IMMUNOLOGY OF TUBERCULOSIS—AN OVER VIEW

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Introduction

Over a hundred years have passed since Robert Koch has isolated the tubercle bacillus, but still the immune mechanisms in tuberculosis are not yet completely unravelled. The information explosion in the field of immunology during the last ten years has resulted in a modest beginning in the knowledge of tuberculoimmunity at the cellular and molecular level. It is necessary to understand the host responses to mycobacterium tuberculosis in order to appreciate the clinical consequences, diagnosis and prophylaxis of tuberculous infection. In the following sections the progress in our understanding of host's immune response to M. tuberculosis is considered with special emphasis on immunopathogenesis, immunosuppression and immunodiagnosis.

The clinical and histologic features of any infectious disease are not only the result of the toxic and invasive properties of the pathogen but also of the immune reactions of the host. The first step in the complex phenomenon is the recognition of the antigens *present* on the bacteria as being foreign. There are two main classes of lymphocytes–the T-lymphocytes (thymus derived) and the B-lymphocytes Bursa or bursa equivalent derived). The B-ceils are the antibody producing cells. Upon contact with specific antigen. the B-ceil divides, matures and differentiates into a plasma cell and synthesizes antibodies (immunoglobulins). The T-cells on activation by antigen produce lymphokines. Lymphokines are biologically active mediators of several immune responses. Some of the well studied lymphokines are chemotactic factors, migration inhibitory factors, mitogenic factors, interferons etc. These lymphokines are apparently involved in activation of macrophages. The macrophage is the phagocytic cell which phagocytoses bacteria and particulate matter and thus plays an important role in aliminating the pathogen. There is perfect co-ordination among the three types of cells (macrophage, T-cell and B-cell) for maintenance of homeostasis.

The antibody response is referred to as the humoral response whereas that involving T-cells is said to be cell mediated. Tuberculosis is a classic example of a disease that is controlled entirely by ceil mediated immunity involving the macrophage as the effector ce!l and the lymphocyte as the immunoresponsive cell.

Immunopathogenesis

The complexity of tuberculoimmunity is at least in part due to the unique features of the bacteria itself. It produces neither an exotoxin nor an endotoxin. Its thick lipid coat is responsible for its resistance to killing by adverse environment–be it inside of the macrophage or antibodies or drugs. The sulfolipids of the bacteria is believed to prevent the release of lysosomal enzymes into the phagosomes of the macrophage. Its virulence relates to its ability to survive and proliferate in mononuclear phagocytes.

Several of the bacterial constituents appear to influence the pattern of host response[1].

(1) **Cord factors:** (trehaloss-6, 6'-dimycolic acid), the material responsible for the *in vitro* serpentine cord like growth of M. tuberculosis, inhibits migration of leucocytes and stimulates granuloma formation.

(2) High molecular weight lipids and waxes are probably responsible for much of the tissue reaction to the bacteria: this may account for its resistance to killing by lysosomal enzymes and other agents.

(3) Wax-D and tuberculoproteins are associated with tuberculin hypersensitivity and skin test positivity.

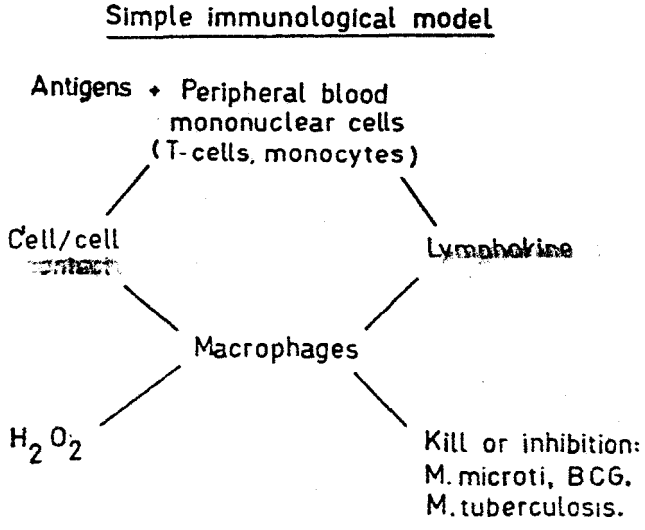
M. tuberculosis is a facultative intra-cellular organism. Essentially tuberculosis is a struggle between the macrophage and the bacillus. Upon entry into the human host, which is usually by droplet inhalation, the bacteria is phagocytosed by the alveolar macrophages. The precise biochemical events in antigen processing are not understood. The antigens are then presented to the T-lymphocytes with the major histocompatibility antigens on the macrophage surface. The T-cells recognize the antigen and in turn produce lymphokines which are soluble mediators (Table-1, see appendix) which through their biologic activity are capable of recruiting host inflammatory cells, activating them and keeping them at the site. This leads to the arrival of a large number of activated macrophages. Some of the

characteristics of activated macrophages are listed in Table-2. (see appendix).

The mechanism of intracellular killing of the bacteria by the macrophage is probably by more than one way. There are several evidences to show that hydrogen peroxide and reactive oxygen intermediates (Superoxide, hydroxylion, Singlet oxygen, etc.), produced by the macrophage during its metabolism are detrimental to the organism[2,3,4]. Gately et al[5] have isolated a lymphokine which is capable of increasing the intracellular concentration of hydrogen peroxide. Walker and Lowrie[6] have shown increased intracellular killing by mouse peritoneal macrophages by hydrogen peroxide which was markedly diminished upon addition of catalase, an enzyme known to detoxify hydrogen peroxide.

Interferon- [8], a lymphokine produced by T-lymphocytes plays an important role in immune response[7]. It has been shown by Murray et al[8] that interferon- [8] enhances intracellular killing of Leishmania donovani by macrophages both by a hydrogen peroxide dependent and a hydrogen peroxide independent mechanism. It would seem reasonable to determine if interferon- [8] (affects killing of M. tuberculosis also.

The cell mediated immunity in tuberculosis is represented in a simplified form in the figure[9] below:



Immunosuppression

Recently substantial evidence has appeared indicating that the depressed cellular immunity observed in mycobacterial diseases is in some instances due to over activity of suppressor cell systems. The existence of sub-populations of lymphocytes which decrease rather than increase certain immune responses was first suggested in 1970[10].

There are two types of suppressor cells: the first is a T-cell and can be found following large intravenous doses of BCG[11], M. lepremurium[12] or several M. avium like organisms[11]. The second kind of suppressor cell appears to be the macrophage which is found in the presence of heavy systemic loads of mycobacteria or mycobacterial products and chronic granulomatous lesions[12,13,14]. Ellner[15] has reported on the suppressive role of monocytes in tuberculosis. Macrophages produce factors such as prostaglandins, arginase and thymidine which *in vitro* produce suppression of lymphoproliferation. However, in tuberculosis it appears to be tuberculin specific, and acts via cellular interaction with responding cells apparently by mechanisms not involving the factors mentioned above.

Immunosuppression has also been attributed to mycobacterial components. Ellner[16] has shown that mycobacterial D-arabino-D-mannan suppresses antigen induced radiolabelled thymidine incorporation and migration inhibition factor production by mononuclear cells. Wadee et al[17] have performed elegant *in vitro* experiments to show that both the lipid components of the tubercle bacillus as well as the suppressor monocytes can cause immunosuppression.

According to Katz et al[18] tuberculous patients have profound impairment of antibody synthesis following polyclonal activation with pok weed mitogen *in vitro.* This depression appears to be due in part to the presence of circulating suppressor monocytes which are capable of suppressing antibody production.

We must admit that the rote for suppressor cells in the pathogenesis of tuberculosis is poorly understood. We need to demonstrate the direct involvement of these suppressive factors in the intracellular killing of M. tuberculosis. More in-depth studies on specific immunosuppression is required before one can interpret the present *in vitro* experiments in terms of immunosuppression in disease.

Immunodiagnosis

A definitive diagnosis of tuberculous infection requires the direct demonstration of the bacteria in the host. Though this is possible in smear positive pulmonary tuberculosis patients; in smear negative pulmonary tuberculosis and extra pulmonary tuberculosis, it is quite difficult to demonstrate the bacilli either because they are present in insufficient numbers. or because of the inaccessibility to the site of lesion as happens in extra pulmonary tuberculosis.

The fact that no serodiagnostic test is extensively carried out at present is a sure indication that all the tests available have some drawback or the other, the most important of which is that they are unable to discriminate between a past. infection or exposure and active infection. Most of the tests devised so far have aimed at assaying either a supposedly specific antibody or less often, antigen. The reason for the failure of the tests is mainly due to the non-specificity of the detection systems. This is because many of the antigens of the tubercle bacillus are shared by various mycobacterial species and to a lesser extent by other genera.

The endeavour to serodiagnosis of tuberculosis dates back to the last century. The important ones are listed in Table-3 (see appendix). All these tests, in spite of several modifications; face the problem of overlap between infected patients and skin test positive healthy subjects and could not stand the test of time. With the introduction of enzyme linked immunosorbent assay and radio immuno assay the sensitivity of the tests has been well taken care of i.e., the antigen or antibody can be detected at nanogram or even picogram levels. The authors' studies with enzyme linked immunosorbent assay using PPD as antigen have not been rewarding. The overlap of antibody levels between the patients and the control groups was so much that it was not possible to discriminate the two groups[19].

By applying the technique of radio immuno assay Kadival[20] was able to show a significant difference in the antigen concentration in the sputum of tuberculous and non-tuberculous patients. Sada[21] reports 95% specificity and 81.25% sensitivity of detection of mycobacterial antigens in the cerebrospinal fluid of tuberculous meningitis patients by enzyme linked immunosorbent assay.

The prospects of developing more specific tests for immunodiagnosis are discussed in the section on scope for future research.

Scope for future research

The immunodeficiency in tuberculosis at the molecular level needs to be known. Only then chemotherapy can be coupled with a suitable immunopharmacologic agent and make treatment more efficient. At present, it is not known whether the defect lies in the macrophage per se, which lacks the capacity to destroy M. tuberculosis or in the T-lymphocyte which is unable to produce the right lymphokine to stimulate the macrophage to perform its function. Modern technology offers a number of tools to study these functions *in vitro.* With the advent of the fibre-optic bronchoscope, macrophages from the lesion can be obtained which will yield more direct information about the disease, than peripheral blood cells.

Kohler and Milestein[22] have provided us with a very promising research tool. Monoclonal antibodies produced by the hybridoma technique are capable of recognising a single epitope in an antigen molecule. A crude extract of the organisms containing a large number of various antigens when passed through an affinity column of a monoclonal antibody will exclude all the nonspecific ones and retain only one specific antigen, which can be used for serodiagnosis or skin testing. In 1981 Coates et al[23] produced eight such monoclonal antibodies and only one of them (TB-72) has been found specific for M. tuberculosis $H_{37}Rv$. TB-68 is specific for M. tuberculosis $H_{37}Rv$ as well as BCG. TB-72 has been tried for antibody detection by Ivany[24] and found to detect 74% of culture proved tuberculosis with negligible false negativity. Application of the recently developed Hybridoma and DNA cloning techniques in the field of tuberculosis opens up a new era which may solve many of the unresolved issues.

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APPENDIX

Products of activated lymphocytes (Table-l) [1]

Migration inhibitory factor.
Macrophage activating factor.
Chemotactic factor for macrophages.
Mitogenic factors.
Factors affecting antibody production, lnterferon.

Activated Macrophages (Table-2) [25]

Increase in size.
Increase in cytoplasmic organelles involving increases in enzymatic activity of mitochondria and lysosomes.
Increase in membrane mobility
Adherence and spreading
Increase in phagocytosis and pinocytosis
Increase in ability to destroy bacteria
Production of mediators affectors affecting lymphocytes.

Table-3

Agglutination test
Complement fixation test
Hemagglutination
Agglutination of inert particle coated with antibody
Precipitation and gel diffusion
Fluorescence antibody test
Enzyme linked immunosorbent assay
Radio immuno assay

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