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Correlates of Protective Immunity to *Mycobacterium tuberculosis* in Humans

Jerrold J. Ellner[1], **Christina S. Hirsch**[2], and **Christopher C. Whalen**[2]

[1]University of Medicine and Dentistry of New Jersey, Newark

[2]Case Western Reserve University and University Hospitals, Cleveland, Ohio

Abstract

Correlates of protective immunity to *Mycobacterium tuberculosis* in humans are desirable for identifying protective antigens, demonstrating the immunogenicity of a vaccine candidate and its potential efficacy, and permitting optimization of the dose, vehicle, adjuvant, and schedule of immunization. Potential correlates can be proposed on the basis of animal models and ex vivo/in vitro studies in humans. Most critical is their validation; ultimate validation will require correlation with protection in a phase III efficacy trial of an effective vaccine. Other approaches, however, can allow selection of the most promising correlates for inclusion in phase I and II and, ultimately, phase III vaccine trials. Current data from experimental models and studies of patients with pulmonary tuberculosis and their household contacts indicate that *Mycobacterium tuberculosis*–stimulated whole-blood production of interferon-γ, although imperfect, is the best available correlate. Nonetheless, further refinement of this assay and additional studies of more complex assays that model *M. tuberculosis* killing and cytotoxic T lymphocyte activity are warranted. During planning of a vaccine trial, the best available correlates of immunity can be selected for inclusion.

The availability of correlates of protective immunity in humans is generally useful for the development of vaccines and may be critical for an effective tuberculosis vaccine. Efficacy trials of BCG vaccine in the past have enrolled >1250,000 subjects who have been followed for 2 decades. Both to encourage commercial interest in tuberculosis vaccine development and to increase the likelihood of success, alternative study designs should be developed that capitalize on correlates of protection as surrogate end points for vaccine immunologic activity and efficacy.

Although many vaccines have been developed without correlates of protection, the issues raised by the complexity of evaluating tuberculosis vaccines indicate the particular need for a correlate of protection that can provide early indication of potential efficacy. Correlates of protection, chiefly antibody levels, have been used to advantage in the development of vaccines, mostly against bacterial disease (e.g., that caused by *Streptococcus pneumoniae*) and viral disease (e.g., hepatitis B). Furthermore, both neutralizing antibody activity and

Reprints or correspondence: Jerrold J. Ellner, Division of Infectious Diseases, Dept. of Medicine, 185 S. Orange Ave., MSB I-510, Newark, NJ 07103-2714 (ellnerjj@umdnj.edu)..

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cytotoxic T lymphocyte activity have been proposed as correlates of protection and end points for the evaluation of vaccines against HIV.

Once validated, correlates of protective immunity could be used to identify or to prioritize protective antigen and vaccine candidates; to optimize dose, vehicle, adjuvant, and schedule of immunizations; and to provide preliminary evidence of immunogenicity and/or probable protective efficacy. Although promising correlates of protection can be proposed on the basis of current understanding and dogma, validation is the essential issue. Definitive validation must await a phase III trial of an effective vaccine. Evaluation of a potential candidate in an initial vaccine efficacy trial would validate that correlate for use as a surrogate end point in future vaccine trials.

Fortunately, a number of approaches can be taken to select correlates for application in initial vaccine trials (table 1). It should be noted that potential correlates need to be studied in phase I/II trials of vaccines to demonstrate that they are modulated by the vaccine under study before they are incorporated in the design of a phase III trial. As indicated, there are shortcomings to each of the “half-steps.” Ultimately, an assay that looks promising from multiple standpoints can be advanced as a credible end point for phase III trials.

Two general considerations are germane before a discussion of specific correlates. First, a correlate of protection is not necessarily part of the mechanism of protection. In fact, a new vaccine may induce protective immunity without engaging the pathways constituting “natural” protection. Second, support for particular correlates will accrue with time, and the pace of interest and activity concerning new vaccines is quickening. Selection of a correlate or correlates therefore must be decided by reviewing the currently available data at the time a vaccine trial is about to start.

Whole-Blood Production of IFN-γ (WB–IFN-γ)

The most attractive available correlate of protection is WB–IFN-γ after stimulation with *Mycobacterium tuberculosis* antigens [1]. This is a low-technology, inexpensive approach, and in case-control studies it has correlated well with IFN-γ production by peripheral blood mononuclear cells (Hirsch et al., manuscript submitted). The rationale and evidence supporting the use of assays for determining IFN-γ production as a correlate of protection are substantial; they can be summarized as follows.

Studies in genetically disrupted mice indicate that IFN-γ is essential for protective immunity against *M. tuberculosis* [2, 3]. However, the mouse model is not particularly reflective of human tuberculosis; moreover, IFN-γ effects in mice are mediated in large part by nitric oxide pathways, which may not be as critical to protection in humans.

Humans with an IFN-γ–receptor abnormality show marked susceptibility to infection with atypical mycobacteria [4], disseminated infection with the BCG strain of *Mycobacterium bovis*, and infection with *M. tuberculosis* [5, 6].

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Patients with moderate and far-advanced tuberculosis have depressed *M. tuberculosis*–stimulated WB–IFN-γ [7, 8], although it is not certain whether this is the cause or the effect of the tuberculosis.

IFN-γ immunotherapy may provide some clinical and bacteriologic benefit in the treatment of multidrug-resistant tuberculosis [9].

In a household contact study, WB–IFN-γ was increased in individuals with recent or remote *M. tuberculosis* infection but no disease (Whalen et al., unpublished data; table 2), individuals who may be relatively resistant to exogenous reinfection.

WB–IFN-γ increases in subjects who have a tuberculin skin test conversion (Whalen et al., unpublished data).

In pleural tuberculosis, IFN-γ concentrations and mRNA for IFN-γ in pleural fluid, as well as IFN-γ production by *M. tuberculosis*–stimulated pleural fluid cells, all are increased relative to similar measurements in blood [10]. The observation that pleural tuberculosis has the potential to resolve without therapy suggests that the local immune response is protective in these patients.

Overall, support for IFN-γ as a correlate of protection thus is derived from four of the five approaches listed in table 1. Recent evidence suggests, nonetheless, that WB–IFN-γ may not be the ideal correlate of protection. Adults with minimal tuberculosis and children with progressive primary tuberculosis show high WB–IFN-γ responses (Whalen et al., unpublished data; table 2). Therefore, WB–IFN-γ is imperfect in distinguishing *M. tuberculosis* infection from disease. On balance, however, some cases of progressive primary tuberculosis in children and some cases of minimal disease in adults self-cure without specific chemotherapy.

In addition, IFN-γ fails to activate the killing of *M. tuberculosis* by human monocytes and in certain circumstances may promote intracellular replication [11].

Improved WB–IFN-γ

One approach to address the shortcomings of *M. tuberculosis*–stimulated WB–IFN-γ as a correlate of protection is to refine the assay. Thus far, crude antigens have been used as stimuli and crude cell populations as producers of IFN-γ. The stimulus can be refined by the use of purified *M. tuberculosis* antigens that may be *M. tuberculosis*–specific and/or vaccine candidates. Several populations of lymphocytes in whole blood can produce IFN-γ. A focus on production by CD4 cells, for example, may provide an improved correlate. Flow cytometry performed on whole blood can be used for this analysis.

Another approach would be to assess the ratio of IFN-γ responses induced by a purified antigen to the responses induced by crude antigenic preparations. Ideally, the purified antigen would be restricted in its species distribution to *M. tuberculosis* and also would be a vaccine candidate. An alternative ratio of interest would be that of WB–IFN-γ and TNF-α. TNF-α levels are increased by inflammation during modulation of *M. tuberculosis* infection

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or disease (table 2). Preserved IFN-γ production in the presence of active inflammation may have a different significance than production in the absence of inflammation.

The lack of an absolute correlation between WB–IFN-γ and disease indicates both the need for and the potential approach to evaluating new potential correlates. In fact, use of purified antigens and/or assessment of expression of IFN-γ by lymphocyte subpopulations may provide a correlate that can be evaluated to determine whether it more reliably distinguishes infection from disease.

Alternative Correlates of Protection

If the refined assay of WB–IFN-γ fails to distinguish infection from disease, then alternative approaches should be developed and evaluated. In general, these may represent more complex assays that indicate effector functions against *M. tuberculosis*. Several assays have been developed to assess the killing of intracellular mycobacteria. Studies can be performed with use of isolated monocytes or in a T cell–dependent monocyte system [11]. Although representing an in vitro model of effector function, this assay system is technically demanding and requires the use of a containment facility if virulent *M. tuberculosis* is used as target. An interesting alternative now under development evaluates killing in a whole-blood system and assesses viable organisms by measuring the luminescence of reporter mycobacteria that express luciferase genes (B. Kampmann et al., manuscript submitted).

CD4- and CD8-dependent cytotoxic effector mechanisms also contribute to anti–*M. tuberculosis* immune responses [12–15]. The standard chromium-release assay is laborious and insensitive and requires working with radioactive substances, a particular problem in developing countries. Alternatives include using quantitative controlled reverse-transcriptase PCR to measure the release of endogenous molecules, such as lactate dehydrogenase, and to measure the expression of RNA for effector molecules that mediate CD4- and CD8-dependent cytotoxicity (FasL, Perforin, Granzyme B) [16]. Fas/FasL interactions are associated with killing of *M. tuberculosis*-infected macrophages [17] and potentially represent another approach to monitoring effector function.

Conclusions

New assays need to be developed as correlates of protective immunity and to be compared with the flawed current “gold standard,” *M. tuberculosis*-stimulated whole blood IFN-γ production. The household-contact study design offers a window of opportunity in which a new assay can demonstrate superiority in comparison with WB–IFN-γ by allowing the distinction between protection and certain forms of disease (minimal pulmonary tuberculosis in adults and coprevalent tuberculosis in children). These issues must be tackled now to ensure that the best available correlate can be included in future vaccine trials.

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References

1. Weir RE, Morgan AR, Britton WJ, Butlin CR, Dockrell HM. Development of a whole blood assay to measure T cell responses to leprosy: a new tool for immuno-epidemiological field studies of leprosy immunity. J Immunol Methods. 1994; 176:93–101. [PubMed: 7963598]
2. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for IFN-γ in resistance to *Mycobacterium tuberculosis* infection. J Exp Med. 1993; 178:2249–54. [PubMed: 7504064]
3. Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. Disseminated tuberculosis in IFN gene–disrupted mice. J Exp Med. 1993; 178:2243–7. [PubMed: 8245795]
4. Levin M, Newport MJ, D'Souza S, et al. Familial disseminated atypical mycobacterial infection in childhood: a human mycobacterial susceptibility gene? Lancet. 1995; 345:79–83. [PubMed: 7815885]
5. Jouanguy E, Altare F, Lamhamedi S, et al. Interferon-γ–receptor deficiency in an infant with fatal bacille Calmette-Guérin infection. N Engl J Med. 1996; 335:1956–61. [PubMed: 8960475]
6. Jouanguy E, Lamhamedi-Cherradi S, Altare F, et al. Partial interferon-γ receptor 1 deficiency in a child with tuberculoid bacillus Calmette-Guérin infection and a sibling with clinical tuberculosis. J Clin Invest. 1997; 100:2658–64. [PubMed: 9389728]
7. Hirsch C, Hussain R, Toossi Z, Dawood G, Shahid F, Ellner J. Cross-modulation by transforming growth factor β in human tuberculosis: suppression of antigen-driven blastogenesis and interferon-γ production. Proc Natl Acad Sci. 1996; 93:3193–8. [PubMed: 8622912]
8. Hirsch C, Ellner J, Blinkhorn R, Toossi Z. In vitro restoration of T-cell responses in tuberculosis and augmentation of monocyte effector function against *Mycobacterium tuberculosis* by natural inhibitors of transforming growth factor β. Proc Natl Acad Sci. 1997; 94:3926–31. [PubMed: 9108081]
9. Condos R, Rom WN, Schluger NW. Treatment of multidrug-resistant pulmonary tuberculosis with interferon-γ via aerosol. Lancet. 1997; 349:1513–5. [PubMed: 9167461]
10. Barnes PF, Lu S, Abrams JS, Wang E, Yamamura M, Modlin RL. Cytokine production at the site of disease in human tuberculosis. Infect Immun. 1993; 61:3482–9. [PubMed: 8335379]
11. Silver R, Li Q, Boom W, Ellner J. Lymphocyte-dependent inhibition of growth of virulent *Mycobacterium tuberculosis* H37Rv within human monocytes: requirement for $CD4^+$ T cells in purified protein derivative–positive, but not in purified protein derivative–negative subjects. J Immunol. 1998; 160:2408–17. [PubMed: 9498784]
12. Boom WH, Balaji KN, Nayak R, Tsukaguchi K, Chervenak KA. Characterization of a 10- to 14-kilodalton protease-sensitive *Mycobacterium tuberculosis* H37Ra antigen that stimulates human γ^{δ} T cells. Infect Immun. 1994; 62:5511–8. [PubMed: 7960133]
13. Tan JS, Canaday DH, Boom WH, Balaji KN, Schwander SK, Rich EA. Human alveolar T lymphocyte responses to *Mycobacterium tuberculosis* antigens: role for $CD4^+$ cytotoxic T cells and relative resistance of alveolar macrophages to lysis. J Immunol. 1997; 159:290–7. [PubMed: 9200465]
14. Stenger S, Hanson D, Teitelbaum R, et al. An antimicrobial activity of cytolytic T cells mediated by granulysin. Science. 1998; 282:121–5. [PubMed: 9756476]
15. Tsukaguchi K, Balaji KN, Boom WH. $CD4^+$ αβ T cell and γ^{δ} T cell responses to *Mycobacterium tuberculosis*: similarities and differences in Ag recognition, cytotoxic effector function, and cytokine production. J Immunol. 1995; 154:1786–96. [PubMed: 7836763]
16. Hockett RD Jr, Janowski KM, Bucy RP. Simultaneous quantitation of multiple cytokine mRNAs by RT-PCR utilizing plat- based EIA methodology. J Immunol Methods. 1995; 187:273–85. [PubMed: 7499887]
17. Lewinsohn DM, Bement TT, Xu J, et al. Human purified protein derivative–specific $CD4^+$ cells use both CD95-dependent and CD95-independent cytolytic mechanisms. J Immunol. 1998; 160:2374–9. [PubMed: 9498779]

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Table 1

Approaches to partially validating correlates of protective immunity.

Approach	Shortcoming
Animal models	Relevance to humans uncertain
Human studies	
Tuberculosis	More relevant to susceptibility than protection; findings may be secondary to disease and/or be nonspecific
Household contacts	Difficult to definitively characterize protected populations
Immunogenetics	More relevant to disease susceptibility; need to identify common rather than rare genetic traits
Trials of immunotherapy	Augmentation of immunity to clear bacteria from sputum may not equate to protection against tuberculosis
Modulation in a phase I/II trial (e.g., BCG)	BCG vaccination is not uniformly effective; data may not be relevant to other types of vaccines

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Table 2

Stages of *Mycobacterium tuberculosis* infection.

Stage of infection	Patients, condition	Immunologic profile	
		IFN-γ	**TNF-α**
Initial infection	Skin test converters (before skin test conversion)	Low	High
Progressive primary tuberculosis	Children aged 5 y; diseased, PPD^+	High	High
Protective immunity	Children aged 5 y; healthy, PPD^+	High	Low
Latent tuberculosis	Adults aged 15 y; healthy, PPD^+	High	Low
Reactivated tuberculosis	Adults aged 15 y; minimal tuberculosis	High	Low/high
Reactivated tuberculosis	Adults aged 15 y		
	Moderate tuberculosis	Low	High
	Far-advanced tuberculosis	Lowest	Highest

NOTE. Table is based on unpublished data of Whalen et al.