TUBERCULOSIS AND THE IMMUNE RESPONSE IN CHILDREN

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Tuberculosis is one of the major killers in the world today and has been described as a "global emergency" by WHO. In India there are approximately 1 million new cases every year and 500,000 deaths. Tuberculosis also affects children who get the infection from adults. It is important to study the natural history, clinical manifestations and immunological profile of children in order to diagnose them earlier and treat them effectively.

The clinical expression of infection with Mycobacterium tuberculosis (M.tb) is quite varied and depends on a number of factors (Table 1). Among healthy persons, infection with M.tb is generally asymptomatic as infection is contained by the host immune system. Only a positive tuberculin skin test indicates the presence of the organism in persons with a latent infection. The lifetime risk of developing clinical disease is approximately 10% (1). However, in subjects who are immunosuppressed for any reason e.g. infancy or HIV infection, the proportion who develop disease is much greater (2). Age plays a major role and infants who are infected by a close contact are estimated to have a 40% chance of developing disease as opposed to adolescents whose risk is around 15%.

Table 1
FACTORS INFLUENCING THE CLINICAL FEATURES OF TUBERCULOSIS

Host factors	Microbial factors	Host-microbe interaction
Age	Virulent of org?	Sites of involvement
Immune status	Predilection for specific	
Specific immunodeficiency states	tissues	Severity of disease
Malnutrition	Size of infect particle	
Genetic factors ?		
Coexisting diseases	No. of tubercle bacilli inhaled	
BCG immunisation		

When Robert Koch described the etiologic agent of TB in 1882, he had noted the intracellullar location of M. tb within giant cells in granulomatous lesions. Metchnikoff was the first to fully realise the importance of macrophages in antibacterial immunity particularly against TB. Mackaness showed much later that activation of antimycobacterial macrophage functions is controlled by lymphocytes (3). That this activation is mediated by soluble substances, now called cytokines was noted by B. R. Bloom (4) and J.R.David (5).

THE IMMUNE SYSTEM

There are two arms of the immune system : cellular and humoral. Cellular immunity is more important in dealing with intracellular organisms like mycobacteria, leishmania, listeria etc. However, the exact mechanism of protective immunity against TB is not known. The major cells that play a role are mononuclear phagocytes (macrophages) and T

lymphocytes. While macrophages are the principal effector cells that kill bacteria, T lymphocytes are the inducers of protection. Both cells also play a dual role in TB contributing not only to protection but also to survival of the pathogen (macrophages) and pathogenesis of the disease (T cells). Coordination between these two cell types is essential for protection. Inspite of this, full eradication of the pathogen may not be achieved and it remains within the grandulomas in a dormant state, which can produce reactivation disease at a later time.

Cluster of differentiation (CD) antigens

Numerous glycoproteins, known as CD antigens are present on the surface of lymphocytes and on other cells of the immune system and can be used as phenotypic surface markers to further subcategorize immunocompetent cells. All native T cells express the CD2 and CD3 determinants in addition to other specific CD antigens.

Though the functional property of a given T cell can generally be inferred from its phenotypic surface markers, dissociations between classic cell markers and functions are known to occur. For instance, though most cytotoxic T cells are CD8+, clones of CD4+ cytotoxic T cells are known to occur. Thus, cells with the same phenotype can perform overlapping or antagonistic functions and cells with different phenotypes can have the same functional properties.

T cells recognize antigens through an antigen receptor composed of alpha and beta or gamma and delta polypeptide chains known as the T cell receptor (TCR)-CD3 complex. Changes in the amino acid sequences of the variable regions of these receptors alter their shape and confer antigen specificity. Once antigen is bound to the receptor, signal transduction into the cytoplasm triggers functions such as cytokine production and cytolytic activity. Most alpha beta T cells bear the CD4 or CD8 determinants. CD4+ T cells recognise antigens associated with self MHC class II gycoproteins whereas CD8+ alpha beta T cells recognise antigen in the context of MHC class I products.

MICROBICIDAL MECHANISMS IN TB

The various cells and their products that are involved in the killing of M.tb are known but the interplay and interaction between them is complex. The chief players are the macrophages and T lymphocytes.

a) ACTIVATED MACROPHAGES

Macrophages must be activated before they can destroy tubercle bacilli. Activated macrophages contain many mitochondria and lysosymes and have high levels of oxidative and digestive enzymes. In the mouse model, murine macrophages can be activated to inhibit or destroy virulent M.tb in vitro. Stimulation with alpha-interferon and either LPS or TNF causes release of nitric oxide from macrophages which is required for antimycobacterial activity (6). Nitric oxide (NO) which is produced from arginine is now thought to be more important than free oxygen radicals in killing of M.tb.

However majority of the work done with human macrophages has not been able to demonstrate killing of M.tb. even after activation with various substances. At best a slowing of the intracellular replication could be achieved following addition of IFN-gamma and calcitriol. It has also not been able to demonstrate NO production in vitro by human macrophages (7) probably because they are unable to generate tetrahydrobiopterin, an essential cofactor for arginine dependent NO synthesis.

When tubercle bacilli are inhaled into the lung, they are engulfed by alveolar macrophages which attempt to kill the bacteria within the phagolysosome. Macrophages also produce certain specific cytokines or medaitors including IL-1, IL-6, IL-10, TNF alpha and TGF beta (8). These cytokines have immunoregulatory effects and also mediate some of the clinical manifestations of tuberculosis, e.g. IL-1 contributes to fever, IL-6 may mediate the hyperglobulinaemia and TNF is essential for granuloma formation. TNF also causes fever, weight loss and tissue necrosis. IL-10 inhibits cytokine production by lymphocytes and TGF-beta suppresses T cell proliferation. There two cytokines may prevent excessive inflammation and tissue damage.

b) ROLE OF T CELLS

i) CD4+ T cells : CD4+ T cells play a dominant but not exclusive role in immune defense against TB. Transfer of CD4+ T cells from sensitised animals confers protection against tuberculosis. In humans, CD4+ T cells are selectively expanded at the site of disease in patients with a resistant immune response e.g. tuberculous pleuritis (10). In patients with HIV infection, CD4+ depletion increases the risk of disease and as the CD4+ counts fall clinical indicators of severe disease increase.

CD4+ cells can be divided into "naiva" and "memory" cells based on their expression of CD45. Memory cells (with prior) exposure to antigen) express the CD45 RO isoform and are concentrated in the pleural fluid of patients with TB pleurisy whereas naive T cells are not (10). Memory T cells also proliferate in response to M.tb antigens and produce IFN-gamma, a macrophage activating cytokine suggesting that they are important in local immune defenses against tuberculosis.

Subtypes of CD4+ T cells: Th1 and Th2 cells

A few years ago, two distinct sub-populations of CD4+ T cells were described in the mouse that produce different patterns of cytokines (Table 2). Th1 cells produce gamma interferon, IL-2 and lymphotoxin and augment DTH responses and microbicidal capacity of macrophages. Th2 cells produce IL-4, IL-5, IL-6 and IL-10, support B cell growth and aument humoral immune responses (11). Th1 cells are considered important in mediating immunity against intracellular pathogens whereas Th2 cells play a role in atopic and parasitic diseases. The two subtypes exert cross regulatory influences, that favour predominance of one subtype, in response to a specific pathogen, IL-12 produced by macrophages and IFN-gamma produced by natural killer (NK) cells favour development of a Th1 response whereas IL-10 produced by macrophages and IL-4 produced by CD4+ NK1.1+ cells bias towards a Th2 response (12). In murine leishmaniasis, Th1 cells mediate immunologic resistance to infection whereas Th2 exacerbate disease (13).

Table 2
CYTOKINES PRODUCED BY MACROPHAGES AND BY TH1 AND TH2 CELLS

Cytokine	Production by		
	Macrophage	Th1	Th2
IL-2		+	
IFN-gamma		+	
Lymphotoxin		+	
IL-4			+
IL-5			+
IL-6	+		+
IL-10	+	+	+
IL-3		+	+
GM-CSF	+	+	+
TNF	+	+	+
IL-1	+		
IL-8	+		
TGF-beta	+		
Elicits DTH		+	
IgE production and eosinophilia			+

Human T cells also exhibit patterns of cytokine production similar to those of murine Th1 and Th2 cells. IL-10 is produced by human Th1 and Th2 cells both as well as mononuclear phagocytes and inhibits proliferation and cytokine production by both subpopulation. Pattern of cytokine production in humans correlate with clinical manifestations of infectious disease eg CD4+ T cells from patients with helminthic infections perferentially produce the Th2 cytokines IL-4 and IL-5 which stimulate IgE production and eosinophil growth respectively. In patients with leprosy the Th1 cytokines IL-2 and IFN gamma predominate in skin lesions of tuberculoid leprosy patients (resistant immune response) whereas the Th2 cytokines IL-4 and IL-10 are prominent in lepromatous leprosy patients with ineffective immunity (14).

In patients with tuberculosis also, there is a spectrum of immune response which correlates broadly with clinical manifestations. Healthy tuberculin reactors have protective immunity against disease and peripheral blood monocytes from such individuals produce high concentrations of IFN-gamma when stimulated with M.tb. Monocytes from patients with pulmonary TB however produce significantly lower amount of IFN-gamma (15). Finally, in patients with HIV and tuberculosis who often have extrapulmonary dissemination, IFN-gamma production is markedly depressed. Though Th1 responses are depressed in patients with TB, there is no evidence that Th2 responses are enhanced either systemically or at the site of disease (16). Recent work has shown that IL-10 is responsible for the depressed IFN-gamma response and acts by inhibiting IL-12 production (15). Neutralization of IL-10 or addition of IL-12 enhances IFN-gamma production by PBMC from TB patients. However IL-10 and IL-12 production by antigen stimulated PBMC from TB patients is normal, suggesting that rather than cytokine concentrations, there may be changes in receptor expression or signalling pathways.

Cytolytic CD4+ T cells

An alternative mechanism by which T cells contribute to immune defence is through direct cytolysis of macrophages and non-phagocytic cells infected with M.tuberculosis. M.tb specific cytolytic activity of CD4+ cells at the site of disease is greatly enhanced compared to that of peripheral blood cells. Cytolytic T cells that recognise mycobacterial antigens can lyse infected macrophages releasing bacilli which can be engulfed and killed by macrophages with greater antimycobacterial activity. They are also thought to contribute to immunopathology by destroying infected macrophages which in turn release toxic products that result in caseous necrosis (17).

ii) CD8+ T lymphocytes :

CD8+ T cells contribute the major cytolytic T cell population in defenses against many intracellular pathogens. CD4+ and CD8+ T cells play complementary roles in the immune response to M.tb. CD4+ T cells may produce cytokines that activate macrophages to kill most mycobacteria and produce sufficient IL-2 to activate CD8+ T cells, which in turn lyse additional mycobacterium infected cells.

However, the role of CD8+ cells in human antimycobacterial defenses remains uncertain. CD8+ T cells are not selectively concentrated at the site of disease and the severity of TB in HIV infected patients is unaffected by the CD8 cell count. More comprehensive studies are needed to assess the role of CD8+ T cells in human anti TB defenses.

iii) Gamma Dalta T cells :

T cells bearing the gamma delta receptor account for a minority (<5%) of cells in peripheral blood, the majority being alpha beta positive. Gamma delta T cells proliferate in response to mycobacterial antigens, are cytotoxic and secrete a pattern of cytokines similar to Th1 cells (18). The percentage of gamma delta T cells is greatly increased in draining lymphnodes and lungs of mice after primary infection with M.tb. Expansion of M.tb-reactive gamma delta T cells is greater in healthy tuberculin reactors and in patients with tuberculous pleuritis than in those with advanced plumonary and miliary TB suggesting that they contribute to immune resistance. although the exact role played by these cells is not known, it is thought that they contribute to control of primary infection

and may be important in the early phase of the immune response before the alpha beta T cell response has become established.

The study of tuberculous infection in children provides a unique opportunity to evaluate the primary human immune response to infection. Children infected with tubercle bacilli can remain healthy tuberculin reactors, develop uncomplicated primary tuberculosis or suffer severe complications such as miliary or meningeal tuberculosis. We have evaluated the lymphocyte subsets in blood and BAL fluid in children with primary tuberculosis (Table 3 and 4). In the blood, we found the total lymphocyte count as well as the CD3 and CD4 count and percentage to be decreased in children with TB compared to healthy tuberculin positive controls. This could indicate that either the T cells are sequestrated at the site of disease (in the lung) or that there is a suppression of cell mediated immune response during active TB.

In the bronchoalveolar lavage fluid (which represents the alveolar environment), the percentage of lymphocytes and eosinophils was significantly increased and macrophages proportionately reduced in children with TB. This adds weight to the hypothesis that activated lymphocytes are sequestered at the site of disease. Further studies are required to determine which subtype these cells belong to and what cytokines they produce.

Table 3
LYMPHOCYTE SUBSETS IN BLOOD

	CD3*	CD4*	CD8*	Gamma Delta
TB (n=22)	63±13%	33±10%	27±8%	7±4%
PPD+ (n=17)	70±7%	38±9%	21±5%	8±6%

*p<0.05

Table 4
CELLULAR PROFILE OF BAL FLUID IN PULMONARY TUBERCULOSIS

	Total Count (10^6/100ml	Macrophages %	Lymph %	Eosin %
TB (n=18)	75±45	56±25*	22±17*	10±17.5*
CONTROLS (Ronchetti et al)	80±84	84±8	10±6	0

*p<0.02

c) HUMORAL IMMUNE RESPONSE

The humoral immune response in tuberculosis does not play a role in controlling the infection. However, immunodiagnosis using different antigens from M. Tuberculosis has been attempted for the last 100 years. There are a number of crude, semipurified and, highly purified antigens that have been proposed as useful antigens for diagnosis. The only M.tb specific antigens shown to date are the 38kDa of Ivanyi (19) and 14kDa (20). Only a small number of monoclonal antibodies are M. tuberculous specific (TB 23, 68, 71, 78) (28). The value of a serodiagnostic test depends on its sensitivity (number of positive tests out of total number of true cases) and specificity (number of negative tests out of the total number of control samples). Whether antibodies develop to a particular antigen will depend on its immunogenecity, dose and duration of exposure and possibly the genetic make up of the host. Also, with "natural exposure" to environmental mycobacteria antibodies develop to common mycobacterial antigens which could cross react with the test antigen (21).

Reported studies on serodiagnostic tests for TB in children have given conflicting results (Table 5) Turneer et al did not find the anti A60 IgM and IgG measurements useful in the diagnosis of primary TB or mycobacterial adenitis in children (22). Delacourt et al found that using the anti-A60-IgG test and a specificity of 98% a positive serodiagnosis was observed in 68% of children with clinically active TB (23). The IgM test however had a low sensitivity of 19%. They also found that the IgG antibody values were influenced by BCG vaccination and age, increasing with both.

Table 5
SERODIOAGNOSTIC STUDIES FOR TB IN CHILDREN

Reference	Test	Population	Sensitivity	Specificity
Delacourt (1993)	Antigen 60 (IgG)	Pulm. TB	0.67	0.98
Rosen (1990)	Mycobact. Sonicates	Pulm. TB	0.21	0.40
Barrera (1989)	PPD	Bacteriologically confirmed cases	0.51	0.98
Alde (1989)	Angigen 5		0.85	1.00
Turneer (1994)	Antigen 60 (IgG)	Pulm. TB.	0.14	0.95
Seth (1997)	PPD80	Pulm. Primary complex	0.86	0.92
Swaminathan (1997)	38kDa (IgG)	Pulm. TB	0.47	0.73
	Antigen 60		0.28	0.86

Rosen used mycobacterial sonicates but sensitivity and specificity were poor, essentially due to cross reactivity with BCG (24). Barrea et al had better results using purified protein derivative and showing a specificity of 98% and a sensitivity of 51% in bacteriologically confirmed cases (25). The best results were obtained by Alde et al using antigen 5 which is the same as the 38kDa antigne (26). They found a specificity of 100% and sensitivity of 85.7% in bacteriologically confirmed tuberculous children but the number of control subjects was low in that study. We studied the role of Anda (A60) antigen and the Pathozyme TB Complex (38kDa) antigen in the diagnosis of children with TB and got disappointing results. Even in children with culture proved tuberculosis, the A60 IgG test was positive only in 30% and the 38 kDa antibody test was positive in 50% of cases (27).

In children antibody. levels to M.tb antigens are generally lower than in adults. Such a hyporesponsiveness could be due to the nature of exposure, extent of disease, immuno competence and genetic background of the children[1]6. The presence of cross reacting antibodies will also depend on the enviornment the child lives in. Hence studies done in commercially available serodiagnostic kits can be answered only by testing children in whom the diagnosis has been confirmed. In our study the kits tested did not diagnose all those children in whom TB was bacteriologically confirmed. At the same time the kits gave positive results in a significant number of healthy (control) children. Hence they cannot be recommended for routine diagnostic use.

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