# 5 THE IMMUNOPHYSIOLOGY AND IMMUNOPATHOLOGY OF TUBERCULOSIS

*J.M. Grange*

The course, characteristics and outcome of tuberculosis vary enormously from patient to patient. Almost without exception, these variations are attributable to the immune responses of the host rather than to differences in the virulence of the causative organism. The mechanism of virulence of the tubercle bacillus remains shrouded in mystery but it has been apparent for almost a century that it does not owe its virulence to the synthesis of toxic substances but to its ability to survive the host's various immune defence mechanisms. More recently, it has become clear that virtually all the clinical and pathological manifestations of tuberculosis, as well as the infectivity of some patients, are the result of inappropriate, tissue-damaging immune reactions. Thus, in tuberculosis, the host's immune response is a two-edged sword – mediating protective responses but also facilitating progression of the disease in the patient and in the community.

The characteristic lesion of tuberculosis, and indeed of most chronic infections, is the granuloma (Plate 6). This consists of a compact aggregate, many layers thick, of macrophages in an activated form around the pathogen and a peripheral zone containing lymphocytes responsible for macrophage activation. The closely interdigitated macrophages bear a resemblance to epithelial cells and are therefore termed 'epithelioid cells'. Some of the macrophages fuse to form multinucleate giant cells (Langerhans cells) which, though not unique to tuberculosis, strongly support the histological diagnosis.

## 5.1 THE NATURAL HISTORY OF TUBERCULOSIS

Despite the enormous variation in the clinical features of tuberculosis, the disease nevertheless tends to follow a common pattern or 'timetable' of events (Table 5.1)[1]. Most cases of human tuberculosis are the result of inhalation of small, moist, expectorated droplets containing tubercle bacilli. These lodge in the alveoli or terminal air passages of the lung and establish a local focus of disease termed the Ghon focus. Bacilli are transported to the lymph nodes at the hilum of the lung where additional foci of disease develop. The Ghon focus together with the hilar lymphadenopathy is termed the primary complex (of Ranke). Bacilli disseminate further by the lymphatic and blood streams and lodge in many organs of the body. Thus, primary tuberculosis is a systemic infection. Primary complexes may also be acquired by ingesting tubercle bacilli, usually *M. bovis* in milk, in which case the implantation focus

*Clinical Tuberculosis*. Edited by P.D.O. Davies. Published in 1994 by Chapman & Hall, London. ISBN 0 412 48630 X

**Table 5.1** The 'timetable of tuberculosis'

| *Stage* | *Duration* | *Principal features* |
|---|---|---|
| 1 | 3–8 weeks | Development of primary complex. Conversion to tuberculin positivity |
| 2 | 3 months | Serious forms of tuberculosis due to haematogenous dissemination: miliary and meningeal disease |
| 3 | 3–4 months | Tuberculous pleurisy due to haematogenous spread or direct spread from enlarging primary lesion |
| 4 | Up to 3 years | Resolution of primary complex. Appearance of more slowly developing extrapulmonary lesions: bone and joint and renal tuberculosis |

Adapted from A. Wallgren, *Tubercle*, 1948, **29**, 245–51.

will be in the tonsil or intestinal wall and the lymphatic lesion will be in the cervical or mesenteric nodes. A minority of primary lesions follow traumatic inoculation, most typically as an occupational hazard of anatomists and pathologists – the lesion being termed 'prosector's wart'[2].

In most cases, the host's immune defences overcome the primary infection, which often passes unnoticed. In the minority of cases, the Ghon focus may enlarge progressively and possibly rupture into the pleural cavity, causing pleurisy. The hilar lymph node enlargement may be sufficient to compress a bronchus, causing collapse of a lobe of the lung, or it may erode into the pericardial space, causing tuberculous pericarditis. Alternatively, one of the foci of infection in more distant organs may progress, leading to the serious non-pulmonary sequelae of primary tuberculosis including bone and joint, renal and meningeal disease.

Healed primary complexes may remain dormant; in about 10% of infected persons, reactivation eventually occurs, resulting in post-primary tuberculosis. Exogenous reinfection may, of course, also cause this form of tuberculosis.

For reasons that are not known, post-primary tuberculosis of whatever origin tends to occur in the upper parts of the lung. The necrotic element in the post-primary lesion is much more evident than in primary disease, resulting in very large lesions, which often rupture and discharge their necrotic contents into the bronchi, thereby forming pulmonary cavities (Fig. 5.1). Unlike primary disease, the regional lymph nodes are rarely involved and associated disease in other organs is uncommon. Post-primary tuberculosis is therefore more localized and contained than the primary form of the disease. On the other hand, secondary lesions may develop in the same and opposite lung and the larynx due to spread of bacilli through the bronchi and trachea. Bacilli may be swallowed and cause secondary indurated lesions in the alimentary tract. This spread of disease is, however, quite distinct from the haematogenous spread in primary tuberculosis.

The cavity formation and containment of disease in post-primary tuberculosis is the result of active immune responses. Old patients and those whose immunity is suppressed by, for example, AIDS, renal failure and post-transplant immunosuppressive therapy, tend to develop spreading pulmonary lesions with little or no cavity formation and widespread haematogenous dissemination of the disease.

## 5.2 HOST RESPONSES IN EARLY INFECTION

Little is known of the events occurring in the first few days after primary infection of human beings by the tubercle bacillus: our

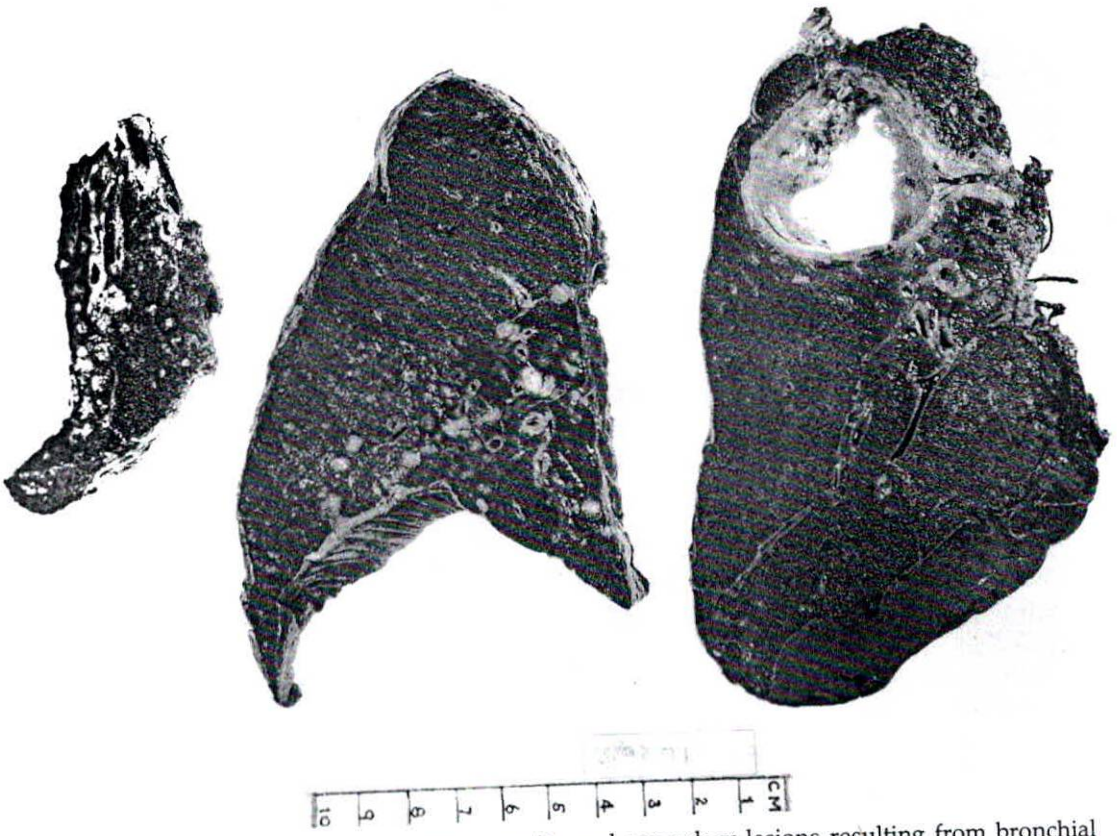

Fig. 5.1 Post-primary tuberculosis showing a cavity and secondary lesions resulting from bronchial spread of bacilli.

knowledge of these events comes mostly from studies on the rabbit[3]. An inflammatory reaction develops at the site of implantation and initially consists of an accumulation of blood-derived white cells, principally polymorphonuclear leucocytes. Subsequently, macrophages infiltrate the lesion leading first to a mixed appearance of acute pyogenic and chronic granulomatous inflammation and eventually to a distinct epithelioid cell granuloma. The early inflammation and granuloma formation is induced non-specifically by various components of the mycobacterial cell wall and probably inhibits the spread of the infection. There is also evidence that a population of T cells known as gamma-delta T cells is able to respond non-specifically to a limited range of bacterial components and cause macrophage aggregation and activation before the specific immune responses have developed[4].

The non-specific protective reactions in the early stages of the infection are unable to prevent dissemination of the bacilli by the lymphatic system to the regional lymph nodes, resulting in formation of the primary complex, or further dissemination by the blood stream to more distant organs.

## 5.3 INDUCTION OF IMMUNE RESPONSIVENESS IN TUBERCULOSIS

Antigens of *M. tuberculosis* are taken up and processed by the antigen-presenting cells and presented in close association with products of the major histocompatibility complex (MHC) genes to antigen-specific T cells. There are two classes of MHC gene products and these determine the subsets of T cells to which the antigen is presented. The T cells that induce and help immune functions ($CD4^+$ T cells) recognize antigens in association with the Class II MHC antigens, coded for by the HLA-D genes, while T cells with suppressor and cytotoxic functions ($CD8^+$) recognize antigen in association with Class I MHC antigens, coded for by the HLA-A and -B genes.

The 'repertoire' of antigens that may be presented on the surface of the antigen-presenting cell is affected by genetically determined factors and varies from one person to another. This genetic polymorphism affecting antigen recognition is thought to be an evolutionary mechanism to ensure that no single pathogen can eliminate an entire mammalian species[5].

After binding to the antigen/MHC complex, the antigen-specific T cells undergo activation and clonal expansion and then participate in the wide range of possible immune reactions. Thus T cells responsible for the induction and suppression of protective immunity, delayed hypersensitivity, cytolysis and antibody production as well as memory cells, with varying kinetics of appearance and disappearance, are produced in response to challenges by *M. tuberculosis* [6]. Although clones of T cells capable of recognizing mycobacterial antigens have been produced *in vitro*, it has not been easy to relate the subset of T cells to the type of immune response, whether protective or tissue-damaging, that they facilitate. This may be easier in the future as there is now evidence that the $CD4^+$ and $CD8^+$ cells may be further divided into distinct functional subclusters. In the mouse, helper T cells are divisible into two such subclusters, one of which ($T_H1$) secretes interleukin 2 and gamma interferon and helps cell-mediated immunity reactions and the other ($T_H2$) secretes interleukins 4 and 5 and helps antibody production[7]. Human helper T cells appear to be divisible into analogous subclusters[8].

## 5.4 GENETIC CONTROL OF IMMUNE RESPONSES IN TUBERCULOSIS

The existence of genes that determine resistance to tuberculosis has long been suspected. In the mouse, a *bcg* gene confers resistance to early stages of infection by BCG and other intracellular pathogens[9], apparently by affecting the innate ability of the macrophages to inhibit or kill the pathogens. There is suggestive evidence for a similar gene determining disease susceptibility in man.

There have been many unsuccessful attempts to find linkages between susceptibility to tuberculosis and the class I HLA genes (HLA-A and -B). Studies on class II (HLA-D) genes have been more promising and have revealed that the HLA-DR2 gene appears to predispose to the development of tuberculosis, particularly radiologically advanced, smear-positive disease[10–12]. The HLA-DR2 specificity may affect antigen recognition as persons of this genotype have higher levels of antibody to epitopes on a 38 kilodalton protein unique to *M. tuberculosis* than those lacking this genotype[10].

It has been suggested that the class II genes may determine the functional type of T cell (e.g. $T_H1$ or $T_H2$) to which mycobacterial antigen is presented. On the other hand, the lack of a very close linkage between tuberculosis and HLA has led to the concept that the selection of the type of immune response by mycobacteria is a multifactorial event based not only on specific antigen recognition but also on more primitive systems that

recognize a range of common bacterial components[8]. A predetermined tendency to an induction of predominantly $T_H1$- or $T_H2$-mediated responses to mycobacterial antigens in man is suggested by a study of healthy hospital workers exposed to tuberculosis patients. Some of these workers reacted strongly to PPD and had low levels of antibody to *M. tuberculosis* (suggesting a $T_H1$ response) while others reacted poorly to PPD but had higher antibody levels (suggesting a $T_H2$ response)[13].

Class II genes also affect reactivity to skin testing with tuberculin. Thus, skin testing with a range of mycobacterial sensitins revealed that persons lacking the HLA-DR3 gene tend to respond poorly to all sensitins, while those of HLA-DR4 phenotype respond relatively strongly to species-specific antigens of *M. tuberculosis*[14].

## 5.5 PROTECTIVE IMMUNITY AND THE ROLE OF THE MACROPHAGE

In the classical theory of cell-mediated immunity to mycobacteria and other intracellular pathogens, antigens of the pathogens are specifically recognized by helper T cells, which then activate macrophages non-specifically so that they are then able to destroy a wide range of intracellular pathogens[15]. The experiments that led to this theory were conducted principally with mice. Problems have been encountered with this theory in respect to human tuberculosis as this differs considerably from the disease in the mouse. Thus the latter is more resistant than human beings to tuberculosis and the disease is principally an intracellular one. Furthermore, although activated mouse macrophages undoubtedly kill tubercle bacilli, attempts to demonstrate such killing by human macrophages have, with few exceptions, been unsuccessful[16]. Mouse macrophages may owe their greater mycobactericidal powers to their ability to generate toxic nitrogen metabolites, i.e. nitric oxide and nitrogen dioxide. Activated human macrophages, unlike those of the mouse, are able to utilize vitamin D to induce further activation and, as outlined below, this phenomenon may account for further differences between immune responses in mice and man.

While the isolated human macrophage may be of limited effectiveness against tubercle bacilli, collectively they may form a powerful defence mechanism in the form of the granuloma. Being metabolically very active, the macrophages consume oxygen diffusing into the granuloma so that the interior region becomes anoxic and necrotic – a process termed caseation on account of the cheese-like appearance of the necrotic material. The acidic and anoxic conditions within the granuloma inhibit the growth of mycobacteria and may be bactericidal. This, together with bacterial inhibition by the activated macrophages, leads to quiescence. The granuloma becomes dormant and is entombed in fibrous scar tissue, which may become calcified. Unfortunately, a few mycobacteria may remain viable within these biological sarcophagi and re-emerge as the cause of disease years or decades later.

## 5.6 MYCOBACTERIAL PERSISTENCE

The nature of the mycobacteria that persist for many years within the tissues is one of the mysteries of mycobacteriology[17]. It has been shown that mycobacteria may remain viable for long periods without replication under anaerobic conditions[18]. This could explain dormancy, but several authors have advanced more elaborate theories, including the existence of cell-wall-free forms or microspores (Much's granules)[17,19]. Host immunity certainly plays a part in maintaining dormancy as reactivation is often associated with a weakening of immune defences. This suggests that persisting bacilli are not truly dormant but undergo replication, perhaps intermittently and slowly, at a

rate that is matched by their destruction by immune or other mechanisms. Overt disease would then develop if the rate of host-mediated bacterial destruction failed to keep up with the replication rate. This possibility is suggested by the fact that a 6–12 month course of isoniazid, a drug that is reported to kill only those tubercle bacilli that are actively replicating, eliminates these persistors in a high proportion of infected people.

## 5.7 POST-PRIMARY TUBERCULOSIS

This form of tuberculosis usually occurs in the upper part of the lung. The immunological reaction with granuloma formation is initially similar to that seen in primary disease but tissue necrosis is much more evident, resulting in very large caseous lesions termed tuberculomas. Proteases released by activated macrophages cause softening or liquefaction of the caseous material. The acidic and anoxic conditions within the lesion, together with free fatty acids in the softened caseous material, do not favour mycobacterial growth and relatively few acid-fast bacilli are present. Many lesions eventually erode into bronchi and their softened contents are discharged, resulting in the formation of cavities. The environment of the cavity wall is quite different from that of the solid tuberculoma. Air enriched with carbon dioxide, the ideal atmosphere for cultivation of tubercle bacilli, enters the cavity and neutralizes the previously acidic conditions. As a result, there is a massive increase in the numbers of acid-fast bacilli in the cavity wall and many gain access to the sputum, rendering the patient infectious. Bacilli are also able to spread to other parts of the lung through the bronchial tree and to set up additional foci of disease. In the days before effective chemotherapy, surgical procedures designed to obliterate pulmonary cavities appeared to limit the progression of the disease and to encourage resolution.

It is generally assumed that cavity formation and the other manifestations of post-primary tuberculosis are a consequence of the necrotizing reaction known as delayed hypersensitivity. Although obviously causing extensive tissue damage, this reactivity may have some protective value. Thus, in contrast to primary tuberculosis, bacilli rarely spread from the site of disease via the lymphatic or blood streams. (As mentioned earlier, they may spread to other parts of the lung through the bronchial tree.) Also, the tissue destruction may lead to massive fibrosis and scarring that, in turn, may wall off the active lesions, leading to quiescence. In the pre-chemotherapeutic era, spontaneous resolution occurred in about one-fifth of patients with cavitary post-primary tuberculosis[20]. In the chemotherapeutic era, such excessive scarring may be distinctly disadvantageous by favouring bacillary dormancy and inhibiting the diffusion of antituberculosis agents into the lesion[21].

To understand the immunological phenomena responsible for the extensive tissue necrosis and other characteristics of post-primary tuberculosis it is necessary to look back to the studies of Robert Koch, who discovered the tubercle bacillus in 1882.

## 5.8 THE KOCH PHENOMENON AND DELAYED HYPERSENSITIVITY

In 1891 Koch described the series of studies that had, in the previous year, led him to claim that he had discovered a cure for tuberculosis[22]. During these studies, he inoculated guinea-pigs with cultures of virulent tubercle bacilli by intradermal injection and observed the development of disease (Fig. 5.2). After 10–14 days, a small nodule developed at the inoculation site. This subsequently ulcerated and remained open until the animal died. The regional lymph nodes were grossly involved about 1 month later, after which the disease spread to many organs and the animal died between 3 and 4 months after inoculation. It was found,

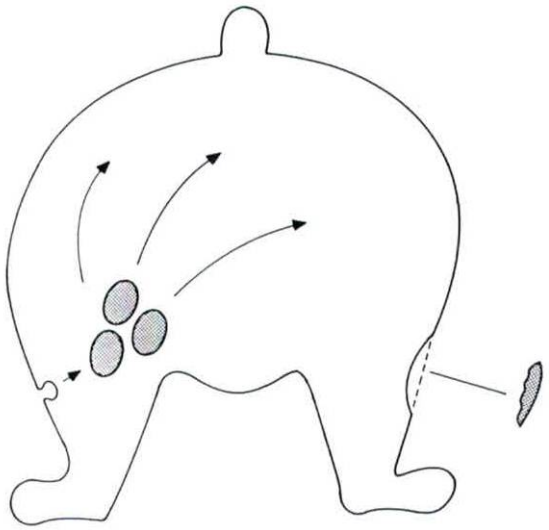

Fig. 5.2 A diagrammatic representation of the Koch phenomenon in the guinea-pig. The first challenge (left side) leads to an ulcer at the inoculation site and enlarged draining lymph nodes (primary complex) and further haematogenous dissemination. Subsequent challenge (right side) leads to a flat ulcer with sloughing off of the bacilli-laden dermis and no involvement of the draining lymph nodes.

however, that if infected guinea-pigs were re-inoculated at another site 4–6 weeks after the initial inoculation, the ensuing reaction was quite different. Within a day or two, an area of skin 0.5 to 1 cm across at the inoculation site became darkened and, after a further few days, it became necrotic and sloughed off, leaving a shallow ulcer that rapidly healed. Regional lymph nodes were not involved and it appeared that the second infection had been successfully eliminated. Koch then found that an identical reaction occurred after injection of killed tubercle bacilli and also of a filter-sterilized broth culture of the bacilli concentrated by evaporation. Koch named this preparation Old Tuberculin and administered it to tuberculosis patients by subcutaneous injection in the belief that it would induce a systemic protective reaction. This, unfortunately, did not occur: the remedy was ineffective for pulmonary disease and a few patients died of 'tuberculin shock' but some patients with long-standing skin tuberculosis made dramatic recoveries. These findings suggested that the necrotic 'Koch phenomenon' was protective when the disease was confined to the skin and could be sloughed off but was ineffective in those cases in which the disease involved internal organs.

Tissue damage may be an unavoidable consequence of a protective immune reaction. Nevertheless, some tissue-damaging immune processes appear to confer no benefit to the host and are referred to as hypersensitivity reactions. Four types were defined by Gell and Coombs – the first three are relatively rapid in onset and are the result of various forms of antigen-antibody reactions[23]. Type IV reactions, of which the Koch phenomenon is the classical example, appears to be mediated by cells rather than antibody and is of more delayed onset than the former three. Thus it is usually termed delayed type hypersensitivity (DTH).

## 5.9 THE TUBERCULIN REACTION

Although abandoned as a therapeutic agent, Koch's Old Tuberculin was used as a skin-testing reagent by the Austrian physician Clemens von Pirquet. On the basis of extensive clinical and post-mortem studies, von Pirquet established that reactivity to tuberculin indicated that the person had previously been infected by the tubercle bacillus[24]. The procedure for skin testing has been somewhat modified over the ensuing decades. In the original test, a drop of Old Tuberculin was placed on the skin, which was scratched through the drop, but reagents are now administered by intradermal injection (Mantoux method) or by multiple pronged devices (Heaf and tine tests). Old Tuberculin has been replaced by Purified Protein Derivative (PPD), the reagents are now standardized

and their strength is expressed in International Units (previously termed Tuberculin Units). Nevertheless, the principle of the test remains unchanged and, in countries where tuberculin reactivity has not been artificially induced by BCG vaccination, provides a useful indication of the extent of transmission of tuberculosis in the community[25].

Histological examination of biopsies of the tuberculin reaction reveals a dense infiltration of blood-derived white cells around the capillaries, hair follicles and sweat glands[26,27]. Some of the mononuclear cells (macrophages and lymphocytes) migrate from these inflammatory foci into the intervening dermis, especially into the subepidermal region. This migration is, at least in part, in response to specific mycobacterial antigens as more migration occurs in reactions to tuberculin than to leprosin in tuberculosis patients and *vice versa* in leprosy patients. There is no correlation between the number of cells in the test site, estimated as the percentage of the dermis occupied by the perivascular and periappendicular inflammatory foci, and the presence or extent of clinically evident swelling and induration. Indeed, subjects who are clinically tuberculin-negative may have an intense cellular infiltrate in the dermis. Thus, although the reaction is cell-mediated, it is not a direct consequence of the bulk of the cellular infiltrate.

The greatly increased cellularity of the dermis at the tuberculin test site leads to increased oxygen consumption and a compensatory increase in blood flow, accounting for the zone of erythema that surrounds the area of induration. Blood flow measurements have, however, revealed that in many tuberculin reactions, notably the larger and more obviously indurated ones, there is a central slowing of the blood flow[28]. This results in tissue anoxia, acidosis and, in a few reactions, overt tissue necrosis. The mechanism of this central relative slowing of blood flow is unknown but it is likely that it is related to the mechanisms, discussed below, that are responsible for the tissue necrosis and pulmonary cavity formation in post-primary tuberculosis.

Each mycobacterial species contains antigens unique to that species and also those that are common to all mycobacteria. As both groups of antigens may elicit tuberculin reactions, exposure to other mycobacterial species in the environment may induce cross-reactive responses on skin testing with tuberculin. In some countries or regions, cross-reactions are clearly differentiated by their size from genuine responses to tuberculin but in others the distinction between genuine and cross-reactions is not clear. For this reason, the diameter of a tuberculin reaction considered as being diagnostically significant varies from region to region. In veterinary practice, simultaneous testing of cattle with reagents prepared from *M. bovis* and from a common environmental mycobacterium (*M. avium*) is used to distinguish specific reactivity from cross-reactivity but this technique is rarely used in human studies.

Skin testing studies with reagents prepared from filter-sterilized ultrasonicates of many mycobacterial species (new tuberculins) have revealed three categories of reactor[29,30]. Persons in Category 1 fail to react to any reagent, even if they have been infected by *M. tuberculosis* or have received BCG vaccine. This non-reactivity appears to have a genetic basis (p. 59). Category 2 responders react to any mycobacterial species, even if it is not present in the environment, indicating that these persons respond to common mycobacterial antigens. Category 3 responders only react to certain mycobacteria, indicating that they recognize species-specific, but not common, mycobacterial antigens. While most healthy people are Category 2 responders, those with overt tuberculosis or other mycobacterial disease are mostly Category 3 responders, suggesting that they have lost the ability to recognize the common mycobacterial antigens, which may include

protective epitopes. The significance of this finding to the development of effective immunotherapy is discussed in section 5.14 on p. 66.

## 5.10 PROTECTIVE IMMUNITY AND DELAYED HYPERSENSITIVITY

The relation between protective and non-protective immune reactions in tuberculosis, and the relation of both to tuberculin reactivity, has been the topic of considerable debate and confusion for many decades[31]. Much of the confusion is due to nomenclature, as both types of reaction have been grouped under the umbrella title of 'cell-mediated immunity' (CMI).

During his pioneering studies on tuberculin testing, von Pirquet observed that patients with very advanced tuberculosis were often tuberculin-negative[32]. He thus concluded that a positive tuberculin reaction was a correlate of protective immunity. This idea has been challenged on many occasions and there is still controversy as to whether protective immune responses and necrotic DTH reactions are quite distinct or whether they are manifestations of the same response, but differing in intensity. This controversy has been extensively reviewed[31,33–35] but, although several questions remain unanswered, modern immunological and molecular biological approaches are close to permitting a resolution of the issue.

## 5.11 THE NATURE AND MECHANISM OF DELAYED HYPERSENSITIVITY

Following their activation and clonal expansion, helper T cells secrete gamma interferon (IFN-g) and other cytokines that activate the macrophages (Fig. 5.3). *In vitro* studies show that IFN-g *per se* does not increase the resistance of human macrophages to *M. tuberculosis* but that it has another important effect. It induces a 1-hydroxylase in human macrophages, which converts the inactive 25-OH vitamin D3 to the active 1,25 $(OH)_2$ vitamin D3 (calcitriol)[36]. This increases the ability of the macrophages to inhibit the intracellular replication of *M. tuberculosis* but it also sensitizes them to the triggering of the release of tumour necrosis factor (TNF) and other cytokines[16]. One potent trigger of TNF release from such sensitized macrophages is *M. tuberculosis* and the active substance is a cell wall component termed lipoarabinomannan B (LAM)[37].

Under normal circumstances, TNF plays a protective role in infections by rapidly activating phagocytic cells and contributing to the process of granuloma formation. By contrast, excessive release as occurs, for example, in Gram-negative septicaemia, causes the toxic shock syndrome. TNF is also termed cachectin and is said to be responsible for the severe wasting (consumption, phthisis or cachexia) seen in advanced untreated tuberculosis. TNF is, however, undetectable in sera from tuberculosis patients without such advanced disease and, indeed, these patients have a circulating inhibitor of the toxic effects of TNF. The question has thus been raised as to whether the relatively low levels of TNF released from tuberculosis granulomata are protective or lead to necrosis.

Rook and his colleagues have shown that infection of cell lines by *M. tuberculosis*, or indeed the mere addition of crude culture supernatants of this bacillus, greatly enhances the susceptibility of the cells to killing by TNF[16,38]. *In vivo*, injection of tuberculin followed 24 h later by an injection, at the same site, of a minute amount of TNF leads to a necrotic reaction. This sensitization appears to be T cell-dependent.

Thus the sequence of events in a necrotic tuberculin reaction or tuberculous lesion could be as follows. Mycobacterial antigen is recognized by T cells, which then release gamma interferon and other cytokines that activate macrophages and induce the 1-hydroxylase enzyme, which, by generating

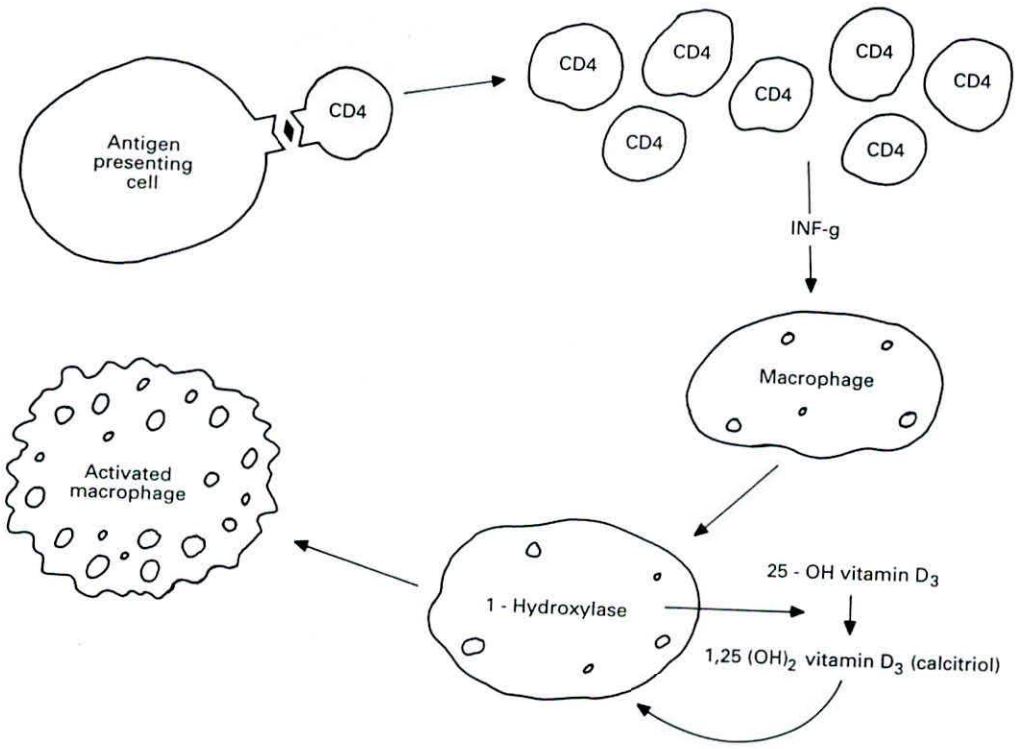


**Fig. 5.3** The pathway of macrophage activation. Mycobacterial antigen is presented by the antigen presenting cell to an inducer T cell (CD4+). This undergoes clonal expansion and the resulting cell population secretes gamma interferon and other cytokines that activate the macrophage. Induction of a 1-hydroxylase enables the macrophage to convert inactive 25-OH vitamin $D_3$ to the active 1,25 $(OH)_2$ vitamin $D_3$, resulting in further activation.

calcitriol, primes these cells for TNF release. Other T cell products sensitize cells at the site to the toxic effects of TNF. Mycobacterial components, notably LAM, then trigger TNF release from the primed macrophages and this kills the sensitized cells in the neighbourhood (Fig. 5.4). (In this context it is noteworthy that sarcoid granulomata produce large quantities of 1,25 $(OH)_2$ vitamin D3, enough indeed to induce hypercalcaemia, but necrosis of the lesions is very uncommon, presumably as there is no LAM or other TNF releasing factor of bacterial origin.)

At first view, it might seem that this explanation of necrosis occurring in tuberculosis would imply that all reactions, whether lesions or tuberculin test sites, would be necrotic and counter-protective. Non-necrotic reactions may be explained by postulating that, while T cell products may sensitize cells to the toxic effects of TNF, this is the property of a particular subset of T cells. Antigen recognition by other subsets might have the opposite effect. Although the mechanism is unknown, there is very strong evidence that the immune system may make a 'decision' between a necrotic and non-necrotic response to infection by *M. tuberculosis* and that an inappropriate decision may be reversed by an appropriate immunotherapeutic intervention.

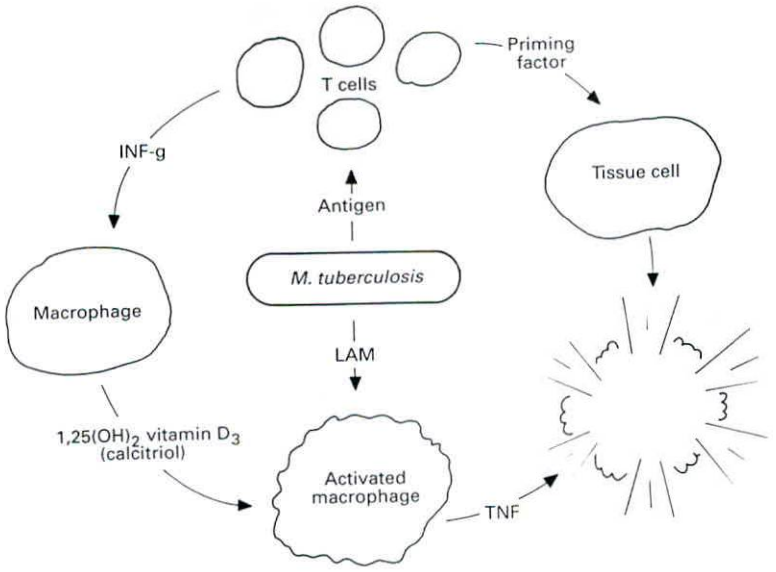


Fig. 5.4 The role of tumour necrosis factor (TNF). Macrophages are primed to release TNF and T cell factors either protect from, or enhance susceptibility of cells to killing by TNF. Mycobacterial lipoarabinomannan B (LAM) triggers the release of TNF from macrophages. (Adapted from G.A.W. Rook and R. Al Attiyah, *Tubercle*, 1991, **72**, 13–20.)

## 5.12 'SPECTRUM' OF IMMUNE REACTIVITY IN TUBERCULOSIS

Attempts have been made to classify cases of tuberculosis according to a 'spectrum' of immune reactivity similar to that evident in leprosy. There are, however, fundamental differences between the two diseases that render such a comparison difficult[39]. In leprosy, there is a hyper–reactive (tuberculoid) form with extensive granuloma formation but very few bacilli and an anergic (lepromatous) form in which there are huge numbers of bacilli and a very specific suppression or absence of cellular immune responses to the leprosy bacillus and various borderline forms in which the immune responsiveness is unstable and liable to cause severe reactions. The nearest equivalent in tuberculosis to tuberculoid leprosy is lupus vulgaris, a very chronic cutaneous form of tuberculosis in which there are well-organized, non-necrotic epithelioid cell granulomata and very few bacilli.

There is no real form of tuberculosis equivalent to anergic lepromatous leprosy. Although disseminated, multibacillary tuberculosis may occur, this is usually a consequence of generalized immunosuppression rather than the specific failure to recognize antigens of the pathogen. Also, *M. tuberculosis* is much more toxic and rapidly growing than *M. leprae* so that, unless treated, disseminated tuberculosis rapidly progresses to a fatal outcome.

Ridley and Ridley thus described a three-group 'spectrum' of tuberculosis, with group 1 corresponding to chronic cutaneous disease and group 3 to disseminated disease in immunosuppressed persons. Most cases of tuberculosis are of the localized pulmonary and non-pulmonary types and belong to group 2[40].

## 5.13 IMMUNOSUPPRESSION AND TUBERCULOSIS

It has long been known that suppression of immune reactivity may lead to endogenous

reactivation of tuberculosis and this fact has been particularly evident since the advent of the HIV pandemic[41]. Persons dually infected by HIV and *M. tuberculosis* have a much greater chance of developing reactivation tuberculosis than those only infected by the latter, i.e. an increase in the annual reactivation rate from 1% to 10%. Tuberculosis in HIV-positive patients differs from that in non-immunocompromised patients in that the disease is much less contained and cavity formation is less apparent. Thus, the disease may present as a spreading pulmonary lesion with rather non-specific radiological features or as disseminated disease. This emphasizes that both cavity formation and the 'walling-off' process seen in post-primary disease have an immunological basis.

Not only does HIV infection predispose to tuberculosis, the latter may adversely affect the progress of the former. Tuberculosis, even if effectively treated, often leads to a rapid progression of HIV infection to AIDS. This appears to be due to tumour necrosis factor, which induces the production of a nuclear factor, which in turn activates the transcription of the DNA provirus of the HIV leading to viral replication[42].

Tuberculosis itself may induce a degree of immunosuppression, which reverts to normal after successful therapy of the disease. Various defects in immune function have been described in tuberculosis and, in particular, HIV–negative patients with active disease may show a $CD4^+$ T cell lymphopenia[43].

Relatively high numbers of CD8 cells were found in broncho-alveolar lavage (BAL) fluid from patients with miliary tuberculosis. The time taken for the radiographs to clear on therapy was related to the number of these cells, suggesting that they had an adverse effect on protective immunity. The number of lymphocytes in BAL fluid was much higher after 8 weeks of therapy and there was a distinct shift from $CD8^+$ to $CD4^+$ dominance [44].

## 5.14 VACCINATION AND IMMUNOTHERAPY

Bacille Calmette-Guerin (BCG) was produced from a tubercle bacillus of bovine origin by repeated subculture on potato-bile medium. This approach to vaccine development was based on the finding that children who developed, and recovered from, tuberculous cervical lymphadenopathy (scrofula) as a result of consuming milk contaminated with *M. bovis* appeared to be protected against the more serious pulmonary forms of tuberculosis later in life (Marfan's law)[45]. In accord with this theory, BCG was initially given as an oral suspension to infants although, for reasons of safety and standardization, it is now given by intradermal injection. The mode of action of BCG is unknown. It is particularly effective in preventing the serious non-pulmonary forms of primary tuberculosis such as meningitis. Thus its principal effect may be to prevent dissemination of bacilli from the primary infection.

BCG protects, to some extent, against leprosy[46] and against lymphadenitis due to environmental mycobacteria in children[47], indicating that some, or all, of the determinants of protection are to be found among the antigens common to all mycobacteria.

The efficacy of BCG varies greatly from region to region (see Fig. 14b.1, p. 299). In regions where the vaccine is relatively ineffective, greater protection is obtained by vaccinating children shortly after birth[48]. Various explanations have been given for the regional variation: one of the more plausible ones is that immunity to mycobacterial disease may be conferred by exposure to environmental mycobacteria and that subsequent BCG vaccination cannot add substantially to the level of protection. Alternatively, some species or populations of environmental mycobacteria may induce inappropriate tissue-damaging responses that BCG cannot counteract or may even boost[49]. In this respect, tuberculin reactions may be divided

into non-necrotic 'Listeria-type' and necrotic 'Koch-type' responses by careful clinical inspection[50] and detection of central slowing of the blood flow by laser Doppler velicometry[51].

There is therefore abundant evidence that, depending on various factors, immune responses may confer protection or cause excessive tissue damage and permit bacillary replication in the cavity wall. Any means of switching from the latter to the former would be of great therapeutic benefit. Skin testing with mixtures of sensitins prepared from various mycobacteria showed that necrotic Koch-type reactions are converted to non-necrotic reactions by the inclusion of antigens of the non-pathogenic, rapidly growing species such as *M. vaccae*[52]. Subsequent extensive studies revealed that an injection of $10^9$ killed *M. vaccae* has a systemic effect in replacing Koch-type reactivity with a protective response[21]. It also restores immune recognition of the common mycobacterial antigens, which, as described above, is absent in patients with active mycobacterial disease. The precise mode of action of *M. vaccae* has not been determined but clinical studies indicate that it is a valuable adjunct to short-course chemotherapy, possibly permitting the duration of therapy to be reduced from 5 months or more to 2 months or less[53].

## 5.15 OTHER IMMUNOLOGICAL PHENOMENA IN TUBERCULOSIS

Attention has focused recently on the possibility that immunity to mycobacteria may, to some extent, involve lysis of cells harbouring mycobacteria, a process that would also contribute to the immunopathological features of the tissue reactions. Such cell killing may be due to antigen-specific cytotoxic ($CD8^+$) T cells as, in the mouse, these have been shown to confer resistance to infection by *M. tuberculosis* by *in vivo* depletion and adoptive transfer studies[54]. As yet, however, there is no evidence for the involvement of cytotoxic $CD8^+$ cells in human tuberculosis[44]. In addition, natural killer (NK) and $CD4^-$ $CD8^-$ T cells have been implicated in non-specific intracellular killing of mycobacteria, as well as killing of infected macrophages[55]. Large numbers of cytolytic gamma-delta T cells have been found in the necrotic lesions of tuberculous lymphadenitis[56], although their precise role in such lesions is not clear.

There have been extensive studies on antibody assay in tuberculosis in the hope of developing a serological test for this disease. Unfortunately no test has proved sensitive or specific enough to justify its introduction into routine diagnostic services[57]. Serological studies have been used to relate immune responses to various mycobacterial epitopes to susceptibility to tuberculosis in the hope of delineating those antigens that confer protective immunity. Thus, for example, healthy subjects exposed to open tuberculosis have high levels of antibody to a 14 kDa protein of *M. tuberculosis* while those with progressive tuberculosis have low levels of that antibody[58] (Chapter 17a, p. 369).

A characteristic of tuberculosis is an increase in the proportion of a form of immunoglobulin in the IgG class that lacks a terminal galactose from a sugar component of this macromolecule. Raised levels of this so-called 'agalactosyl IgG' also occur in other diseases, including rheumatoid arthritis (RA) and Crohn's disease, which are characterized by tissue damage due to cytokines released by T cells and an acute-phase protein response[16]. A tenuous connection between tuberculosis and RA has emerged from studies on the so-called heat-shock proteins (HSP). These form a class of structurally highly conserved proteins found in all living creatures and are also termed 'chaperone' or 'nurse-maid' proteins as they assist in the folding and assembly of other protein macromolecules. It has been shown that T cells from patients with RA react to mycobacterial HSPs and to the human homologues and that

adjuvant arthritis in rats is adoptively transferable by T cell clones reacting to a 65 kDa mycobacterial HSP[59]. Clearly, tuberculosis is not a regular cause of RA (although a very few patients develop an arthritis-like condition termed Poncet's disease), but mycobacteria could, by antigen mimicry, be one of the triggers for the onset of RA. Whether or not this proves to be the case, mycobacteria may, by antigen mimicry, induce autoimmune phenomena that contribute to tissue damage in both tuberculosis and leprosy[60].

A further factor suggesting a link between the immunopathology of tuberculosis and of RA is the finding that the HLA-DR4 phenotype, which is known to predispose to RA, is associated with large dermal reactions to specific antigens of *M. tuberculosis* but not to those of other mycobacteria[14].

## 5.16 CONCLUSIONS

Immune reactions in tuberculosis are complex and, depending on various genetic and environmental factors, result in either protective immunity or excessive tissue damage. Tuberculosis passes through two stages – primary and post-primary. In the former, bacilli disseminate to regional lymph nodes and to more distant sites. Nevertheless, most primary lesions resolve although a few bacilli may persist within healed lesions and subsequently reactivate. Post-primary disease is characterized by excessive tissue necrosis, which, though having some protective effect by walling off the active lesions, generates cavities that favour massive bacillary multiplication, rendering the patient infectious, and dissemination of disease throughout the lung by bronchial spread. This necrotic hypersensitivity is analogous to the Koch phenomenon in the guinea-pig.

There is evidence that necrotizing immune responses are the result of the sensitization of tissues by T cell-derived factors to killing by tumour necrosis factor (TNF) released by macrophages primed for such release by endogenously generated calcitriol. The TNF release is triggered by mycobacterial cell wall components, especially lipoarabinomannan. In protective immune responses, however, TNF has a more beneficial effect by activating macrophages and facilitating granuloma formation. The factors determining the nature of the response are poorly understood. The immunopathological response is characterized by elevated levels of an abnormal immunoglobulin (agalactosyl IgG), suggesting immune dysregulation. In addition, patients with active tuberculosis often fail to react in skin testing to shared mycobacterial antigens.

Both the protective immune responses and the necrotic hypersensitivity responses responsible for walling off the infection are suppressed in HIV positive and other immunocompromised tuberculosis patients so that progressive disseminated disease is commonly seen. The immune response in tuberculosis activates the HIV so that tuberculosis predisposes to the rapid onset of AIDS.

The mode of action of BCG is not understood. Its efficacy varies enormously from region to region but, in all regions, it is most effective when given neonatally. Thus extrinsic factors, probably exposure to environmental mycobacteria, may induce inappropriate immune reactions that administration of BCG cannot override. There is, however, strong evidence that the administration of killed cells of a rapidly growing mycobacterium, *M. vaccae*, which is rich in shared mycobacterial antigens, replaces a necrotic Koch-type response by a non-necrotic protective one. Such immunomodulation may be the key to future prevention and treatment of tuberculosis.

## REFERENCES

1. Wallgren A. (1948) The 'time-table' of tuberculosis. *Tubercle*, **29**, 245–51.
2. Grange J.M., Noble, W.C., Yates, M.D. and Collins, C.H. (1988) Inoculation mycobacterioses. *Clin. Exp. Dermatol.*, **13**, 211–20.

3. Medlar, E.M. (1955) The behaviour of pulmonary tuberculosis. A pathological study. *Am. Rev. Tuberc.*, **71** (Part 2, Suppl.), 1–244.
4. Inoue, T., Yoshikai, Y., Matsuzaki, G., and Nomoto, K. (1991). Early appearing g/d-bearing T cells during infection with Calmette Guerin bacillus. *J. Immunol.*, **146**, 2754–62.
5. de Vries, R.R.P. (1991) An immunogenetic view of delayed type hypersensitivity. *Tubercle*, **72**, 161–7.
6. Orme, I.M. (1987) The kinetics of emergence and loss of mediator T lymphocytes acquired in response to infection with *Mycobacterium tuberculosis*. J. *Immunol.*, **138**, 293–8.
7. Mossman, T.R. and Moore, K.W. (1991) The role of Il-10 in crossregulation of $T_H1$ and $T_H2$ responses. In *Immunoparasitology Today* (eds C. Ash and R.B. Gallagher), Elsevier, New York A49–A53.
8. Rook, G.A.W. (1991) Mobilising the appropriate T cell subset: the immune response as taxonomist? *Tubercle*, **72**, 253–4.
9. Schurr, E., Malo, D., Radzioch, D. *et al.* (1991) Genetic control of innate resistance to mycobacterial infections. *Immunol. Today*, **12**, A42-A45.
10. Bothamley, G.H., Beck, J.S., Geziena, M. *et al.* (1989) Association of tuberculosis and *M. tuberculosis*-specific antibody levels with HLA. *J. Infect. Dis.*, **159**, 549–55.
11. Brahmajothi, V., Pitchappan, R.M., Kakkanaiah, V.N. *et al.* (1991) Association of pulmonary tuberculosis and HLA in South India. *Tubercle*, **72**, 123–32.
12. Khomenko, A.G., Litvinov, V.I., Chukanova, V.P. and Pospelov, L.E. (1990) Tuberculosis in patients with various HLA phenotypes. *Tubercle*, **71**, 187–92.
13. Pitchappan, R.M., Brahmajothi, V., Rajaram, K. *et al.* (1991) Spectrum of immune reactivity to mycobacterial (BCG) antigens in healthy hospital contacts in South India. *Tubercle*, **72**, 133–9.
14. Ottenhoff, T.H.M., Torres, P., de las Aguas, J.T. *et al.* (1986) Evidence for an HLA-DR4-associated immune response gene for *Mycobacterium tuberculosis*. *Lancet*, **2**, 310–12.
15. Mackaness, G.B. (1967) The immunology of antituberculous immunity. *Am. Rev. Respir. Dis.*, **97**, 337–44.
16. Rook, G.A.W. and Al Attiyah, R. (1991) Cytokines and the Koch phenomenon. *Tubercle*, **72**, 13–20.
17. Grange, J.M. (1992) The mystery of the mycobacterial persistor. *Tubercle Lung Dis.*, **73**, 249–51.
18. Wayne, L.G. and Lin, K-Y. (1982) Glyoxylate metabolism and adaptation of *Mycobacterium tuberculosis* to survival under anaerobic conditions. *Infect. Immun.*, **37**, 1042–9.
19. Stanford, J.L. (1987) Much's granules revisited. *Tubercle*, **68**, 241–2.
20. Springett, V.H. (1971) Ten year results during the introduction of chemotherapy for tuberculosis. *Tubercle*, **52**, 73–87.
21. Stanford, J.L. (1991) Koch's phenomenon: can it be corrected? *Tubercle*, **72**, 241–9.
22. Koch, R. (1891) Weitere Mitteilungen uber ein Heilmittel gegen Tuberculose. *Dtsch. Med. Wochenschr.*, **171**, 101–2.
23. Coombs, R.R.A. and Gell, P.G.H. (1975) Classification of allergic reactions responsible for hypersensitivity and disease. In *Clinical Aspects of Immunology*, 3rd edn, (eds P.G.H. Gell and R.R.A. Coombs), Blackwell, Oxford, pp. 761–81.
24. von Pirquet, C. (1909) Demonstration zur Tuberculindiagnose durch Hautimpfung. *Berl. Klin. Wochenschr.*, **481**, 699.
25. Styblo, K. (1980) Recent advances in epidemiological research in tuberculosis. *Adv. Tuber. Res.*, **201**, 1–63.
26. Beck, J.S., Morley, S.M., Gibbs, J.H., *et al.* (1986) The cellular responses of tuberculosis and leprosy patients and of healthy controls in skin tests to new tuberculin and healthy subjects. *Clin. Exp. Immunol.*, **64**, 484–94.
27. Beck, J.S. (1991) Skin changes in the tuberculin test. *Tubercle*, **72**, 81–7.
28. Beck, J.S., Gibbs, J.H., Potts, R.C. *et al.* (1989) The relation between cutaneous blood flow and cell content in the tuberculin reaction. *Scand. J. Immunol.*, **291**, 33–9.
29. Stanford, J.L., Nye, P.M., Rook, G.A.W., *et al.* (1981) A preliminary investigation of the responsiveness or otherwise of patients and staff of a leprosy hospital to groups of shared or specific antigens of mycobacteria. *Lepr. Rev.*, **52**, 321–7.
30. Kardjito, T., Beck, J.S., Grange, J.M. and Stanford, J.L. (1986) A comparison of the responsiveness to four new tuberculins among Indonesian patients with pulmonary tuberculosis and healthy subjects. *Eur. J. Respir. Dis.*, **69**, 142–5.
31. Bothamley, G.H. and Grange, J.M. (1991) The

Koch phenomenon and delayed hypersensitivity: 1891–1991. *Tubercle*, **72**, 7–11.
32. von Pirquet, C. (1908) Verlauf der tuberculosen Allergie bei einen Falle von Masern und Miliartuberkulose. *Wein. Klin. Wochenschr.*, **21**, 861–5.
33. Youmans, G.P. (1975) Relation between delayed hypersensitivity and immunity in tuberculosis. *Am. Rev. Respir. Dis.*, **111**, 109–18.
34. Lefford, M.J. (1975) Delayed hypersensitivity and immunity in tuberculosis. *Am. Rev. Respir. Dis.*, **111**, 243–46.
35. Salvin, S.B. and Neta, R. (1975) A possible relation between delayed hypersensitivity and cell-mediated immunity. *Am. Rev. Respir. Dis.*, **111**, 373–77.
36. Rook, G.A.W. (1988) The role of vitamin D in tuberculosis. *Am. Rev. Respir. Dis.* **138**, 768–70.
37. Moreno, C., Taverne, J., Mehlert, A. *et al.* (1989) Lipoarabinomannan from *Mycobacterium tuberculosis* induces the production of tumour necrosis factor from human and murine macrophages. *Clin. Exp. Immunol.*, **76**, 240–45.
38. Al-Attiyah, R.J. (1991) The regulation of necrosis in mycobacterial lesions. Doctor of Philosophy Thesis, University of London.
39. Skinsnes, O.K. (1968) Comparative pathogenesis of mycobacterioses. *Ann. NY Acad. Sci.*, **154**, 19–31.
40. Ridley, D.S. and Ridley, M.J. (1987) Rationale for the histological spectrum of tuberculosis. A basis for classification. *Pathology*, **19**, 186–92.
41. Festenstein, F. and Grange, J.M. (1991) Tuberculosis and the acquired immune deficiency syndrome. *J. Appl. Bacteriol.*, **71**, 19–30.
42. Osborn, L., Kunkel, S. and Nabel, G.J. (1989) Tumour necrosis factor and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kappa B. *Proc. Natl Acad. Sci. USA*, **86**, 2236–40.
43. Beck, J.S., Potts, R.C., Kardjito T. and Grange, J.M. (1985) T4 lymphopenia in patients with active pulmonary tuberculosis. *Clin. Exp. Immunol.*, **60**, 49–54.
44. Ainslie, G.M., Solomon, J.A. and Bateman, E.D. (1992) Lymphocyte and lymphocyte subset numbers in blood and in bronchoalveolar lavage and pleural fluid in various forms of human pulmonary tuberculosis at presentation and during recovery. *Thorax*, **47**, 513–18.
45. Savage, W.G. (1929) *The Prevention of Human Tuberculosis of Bovine Origin*, Macmillan, London.
46. Brown, J.A.K., Stone, M.M. and Sutherland, I. (1966) BCG vaccination of children against leprosy in Uganda. *B. Med. J.*, **1**, 7–14.
47. Romanus V. (1988) Swedish experiences 12 years after the cessation of general BCG vaccination of new borns in 1975. *Bull. Union Int. Tuberc. Lung Dis.*, **63**(4), 34–8.
48. Tripathy, S.P. (1987) Fifteen year follow-up of the Indian BCG prevention trial. *Proceedings of the 24th International Union Against Tuberculosis Conference on Tuberculosis and Respiratory Diseases*, Singapore, Professional Postgraduate Services, pp. 69–72.
49. Stanford, J.L., Shield, M.J. and Rook, G.A.W. (1981) How environmental mycobacteria may predetermine the protective efficacy of BCG. *Tubercle*, **62**, 55–62.
50. Stanford, J.L. and Lema, E. (1983) The use of a sonicate preparation of *Mycobacterium tuberculosis* (new tuberculin) in the assessment of BCG vaccination. *Tubercle*, **64**, 275–82.
51. Potts, R.C., Beck, J.S., Gibbs, J.H. *et al.* (1992) Measurements of blood flow and histometry of the cellular infiltrate in tuberculin skin test responses of the Koch-type and Listeria-type in pulmonary tuberculosis patients and apparently healthy controls. *Int. J. Exp. Pathol.*, **73**, 565–72.
52. Nye, P.M., Price, J.E., Revankar, C.R. *et al.* (1983) The demonstration of two types of suppressor mechanism in leprosy patients and their contacts by quadruple skin-testing with mycobacterial reagent mixtures. *Lepr. Rev.*, **54**, 9–18.
53. Stanford, J.L., Grange, J.M. and Pozniak, A. (1991) Is Africa Lost? *Lancet*, **338**, 557–8.
54. Muller, I., Cobbold, S.P., Waldmann, H. and Kaufmann, S.H.E. (1987) Impaired resistance against *Mycobacterium tuberculosis* infection after selective *in vitro* depletion of $L3T4^-$ and $Lyt2^+$ T cells. *Infect. Immun.*, **55**, 2037–41.
55. Restrepo, L.M., Barrera, L.F. and Garcia, L.F. (1990) Natural killer cell activity in patients with pulmonary tuberculosis and in healthy controls. *Tubercle*, **71**, 95–102.
56. Modlin, R.L., Pirmez, C. Hofman, F.M. *et al.*, (1989) Lymphocytes bearing antigen-specific gd T-cell receptors accumulate in human infectious disease lesions. *Nature*, **339**, 544–8.
57. Grange, J.M. and Laszlo, A. (1990) Serodiag-

nostic tests for tuberculosis: a need for assessment of their predictive accuracy and acceptability. *Bull. WHO*, **681**, 571–6.

58. Bothamley, G.H., Beck, J.S., Potts, R.C. *et al.*, (1992) Specificity of antibodies and tuberculin response following occupational exposure to tuberculosis. *J. Infect. Dis.*, **166**, 182–6.

59. Rook, G.A.W. (1988) Rheumatoid arthritis, mycobacterial antigens and agalactosyl IgG. *Scand. J. Immunol.*, **28**, 487–93.

60. Das, P.K. and Grange, J.M. (1993) Mycobacteria in relation to tissue immune response and pathogenesis. *Rev. Med. Microbiol.*, **4**, 15–23.