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Chapter 5: Immunology, Pathogenesis, Virulence

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5.1. Immune response against *Mycobacterium tuberculosis*

The immune response against tuberculosis (TB) plays a fundamental role in the outcome of *M. tuberculosis* infection. It is clear that the immune system reacts efficiently in the vast majority of infections. This is particularly evident in the case of TB, where most people infected by the tubercle bacillus (~ 90 %) do not develop the disease throughout their lifetimes. Nevertheless, the risk of developing the disease increases considerably when TB infection co-exists with an alteration in the immune system, such as co-infection with human immunodeficiency virus (HIV).

Also, it is well known that bacille Calmette-Guérin (BCG) vaccination has not been completely efficient in the prevention of pulmonary TB. Thus, the design of vaccines against TB is a field in which much effort has been invested with the aim of fighting this disease. Recently, it has become clear that, in order to develop a more efficient vaccine, a better understanding of the relation between the immune response of the host and the tubercle bacillus is needed.

In view of this, the present chapter provides an updated overview of the cellular and molecular immune mechanisms involved in the development of the disease.

5.1.1. Innate immune response

5.1.1.1. Neutrophil leukocytes

Even though macrophages are considered the main targets for infection by *Mycobacterium tuberculosis*, it has been recently proposed that other cell populations can also be infected by mycobacteria and therefore may be important in the development of the disease. Neutrophils are found within this group of cells (Figure 5-1). Characteristically, they are among the earliest cells recruited into sites where any noxious agent enters into the body and/or inflammatory signals are triggered. They also have well-characterized microbicidal mechanisms such as those dependent on oxygen and the formation of neutrophil extracellular traps (Urban 2006).

Using the murine experimental model, the role played by neutrophils in TB is controversial. These cells have been detected at the beginning of infection as well as several days after infection (Pedrosa 2000, Fulton 2002) and were thought to have

an important role in the control of mycobacterial growth. Indeed, if neutrophils are eliminated before infection, mycobacterial growth increases in the lungs of experimentally infected animals; and conversely, if mice are treated with an agent that increases neutrophils, the bacillary growth rate decreases (Appelberg 1995, Fulton 2002). However, when the microbicidal ability of neutrophils against mycobacteria was analyzed, controversial results were obtained. There are reports of neutrophils being able to kill mycobacteria (Jones 1990) and other reports where this phenomenon was not observed (Denis 1991). Nevertheless, it is believed that the function of neutrophils goes beyond their microbicidal ability. Therefore, these cells are thought to contribute to the control of infection through the production of chemokines (Riedel 1997), the induction of granuloma formation (Ehlers 2003) and the transference of their own microbicidal molecules to infected macrophages (Tan 2006).



Figure 5-1: Neutrophils ingest *Mycobacterium tuberculosis*. Human purified neutrophils were incubated with *Mycobacterium tuberculosis* H37Rv and DNA was stained with SYTOX™ Green. Fluorescent rods on the left are intracellular bacilli.

On the other hand, neutrophils have recently been ascribed a role in the development of the pathology, rather than the protection of the host. TB susceptible animals were found to have a larger and longer accumulation of neutrophils in TB lesions compared to TB resistant animals (Eruslanov 2005). This event seems to be influenced by the differential expression of molecules which are chemoattractant to

neutrophils (Keller 2006). The different susceptibility of the hosts may explain the discrepancies in the results of these recent studies and those of earlier ones, which suggested a protective role of neutrophils in the control of TB infection. While those early studies showing protection were conducted in mouse strains that were naturally resistant to TB, the later studies mainly focused on the role of these cells in TB susceptible mouse strains. Evidently, a more precise definition of the role played by neutrophils during infection will depend on an evaluation of the kinetics and magnitude of the response that these cells have in the early stages of the disease.

5.1.1.2. Mast cells

Mast cells are effector cells with a relevant role in allergic reactions (Woodbury 1984, Miller 1996, Galli 1999, Williams 2000); and are also critical for the development of a T helper 2 (Th2) response (Galli 1999, Metcalfe 1997). They are found in the mucosa of the respiratory, gastrointestinal, and urinary tracts and can also be observed in the vicinity of blood and lymph vessels.

These cells express a receptor with high affinity for IgE (FcεRI) and therefore this immunoglobulin is bound to their membrane. Upon the union of the antigen to the active sites of FcεRI-bound IgE, mast cells liberate several molecules, including preformed mediators and mediators synthesized *de novo* (Metzger 1992, Turner 1999, Williams 2000). Among the preformed mediators contained in mast cell granules are histamine, tryptase, chymase, carboxypeptidase, and heparin, while mediators synthesized *de novo* include leukotriene C4, prostaglandin D2, platelet-activating factor (PAF), tumor necrosis factor alpha (TNF-α), transforming growth factor (TGF-β), fibroblast growth factor 2 (FGF-2), vascular endothelial growth factor (VEGF), and interleukins IL-4, IL-5 and IL-8 (William 2000, Turner 1999, Sayama 2002).

Besides this interaction between IgE and the antigen, other agents are able to induce the activation of mast cells and the liberation of cytokines and other mediators. For instance, microbial products (Di Nardo 2003, Feger 2002) stimulate mast cells via two members of the toll-like receptor (TLR) family, TLR-2 and TLR-4 (Supajatura 2002, Sabroe 2002, McCurdy 2003).

The locations where mast cells are usually found are common gateways for infectious agents and there is evidence of these cells being excellent mediators of the inflammatory response (Williams 2000, Metcalfe 1997). At least in bacterial infections by *Klebsiella pneumoniae* and *Escherichia coli*, mast cells are required for the triggering of innate immunity (Malaviya 1996, Malaviya 2001). In addition, due to their strategic distribution within the lung, mast cells have a fundamental role in the

defense of the host against mycobacteria. An early study showed an increased number of mast cells and their degranulation in the lungs of animals experimentally infected with *M. tuberculosis* (Ratnam 1977). The presence of mast cells has also been described in the duodenum and the ileum of cows infected with *Mycobacterium paratuberculosis*, a microorganism that causes granulomatous enteropathic lesions (Lepper 1988). Muñoz *et al* (2003) demonstrated that there is an interaction between mast cells and *M. tuberculosis* through the CD48 molecule. This interaction triggers the release of preformed mediators, such as histamine and β -hexosamidase, and the liberation of *de novo* synthesized cytokines, such as IL-6 and TNF- α , which are involved respectively in the activation of neutrophils and the maintenance of the integrity of the granuloma (Muñoz 2003, Law 1996, Adams 1995). The secretory proteins *Mycobacterium tuberculosis* secreted antigen (MTSA-10) and 6-kiloDalton (kDa) early secretory antigenic target (ESAT-6) contribute to the activation not only of macrophages and dendritic cells but also of mast cells for the liberation of their pro-inflammatory mediators (Muñoz 2003, Trajkovic 2004).

5.1.1.3. Macrophages

The macrophage is the paradigmatic cell with regard to *M. tuberculosis* infection. Indeed, alveolar macrophages have been shown to play an essential role in the elimination of particles that enter the organism through the airways; and have long been considered the first cell population to interact with the tubercle bacillus. More macrophages are recruited afterwards from the bloodstream, and are in charge of maintaining the infection in the host (Dannenberg 1991, Dannenberg 1994).

The initial interactions of the bacilli with the macrophage take place through cellular receptors, such as receptors for Fc, complement (Schlesinger 1990), mannose (Schlesinger 1993), surfactant protein (Zimmerli 1996), CD14 (Peterson 1995), and CD43 (Randhawa 2005). Though it is unknown if the bacteria interact with one or more of these receptors during *in vivo* infection, the results of *in vitro* experiments suggest that the macrophage response depends on the type of receptor with which the bacteria interact. Their interaction with Fc receptors increases the production of reactive oxygen intermediates and allows the fusion of the bacteria-containing phagosomes with lysosomes (Armstrong 1975). On the other hand, interaction of the bacteria with the complement receptor 3 (CR3) prevents the respiratory burst (LeCabec 2000) and blocks the maturation of phagosomes harboring the bacteria, thus preventing fusion with lysosomes (Sturgill-Koszycki 1996).

The interactions of mycobacteria with members of the Toll-like receptor family have been studied for some years. TLR-2 (Brightbill 1999) and TLR-4 (Jeans

1999) are activated by several *M. tuberculosis* components. Among others, the 19-kDa lipoprotein and lipoarabinomannan (LAM) activate macrophages through TLR-2, promoting the production of IL-12 and inducible nitric oxide synthase (iNOS) (Brightbill 1999).

Regardless of the receptor with which the bacteria interact, it has been observed that the cellular cholesterol present in the macrophage cell membrane is an essential molecule for the internalization of the bacteria (Gatfield 2000). It is believed that cellular cholesterol works as a direct anchorage point for the bacterium and stabilizes its interaction with the macrophage membrane. Afterwards, the bacterium is efficiently internalized (Pieters 2001).

Once the bacteria enter the macrophage, they generally locate themselves in the mycobacterial phagosome (Armstrong 1971, Armstrong 1975). This structure derives from the plasma membrane and presents some cell surface receptors (Russell 1996, Hasan 1997). In contrast to normal phagocytosis, during which the phagosomal content is degraded upon fusion with lysosomes, the mycobacteria block this process (Armstrong 1971, Armstrong 1975).

This inhibition depends on an active process induced by viable mycobacteria, since dead bacilli can be easily found in lysosomal compartments (Armstrong 1971, Armstrong 1975). Besides having a different morphology, the vacuoles in which the bacteria reside present “early” endosomal compartment markers instead of the characteristic “late” endosomes (Hasan 1997, Clemens 1996, Baker 1997). In addition, these mycobacterial phagosomes retain “early” markers, such as Rab5 and Rab14 GTPases, and do not acquire the “late” Rab7 molecule; a finding which is also consistent with a blockage of the maturation process from early to late endosome (Via 1997, Kyei 2006).

Another characteristic of the mycobacterial phagosome is its limited acidification (Crowle 1991). Normally, material transported through an endosomal route finds an acidic medium due to the action of the vesicular proton-pump adenosine triphosphatase (V-ATPase) in the late endosome. It is suggested that such reduced acidification is the result of a low or zero concentration of V-ATPase in the mycobacterial phagosome (Sturgill-Koszycki 1994). A more recently described property is that this mycobacterial phagosome can not physically associate with iNOS (Miller 2004).

The inability of the mycobacterial phagosome to mature has been attributed to the active retention of a protein present in phagosomes, known as tryptophan aspartate coat protein (TACO), which was elegantly demonstrated by Ferrari *et al.* When these authors infected TACO-deficient cells, the maturation of mycobacterial

phagosomes was not arrested and therefore these cells were able to eliminate bacilli by fusion of phagosomes with lysosomes (Ferrari 1999). It is also worth noting that TACO binds itself to the plasmatic membrane of macrophages through cholesterol, which also plays an essential role in mycobacterial uptake by macrophages. These events show both molecules to be importantly associated in the mycobacterial mechanisms for survival (Gatfield 2000).

The inhibition of phagosome maturation by mycobacteria may be reverted by cytokines, such as interferon-gamma (IFN- γ) and TNF- α , which also stimulate microbicidal mechanisms, including the production of reactive oxygen and nitrogen intermediates (Flesch 1990, Chan 1992). The protective role of nitrogen intermediates has been demonstrated in different murine models (MacMicking 1997, Flynn 1998), and a similar function has been suggested for these molecules in human TB (Nicholson 1996). In contrast, the role played by the reactive oxygen intermediates during infection has not been completely explained, though it is known that hydrogen peroxide produced by macrophages activated by cytokines has a mycobactericidal activity (Walter 1981). Also, it has been found that the tubercle bacillus presents molecules, such as LAM and phenolic glycolipid I, which work as oxygen radical scavenger molecules (Chan 1989, Chan 1991).

5.1.1.4. Dendritic cells

Dendritic cells are clearly involved in the protective immune response to *M. tuberculosis* infection. As explained above, when *M. tuberculosis* bacilli are inhaled and phagocytosed by the pulmonary macrophages, they remain, and even replicate, within the cell phagosome. Dendritic cells recruited from blood, and probably also from lung tissues, may play a role in protective immunity since they are found in increased numbers in TB lesions (Sturgill-Koszycki 1994, Pedroza-González 2004, García-Romo 2004).

Dendritic cells recognize, capture and process antigens, thus being able to present them in the context of major histocompatibility complex (MHC) molecules, as well as through CD1 (Banchereau 1998, Gumperz 2001). Dendritic cells bind antigens via C-type lectin receptors and Fc γ /Fc ϵ receptors, and internalize them by endocytosis (Engering 1997, Fanger 1996, Jiang 1995). *M. tuberculosis* endocytosis is carried out through known C-type lectin receptors, such as dendritic cell-specific intercellular-adhesion-molecule-grabbing non-integrin (DC-SIGN) (Geijtenbeek 2003, Tailleux 2003). This molecule interacts with mannose capped-LAM, a component of the mycobacterial cell wall (Geijtenbeek 2003, Figdor 2002). In addition, peripheral blood dendritic cells and immature dendritic cells derived from monocytes express TLR-2 and TLR-4 (Jarrossay 2001, Kadowaki 2001), two Toll-like

receptors with which mycobacteria seem to interact. Thus, it can be assumed that a protective host response may be induced through these signals. Additional signals generated by the association of mannose capped-LAM to DC-SIGN induce IL-10 production (Geijtenbeek 2003), while the union of a 19 kDa *M. tuberculosis* lipoprotein to TLR-2 induces production of IL-12, TNF- α , and IL-6 (Means 2001, Means 1999, Underhill 1999).

Once the antigens have been captured and internalized, dendritic cells become mature (indicated by phenotypical and functional changes) and efficiently migrate to peripheral lymph nodes. There is evidence of *in vivo* *M. tuberculosis* and BCG transport from lung tissues to the lymph nodes inside infected dendritic cells (Dieu 1998). This migration of infected dendritic cells requires the expression of the chemokine receptor 7 (CCR7) on their surface, which makes them sensitive to chemokines (CC) CCL19 and CCL21 (Dieu 1998, Gunn 1998, Kriehuber 2001, Bhatt 2004). It is important to mention that maturation of dendritic cells is not only accompanied by an increased synthesis of MHC class I and II, but also by the expression of co-stimulating molecules, such as CD80 and CD86 (Turley 2000), and the production of IL-12 (Steinmann 2001).

The internalization of *M. tuberculosis* into human and murine dendritic cells has been observed in several *in vitro* (Bodnar 2001, Fortsh 2000, Giacomini 2001, Hanekom 2002, Henderson 1997, Inaba 1993) and *in vivo* (Jiao 2002, Pedroza-González 2004, García-Romo 2004) studies. Reportedly, when dendritic cells derived from monocytes are infected with *M. tuberculosis*, their ability to present lipidic antigens is impaired and thus the expression of CD1 decreases (Stenger 1998). Components of the mycobacterial cell wall were also shown to inhibit the phenotypical maturation of dendritic cells induced by lipopolysaccharides. Different lineages of *M. tuberculosis* may vary in the degree by which they affect the dendritic cells. In particular, the enhanced virulence ascribed to Beijing strains might well be related to their inability to stimulate dendritic cell maturation (Lopez 2003, Ebner 2001).

In a protective immune response, dendritic cells induce maturation of T cells towards a T helper 1 (Th1) profile by secreting cytokines, such as IL-12, IL-18, IL-23, and probably IFN- α and β , but not IFN- γ (Wozniak 2006, Kadowaki 2001, Kalinski 1999, Thurnher 1997). Th1 cells expand in response to the BCG antigens presented by the dendritic cells in the lymphoid nodules and migrate toward infection sites, such as the lung tissue, where they liberate IFN- γ , thus activating local macrophages that control bacilli replication (Humphreys 2006).

5.1.1.5. Natural killer cells

Natural killer cells play a very important role in the development of the innate immune response. Their main function has been associated with the development of cytotoxicity to target cells and they are among the first cell populations to produce IFN- γ during the immune response. For a long time, the study of this cell population was focused on their role in viral and tumoral diseases. More recently, however, increasing interest has arisen in their eventual function in several bacterial infections.

The number of natural killer cells was shown to increase in the lungs of C57BL/6 mice during the first 21 days after aerosol infection with *M. tuberculosis* complex strains. This cell expansion was associated with an increased expression of activation and maturation markers, and IFN- γ production. However, the depletion of natural killer cells had no influence on the lung's bacterial load, indicating that although these cells become activated during the early response in pulmonary TB, they are not essential for host resistance (Junqueira-Kipnis 2003). Natural killer cells also play an important role in human TB by regulating different aspects of the immune response. Human natural killer cells have been shown to have an enhanced cytotoxicity for macrophages infected with *M. tuberculosis*. They also optimize the ability of CD8⁺ T lymphocytes to produce IFN- γ and to lyse *M. tuberculosis* infected cells, thus joining innate to adaptive immune responses (Vankayalapati 2002, Vankayalapati 2004).

5.1.1.6. CD1d-restricted natural killer T cells

These are a unique subset of human natural killer T cells characterized by the expression of an invariant V α 24 T cell receptor that recognizes the nonclassical antigen-presenting molecule CD1d. The activity of CD1d-restricted killer cells is notably enhanced by the marine glycolipid alpha-galactosylceramide derived from sponges. Once activated by alpha-galactosylceramide, CD1d-restricted natural killer T cells contribute to human host defense against *M. tuberculosis* infection. Human monocyte-derived macrophages expressing CD1d can induce effector functions of natural killer T cells against cells infected with *M. tuberculosis* when activated with alpha-galactosylceramide. These functions include IFN- γ secretion, proliferation, lytic activity, and anti-mycobacterial activity; this latter via the antimicrobial peptide granulysin, which damages the mycobacterial surface. There is further support of the potential interaction of natural killer T cells with CD1d-expressing cells at the site of disease, since CD1d can be readily detected in granulomas of TB patients (Gansert 2003). Such a role has not been proved in *M. tuberculosis* infected mice. Rather, natural killer T cells have been shown to play a

detrimental role, at least in the late phase of mouse experimental infection (Sugawara 2002).

5.1.1.7. Epithelial cells

Alveolar macrophages have been considered for a long time to be the first cell population to interact with *M. tuberculosis*. However, the number of epithelial cells in the alveoli is 30 times higher than the number of macrophages and thus, the likelihood that they are the first cells exposed to the infecting bacilli is similarly higher. The first indication of the involvement of epithelial cells in *M. tuberculosis* infection was derived from a study where the presence of mycobacterial DNA was detected in necropsy specimens from people who had died from diseases other than TB. In that study, *M. tuberculosis* DNA was detected in macrophages, type II pneumocytes, fibroblasts, and endothelial cells (Hernandez-Pando 2000). In addition, several *in vitro* studies have characterized the interaction between epithelial cells and *M. tuberculosis*. These cells can host *M. tuberculosis* bacilli and allow their replication (Bermudez 1996). Moreover, epithelial cells are able to establish an initial pro-inflammatory environment by secreting IL-8 (Wickremashinge 1999) and inducing the production of nitric oxide (NO) (Roy 2004). Obviously, *in vivo* experiments are necessary to better understand the role played by alveolar epithelial cells in *M. tuberculosis* infection.

5.1.1.8. Defensins

A conspicuous element of the innate immune response against microorganisms is a group of small endogenous antimicrobial peptides known as defensins (Diamond 1998). These cationic peptides, consisting of approximately 30 to 50 amino acids, are present in myeloid and epithelial cells of all animal species. They were shown to display antibacterial (Gabay 1989, Ganz 1985, Selsted 1987), antifungal (Selsted 1985), and antiviral (Daher 1986) activities. These molecules are classified as alpha, beta, and theta defensins based on the position of cysteine residues and the number of disulfur bonds (Bals 2000, Hoover 2000, Lehrer 1993). In phagocytic cells, defensins represent the main microorganism destruction components independent of oxygen metabolism (Miyakawa 1996, Ogata 1992). Allegedly, these peptides break the membrane of several microorganisms and some of them are even able to pass through the cytoplasmic membrane and enter the infected cell (Ganz 2003, Rivas-Santiago 2006).

Defensins were first described in guinea pig and rabbit neutrophils (Zeya 1963, Zeya 1966). There is no report of human monocytes and macrophages having defensins, although neutrophils have been reported to have four known human neutrophil defensin peptides (Ganz 1990), of which three (HNP-1, HNP-2 and HNP-3) were found to be active against *Mycobacterium avium-intracellulare* and *M. tuberculosis* (Ogata 1992, Miyakawa 1996).

In vitro, the human alpha defensins present in human neutrophils directly attracts CD4+/CD45RA+ T cells, CD8+ cells, and dendritic cells. The expression of human beta-defensin 1 is constitutive in epithelial cells but the expression of human beta-defensins 2 and 3 is inducible by IL-1, TNF- α , and by Toll-like receptor recognition of bacteria and fungi (Kaiser 2000, Lehrer 1993, Stolzenberg 1997). Human beta-defensins are also chemoattractants for T CD4+/CD45RO+ cells through receptor CCR6 (Chertov 2000).

Mice infected with *M. tuberculosis* express murine beta defensins mBD-3 and mBD-4 n. In the first stages of infection, the epithelial cells of the respiratory tract express both defensins, which correlates to the early control of bacterial proliferation. However, their expression decreases as the disease progresses. In the latent infection model, mBD-3 and MBD-4 are continuously expressed, but their expression is suppressed if the infection is reactivated (Rivas-Santiago 2006).

Genetic expression of human beta-defensin 2 (HBD-2) has been identified in epithelial cells of the skin, lung, trachea, and urogenital system (Bals 1998, Kaiser 2000, Lehrer 1993, Linzmeier 1999, Singh 1998, Stolzenberg 1997). This defensin was also detected in bronchial lavage cells from patients infected with *M. avium-intracellulare* (Ashitani 2001). Peripheral blood monocytes transfected with human beta-defensin HBD-2 have a better control of *M. tuberculosis* growth than non-transfected monocytes (Kishik 2001). Human alveolar epithelial cells infected with *M. tuberculosis* were also found to express human beta-defensin HBD-2 (Rivas-Santiago 2005).

M. tuberculosis infected mice that have been treated with the defensin peptide HNP-1 show a reduction of bacterial load in the lungs, liver, and spleen (Sharma 2001). This observation suggests that defensins could represent important components of the innate response mechanisms against *M. tuberculosis* and could be used as new therapeutic tools.

5.1.2. Acquired immune response against *M. tuberculosis*

In contrast to innate mechanisms, the specific or adaptive immune response requires the specific recognition of foreign antigens. The innate immune system has a profound influence on the type of acquired immune mechanisms generated, and *vice versa*, the specific immune response executes several of its effector functions via the activation of components of the innate immunity. Specific immune responses can be divided into cell-mediated mechanisms, which include T-cell activation and effector mechanisms, and the humoral immune response, consisting of B-cell maturation and antibody production. Both mechanisms are not mutually exclusive, and T helper cells are required for antibody maturation, isotype switching and memory. B cells also function as antigen presenting cells by activating T cells in a specifically driven manner. In the following pages we will focus on the generation of both humoral and cellular immune responses against *M. tuberculosis*.

M. tuberculosis is the most conspicuous example of an intracellular bacterium that persists for long periods within the host, causing a latent infection, namely a chronic asymptomatic infection without tissue damage. This is best illustrated by the fact that two billion people worldwide are infected with *M. tuberculosis*, but more than 90 % of them remain healthy and free of clinical disease and the tubercle bacilli remain within them in a state of dormancy. Therefore, although the host cell-mediated immunity is enough to control the progression of disease, it fails to exert sterile eradication and hence, those two billion infected persons suffer the latent form of TB (Collins 2002).

As for other intracellular infections, the primary protective immune response is cell mediated rather than antibody mediated. *M. tuberculosis* resides inside the macrophage and is relatively resistant to microbicidal mechanisms that efficiently eliminate other phagocytosed bacteria. This is due in part to the ability of the tubercle bacilli to hinder macrophage activation by IFN- γ and IL-12. Several studies have confirmed the critical importance of these cytokines in both human and mice *M. tuberculosis* infection. In addition, deficiencies in IL-12 or IFN- γ , or their receptors, render the individual more susceptible to mycobacterial infections (Jouanguy 1999, Alcais 2005). For the last 20 years, it has been assumed that the induction of a Th1-type immune response affords the host the greatest protective capacity.

Despite the fact that there are hundreds of studies published on TB immunity, still there is a lack of information regarding important issues, such as the role of lung antigen presenting cells *in vivo* during pulmonary TB (Pedroza-González 2004). This type of information would allow a better understanding of the induction of

specific immune responses against *M. tuberculosis*, and therefore the development of tools that could control the disease more effectively.

5.1.2.1. Humoral immune response

Because of their intracellular location, it is frequently assumed that tubercle bacilli are not exposed to antibody and therefore this type of immune response is considered to be non-protective. However, during the initial steps of infection, antibodies alone or in conjunction with the proper cytokines may provide important functions, such as prevention of entry of bacteria at mucosal surfaces. Even though the issue remains controversial, the role of antibodies in intracellular bacterial infections has gained renewed attention. Lately, their participation in the control of acute infections, such as chlamydial respiratory infection (Skelding 2006), and chronic infections produced by Actinomycetes, including *M. tuberculosis* (Salinas-Carmona 2004, Williams 2004, Reljic 2006), was explored.

Antibodies can be exploited in two ways in the clinical management and control of TB: as serological diagnostic tools; and as active participants in protection. Serological methods have been regarded for a long time as attractive tools for the rapid diagnosis of TB due to their simplicity, rapidity, and low cost. As early as 1898, Arlöing showed that sera from TB patients could agglutinate tubercle bacilli (cited in Daniel 1987). With the introduction of the enzyme-linked immunosorbent assay in the '70s, interest was renewed and several groups of investigators committed themselves to finding an optimum antigen for TB serodiagnosis. At that time, complex antigens were used in most cases, such as whole bacteria, culture filtrates, bacterial extracts, tuberculins and their purified derivatives (PPD). More recently, individual purified antigens have also been assayed, including proteins, lipopolysaccharides and glycolipids, *i.e.*, Ag 85, 38-kDa protein, LAM or diacylthreosides. To date, however, no test has shown sufficiently high sensitivity and specificity values for diagnostic purposes (Al Zahrani 2000, Bothamley 1995, Singh 2003, Raqib 2003, Julián 2004, Lopez-Marin 2003, see also chapter 13).

As for their use in protection against TB, antibodies could enhance immunity through many mechanisms including neutralization of toxins, opsonization, complement activation, promotion of cytokine release, antibody-dependent cytotoxicity, and enhanced antigen presentation. In this sense, data from several laboratories indicate that anti-mycobacterial antibodies play an important role in various stages of the host response to TB infection (Costello 1992, Hoft 1999, Hoft 2002, Teitelbaum 1998, Williams 2004, De Vallière 2005). In particular, De Vallière *et al.* showed that specific antibodies increased the internalization and killing of BCG by neutrophils and monocytes/macrophages. Moreover, antibody-coated BCG bacilli

were more effectively processed and presented by dendritic cells for stimulation of CD4⁺ and CD8⁺ T-cell responses.

This enhanced anti-mycobacterial activity of phagocytes by antibody-coated bacilli is extremely important in the context of mucosal immunity. IgG and IgA antibody classes have been shown to be present in the mucosal secretions of the human lower respiratory tract (Boyton 2002). The specific mycobacterial targets for antibody-mediated enhanced interiorization and/or killing are not known, but surface antigens such as LAM or proteins expressed under stress conditions, such as alpha crystallin protein, may be relevant. In an experiment where 17 recombinant mycobacterial protein antigens, native Ag85 complex, LAM, and *M. tuberculosis* lysate were used to detect antibody responses induced by BCG vaccination, only LAM-reactive serum IgG responses were significantly increased in both BCG vaccinated individuals and active TB patients. As expected, oral BCG vaccination leads to a significant increase in LAM-reactive secretory IgA (Brown 2003).

A new approach toward protection against TB, using passive inoculation with IgA antibodies, was tested in an experimental mouse model of TB lung infection (Williams 2004). Intranasal inoculation of mice with an IgA monoclonal antibody against alpha crystallin protein reduced the *M. tuberculosis* colony up to 10-fold, forming units (cfu) in the lungs nine days after either aerosol or intra-nasal challenge. Both monomeric and polymeric IgA reduced cfu to the same extent, suggesting that the antibody may target the Fc alpha receptor (Fc- α R) rather than polymeric immunoglobulin receptor (poly-IgR) in infected lung macrophages. As expected, protection was of short duration, probably due to the rapid degradation of the intranasally-applied IgA.

More recently, in a follow-up of this study (Reljic 2006), the duration of protection was extended by inoculation of IFN- γ three days before infection, and further co-inoculation with IgA at different time points (2 h, 2 and 7 days) after aerosol infection with *M. tuberculosis* H37Rv. Instead of a 10-fold reduction in cfu, a 17-fold reduction was observed, as well as lower granulomatous infiltration of the lungs. Thus, the combined administration of IFN- γ and IgA shows promise as a prophylactic treatment of immunodeficient patients or as an adjunct to chemotherapy.

Taken all together, these findings suggest an urgent need to reassess the role of antibody responses in TB. In particular, the mechanism involved in antibody-mediated enhancement of innate and cell-mediated immunity should be addressed, in order to analyze whether these mechanisms could be exploited to develop better TB vaccines or to design alternative immunotherapeutic tools.

5.1.2.2. Cellular immune response

Since the tubercle bacilli reside inside a compartment within the macrophage, their antigens are presented by MHC class II molecules to CD4+ T lymphocytes. These cells play an important role in the protective response against *M. tuberculosis* and, when they are absent, growth of the bacilli cannot be controlled (Caruso 1999, Muller 1987). This is the case in patients with an immunodeficiency, such as that caused by HIV infection.

The main function of CD4+ T cells is the production of cytokines including IFN- γ , which activates macrophages and promotes bacilli destruction. Recently, another function has been ascribed to these cells, i.e., helping to develop the CD8+ T cell-mediated response (Scanga 2000, Serbina 2001). In the same way, CD4+ T cells may participate in the induction of apoptosis of infected cells and the subsequent reduction of bacterial viability through the CD95 Fas ligand system (Oddo 1998).

The participation of CD8+ T cells in the control of the infection is well recognized. Mice deficient in molecules such as CD8 α , transporter associated with antigen processing (TAP), and perforin, were shown to be more susceptible to *M. tuberculosis* infection than animals which produced these molecules (Flynn 1992, Behar 1999). The mechanisms used by these cells for the control of TB seem to be mainly cytokine production and bacterial lysis.

In the lungs of infected mice, CD8+ T cells showed to be able to secrete IFN- γ through activation of the T-cell receptor or by interaction with infected dendritic cells (Serbina 1999). Once again, the function performed by this IFN- γ is the activation of the macrophage and promotion of bacterial destruction.

In addition, CD8+ T cells proved to be efficient in lysing infected cells and in reducing the number of intracellular bacteria (Stenger 1997, Cho 2000). The mechanisms of control of the bacterial load seem to be associated with granular exocytosis involving perforin and granzymes. Still, granulysin, which is found in CD8+ T granules, is the molecule responsible for killing the bacterium (Stenger 1998).

5.1.2.3. Gamma/delta T cells

Previously, the CD4- CD8- T cells, known as $\gamma\delta$ T cells, were considered to play a low-profile role in the immune response. They were believed to proliferate only in response to non-peptidic antigens (Schoel 1994). As they were found in early lesions, they were thought to react to infected macrophages only through the production of IFN- γ (Ferrick 1995). In the last few years, however, $\gamma\delta$ T cells proved to be relevant for the regulation of the immune response.

High levels of $\gamma\delta$ T cells are usually found in the peripheral blood of TB patients (Ito 1992). *Ex vivo*, $\gamma\delta$ T cells from human TB patients display a lytic activity that is independent of the MHC. While the lytic activity of CD4+ and CD8+ T cells decreases gradually as the disease becomes more severe, $\gamma\delta$ T cells increase their activity, lysing target cells infected with *M. tuberculosis* through the Fas-FasL mechanism and the perforin pathway (De la Barrera 2003).

In a murine model of TB infection, $\gamma\delta$ T cells were shown to contribute to the elimination of *M. tuberculosis* and to have an anti-inflammatory effect. Indeed, when these cells are eliminated by genetic manipulation or by using a specific monoclonal antibody, inflammatory damage is accelerated in the lungs of mice infected with *M. tuberculosis* (D'Souza 1997, Ladel 1995). In addition, $\gamma\delta$ T cells produce IL-17 during early infection, which probably promotes the flow of cells towards infection sites. This IL-17 secretion may be produced in response to IL-23 secretion by dendritic cells infected with *M. tuberculosis* (Lockhart 2006).

The role of $\gamma\delta$ T cells in protective immunity is not limited to cytokine secretion (such as IL-17 and IFN- γ) and cytotoxic activity. These cells also behave as antigen-presenting cells. Like dendritic cells, they can process and efficiently present antigens and give the co-stimulating signals needed to induce proliferation of $\alpha\beta$ T cells (Brandes 2005). As noted, $\gamma\delta$ T cells act as a link between the innate immune response and the adaptive immune response, although other roles played by these cells still remain unknown.

5.2. Tuberculosis pathogenesis and pathology related to the immune response

An important reason for the current failure to control TB is that, even when applying the best available chemotherapy, treatment must be continued for at least six months. This treatment regimen is not a realistic option in limited-resource countries, or even in large cities of developed countries, because after a few weeks of treatment the patients start to feel well again and stop taking the drugs.

There are two main reasons for prescribing such long-term treatments. The first is that the antibiotics kill the vast majority of the bacilli within a few days, but persisting bacteria are not killed by the drugs. These persisting bacilli may be in a true stationary phase with very low metabolism, and may be non-replicating or replicating very slowly (latent infection). The other reason is the necrotizing tissue response that is analogous to the Koch phenomenon (Koch 1891). Robert Koch

demonstrated that the intradermal challenge of guinea pigs with whole organisms or culture filtrate, four to six weeks after the establishment of infection, resulted in necrosis at both the inoculation site and the original tuberculous lesion site. A similar phenomenon occurs in persons with active TB, in whom the PPD test site may become necrotic. Koch tried to exploit this phenomenon for the treatment of TB and found that subcutaneous injections of large quantities of culture filtrate (old tuberculin) into TB patients evoked necrosis in their tuberculous lesions. In fact, this treatment was shown to have extremely severe consequences associated with extensive tissue necrosis and was discontinued (Anderson 1891). Still today, the task for the researchers working in this field is to understand the differences between protective immunity and progressive disease, including the Koch phenomenon (Rook 1996). The final aim is to learn how to replace the pathological immune response by the protective one, and with such knowledge, to design short-course chemotherapy schemes supplemented with immunotherapy, which would enable TB control worldwide. These important topics on immunopathology and immunotherapy are revised in the following sections.

The role of individual immune cells in the protection against *M. tuberculosis* has been described in previous sections of this chapter. The next paragraphs of the 5.2 section describe patterns of immune response to infection and disease.

5.2.1. Mechanism of protective immunity

In the experimental murine TB model, the protective response has a distinct Th1 type of cytokine pattern, as demonstrated by manipulation of the immune system through genetic knockout or the administration of specific monoclonal antibodies (Cooper 1995, Dalton 1993, Flynn 1993). The same cytokine pattern seems to be protective in humans because children with defective receptors for IFN- γ or IL-12 are susceptible to mycobacterial disease (Jouanguy 1999, Alcaïs 2005).

In addition to these Th1-type cytokines, TNF- α is also essential for immunity to *M. tuberculosis* in mice (Kindler 1989) as well as in humans (Keane J 2001). These observations further suggest a role for NO, because TNF- α triggers the release of NO from IFN- γ -activated cells. In fact, iNOS expression is essential to control infection in mice (Chan 1992) and is also highly expressed in human tuberculous lesions (Schon 2004).

5.2.2. The immune response related to progressive disease: mechanisms of immunopathology

In the early '90s, some researchers reported an increased expression of Th2 cytokines, especially IL-4, in patients with advanced pulmonary TB (Schauf 1993, Sanchez 1994). Later, other authors failed to detect IL-4 and the issue remained controversial (Lin 1996). More recent data indicate that these failures could be attributed to technical problems (Rook 2004), and now there is substantial evidence that demonstrates a Th2 response in human TB (Seah 2000, Van Crevel 2000, Marchant 2001, Leinhardt 2002). Interestingly, IL-4 levels tend to be higher in patients living close to the Equator, possibly as a consequence of simultaneous infection with helminths and/or higher mycobacterium inoculum (Malhotra 1999). Indeed, TB patients have several IL-4-dependent manifestations, including high IgE anti-mycobacterial antibodies (Yong 1989), antibodies against cardiolipin (Fisher 2002), and high expression of DC-SIGN in dendritic cells (Relloso 2002). IL-4 has been detected in human TB lung lesions by in-situ hybridization (Fenhalls 2000). Also, IL-4 messenger ribonucleic acid (mRNA) and T-cells containing IL-4 are increased in human pulmonary TB. This high IL-4 expression correlates significantly with serum IgE, serum soluble CD-30, and also with the extent of cavitation (Seha 2000, van Crevel 2000, Lienhardt 2002). It has been demonstrated that CD8+ cells are another source of IL-4, and this correlates with cavitation (van Crevel 2000). The presence of IL-4 at late stages of the disease has a direct pathogenic role because it downregulates the protective Th1 responses (Biedermann 2001).

Similar abnormalities are also observed in the lungs of Balb/c mice, which have been experimentally infected via the trachea with a high dose of *M. tuberculosis* H37Rv (Hernandez- Pando 1996, Hernandez-Pando 1998). In this model, there is an initial phase of partial resistance dominated by Th1 cytokines plus activated macrophages that produce TNF- α and express iNOS. The phase of progressive disease starts after one month of infection. This late phase is characterized by a drop in the number of cells expressing INF- γ , IL-2, TNF- α , and iNOS, progressive pneumonia, extensive interstitial fibrosis, high bacillary counts and very high levels of IL-4 and TGF- β , produced by a distinctive type of macrophage with numerous cytoplasmic vacuoles (foamy macrophages) (Figures 5-2 and 5-3). It is important to point out that this animal model resembles the disease in developing countries, where the infecting dose is usually high and the progressive disease tends to show a high IL-4 response (Rook 2004). This Balb/c model can be considered a suitable model for human TB because it mimics the well-characterized response observed in the progressive human disease that consists of an increased expression of Th2

cytokines and TGF- β . This switch to Th2 cytokine production seen in Balb/c mice and, albeit to a lesser extent, in humans, is absent in the experimental TB model developed in C57Bl mice, which is characterized instead by progressive lung granulomas and extensive lung consolidation with a strong and sustained Th1 response (Flynn 2006).

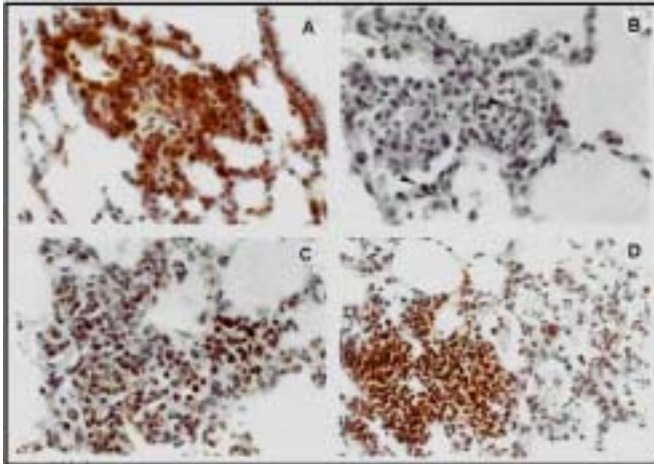


Figure 5-2: Immunohistochemical detection of IFN- γ (Th1 cells) and IL-4 (Th2 cells) in lung granulomas during the course of experimental TB in Balb/c mice infected by the intratracheal route. (A) Numerous IFN- γ immunostained cells (brown staining) exist in early granulomas, after 2 weeks of infection. (B) In contrast, few IL-4 positive cells (arrows) are seen in a serial section from the same granuloma exhibited in A. (C) After 4 months of infection, during active and advanced disease, there are some IFN- γ immunostained cells in granulomas. (D) Numerous IL-4 immunostained cells are seen in the same late granuloma exhibited in C. Thus, during early infection (first month) there is a predominance of Th1 cells, while during progressive disease a mixed Th1/Th2 pattern exist in this animal model.

In the Balb/c model of progressive pulmonary TB described above, the mixed Th1/Th2 cytokine pattern is associated with the pathology and reduced protection (Rook 1996). When pre-sensitized with 10^7 cfu of *Mycobacterium vaccae*, a saprophytic, highly immunogenic mycobacteria, mice infected with *M. tuberculosis* mount a strong Th1 response and are partially protected. In sharp contrast, when pre-immunized with a higher dose of the same mycobacterial preparation (10^9 cfu), mice develop a response with a mixed Th1/Th2 pattern that leads to increased severity of infection with the disease, and death (Hernandez-Pando 1994, Hernandez-Pando 1997). Thus, pre-exposure to saprophytic mycobacteria can determine either

resistance or susceptibility to *M. tuberculosis* infection, and the effect seems to be dose dependent. The nature, route, and dose of mycobacterial exposure depend on where and how an individual lives, because mycobacteria are not part of the usual commensal flora of human beings. This variable priming of antimycobacterial responses by saprophytes can either protect or predispose to infection, and might well be responsible for the uneven efficacy of BCG vaccination in different parts of the world (Rook 2005).

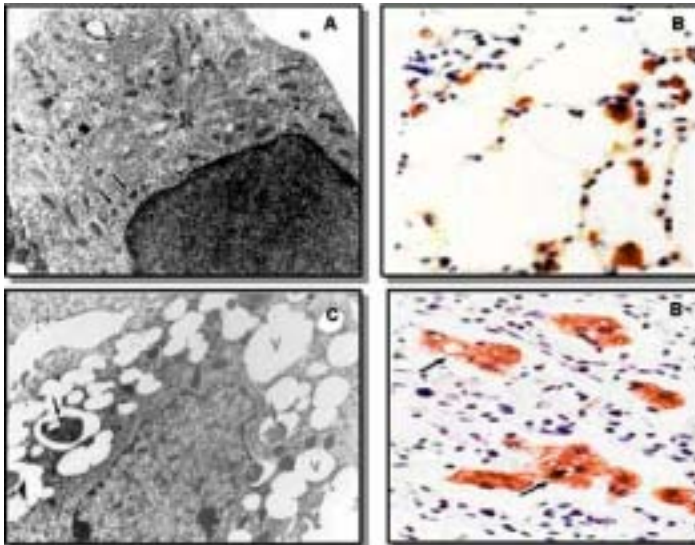


Figure 5-3: Representative immunohistochemical and electron microscopic features of lung macrophages during experimental pulmonary TB in Balb/c mice. (A) Subcellular structure of activated macrophage during early infection, numerous primary lysosomes (arrows) and occasional bacteria (asterisk) are distinctive elements in the abundant cytoplasm of this cell type. (B) These activated macrophages show strong TNF- α immunostaining. (C) During progressive disease, vacuolated or foamy macrophages containing numerous cytoplasmic vacuoles (V) and bacilli (arrows) are the predominant cells in the areas of pneumonia (E) These foamy macrophages show strong TGF- β immunostaining. Thus, activated macrophages are efficient producers of pro-inflammatory cytokines, such as TNF- α , and contribute to the control of the infection, while vacuolated macrophages are severely infected cells and efficient producers of anti-inflammatory cytokines that enable bacilli growth, such as TGF- β .

Various symptoms of TB, such as fever, weight loss and tissue damage, resemble the pathological effects of TNF- α . Evidence that such symptoms may be produced by this cytokine has come from experiments with thalidomide, a compound that

decreases the half-life of TNF- α mRNA (Moreira 1993). TB patients treated with this drug show rapid symptomatic relief and weight gain (Kaplan 1994). Thus, TNF- α has a paradoxical participation in the immunopathology of TB: it plays an essential role in protection but may also be a significant factor in its pathology. This ambiguous activity of TNF- α might be defined on site in the light of the predominant cytokine pattern, Th1 or Th2. In fact, the sensitivity of a given inflammatory site to TNF- α is dependent on the cytokine profile of the prevailing CD4+ T cells.

This ambiguous effect of TNF- α was also observed in Balb/c mice immunized with different doses of *M. vaccae* two months before challenge with fully pathogenic *M. tuberculosis*. Only a Th1 cytokine response was elicited in response to 10^7 cfu of *M. vaccae*, as demonstrated by high INF- γ production by splenocytes and high cutaneous delayed type hypersensitivity (DTH) against mycobacterial antigens. If 1 μ g of TNF- α was injected into the site of the DTH response (right footpad) that had been elicited 24 h earlier in such animals, no necrosis was caused. However, in mice that had been sensitized with a 100-fold higher dose (10^9) of *M. vaccae*, a mixed Th1/Th2 cytokine pattern was evoked, with very high IL-4 production that elicited much lower DTH reactions. In these animals, the injection of TNF- α at the site of DTH response elicited massive inflammation and local necrosis (Hernandez-Pando 1997). Therefore, a possible explanation of the TNF- α paradox can be proposed: it seems that the TNF- α was released into a relatively pure Th1-mediated inflammatory site, where it may act merely as a supplementary macrophage-activating cytokine. But, when released into a mixed Th1/Th2 site with high IL-4 concentration, it causes damage. These observations were confirmed in a further study using the Balb/c model of progressive pulmonary TB (Hernandez-Pando 2004). In the early stage of infection (21 days after infection), while the Th1 cytokine response predominated and controlled the growth of bacilli, the DTH response was the highest and DTH sites were not vulnerable to necrosis by TNF- α . In contrast, during the progressive phase of the disease (50 days after infection) extensive tissue damage and high IL-4 production are manifested, the DTH response was very low, and TNF- α administration in the DTH sites provoked extensive inflammation with necrosis. Moreover, mice that have been preimmunized with a high dose (10^9) of killed *M. vaccae*, which induces mixed Th1/Th2 cytokine responses with high IL-4 production, developed higher and more rapid TNF- α -sensitive DTH response and became more susceptible to intratracheal *M. tuberculosis* challenge than unimmunized control animals (Hernandez-Pando 1997). Thus, this immunopathological response is a clear reminder of the Koch phenomenon. Another experimental confirmation of the TNF- α -mediated immunopathology associated with IL-4 comes from IL-4 gene knockout of tuberculous Balb/c mice that exhib-

ited not only diminished bacterial proliferation, but also complete absence of TNF- α -mediated toxicity following a TNF- α challenge in the DTH sites (Hernandez-Pando 2004).

We have explained above that an inappropriate Th2 component is present in both murine and human TB. Its effect becomes more striking when the disease becomes more severe. What then are the likely causes of this shift in cytokine profile, and what is the participation of other factors that deregulate the protective immunity against TB? These questions will be addressed in the next section, but it is certain that there are many significant participant factors that we do not yet know about, and their characterization will contribute significantly to the knowledge of the immunopathology and control of this significant infectious disease.

5.2.3. Factors that deregulate the protective type 1 response

Figure 5-4 graphically summarizes the participating factors in protection and progression of pulmonary TB. An increase in antigen load is clearly a participating factor, as shown by the striking linkage of the Th1/Th2 balance to the dose after immunization with particulate antigens such as mycobacteria (Hernandez-Pando 1994) or *Leshmania* (Bretscher 1992). Thus, low antigen loads, such as the low dose of *M. vaccae* (10^7 cfu) used to presensitize mice in the above-mentioned experiments, or the relatively low bacterial lung burden during early infection in the Balb/c model of progressive pulmonary TB, prime the Th1 response. In contrast, high antigen loads, for example the 10^9 cfu of *M. vaccae* or the high bacillary loads produced in the lungs during the progressive phase of the Balb/c model, efficiently induce the Th2 response.

Another mechanism that participates in the declination of the Th1 cytokine pattern during progressive disease in the Balb/c model is the selective apoptosis of CD4+/Th1 cells (Rios Barrera 2006). Indeed, Th1 cell apoptosis can partly be induced by foamy macrophages through a Fas/Fas ligand mechanism. Foamy macrophages predominate in advanced TB, they contain numerous bacilli, and their cytoplasmic vacuoles display strong immunostaining for mycobacterial lipids such as LAM. Vacuolated macrophages show little immunostaining for TNF- α or iNOS, but strong TGF- β immunoreactivity (Hernandez-Pando 1997, Hernandez-Pando 2001), and also express high levels of the anti-apoptotic Bcl2 molecule. Due to these properties, foamy macrophages are long-lived cells that harbor mycobacteria for long periods, and at the same time are a significant source of immunosuppressing cytokines that facilitate bacilli proliferation.

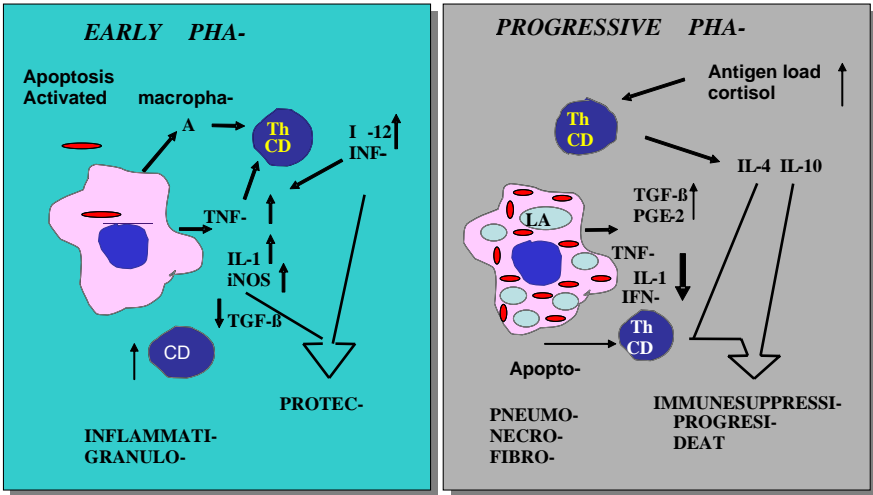


Figure 5-4: Left: Relevant immunopathological events during experimental pulmonary TB in Balb/c mice. During early infection, bacilli are efficiently phagocytosed by lung macrophages, which also secrete proinflammatory cytokines such as TNF- α and IL-1, and present bacillary antigens to Th1 cells. Th1 cells thus activated produce IL-12. Right: During progressive disease, Th2 cells emerge to deactivate Th1 cells, which together with overproduction of anti-inflammatory and immunosuppressive molecules such as cortisol, prostaglandin E (PGE) and TGF- β , deactivate macrophages, enabling bacilli growth and progressive tissue damage that causes death.

Another factor that deregulates the protective immune response against TB is prostaglandin production. Prostaglandins, in particular PGE-2, are potent mediators of intercellular communication. Indeed, at high concentrations PGE-2 is immunosuppressive for T-cell-mediated immunity (Phipps 1991). In the Balb/c model of *M. tuberculosis* infection, pulmonary PGE-2 concentrations remain relatively low and stable during the early phase, and if mice are promptly treated with niflumic acid, a potent and specific blocker of cyclo-oxygenase 2 (the rate limiting enzyme of prostaglandin production), infected lungs show a higher degree of inflammation and expression of TNF- α , IL-1 α and IFN- γ , but almost complete disappearance of iNOS expression, which coexists with a significant increment of the bacterial load. Interestingly, during the late progressive phase in this experimental model, foamy macrophages from the pneumonic areas exhibit strong PGE-2 immunostaining, and PGE-2 concentrations four fold higher than those of the early phase. When prostaglandin production was suppressed in animals suffering from advanced disease, a significant reduction of pneumonia and bacillary load, with a striking increment in

the size of the granuloma was seen, and the expression of IFN- γ , TNF- α and iNOS was also improved. Therefore, PGE-2 is a significant factor participating in the pathogenesis of pulmonary TB and has contrasting functions depending on its concentrations. During the early phase of the infection, the low PGE-2 concentrations contribute to iNOS expression, permitting the temporal control of bacilli growth; while the high PGE-2 concentrations during the late phase contribute to the down-regulation of cell-mediated immunity, allowing disease progression (Rangel 2002).

Excessive release of TGF- β has been implicated in the pathogenesis of human and murine TB. Blood mononuclear cells from TB patients were found to release increased levels of TGF- β , which was found in abundance in tuberculous lung lesions (Tossi 1995). Serum levels of TGF- β were strikingly raised in patients with advanced disease (Fiorenza 2005). The mannose-capped lipoarabinomannan from the cell wall of *M. tuberculosis* is a potent inducer of TGF- β (Dahl 1996). Interestingly, human blood monocytes and alveolar macrophages produced bioactive TGF- β upon stimulation with *M. tuberculosis*, so a signal from the organism was causing activation of TGF- β , as well as its secretion (Aung 2005). Several reports indicate that TGF- β also suppresses protective immunity to TB *in vivo*. In a mouse model, latency-associated peptide, a natural modulator of TGF- β function, decreased BCG growth in the lung and enhanced expression of IFN- γ (Wilkinson 2000). When mycobacterial infected guinea pigs were given intraperitoneal injections of recombinant human TGF- β , mycobacterial loads increased significantly (Dai 1999). In the Balb/c mouse model, high expression of TGF- β during the progressive phase of the infection was seen, and when TGF- β was blocked by recombinant β -glycan (type III TGF- β receptor) expression of IFN- γ and IL-2 increased with strong downregulation of IL-4, in co-existence with a significant reduction in bacterial counts in the lungs (Hernandez-Pando 2006), but with more lung consolidation by pneumonia than in non-treated control animals. Thus, TGF- β is a downregulator of cell-mediated immunity and a suppressor of excessive inflammation preventing tissue damage. This implies that the participation of classical regulatory cytokines, such as IL-10 or TGF- β , is necessary in order to avoid excessive inflammation, and to preserve the architecture and function of the lungs. Therefore, the fine balance between proinflammatory and anti-inflammatory cytokines seems to be a key factor in the immunopathogenesis of TB.

Fibrosis is a major cause of permanent respiratory dysfunction in TB. In human TB, fibrosis might be related to a high production of the potent fibrogenic cytokine TGF- β and to the presence of the Th2 response. Interestingly, pulmonary fibrosis in systemic sclerosis is associated with CD8⁺ cells secreting IL-4 (Atamas 1999).

Also, when IL-4 genes are knocked-out in Balb/c mice, the subsequent absence of IL-4 is associated with very low TGF- β production during the progressive phase of the disease, with lesser fibrosis and diminished bacterial growth (Hernandez-Pando 2004), confirming that Th2 cytokines are directly involved in the development of fibrosis, probably by inducing TGF- β production (Lee 2001).

Adrenal steroids may also contribute to the dysfunction of Th1 responses in TB. Reactivation or progression of infection is sensitive to activation of the hypothalamic-pituitary adrenal axis. The exposure of humans to the stress of war or poverty (Spence 1993), or cattle to the stress of transportation, is efficient in causing reactivation of latent infection. In mice, it has been demonstrated that this is due to glucocorticoid release (corticosterone in mice) (Brown 1995, Tobach 1956), which reduces macrophage activation and Th1-cell activity (Daynes 1991), while synergizing with some Th2 functions (Rook 1994). Tuberculous patients lose the circadian glucocorticoid rhythm, provoking constant exposure of peripheral lymphocytes to cortisol (Sarma 1990). In addition, the total output of cortisol derivatives and of androgens is frequently reduced (Rook 1996). Cortisol undergoes reduced conversion to inactive cortisone, producing normal serum cortisol concentrations in TB patients (Post 1994). The lung enzyme 11-beta-hydroxysteroid dehydrogenase converts inactive cortisone to active cortisol, producing higher concentrations of cortisol in the tuberculous lung (Rook 2000). In the Balb/c model the high production of TNF- α by activated macrophages in mature granulomas during the early phase of the infection (day 21), activates para-ventricular neurons in the hypothalamus, inducing higher expression of corticotrophin-releasing factor (Hernandez-Pando 1998). This factor induces adrenocorticotrophic hormone production in the pituitary and in turn, this hormone stimulates the adrenals to produce glucocorticoid. The stimulus is so strong that both adrenals duplicate their weight due to nodular and diffuse hyperplasia (Hernandez-Pando 1995). In consequence, high concentrations of corticosterone are produced, contributing to the activation of Th2 cells and bacilli cell growth. Perhaps this immuno-endocrine response is another mechanism to avoid excess lung inflammation due to the well-known anti-inflammatory activity of glucocorticoids, but at the same time, this response contributes to deregulation of the protective immunity and bacilli growth. Interestingly, during experimental late progressive disease, a striking adrenal atrophy is produced (Hernandez-Pando 1995). This situation is similar to the reduced adrenal reserve observed in patients with severe TB, that die suddenly and without obvious cause during treatment (Onwubalili 1986, Scott 1990). Occasionally, the adrenals are destroyed by TB, but there are patients whose adrenals are found on postmortem examination to be small and without evidence of direct infection, as in tuberculous mice. Interestingly, TNF- α and IL-1 are much more toxic in adrenalectomized than

in control animals (Zuckerman 1989, Bertini 1998). Thus, a reduced adrenal reserve could play a role in the above-described toxicity of TNF- α , and in the toxicity of TNF- α in tuberculous mice once they have entered the phase of adrenal atrophy in the late progressive stage of the disease (Hernandez-Pando 1995). It is also important to consider that the function of cortisol within lymphoid tissue is regulated by local production of the metabolites of dehydroepiandrosterone sulfate, an androgenic adrenal steroid that has “anti-glucocorticoid effects”, inducing strong activation of Th1 cells (Hernandez-Pando 1998). Administration of dehydroepiandrosterone or its derivative 3,17-androstenediol causes a Th1 bias, so this could be an efficient form of immunotherapy, as discussed below.

5.2.4. Susceptibility to tuberculosis: the importance of the pathogen

From the first exposure to *M. tuberculosis*, the host immune system triggers a series of responses which define the course of infection. However, this defense is not uniform in exposed persons. As mentioned above, the vast majority never develop active disease (Bloom 1992), but in those persons that become sick, a wide spectrum of possible clinical manifestations may occur, and the immune response, as seen for example in *in vitro* T- and B-cell reactivity against mycobacterial antigens, differs significantly from person to person. Thus, the clinical course of the infection and its epidemiological consequences depend on a complex interplay of host, environmental and bacterial factors (Nardell 1993, Hill 1998, Bellamy 1998, Stead 1992, Kramnik 2000).

As mentioned above, environmental factors that determine TB susceptibility include poverty, malnutrition, stress, overcrowding, and exposure to mycobacterial saprophytes. In the host, there is evidence of multifactorial genetic factors that influence susceptibility to *M. tuberculosis* (see Chapter 6). Mouse genes that participate in the control of early mycobacterial multiplication or TB progression in the lungs have been distinguished (Kramnik 2000). However, it seems that the independent participation of these genes is not sufficient to confer full protection against virulent *M. tuberculosis*.

As illustrated in this chapter, the host immune response against mycobacterial infection is the most investigated factor; but recent studies indicate that the genetic variability of *M. tuberculosis* has a significant role in the outcome of the infection. For many years, *M. tuberculosis* was considered to be highly conserved with a high degree of sequence homology and lack of antigenic diversity (Kapur 1994, Kremer 1999). Therefore, most of the immunological research has been done with a limited number of laboratory strains, including H37Rv or Erdman. However, DNA finger-

printing techniques have demonstrated a high degree of DNA polymorphism in the genome of *M. tuberculosis*, associated with repetitive DNA sequences and insertion elements (van Embden 1993). This genetic variability is related to recent epidemiological data indicating striking differences in virulence and transmissibility (Valway 1998, Caminero 2001). Particular outbreak strains were found to elicit distinct immune paths and mortality rates in the course of experimental infection. The investigation of an outbreak produced by a newly identified, genetically distinct *M. tuberculosis* strain named CDC1551 revealed that this strain produced a high rate of transmission in humans; in mice, it induced higher levels of TNF- α , IL-10, IL-6 and IFN- γ (Valway 1998). The genetic comparison between *M. tuberculosis* H37Rv and CDC1551 demonstrated single nucleotide polymorphism in many different genes. The clinical and epidemiological differences in this strain have therefore now been linked with immunological and genetic differences (McShane 2003). In another study, the clinical isolate HN878 was found to be hypervirulent and mice infected with this strain failed to induce a Th1 response, with lower levels of IFN- γ and TNF- α in the infected lungs (Manca 1999).

Using the Balb/c mouse model of progressive pulmonary TB, 12 distinct strains of *M. tuberculosis*, defined on the basis of IS6110 RFLP patterns, and representing four major genotype families found throughout the world, were studied (Lopez 2003). This study demonstrated marked differences in virulence, cytokine induction and immunopathology among the different strains. In comparison, with animals infected with the laboratory strain H37Rv used as a control, mice infected with Beijing strains induced significantly high mortality, high bacillary load and a non-protective pattern of cytokine production (low IFN- γ expression with high but ephemeral TNF- α and iNOS production). “*M. canettii*” strains induced long survival with low bacillary load and significantly fewer areas of pneumonia in coexistence with constant and stable expression of IFN- γ , TNF- α and iNOS. The differences among other strains were less marked and showed intermediate rates of survival. Interestingly, the protective efficacy of BCG against the different strains of *M. tuberculosis* was found to vary and BCG was least protective against the hypervirulent Beijing strain 9501000 (Lopez 2003). This is important, considering that the Beijing genotype is the predominant strain in several distinct geographical areas, presumably due to a selective advantage over other strains (van Soolingen 1995).

The use of microarrays would help to characterize the genetic differences between these strains. Perhaps specific SNPs could be identified facilitating the identification of virulence genes, which will allow the development of attenuated strains and potential vaccine candidates (Hernandez-Pando 2006). Thus, further animal studies

using different clinical isolates and mutant strains are necessary to evaluate how the genetic differences translate into functional differences.

5.3. Latency and maintenance of the immune response

M. tuberculosis is a pathogen capable of producing both progressive disease and latent infection (Parrish 1998). The initial infection usually occurs in the lungs and in most cases is controlled by the immune system. Only 10 % of these infections lead to progressive disease (Sudre 1992, Parrish 1998). Even after successful control of the primary TB infection, some bacilli remain in a non-replicating or slowly replicating dormant state for the rest of the life of the individual. This infectious state, termed latent TB infection, is clinically asymptomatic, and most active TB cases arise as a result of reactivation of dormant bacilli (Parrish 1998, Dolin 1994). Up to one third of the world's population is estimated to carry latent *M. tuberculosis* infection, and hundreds of millions of TB reactivations are anticipated specifically in areas of low or moderate endemicity, where most cases of active TB result from reactivation of latent infection (Parrish 1998, Fine 1999).

It has been established that the low concentrations of oxygen and nutrients in chronic granulomas that remain after efficient control of the primary infection, as well as the local production of TNF- α and NO, are significant factors for the induction and maintenance of latent infection (Parrish 1998, Voskuil 2003, Arriaga 2002, Flynn 1998). Indeed, immunological studies in animal models of latent TB have demonstrated that cytokines such as TNF- α and IFN- γ , as well as NO contribute significantly to maintaining infection in the latent state (Arriaga 2002, Flynn 1998). These types of immune responses, of which the tuberculin skin test is a conspicuous exponent, is also crucial for protection in latently infected individuals.

It has been shown that in a well-characterized experimental latent infection model, as well as in necropsy tissues from humans with latent TB, mycobacterial DNA can be detected by *in situ* PCR in a variety of cell types in the histologically normal lung, including epithelial cells (Hernandez-Pando 2000, Arriaga 2002). This could be an efficient mechanism of the bacilli to evade elimination. As these cells are considered to be non-professional phagocytes, they can not destroy ingested bacilli or present antigens in the MHC II context. Interestingly, in the mouse model of latent infection, we occasionally observed intracytoplasmic bacilli in bronchial cells and type 2 pneumocytes, suggesting that, as happens *in vitro* (Bermudez 1996), mycobacteria can also infect the lung epithelium during experimental latent infection, but in a microenvironment that is completely different from that found in

chronic granulomas. Infected lung epithelium is directly exposed to the air, so perhaps the efficient growth control or elimination of these bacilli during latent infection could be mediated by natural antimicrobial peptides such as NO or β -defensins. It is also important to consider that epithelial cells have a short life span, thus the maintenance of latent bacilli in this cell type should be different from those located in chronic granulomas.

Apart from the lung and the lymph nodes, other organs and tissues are likely to host persistent bacilli during TB latency. Indeed, nearly 15 % of the cases of reactivated TB occur at extrapulmonary sites, without apparent pathology in the lungs (Farer 1979, Hopewell 1994). In those cases, it is likely that the growth of the bacilli resumes directly from the reactivation site rather than from pulmonary sites. In this regard, it has recently been demonstrated that adipose cells could be one of these sites. *M. tuberculosis* can infect mature adipocytes by interaction with CD36, and intracellular bacilli cannot replicate and are not accessible to antibiotics. Moreover, by in-situ PCR, it was demonstrated that adipose tissue from individuals that died from causes other than TB frequently showed mycobacterial DNA, suggesting dormant bacilli infection (Neyrolles 2006).

5.4. Immunotherapy for tuberculosis

The current six-month regimens for TB treatment are too long, causing problems in logistics and compliance.; and they often lead to the development of drug resistance, which can be extreme, as demonstrated by the currently emerging extensively drug resistant TB (XDR-TB) (CDC 2006). These are the most important reasons for seeking efficient immunotherapeutic regimens in TB treatment. Immunotherapy aims at reverting the non-protective immune response, which is usually elicited during the progressive phase of the disease, to the protective Th1 response, and thus to cure TB or act as an adjunct to shorten conventional chemotherapy. Animal work suggests that it might be possible to potentiate the immune system to destroy the organisms more efficiently and even to eliminate bacilli that persist in latently infected tissues.

Following Robert Koch's discovery of *M. tuberculosis*, attempts at immunotherapy were made by Koch himself using subcutaneous injections of *M. tuberculosis* culture filtrate, and by other researchers, such as Macassey and Jousset in 1934, who used antisera raised in animals. As mentioned before, the injection of culture filtrate led to necrotic reactions, both at the site of injection and in distant lesions, and was abandoned (Anderson 1891). However, during the last decade, several studies have demonstrated striking therapeutic effects in experimental animal models by

manipulating the immune response. In general, two approaches have been used: the first one consists of direct stimulation of the Th1 response; and the second aims at the inactivation of factors that suppress the cellular immune response, which are also, usually natural anti-inflammatory factors.

5.4.1. Immunotherapy induced by direct stimulation of the protective Th1 response

The adrenal steroid dehydroepiandrosterone and its derivative, $3\beta,17\beta$ androstenediol, are efficient inducers of Th1 cell activation, favoring the production of the cytokines IFN- γ and TNF- α ; both essential for protection against TB. Administration of either hormone has previously been shown to improve the course of pulmonary TB in Balb/c mice (Hernandez-Pando 1998). Both compounds were protective, particularly $3\beta,17\beta$ androstenediol, which caused reduction in bacterial counts and prolonged survival. The effects correlated with reduced expression of IL-4, and increased expression of IL-2 and TNF- α . However, these hormones have androgenic activities precluding their use in human TB. More recently, the efficacy of dehydroepiandrosterone synthetic analogs, such as 16α -bromo- 5α -androstan- 3β -ol-17-one (HE2000), were tested in the same model. HE2000 is a dehydroepiandrosterone derivative that does not enter sex steroid pathways, and therefore is more suitable for prolonged administration. When tuberculous Balb/c mice suffering from extensive disease were treated with HE2000, a significant inhibition of bacterial proliferation, as well as an increased expression of TNF- α , IFN- γ , and iNOS were observed, while expression of IL-4 was decreased. Moreover, when given as an adjunct to conventional chemotherapy, HE2000 further enhanced bacterial clearance (Hernandez-Pando 2004). The immunological mechanisms underlying the effects of HE2000 are not understood. It is also interesting that when HE2000 is administered as monotherapy in treatment-naïve patients with HIV-1, a significant increase is observed in the number of circulating IFN- γ + CD8+ T cells and in the CD8+ T-cell response against two distinct GAG peptide pools (Reading 2006).

Transfer factors or leukocyte dialyzates are subcellular leukocyte components that appear to be able to transmit information for specific immune responses from experienced or memory leukocytes to naïve leukocytes (Lawrence 1955). The chemical nature of transfer factors has been difficult to elucidate, because they contain many small molecular weight components (Rozzo 1992). It seems that some transfer factor peptides correspond to the amino terminal ends of enkephalins (Sudhir

1988), being very efficient factors to enhance cell-mediated immune responses (Fudenberg 1993).

Since the discovery of transfer factors, 50 years ago, the most important and interesting aspect has their therapeutic applications (Fudenberg 1993). There are many clinical reports that show the usefulness of transfer factors as efficient immunotherapeutic agents in clinical conditions characterized by inappropriate or deficient cell-mediated immune response, including different infectious diseases (Bullock 1972), some neoplastic diseases, and primary immunodeficiencies (Levin 1970, Whyte 1992). When treated with transfer factors obtained from spleen cells or peripheral blood cells of tuberculous mice or humans, Balb/c mice in a late phase of progressive pulmonary TB were able to restore the expression of Th1 cytokines, TNF- α and iNOS, to inhibit bacterial proliferation, increase DTH response, and prolong survival. This beneficial effect was dose dependent and had a synergistic effect when combined with conventional chemotherapy. Indeed, in the combined treatment, murine transfer factors eliminated bacteria from the lungs significantly faster than chemotherapy alone (Fabre 2004).

Heat-shock protein 65 (Hsp65), the mycobacterial homolog of a human stress protein, heat-shock protein 60, evokes a marked immune response in infected animals, in spite of also being highly homologous to the host stress protein (Lamb 1989, Kaufmann 1991). In addition to any putative regulatory role, a major component of the response to Hsp65 is the effect of CD8⁺ cytotoxic T lymphocytes that are protective in animal models (Bonatto 1998). Indeed, Hsp65-responsive CD8⁺ cytotoxic T lymphocytes can lyse *M. tuberculosis* infected macrophages (Silva 2000). A DNA vaccine containing the *M. leprae hsp65* sequence was therapeutic in tuberculous mice (Lowry 1999). Some researchers have not reproduced all these effects in the absence of any simultaneously administered chemotherapy, but a striking synergy with chemotherapy has been demonstrated repeatedly (Silva 2005, Nuermberger 2005). Another significant effect of the vaccine was the downregulation of IL-4-secreting T cells (Lowry 1999).

Many of the properties of the *hsp65* DNA vaccine are shared by the highly immunogenic saprophytic mycobacteria *M. vaccae*, including the induction of CD8⁺ cytotoxic T lymphocytes, and the downregulation of IL-4. Recombinant *M. vaccae* is a Th1 adjuvant for antigens expressed within it (Hetzel 1998, Abou-Zeid 1997), and immunization with heat-killed *M. vaccae* results in the generation of CD8⁺ T cells which kill syngeneic macrophages infected with *M. tuberculosis* (Skinner 1997). This property was associated with Hsp65 (Skinner 2001). Most antigens of *M. vaccae* are cross-reactive with those of *M. tuberculosis*, so it is not surprising that *M. vaccae* is able to induce a cytotoxic T cell response to *M. tuberculosis*. In

fact, the therapeutic effect of heat-killed *M. vaccae* in a Balb/c model of pulmonary TB was first published in 1996 (Rook 1996). Then in a more detailed study, it was shown that when given on days 60 and 90 after intrapulmonary infection, without any chemotherapy, *M. vaccae* caused a 1-2 log fall in bacterial counts, more granuloma, less pneumonia, and a large reduction in expression of IL-4 in granulomas (Hernandez-Pando 2000). There was an increase in IL-2 and TNF- α (Hernandez-Pando 2000).

RUTI is an experimental therapeutic vaccine made of fragmented *M. tuberculosis* delivered in liposomes made of phosphatidyl choline and sodium cholate (Cardona 2005). RUTI might be given after the initial phase of chemotherapy, when the bacterial load is greatly reduced, in order to accelerate destruction of the remaining organisms. Bacillary loads were significantly reduced with the administration of RUTI to experimentally infected animals after the termination of chemotherapy. This therapeutic effect was most likely due to the induction of CD8+ IFN- γ + T cells in the lungs of treated animals (Cardona 2006). RUTI also induced a strong antibody response. Indeed, when these antibodies were passively transferred to SCID mice that had been infected with *M. tuberculosis* H37Rv and treated with a non-sterilizing drug regimen, sera from RUTI-treated tuberculous animals showed a reduction in the growth rate of the bacilli. In the absence of chemotherapy, however, RUTI had no therapeutic effect on late progressive disease.

5.4.2. Immunotherapy induced by suppression of the immunomodulatory anti-inflammatory response

As described before, excessive Th2 cytokine production and release of prostaglandin E and TGF- β have been implicated in the pathogenesis of TB. Thus, in addition to induction of Th1 or CD8+ cytotoxic T cell lymphocytes, downregulation of IL-4 or TGF- β is emerging as a promising immunotherapeutic protocol for established disease, with or without concomitant chemotherapy.

Evidence of the role of the Th2 response in corrupting protective functions and leading to immunopathology and fibrosis has already been described and reviewed elsewhere (Rook 2005). It is important to mention that IL-4 levels are higher in developing countries where BCG fails (Rook 2004). This may be due to both genetic (Flores Villanueva 2005) and environmental reasons, for example other tropical infections such as Th2-inducing helminthiases (Malhotra 1999). The production of IL-4 has detrimental effects in TB (Rook 2004, Rook 2005), including inhibition of apoptosis of macrophages infected with mycobacteria, and increasing intracellular availability of iron (Khanert 2006). Therefore, the effect of injecting neutral-

izing antibodies against IL-4 has been tested as a therapy for TB in Balb/c mice (Lowry 2005). A striking therapeutic effect was seen (Lowry 2005), even when monoclonal anti-IL-4 antibodies were administered as late as day 110 after infection. In this regard, it has been demonstrated that another property of *M. vaccae* is that it downregulates Th2 responses (Tukenmenz 1999, Ozdemir 2003, Zuany Amorim 2002, Hopfenspirger 2001). This ability to suppress Th2-mediated pathology is also seen when killed *M. vaccae* are given after the induction of the Th2 response has taken place (Zuany Amorim 2002, Wang 1998, Hunt 2005). The mechanism by which *M. vaccae* causes Th2 suppression is through the induction of CD25⁺ CD45RB^{low} regulatory T cells (Zuany Amorim 2002). This saprophyte microorganism can also inhibit an ongoing Th2 response in an allergy model, and it is at least as potent by the oral route as it is by the subcutaneous route (Hunt 2005). Therefore, the oral route should be effective in the mouse TB model. As shown for *M. vaccae* given by the subcutaneous route (Hernandez-Pando 2000), when administered by the oral route, killed *M. vaccae* organisms showed a high therapeutic value when given at the start of the infection and a less pronounced, but still significant, effect when administered after day 60 of infection (Hernandez-Pando and Rook, manuscript in preparation).

TGF- β , a potent cell-mediated immune response suppressant and anti-inflammatory cytokine, has also been implicated in the pathogenesis of TB. Blood mononuclear cells from TB patients were shown to release increased levels of TGF- β (Toossi 1995, Dluogovitzky 1999); and Balb/c mice infected by the intratracheal route showed very high expression of TGF- β during the progressive phase of the infection. Treatment with recombinant β -glycan (type III TGF- β receptor) a potent inhibitor of TGF- β , caused increased expression of IFN- γ and IL-2, with strong downregulation of IL-4, and a significant reduction in lung bacterial counts to an extent similar to that achieved by conventional antimicrobial treatment (Hernandez-Pando 2006), but with more lung pneumonic consolidation than non-treated control animals. Thus, as discussed before, TGF- β is a downregulator of cell-mediated immunity and a suppressor of excessive inflammation, thus preventing tissue damage.

TGF- β exhibits a similar immunosuppressive function in the presence of high concentrations of PGE-2, and indeed, high amounts of this molecule are produced in the lungs during late phase TB, which contributes to the modulation of the cellular immune response (Rangel 2002). What is more, the combination of soluble beta glycan and the anti-inflammatory drug niflumic acid, which blocks PGE-2 synthesis, produces a significant reduction in bacillary loads and has a significant synergistic effect on TNF- α , controlling inflammation. When this combination is used

together with chemotherapy, the effects are partly additive (Hernandez-Pando 2006).

5.5. Concluding remarks

Studies on the mechanisms of disease caused by infectious agents demand a broad understanding across many specialized areas, as well as much co-operation between clinicians and experimentalists. In fact, *M. tuberculosis* infection is a fascinating model to study diverse immunopathological mechanisms because it causes a chronic disease, which provokes substantial abnormalities in the function and regulation of the immune system. Moreover, investigation of TB has also contributed seminal concepts to basic immunology. In the mid '40s, Merrill Chase demonstrated that tuberculin hypersensitivity could not be transferred by serum from skin-positive to skin-negative guinea pigs, but only by means of cells, setting the basis of cellular immunology. In the late '60s, one of Merrill Chase's graduate students, Barry Bloom, who was using mycobacterial antigens, discovered cellular mediators produced by the immune cells, such as the migration inhibitory factor, the first described lymphokine. This set the basis for the study of cytokines as essential mediators in the immune response. Thus, investigation of TB has been extremely useful in the development of immunology and immunopathology, and many concepts emerging as a consequence of ongoing research of this type will eventually contribute to novel approaches for better control of this significant infectious disease.

References

1. Abou-Zeid C, Gares M-P, Inwald J, et al. Induction of a type 1 immune responses to a recombinant antigen from *Mycobacterium tuberculosis* expressed in *Mycobacterium vaccae*. *Infect Immun* 1997; 65: 1856-62.
2. Adams LB, Mason CM, Kolls JK, Scollard D, Krahenbuhl JL, Nelson S. Exacerbation of acute and chronic murine tuberculosis by administration of a tumor necrosis factor receptor-expressing adenovirus. *J Infect Dis* 1995; 171: 400-5.
3. Al Zahrani K, Al Jahdali H, Poirier L, Rene P, Gennaro ML, Menzies D. Accuracy and utility of commercially available amplification and serologic tests for the diagnosis of minimal pulmonary tuberculosis. *Am J Respir Crit Care Med* 2000; 162: 1323-9.
4. Alcais A, Fieschi C, Abel L, Casanova JL. Tuberculosis in children and adults: two distinct genetic diseases. *J Exp Med* 2005; 202: 1617-21.
5. Anderson MC. On Koch's treatment. *Lancet* 1891; 1: 651-2.
6. Appelberg R, Castro AG, Gomes S, Pedrosa J, Silva MT. Susceptibility of beige mice to *Mycobacterium avium*: role of neutrophils. *Infect Immun* 1995; 63: 3381-7.

190 Immunology, Pathogenesis, Virulence

7. Armstrong JA, Hart PD. Phagosome-lysosome interactions in cultured macrophages infected with virulent tubercle bacilli. Reversal of the usual nonfusion pattern and observations on bacterial survival. *J Exp Med* 1975; 142: 1-16.
8. Armstrong JA, Hart PD. Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. *J Exp Med* 1971; 134: 713-40.
9. Arriaga AK, Orozco EH, Aguilar LD, Rook, GAW, Hernandez-Pando R. Immunological and pathological comparative analysis between experimental latent tuberculosis infection and progressive pulmonary tuberculosis. *Clin Exp Immunol* 2002; 128: 229-37.
10. Ashitani J, Mukae H, Hiratsuka T, Nakazato M, Kumamoto K, Matsukura S. Plasma and BAL fluid concentrations of antimicrobial peptides in patients with *Mycobacterium avium-intracellulare* infection. *Chest* 2001; 119: 1131-7.
11. Atamas SP, Yurovski VV, Wise R, et al. Production of Type 2 cytokines by CD8+ lung cells is associated with greater decline in pulmonary function in patients with systemic sclerosis. *Arthritis Rheum.* 1999; 42: 1168-79.
12. Aung H, Wu M, Johnson JL, Hirsch CS and Toossi Z. Bioactivation of latent transforming growth factor beta 1 by *Mycobacterium tuberculosis* in human mononuclear phagocytes. *Scand J Immunol* 2005; 61: 558-65.
13. Bals R, Wang X, Wu Z, et al. Human beta-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung. *J Clin Invest* 1998; 102: 874-80.
14. Bals R. Epithelial antimicrobial peptides in host defense against infection. *Respir Res* 2000; 1: 141-50.
15. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998; 392: 245-52.
16. Behar SM, Dascher CC, Grusby MJ, Wang CR, Brenner MB. Susceptibility of mice deficient in CD1D or TAP1 to infection with *Mycobacterium tuberculosis*. *J Exp Med* 1999; 189: 1973-80.
17. Bellamy RC, Ruwende TC, Corrah TKP, Mc Adams KP, Whittle HC, Hill AV. Variation in the NRAMP1 gene and susceptibility to tuberculosis in West Africans. *N Engl J Med* 1998; 338: 640-4.
18. Bermudez LE, Goodman J. *Mycobacterium tuberculosis* invades and replicates within type II alveolar cells. *Infect Immun* 1996; 64: 1400-6.
19. Bertino R, Bianchi M, Ghezzi P. Adrenalectomy sensitizes mice to the lethal effects of interleukin 1 and tumor necrosis factor. *J Exp Med* 1988; 167: 1708-12.
20. Bhatt K, Hickman SP, Salgame P. Cutting edge: a new approach to modeling early lung immunity in murine tuberculosis. *J Immunol* 2004; 172: 2748-51.
21. Biedermann T, Zimmermann S, Himmelrich H, et al. IL-4 instructs TH1 responses and resistance to *Leishmania major* in susceptible Balb/c mice. *Nat Immunol* 2001; 2: 1054-60.
22. Bloom, B. R. and Murray, C. J., Tuberculosis commentary on a reemergent killer. *Science* 1992; 257:1055-64.
23. Bodnar KA, Serbina NV, Flynn JL. Fate of *Mycobacterium tuberculosis* within murine dendritic cells. *Infect Immun* 2001; 69: 800-9.
24. Bonatto VL, Lima VM, Tascon RE, Lowrie DB, Silva CL. Identification and characterization of protective T cells in hsp65 DNA-vaccinated and *Mycobacterium tuberculosis*-infected mice. *Infect Immun* 1998; 66: 169-75.
25. Bothamley GH. Serological diagnosis of tuberculosis. *Eur Respir J Suppl* 1995; 20: 676s-688s.

26. Boyton RJ, Openshaw PJ. Pulmonary defences to acute respiratory infection. *Br Med Bull* 2002; 61: 1-12.
27. Brandes M, Willmann K, Moser B. Professional antigen-presentation function by human $\gamma\delta$ T Cells. *Science* 2005; 309: 264-8.
28. Bretscher P, Wei G, Menon JN, Bielefeldt-Ohmann. Establishment of stable cell-mediated immunity that makes "susceptible" mice resistant to *Leshmania major*. *Science* 1992; 257: 539-42.
29. Brightbill HD, Libraty DH, Krutzik SR, et al. Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. *Science* 1999; 285: 732-6.
30. Brown DH, LaFuse W, Zwilling BS. Cytokine mediated activation of macrophages from *Mycobacterium bovis* BCG resistant and susceptible mice, differential effects of corticosterone on antimicrobial activity and expression of the Bcg gene. *Infect Immun* 1995; 63: 2983-8.
31. Brown RM, Cruz O, Brennan M, et al. Lipoarabinomannan-reactive human secretory immunoglobulin A responses induced by mucosal bacille Calmette-Guerin vaccination. *J Infect Dis* 2003; 187: 513-7.
32. Bullock WE, Fields JP, Bandvias MW. An evaluation of transfer factor as immunotherapy for patients with lepromatous leprosy. *N Engl J Med* 1972; 287: 10-53.
33. Caminero J, Pena MJ, Campos-Herrero MI, et al. Epidemiologic evidence for the spread of a *Mycobacterium tuberculosis* strain of the "Beijing" genotype on Gran Canaria Island. *Am J Respir Crit Care Med* 2001; 164: 1165-70.
34. Cardona PJ, Amat I, Gordillo S, et al. Immunotherapy with fragmented *Mycobacterium tuberculosis* cells increases the effectiveness of chemotherapy against a chronic infection in a murine model of tuberculosis. *Vaccine* 2005; 23:1393-8.
35. Cardona PJ. RUTI: a new chance to shorten the treatment of latent tuberculosis infection. *Tuberculosis (Edinb)* 2006; 86: 273-89.
36. Caruso AM, Serbina N, Klein E, Triebold K, Bloom BR, Flynn JL. Mice deficient in CD4 T cells have only transiently diminished levels of IFN- γ , yet succumb to tuberculosis. *J Immunol* 1999; 162: 5407-16.
37. Centers for Disease Control. Emergence of *Mycobacterium tuberculosis* with extensive resistance to second-line drugs--worldwide, 2000-2004. *MMWR*.2006; 55: 301-5.
38. Chan J, Fan XD, Hunter SW, Brennan PJ, Bloom BR. Lipoarabinomannan, a possible virulence factor involved in persistence of *Mycobacterium tuberculosis* within macrophages. *Infect Immun* 1991; 59: 1755-61.
39. Chan, X, Xing Y, Magliozzo RS, Bloom BR. Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated macrophages. *J Exp Med* 1992; 175: 1111-22.
40. Chertov O, Yang D, Howard OM, Oppenheim JJ. Leukocyte granule proteins mobilize innate host defenses and adaptive immune responses. *Immunol Rev* 2000; 177: 68-78.
41. Clemens DL. Characterization of the *Mycobacterium tuberculosis* phagosome. *Trends Microbiol* 1996; 4: 113-8.
42. Collins HL, Kaufmann SHE. Chapter 15: Acquired Immunity against Bacteria. In: Immunology of Infectious Diseases. Eds: SHE Kaufmann, A Sher & R Ahmed. ASM Press, Washington DC pp 207-21.
43. Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russel DG, Orme IM. Disseminated tuberculosis in interferon γ disrupted mice. *J Exp Med* 1993; 178: 2243-7.

44. Costello AM, Kumar A, Narayan V, et al. Does antibody to mycobacterial antigens, including lipoarabinomannan, limit dissemination in childhood tuberculosis? *Trans R Soc Trop Med Hyg* 1992; 86: 686-92.
45. Crowle AJ, Dahl R, Ross E, May MH. Evidence that vesicles containing living, virulent *Mycobacterium tuberculosis* or *Mycobacterium avium* in cultured human macrophages are not acidic. *Infect Immun* 1991; 59: 1823-31.
46. D'Souza CD, Cooper AM, Frank AA, Mazzaccaro RJ, Bloom BR, Orme IM. An anti-inflammatory role for gamma delta T lymphocytes in acquired immunity to *Mycobacterium tuberculosis*. *J Immunol* 1997; 158: 1217-21.
47. Daher KA, Selsted ME, Lehrer RI. Direct inactivation of viruses by human granulocyte defensins. *J Virol* 1986; 60: 1068-74.
48. Dahl KE, Shiratsuchi H, Hamilton BD, Ellner JJ and Toossi Z. Selective induction of transforming growth factor beta in human monocytes by lipoarabinomannan of *Mycobacterium tuberculosis*. *Infect Immun* 1996; 64: 399-405.
49. Dai G, McMurray DN. Effects of modulating TGF-beta 1 on immune responses to mycobacterial infection in guinea pigs. *Tuber Lung Dis* 1999; 79: 207-14.
50. Dalton DK, Pitts-Meek S, Keshav S, Figueri IS, Bradley A, Stewart TA. Multiple defects of immune cell function in mice with disrupted interferon gamma genes. *Science* 1993; 259: 1739-42.
51. Daniel TM, Debanne SM. The serodiagnosis of tuberculosis and other mycobacterial diseases by enzyme-linked immunosorbent assay. *Am Rev Respir Dis* 1987; 135: 1137-51.
52. Dannenberg AM Jr. Delayed-type hypersensitivity and cell-mediated immunity in the pathogenesis of tuberculosis. *Immunol Today* 1991; 12: 228-33.
53. Daynes RA, Meikle AW, Araneo BA. Locally active steroids hormones may facilitate compartmentalization of immunity by the type of lymphokines produced by helper T cells. *Res Immunol* 1991; 142: 40-5.
54. De La Barrera SS, Finiasz M, Frias A, et al. Specific lytic activity against mycobacterial antigens is inversely correlated with the severity of tuberculosis. *Clin Exp Immunol* 2003; 132: 450-61.
55. de Valliere S, Abate G, Blazevic A, Heuertz RM, Hoft DF. Enhancement of innate and cell-mediated immunity by antimycobacterial antibodies. *Infect Immun* 2005; 73: 6711-20.
56. Denis M. Human neutrophils, activated with cytokines or not, do not kill virulent *Mycobacterium tuberculosis*. *J Infect Dis* 1991; 163: 919-20.
57. Di Nardo A, Vitiello A, Gallo RL. Cutting edge: mast cell antimicrobial activity is mediated by expression of cathelicidin antimicrobial peptide. *J Immunol* 2003; 170: 2274-8.
58. Diamond G, Bevins CL. beta-Defensins: endogenous antibiotics of the innate host defense response. *Clin Immunol Immunopathol* 1998; 88: 221-5.
59. Dieu MC, Vanbervliet B, Vicari A, et al. Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J Exp Med* 1998; 188: 373-86.
60. Dlugovitzky D, Bay ML, Rateni L, et al. In vitro synthesis of interferon-gamma, interleukin-4, transforming growth factor-beta and interleukin-1 beta by peripheral blood mononuclear cells from tuberculosis patients: relationship with the severity of pulmonary involvement. *Scand J Immunol* 1999; 49: 210-7.
61. Dolin PJ, Ravigliani MC, Kochi A. Global tuberculosis incidence and mortality during 1990-2000. *Bull WHO* 1994; 72: 213-20.

62. Ebner S, Ratzinger G, Krosbacher B, et al. Production of IL-12 by human monocyte-derived dendritic cells is optimal when the stimulus is given at the onset of maturation, and is further enhanced by IL-4. *J Immunol* 2001; 166: 633-41.
63. Engering AJ, Cella M, Fluitsma D, et al. The mannose receptor functions as a high capacity and broad specificity antigen receptor in human dendritic cells. *Eur J Immunol* 1997; 27: 2417-25.
64. Eruslanov EB, Lyadova IV, Kondratieva TK, et al. Neutrophil responses to *Mycobacterium tuberculosis* infection in genetically susceptible and resistant mice. *Infect Immun* 2005; 73: 1744-53.
65. Fabre A, Perez TM, Aguilar LD, et al. Transfer factors as immunotherapy and a supplement of chemotherapy in experimental pulmonary tuberculosis. *Clin Exp Immunol* 2004; 136: 215-23.
66. Fanger NA, Wardwell K, Shen L, Tedder TF, Guyre PM. Type I (CD64) and type II (CD32) Fc gamma receptor-mediated phagocytosis by human blood dendritic cells. *J Immunol* 1996; 157: 541-8.
67. Farel LS, Lowell AM, Meador MP. Extrapulmonary tuberculosis in the United States. *Am J Epidemiol* 1979; 109: 205-17.
68. Feger F, Varadaradjalou S, Gao Z, Abraham SN, Arock M. The role of mast cells in host defense and their subversion by bacterial pathogens. *Trends Immunol* 2002; 23: 151-8.
69. Fenhalls G, Wong A, Bezuidenhout J, van Helden P, Bardin P, Lukey PT. In situ production of gamma interferon, interleukin-4, and tumor necrosis factor alpha mRNA in human lung tuberculous granulomas. *Infect Immun* 2000; 68: 2827-36.
70. Ferrari G, Langen H, Naito M, Pieters J. A coat protein on phagosomes involved in the intracellular survival of mycobacteria. *Cell* 1999; 97: 435-47.
71. Ferrick DA, Schrenzel MD, Mulvania T, Hsieh B, Ferlin WG, Lepper H. Differential production of interferon-gamma and interleukin-4 in response to Th1- and Th2-stimulating pathogens by gamma delta T cells in vivo. *Nature* 1995; 373: 255-7.
72. Figdor CG, van Kooyk Y, Adema GJ. C-type lectin receptors on dendritic cells and Langerhans cells. *Nat Rev Immunol* 2002; 2: 77-84.
73. Fine PE, Small PM. Exogenous reinfection in tuberculosis. *N Engl J Med* 1999; 341: 1226-7.
74. Fiorenza G, Rateni L, Farroni MA, Bogue C, Dlugovitzky DG. TNF-alpha, TGF-beta and NO relationship in sera from tuberculosis (TB) patients of different severity. *Immunol Lett* 2005; 98: 45-8.
75. Fischer K, Collins H, Taniguchi M, Kaufmann SH, Schaible UE. IL-4 and T cells are required for the generation of IgG1 isotype antibodies against cardiolipin. *J Immunol* 2002; 168: 2689-94.
76. Flores-Villanueva PO, Ruiz-Morales JA, Song CH, et al. A functional promoter polymorphism in monocyte chemoattractant protein-1 is associated with increased susceptibility to pulmonary tuberculosis. *J Exp Med* 2005; 202: 1649-58.
77. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role of interferon gamma in resistance to *Mycobacterium tuberculosis* infection. *J Exp Med* 1993; 178: 2249-54.
78. Flynn JL, Scanga CA, Tanaka KE, Chan J. Effects of aminoguanidine on latent murine tuberculosis. *J Immunol* 1998; 160: 1796-806.
79. Flynn JL, Goldstein MM, Triebold KJ, Koller B, Bloom BR. Major histocompatibility complex class I-restricted T cells are required for resistance to *Mycobacterium tuberculosis* infection. *Proc Natl Acad Sci U S A* 1992; 89: 12013-7.

194 Immunology, Pathogenesis, Virulence

80. Flynn JL. Lessons from experimental *Mycobacterium tuberculosis* infections. *Microbes Infect* 2006; 8: 1179-88.
81. Fortsch D, Rollinghoff M, Stenger S. IL-10 converts human dendritic cells into macrophage-like cells with increased antibacterial activity against virulent *Mycobacterium tuberculosis*. *J Immunol* 2000; 165: 978-87.
82. Fudenberg HH. Transfer factor 1993. *New frontiers. Prog Drug Res* 1993; 42: 309-400.
83. Fulton SA, Reba SM, Martin TD, Boom WH. Neutrophil-mediated mycobacteriocidal immunity in the lung during *Mycobacterium bovis* BCG infection in C57BL/6 mice. *Infect Immun* 2002; 70: 5322-7.
84. Gabay JE, Scott RW, Campanelli D, et al. Antibiotic proteins of human polymorphonuclear leukocytes. *Proc Natl Acad Sci U S A* 1989; 86: 5610-4.
85. Galli SJ, Maurer M, Lantz CS. Mast cells as sentinels of innate immunity. *Curr Opin Immunol* 1999; 11: 53-9.
86. Gansert JL, Kiessler V, Engele M, et al. Human NKT cells express granulysin and exhibit antimycobacterial activity. *J Immunol* 2003; 170: 3154-61.
87. Ganz T, Selsted ME, Lehrer RI. Defensins. *Eur J Haematol* 1990; 44: 1-8.
88. Ganz T, Selsted ME, Szklarek D, et al. Defensins. Natural peptide antibiotics of human neutrophils. *J Clin Invest* 1985; 76: 1427-35.
89. Ganz T. Defensins: antimicrobial peptides of innate immunity. *Nat Rev Immunol* 2003; 3: 710-20.
90. Garcia-Romo GS, Pedroza-Gonzalez A, Aguilar-Leon D, et al. Airways infection with virulent *Mycobacterium tuberculosis* delays the influx of dendritic cells and the expression of costimulatory molecules in mediastinal lymph nodes. *Immunology* 2004; 112: 661-8.
91. Gatfield J, Pieters J. Essential role for cholesterol in entry of mycobacteria into macrophages. *Science* 2000; 288: 1647-50.
92. Geijtenbeek TB, Van Vliet SJ, Koppel EA, et al. Mycobacteria target DC-SIGN to suppress dendritic cell function. *J Exp Med* 2003; 197: 7-17.
93. Giacomini E, Iona E, Ferroni L, et al. Infection of human macrophages and dendritic cells with *Mycobacterium tuberculosis* induces a differential cytokine gene expression that modulates T cell response. *J Immunol* 2001; 166: 7033-41.
94. Gumperz JE, Brenner MB. CD1-specific T cells in microbial immunity. *Curr Opin Immunol* 2001; 13: 471-8.
95. Gunn MD, Tangemann K, Tam C, Cyster JG, Rosen SD, Williams LT. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. *Proc Natl Acad Sci U S A* 1998; 95: 258-63.
96. Hanekom WA, Mendillo M, Manca C, et al. *Mycobacterium tuberculosis* inhibits maturation of human monocyte-derived dendritic cells in vitro. *J Infect Dis* 2003; 188: 257-66.
97. Hasan Z, Schlaw C, Kuhn L, et al. Isolation and characterization of the mycobacterial phagosome: segregation from the endosomal/lysosomal pathway. *Mol Microbiol* 1997; 24: 545-53.
98. Henderson RA, Watkins SC, Flynn JL. Activation of human dendritic cells following infection with *Mycobacterium tuberculosis*. *J Immunol* 1997; 159: 635-43.
99. Hernandez-Pando R, De La Luz Streber M, Orozco H, et al. The effects of androsterone and dehydroepiandrosterone on the course of tuberculosis in Balb/c mice. *Immunology* 1998; 95: 234-41.

100. Hernandez-Pando R, Aguilar LD, Garcia HLM, Orozco EH, Rook GAW. Pulmonary tuberculosis in Balb/c mice with non-functional IL-4 genes, changes in the regulation of fibrosis and in the inflammatory effects of TNF α . *European Journal of Immunology* 2004; 34: 174-83.
101. Hernandez-Pando R, Aguilar LD, Infante E, et al. The use of mutant mycobacteria as new vaccines to prevent tuberculosis. *Tuberculosis (Edinb)* 2006; 86: 203-10.
102. Hernandez-Pando R, Orozco H., Honour J, Silvia P, Rook GAW: Adrenal changes in murine pulmonary tuberculosis a clue to pathogenesis?. *FEMS Microbiology Immunology* 1995; 12: 63-72.
103. Hernandez-Pando R, Pavon L, Arriaga K, Orozco EH, Madrid-Marina V, Rook G. Pathogenesis of tuberculosis in mice exposed to low and high doses of an environmental mycobacterial saprophyte before infection. *Infect Immun* 1997; 6: 84-90.
104. Hernandez-Pando R, Rook GAW. The role of TNF alpha in T-cell mediated inflammation depends on the Th1/Th2 cytokine balance. *Immunology* 1994; 82: 591-5.
105. Hernandez-Pando R, Schön T, Orozco EH, Serafin M, Estrada-Garcia I. Expression of nitric oxide synthase and nitrotyrosine during the evolution of experimental pulmonary tuberculosis. *Exp Toxicol Pathol* 2001, 53: 257-65.
106. Hernandez-Pando, de la Luz Streber M, Orozco H, et al. Emergent immunoregulatory properties of combined glucocorticoid and anti-glucocorticoid steroids in a model of tuberculosis. *QJM* 1998; 91: 755-66.
107. Hernandez-Pando R, Aguilar-Leon D, Orozco H, et al. 16alpha-Bromoepiandrosterone restores T helper cell type 1 activity and accelerates chemotherapy-induced bacterial clearance in a model of progressive pulmonary tuberculosis. *J Infect Dis* 2005; 191: 299-306.
108. Hernandez-Pando R, Jeyanathan M, Mengistu G, et al. Persistence of DNA from *Mycobacterium tuberculosis* in superficially normal lung tissue during latent infection. *Lancet* 2000; 356: 2133-8.
109. Hernandez-Pando R, Orozco-Esteves H, Maldonado HA, et al. A combination of a transforming growth factor-beta antagonist and an inhibitor of cyclooxygenase is an effective treatment for murine pulmonary tuberculosis. *Clin Exp Immunol* 2006; 144: 264-72.
110. Hernandez-Pando R, Pavon L, Orozco EH, Rangel J, Rook GAW. Interactions between hormone-mediated and vaccine-mediated immunotherapy for pulmonary tuberculosis in Balb/c mice. *Immunology* 2000; 100: 391-8.
111. Hernandez-Pando R, Orozco H, Arriaga K, Sampieri A, Larriva-Sahd J, Madrid-Marina V. Analysis of the local kinetics and localization of interleukin-1 alpha, tumor necrosis factor-alpha and transforming growth factor-beta, during the course of experimental pulmonary tuberculosis. *Immunology* 1997; 90: 607-17.
112. Hernandez-Pando R, Orozco H, Sampieri A, et al. Correlation between the kinetics of Th1, Th2 cells and pathology in a murine model of experimental pulmonary tuberculosis. *Immunology* 1996; 89: 26-33.
113. Hetzel C, Janssen R, Ely SJ, et al. An epitope delivery system for use with recombinant mycobacteria. *Infect Immun* 1998; 66: 3643-8.
114. Hill AV. The immunogenetics of human infectious diseases. *Annu Rev Immunol* 1998; 16: 593-617.
115. Hoft DF, Kemp EB, Marinero M, et al. A double-blind, placebo-controlled study of *Mycobacterium*-specific human immune responses induced by intradermal bacille Calmette-Guerin vaccination. *J Lab Clin Med* 1999; 134: 244-52.

196 Immunology, Pathogenesis, Virulence

116. Hoft DF, Worku S, Kampmann B, et al. Investigation of the relationships between immune-mediated inhibition of mycobacterial growth and other potential surrogate markers of protective *Mycobacterium tuberculosis* immunity. *J Infect Dis* 2002; 186: 1448-57.
117. Hoover DM, Rajashankar KR, Blumenthal R, et al. The structure of human beta-defensin-2 shows evidence of higher order oligomerization. *J Biol Chem* 2000; 275: 32911-8.
118. Hopewell PC. Overview of clinical tuberculosis. In: Bloom BR. Ed. *Tuberculosis: pathogenesis, protection, and control*. Washington DC ASM Press 1994; pp. 25-46.
119. Hopfenspirger MT, Parr SK, Hopp RJ, Townley RG and Agrawal DK. Mycobacterial antigens attenuate late phase response, airway hyperresponsiveness, and bronchoalveolar lavage eosinophilia in a mouse model of bronchial asthma. *Int Immunopharmacol* 2001; 1: 1743-51.
120. Humphreys IR, Stewart GR, Turner DJ, et al. A role for dendritic cells in the dissemination of mycobacterial infection. *Microbes Infect* 2006; 8: 1339-46.
121. Hunt JR, Martinelli R, Adams VC, Rook GAW, Rosa Brunet L. Intragastric administration of *Mycobacterium vaccae* inhibits severe pulmonary allergic inflammation in a mouse model. *Clin Exp Allergy* 2005; 35: 685-90.
122. Inaba K, Inaba M, Naito M, Steinman RM. Dendritic cell progenitors phagocytose particulates, including bacillus Calmette-Guerin organisms, and sensitize mice to mycobacterial antigens in vivo. *J Exp Med* 1993; 178: 479-88.
123. Ito M, Kojiro N, Ikeda T, Ito T, Funada J, Kokubu T. Increased proportions of peripheral blood gamma delta T cells in patients with pulmonary tuberculosis. *Chest* 1992; 102: 195-7.
124. Jarrossay D, Napolitani G, Colonna M, Sallusto F, Lanzavecchia A. Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. *Eur J Immunol* 2001; 31: 3388-93.
125. Jiang W, Swiggard WJ, Heufler C, et al. The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. *Nature* 1995; 375: 151-5.
126. Jiao X, Lo-Man R, Guermontez P, et al. Dendritic cells are host cells for mycobacteria in vivo that trigger innate and acquired immunity. *J Immunol* 2002; 168: 1294-301.
127. Jones GS, Amirault HJ, Andersen BR. Killing of *Mycobacterium tuberculosis* by neutrophils: a nonoxidative process. *J Infect Dis* 1990; 162: 700-4.
128. Jouanguy E, Doffinger R, Dupuis S, Pallier A, Altare F, Casanova JL. IL-12 and IFN-gamma in host defense against mycobacteria and salmonella in mice and men. *Curr Opin Immunol* 1999; 11: 346-51.
129. Julian E, Matas L, Alcaide J, Luquin M. Comparison of antibody responses to a potential combination of specific glycolipids and proteins for test sensitivity improvement in tuberculosis serodiagnosis. *Clin Diagn Lab Immunol* 2004; 11: 70-6.
130. Junqueira-Kipnis AP, Kipnis A, Jamieson A, et al. NK cells respond to pulmonary infection with *Mycobacterium tuberculosis*, but play a minimal role in protection. *J Immunol* 2003; 171: 6039-45.
131. Kadowaki N, Ho S, Antonenko S, et al. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med* 2001; 194: 863-9.
132. Kahnert A, Seiler P, Stein M, et al. Alternative activation deprives macrophages of a coordinated defense program to *Mycobacterium tuberculosis*. *Eur J Immunol* 2006; 36: 631-47.

133. Kaiser V, Diamond G. Expression of mammalian defensin genes. *J Leukoc Biol* 2000; 68: 779-84.
134. Kalinski P, Schuitemaker JH, Hilkens CM, Wierenga EA, Kapsenberg ML. Final maturation of dendritic cells is associated with impaired responsiveness to IFN-gamma and to bacterial IL-12 inducers: decreased ability of mature dendritic cells to produce IL-12 during the interaction with Th cells. *J Immunol* 1999; 162: 3231-6.
135. Kaplan G. Cytokine regulation of disease progression in leprosy and tuberculosis. *Immunobiology* 1994, 191: 564-8.
136. Kapur V, Whitman TS, Musser JM. Is *Mycobacterium tuberculosis* 15 000 years old? *J Infect Dis* 1994; 170: 1348-9.
137. Kaufmann SH, Schoel B, van Embden JD, et al. Heat-shock protein 60: implications for pathogenesis of and protection against bacterial infections. *Immunol Rev* 1991; 121: 67-90.
138. Keane J. Tuberculosis associated with infliximab a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* 2001; 1098-104.
139. Keller C, Hoffmann R, Lang R, Brandau S, Hermann C, Ehlers S. Genetically determined susceptibility to tuberculosis in mice causally involves accelerated and enhanced recruitment of granulocytes. *Infect Immun* 2006; 74: 4295-309.
140. Kindler V, Sappino AP, Grau GE, Piguet PF, Vassali P. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. *Cell* 1989; 56: 731-40.
141. Kisich KO, Heifets L, Higgins M, Diamond G. Antimycobacterial agent based on mRNA encoding human beta-defensin 2 enables primary macrophages to restrict growth of *Mycobacterium tuberculosis*. *Infect Immun* 2001; 69: 2692-9.
142. Koch R. Forsetzung uber ein Heilmittel gegen Tuberculose. *Dtsch Med Wochenschr* 1891; 17: 101-2.
143. Kramnik I, Dietrich WF, Demant P, Bloom BR. Genetic control of resistance to experimental infection with virulent *Mycobacterium tuberculosis*. *Proc Natl Acad Sci U S A* 2000; 97: 8560-5.
144. Kremer K, van Soolingen D, Frothingham R. Comparison of methods based in different epidemiological markers for typing of *Mycobacterium tuberculosis* complex strains: interlaboratory study of discriminatory power and reproducibility. *J Clin Microbiol* 1999; 37: 2607-18.
145. Kriehuber E, Breiteneder-Geleff S, Groeger M, et al. Isolation and characterization of dermal lymphatic and blood endothelial cells reveal stable and functionally specialized cell lineages. *J Exp Med* 2001; 194: 797-808.
146. Kyei GB, Vergne I, Chua J, et al. Rab14 is critical for maintenance of *Mycobacterium tuberculosis* phagosome maturation arrest. *EMBO J* 2006; 25: 5250-9.
147. Ladel CH, Blum C, Dreher A, Reifenberg K, Kaufmann SH. Protective role of gamma/delta T cells and alpha/beta T cells in tuberculosis. *Eur J Immunol* 1995; 25: 2877-81.
148. Lamb JR, Bal V, Rothbard JB, Mehlert A, Mendez-Samperio P, Young DB. The mycobacterial GroEL stress protein: a common target of T-cell recognition in infection and autoimmunity. *J Autoimmun* 1989; 2 Suppl: 93-100.
149. Law K, Weiden M, Harkin T, Tchou-Wong K, Chi C, Rom WN. Increased release of interleukin-1 beta, interleukin-6, and tumor necrosis factor-alpha by bronchoalveolar cells lavaged from involved sites in pulmonary tuberculosis. *Am J Respir Crit Care Med* 1996; 153: 799-804.

198 Immunology, Pathogenesis, Virulence

150. Lawrence HS. The transfer in humans of delayed skin sensitivity to Streptococcal M substances and tuberculin with disrupted leukocytes. *J Clin Invest* 1955; 34: 219-30.
151. Le Cabec V, Cols C, Maridonneau-Parini I. Nonopsonic phagocytosis of zymosan and *Mycobacterium kansasii* by CR3 (CD11b/CD18) involves distinct molecular determinants and is or is not coupled with NADPH oxidase activation. *Infect Immun* 2000; 68: 4736-45.
152. Lee CG, Homer R, Zhu Z, *et al.* Interleukin-13 induces tissue fibrosis by selectively stimulating and activating transforming growth factor beta (1). *J Exp Med* 2001; 194: 809-21.
153. Lehrer RI, Lichtenstein AK, Ganz T. Defensins: antimicrobial and cytotoxic peptides of mammalian cells. *Annu Rev Immunol* 1993; 11: 105-28.
154. Lepper AW, Wilks CR. Intracellular iron storage and the pathogenesis of paratuberculosis. Comparative studies with other mycobacterial, parasitic or infectious conditions of veterinary importance. *J Comp Pathol* 1988; 98: 31-53.
155. Levin AS, Splitter LE, Stites DP, Fudenberg HH. Wiscott Aldrich syndrome, a genetically determined cellular immunologic deficiency: clinical and laboratory responses to therapy with transfer factor. *Proc Natl Acad Sci U S A* 1970; 67: 821-8.
156. Lienhardt C, Azzurri A, Amedei A, *et al.* Active tuberculosis in Africa is associated with reduced Th1 and increased Th2 activity in vivo. *Eur J Immunol* 2002; 32: 1605-13.
157. Lin Y, Zhang M, Hofman FM, Gong J, Barnes PF. Absence of a prominent Th2 cytokine response in human tuberculosis. *Infect Immun* 1996; 64: 1351-6.
158. Linzmeier R, Ho CH, Hoang BV, Ganz T. A 450-kb contig of defensin genes on human chromosome 8p23. *Gene* 1999; 233: 205-11.
159. Lockhart E, Green AM, Flynn JL. IL-17 production is dominated by gammadelta T cells rather than CD4 T cells during *Mycobacterium tuberculosis* infection. *J Immunol* 2006; 177: 4662-9.
160. Lopez B, Aguilar D, Orozco H, *et al.* A marked difference in pathogenesis and immune response induced by different *Mycobacterium tuberculosis* genotypes. *Clin Exp Immunol* 2003; 133: 30-7.
161. Lopez-Marin LM, Segura E, Hermida-Escobedo C, Lemassu A, Salinas-Carmona MC. 6,6'-Dimycoloyl trehalose from a rapidly growing *Mycobacterium*: an alternative antigen for tuberculosis serodiagnosis. *FEMS Immunol Med Microbiol* 2003; 36: 47-54.
162. Lowrie DB, Tascon RE, Bonato VL, *et al.* Therapy of tuberculosis in mice by DNA vaccination. *Nature* 1999; 400: 269-71.
163. Lowrie DB. Potential of Immunotherapy Revealed in Mice. In: Proceedings of 6th International Conference on Pathogenesis Mycobacterial Infections, June 30 to July 3. Stockholm, Sweden, 2005.
164. Macassey SLL, Saleeby CW. Spahlinger Contra Tuberculosis, 1908-1934; an International Tribute. London: Bale & Danielsson 1934.
165. MacMicking JD, North RJ, LaCourse R, Mudgett JS, Shah SK, Nathan CF. Identification of nitric oxide synthase as a protective locus against tuberculosis. *Proc Natl Acad Sci U S A* 1997; 94: 5243-8.
166. Malaviya R, Navara C, Uckun FM. Role of Janus kinase 3 in mast cell-mediated innate immunity against gram-negative bacteria. *Immunity* 2001; 15: 313-21.
167. Malaviya R, Twisten NJ, Ross EA, Abraham SN, Pfeifer JD. Mast cells process bacterial Ags through a phagocytic route for class I MHC presentation to T cells. *J Immunol* 1996; 156: 1490-6.

168. Malhotra I et al. Helminth and bacillus Calmette-Guerin induced immunity in children sensitized in utero to filariasis and schistosomiasis. *J Immunol* 1999; 162: 6843-8.
169. Manca C, Tsenova L, Barry C, et al. *Mycobacterium tuberculosis* CDC1551 induces a more vigorous host response *in vivo* and *in vitro*, but is not more virulent than other clinical isolates. *J Immunol* 1999; 162: 6740-6.
170. Marchant A, Amedei A, Azzurri A, et al. Polarization of PPD-Specific T-Cell response of patients with tuberculosis from Th0 to Th1 profile after successful antimycobacterial therapy or *in vitro* conditioning with interferon-alpha or interleukin-12. *Am J Respir Cell Mol Biol* 2001; 24: 187-94.
171. McCurdy JD, Olynych TJ, Maher LH, Marshall JS. Cutting edge: distinct Toll-like receptor 2 activators selectively induce different classes of mediator production from human mast cells. *J Immunol* 2003; 170: 1625-9.
172. McShane H. Susceptibility to tuberculosis-the importance of the pathogen as well as the host. *Clin Exp Immunol* 2003; 133: 20-1.
173. Means TK, Jones BW, Schromm AB, et al. Differential effects of a Toll-like receptor antagonist on *Mycobacterium tuberculosis*-induced macrophage responses. *J Immunol* 2001; 166: 4074-82.
174. Means TK, Wang S, Lien E, Yoshimura A, Golenbock DT, Fenton MJ. Human toll-like receptors mediate cellular activation by *Mycobacterium tuberculosis*. *J Immunol* 1999; 163: 3920-7.
175. Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev* 1997; 77: 1033-79.
176. Metzger H. The receptor with high affinity for IgE. *Immunol Rev* 1992; 125: 37-48.
177. Miller BH, Fratti RA, Poschet JF, et al. Mycobacteria inhibit nitric oxide synthase recruitment to phagosomes during macrophage infection. *Infect Immun* 2004; 72: 2872-8.
178. Miller HR. Mucosal mast cells and the allergic response against nematode parasites. *Vet Immunol Immunopathol* 1996; 54: 331-6.
179. Miyakawa Y, Ratnakar P, Rao AG, et al. *In vitro* activity of the antimicrobial peptides human and rabbit defensins and porcine leukocyte proteoglycan against *Mycobacterium tuberculosis*. *Infect Immun* 1996; 64: 926-32.
180. Moreira AL, Sampaio EP, Zmuidzinis A, Frindt P, Smith KA, Kaplan G. Thalidomide exerts its inhibitory effect on tumor necrosis factor by enhancing mRNA degradation. *J Exp Med* 1993; 177: 1675-80.
181. Muller I, Cobbold SP, Waldmann H, Kaufmann SH. Impaired resistance to *Mycobacterium tuberculosis* infection after selective *in vivo* depletion of L3T4+ and Lyt-2+ T cells. *Infect Immun* 1987; 55: 2037-41.
182. Munoz S, Hernandez-Pando R, Abraham SN, Enciso JA. Mast cell activation by *Mycobacterium tuberculosis*: mediator release and role of CD48. *J Immunol* 2003; 170: 5590-6.
183. Nardell EA. Environmental control of tuberculosis. *Med Clin North Am* 1993; 77: 1315-34.
184. Neyrolles O, Hernandez-Pando R, Pietri-Rouxel F, et al. *Mycobacterium tuberculosis* persistence in adipose tissue. *PLOS Med*. 2006; 1: e43.
185. Nicholson S, Bonecini-Almeida MG, Lapa e Silva JR, et al. Inducible nitric oxide synthase in pulmonary alveolar macrophages from patients with tuberculosis. *J Exp Med* 1996; 183: 2293-302.
186. Nuernberger E, Tyagi S, Williams KN, Rosenthal I, Bishai WR, Grosset JH. Rifapentine, moxifloxacin, or DNA vaccine improves treatment of latent tuberculosis in a mouse model. *Am J Respir Crit Care Med* 2005; 172: 1452-6.

200 Immunology, Pathogenesis, Virulence

187. Oddo M, Renno T, Attinger A, Bakker T, MacDonald HR, Meylan PR. Fas ligand-induced apoptosis of infected human macrophages reduces the viability of intracellular *Mycobacterium tuberculosis*. *J Immunol* 1998; 160: 5448-54.
188. Ogata K, Linzer BA, Zuberi RI, Ganz T, Lehrer RI, Catanzaro A. Activity of defensins from human neutrophilic granulocytes against *Mycobacterium avium-Mycobacterium intracellulare*. *Infect Immun* 1992; 60: 4720-5.
189. Onwubalili JK, Scott GM, Smith H. Acute respiratory distress related to chemotherapy of advanced pulmonary tuberculosis a study of two cases and review of the literature. *QJ M* 1986; 59: 599-61.
190. Ozdemir C, Akkoc T, Bahceciler NN, Kucukercan D, Barlan IB, Basaran MM. Impact of *Mycobacterium vaccae* immunization on lung histopathology in a murine model of chronic asthma. *Clin Exp Allergy* 2003; 33: 266-70.
191. Parrish NM, Dick JD, Bishai WR. Mechanism of latency in *Mycobacterium tuberculosis*. *Trends Microbiol* 1998; 6: 107-12.
192. Pedrosa J, Saunders BM, Appelberg R, Orme IM, Silva MT, Cooper AM. Neutrophils play a protective nonphagocytic role in systemic *Mycobacterium tuberculosis* infection of mice. *Infect Immun* 2000; 68: 577-83.
193. Pedroza-Gonzalez A, Garcia-Romo GS, Aguilar-Leon D, et al. In situ analysis of lung antigen-presenting cells during murine pulmonary infection with virulent *Mycobacterium tuberculosis*. *Int J Exp Pathol* 2004; 85: 135-45.
194. Peterson PK, Gekker G, Hu S, et al. CD14 receptor-mediated uptake of nonopsonized *Mycobacterium tuberculosis* by human microglia. *Infect Immun* 1995; 63: 1598-602.
195. Phipps RP, Stein SH, Roper RL. A new view of prostaglandin E regulation of the immune response. *Immunol Today* 1991; 12: 349-52.
196. Pieters J. Entry and survival of pathogenic mycobacteria in macrophages. *Microbes Infect* 2001; 3: 249-55.
197. Post FA, Soule SG, Willcox PA, Levitt NS. The spectrum of endocrine dysfunction in active tuberculosis. *Clin Endocrinol* 1994; 40: 367-71.
198. Randhawa AK, Ziltener HJ, Merzaban JS, Stokes RW. CD43 is required for optimal growth inhibition of *Mycobacterium tuberculosis* in macrophages and in mice. *J Immunol* 2005; 175: 1805-12.
199. Rangel MJ, Estrada García I, García HML, Aguilar LD, Marquez VR, Hernandez-Pando R. The role of prostaglandin E-2 in the immunopathogenesis of experimental pulmonary tuberculosis. *Immunology* 2002; 106: 257-66.
200. Raqib R, Rahman J, Kamaluddin AK, et al. Rapid diagnosis of active tuberculosis by detecting antibodies from lymphocyte secretions. *J Infect Dis* 2003; 188: 364-70.
201. Ratnam S, Ratnam S, Puri BK, Chandrasekhar S. Mast cell response during the early phase of tuberculosis: an electron-microscopic study. *Can J Microbiol* 1977; 23: 1245-51.
202. Reading C, Dowding C, Schramm B, et al. Improvement in immune parameters and human immunodeficiency virus-1 viral response in individuals treated with 16alpha-bromoepiandrosterone (HE2000). *Clin Microbiol Infect* 2006; 12: 1082-8.
203. Reljic R, Clark SO, Williams A, et al. Intranasal IFN γ extends passive IgA antibody protection of mice against *Mycobacterium tuberculosis* lung infection. *Clin Exp Immunol* 2006; 143: 467-73.
204. Relloso M, Puig-Kroger A, Pello OM, et al. DC-SIGN (CD209) expression is IL-4 dependent and is negatively regulated by IFN, TGF-beta, and anti-inflammatory agents. *J Immunol* 2002; 168: 2634-43.

205. Riedel DD, Kaufmann SH. Chemokine secretion by human polymorphonuclear granulocytes after stimulation with *Mycobacterium tuberculosis* and lipoarabinomannan. *Infect Immun* 1997; 65: 4620-3.
206. Rios Barrera V, Campos Peña V, Aguilar Leon D, et al. Macrophage and T lymphocyte apoptosis during experimental pulmonary tuberculosis: Their relationship to mycobacterial virulence. *Eur J Immunol* 2006; 36: 345-53.
207. Rivas-Santiago B, Sada E, Tsutsumi V, Aguilar-Leon D, Contreras JL, Hernandez-Pando R. beta-Defensin gene expression during the course of experimental tuberculosis infection. *J Infect Dis* 2006; 194: 697-701.
208. Rivas-Santiago B, Schwander SK, Sarabia C, et al. Human {beta}-defensin 2 is expressed and associated with *Mycobacterium tuberculosis* during infection of human alveolar epithelial cells. *Infect Immun* 2005; 73: 4505-11.
209. Rook GAW, Baker R, Walker B, et al. Local regulation of glucocorticoid activity in sites of inflammation. Insights from the study of tuberculosis. *Ann N Y Acad Sci* 2000; 917: 913-22.
210. Rook GAW, Deehda K, Zumla A. Immune responses in developing countries; implications for new vaccines. *Nat Rev Immunol* 2005; 5: 661-7.
211. Rook GAW, Hernandez-Pando R, Dheda K, Teng Seah G. A new look at the role of IL-4 in tuberculosis: implications for vaccine design. *Trends Immunol* 2004; 25: 483-8.
212. Rook GAW, Hernández Pando R, Lightman S: Hormone, peripherally activated pro hormones and regulation of the Th1/Th2 balance. *Immunology Today* 1994; 15: 301-3.
213. Rook GAW, Hernandez-Pando R. The pathogenesis of tuberculosis. *Ann Rev Microbiol* 1996; 50: 259-84.
214. Rook GAW, Honour J, Kon OM, Wilkinson RJ, Davidson R, Shawn RJ. Urinary metabolites in tuberculosis; a new clue to pathogenesis. *QJ Med* 1996; 88: 333-41.
215. Roy S, Sharma S, Sharma M, Aggarwal R, Bose M. Induction of nitric oxide release from the human alveolar epithelial cell line A549: an in vitro correlate of innate immune response to *Mycobacterium tuberculosis*. *Immunology* 2004; 112: 471-80.
216. Rozzo SJ, Kirkpatrick CH. Purification of transfer factors. *Molec Immunol* 1992; 29: 167-82.
217. Sabroe I, Jones EC, Usher LR, Whyte MK, Dower SK. Toll-like receptor TLR2 and TLR4 in human peripheral blood granulocytes: a critical role for monocytes in leukocyte lipopolysaccharide responses. *J Immunol* 2002; 168: 4701-10.
218. Salinas-Carmona MC, Perez-Rivera I. Humoral immunity through immunoglobulin M protects mice from an experimental actinomycetoma infection by *Nocardia brasiliensis*. *Infect Immun* 2004; 72: 5597-604.
219. Sanchez FO, Rodriguez JI, Agudelo G, Garcia LF. Immune responsiveness and lymphokine production in patients with tuberculosis and healthy controls. *Infect Immun* 1994; 62: 5673-8.
220. Sarma GR, Chandra I, Ramachandran G. Adrenocortical function in patients with pulmonary tuberculosis. *Tubercle* 1990; 71: 277-82.
221. Sayama K, Diehn M, Matsuda K, et al. Transcriptional response of human mast cells stimulated via the Fc(epsilon)RI and identification of mast cells as a source of IL-11. *BMC Immunol* 2002; 3: 5.
222. Scanga CA, Mohan VP, Yu K, et al. Depletion of CD4(+) T cells causes reactivation of murine persistent tuberculosis despite continued expression of interferon gamma and nitric oxide synthase 2. *J Exp Med* 2000; 192: 347-58.

202 Immunology, Pathogenesis, Virulence

223. Schauf V, Rom WN, Smith KA, et al. Cytokine gene activation and modified responsiveness to interleukin-2 in the blood of tuberculosis patients. *J Infect Dis* 1993; 168: 1056-9.
224. Schlesinger LS, Bellinger-Kawahara CG, Payne NR, Horwitz MA. Phagocytosis of *Mycobacterium tuberculosis* is mediated by human monocyte complement receptors and complement component C3. *J Immunol* 1990; 144: 2771-80.
225. Schlesinger LS. Macrophage phagocytosis of virulent but not attenuated strains of *Mycobacterium tuberculosis* is mediated by mannose receptors in addition to complement receptors. *J Immunol* 1993; 150: 2920-30.
226. Schoel B, Sprenger S, Kaufmann SH. Phosphate is essential for stimulation of V gamma 9V delta 2 T lymphocytes by mycobacterial low molecular weight ligand. *Eur J Immunol* 1994; 24: 1886-92.
227. Schön T, Kimberger G, Nagese Y, Hernández Pando R, Sundqvist T, Britton S. Local production of nitric oxide in patients with tuberculosis. *Int J Tuberc Lung Dis* 2004; 8: 1134-7.
228. Schuller S, Neefjes J, Ottenhoff T, Thole J, Young D. Coronin is involved in uptake of *Mycobacterium bovis* BCG in human macrophages but not in phagosome maintenance. *Cell Microbiol* 2001; 3: 785-93.
229. Scott GM, Murphy PG, Gemidjoglu ME. Predicting deterioration of treated tuberculosis by corticosteroids reserve and C-reactive protein. *J Infect* 1990; 21: 61-9.
230. Seah GT, Scott GM, Rook GA. Type 2 Cytokine gene activation and its relationship to extent of disease in patients with tuberculosis. *J Infect Dis* 2000; 181: 385-9.
231. Seiler P, Aichele P, Bandermann S, et al. Early granuloma formation after aerosol *Mycobacterium tuberculosis* infection is regulated by neutrophils via CXCR3-signaling chemokines. *Eur J Immunol* 2003; 33: 2676-86.
232. Selsted ME, Harwig SS. Purification, primary structure, and antimicrobial activities of a guinea pig neutrophil defensin. *Infect Immun* 1987; 55: 2281-6.
233. Selsted ME, Szklarek D, Ganz T, Lehrer RI. Activity of rabbit leukocyte peptides against *Candida albicans*. *Infect Immun* 1985; 49: 202-6.
234. Serbina NV, Flynn JL. Early emergence of CD8(+) T cells primed for production of type 1 cytokines in the lungs of *Mycobacterium tuberculosis*-infected mice. *Infect Immun* 1999; 67: 3980-8.
235. Serbina NV, Lazarevic V, Flynn JL. CD4(+) T cells are required for the development of cytotoxic CD8(+) T cells during *Mycobacterium tuberculosis* infection. *J Immunol* 2001; 167: 6991-7000.
236. Sharma S, Verma I, Khuller GK. Therapeutic potential of human neutrophil peptide 1 against experimental tuberculosis. *Antimicrob Agents Chemother* 2001; 45: 639-40.
237. Silva CL, Bonato VL, Coelho-Castelo AA, et al. Immunotherapy with plasmid DNA encoding mycobacterial *hsp65* in association with chemotherapy is a more rapid and efficient form of treatment for tuberculosis in mice. *Gene Ther* 2005; 12: 281-7.
238. Silva CL, Lowrie DB. Identification and characterization of murine cytotoxic T cells that kill *Mycobacterium tuberculosis*. *Infect Immun* 2000; 68: 3269-74.
239. Singh KK, Dong Y, Hinds L, et al. Combined use of serum and urinary antibody for diagnosis of tuberculosis. *J Infect Dis* 2003; 188: 371-7.
240. Singh PK, Jia HP, Wiles K, et al. Production of beta-defensins by human airway epithelia. *Proc Natl Acad Sci U S A* 1998; 95: 14961-6.
241. Skelding KA, Hickey DK, Horvat JC, et al. Comparison of intranasal and transcutaneous immunization for induction of protective immunity against *Chlamydia muridarum* respiratory tract infection. *Vaccine* 2006; 24: 355-66.

242. Skinner MA, Prestidge R, Yuan S, Strabala TJ, Tan PL. The ability of heat-killed *Mycobacterium vaccae* to stimulate a cytotoxic T-cell response to an unrelated protein is associated with a 65 kilodalton heat-shock protein. *Immunology* 2001; 102: 225-33.
243. Skinner MA, Yuan S, Prestidge R, Chuk D, Watson JD, Tan PLJ. Immunization with heat-killed *Mycobacterium vaccae* stimulates CD8+ cytotoxic T cells specific for macrophages infected with *Mycobacterium tuberculosis*. *Infect Immun* 1997; 65: 4525-30.
244. Spence DP, Hotchkins J, Williams CS, Davies PD. Tuberculosis and poverty. *Br Med J* 1993; 307: 759-61.
245. Stead, WW. Genetics and resistance to tuberculosis. Could resistance be enhanced by genetic engineering? *Ann Intern Med* 1992; 116: 937-41.
246. Steinman RM. Dendritic cells and the control of immunity: enhancing the efficiency of antigen presentation. *Mt Sinai J Med* 2001; 68: 160-6.
247. Stenger S, Hanson DA, Teitelbaum R, et al. An antimicrobial activity of cytolytic T cells mediated by granulysin. *Science* 1998; 282: 121-5.
248. Stenger S, Mazzaccaro RJ, Uyemura K, et al. Differential effects of cytolytic T cell subsets on intracellular infection. *Science* 1997; 276: 1684-7.
249. Stenger S, Niazzi KR, Modlin RL. Down-regulation of CD1 on antigen-presenting cells by infection with *Mycobacterium tuberculosis*. *J Immunol* 1998; 161: 3582-8.
250. Stolzenberg ED, Anderson GM, Ackermann MR, Whitlock RH, Zasloff M. Epithelial antibiotic induced in states of disease. *Proc Natl Acad Sci U S A* 1997; 94: 8686-90.
251. Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, et al. Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. *Science* 1994; 263: 678-81.
252. Sudhir KS, Sizemore RS, Gottlieb AA. Immunomodulatory components present in IM-REG 1, an experimental immunosupportive biologic. *Biotechnol* 1988; 6: 810-5.
253. Sudre P, ten Dam G, Kochi A. Tuberculosis: a global overview of the situation today. *Bull WHO* 1992; 70: 149-59.
254. Sugawara I, Yamada H, Mizuno S, Li CY, Nakayama T, Taniguchi M. Mycobacterial infection in natural killer T cell knockout mice. *Tuberculosis (Edinb)* 2002; 82: 97-104.
255. Supajatura V, Ushio H, Nakao A, et al. Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. *J Clin Invest* 2002; 109: 1351-9.
256. Tailleux L, Schwartz O, Herrmann JL, et al. DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells. *J Exp Med* 2003; 197: 121-7.
257. Tan BH, Meinken C, Bastian M, et al. Macrophages acquire neutrophil granules for antimicrobial activity against intracellular pathogens. *J Immunol* 2006; 177: 1864-71.
258. Teitelbaum R, Glatman-Freedman A, Chen B, et al. A mAb recognizing a surface antigen of *Mycobacterium tuberculosis* enhances host survival. *Proc Natl Acad Sci U S A* 1998; 95: 15688-93.
259. Thurnher M, Ramoner R, Gastl G, et al. Bacillus Calmette-Guerin mycobacteria stimulate human blood dendritic cells. *Int J Cancer* 1997; 70: 128-34.
260. Tobach E, Bloch H. Effects of crowding prior to and following tuberculous infection. *Am J Physiol* 1956; 187: 399-402.
261. Toossi Z, Gogate P, Shiratsuchi H, Young T, Ellner JJ. Enhanced production of TGF-beta by blood monocytes from patients with active tuberculosis and presence of TGF-beta in tuberculous granulomatous lung lesions. *J Immunol* 1995; 154: 465-73.
262. Trajkovic V. [The role of mycobacterial secretory proteins in immune response in tuberculosis] *Med Pregl* 2004; 57 Suppl 1:25-8.

263. Tukenmez F, Bahceciler NN, Barlan IB, Basaran MM. Effect of pre-immunization by killed *Mycobacterium bovis* and *vacciae* on immunoglobulin E response in ovalbumin-sensitized newborn mice. *Pediatr Allergy Immunol* 1999; 10: 107-11.
264. Turley SJ, Inaba K, Garrett WS, et al. Transport of peptide-MHC class II complexes in developing dendritic cells. *Science* 2000; 288: 522-7.
265. Turner H, Kinet JP. Signalling through the high-affinity IgE receptor Fc epsilonRI. *Nature* 1999; 402 (6760 Suppl): B24-30.
266. Underhill DM, Ozinsky A, Smith KD, Aderem A. Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *Proc Natl Acad Sci U S A* 1999; 96: 14459-63.
267. Urban CF, Lourido S, Zychlinsky A. How do microbes evade neutrophil killing? *Cell Microbiol* 2006; 8: 1687-96.
268. Valway SE, Sanchez MPC, Shinnick TF, et al. An outbreak involving extensive transmission of a virulent strain of *Mycobacterium tuberculosis*. *N Engl J Med* 1998; 338: 633-9.
269. van Crevel R, Karyadi E, Preyers F, et al. Increased production of interleukin 4 by CD4+ and CD8+ T cells from patients with tuberculosis is related to the presence of pulmonary cavities. *J Infect Dis* 2000; 181: 1194-7.
270. van Embden JD, Cave MD, Crawford J, et al. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting recommendation for a standardized methodology. *J Clin Microbiol* 1993; 31: 406-9.
271. Vankayalapati R, Klucar P, Wizel B, et al. NK cells regulate CD8+ T cell effector function in response to an intracellular pathogen. *J Immunol* 2004; 172: 130-7.
272. Vankayalapati R, Wizel B, Weis SE, et al. The Nkp46 receptor contributes to NK cell lysis of mononuclear phagocytes infected with an intracellular bacterium. *J Immunol* 2002; 168: 3451-7.
273. van Soolingen D, Qian L, de Haas, et al. Predominance of a single genotype of *Mycobacterium tuberculosis* in countries of East Asia. *J Clin Microbiol* 1995; 33: 3234-8.
274. Via LE, Deretic D, Ulmer RJ, Hibler NS, Huber LA, Deretic V. Arrest of mycobacterial phagosome maturation is caused by a block in vesicle fusion between stages controlled by rab5 and rab7. *J Biol Chem* 1997; 272: 13326-31.
275. Voskuil ML, Schnappinger D, Visconti KC, et al. Inhibition of respiration by nitric oxide induces a *Mycobacterium tuberculosis* dormancy program. *J Exp Med* 2003; 198: 705-13.
276. Walker L, Lowrie DB. Killing of *Mycobacterium microti* by immunologically activated macrophages. *Nature* 1981; 293: 69-71.
277. Wang CC, Rook GAW. Inhibition of an established allergic response to ovalbumin in Balb/c mice by killed *Mycobacterium vaccae*. *Immunology* 1998; 93: 307-13.
278. Whyte RI, Schorke MA, Sloan H, Orringer MB, Kirsh MM. Adjuvant treatment using transfer factor for bronchogenic carcinoma: long term follow up. *Ann Thorac Surg* 1992; 53: 391-6.
279. Wickremasinghe MI, Thomas LH, Friedland JS. Pulmonary epithelial cells are a source of IL-8 in the response to *Mycobacterium tuberculosis*: essential role of IL-1 from infected monocytes in a NF-kappa B-dependent network. *J Immunol* 1999; 163: 3936-47.
280. Wilkinson KA, Martin TD, Reba SM, et al. Latency-associated peptide of transforming growth factor beta enhances mycobacteriocidal immunity in the lung during *Mycobacterium bovis* BCG infection in C57BL/6 mice. *Infect Immun* 2000; 68: 6505-8.

281. Williams A, Reljic R, Naylor I, et al. Passive protection with immunoglobulin A antibodies against tuberculous early infection of the lungs. *Immunology* 2004; 111: 328-33.
282. Williams CM, Galli SJ. The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. *J Allergy Clin Immunol* 2000; 105: 847-59.
283. Woodbury RG, Miller HR, Huntley JF, Newlands GF, Palliser AC, Wakelin D. Mucosal mast cells are functionally active during spontaneous expulsion of intestinal nematode infections in rat. *Nature* 1984; 312: 450-2.
284. Wozniak TM, Ryan AA, Triccas JA, Britton WJ. Plasmid interleukin-23 (IL-23), but not plasmid IL-27, enhances the protective efficacy of a DNA vaccine against *Mycobacterium tuberculosis* infection. *Infect Immun* 2006; 74: 557-65.
285. Yong AJ, Grange JM, Tee RD, et al. Total and anti-mycobacterial IgE levels in serum from patients with tuberculosis and leprosy. *Tubercle* 1989; 70: 273-9.
286. Zeya HI, Spitznagel JK. Antibacterial and enzymic basic proteins from leukocyte lysosomes: separation and identification. *Science* 1963; 142: 1085-7.
287. Zeya HI, Spitznagel JK. Cationic proteins of polymorphonuclear leukocyte lysosomes. I. Resolution of antibacterial and enzymatic activities. *J Bacteriol* 1966; 91: 750-4.
288. Zimmerli S, Edwards S, Ernst JD. Selective receptor blockade during phagocytosis does not alter the survival and growth of *Mycobacterium tuberculosis* in human macrophages. *Am J Respir Cell Mol Biol* 1996; 15: 760-70.
289. Zuany-Amorim C, Sawicka E, Manlius C, et al. Suppression of airway eosinophilia by killed *Mycobacterium vaccae*-induced allergen-specific regulatory T-cells. *Nat Med* 2002; 8: 625-9.
290. Zuany-Amorim C, Manlius C, Trifilieff A, et al. Long-term protective and antigen-specific effect of heat-killed *Mycobacterium vaccae* in a murine model of allergic pulmonary inflammation. *J Immunol*. 2002; 169: 1492-9.
291. Zuckerman SH, Shelhaas J, Butler LD. Differential regulation of lipopolysaccharide-induced interleukin 1 and tumor necrosis factor synthesis; effect of endogenous and exogenous glucocorticoids and the role of the pituitary-adrenal axis. *Eur J Immunol* 1989; 19: 301-5.

