

Review

Recent advances in our understanding of human host responses to tuberculosis

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Abstract

Tuberculosis remains one of the world's greatest public health challenges: 2 billion persons have latent infection, 8 million people develop active tuberculosis annually, and 2–3 million die. Recently, significant advances in our understanding of the human immune response against tuberculosis have occurred. The present review focuses on recent work in macrophage and T-cell biology that sheds light on the human immune response to tuberculosis. The role of key cytokines such as interferon- γ is discussed, as is the role of CD4⁺ and CD8⁺ T cells in immune regulation in tuberculosis, particularly with regard to implications for vaccine development and evaluation.

Keywords: CD4⁺ cell, CD8⁺ cell, immunity, interferon, tuberculosis

Introduction

Tuberculosis remains one of the most important infectious diseases in the world [1]. It is estimated that 2 billion persons on the planet harbor latent tuberculosis infection. Eight to 12 million new cases of active tuberculosis occur each year, and at any given time there are approximately 16 million persons with active tuberculosis in the world. These cases result in 2–3 million deaths annually, making tuberculosis the single leading cause of death of any infectious disease. These figures are even more staggering when one realizes that the vast majority of cases of tuberculosis are curable with currently available medications.

Although there have been some notable success stories in recent years in controlling tuberculosis and reducing case rates (mostly in wealthy countries such as the USA), there is little cause for optimism in parts of the world where poverty, political disorganization, and access to care remain major obstacles to global tuberculosis control. In

fact, with the continued spread of the human immunodeficiency virus (HIV) epidemic, particularly in Africa and Asia, and the emergence of multidrug-resistant tuberculosis in many parts of the world, there will continue to be significant upwards pressure on the number of tuberculosis cases in the world for the next several years. This increasing pressure occurs in the context of what has been a long stagnant period in tuberculosis drug development. No new class of antituberculosis drugs has been introduced since the rifamycins came into use 30 years ago.

The clinical manifestations of infectious disease are caused by the balance between virulence factors that are elaborated by the invading microbe, and the host immune response that the body mounts to defend itself. In many infectious syndromes, virulence factors are responsible for most of the disease manifestations. Examples include toxic shock syndrome and Gram-negative sepsis, in which lipopolysaccharide released by invading bacteria sets off a

whole cascade of inflammatory events. On the other hand, in many infectious syndromes relatively avirulent organisms cause disease mainly by forcing the host to respond in one or more of a variety of ways that result in specific manifestations of disease. Such would seem to be the case in leprosy, for example. *Mycobacterium leprae* appears capable of eliciting two distinct host immune responses that result in the two different clinical manifestations of the disease [2,3]: tuberculoid leprosy and lepromatous leprosy.

Little is known about virulence factors of *Mycobacterium tuberculosis*. Interesting studies have recently been reported that describe the mechanisms that underlie behaviors such as cording, and a body of work describing the relationship between sigma factors and mycobacterial latency has also been done [4–8]. During the next few years it is likely that significant advances will be made in our understanding of mycobacterial virulence as a result of projects such as the sequencing of the *M tuberculosis* genome (now already complete for several laboratory and clinical isolates), as well as advances in the field of mycobacterial genetics. Such advances in our understanding of the basic biology of *M tuberculosis* should aid in the design and evaluation of new therapeutic drugs.

Understanding human host immunity to tuberculosis is important for several reasons. Paramount among these, however, must be that only through a thorough knowledge of how tuberculosis is recognized and controlled by the immune system will we be able to design and evaluate new vaccine candidates. In the long run, vaccination still represents an important goal in tuberculosis control, and is perhaps the best hope for ultimate eradication of this disease.

The challenges in tuberculosis vaccine development are enormous. Two major features of clinical tuberculosis frame the challenge of vaccine development. The first is that, as noted above, 2 billion persons are already infected with *M tuberculosis*, so that a vaccine might need to protect against reactivation rather than infection. The second is that, unlike many other infections (particularly viral infections), the extent to which natural immunity to tuberculosis exists is not clear. Whereas patients who recover from chicken pox have lifelong immunity against reinfection, patients who have recovered from tuberculosis may be subject to reinfection. This has been demonstrated in patients with HIV who clearly are significantly immunocompromised, but recent data indicate that reinfection may also occur in patients without HIV infection or apparent immunosuppression [9,10]. This may be an infrequent occurrence, but it does raise the possibility (as recently pointed out by Kaufmann [11]) that we may be faced with the challenge of designing a vaccine that needs to provide better than natural immunity!

In addition to aiding the effort to develop a novel vaccine for tuberculosis, understanding the human immune response might also point to novel immunotherapeutic approaches to treatment of tuberculosis, particularly in the setting of multidrug resistance, in which there are often no viable chemotherapy options.

During the past several years much has been learned about the human immune response to tuberculosis. In fact, the conduct of direct experiments using human tissues, as well as *in vivo* studies of human immune responses to tuberculosis, represents a major advance in our understanding of the pathogenesis of this disease. Although animal models of tuberculosis have taught us (and will continue to teach us) a great deal about the pathogenesis of this disease, it remains the case that there is no completely satisfactory animal model of human tuberculosis. The present review focuses wherever possible on studies of the human host response to tuberculosis, making reference to animal studies only when they are particularly instructive or are the only good data available. Attention is directed mainly to macrophage and T-lymphocyte biology.

Genetic susceptibility to tuberculosis infection and disease

Several observational studies indicate that certain populations appear to exhibit unusual susceptibility to tuberculosis, and it is likely that to a certain degree this susceptibility has a genetic basis. It appears that tuberculosis was a disease that may have originated largely in Western Europe, and was then transmitted to other parts of the world through migration, exploration, and colonization. If this indeed happened, then there would have been relatively little selection pressure favoring resistance to tuberculosis infection and disease in regions such as Africa and Asia, and the disease would have spread easily among these populations. There is relatively convincing evidence that Eskimos, African-Americans, and other populations do seem to exhibit heightened susceptibility (or conversely, that populations descended from white European stock have a degree of innate resistance) [12]. The most recently described example of this susceptibility may be that of the Yanomami Indians of Peru [13], a tribe that had remained extremely isolated for thousands of years until being 'discovered' by anthropologists a few decades ago. It is almost certain that tuberculosis had not occurred among the Yanomami previously, but soon after contact with outsiders a tuberculosis epidemic swept through the tribe with unusual ferocity and lethality.

Despite the apparent susceptibility of various populations, the actual genetic basis for vulnerability to tuberculosis is obscure; susceptibility is probably a polygenetic predisposition that has major interactions with the environment. Furthermore, whereas animal models allow the study of risk of actual infection, human studies necessarily focus on

the risk of progression to active disease in patients who have already been infected, because it is difficult if not impossible to determine clinically when and how an individual initially developed latent infection.

Polymorphisms in several candidate genes have been linked to relatively increased risk for tuberculosis disease [14]. These genes include several human leukocyte antigen loci, vitamin D receptors, and the gene for the natural resistance-associated macrophage protein (*NRAMP*). *NRAMP* presents an intriguing although somewhat confusing story. Originally, *NRAMP* was determined to control susceptibility to infection in a strain of mice known as *lty/Lsh/Bcg*, which are extremely vulnerable to disease caused by intracellular pathogens such as salmonella, leishmania, and *Mycobacterium bovis*. However, later work [15–18] cast doubt on the importance of this gene (which produces a membrane-bound transport protein of uncertain function) in protection against murine tuberculosis. A human homolog for *NRAMP* was quickly identified, and a study was reported that implicated a link between certain *NRAMP* polymorphisms and the risk of developing active tuberculosis among patients with latent infection in West Africa [19]. The relative risk of developing active tuberculosis in among those with susceptible genotypes was about 1.8, and interestingly the dominant genotype in humans appeared to be susceptible, whereas in mice it was resistant.

Immunologic defense against infection and disease caused by *M tuberculosis*

It is generally felt that only the cellular immune system plays a significant role in host defense against *M tuberculosis* [20]. Although antibodies are made against several mycobacterial products, including cell-wall components, there is as yet little evidence that humoral immunity is clinically important. Studies of antibody production in response to tuberculosis mainly have application in the continuing effort to develop a serologic test for tuberculosis, although some still feel that humoral immunity does in fact play a significant role in actual host defense [21–23].

Initial defense against *M tuberculosis* infection: phagocytosis by macrophages

After evading the mechanical defenses of the upper respiratory tree, respiratory droplet nuclei that contain viable *M tuberculosis* organisms make their way into the distal regions of the lung. Here, they come into contact with the resident immune cell of the lung: the alveolar macrophage. Uptake of *M tuberculosis* by macrophages represents the first major host–pathogen interaction in tuberculosis. Presumably, there are persons in whom macrophages, upon initial contact with *M tuberculosis*, are able to kill the pathogen directly and completely eliminate it, never allowing a latent stage of infection to develop. Although there is of course no direct evidence of this, there are persons

with repeated exposure to cases of active tuberculosis who neither develop positive tuberculin skin tests or active tuberculosis, providing indirect evidence that such an outcome is possible.

Binding of *M tuberculosis* to macrophages can be accomplished by a number of mechanisms. It has been demonstrated [24–28] that complement receptors, mannose receptors, surfactant receptors, scavenger receptors, and others are capable of mediating this initial interaction. Most recently, attention has focused on the role of toll-like receptors (TLRs) in mediating the uptake of mycobacteria by macrophages. Brightbill *et al* [29] demonstrated that, when TLRs are activated by lipoproteins that are contained within the *M tuberculosis* cell wall, interleukin-12 production is stimulated in THP-1 cells, a human macrophage-like cell line. Interleukin-12 is an important pro-inflammatory cytokine in host responses against tuberculosis, and the TLR-mediated stimulation of interleukin-12 was itself mediated through the transcription factor nuclear factor- κ B. Furthermore, TLR-mediated interleukin-12 production also resulted in increased production of nitric oxide synthase and nitric oxide production, which are important steps in intracellular killing of *M tuberculosis*. Thus, engagement of TLRs may be an important triggering step in the host response against *M tuberculosis*.

The complexities of the TLR system have been amplified by the work of Means *et al* [30] and Underhill and coworkers [31,32]. These groups have carried out experiments that dissect the relative contributions of the TLR2 and TLR4 subsets of receptors when they are activated by different mycobacterial components. Both of these receptors can be activated by live *M tuberculosis*, although different bacterial components can activate each, and it is possible that the different subclasses of receptors in turn stimulate different facets of the immune response. For example, the work of Underhill and coworkers [31,32] showed that arabinogalactan and peptidoglycan can increase tumor necrosis factor (TNF)- α production through activation of TLR2, but that mannose lipoarabinomannan did not. Interestingly, nonreceptor surface molecules, such as CD43 (leukosialin/sialophorin), may also be key components of the initiation of the immune response, because it has recently been demonstrated that CD43 is involved in the stable interaction of mycobacteria with other cell-surface receptors that increase TNF- α production.

A major puzzle in the biology of tuberculosis is the establishment and persistence of the latent state of infection, in which a small number of mycobacteria can remain dormant but viable for many years. An interesting recent study sheds light on this phenomenon. Gatfield and Pieters [33] demonstrated that cholesterol is required for uptake of mycobacteria into cells. After demonstrating that cholesterol accumulated at the site of phagocytosis in

(murine) macrophages, the investigators depleted cellular cholesterol by inhibiting cholesterol synthesis and extracting residual cholesterol from the plasma membrane. After this, uptake of mycobacteria was reduced by 85%. Cholesterol was also important in mediating the phagosomal association of a molecule termed tryptophane aspartate-containing coat protein, which prevents the degradation of mycobacteria in lysosomes.

Many bacterial factors are also involved in establishing the latent state of infection. Although a thorough description of mycobacterial biology related to latency and persistence is beyond the scope of the present review, it is likely that a balance of host immune responses and microbial factors are involved in initiating and maintaining the dormant state.

In addition to phagocytosis, macrophages contribute further to the immune response both by inhibiting growth or by killing mycobacteria (see below), and by secreting cytokines that further amplify the immune response. One such cytokine is TNF- α , which appears to play a central role in granuloma formation. Although the importance of TNF in granuloma formation (and the exact role of the granuloma itself in host defense) in humans has not been directly assessed, animals that lack TNF showed markedly impaired granuloma formation and died from overwhelming mycobacterial disease soon after becoming infected [34–36].

T cells in the host immune response

During the past several years it has become apparent that T cells play a major role in the tuberculosis host response in humans. This is nowhere more obvious than in patients with HIV infection, in whom tuberculosis is a major pathogen and often represents the acquired immune deficiency disease-defining illness. Patients with HIV infection and latent tuberculosis infection develop active tuberculosis at a rate that approaches 10% per year, as opposed to 10% over the lifetime of a person with an intact immune system.

Initial studies in humans focused on the role of CD4⁺ T cells in tuberculosis host defense, but in recent years a great deal of attention has been devoted to CD8⁺ T cells, particularly with regard to protective immunity.

CD4⁺ T lymphocytes

CD4⁺ T cells, also called T-helper (Th) cells, provide T-cell help to other immune cells, and thus amplify the immune response. There is now substantial evidence in humans that Th cells can display at least two phenotypes – Th1 and Th2 – which can be described mainly by the pattern of cytokines secreted [3]. The hallmark of Th1 cells is the production of interferon- γ , which has been shown during the past several years to be a key effector cytokine in the host response against tuberculosis. Th2 cells primarily secrete the cytokines interleukin-4, interleukin-5, and inter-

leukin-10, cytokines that in general have not been shown to play a major role in tuberculosis host immunity.

A major aspect of the importance of Th1 cells in tuberculosis host defense is their ability to secrete interferon- γ . Although CD8⁺ T cells (and perhaps macrophages as well) also secrete this cytokine (see below), CD4⁺ cells probably constitute the major source of this protein. A substantial body of evidence now exists that demonstrates that interferon- γ plays a key role in defense against tuberculosis [20]. This pro-inflammatory cytokine has multiple beneficial actions, many of which are centered on its effects on macrophage biology. Production of both reactive oxygen intermediates and reactive nitrogen intermediates by macrophages are stimulated by interferon- γ . Both reactive oxygen intermediate and reactive nitrogen intermediate pathways have been implicated in intracellular growth inhibition and/or killing of mycobacteria, although there is debate about which pathway, if either, is critical in this regard [37–40].

Interferon- γ has several other effects on macrophages, including the increased expression of major histocompatibility complex (MHC) class II molecules, thus leading to increased antigen presentation and further amplification of the immune response. Interferon- γ may also upregulate expression and secretion of TNF from macrophages.

In human tuberculosis, the importance of interferon- γ is clear. Individuals who lack the ability to produce interferon- γ on a genetic basis, or who cannot respond to it or lack the receptor for it, are susceptible to severe systemic infection with mycobacterial species that do not usually cause significant disease in immunocompetent individuals [41,42]. In humans with pulmonary tuberculosis, we have shown [43] that a lymphocytic alveolitis characterized by significant local production of interferon- γ is associated with clinically and radiographically mild disease, whereas patients who do not mount this type of response are much more likely to have sputum smear-positive, cavitary disease. In addition, when exogenous interferon- γ was administered by aerosol to a group of patients with multidrug-resistant tuberculosis who had failed medical therapy, clinical and radiographic improvement was consistently noted [44]. The improvement seemed to persist only as long as the course of treatment.

Because an interferon- γ -producing Th1 response is clearly crucial in effective tuberculosis host defense, the generation and maintenance of such a response has generated considerable interest. It is in the generation of this response that the keys to understanding and development of an effective vaccine are likely to be hidden. Recent studies using *Leishmania major* (an intracellular pathogen that is controlled by a Th1 response in a manner similar to that for tuberculosis) as a model pathogen have implicated

the involvement of some key cytokines and antigens [45]. In a murine model of leishmaniasis, it appears that only certain *L major* antigens (in this instance a protein termed *Leishmania* homolog of receptors for activated C-kinase [LACK]) are capable of stimulating protective immunity when given in the form of a DNA vaccine before challenge with live pathogen. In addition, LACK DNA vaccination alone does not confer protective immunity; a persistent source of interleukin-12 was also required to establish a Th1-type protective immune response. Interleukin-12 has previously and definitely been shown to be a potent inducer of interferon- γ from many different cell types.

As bacille Calmette–Guérin (BCG) vaccine provides only limited protection in humans, eliciting longer lived immunity by linking interleukin-12-inducing strategies with immunogenic mycobacterial proteins in a vaccine may be efficacious. Indeed, several groups are pursuing this. When interleukin-12 was used as an adjuvant to BCG vaccine in a murine model, enhanced protection was noted [46]. BCG vaccination alone resulted in a 1–2 log decrease in bacterial burden after challenge with virulent *M tuberculosis*, as compared with unvaccinated animals. Adding interleukin-12 to the vaccine decreased bacterial loads twofold to fivefold further. In addition, interferon- γ gene expression and protein production in spleen cells were increased when interleukin-12 was added to BCG alone. In work by Russo *et al* [47], tuberculosis-naïve T cells were primed *in vitro* with intact mycobacteria or only the antigenic protein Ag85. This elicited a Th1 response when cells were rechallenged with mycobacteria. Adding interleukin-12 to the priming enhanced the magnitude of the Th1 response; interestingly, however, antibody to interleukin-12 did not eliminate the response. This reflects the complex nature of this phenomenon. Most recently, Marchant *et al* [48] demonstrated that Th-null (Th0), purified protein derivative-specific T-cell clones from patients with active tuberculosis could be coaxed into a Th1 phenotype by the *in vitro* administration of interleukin-12. This provides further evidence for the importance and potential clinical utility of this cytokine in vaccine development, or perhaps as adjunctive immunotherapy.

CD8⁺ T lymphocytes

During the past 3–4 years, increasing attention has turned to CD8⁺ T cells and their involvement in mycobacterial host defense, and there is substantial evidence that these cells play a major role [49,50]. CD8⁺ T cells are capable of producing significant amounts of interferon- γ , and a Tc1 phenotype, similar to a Th1 phenotype, is the predominant type of CD8⁺ cell; in addition, they are cytotoxic T cells and probably play a significant role in true protective immunity of the type conferred by vaccination. CD8⁺ T cells recognize processed peptide fragments that are presented on cell surfaces in the context of MHC class I molecules (expressed on most cells in the body), which

then bind to the T-cell receptor. CD8⁺ T cells can also bind the CD1 molecule, a more recently described mode of antigen presentation, which is present on the surface of professional antigen-presenting cells. CD8⁺ T cells have clearly been identified that recognize alveolar macrophages and dendritic cells, which are of course professional antigen-presenting cells.

CD8⁺ cells can be both cytotoxic, causing lysis of infected target cells such as monocytes and macrophages, and microbicidal, causing death of intracellular pathogens directly. When macrophages release the intracellular pathogens, those pathogens can then be killed by activated effector cells that have been recruited through a variety of signals. This is an example of cell-mediated cytotoxicity. Direct microbicidal activity is also possessed by CD8⁺ T cells. A granule-associated T-cell protein called granulysin can be secreted by CD8⁺ cells, and can kill extracellular *M tuberculosis* directly. In concert with perforin, another T-cell product, it may also kill intracellular mycobacteria.

Lewinsohn *et al* [51] characterized human CD8⁺ T cells that are reactive with *M tuberculosis*-infected antigen-presenting cells. These investigators showed that *M tuberculosis*-reactive CD⁺ T cells are found mainly in persons with latent tuberculosis infection, and in response to stimulation with *M tuberculosis*-infected target cells produced significant amounts of interferon- γ . Interestingly, recognition of infected cells by CD8⁺ T cells was not restricted by MHC class I A, B, or C alleles, or by CD1. These tuberculosis-specific CD8⁺ cells recognized an antigen that is generated in the proteasome, although it is not transported through the Golgi–endoplasmic reticulum apparatus.

Smith *et al* [52] further characterized the role of tuberculosis-specific CD8⁺ T cells in humans. When taken from persons who had received BCG vaccination and restimulated with live *M bovis* BCG, CD8⁺ T cells produced substantial amounts of interferon- γ and TNF- α . In fact, more of these cytokines were produced when cells were stimulated with *M bovis* BCG than with purified protein derivative. Perforin was also expressed by these CD8⁺ cells, which demonstrated marked cytotoxic ability against cells infected with *M bovis* BCG, *M tuberculosis* antigens 85A and B, and to a lesser extent the 19 kDa or 38 kDa tuberculosis proteins. No significant cytotoxic activity of CD8⁺ cells from BCG-vaccinated persons was directed against target cells infected with the early secreted *M tuberculosis* antigen (ESAT)-6. This is of interest because recent data from the genomic sequences of several mycobacteria indicate that the gene for ESAT-6 is absent in the vaccine strain of BCG, and is highly specific for *M tuberculosis*. This finding provides further evidence for the specificity of CD8⁺ T-cell responses. Unlike Lewinsohn *et al* [51], Smith *et al* [52] determined that the cytotoxic activity of the

CD8⁺ cells was in fact mediated through MHC class I pathways. As Lewinsohn *et al* studied presentation of *M tuberculosis* rather than that of *M bovis*, the circumvention of MHC class I pathways might represent a virulence strategy of *M tuberculosis*.

Pathan and coworkers [53,54] studied tuberculin skin test-positive household contacts and persons with inactive 'self-healed' pulmonary tuberculosis, and showed that a nonamer epitope from the *M tuberculosis* protein ESAT-6 could be recognized by CD8⁺ T cells for long periods of time after infection. These data suggest that long-lived specific CD8⁺ T-cell responses are associated with apparent control of *M tuberculosis* infection in humans.

Conclusion

Recent years have seen an explosion in knowledge regarding the human host response to tuberculosis and disease. The importance of cytokines such as interferon- γ has been established beyond doubt, and the role of important lymphocyte effector cells has begun to be elucidated. Major unanswered questions remain, however. The relative importance of CD4⁺ and CD8⁺ T cells in the host response must be clarified, although gathering evidence suggests that the former play a greater role in the immune response against active disease and that the latter are more involved in controlling latent infection. The specific *M tuberculosis* antigens that are capable of provoking the most protective immune responses remain to be identified, although progress is being made, and elucidation of the entire genomic sequences of several pathogenic strains of *M tuberculosis* and related strains should aid this effort [55–57]. The reasons why some persons are able to quickly develop an effective cytotoxic response (ie effective CD8⁺ T-cell function) and others are not remain almost a complete mystery.

As these issues are clarified, rational strategies for developing and testing novel candidate vaccines can be formed. The ability to identify reliable surrogates of immunity (possibly *in vivo* assays of CD8⁺ T-cell mediated cytotoxicity) will be crucial to the testing of novel vaccine candidates. Actual field trials will require huge numbers of persons to be followed for considerable periods of time, and are an inefficient way to screen interesting new vaccine candidates.

If true immunity is the ability to resist infection, then it is more accurate to state that we currently know a good deal more about aspects of the host immune response to tuberculosis than about genuine immunity against *M tuberculosis*. However, the work summarized in the present review makes it possible to begin to approach a deeper understanding of the problem.

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