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Recent trends in the immunopathogenesis of tuberculosis

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PREAMBLE

Tuberculosis kills about 500,000 Indians every year, ie, one person every minute. For every patient who dies of the disease, there are at least two individuals who develop disability. Further, the advent of infection by the human immunodeficiency virus and drug resistant forms of *Mycobacterium tuberculosis* have made the management and control of tuberculosis more difficult. Although the genome of *M. tuberculosis* has been sequenced, diagnosis of the infectious sputum positive case is straightforward, simple and inexpensive and effective short course chemotherapy is available, morbidity and mortality due to the disease have not appreciably decreased.

Among the several reasons cited for this failure are the inadequacy of the BCG vaccine in preventing post primary tuberculosis and the relatively long duration (six months) of chemotherapy which encourages irregularity and incomplete treatment. Furthermore, two more aspects which deserve consideration are a) lack of appropriate tests to identify the individual who is likely to develop clinical illness and b) disability caused by the disease process.

This presentation will limit itself to two major areas of the immunopathology of the disease. These are a) host immune mechanisms underlying the varied clinical and pathological manifestations and b) events that lead to tissue damage and disability

HOST IMMUNE MECHANISMS

The histopathology of tuberculosis presents a pleomorphic picture ranging from a well-organised, non-necrotic, epithelioid cell granuloma to a poorly-organised granuloma with necrosis and macrophages with very few epithelioid cells (Ramanathan *et al.,* 1999). Further, it is known that the predominant histological form also varies from organ to organ. While necrosis is common in pulmonary and lymphoidal lesions, it is rare in the skin especially in verrucosa cutis.

The immunopathology of tuberculosis is best understood by following its natural history. The evolution of the disease when the organism enters a host for the first time is different from that when it invades subsequently. The sequence of events following the first infection leads to what is known as the primary complex. Post primary tuberculosis is the term given to the disease when an individual comes down with the disease subsequently.

Primary complex

The first infection with tuberculosis occurs usually in childhood especially in our country where the disease is endemic. In a majority of cases, the primary complex involves areas in the lung and the draining hilar lymph nodes. Occasionally, this may be seen in the tonsils and the cervical lymph nodes or the intestine and the mesenteric lymph nodes. Very rarely, this can occur in the skin in the form of a tuberculous chancre.

The initial infection in the lung occurs by deposition of the bacilli in the acini at one or more places. *M.tuberculosis*

encounters the complement system initially which it activates (Schlesinger *et al.,* 1990). A consequence of this is an exudative lesion with local oedema and recruitment of neutrophils which phagocytose the bacilli though this does not lead to killing of the organisms. Instead. the bacilli multiply and monocytes are recruited into the lesion now and the infection proceeds to the next granulomatous phase with infiltration additionally by T lymphocytes also. At this stage gd T cells appear to play a prominent role (Dieli *et al.,* 2000).

Although this primary or Ghon's focus can occur anywhere in the lung, it is more often seen in a lower lobe where the ventilation is greater. Pathogenetically, this results in a tuberculous pneumonia with oedema and deposition of fibrin along with the monocytes. Bacilli or bacilli-laden phagocytes then reach the draining lymph node via lympahtics and a secondary focus arises. The primary focus in the lung usually remains small and develops central caseation and transforms into a typical granuloma.

During the early evolution of the primary complex, spread along the lymphatics may extend up to the superior mediastinal nodes or even to those in the upper abdomen. From the lymphatics, a few bacilli may escape into the venous system from where they filter into the organs of the reticulo endothelial system viz. the spleen, liver or bone marrow. Even the lung can be a secondary focus through haematogenous spread and this is seen more often in the apical areas of the lung and is known as the Simon's focus.

Over a period ranging from several weeks to a few months, the lesion becomes encapsulated. Eventually healing occurs by fibrosis with the area of caseation becoming calcified or rarely even ossified. The secondary lymph node focus also follows the same course although the lesion may be bigger and healing slower than the primary focus.

Approximately about six to eight weeks after the initial infection, antigen reactive ab T lymphocytes appear in the lesion and this is characterised systemically by the development of cutaneous hypersensitivity to PPD and the Mantoux test becomes positive. At this stage, the host develops the ability to kill the organisms. However, a few bacilli may remain alive a in a metabolically inactive state in some of the caseous areas for months, years or even decades becoming activated at any time by a breakdown in immunity.

Progressive primary complex

The course of events described above, namely, a transient growth of the bacilli and their spread to lymph nodes flowed by encapsulation of the lesion and healing occurs in a very large proportion of the infected individuals. However, in about 10% of the patients, owing to a combination of increased bacterial virulence, increased hypersensitivity and lowered resistance to the infection, instead of healing, the lesion progresses into a more serious form.

The caseous material may liquefy and live bacilli may become disseminated through the bronchi to other regions of the lung or even to the opposite lung. Often, this is seen when the enlarged hilar node erodes a bronchus and discharges the bacilli. If hypersensitivity against the organisms is marked, further destruction of normal lung and dissemination of the bacilli are inevitable. In a study in children suffering from progressive primary complex, it has been shown that the production of IFN-**g** by their peripheral blood lymphocytes was significantly greater than normal children (Swaminathan *et al.,* 1999). This probably indicates that though these children are able to react by producing IFN-**g,** either it is not enough or additional factor(s) may be required to contain the illness.

Instead of the erosion of the bronchus, if large numbers of organisms are discharged into a blood vessel, miliary tuberculosis results with multiple granulomata in the lung, liver, spleen, kidneys, brain and bone marrow. Usually, the Mantoux test is negative in these patients.

Post primary tuberculosis

Post primary form of tuberculosis is the predominant type of disease seen in our country since a majority of the individuals would have been exposed to the tubercle bacilli in their childhood itself.

In most cases, post primary tuberculosis is first detected in the subapical region of one or both lungs, probably caused by spread of viable bacilli from a Simon's focus. The disease arises as a small tuberculous pneumonia. At this stage, the lesion is characterised by exudation of fibrin and recruitment of neutrophils and monocytes in the alveoli. As mentioned earlier, the recruitment of neutrophils at an early stage can be attributed to activation of complement and release of C5a, a potent neutrophil chemotactic factor. However, experiments in mice have shown that a persist-

ent neutrophilic influx may be attributed to T lymphocytes (Appelberg, 1992).

The lesion, which is predominantly intra alveolar at this stage, may. still heal spontaneously. On the other hand, it may progress slowly with well-developed epithelioid cell granuloma and central area of caseation. Alternatively, some of the granulomata may heal while others might develop in the same organ. Usually, however, the lesion undergoes extensive necrosis and liquefaction and then sloughing occur, especially through a patent bronchus, giving rise to a cavity. Viable *M.tuberculosis* is freely excreted through the sputum into the environment in this productive stage of the disease.

The formation of cavity is a major event in the natural history of tuberculosis. Since the cavity opens out into the airway, the bacteria proliferate more rapidly due to higher oxygen availability. Further, as the cavity communicates into the bronchial tree, bacilli can spread to the other parts of the lungs on the one hand and on the other, can be excreted out into the environment and thereby infect fresh individuals in the community.

Depending on the number of organisms being released from the cavity, lesions of varying sizes are produced elsewhere in the lung. Tuberculous cavities are of great pathogenic significance for two more reasons. The first is the formation of aneurysms of the arteries that cross the cavity. Although most of the vessels become fibrosed, in some, the destruction of the wall leads to the formation of aneurysms which then become vulnerable any time to cause haemoptysis and even fatal haemorrhage. The second reason is that widespread cavity formation destroys a large mass of the lung and when the disease is treated with anti tuberculous drugs, it heals with extensive fibrosis leading to considerable disability even after the primary process of the disease is controlled.

IMMUNOLOGICAL CORRELATES OF THE PATHOLOGICAL AND CLINICAL MANIFESTIONS OF TUBERCULOSIS

The two main outcomes of the interaction of *M. tuberculosis* with human beings, protection and immunopathology (which can eventual!y lead to disability or death) can be understood by looking at the immunological correlates of the various manifestations. Our current knowledge is derived from two major approaches: 1) studies on experimental infection in laboratory animals, especially mice where the disease can be dissected out using gene disrupted mice and 2) correlative studies in natural human infections where the immunological response patterns of peripheral blood leucocytes and tissue pathology can be undertaken.

There are obvious advantages and disadvantages when looking at each of the above mentioned approaches separately. For example, it is well known that the mouse is a very poor model to study human tuberculosis and guinea pigs are more appropriate (Mc Murray *et al.,* 1996). However, lack of gene disrupted guinea pigs and paucity of immunological reagents against guinea pig host cells and products have hindered a wide spread use of this species to understand the host response to *M.tuberculosis.* Thus caution must be exercised while extrapolating mouse data to human tuberculosis. On the other hand, the disadvantages while studying human disease are a) the *in vivo* immunological correlates of an evolving lesion cannot be studied and b) it is often difficult to get the affected tissues to examine the in situ immunological parameters and immunological profiling of peripheral blood cells may not be representative of what goes on at the site of lesion. However, occasionally, 'experiments of nature' have shown the crucial role of IFN-**g** (Jouanguy *etal.,* 1996; Newport *et al.,* 1996) and IL-12 (Altare *etal.,* 1998; de Jong *et al.,* 1998). In addition, the vital importance of the CD4 T cells in controlling *M.tuberculosis* has been amply demonstrated in HIV infected individuals.

Notwithstanding the obvious disadvantages of these two approaches, considerable progress has been made in our understanding the immunological basis of the disease and will be reviewed in the following section.

Protection

While initial complement activation and subsequent mediation of phagocytosis through the various complement receptors are important in the establishment of the infection, interaction with the complement system does not lead to killing of mycobacteria (Schlesinger *et al.,* 1990). Early in the infection, gd T cells appear to play a role in killing infected cells and the bacterial by the granule pathway (Dieli *et al.,* 2000). These cells decline after treatment suggesting that probably these are driven by the presence of viable tubercle bacilli.

It is presently held that anti tuberculous immunity is mainly mediated by T lymphocytes belonging to CD4, CD8 and gd subsets (Stenger and Modlin, 1999). As has been shown through a number of animal experiments and data

from human tuberculosis (Reviewed in Rook and Hernandez-Pando, 1996; Rook and Zumla, 2001), Type 1 response appears to be vital for protective immunity in tuberculosis. This involves the release of IL-12 from antigen presenting cells and IL-2 and IFN-**g** from T lymphocytes. Apart from CD 4 cells, CD 8 cells (Cho *et al.,* 2000; Pathan *et al.,* 2000) and CD 68 bearing macrophages (Fenhalls *et al..* 2000) have also been shown to produce IFN-**g.** It is believed that apart from these cytokines. the other pro inflammatory cytokine TNF-**a** also is critical in inducing protective immunity in tuberculosis (Kindler *et al.,* 1989; Hernandez-Pando *et al.,* 1997).

Immunopathology and tissue damage

The ideal response of a host to *M.tuberculosis* should be such that it is able to contain and destroy the bacilli without damaging the host tissue. This could be the case in the majority of individuals who have been infected but have not developed clinical disease. However, in all patients who have clinical disease, it can be safely assumed that this ideal response has not taken place. A major cause of the morbidity can be attributed to the nature of host response itself.

A number of cytokines appear to be important in producing the clinical manifestations of the disease. For example, it is known that IL-1 is responsible for the fever while TNF-**a** causes loss of appetite and loss of weight seen in tuberculosis. Similarly, IL-4, a cytokine associated with Type 2 response appears to play a role in inducing cavitary tuberculosis (van Cravel *et al.,* 2000). Additionally, murine experiments have shown that IL-4 is important for the toxicity of TNF-**a** and the fibrogenic effects of TGF-B (Hernandez-Pando *et al.,* 2004).

In studies involving experimental tuberculosis in the guinea pig it was shown that in the initial phase of infection there was a gross destruction of the affected tissue followed by fibrosis as indicated by an increase in the content of hydroxyproline (Jayasankar *et al.,* 1999). Similarly, in human cutaneous tuberculosis, it was shown that the TGF-**b** and collagen which were elevated in the active disease regressed with chemotherapy (Jayasankar *et al.,* 2002) thus demonstrating the importance of fibrogenic cytokines.

The role of B cells in tuberculosis

Traditionally, B lymphocytes and antibodies have been thought to be of marginal significance in tuberculosis. However, the data currently available suggests a more complex picture. We have seen the presence of large numbers of B lymphocytes (even up to 20% of the lymphocyte population in the granuloma) in cutaneous tuberculosis (Shakila *et al.,* manuscript under preparation). Similarly, evidence from animal studies using B cell-deficient mice has indicated that these cells could play an active role in containing the spread of the tubercle bacilli (Bosio *et al.,* 2000). Further, since B cells are potent antigen presenting cells, these could be of relevance in inducing the appropriate T cell response also. Additionally, in situ antibody production (Ridley and Ridley, 1986) especially in necrotic areas implies that these could be involved in immunopathology, It has been shown that in tuberculous lymph adenitis that plasma cells were more numerous in necrotic lesions compared to non necrotic ones (Ramanathan *et al.,* 1999).

STRESS AND IMMUNE RESPONSE IN TUBERCULOSIS

Early in the last century it was shown that phagocytes from individuals undergoing emotional stress were able to phagocytose less number of tubercle bacilli (Khansari *et al.,* 1990). It is well known that stress releases glucocorticoid hormones and these are capable of suppressing subsequent immune response (Rook and Zumla, 2001). These appear to act by a switch over to Type 2 cytokine response (Ramirez *et al.,* 1996) by increasing the production of IL-10 and reducing the release of IL-12 (Visser *et al.,* 1998) and down regulating the antimycobacterial effects of macrophages (Rook *et al.,* 1987). Thus, any efforts to understand the immunology and pathology of tuberculosis in human beings will have to include a systematic study of the effects of stress in precipitating latent tuberculosis - an area sadly neglected so far.

CONCLUDING REMARKS

It is clear from the foregoing that immuno protection and immuno pathology are finely balanced by a complex interplay of cells and cytokines (Fig 2). Further, a clear understanding of these factors would help answer the question as to why only a small fraction of infected individuals in an endemic area actually come down with the disease. Additionally, efforts are needed to institute adequate measures to prevent and revert the tissue damage inflicted by host responses. A major consequence of tissue damage is the permanent disability that a large number of patients suffer from which adds to the gross post treatment morbidity in tuberculosis. It is certainly clear that the issues

mentioned above need to be addressed to pave the way for devising early diagnostic tests and developing suitable strategies for prevention of this 'captain of all men of death'.

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