Tuberculosis Immunology*

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Despite the availability of excellent treatment regimens, tuberculosis continues to be one of the most important global diseases. Table 1 depicts estimates of the respiratory tuberculosis rates of some Asian Pacific countries and other selected countries of the world[1,2] which contribute to the over 10 million new cases of tuberculosis occurring annually. Twenty years after the formulation of the World Health Organisation (WHO) comprehensive policy for tuberculosis control programs, in many developing countries no improvement has been noted in the epidemiologic situation despite a continuing steep decline in developed countries.[3] This may be attributed in large measure to inadequte treatment programs and the high cost of drugs, such as rifampicin.[4] In the projected program of work for tuberculosis control for the period 1984-1989, the Tuberculosis and Respiratory Infections Branch of the World Health Organisation (WHO) proposed a multifaceted approach to the problem.[3] Included in the program was the recognition of a need for more immunological research into the pathogenesis of tuberculosis and the development of tests for a more rapid diagnosis, immunotherapeutic agents, and more effective vaccines.

Table 1 Incidence of respiratory tuberculosis in selected countries[1,2]

Country (ies)	Rate per 100,000*
Botswana	475
Macau	382
Republic of Korea	378
Vietnam	327
Bolivia	189
Hong Kong	153
Brunei	115
Singapore	114
Thailand, Morocco, Sri Lanka, Pakistan, Malaysia, Chile, Peru, Greece, Burma, Papua New Guinea, New Caledonia	76-100
Guam, India, Argentina, Japan, Nicaragua, Austria, Ghana, Lao Peoples Democratic Republic	51-75
Indonesia, Israel, Nepal, Brazil, Kenya, New Zealand, Fiji, Pacific Islands, Samoa	11-50
Australia, Denmark, Egypt, USA	10 or less
World (for all forms of reported tuberculosis)	68.4

*Rates for 1976-7 or latest available figures.

This review will provide an update on selected areas of tuberculosis immunology relevant to our understanding of the pathogenesis of the diseases, the immune responses of the host during the different phases of infection and disease, vaccination, and diagnosis of mycobacteria which produce disease.

Eighteen species of the genus *Mycobacterium* have been reported to be capable of inducing disease in human beings (Table 2). However, for most of these species, disease induction is a rare event even though persons become infected. For example, persons who are skin test sensitive to tuberculin because of infection with the *M. avium-intracellulare-scrofulaceum* complex of organisms have a much lower risk of developing disease than those whose sensitivity is caused by infection with *M. tuberculosis*. Such infections complicate the diagnostic

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Table 2 Disease caused by mycobacterial species

	Runyon group	Primary disease induced
I.	Photochromogens: produce pigment when exposed to light	
	M. kansasii	Tuberculosis-like
	M. asiaticum	Tuberculosis-like
	M. simiae	Tuberculosis-like
	M. marinum	Swimming pool granuloma of skin
II.	Scotochromogens: produce pigment in light or darkness	
	M. scrofulaceum	Lymph node, esp. in children rarely pulmonary involvement
	M. xenopi	Pulmonary
	M. szulgai	Pulmonary, lymph nodes
III.	Nonphotochromogens: produce little or no pigment in light or darkness	
	M. hemophilum	Extrapulmonary
	M. avium, M. intracellulare	Pulmonary, lymph nodes in children
	M. malmoense	Pulmonary, lymph nodes in children
IV.	Rapid growers	
	M. fortuitum	Skin abscesses or in areas
	M. chelonei	secondary to surgical or other trauma
Other.	*M. tuberculosis-africanum-bovis* complex	Pulmonary, may become systemic; extrapulmonary
	M. leprae	Predilection for skin, nerves, extremities may become generalised
	M. ulcerans	Skin and underlying tissue

value of a tuberculin skin test and provide a "natural" immunity against tuberculosis and leprosy.[5-8]

Initial exposure to *Mycobacterium tuberculosis* is usually via the respiratory route. Inhaled organisms which are not trapped in the nares may proceed into bronchioles or alveoli beyond the mucociliary layer. Organisms become phagocytosed and may remain viable and reproduce. Haematogenous and lymphatic spread may occur to other organs and other regions of the lung. In most persons affected sites heal as immunity develops, however, in a small percentage disease may follow. In some persons tubercle bacilli may persist without any symptoms for many years and present themselves in the form of disease under conditions of suppressed immunity. Whether or not disease develops depends largely on virulence factors possessed by the organism, the immune status of the host and how he responds to components of the organism, and on the public health and economic conditions of the region.

Mycobacterium tuberculosis cell wall

Tubercle bacilli possess a thick lipoidal cell wall which renders the organism impervious to many drugs, antibody, potentially harmful products of lymphocytes and macrophages, and to the intracellular barrage of lysosomal enzymes found within macrophages. Additionally, the cell wall components activate immune and nonimmune systems which ultimately mobilise anti-tubercular defense mechanisms.

When mycobacteria become internalised by phagocytic cells they can be visualised within a vesicle called a phagosome. Normally lysosomes within the phagocyte attach to and fuse with the phagosomal membrane permitting the lysosomal enzymes to digest the microorganisms in an orderly fashion without damage to the host cell. Virulent tubercle bacilli may release sulfatides from the cell wall which prevent such fusion from occurring and thus the mycobacteria may persist or multiply.

Cell wall substances including Wax D, N-glycolyl muramyl dipeptide and trehalose dimycolate (cord factor) have adjuvant, granulomagenic, and macrophage activating properties. These activities contribute considerably to the tissue damage seen in tuberculosis and dissemination of the organism to other parts of the body. For a detailed review of the immunoreactive substances of *M. tuberculosis* see the article by Goren.[9]

The host must restrict and eliminate the invading tubercle bacilli. The consequences of an accumulating large bacterial mass may cause a) the release of toxic components into the tissues; b) the induction of a high degree of cellular hypersensitivity and an exaggerated response to the bacterial antigens with tissue damage, caseation and further dissemination of the organisms; c) eventually a large suppressor population of cells may emerge resulting in anergy and a poor prognosis. Successful resistance is not easily predicted even in the presence of a strong immune response. It will depend in large measure on the ability of the microorganisms to evade the host defenses and on the net balance of helper and suppressor cells and substances. These apects will be discussed below.

The immune response in tuberculosis

In progressive tuberculosis an immunological spectrum is traversed which ranges from a state of high resistance to one of very low resistance.[10] The spectrum is divided into 3 regions: RR, which represents a reactive region with well developed cellular hypersensitivity, with low antibody levels and only few bacilli in the tissues; UU, a polar region in which T-helper lymphocyte (T_H) activities have been nullified, in which for a number of subjects there is a tendency for an increase in antibody levels, and in which large numbers of bacilli are found in the tissues; a third intermediate region is subdivided into RI which leans towards the RR end of the scale and UI which leans toward the UU end.

Figure 1 outlines the pathways and interactions that may follow exposure to tubercle bacilli. The macrophage first encounters the bacilli and processes and presents antigen to lymphocytes. Presentation by the macrophage is restricted by the chromosomal HLA-D region. The lymphocyte requires not only recognition of the antigen determinant but also the self-coded product of the macrophage.[11] Normally, a tuberculosis infection stimulates T-lymphocytes in the direction of help which favours activation of macrophages so that they more effectively destroy tubercle bacilli. Activated macrophages also produce interleukin 1 (IL1) which in turn stimulates T-lymphocytes.[12] T-lymphocytes produce interleukin 2 (IL2) which further activates T-lymphocytes to proliferate, mature, and to better respond to antigens.[13]

Suppressor T-lymphocytes (Ts) play a role in a complex series of immune regulatory circuits that control the immune balance. If suppressor cells are excessively stimulated, as happens in progressive tuberculosis, the delicate immune balance may be tipped toward the UU end of the scale which may result in anergy and a poor prognosis.

Suppressor T-lymphocytes with surface receptors for the Fc portion of IgG (Tγ+) appear to be the predominant suppressor cells in tuberculin reactive persons. In anergic patients an adherent monocyte appears to be the dominant suppressor cell.[14] Suppressor cells function by releasing soluble suppressor substances that modulate the production of B-cells, T-cells and the action of their mediators. Prostaglandin E_1 production during mycobacterial infection can induce a suppression of cellular hypersensitivity.[15] Additionally, monocytes from patients with tuberculosis display a defective chemotaxis[16] which further compounds problems in immunity.

Alleviation from the heavy bacterial load with successful chemotherapy results in a shift of the immune status of the patient towards the reactive RR end and a restoration of tuberculin skin reactivity. In drug resistant tuberculosis we are faced with a more complex problem and novel approaches need to be assessed. Can the human immune response be modified by exogenous application of lymphokines or monokines to push the patient's own immunity in a more desirable direction to cope with the tubercle bacillus? Some encouragement for this approach has been reported[17] by the successful use of transfer factor in a patient with progressive primary tuberculosis caused by drug resistant organisms. Human T-cell hybridomas are able to produce interleukin 1 (IL1) and other lymphokines which provide a tremendous potential for the management of tuberculosis and other diseases in the future. Human (or even nonhuman) B-cell hybridomal antibodies directed towards specific surface antigens for the various T-lymphocyte subsets, such as T-suppressor cells, may permit the reduction or elimination of cell types unfavourable to the patient's recovery from disease.

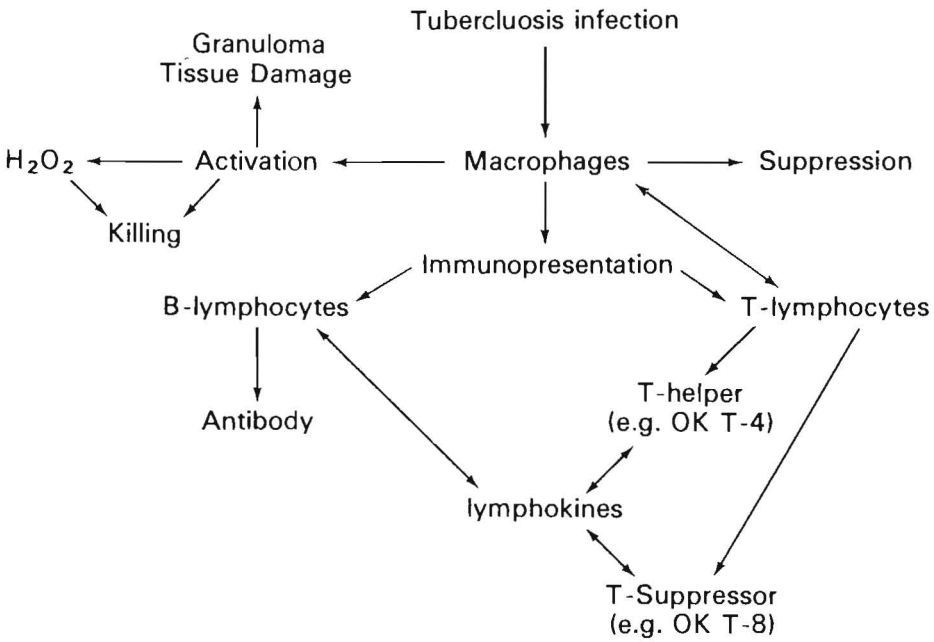

Fig. 1 Immunological pathways during infection with *Mycobacterium tuberculosis*

How is the tubercle bacillus killed?

Immune macrophages are more effective than normal macrophages in inhibiting tubercle bacilli.[18-20] The precise machanism of killing is not clearly understood. Figure 1 shows a pathway of macrophage activation which indicates two alternative means to killing, one by

an oxidative route and peroxide formation and another by some other means. Cohn[21] considered that macrophage activation occurred in a sequence of events, which were influenced by a number of humoral or cellular products. Activation of macrophages is accompanied by an increase in oxidative metabolism which results in the release of bactericidal compounds such as superoxide (O_2^-) anions, hydrogen peroxide (H_2O_2), hydroxyl radicals (OH°), and hypohalites (OCl^-). Disruption of bacterial cell membranes and cell walls occurs. Together with lysosomal enzymes or mediators these oxygen metabolites can kill some species of mycobacteria.[22,23]

Some mycobacteria are able to persist and continue to activate the phagocytic cell. Toxic products of the phagocyte may then result in an auto-intoxication of the macrophage and release of the organism which can reinfect new phagocytes.[24] Prostaglandin E_2 (PGE^2) can inhibit early steps in the production of oxygen radicals by activated macrophages.[24] PGE^2 may also suppress the release of lysosomal enzymes, gamma interferon production; phospholipase A_2 activity, and the effects of migration inhibitory factor.[25]

Many virulent strains of *M. tuberculosis* are endowed with properties which permit them to escape the deleterious influences of oxygen metabolites or other destructive substances. The thick lipoidal wall provides an effective shield. Some mycobacteria produce catalase which breaks down H_2O_2 produced by macrophages and prevents the killing of the bacilli.[26] Sulfatides and other polyanions produced by mycobacteria may prevent the fusion of lysosomes to phagosomes thus sequestering *M. tuberculosis* organisms from the harmful effects of lysosomal enzymes.[23] Mycobacteria may elicit substances which may have an immunosuppressive effect on the host such as arabinomannan,[27] dialysable components[28] and excessive amounts of soluble antigens.

Whether a tubercle bacillus is killed or not and whether a disease progresses depends on a delicate balance involving the virulence of the infecting strain and an unpredictable direction of the immune response. This may result in a predominance of either suppressor or inducer influences.

Vaccination

Vaccination with the Bacillus of Calmette and Guerin (BCG) does not prevent tuberculosis infection but apparently does prevent the spread of the infection and the development of disease within a host.[29] The self-limiting infection of BCG vaccination imparts to a person a modest cellular hypersensitivity toward antigens of *Mycobacterium tuberculosis* as well as to most other mycobacterial species. Conversely, significant infection with mycobacterial species which are capable of infecting human beings provides a cross sensitivity and, therefore, "natural vaccination," towards other species of the genus including *M. tuberculosis.* There is no evidence that BCG vaccination of humans significantly increases this type of naturally acquired immunity. The concept is, however, supported by extensive studies in experimental animals.[30]

Both BCG and "natural" vaccination result in a population of lymphocytes capable of responding anamnestically to inhaled or otherwise acquired tubercle bacilli. Future exposure to and multiplication of tubercle bacilli, induce the thrust of the immune response which in most cases will limit multiplication and spread of the organism. In the absence of a recall immunity there is a delay in an adequate immune response, a greater chance of multiplication and spread of the organisms, and a greater chance of developing disease. From studies in the Netherlands it has been estimated that unvaccinated persons upon their primary infection with the tubercle bacillus have a 5.5 per cent annual risk of developing disease in the absence of preventive treatment.[31] Exogenous reinfection of those infected who did not develop disease posed an annual risk of 1.9 per cent for males and 1.1 per cent for females. Thus primary infection provides an order of protection approximating that seen in successful BCG vaccination trials.

The effectiveness of BCG vaccine has been found to range from ineffective or poorly effective to about 80 per cent effective. The eight trials generally accepted as adequately designed and executed have been reviewed recently[5,6] and summarised in Table 3. The areas in

Table 3 BCG vaccination trials for the prevention of tuberculosis

Population	Prevalence of NTM	Vaccinated		Nonvaccinated		% Effectiveness
		No. cases	Case rate*	No. cases	Case rate	
		Highly effective				
Northern USA Indian population	Low	64	320	238	1,563	80
Chicago, Illinois	Low	17	57	65	223	74
Great Britain	Low	56	28	260	128	78
		Moderately effective				
Puerto Rico	High	186	20	141	28	29
South India	High	56	61	46	89	31
		Poorly or not effective				
Georgia, Alabama	High	26	11	32	13	14
Georgia	High	5	17	3	11	0
South India	High	74	—	28	—	0

NTM = Nontuberculous mycobacteria *Case rate per 100,000

which BCG appeared to be ineffective, poorly effective, or modestly effective were higher in the prevalence of nontuberculous mycobacterial infection and the case rate in nonvaccinated persons in these areas was also lower than in populations where BCG vaccine was found to be highly effective. These findings are consistent with the concept that endemic nontuberculous mycobacteria provide a "natural" vaccination and BCG adds little or no protection over that which they have already induced. The BCG study in Chingleput, South India is suggestive of an environment in which nontuberculous mycobacteria induce a natural immunisation because many of the tested subjects had small reactions to tuberculin. Ten Dam and Pio[32] have analysed the Chingleput study in detail and provide further explanations for the apparent ineffectiveness of BCG vaccine in this region.

Future vaccine trials with BCG should consider the potential of nontuberculous mycobacteria in conveying protection in the target population. The prevalence of nontuberculous mycobacteria may be determined by microbiological surveys of soil and water samples[33] or by skin test surveys as will be discussed below. At this point it would appear prudent where tuberculosis is still a problem to continue BCG vaccination programs especially in very young children. If BCG is given soon after birth the primary infection will have been a harmless species which primes immune cells and provides a recall mechanism for resisting exposure to virulent *M. tuberculosis.*

The tuberculin test

A positive tuberculin test is an indication that an individual possesses a most important cellular hypersensitivity which provides a mechanism for combating infection with *Mycobacterium tuberculosis* or other mycobacteria. This immune mechanism can be overcome by virulent tubercle bacilli if they have an opportunity to become established. The intracellular environment of the organism and its impermeable lipid cell wall help it to resist adverse conditions. A high degree of sensitivity (reflected by a large tuberculin reaction) reflects considerable multiplication and dissemination of the organism with stimulation of lymphoid elements throughout the body. In spite of a heightened cellular immunity virulent strains may continue to multiply and eventually outstrip the body's defence efforts at containment which leads to progressive tuberculosis with the eventual development of anergy and a poor prognosis. On the other hand, BCG vaccine, because of its avirulence, multiplies to a limited extent and induces generally a modest tuberculin sensitivity. The sensitivity is adequate in most cases to limit the infection to a few virulent tubercle bacilli that one is usually exposed to. Thus, for BCG infection a resultant small tuberculin reaction reflects a controlled infection.

In populations where the tuberculous infection rate is high or in populations where infection with endemic nontuberculous mycobacteria is of a low order, sensitivity is most likely to have been induced by *M. tuberculosis.* In regions where endemic nontuberculous mycobacteria are highly prevalent and the tuberculosis rate is low much of the sensitivity to tuberculin is not due to the tubercle bacillus and the risk of developing disease is only one-fortieth that of sensitivity due to *M. tuberculosis.*[34]

Several patterns of curves may be discerned for different population groups based on the distribution of reaction sizes to a five tuberculin unit (TU) dose (or equivalent) of tuberculin. These are shown in Figure 2. The dash-lined curve in panel A shows typical distribution of reaction in sizes for patients with tuberculosis. The solid line in panel A shows a bimodal distribution of reaction with most persons having

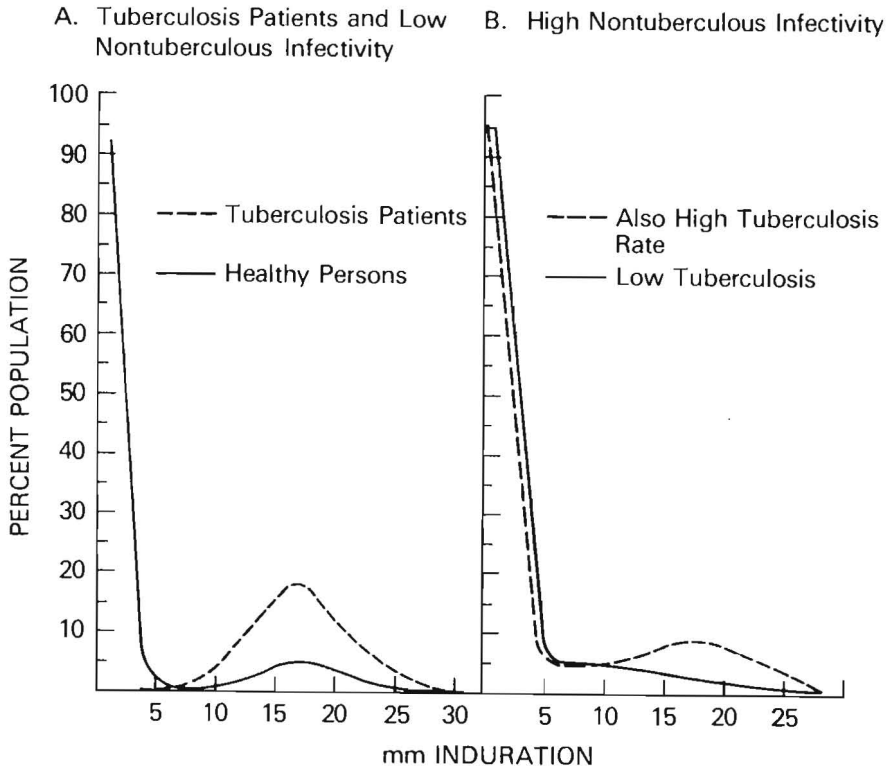

Fig. 2 Distribution of reaction sizes to tuberculin for various population groups

reactions less than 5 mm and in which the curve dips close to the baseline for reactions measuring about 5 to 10 mm. The shape of the curve for the larger reactions is similar to that for patients. This type of distribution is characteristic for regions in which very little sensitivity to tuberculin is induced by nontuberculous mycobacteria. Almost all reactors can be presumed to be infected with *M. tuberculosis*. In panel B of Figure 2 are seen curves representing distribution of reactions for healthy persons in regions in which the prevalence of nontuberculous mycobacteria is high. The solid line represents a region also low in tuberculosis infection. In contrast to the solid line in panel A there are a significant number of persons giving reactions between 5 to 10 mm resulting in the elimination of the dip in the curve. These reactions are most likely due to a cross-sensitisation to tuberculin. If the tuberculosis rate is also high a bimodal distribution may be seen similar to the curve constructed with dashed lines in panel B. Note that the curve does not dip close to the baseline as it did for the solid line in panel A. The role of endemic non-tuberculous mycobacteria in populations depicted in panel B in conveying protection against tuberculosis and the effectiveness of BCG in these populations needs to be elaborated.

In areas of the world where the incidence of tuberculosis is low and infection with nontuberculous mycobacteria is high, the tuberculin skin test with 3 or 5 TU often poses problems in interpretation. About 27 per cent of patients with tuberculosis who display a 10 mm or greater reaction with 5 TU of tuberculin will also display a 10 mm or greater reaction size to an *M. intracellulare* (Battey) antigen preparation.[35] For patients with non-tuberculosis mycobacterial disease, 55 per cent of those who give a 10 mm reaction with Battey antigen also give a 10 mm or greater reaction with tuberculin. Considerable similar cross reactivity also exists in sensitivity found in healthy persons in areas endemic with mycobacteria other than *M. tuberculosis*.[36] A completely monospecific skin test which will react only in patients who have been infected with *M. tuberculosis* would be extremely valuable in attributing sensitivity due to this organism. Despite decades of effort monospecific antigens for skin testing have not been isolated.[37,38] This failure may be due to the presence of multiple determinants on single antigenic molecules some of which may be species specific but which are accompanied also with genus specific determinants. For further explanation see reference 7.

Dual skin tests utilizing tuberculin PPD at 5 TU in conjunction with Battey or Gause *(M. scrofulaceum)* antigen[39] have been able to resolve whether sensitivity expressed by a subject is likely to be due to *M. tuberculosis* or to another mycobacterial species. Occasionally skin testing with a PPD prepared from a suspected species can strengthen a diagnosis. However, such antigens should be standardised in experimental systems to approximate in the homologously sensitised species a reaction equal to that of 5 TU of tuberculin in an *M. tuberculosis* sensitised species. The two reagents should be used in humans simultaneously and the antigen from the suspected species should yield a reaction greater than the cross-reacting tuberculin. Short of the isolation and identification of the aetiologic agent it is not possible to draw a definitive diagnostic conclusion on the basis of a skin test alone.

Current directions of tuberculosis research

The World Health Organisation has organised a comprehensive research plan to encourage research in several promising areas which should lead to a better understanding of the disease process and should offer new approaches for management of tuberculosis patients. Included in this plan are a) the generation of T-lymphocyte clones which are capable of producing regulators of suppressor and inducer elements such as transfer factor and interleukin 2 which have shown a therapeutic potential. Such clones can also provide valuable information on pathogenesis; b) monoclonal antibodies also offer promise as immune modifiers and have already helped to sort out the roles of various subclasses of lymphocytes in the various stages of disease. They may also be used to isolate single antigens which may be more specific than currently available skin test and serologic test reagents; c) in molecular biology the genetic basis for virulence and DNA probes should provide a better understanding about the pathogen's basis for virulence and immunogenicity. The next decade should provide many answers to problems that have been perplexing for over a century.

Summary

Today as throughout the history of mankind tuberculosis remains a major world health problem. This review is an update of current knowledge and thoughts about the immune response in tuberculosis, and factors which permit the disease organisms to often escape the body's defenses. Lymphocytes and macrophages are key elements in the resistance to tuberculosis. The various subclasses of lymphocytes and the factors they produce which regulate the immune response in tuberculosis are examined. Killing of tubercle bacilli occurs within macrophages; how this may be accomplished is reviewed. The significance of nontuberculous mycobacteria in the environment and how they affect interpretations of tuberculin reactions and concepts of vaccination are discussed. Current trends and future directions in reasearch in tuberculosis are outlined.

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