Review: The Immune Response in Human Tuberculosis—Implications for Tuberculosis Control

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In March 1993, the World Health Organization (WHO) designated tuberculosis (TB) a global public health emergency. In the United States, the transient increase in the incidence of TB coupled with the emergence and nosocomial spread of multidrug-resistant disease caused alarm and an outpouring of resources to support TB control and research. The failure to achieve global control of TB stems largely from impediments to the implementation of existing effective measures; these impediments, in turn, are economic, political, and behavioral rather than strictly biologic. Nonetheless, renewed interest and recent progress in basic research on the microbiology and biochemistry of *Mycobacterium tuberculosis,* host defenses against the organism, and the pathogenesis of TB is expected to accelerate development of interventions that will facilitate TB control. The current sequencing of the genome of *M. tuberculosis,* likewise, will represent a quantum leap forward in understanding the organism and has tremendous potential to lead to new interventions.

This review will focus on current understanding of the human immune response to *M. tuberculosis* infection and disease and its potential implications for new approaches that will facilitate TB control.

Protective Immunity and an Improved Vaccine

Bacille Calmette-Guérin (BCG) is an attenuated strain of *Mycobacterium bovis* first administered to humans as a vaccine to prevent TB in 1921. At that time, there was no knowledge of the protective immune response required to prevent TB. The rationale for the use of BCG as a vaccine was that an attenuated organism would provide immunity against virulent challenge through cross-reactive immunogenic determinants. This empiric notion, although the basis for the eradication of smallpox and control of other viral diseases, may not be entirely relevant for a facultative intracellular organism such as *M. tuberculosis.*

Durability of protection. The efficacy of BCG in the prevention of pulmonary TB has shown considerable variation in different populations and trials [1]. There has, however, been consistent efficacy (~80%) in preventing extrapulmonary TB (TB meningitis and miliary TB) in infants and children. Two explanations have been offered for the differential effectiveness in children and adults. The finding that efficacy is most apparent shortly after BCG vaccination may reflect the fact that protective immunity is only transitory. Alternatively, vaccination with BCG may not prevent primary infection following exposure to virulent *M. tuberculosis* but abort hematogenous dissemination. In this case, BCG would be most effective in averting extrapulmonary disease, including meningitis and miliary disease in children. If BCG efficacy is expressed through prevention of bacillemia, vaccination also would be less effective in areas of high transmission, where reinfection and progressive primary TB may be more relevant to the pathogenesis of pulmonary TB than reactivation of latent foci. In fact, the lack of effectiveness of BCG as a vaccine in certain geographic areas, particularly near the equator, is consistent with this thesis.

Ideally, a vaccine to prevent TB would confer lifelong protection. Is this feasible? A related question is, Does natural infection with virulent *M. tuberculosis* confer lifelong immunity? In a study of persons known to be tuberculin-reactive 19 years previously, we demonstrated excellent retention of skin test reactivity and in vitro evidence of circulating PPD (purified protein derivative)-reactive memory immune T cells [2]. This high level of retained sensitization could result from repeated breakdown of infectious foci with in situ boosting. In fact, the high incidence of reactivation TB in persons coinfected with human immunodeficiency virus type 1 (HIV-1) supports this contention, as will be discussed.

It is the persistence of the infectious agent in tissue and the capacity for in vivo boosting that most likely accounts for the strong, sustained immunogenicity resulting from natural infection. The persistence in tissues is a property of virulent strains of *M. tuberculosis.* The latent focus of infection with virulent *M. tuberculosis* provides not just the basis for boosting DTH responses, but also the substrate for reactivation diseases. In turn, the limited efficacy of BCG as a vaccine could reflect overattenuation and diminished capacity for replication and persistence. This creates a ''Catch 22'' situation—the requirement for development of a vaccine strain with sufficient virulence to evoke lifelong protection but not enough virulence to itself cause disease, particularly in the immunosuppressed host. The development of a rationally attenuated library of vaccine strains of *M. tuberculosis* (e.g., auxotrophic mutants [3]) may permit more rational selection of a candidate vaccine strain. Other approaches to enhance the duration of protection include repeated immunizations with BCG or primary immunization

Received 3 December 1996; revised 24 June 1997.
Grant support: NIH (AI-45244).
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The Journal of Infectious Diseases 1997; 176:1351–9

0022–1899/97/7605–0030$02.00

with BCG followed by boosting, possibly with a subunit vaccine.

Basis for protective immunity. Understanding the immunologic basis for protective immunity would provide a means for screening of potentially protective antigens and also a surrogate marker for rapid evaluation of the potential efficacy of a new vaccine. The natural history of *M. tuberculosis* infection indicates that the emergence of delayed-type hypersensitivity (DTH) and presumably specific acquired resistance is associated with control of the initial infection in 95% of normal hosts; the other 5% develop progressive primary TB. In addition, 5% of infected persons eventually will reactivate latent pulmonary or extrapulmonary foci. It appears, therefore, that protective immunity results in the initial containment of endogenous infectious foci in most immunologically intact humans. Epidemiologic studies further indicate that cohorts of young, otherwise healthy, *M. tuberculosis*-infected persons are relatively immune to exogenous reinfection and the development of TB upon exposure to infectious cases. The immunity against exogenous reinfection may not, however, persist for the lifetime of the host. Susceptibility to exogenous reinfection over time becomes a particular issue in areas of high transmission, where reexposure is common. Immunity against endogenous reactivation also presumably does not endure for a lifetime. Endogenous reactivation represents the predominant pathogenetic mechanism in areas of low transmission.

Ideally, DTH to PPD could be used as a surrogate marker of protection. Unfortunately, although DTH and protective immunity emerge in parallel following primary infection with *M. tuberculosis,* analysis of BCG trials indicates that DTH is not predictive of protection [4]. Abundant experimental data also demonstrate that DTH and protective immunity are dissociable phenomena, elicited by different antigens and vaccines (as will be discussed below) and effected by different populations of lymphocytes.

In experimental murine models, CD4 lymphocytes, CD8 lymphocytes, and $\gamma\delta$ T cells each play a role in protective immunity in the relative order: CD4 $>$ CD8 $>$ $\gamma\delta$ T cells. A recent study indicates that $\gamma\delta$ T cells, in fact, have little role in the protective immune response of mice to low-dose aerosol challenge [5]. They appear, however, to limit the trafficking of potentially injurious inflammatory cells to pulmonary granulomas. At the cytokine level, elegant experiments in mice with genetically disrupted cytokine genes or treated with neutralizing anticytokine antibodies indicate that both interferon (IFN)-γ and tumor necrosis factor (TNF)-α are essential for protection [6–8]. Inducible nitric oxide (NO) synthase and NO also are required for bacterial killing [9]. In fact, the role of TNF-α and IFN-γ may be partly to activate this effector pathway in murine mononuclear phagocytes. Exogenous interleukin (IL)-12 transiently increases murine resistance to *M. tuberculosis* [10] and increases survival [11], although no effects are seen in mice with T cell deficiencies or disrupted IFN-γ genes. IL-12 promotes differentiation of CD4 cells that produce IFN-γ and also induces NK cells to produce this cytokine, which is key to protective immunity in the mouse.

In humans, the remarkable susceptibility of patients with AIDS to TB has demonstrated the critical role of CD4 lymphocytes in protective immunity. The dually infected individual (PPD skin test–positive, HIV-1–infected) in most cases is infected with *M. tuberculosis* before HIV-1 and has a 170-fold increased risk (5%–10%/year) of reactivation TB. This indicates, somewhat surprisingly, that in the absence of immunosurveillance by CD4 cells, 5%–10% of persons with latent foci of TB have the potential to break down and reactivate each year. Therefore, the CD4 cell axis has a previously unrecognized role in maintaining the inactivity of latent foci through active immunologic surveillance. In fact, ''latency'' may be a misnomer, as these foci appear to activate repeatedly, requiring CD4 cells to restore quiescence. Recent studies by Orme in a mouse model also suggest that ''latency'' really reflects a dynamic balance between the organism and host cells (Orme IM, personal communication). The role of NK cells and ''double negative'' ($CD4^-CD8^-$) $\alpha\beta$ T cells (which display reactivity to lipoarabinomannan [LAM] and mycolic acids) in protective immunity is uncertain. Observations in HIV-1 infection suggest, however, that the role of non-CD4 cells, individually and collectively, is at best secondary.

In healthy persons, CD4 cells reactive with mycobacterial antigens produce cytokines, chiefly of the Th1 pattern, and also engage in major histocompatibility complex (MHC) class II–restricted killing of antigen-presenting cells infected with *M. tuberculosis* or pulse-exposed to soluble antigens [12]. The relative importance of cytokine production versus cytotoxic T lymphocyte activity in the protective function of CD4 lymphocytes is unclear.

Two approaches have been taken to identify the cytokines that mediate protective immunity: characterization of the cytokine milieu at a local site of effective immunity, and identification of cytokines that activate intracellular killing by mononuclear phagocytes. Pleural TB self-cures. The pleural fluid and the contained mononuclear cells show a biasing toward overexpression of IFN-γ, IL-2, and IL-12, with depressed expression of IL-4, the latter only seen at the mRNA level [12]. This pattern resembles dominance of Th1 versus Th2 CD4 cells in the murine paradigm. Th1 CD4 T cells produce IL-2 and IFN-γ and promote cell-mediated immunity. Th2 CD4 T cells produce IL-4, IL-5, and IL-10 and promote humoral immunity. IL-12 drives differentiation of uncommitted Th0 cells into the Th1 pathway. Th1 and Th2 cytokines cross-regulate. For example, as will be seen, IL-10 can depress IFN-γ production.

Studies of the capacity of cytokines to activate intracellular growth inhibition (not to mention killing) of *M. tuberculosis* by human mononuclear phagocytes have failed to show convincing activity of IFN-γ. Rather, TNF-α and transforming growth factor (TGF)-β are induced by infection of mononuclear phagocytes with *M. tuberculosis* and function in autocrine pathways. TNF-α has a positive and TGF-β has negative ef-

fects on limiting intracellular growth of the avirulent strain of *M. tuberculosis,* H37Ra, in human monocytes [13]. As noted, in contrast to murine models, IFN-γ does not display macrophage-activating factor activity against *M. tuberculosis* in humans. Two explanations have been offered. First, ingestion of *M. tuberculosis* may induce deactivating cytokines that interfere with the action of IFN-γ. Indeed, some enhancement of the macrophage-activating factor activity of IFN-γ is observed in the presence of neutralizing antibody to TGF-β [14]. Second, the chief activity of IFN-γ in resistant species, such as the mouse, is to induce NO production. This mechanism is not operant or expressed more stringently in susceptible species, such as humans and guinea pigs.

The relevant product(s) of sensitized CD4 lymphocytes that mediate protective immunity in humans, therefore, is uncertain. Nonetheless, blastogenesis and the production of cytokines such as INF-γ and IL-2 are convenient readouts for the antigenic repertoire of CD4 cells and well may reflect recognition by the CD4 population that is critical to protection.

Protective antigens of M. tuberculosis. Immunization of mice with live *M. tuberculosis* induces protection and DTH, whereas heat-killed organisms induce DTH only [15]. This experimental observation has sparked interest in antigens released or secreted by viable mycobacteria into culture filtrates as vaccine candidates. The antigenic repertoire of humans is surprisingly diverse and heterogeneous, with recognition of multiple culture filtrate antigens. It is possible, nonetheless, that one or a few antigens could elicit a protective immune response. Current focus is on two antigens, ESAT-6 and the 30-kDa α or 85B antigen.

ESAT-6 is a low-molecular-mass product of early culture filtrates that induces IFN-γ–producing T cells in immunized mice [16] and guinea pigs [17]. The 85B antigen is recognized by T cells from most *M. tuberculosis*–infected persons but not TB patients [18]. Further, immunization of guinea pigs with the 85B antigen confers protection against virulent challenge [19]. On the other hand, immunization of mice with culture filtrate antigens induced protective immunity that was not sustained at the same level as that induced by a live vaccine [20]. Multiple antigens were recognized during the initial expression of acquired immunity. In fact, immunization of mice with plasmid DNA constructs that encode the 85A antigen conferred protection [21], as did plasmid DNA that encoded the 65-kDa heat-shock protein and the 36-kDa proline-rich antigen [22]. Epitope mapping of the α antigen in humans reveals that the dominant T cell epitopes demonstrate ''promiscuous reactivity,'' that is, donors with different MHC class II demonstrate responses, a property desirable for a vaccine [23]. Similarly, individual epitopes of ESAT-6 are recognized by 25%–35% of all culture filtrate–reactive T cells recruited to the site of infection in a mouse model [24]. Nonetheless, rather than individual epitopes and purified antigens, the protective immune response appears more and more likely to represent a summation of reactivity to a large number of antigens, so a vaccine will need to be multivalent.

Once a consensus emerges as to the identity of protective antigens, the next issue is their formulation as a vaccine. There are potential advantages for each of subunit vaccines, DNA vaccines, recombinant BCG overexpressing selected antigens, and *M. tuberculosis* of attenuated virulence. Vaccination with BCG followed by a boost with a subunit vaccine also deserves further consideration. In addition to being effective, a new TB vaccine will need to be responsive to the issue of safety, particularly in the HIV-1–infected host. It is quite apparent that a subunit or killed vaccine would be ideal. Some questions have been raised about the safety of DNA vaccines, but their development for prevention of a number of infectious diseases, including HIV-1 infection, makes it likely that the generic concerns will be addressed before a TB-specific DNA vaccine is ready for human trials. It is the attenuated *M. tuberculosis* auxotrophic mutants, for example, that are likely to incite the most controversy. Genetic safeguards may be helpful in gaining support for human trials with auxotrophic mutants. Efforts are underway to produce double mutations that will make reversion to a virulent phenotype more unlikely and to remove the antibiotic resistant cassette.

A practical issue concerns the ethics of testing new vaccines. As vaccination with BCG provides protection of infants and children against the lethal forms of extrapulmonary TB, it may be unethical to conduct a trial in newborns in which a new vaccine is compared with BCG. Alternative study designs, such as the vaccination or revaccination of adult populations with a high incidence of TB, clearly will be required. The performance of trials in BCG-immunized adults in geographic areas in which BCG has failed to confer immunity has particular appeal.

Surrogates for protective immunity. The Chingleput trial of BCG vaccination in South India followed >250,000 vaccinees for 20 years and ultimately showed no clear efficacy. Ideally, surrogate markers of the protective immune response would permit rapid preliminary evaluation of efficacy in small to medium-sized phase II trials. This would allow selection of the most promising from among competing candidate vaccines and the optimization of vehicles, adjuvants, schedules, etc. It is true, however, that most existing effective vaccines were developed without surrogate end points. The natural history of TB and extraordinary costs for an efficiency trial in terms of resources and time almost require, nonetheless, that some criteria be applied before committing to a phase III trial of a new vaccine.

Characterization of surrogates for vaccine efficacy in humans is not a simple and straightforward task. Demonstration of protection in animal models is helpful, but may not be sufficient, to establish likely efficacy in humans. Clearly, available experimental models have their shortcomings. They do not adequately model reactivation TB, nor have they been used to address the potential for vaccination of infected persons. In fact, in animal models, BCG is the most potent of the vaccine candidates evaluated to date.

At this time, there is no validated human surrogate for protective immunity. As noted, the logical in vivo surrogate, DTH, clearly dissociates from protection in BCG trials. On the other hand, DTH and protection parallel each other following infection with *M. tuberculosis*. It is conceivable, therefore, that DTH could be used to monitor trials of certain vaccines. A recent WHO workshop supported the initial standardization of assays for whole blood IFN-γ and IL-5 as surrogates for Th1 and Th2 responses, pending the validation of these or other assays.

The critical question is how to establish the validity of a surrogate marker. It is essential that the proposed surrogate not be totally empirically derived but have some scientific basis. Ideally, validation would be possible in the context of epidemiologic studies that prospectively establish the association of a proposed marker with resistance to the development of TB. Another approach would be to demonstrate that the surrogate marker of interest was induced by administration of an effective immunotherapy. Neither approach, however, addresses the issue of whether the immune response that contains the initial infectious focus is relevant to long-standing protection and immunologic memory. A third approach, genetic, is considered below. Ultimately, the validity of any proposed surrogate will in fact be corroborated in a phase III trial with clinical end points such as vaccine efficacy.

Immunogenetics of TB

Epidemiologic data derived from twin studies clearly indicate that the susceptibility to TB has genetic determinants. Studies of the association between HLA and TB generally have resulted in variable conclusions. DR2 was, however, associated with increased risk of progression to advanced pulmonary disease [25]. It is the DRB1*1501 allele that is associated with advanced disease and failure to respond to drug therapy, whereas the DRB1*1502 allele was associated with decreased risk [26]. Recent interest also has focused on the role of polymorphisms in cytokine promoters (e.g., TNF-α) and cytokine receptors (e.g., IFN-γR) in susceptibility to mycobacterial disease. The sequencing of both the human and *M. tuberculosis* genome, development of efficient approaches to screen the genome for disease susceptibility genes, and availability of suitable human populations for evaluation promise rapid progress in defining human susceptibility genes. Identification of the pattern of immune responses in subjects with the susceptible versus the resistant genotype also may provide insights into protective immunity and avenues for immunotherapy.

Studies of the genetics of resistance to BCG in the mouse have led to characterization of the *BCG* resistance gene *(NRAMP)* as well as a human equivalent on chromosome 2q. Recent studies suggest, however, that the *BCG* gene does not affect resistance of mice to virulent *M. tuberculosis* [27]. Family studies should determine whether the *BCG* gene has a role in human susceptibility and resistance. Other potential loci such as the MHC deserve reexamination using current approaches. Broad genetic screens for genes linked to susceptibility also are feasible and will be facilitated by mapping of the human genome.

If convincing links are demonstrated between certain alleles and susceptibility to TB, immunogenetic analysis may allow characterization of immunologic correlates of the resistant phenotype; these would become prime candidates as the required surrogates for protective immunity. Characterization of the immunologic deficit associated with a ''susceptibility'' gene also may indicate what is missing in the susceptible host and afford a rationale for certain forms of immunotherapy.

Immunoregulation and Cytokine Therapy

Insights into potential surrogates for protective immunity also derive from comparative in vitro studies of tuberculin reactivity in skin test–positive ''resistant'' persons and ''susceptible'' patients with TB. Direct study of the diseased patient is, however, more directly relevant to pathogenesis and immunotherapy.

Regulation of the systemic immune response. The profile of systemic immune reactivity in patients with TB is one of depressed tuberculin DTH, depressed in vitro PPD-stimulated blastogenesis, and expression of IL-2 and IFN-γ [28]. The depression of blastogenesis extends to nonmycobacterial antigens, although the defect in IL-2 production demonstrates more selectivity. There is concurrent elevation of IgM, IgG, and IgE specific for culture filtrate antigens as well as polyclonal B cell activation [29]. This pattern of increased B cell and depressed T cell function resembles experimental paradigms in which there is an excess of Th2 relative to Th1 T cells and cytokines.

Understanding of immunoregulation in human TB has advanced beyond that of many other infectious diseases. The data indicate a novel regulatory mechanism. The response of peripheral blood mononuclear cells (PBMC) to PPD is depressed by monocytes that overproduce inhibitory cytokines. The apparent restriction of the specificity of monocyte-dependent suppression to mycobacterial antigens results from the unique ability of these antigens to stimulate expression of inhibitory cytokines. PPD is not just a stimulus for T cells but, in fact, a direct stimulus for monocytes activated in situ to overexpress IL-1, IL-6, IL-10, TNF-α, and TGF-β [30]. IL-10 and TGF-β function as cross-modulatory cytokines depressing blastogenesis and IFN-γ production by T cells [31]. These cellular interactions are displayed in figure 1.

Recent studies have addressed the relevant mycobacterial stimuli and the mechanism by which they activate mononuclear phagocytes. PPD consists of proteins and nonprotein constituents. LAM is the major cell wall lipoglycan of *M. tuberculosis* and an important constituent/contaminant of PPD. LAM derived from the virulent strain H37Rv induces little TNF-α production compared with avirulent rapidly growing mycobacteria, but LAM from virulent organisms retains the ability to induce TGF-β expression that is comparable in magnitude to that of

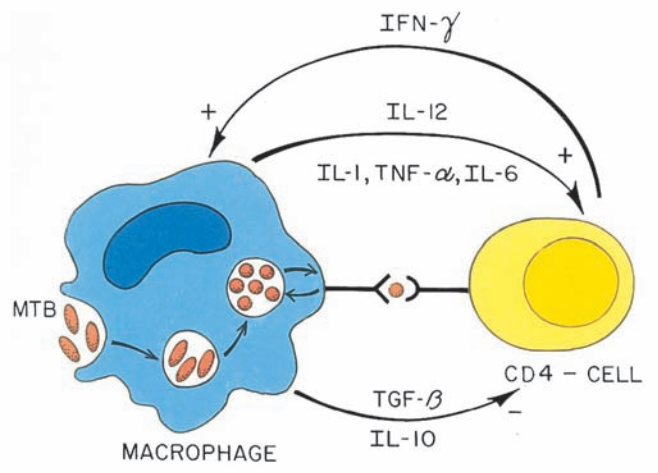

Figure 1. Regulation of human immune response in tuberculosis. Mononuclear phagocytes from TB patients are primed so that, when exposed to *M. tuberculosis* (MTB) or its soluble products, they overproduce cytokines. CD4 cells are activated by expression of mycobacterial antigens on monocytes in context of class II major histocompatibility component determinants. Costimulatory factors produced by monocytes amplify this activation (interleukin [IL]-1, IL-6, tumor necrosis factor [TNF]-α), as does interaction of certain cell-surface ligands on each cell with receptors on other (not shown). Level of T cell activation is modulated, however, by transforming growth factor (TGF)-β and IL-10. TGF-β depresses production or action (or both) of Th1 cytokines interferon (IFN)-γ and IL-2 and also blocks activity of IL-12. TGF-β amplifies its own production and serves to deactivate mononuclear phagocyte effector function. Result is profound suppression of Th1-like responses. Same cross-modulatory cytokines (TGF-β and IL-10) also may enhance antibody production.

avirulent organisms [32]. Therefore, the bias toward expression of TGF-β and the spontaneous expression of this cytokine by blood monocytes and in granulomas [33] may relate to nonprotein constituents of the tubercle bacillus.

Culture filtrate proteins also induce cytokine production by mononuclear phagocytes, but in this case, there is balanced expression both of stimulatory and inhibitory cytokines. The 85B antigen, a major culture filtrate protein and candidate for protective antigen, induces expression of cytokines by binding to fibronectin on the monocyte cell surface [34]. A 58-kDa culture filtrate protein [35] with glutamate synthetase activity [36] is another and more potent inducer of cytokines. The induction of cytokines by mycobacterial constituents may be a factor in the immunopathogenesis of TB. The proteins that induce cytokines also may possess an adjuvant-like property that enhances their immunogenicity and thus is potentially relevant to their use as vaccines.

Depressed tuberculin skin test responses and the suppressed PPD-stimulated blastogenesis are transient phenomena, largely reversed within 1 month of the start of treatment. Recent data indicate, however, that cytokine production is regulated differently. The depressed PPD-stimulated production of IFN-γ persists for at least 12 months after the diagnosis of TB (and 6 months after completion of treatment) (Hirsch CS, personal communication). Increased expression of IL-10 and TGF-β by PPD-stimulated monocytes is a by-product of immune activation that reverses slowly during the treatment of pulmonary TB. Expression of the immunosuppressive cytokines is more pronounced in far-advanced than moderately advanced pulmonary TB and virtually nil in patients with minimal disease. The addition of exogenous IL-12 to cultures normalizes PPD-stimulated IFN-γ production. It is not clear, however, whether this is the result of reversing the defect so that CD4 cells produce normal amounts of IFN-γ or, as may be more likely, bypassing the defect by stimulating IFN-γ production by NK and other non-CD4 cell populations. Initial studies in HIV-infected TB patients also indicate profound suppression of IFN-γ responses [37, 38], a negative regulatory role for IL-10 [37, 39], and restitution by IL-12 [37, 39].

The persistent depression of IFN-γ production in TB suggests that the cross-modulation by IL-10 and TGF-β is superimposed on a primary T cell dysfunction. Our recent data, in fact, suggest that spontaneous and PPD-induced apoptosis may have a role in the depressed IFN-γ responses.

It is not yet clear whether suppression of T cell responses is the cause or the effect of active TB. In either case, it clearly may contribute to a vicious cycle of immunosuppression, deactivation of macrophage effector function, and disease progression.

Regulation of the local immune response in TB. In humans, studies of the systemic immune response are more easily conducted than studies of local immunity. The goal of immunotherapy is, however, to activate protective immunity at local sites of disease. Therefore, it is essential to address the question of the relevance of studies of the blood to the local immune response. Initial studies of the pulmonary immune response by bronchoalveolar lavage indicate that there is an alveolitis comprising predominantly T lymphocytes and macrophages, with an increase in immature peroxidase-positive cells [40] (figure 2). The local inflammatory response appears to develop by chemotaxis of circulating T cells and monocytes to the site of disease. Therefore, understanding the function and regulation of blood mononuclear cell responses to PPD is extremely relevant to the understanding of the local immune response as well as selection of immunotherapy. Preliminary functional studies indicate, however, that alveolar lymphocytes from patients with tuberculosis show increased PPD-induced blastogenesis and an increased frequency of IFN-γ–producing cells. Also, alveolar macrophages from TB patients appear to fail to suppress PPD-stimulated blastogenesis (Schwander SK, personal communication). Obviously, further studies will be needed to clarify the relationship between regulation of the local and systemic immune response in tuberculosis. It should be noted that it is the systemic response that is relevant to protective immunity induced by vaccines. On the other hand, immunotherapy of active disease ideally is directed at enhancing the local response at the site of disease.

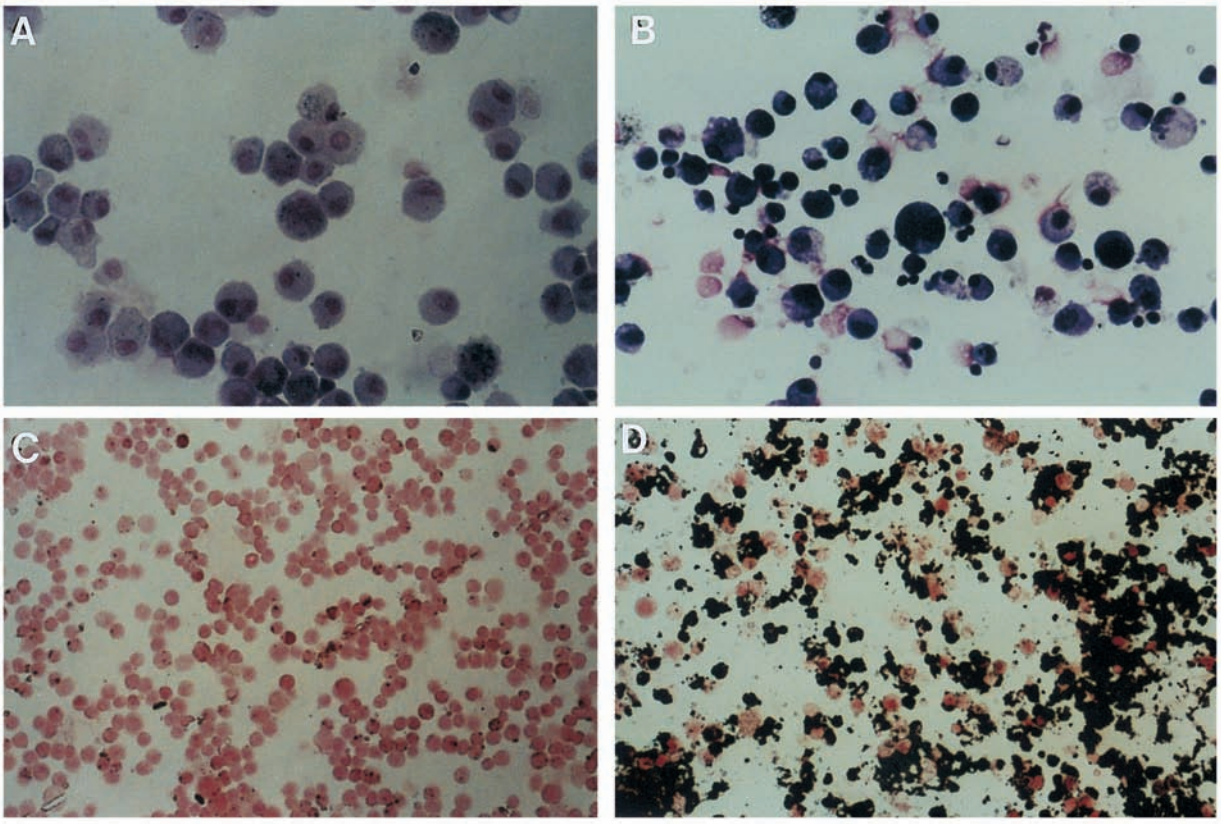

Figure 2. Bronchoalveolar cells (BAC) in health and in pulmonary tuberculosis. Cytocentrifugation preparations of BAC were stained with Wright's stain (**A** and **B**, ×100) or peroxidase stain (**C** and **D**, ×50). **A**, BAC from healthy subject showing predominantly large alveolar macrophages. **B**, BAC from affected lung of patient with pulmonary tuberculosis. Macrophages are heterogeneous in size, and small mononuclear cells with morphology of lymphocytes are present. **C**, BAC from healthy subject demonstrating few peroxidase-positive cells. **D**, BAC from affected lung of patient with pulmonary tuberculosis. Many peroxidase-positive mononuclear cells (dark stain) are present. Among 15 tuberculosis patients, mean % of peroxidase-positive mononuclear BAC was ~20%. Courtesy of S. Schwander and E. Rich (Case Western Reserve University, Cleveland).

Implications for immunotherapy. The potential goals of immunotherapy are 2-fold: in multidrug-resistant disease, as an adjunct to available chemotherapy in an attempt to improve on the prevailing 50% cure rates, and in drug-sensitive TB, to shorten the duration of treatment by bolstering the immune response, thereby accelerating the eradication of persisting organisms. The traditional approach to immunotherapy of TB would be to administer the cytokines that are essential to protective immunity. There are several problems with this approach. First, the cytokines most relevant to protective immunity in humans are unknown. Data from murine models may be misleading, as the cytokines of interest function to activate inducible NO, which has not been shown convincingly to be a mediator of macrophage effector function in humans. Second, given the bias in TB toward immunosuppression, the provision of exogenous cytokines may merely ''fuel the fire'' by activating more endogenous expression of suppressive mediators. In such a case, the main result of cytokine therapy would be toxicity.

Purified recombinant cytokines that are potentially available and relevant as immunotherapy for TB include IL-2, IFN-γ, and IL-12. Recent data with low-dose IL-2 immunotherapy suggest accelerated clearance of acid-fast bacilli from the sputum in drug-resistant TB [41]. IFN-γ has been used with some success to treat drug-resistant *Mycobacterium avium* disease in non-AIDS patients [42]. IL-12 is of particular interest because, as noted, it induces expression of IFN-γ by NK cells and also the differentiation of Th0 from Th1 cells. Exogenous IL-12 achieves a remarkable reconstitution of PPD-stimulated IFN-γ production in PBMC from TB patients (Hirsch CS, personal communication). The use of recombinant cytokine therapy, although promising for drug-resistant disease, may be too difficult to administer, toxic, and expensive for use in the treatment of drug-sensitive disease. Another potential approach would be to administer pharmacologic cytokine inducers as appropriate agents became available.

Inhibition of negative regulators deserves further consideration as immunotherapy for TB, particularly as naturally oc-

curring inhibitors of TGF-β, L-decorin, and latency-associated protein have been described. Initial studies indicate that both L-decorin and latency-associated protein increase PPD-stimulated blastogenesis and IFN-γ production by PBMC from patients with pulmonary TB [43]. Blocking the negative cytokine mediators could be used as a sole immunotherapeutic strategy or in addition to providing a positive signal.

Another approach would be to modulate the immune response by administering a therapeutic vaccine. Uncontrolled trials and recently a controlled trial suggest that administration of heat-killed *Mycobacterium vaccae* as a therapeutic vaccine accelerates sputum bacteriologic conversion and provides clinical benefit in pulmonary TB. It has been proposed that *M. vaccae* shifts the T cell response from a Th2 to a Th1 pattern. *M. vaccae* is easy to prepare and administer, appears to be nontoxic, and should be inexpensive. Clearly, further study is warranted. In fact, controlled clinical trials are in progress in South Africa and Uganda. If *M. vaccae* is effective as immunotherapy, parallel studies of immune reactivity may identify surrogates for an effective or protective (or both) response.

TB-HIV Interactions

TB and HIV-1 often occur in the same geographic area, in the same person, and even in the same cell. It is not surprising, therefore, that they have important and bidirectional interactions. The impact of HIV on TB is profound. As indicated, the immunosuppression of HIV dramatically increases the risk of reactivation of a latent focus of infection and progression of primary infection. The rapid progression of primary infection to infectious TB catalyzes the spread of the strains of *M. tuberculosis* that are prevalent in the community, including multidrug-resistant TB. HIV-1 also alters the clinical expression of TB, as there is less cavitary disease and more atypical, disseminated, and extrapulmonary manifestations.

The impact of TB on HIV may prove to be equally profound. TB in the HIV-infected is associated with shortened survival not attributable to death from TB [44, 45]. Rather it appears that patients are dying of progressive immunodeficiency. Does TB accelerate the course of HIV? Recent data support this notion. TB-HIV coinfection is associated with a higher plasma virus load (RNA) than is found in CD4 cell–matched HIV-infected controls. Moreover, virus load actually increases during the course of treatment of the TB [46]. The development of TB also is associated with accelerated rates of decline in numbers of CD4 lymphocytes, a process that continues as the TB is treated [47].

The basis for increased viral replication and more rapid decline in numbers of CD4 cells in TB-HIV coinfection may relate to activation of cells that harbor latent HIV. In fact, CD4 cells and monocytes display surface markers of activation [48], and there is increased production of TNF-α. Serum TNF-α also is increased. TNF-α is known to activate latent infection with HIV-1 through NFκB mechanisms. Moreover, two markers of macrophage activation, serum neopterin and TNF-αR-II, are increased in TB-HIV coinfection and represent independent predictors of poor prognosis [49]. To complete the story, monocytes from patients with TB show increased susceptibility to a productive infection with HIV-1 [48]. *M. tuberculosis* and PPD also induce HIV replication in the latently infected U1 cell line [50].

Although other opportunistic infections also may shorten survival in HIV, TB may be unique because its impact on HIV is exacted at a higher CD4 cell count and for prolonged periods, when there is more to lose in terms of survival. Further, activation of T cells and macrophages and expression of TNF-α are essential features of the immunopathogenesis and protective immune response of TB but may be deleterious as regards containment of HIV-1.

Trials of cytokine inhibitors. The obvious approach to averting the morbidity of TB-HIV coinfection is prevention, although this may prove difficult to accomplish in developing countries. Cytokine inhibitors also may have a role as an adjunct to chemotherapy, particularly if viral replication increases during treatment of the TB. Both thalidomide, a specific TNF-α inhibitor, and pentoxifylline, a nonspecific inhibitor, have undergone controlled trial in TB-HIV. The results indicate a decrease in virus load and possible clinical benefit (increase in weight gain for thalidomide, increased Karnofsky score and hemoglobin level for pentoxifylline) [52–54]. The impact on survival of these and possibly more potent cytokine inhibitors needs to be assessed.

Predictions

The allure of improved vaccines, immunotherapy, and cytokine inhibitors is apparent. The question is, How close are we (and how great will the impact be on TB control)? It is easy to be skeptical, as the application of immunotherapy to infectious diseases has been talked of since the time of Shaw: ''Stimulate the phagocytes.''

In fact, the era is upon us. Controlled trials of immunotherapy for TB are in progress or planning stages for *M. vaccae* as a therapeutic vaccine and for cytokine therapy with recombinant IL-2. Likewise, controlled trials of the cytokine inhibitors pentoxifylline and thalidomide have been completed in patients with TB-HIV coinfection, and a trial of prednisolone has started. Of these interventions, only the widespread use of a safe and inexpensive agent could impact meaningfully on TB control.

Of course, the single most valuable intervention would be a vaccine that is effective in preventing *M. tuberculosis* infection as well as disease and in aborting progression from infection to disease. Identifying the optimal vaccine and schedule, validating immunologic surrogates as trial end points, and conducting a trial to demonstrate superior efficacy relative to BCG vaccine separately and together pose a formidable challenge,

but recent progress in immunology, microbiology, and molecular genetics will provide fundamental shifts in understanding that promise to have extraordinary impact on the approach to TB control.

Acknowledgments

I gratefully acknowledge the unique and vital contributions of the following persons to the research described in this review: Henry Boom, Christine Hirsch, John Johnson, Elizabeth Rich, Richard Silver, Zahra Toossi, Bob Wallis, and Chris Whalen. I am thankful, as well, for the guidance and support of first John Foulds and Carole Heilman and now Ann Ginsberg and Pam McInnes at the National Institutes of Health.

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