic cells may prevent dissemination of infection. In addition, apoptosis of infected cells reduces viability of interacellular mycobacteria, while necrosis of infected cells does not .TNF-_is required for induction of apoptosis in response to infection with M. tuberculosis. Interestingly, pathogenic M. tuberculosis strains induced significantly less host cell apoptosis than related attenuated strains. This difference was explained by selective induction and release of neutralizing soluble TNF-alpha receptors by pathogenic strains .Release of TNF-alpha receptors in turn was regulated by IL-10 production Thus, pathogenic strains of M. tuberculosis may selectively induce IL-10, leading to decreased TNF-alpha activity and reduced apoptosis of infected cells. Independent of cytokine production, LAM may prevent in vitro apoptosis of M. tuberculosis-infected cells in a Ca2_-dependent mechanism. In addition, increased expression of Fas ligand in infected macrophages may also contribute to decreased macrophage apoptosis.

We briefly want to mention the role of other cell types. Although the precise mechanisms remains to be elucidated, human neutrophils may contribute to killing of M. tuberculosis. However, patients with disorders of neutrophil activity do not show increased susceptibility to tuberculosis. Of more clinical relevance may be the contribution of cytotoxic T cells . Of special interest, granules of cytotoxic T cells and NK cells contain granulysin, a molecule that directly alters the mycobacterial membrane integrity and thereby kills M. tuberculosis.

INITIATION OF ADAPTIVE IMMUNITY TO M. TUBERCULOSIS
It is clear that innate and adaptive immunity are closely connected. Macrophages and dendritic cells, the primary cell types involved in the innate immune response to mycobacteria, play a crucial role in the initiation of adaptive host response to mycobacteria is beyond the scope of this review, we will briefly summarize this subject. In principle, three processes contribute to the initiation of adaptive immunity: antigen presentation,costimulation, and cytokine production. Patients with active tuberculosis may suffer from energy or T-cell unresponsiveness . This may b caused by intrinsic defects or dynamic inhibition of one of these three processes.

Antigen Presentation:

Presentation of mycobacterial antigens by macrophages a compartments in professional antigen-presenting cells. Second, MHC class I molecules, expressed on all nucleated cells, are able to present mycobacterial proteins to antigen specific CD8_ T cells. This mechanism allows for the presentation of cytosolic antigens, which may be important as certain mycobacterial antigens may somehow escape the phagosome . The importance of MHC class I-mediated antigen presentation has been shown in murine models and tuberculosis patients. Third, nonpolymorphic MHC class I molecules such as type I CDI (-a, -b, -c) molecules, which are expressed on macrophages and dendritic cells, are able to present mycobacterial lipoproteins to CDI- restricted T cells. This mechanism of antigen presentation enables the activation of a larger fraction of T cells at an earlier point in the infection, before antigen specificity has developed.
A fourth pathway may involve nonpolymorphic MHC class Ib molecules. The expression of particular class I and class II MHC alleles in an individual determines the ability of that individual to respond to particular (mycobacterial) antigens and epitopes.
Certain allelic human leukocyte antigen (HLA) variants have been associated with tuberculosis . HLA polymorphism may explain the vulnerability of certain isolated populations like Amazonian Indians which have only recently been exposed to tuberculosis. There is a large body of evidence for similar mechanisms in leprosy. The expression of antigen-presenting molecules is also a dynamic process, which is regulated by cytokines. While inflammatory cytokines, primarily IFN-γ stimulate expression of MHC, anti-inflammatory cytokines inhibit its expression. Mycobacteria may modulate the antigen presentation function, but different results have been obtained in vitro with macrophages and dendritic cells. Mycobacteria may downregulate expression of antigen presenting molecules in macrophages, most likely through the production of anti-inflammatory cytokines .On the other hand, MHC expression on dendritic cells is upregulated following M. tuberculosis infection.

Costimulation:

It is well known that antigen presentation only leads to T-cell stimulation in the presence of particular co-stimulatory signals. The most well-known co-stimulatory signals for T-cell stimulation are B-7.1 (CD80) and B-7.2 (CD86). These molecules are expressed on macrophages and dendritic cells and bind to CD28 and to CTLA-4 on T cells. Interestingly, in vitro infection of monocytes with M. tuberculosis leads to diminished expression of B-7.1. On the other hand, M. tuberculosis infection of dendritic cells induces expression of B7.1, CD40, and ICAM-1. In the absence of proper co-stimulatory signals, antigen presentation may lead to increased apoptosis of T cells.

Cytokine Production:

Several cytokines produced by activated macrophages and dendritic cells are essential for stimulation of T lymphocytes. Macrophages and dendritic cells produce the type I cytokines IL-12, IL-18, and IL-23. In patients with recurrent or fatal nontuberculous mycobacterial infections, functional genetic mutations have been found in the genes encoding IL-12p40 , IL-12R_1 IFN-γ receptor 1, and IFN-γ receptor 2, all of which are involved in IFN-γ receptor signaling in macrophages and dendritic cells. Clearly the capacity of these cells to produce or react to Th1- type cytokines is necessary for proper T-cell stimulation (Fig.4). In addition, proinflammatory cytokines like IL-1and TNF-α have important T-cell stimulatory properties. Reduced production of type 1 or proinflanmmatory cytokines may delay or decrease T-cell stimulation and the initiation of antigen-specific T-cell immunity. In this respect, the production of anti-inflammatory cytokines may be relevant as well. For instance, in anergic tuberculosis patients it was recently shown that IL-10 production is constitutively present and that T-cell receptor mediated stimulation results in defective signal transduction . TGF-β may have a similar antagonistic role.

CONCLUDING REMARKS:
The interplay between M. tuberculosis and the human host determines the outcome after infection. With respect to the human host, both innate and adaptive defense mechanisms are involved. After uptake of M. tuberculosis in alveolar macrophages, several possible scenarios may be envisaged. M. tuberculosis may be destroyed immediately, in which case no adaptive T-cell response is developed. When infection is established, however, a focal nonspecific inflammatory response follows. This response is regulated by a network of pro- and anti-inflammatory cytokines and chemokines. Most of the mediators at this point are derived from macrophages or dendritic cells, but IFN-α has several cellular sources, including NK cells, T cells, and CDI-restricted T cells. This initial response determines the local outgrowth of M. tuberculosis (sometimes dissemination) or containment of infection. Phagocytic cells also play a key role in antigen presentation and the initiation of T-cell immunity which follows. At many stages in the host response, M. tuberculosis has developed mechanisms to circumvent or antagonize protective immunity.

The inter individual differences in outcome after infection with M. tuberculosis may in part be explained by the efficiency of various innate host defense mechanisms. Phagocytosis, immune recoginition, cytokine production, and effect mechanisms may all contribute to innate immunity. In this respect, different gene polymorphisms have been found which are associated with increased susceptibility and severity of tuberculosis. Some of these polymorphisms are functional, but for many of these no functional (immunologic) changes have been demonstrated yet, and these associations therefore need further confirmation and investigation.

What remains to be determined is to what extent the encounters between M. tuberculosis and the human host can be FIG.4. Cytokies and cytokine receptors involved in type I immunity in tuberculosis. A mojor effector mechanism of cell-mediated immunity in tuberculosis is the

activation of M. tuberculosis-infected macrophages by IFN-γ. IFN-γ is produced by NK cells and different T-cell subsets, and its production is regulated by TNF-α, IL-1, IL-12, IL-18, and possibly IL-15, all released from activated macrophages and dendritic cells.

In many settings the most cost-effective way to improve disease outcome is to increase patients' access to health care facilities and to strengthen the quality of diagnosis and antimycobacterial treatment. However, in many parts of the world the spread of multidrug-resistant tuberculosis seriously threatens the success of antibiotic treatment. Therefore, more-effective vaccines and new therapeutic strategies (like immunotherapy) are desperately needed. It is expected that increased understanding of disease pathogenesis will help the design of such adjunctive treatment, which will undoubtedly benefit the outcome of individual patients and limit the spread of M. tuberculosis around the world.

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