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THE HUMAN IMMUNE RESPONSE TO *MYCOBACTERIUM TUBERCULOSIS* INFECTION AND DISEASE

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Introduction

The natural history of TB in humans poses a number of questions and challenges to the immunologist. *M. tuberculosis* (MTB) infection is initially contained in over 95% of infected individuals. A latent focus is established in them following primary infection, but there are no manifestations of disease. What is the basis for protective immunity in these individuals that allows them to contain the initial tuberculous focus? This is a particularly relevant era as we contemplate means of boosting the protective immune response either through immunotherapy or an improved TB vaccine. Progressive primary disease is more common in infants and children indicating that protective immunity requires some maturation of the immune system. Patients with latent MTB infection who develop HIV infection/AIDS demonstrate a strikingly increased risk of reactivation of the latent focus. Clearly, the attendant CD4 dysfunction is associated with failure to maintain the "clinical latency" of the tuberculous focus. Therefore, the concept of "latency" probably is a misnomer as low levels of bacterial replication are held in check by active immunologic "surveillance". Understanding the immunologic basis for the protective immunity that contains the initial infection and for the concomitant immunity that maintains the latency of local foci of *M. tuberculosis* is important in its own right and also to establish correlates of protection that can be used as to assess the activity of new TB vaccines both preventive and therapeutic in nature.

In about 5% of individuals with latent MTB infection, the latent focus breaks down after years to decades of clinical quiescence and disease manifestations develop. Reactivation TB often poses a paradox in the ostensibly normal host—unexplained disease progression after years of latency and despite pre-existing delayed type hypersensitivity to MTB. I speculate that the equilibrium between host and parasite is imbalanced transiently either due to increased bacterial replication or depression of the host response. The resulting infectious foci and the intense inflammatory reactions to it in the sensitized host is sufficient to cause clinical disease. The host immune response no longer captures and contains bacterial replication. Rather, unregulated immune activation instead becomes a factor in immunopathogenesis contributing to morbidity and tissue damage.

This review focuses on progress in understanding the human immune response to tuberculous infection and disease based, in large part, on observations made in Cleveland, Ohio by investigators then at Case Western Reserve University and in Kampala, Uganda through a

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Protective Immunity against MTB

In humans, it is not possible to study the protective immune response to tuberculosis directly. Initial attention was placed on the systemic in immune response in patients with pulmonary TB. In them, protective immunity has failed but it is impossible to address the cause and effect conundrum. Do the observed abnormalities in the immune response represent a genetically-determined or acquired factor that predisposed to reactivation disease, or are they a consequence of the disease manifestations?

Three types of observations have provided insights into protective immunity in humans : a study of household contacts of patients with infectious tuberculosis ; long-term follow-up of a cohort of persons with latent TB infection ; and, longitudinal studies of patients with TB.

Household Contact Study

Household contacts of patients with active infectious TB all have been recently exposed ; some are uninfected as evidenced by a negative tuberculin skin test (TST), whereas others are infected tuberculin skin test (TST positive) and some have co-prevalent TB. Further, infection and disease modulate with time. TST conversion is evidence of modulation of primary infection. Some individuals develop incident disease. Ethical considerations, require, however, that contact at greatest risk of developing disease receive treatment with preventive therapy which decreases the number of incident cases.

In Kampala Uganda, we performed serial evaluations of 1206 household contacts of 302 patients with sputum acid fast bacilli smear positive pulmonary tuberculosis in Kampala Uganda. 80% were TST positive. There were 58 (5%) cases of TB (co-prevalent TB) that were diagnosed during the initial ascertainment within households ; 41 in children 5 years of age or younger and 17 in older contacts. All children less than 5 and all HIV-infected contacts were offered treatment of latent TB infection. Nonetheless, 24 (2%) were found to have developed TB (incident TB) in follow-up. 5% (62) of all household contacts and 26% of those initially TST negative underwent tuberculin skin test conversion. At various time points, whole blood was stimulated with MTB culture filtrate and supernates were collected for analysis of interferon (IFN)-gamma, tumor necrosis factor-alpha, transforming growth factor (TGF) beta and IL 10. IFNgamma correlated with MTB infection (Table). It should be noted, however, that over 30% of the TST positive subjects had little IFNgamma production. They may represent the reservoir of patients at increased risk of progressive primary TB. Likewise, 30% of the TST negative individuals manifest significant

Table Whole Blood Interferon gamma Production in a Household Contact Study in Kampala Uganda

	Time 0	3 months	increment
Clinically Well			
TST neg	+	+	0
TST pos	++++	++++	0
TST converters	++	++++	++
TB patients			
Index cases	+	++	+
Co-prevalent			
<5 yrs old	++++	ND	
>5 yrs old	++	ND	
Incident TB	+	++	+

IFNgamma responsiveness. These may be transient, as these individuals are exposed but not infected. Alternatively, their reactions may be contingent on BCG immunization or infection with environmental mycobacteria. Future studies, evaluating temporal changes in IFNgamma responses and using MTB and *Mycobacterium avium-intracellulare* specific antigens should allow distinction among these possibilities.

Two other groups of interest are individuals undergoing TST conversion and those developing active TB. TST conversion is associated with an actual three-fold increment in the IFNgamma response, whereas there is little if any increase in persons who develop TB (Table). As 95% of the TST converters will contain the tuberculous focus without developing disease, the IFNgamma response is a clear concomitant of protection. Once more, 12 of the 33 TST converters do not increase IFNgamma and may represent a group at increased risk of disease. The finding that patients who develop incident TB fail to manifest an IFNgamma response (despite the fact that their TB was minimal) also supports the notion that this assay is a correlate of protection.

Patients with co-prevalent TB pose a paradox. Those cases over 5 years of age have a markedly depressed IFNgamma response; it is slightly greater than that of the index cases, in part because of the relatively preserved response of patients with minimal pulmonary TB. On the other hand, co-prevalent cases younger than 5 had a vigorous IFNgamma response and also high levels of TNFalpha production. At first, the observed retained IFNgamma response concomitant to active disease appears to undermine the support for this assay as a correlate of protective immunity. It should be noted, however, that both children less than 5 with primary TB and older individuals with minimal pulmonary TB have the potential to self-cure.

Although IFNgamma is the best available correlate of protection, refinement of the assay (purified antigens, phenotyping of the producing cell) may allow better distinction of individuals with disease from those with infection only. Functional assays focusing on killing of MTB and cytotoxic T-cell (CTL) activity also need to be evaluated as potential correlates of protection.

Follow-Up of TST Reactors

We assessed TST reactivity and *in vitro* lymphocyte responses in a group of individuals in Cleveland known to be TST positive 19 years previously[1]. 17 of the 22 maintained vigorous TST reactivity with a mean reaction size of over 30 mm of induration as well as high levels of PPD-stimulated blastogenesis (Fig. 1, 2). The 5 TST reverters all had an initial reaction size of <12 mms and none were known contacts of active cases. One individual underwent a booster response (TST increased from 9 to 15 mm of induration) which was associated with a dramatic increase in lymphocyte transformation. It appears that true MTB infection (TST >12 mms, known contacts of cases) is associated with retention of robust responses. This is more likely to be due to endogenous re-boosting by bacteria and antigens in the latent focus rather than re-exposure which is rare in the low prevalence setting. This boosting and retained reactivity is likely to be responsible for the long tenure of protective immunity.

Studies in Pulmonary TB

Pulmonary TB in the adult represents a good model to delineate the pathogenesis and concomitants of reactivation disease and can be used to test concepts concerning immunotherapy. The systemic immune response is most easily sampled and contains the precursors of cells that will be recruited to the inflammatory focus. Bronchoalveolar lavage (BAL) can be used to assess the local immune response and provides insights into immunologic compartmentalization. TB pleurisy represents a vigorous local immune and inflammatory response that can self-cure without specific treatment. Although protective immunity presumably is eclipsed in patients with active disease and may be restored following effective therapy, as noted, additional approaches must be taken to charac-

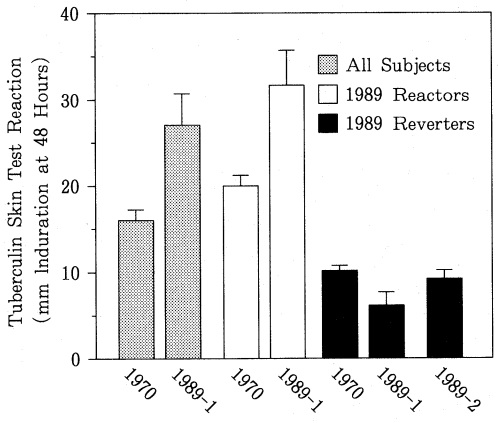

Fig. 1 Tuberculin skin test reactivity in 1970 and 1989. For 1989 reverters, values of the first and second test of two step testing are shown. (Reprinted with permission from Reference 1)

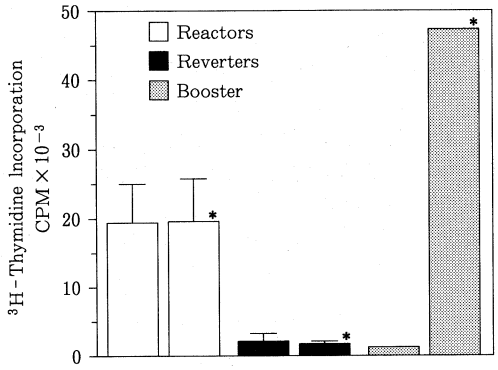

Fig. 2 Blastogenic response to PPD before and one week after tuberculin skin testing. Asterisk designates stimulation after skin testing. (Reprinted with permission from Reference 1)

terize protection in humans.

Systemic Responses

About 20% of adults with PTB have transiently negative TST. Studies of peripheral blood mononuclear cells (PBMC) show depression of PPD-stimulated lymphocyte transformation[2] and of TH1-type cytokines (IL-2, IFNgamma)[3,4]. Initial studies indicated that this is not simply due to deletion or dysfunction of antigen-specific T-cells but that there is selective suppression of the response to PPD by blood monocytes[1]. The selectivity of suppression is important because it argues against total non-specificity of the phenomenon. But it also raises two questions: What is the basis for the antigen specificity of suppression? And what are the mediators of suppression? The antigen specificity of suppression reflects the fact that both crude and purified culture filtrate proteins of MTB as well as lipoglycans have the capacity to directly stimulate monocytes to produce cytokines some of which are immunosuppressive. TGFbeta and IL-10 are the main mediators of suppression[4]. So, the basis of antigen-specific suppression in TB is that monocytes primed *in situ* are restimulated *in vitro* with MTB constituents to overproduce immunosuppressive cytokines. I now will present some of the observations that support these conclusions.

Monocytes (MN) from patients with PTB are activated by numerous criteria—e.g. expression of Fc Receptor Type I and Type III[5]. More fundamentally, NFkB is activated and translocated to the nucleus—this in turn is due to degradation of its cytoplasmic inhibitor IkB[6]. Activation is due to exposure *in situ* to circulating cytokines, immune complexes, inflammatory mediators and bacterial products. Monocyte activation has clear immunologic consequences. For example, there is transcriptional activation of TGFbeta, which is spontaneous released by PBMC in a biologically active form[7]. The basis for the antigen specificity of suppression is the ability of MTB constituents to directly stimulate MN primed *in situ* to overproduce cytokines. For example, MTB cell wall lipoglycans and culture filtrate proteins directly stimulate monocytes to produce cytokines including TNFalpha[8] and TGFbeta[9]. The basis for stimulation of MN by one TB protein, 85B has been determined. 85B is a major secretary product of MTB and a fibronectin-binding protein with mycolyl transferase activity; stimulation of TNFalpha production by 85B follows its binding to plasma fibronectin on the MN surface[10].

Longitudinal studies performed in Kampala, Uganda have provided a number of insights into the mediators and relevance of suppression by MN in PTB. IFNgamma production by MTB-stimulated PBMC is depressed in PTB in association with overproduction of TGFbeta, TNFalpha, and IL-10[11]. This cytokine profile is similar but more pronounced in far-advanced than moderately advanced pulmonary TB. Neutralization of TGFbeta or IL-10 partly reconstitutes IFNgamma responses. Serial studies during treatment show normalization of TGFbeta, TNFalpha, and IL-10 production (and a diminution of reconstituting effects of neutralizing antibody) within 3 months (Fig. 3). However, depression of IFNgamma responses is more sustained. Even at 18 months, one full year after completion of treatment, production of IFNgamma is only 80% of that of controls. These data indicate that suppression by MN is transient and superimposed on a more sustained primary T-cell abnormality.

Recent studies have begun to address the basis for the primary T-cell abnormality. The ELIspot method allows calculation of the frequency of MTB-responsive IFNgamma-producing cells in PBMC. This frequency is decreased in TB patients relative to controls in association with increased spontaneous and MTB-stimulated apoptosis[12]. Apoptosis by cells nonspecifically activated in the cytokine and inflammatory milieu results in selective deletion of IFNgamma producing cells. Both CD4 and nonCD4 T-cells from patients with pulmonary TB showed increases in spontaneous and MTB-stimulated apoptosis (Fig. 4). Compared with the baseline evaluation both spontaneous and MTB-stimulated apoptosis in CD4 and nonCD4 lymphocytes decreased by approximately 50% after 6 months of therapy. sFas and TNFalpha also were increased in supernates of MTB stimulated PBMC at initial evaluation and had normalized by 6 months. These pro-apoptotic molecules may be responsible for the high levels of apoptosis in TB. It is of note that IL-2 and IFNgamma production were depressed initially; the former normalized by 6 months whereas the latter did not.

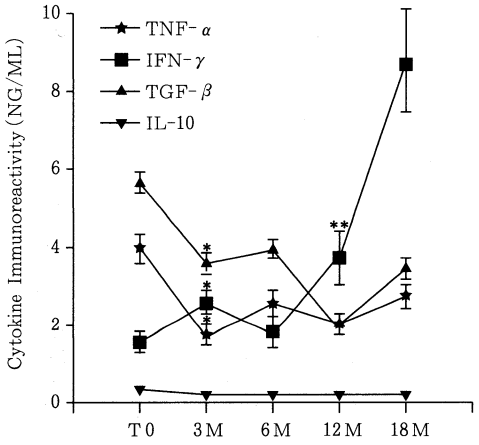

Fig. 3 Cytokine profiles at diagnosis of tuberculosis and during and after completion of antituberculous chemotherapy. Peripheral blood mononuclear cells (PBMC) from human immunodeficiency virus (HIV)-uninfected patients with tuberculosis (n=24) were obtained at time of diagnosis (T0) and at 3-18 months (M) thereafter. Purified protein derivative-induced cytokine production (by ELISA) was assessed in PBMC (1×10^6/mL) cultures. Results represent mean ± SE of cytokine immunoreactivities. *$P \leqslant .04$, when compared with baseline immunoreactivity. **$P \leqslant .001$, when compared with interferon (IFN)-γ levels at baseline evaluation. IL-10, interleukin-10; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α. (Reprinted with permission from Reference 11)

Local Responses

The immune response clearly is compartmentalized. Bronchoalveolar lavage (BAL) from involved segments of lung shows a lymphocytic alveolitis with surface expression of activation markers. There is an increased frequency of MTB-reactive, IFNgamma producing cells in BAL[13]. Alveolar lymphocytes also show increased responses to MTB antigens[14]. As many as 30% of the macrophages in BAL appear to be immature macrophages or MN by cytochemical criteria (peroxidase positive). Therefore,

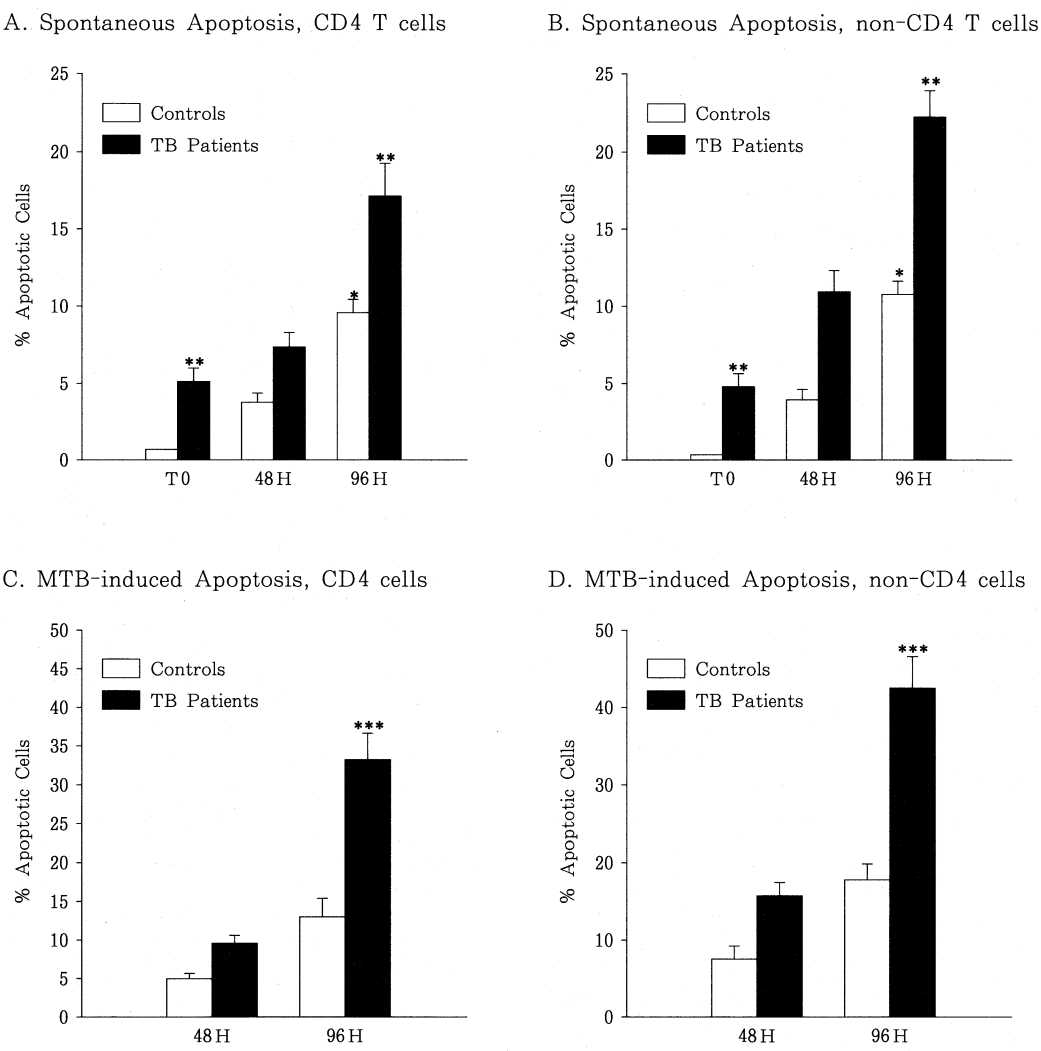

Fig. 4 Spontaneous and *Mycobacterium tuberculosis* (*MTB*)-induced apoptosis in CD4 and non-CD4 T cells of TB patients (n=20) and healthy controls (n=15). Peripheral blood mononuclear cells (PBMC) from TB patients and controls were processed immediately (T0) or incubated ⩽ 96 h in presence or absence of MTB. Proportion of apoptotic cells was assessed by 3-color flow cytometry (TUNEL method). Spontaneous apoptosis in CD4 (*A*) and non-CD4 (*B*) cells both at T0 and after *in vitro* culture for ⩽ 96 h was increased in T cells from TB patients compared with controls. MTB-stimulated T cells from TB patients contained 2-fold (CD4 cells, *C*) and 3-fold (non-CD4 cells, *D*) more apoptotic cells than cells of controls. Bars show mean ± SE apoptotic cell %, $^{*}P \leqslant .0001$ (n=15) for spontaneous apoptosis among T cell subsets (CD4 and non-CD4) of controls at baseline (T0) and after 96 h of culture in absence of MTB. $^{**}P \leqslant .0001$, spontaneous apoptosis in freshly isolated (T0) and cultured (96 h, no MTB) T cells (CD4 and non-CD4 subsets) of TB patients and controls. $^{***}P \leqslant .002$, MTB-induced apoptosis (after 96 h of culture) in CD4 and non-CD4 T cells from TB patients and controls. (Reprinted with permission from Reference 12)

increased spontaneous and antigen-stimulated expression of TGFbeta are to be expected and may block IFNgamma production and responses. In fact, the increased number of IFNgamma producing cells in segments of lung containing viable MTB is suggestive of an acquired or genetic block in the response to IFNgamma.

TB pleurisy is a model of an intense local immune response. Levels of IFNgamma and the pro-apoptotic molecules TNFalpha, FasL and Fas are increased in pleural fluid relative to plasma[15]. Spontaneous apoptosis of T-cells is increased in pleural TB and correlates with loss of MTB-reactive IFNgamma-producing T-cells. In HIV negative pleural TB, this vigorous immune response usually is associated with negative cultures. In HIV positive pleural TB, cultures most often are positive. Therefore, the finding that levels of IFNgamma (as well as the pro-apoptotic molecules, and apoptosis) are increased further in HIV positive pleural TB is further suggestive of a block in the response to IFNgamma.

Implications for immunotherapy

The goals of immunotherapy in TB are to kill persisting organisms so as to shorten the course of chemotherapy in drug sensitive TB and as an adjunct in the treatment of multidrug resistant (MDR)-TB. The primary sustained depression of IFNgamma in PTB warrants the use of IFNgamma or IFNgamma inducers as immunotherapy in TB. The early component of suppression is due to over-expression of TGFbeta suggesting that its naturally occurring inhibitors (latency associated peptide, L-Decorin) may be useful immunomodulators in TB. Several trials of immunotherapy have been completed or are in progress —*Mycobacterium vaccae*, IL-2, aerosolized IFNgamma. In a study conducted in Kampala, Uganda the administration of *M. vaccae* as a therapeutic vaccine increased the proportion of patients with PTB who sterilized their sputum at one month and also was associated with greater radiologic improvement[16]. However, there were no concurrent changes in the systemic immune response suggesting that the effects may be local and strictly compartmentalized to the sites of disease.

TB/HIV

Overlapping and distinctive immunologic alterations in TB and HIV may explain the mutually unfavorable interactions of these diseases[17]. The primary T-cell abnormality manifest by decreased production of IFNgamma is more pronounced in patients with TB and HIV infection as compared to TB alone. CD4 and CD8 cells from both HIV and TB patients show increased expression of activation markers (HLA-DR, CD38); their CD8 cells show more CD95 (pro-apoptotic) and less CD28 (co-stimulatory molecule)[18]. PTB (with or without HIV infection) is characterized by monocytosis, granulocytosis, and increased TGFbeta production and PPD-induced apoptosis. On the other hand, HIV-infected subjects (with or without PTB) demonstrate CD4 T cell depletion, increased spontaneous CD4 apoptosis *in vitro* and defects in the IFNgamma responses of whole blood to antigens. The occurrence of both diseases often is "co-pathogenic" with additivity or synergism of effect.

Dual infection is associated with increased viral load, more rapid decline in CD4 cells and shortened survival warranting trials of cytokine inhibitors and anti-virals. These observations suggest co-pathogenesis of TB and HIV that is particularly relevant in countries that cannot afford anti-virals. Trials are in progress in TB/HIV with agents that depress cytokine production (thalidomide, pentoxifylline, or prednisolone) and may decrease viral load. The soundest approach would be to attempt to avert the morbidity and decreased survival of TB/HIV with preventive therapy of latent TB infection.

Conclusions

1. Immune activation in TB is associated with acquired immunosuppression of IFNgamma due to over-production of TGFbeta and IL-10 by activated monocytes and deletion of IFNgamma producing cells by apoptosis

and trafficking to the lung.

2. The local immune response is characterized by alveolitis, and increased frequency of IFNgamma producing cells (BAL); or increased expression of cytokines and apoptosis (pleural fluid). These observations suggest a block in the response to IFNgamma.
3. Whole blood production of IFNgamma is the best available correlate of protection for monitoring vaccine trials.
4. In children < 5 years of age and adults with minimal TB, concomitant disease occurs despite preserved IFNgamma production. It should be noted, however, that self cure and occur in such cases, and also in pleural TB in the HIV negative so that protective immunity may prevail in them.
5. These observations support the use of immunotherapy in TB (to augment IFNgamma production) and TB/HIV (to block cytokine production) and provide an approach to the initial evaluation of preventive vaccines in clinical trials.

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〈要旨〉ヒトの結核菌感染に対する免疫応答と結核の発病

ヒトにおいて，結核菌感染から直ぐの発病は5％である。免疫系が未成熟の乳幼児の結核発病によく見られる。また，HIV感染に伴って，内因性再燃による結核の発病が起こりやすい。これらの事実からも，ヒトにおける結核感染から発病に，防御免疫が存在することは確かである。ではその結核における防御免疫とは何か。

ツベルクリン反応（ツ反）陽性および陰性の健康接触者，長期にわたる健康既感染者，治療中の結核患者，これらの個体のツ反の推移や末梢血を用いて結核菌抗原特異的刺激によるリンパ球幼若化反応や，サイトカイン産生等の免疫学的解析を行った。その結果，ツ反の成績よりも，IFN−γ活性が宿主の抗結核防御免疫能とよく相関した。結核発病者ではIFN−γ活性は低く，逆にTGF−β，IL−10の抑制性サイトカイン活性が強く見られた。化学療法の結果，IFN−γ活性は回復した。結核患者に見られたIFN−γ活性の低下は，(1) 抑制性サイトカインによる産生のブロック，(2) アポトーシスの亢進による産生T細胞（$CD4^+$，$CD4^-$）の減少に起因することが示唆された。

これらの結果から，末梢血細胞からのIFN−γ産生能が当面，ワクチンの効果判定等での最も良い防御免疫のマーカーとなり得ると考える。一方，われわれの成績は，*M. vaccae* やIL−2を投与することによりIFN−γ産生を誘導したり，IFN−γを直接吸入することが，結核の免疫療法として効果が期待され得ることを支持している。