CHAPTER I

INTRODUCTION

Tuberculosis is one of the most widesprad of the life-threatening infectious disseases still affecting human and animal populations throughout the world (1). In 1882, Koch (2) discovered the tubercle bacillus; Mycobacterium tuberculosis. He described a set of criteria the so called Koch 's postulates, that have been used to relate a suspected pathogen with a given disease (3). Despite the ready availability of a vaccine and existence of chemotherapeutic agents, tuberculosis continues to pose a major health problem, especially for many third world countries (4).

Properties of Mycobacterium tuberculosis.

Mycobacteria are considered transitional forms between Eubacteria and Actienomycetes. The mycobacteria have therefore been classified in the order Actinomycetalese (5). The ones that cause human diseases can be classified into 3 groups (6):

1. TB complex. The mycobacteria in this group are Mycobacterium tuberculosis, Mycobacterium bovis and Mycobacterium africanum.

2. Atypical mycobacteria. The bacteria in this group cause mycobacteriosis in man and can be clasified into 4 groups by Runyon 's classification (7) that contained :

Group I. Photochromogen

Group II. Scotochromogen

Group III. Non-photochromogen

Group IV. Rapid growers

3. Mycobacterium leprae.

Tubercle bacilli are typically slightly bent or curved slender rods, about 2-4 Um. long and 0.2-0.5 Um. wide. In culture media, the cells may vary from coccoid to filamentous. Strains differ in their tendency to grow as discrete rods or as aggregates with their long strands parallel, called serpentine cords. The most virulent strains that grow in cords contain a surface lipid; the cord factor. To stain the acid fast tubercle bacilli in smears or in tissues Ziehl-Neelsen 's method is used. It promotes penetration of the carbolfuchsin dye. Subsequent washing in acid alcohol decolorizes most bacteria in a few second, whereas acid fast organisms retain the red stain . The tubercle bacillus is an obligate aerobe, It can grow in simple synthetic media and generally shows a marked nutritional preference for lipids. Egg yolk has been a constituent of many enriched media which are used for

diagnostic cultures. Growth of tubercle bacilli in culture media and in animals is charecteristically slow. The cell walls of mycobacteria contain a peptidoglycan with diaminopimelate and have a remarkably high lipid content (up to 60 %), much of which is attached to polysaccharide. The glycolipids and proteins are located in a firmly attached outer layer of the wall , and external location of lipid accounts for the hydrophobic charecter of the cells (8).

The thickness and lipid rich cell wall of mycobacteria also accounts for some of the other unusual properties of mycobacteria :

1. Acid fastness.
2. Slow growth and resistance to acid or alkaline environments.
3. Causing granulomatous reaction in host.
4. Resistance to the bactericidal action of antibodies and complement.

Lipid compound of tubercle bacilli.

The most striking chemical feature of the mycobacteria are their extraordinary high lipid content. Among the lipids extracted with neutral organic solvents are (8) :

1. True waxes : esters of fatty alcohol. While many different fatty acids are formed in mycobacteria, mycolic acid appears to be unique to the cell walls of these organisms and to norcardiae and corynebacteria.

2. Mycosides or glycolipids : lipid-soluble compounds with covalently linked lipid and carbohydrate moieties. There are 2 important mycosides in Mycobacterium tuberculosis; cord factor and wax-D.

2.1 Cord factor. This factor, which may be essential for both virulence and serpentine growth, was extracted from virulent cells by petroleum ether (9). It has been identified as a mycoside,6-6-dimycoelyltrehalose. The toxicity of cord factor is demonstrated by its ability to inhibit migration of normal polymorphonuclaer cells in vitro. Virulent tubercle bacilli, and 10 Ug. of cord factor given subcutaneously will kill a mouse. Mice have been protected against tuberculosis by immunization with a complex of cord factor and methylated bovine serum albumin, or by passive transfer of rabbit anti-cord factor serum.

2.2 Wax-D. The high molecular weight wax-D is not a true wax but contains the characteristic amino acid of the basal wall layer, like polymers of hexoses and hexosamines. In a water-in oil emulsion (Freund 's adjuvant) this fraction, like whole tubercle bacilli,

enhances the immunogenicity of a variety of added antigens. Moreover, a mixture of wax-D and protein of tubercle bacilli induces delayed hypersensitivity to tuberculin, whereas the protein alone is poorly immunogenic.

1.3 Phospholipid. The crude phosphatide fraction has the interesting property of evoking a cellular response resembling tubercle formation and caseation necrosis. It contains both saturated and unsaturated fatty acids eg. pulmitic, linoleic, phthioic and tuberculostearic acids.

Pathogenicity.

The agents of human tuberculosis (Mycobacterium tuberculosis and Mycobacterium bovis), are pathogenic for various lower animals, especially guinea pigs and mice. Human tubercle bacilli freshly isolated from pulmonary lesions, produce progressive disease in guinea pigs, These animals die within 1-6 months after infection, depending on the size of the inoculum.

Genetic variation.

As virulence varies quantitatively , serial passage through artificial culture media selects indirectly for less virulent mutants, while animal passage of such strains, selects directly for mutants with restored virulence.

One attenuated bovine strain, carried through several hundred serial cultures on unfavorable (bile-containing) media, is known as bacille Calmette-Guerin or BCG. This strain is used to immunize humans againt tuberculosis (10).

Tuberculoprotein.

1. Old tuberculin.

Tuberculin as originally described by Koch, as old tuberculin (OT), is prepared by autoclaving or boiling a culture of tubercle bacilli, concentrating it ten folds on a steam bath, filtering off the debries, and adding preservative. It is remarkable for its heat stability: after being autoclaved it remains soluble and retains specific determinants of the protein in the infecting bacilli.

2. Purified protein derivative (PPD).

PPD, a slightly more refined tuberculin, is prepared by precipitation several times with 50% saturated ammonium sulfate. The product is mostly a mixture of small proteins (average molecular weight 10,000).

The test procedures for the tuberculin skin test is by intradermal injection of an appropiate

standardized PPD into the most superficial layers of the skin of the forearm (Mantoux test). The average diameter of induration (and not simply erythema) at the injected site is measureed at 48 hours, and reactions less than 10 mm. diameter are recorded as doubtful. In epidermiologic work, the standard test dose generally used is 5 tuberculin units (TU) of PPD (0.1 ml.), corresponding to OT 1:1000.

Pathogenesis of tuberculosis.

Tuberculosis is a granulomatous disease. The granulomas are induced either directly by granulomagenic substances such as wax-D, cord factor etc. or by immunological response to antigens. The cell composition is in a dynamic state and varies throughout the various stages of formation and involution. The tightly packed cells permit the emission and receipt of inhibitory or facilitating signals, although B cells are also present. Macrophages are abundant and undoubtedly continue their phagocytic, affector, and effector roles in the immune response. These cells may be transformed into epithelioid cells, which are less phagocytic but active in pinocytosis and in the elaboration of digestive enzymes. Eventually some of these cells may form the characteristic multinucleate giant cells.

Neutrophils are present in the earliest stages of granuloma formation and also later in liquefying granulomas, where the enzymes they release may further contribute to the liquefaction of the caseous material. Fibroblastic infiltration and collagen production are important in the repair and subsequent fibrosis of the lesion.

Immunology of tuberculosis.

In primary tuberculosis, neutrophils and macrophages are an important first line natural defence mechanism that kills and eliminates the infecting organism by the process of phagocytosis (11). However, Mycobacterium tuberculosis, which is an obligate intracellular parasite, may survive and multiply within phagocytic cells. A certain strain of tubercle bacilli which contains mycobacterial sulfatides may prevent the formation of phagolysosomes. Infected macrophages can initiate the afferent limb of the immune response by processing and presensting tuberculous antigens to specific receptors of lymphocytes in regional lymph nodes. T- and B-lymphocytes which recognized specific antigens can transform and proiliferate to be sensitized T- and B-cells that mediate cell-mediated immune response and synthesis of specific antibodies respectively in the primary immune response.

1. Cell-mediated immune response.

Upon reexposure to the same antigen from a second infection or endogenous proliferation of a focus of tubercle bacilli, specifically sensitized T-lymphocytes come into contact with processed antigen that is released from infected macrophage within tubercle. This results in the production of a large number of soluble mediators or lymphokines such as migration inhibition factor (MIF), macrophages activating factor (MAF), chemotactic factor (CF) etc. and also causes blast transformation for increasing numbers of antigen specific sensitized lymphocyte. The sensitized lymphocytes may directly participate in a cell-mediated immune response by killing cells with specific surface antigens. This is known as lymphhocyte mediated cytoticity. The lymphokines which are released at the site of antigen deposition, initiate and amplify the cell-mediated immunity by their biologic activity in recruiting host inflammatory cells, activating them, and keeping them at the site of infection. Therefore, MAF could activate blood derived monocytes to become activated macrophages that exhibit increasing bactericidal capacity by morphologic, enzymatic and metabolic changes. They become larger and spread more rapidly than normally. MIF can inhibit migration of macrophages and chemotactic factor attracts activated macrophages to infiltrate at the reaction site. They can

then phagocytise more efficiently and kill tubercle bacilli at a markedly enhance rate compared to unstimulated or resident cells. The induction of acquired cellular immunity is immunologically specific, while the enhanced activity of altered macrophages is non-specific (12).

2. Humoral immune response.

The role of B-cells in immunity to tuberculosis is not clear. Serum antibodies do not account for this immunity. Though the tubercle bacilli enhances formation of antibodies to a wide variety of immunogens, antibodies to proteins and polysaccharides of tubercle bacilli are generally found only in low titers in tuberculous individuals, and the levels observed have no diagnostic and prognostic value. These antibodies are not bactericidal in vitro and, althrough they promote phagocytosis of tubercle bacilli in vitro, the organisms multiply within the phagocytes (11). Moreover, increased quantities of serum antibodies specific for mycobacterium antigens of patients with miliary tuberculosis (13) or their circulating antigen-antibody complexes could interfere with an effective cell-mediated immunity. The serum factors in some tuberculous patients can inhibit sheep red cell rosette formation or lymphocyte transformation tests in vitro (14).

Acquired cell mediated immunity; the main characteristic of the antituberculous response.

Koch (3) was the first to describe the reinfection phenomenon that still bears his name; the Koch phenomenon or delayed-type hypersensitivity. He demonstrated an accelerated inflammatory and healing response in the skin of tuberculous guinea pigs re-infected with the homologous organism. The primary dermal infection site was associated with a slowly progressive, localized granulomatous response with extensive lymph node involvement. The secondary infection site developed an early localized indurative response that peaked at 72 hours and was followed by rapid healing. Koch demonstrated that a similar local response could be induced in skin of tuberculous individuals by the intradermal injection of heat-killed or culture filtrate tubercle bacilli. The diagnostic value of this reaction was immediately recognized long before its true nature was known (15). This local response is not transferable to naive recipients by hyperimmune serum. It is ,however, transferable when sensitized lymphoid cells that have been harvested from tuberculin hypersensitive donors are infused. Antituberculous cellular immunity can thus be transferred to normal recipients. In tuberculous experimental animals, mononuclear phagocytes of BCG immunized rabbits can engulf and kill virulent tubercle bacilli at an increased rate compared to normal

macrophages (16). Hyperimmune serum had little enhancing effect in this system. In addition, tuberculosis antisera were not protective in vivo. Antituberculous immunity can be deduced from the inability of T-cell depleted mice (either neonatal thymectomized or congenitally athymic) to express tuberculin hypersensitivity or acquired antituberculous resistance (17). The increasing mononuclear infiltration that is seen within the alveolar tissues of lung of T-cell depleted mice, could be readily reversed by an infusion of normal or immune splenic T-cells (18). The restorative effect by the T-cell infusion provides further evidence that one of the major contributions made by immunocompetent T-cells to the defence of the host againts a tuberculous challenge, is the limitation of secondary spread of the infection to univolved organs. (19). Acquired antituberculous immunity is mediated by a population of immunocompetent T-cells that enter the developing tubercle from the spleen and regional lymph nodes (20).

Acute or subacute inflammation occurs with exudation of fluid and aggregation of polymorphonuclear leukocytes around the bacteria. Granulomas form when the individual becomes hypersensitive to tuberculoprotein. The macrophages then undergo a dramatic modification on contact with tubercle bacilli or their products, becoming concentrically arranged in the form of elongated epithelioid cells. They form the

tubercles that are characteristic of this disease. In the center of the tubercle, some of these cells may fuse to form one or more giant cells, with dozens of nuclei arranged at their periphery and viable baciili often visible in their cytoplasm. Outside the multiple layers of epithelioid cells, is a mantle of lymphocytes and proliferating fibroblasts. This eventally leads to extensive fibrosis. Tuberculin sensitivity is clearly a double-edged sword. It is assosiated with increased resistence, but under some circumstances the response can also lead to harmful reactions that exacerbate symptoms and increase spread of the bacteria (8).

Delayed type hypersensitivity and cell-mediated immunity.

Delayed hypersensitivity can be thought of as an in vivo model for cell-mediated immunity. Its response to specific antigens, reflects the host 's immune status or resistance to that antigen (21). Nevertherless, there are considerable evidences that the cellular mechanism, underlying both delayed hypersensitivity and cellular immunity is identical. Delayed hypersensitivity and cellular immunity usually develop simultaneously. Methods of induction are the same and both require the presence of live bacteria which are able to multiply in vivo. If T-cells are depleted, both delayed hypersensitivity and cellular immunity disappear. Both can be adoptively transferred with T-cells. On the

other hand, there is evidence to suggest that both are unrelated phenomenon. Some of these are: the level of resistance dose not correlate with the degree of delayed hypersensitivity. Resistance can be induced without induction of delayed hypersensitivity or vice versa. RNA and polysaccharide of tubercle bacilli can induce immunity without delayed hypersensitivity. Wax-D can also induce delayed hypersensitivity without immunity. Furthermore, desensitization to tuberculin is not associated with loss of resistance (8). Finally, T-cell subpopulations responsible for the expression of these two cell-mediated responses to the tubercle bacillus can also be differentiated functionally. These T-cell subpopulations can now be identified by Lyt alloantigens (17,23,34).

Lymphokines

The cell-mediated immune response which is mediated by T- lymphocytes plays a major role in antituberculous immunity. The lymphokines that are produced from antigen stimulated lymphocytes have marked biologic effects on a variety of cell types including T-and B-lymphocytes, macrophages, eosinophils, basophils, neutrophils, endothelial cells and fibroblasts. The functional activity of lymphokines on effector cells is to cause them to be nonspecifically recruited to the site of reaction and amplify the inflammatory response of the deleyed

hypersensitivity. Although lymphokines were originally described as in vitro phenomena, increasing evidence suggests that they play an important role in in vivo cellular immunity as well (23). More than 90 lymphokine activities have been identified (24). Lymphokines can be classified functionally according to their effects in; proliferation (Mitogenic factor), differentiation (phenotypic expression), motility (Migration inhibition factor) and activation (Macrophage activating factor) (25). Moreover, they can be categorized from their effects on certain target cells as shown in table 1 (26).

Table 1. Lymphokines.

A. Mediators affecting macrophages.
   1. Migration inhibitory factor (MIF).
   2. Macrophage activating factor (MAF).
   3. Chemotactic factors for macrophages.
   4. Antigen dependent MIF.

B. Mediators affecting polymorphonuclear cells.
   1. Chemotactic factor (CF).
   2. Leukocyte migration inhibitory activity (LIF).
   3. Eosinophil stimulation promotor (ESP).

C. Mediators affecting lymphocytes.
   1. Mitogenic factor (MF).
   2. Factor enhancing antibody formation.
   3. Factor suppressing antibody formation.
   4. Interleukin 2.

D. Mediators affecting other cells.
   1. Lymphotoxin (LT).
   2. Osteoclastic factor (OAF).
   3. Collagen-producing factor.
   4. Colony-stimulating factor.
   5. Interferon.

E. Immunoglobulin-binding factor (IBF).

F. Procoagulant (tissue factor).

1. Migration inhibitory factor.

Specifically sensitized lymphocytes respond to specific antigens by release of lymphyokines, -macrophages migration inhibitory factor (MIF) and -leukocyte migration inhibitory factor (LIF). MIF inhibits the migration of macrophages and monocytes, but LIF selectively inhibits the random movement of polymorphonuclear leukocyte. The distinction between MIF and LIF was first clearified by Rocklin (27). He found that a human MIF rich fraction of molecular weight (MW.) 23,000 selectively inhibited the migration of human monocytes and guinea pig macrophages with no detectable effect on the mobility of polymorphonuclear leukocytes. Moreover, the human LIF rich fraction of MW. 68,000 was responsible for migration inhibition of human buffy coat cells or of purified polymorphonuclear cells. Both mediators appear to act without apparent genetic restricton, since MIF and LIF from one species will be active on allogeneic and even on xenogeneic target cells (28).

1.1 Leukocytes migration inhibitory factor (LIF).

Human LIF is a protein of MW. 68,000 . It is susceptible to chymotrypsin but resistant to treatment with nuraminidase. On disc gel electrophoresis at pH 9.1, the migration velocity of LIF is similar to albumin. LIF

is heat stable when exposed to 56 °C for 1 hour, but it is destroyed at 80 °C for 30 minutes or at pH 7-10 . LIF appears to contain one or several disulfide linkage groups critical for its biologic activity. Exposure of neutrophils to LIF for 1 hour at 37 °C is sufficient to inhibit the motilty of the cells for the next 24 hrs., whereas exposure for shorten periods is less effective (26). The mode of action of LIF on the target cell at molecular level is unknown. LIF may affect microfilament function of polymorphonuclear cells, possibly through changes in the cellular levels of cyclic nucleotides . The nutural substrate for LIF and its relationship to the putative LIF receptor on neutrophils is unknown. The LIF receptor on neutrophil surface appeares to contain sugars (29). Thus, LIF inhibits polymorphonuclear cell migration by a direct action on cells and an amplification pathway that is mediated by low molecular weight chemotactic inhibitors similar to neutrophil inhitory factor (NIF) (30). The expression of LIF activity depends upon the presence of specific antigen, but the addition of non-cross reacting antigens, it is ineffective. However, the LIF production can also be induced by mitogen such as concanavalin A (Con A) or phytohemagglutinin (PHA) with similar physicochemical and immunochemical properties to that relesed by specific antigen stimulation (31).

1.2 Macrophage migration inhibition factor (MIF).

MIF was the first described lymphocyte mediator (26). A spleen cell suspension taken from tuberculous guinea pigs with delayed hypersensitivity , would not migrate out of the capillary tube if old tuberculin was present in the culture medium. The technical reproducible methods for MIF detection were developed for antigen induced migration inhibition. Peritoneal cells from experimental animals that consisted of 70-80% macrophages were used to be the target cells for MIF detection in a capillary tube technique. The production of antigen induced MIF by human or animal lymphocytes is closely associated with the precence of in vivo cellular hypersensitivity of the host to that antigen. Human MIF is a protein of MW. 23,000-55,000. It is heat stable, has an isoelectric end point of about 4.0-6.0 and migrates anodally to the albumin band in electrophoresis. How MIF effects macrophage migration is not known. It would appear that cells which come out of the capillary tube are first retarded in their migration because they clump together and may physically impede the migration of cells behind them. This would result from membrane changes which cause the cells to become more sticky. In addition to the specific antigen induced MIF production by lymphocytes from blood, lymph node, spleen, peritoneal exudate and thymus lymphocytes from several

animal species, including man, can be triggered in a nonspecific manner to produce MIF. Thus, stimulation with plant lectins, such as PHA, Con A, and with antigen-antibody complexes results in production of MIF with apparently similar physicochemical and immunologic properties (32,33).

2. Lymphocyte mitogenic factor (LMF).

Following stimulation by specific antigen, specifically sensitized lymphocytes release into the culture fluid a substance which has mitogenic activity for non-sensitized lymphocytes. It can induce normal lymphocytes to undergo blast transformation and incorporate induced amouts of tritiated thymidine into cellular DNA. The culture fluids from antigen stimulated sensitized lymphocytes after 24-48 hrs. can induce nonsensitized lymphocyte transformation in about 6 days. LMF is a protein of MW. 20,000-30,000. It is a non-dializable macromolecule, and is heat stable at 56 C for 30 mins. LMF, is capable of activating or recruiting nonsensitized lymphocytes, an can furnish a mechanism for expanding a cellular reaction and produce greater amounts of other mediators. The role of LMF in the proliferative response, may be that the first stage is initiated after activated lymphocytes have released this factor. The second stage would be the non-specific activation of other lymphocytes by the material.

Mitogenic stimulation of nonspecific lymphocyte, as well as antigen stimulated specific lymphocytes, can also produce this mediator (26).

3. Transfer factor.

Transfer factor has a molecular weight of less than 4,000, is resistant to treatment by DNase or RNase. The ability to transfer specific delayed hypersensitivity in humans can be accomplished by transfer of a dializable material obtained from disrupting the sensitized lymphocytes or from stimulating them with specific antigen (26).

Tuberculous effusion

Although tuberculous pleurisy is usually a self limited disease, 65% of untreated patients develop active pulmonary or extrapulmonary tuberculosis within five years of the occurence. Tuberculous pleurisy denotes inflammatory disease of the pleura due to Mycobacterium tuberculosis. Usually, it is an unexplained pleural effusion which first suggests the diagnosis. The disease is thought to result from rupture of a subpleural caseous focus in the lung into the pleural space . Hypersensitivity to tubercle bacilli also plays an important role in determining the occurence and extent of pleural effusion. Tuberculous pleurisy most commonly occurs 3 to 7 months following a

primary infection, but may be seen at any time in the natural course of tuberculosis.It is the most common type of extrapulmonary tuberculosis (35).

1. Clinical features.

Tuberculous pleurisy was previously found almost exclusive in young adults. The presently increasing age of patients with tuberculous pleurisy creates diagnostic difficulties since malignancy, congestive heart failure, pneumonia, and pulmonary infarction are common problems in older patients. Cough is usually nonproductive, particularly when active pulmonary or intrabronchial lesions are not present. The chest pain is usually pleuritic. Night sweats, chills, dyspnea, weakness and weight loss are common complaints, but occur less frequently than cough and chest pain. The hemoglobin content and peripheral white blood cell count are usually normal in tuberculous pleurisy. After adequate therapy, these complications are rarely seen.

Pneumonia, pulmonary infarction, malignant disease of the pleura, systemic lupus erythematosus, and subdiaphragmatic abcess as well as other diseases, can be confused with tuberculous pleurisy. Different treatment and prognosis require that a specific diagnosis be made as early as possible.

2. The pleural fluid.

Pleural fluid white blood cell count were between 1,000 and 6,000 cu.mm. in tuberculous pleurisy and more than 95% lymphocytes are usually found. The finding of 95-100% lymphocytes is not specific for tuberculsis, but is also frequently seen in carcinoma, lymphoma and in chronic effusions of many causes (36). Serial pleural fluid differential white blood cell counts may show progression from a predominance of polymorphonuclear cells in early cases to a predominance of lymphocytes later on. Eosinophilia is rare in tuberculous pleurisy.

The pleural fluid protein concentration is usually more than 3 gm.% . Tuberculous pleural fluid is usually an exudate. Elevated lactic acid dehydrogenase (LDH) levels in the pleural fluid were found in 77% (37). Glucose concentration in tuberculous effusions is most commonly decreased when compared to a simultaneous serum glucose level.

3. Roentgenographic findings.

Pleural effusion occur at grater frequency on the right-side but 10% had bilateral effusions. About 37 % of patients had coexisting active pulmonary and pleural tuberculosis. Previous chest X-rays, if available, may show the pulmonary lesion prior to the development of

effusion. Pulmonary disease varies from minimal to far advanced and may be either unilateral or bilateral. The pleural effusion always occurred on the side of a parenchymal infiltration in patients with pulmonary and pleural disease (35).

4. Pleural fluid culture.

Culture of the pleural fluid may reveal tubercle bacilli. In most other series positive culture results were found in less than 30% of cases (38). The percentage of positive cultures can be increased by centrifuging large volumes of pleural fluid (100-500 ml.). Dubos liquid medium has been superior in recovering organisms to Lowenstein-Jensen Medium (35).

5. Pleural biopsy finding.

Needle pleural biopsies have been reported positive in 50-80% of cases of tuberculous effusion (35). A biopsy demonstrating caseating epithelioid granulomas is accepted as tuberculous although only the identification of acid fast bacilli or a positive culture from the specimen is truely diagnostic (38). Pleural biopsies were positive in 69% of cases with caseating epitheioid granulomas, in 26% of noncaseating granulomas and acid-fast bacilli were found in 14% (35). Mestitz et al (40) found that the chances of obtaining diagnostic results from pleural biopsy

were uninfluenced by the size of the white blood count in the pleural effusion, by presence of a parenchymal lung shadow, by anti-tuberculous therapy, or the duration of symptoms. Internal memmary lymph node biopsy has also been reported to be useful in obtaining a diagnosis of tuberculous pleurisy (40). Tubercule bacilli were cultured from pleural biopsy tissue more frequently using Dubos liquid medium (35). Thus, failure to find granulomas on needle biopsy does not exclude a diagnosis of tuberculosis, and repeat biopsies should be performed. Culture of pleural biopsy specimen is as useful a diagnostic tool as histologic examination, and may be positive when microscopy is negative. The triad of pleural biopsy histologic examination, pleural biopsy culture, and pleural fluid culture for tuberculosis should be performed on all patients with pleural effusions. Sputum cultures infrequently yield tubercle bacilli unless visible pulmonary tuberculosis coexists with the pleurisy.

6. Tuberculin skin test.

It was formerly observed that almost all patients with active tuberculosis have a positive intermediate strength tuberculin skin test. A tuberculin test indicates whether the individual shows evidence of cell-mediated immunity as a result of a past or present mycobacterial infection (8). More recently reports stress the frequent occurence of negative skin test in tuberculous pleurisy

(41). These patients had negative PPD intermediate tests, but all had positive reactions when re-evaluated with a repeat intermediate PPD or with a second strength PPD. Falk (38) stated that tuberculous pleurisy can be excluded if the tuberculin test is negative after six to eight weeks. The temporarily negative PPD skin test should be considered as transient hyposensitization due to preferential sequestration of antigen reactive lymphocytes in the pleural space (42). Adherent suppressor cells in anergic patients which can suppress in vitro correlates of cellular immunity (43), or technical failure in the intradermal PPD skin test can cause failures. In addition, reactivity can also be suppressed by rapidly progressive tuberculosis or diseases associated with depressed cellular immunity (8).

Diagnostic problems of tuberculous effusion.

The differential diagnosis of tuberculous pleurisy can be confused with other obvious diseases with pleural effusion such as malignant disease of the pleura, systemic lupus erythematosus with pleural effusion, congestive heart failure etc.. Different treatments and prognosis require that a specific diagnosis be made as early as possible. Definite diagnosis of tuberculous effusion is made by demonstration of tubercle bacilli in pleural fluid either by culture or by AFB smear. However, this will be positive in less than 30 percent (91). Negative culture

or AFB smear cannot exclude the diagnosis of tuberculosis. As the consequence, additional diagnostic criteria are needed to confirm the diagnosis of tuberculous pleural effusion. The exudative pleural fluid that consist of protein concentration over 3.5 gram percent with predominant mononuclear cells may be of either infectious or inflammatory origin. The concomitant lesion in lung parenchymacannot be easily detected by roentgenographic examination due to its usually small size and the obscuring effects of the fluid. Needle biopsy of the parietal pleura has proved to be a useful for histologic examination. It has materially aided the early diagnosis of not only tuberculosis but also other granulomatous diseases and neoplasms of the pleura and lung (92). However, even when pleural biopsy is used, tuberculous effusion may remain undiagnosed due to inadequate biopsy (93). In addition, the diagnosis depends on demonstrating a positive skin reaction to intermediate strength PPD. A tuberculin test indicates whether the individual shows evidence of cell-mediated immunity as a result of a past of present mycobacterial infection (8). The temporary negative PPD skin test should be considered as transient hyposensitization due to preferential sequestration of antigen reactive lymphocytes in the pleural space (42), or technical failure in the intradermal PPD injection. However, these additional diagnostic criteria may increase the diagnostic accuracy to 65-85 %.

There fore, simple and more specific immunologic tests have been sought to confirm the diagnosis of tuberculous effusion and the main objectives of this thesis.

Immunologic tests in tuberculous effusion.

The followings are the in vitro immunologic tests which have been studied as possible diagnostic aids in tuberculous effusion :

1. Lymphocyte transformation test.

Lymphocytes from immunized animals or human can be induced to transform into lymphoblast and divide when cultured with antigen to which they are sensitized (56). Incorporation of radiolabeled thymidine into the newly synthesized DNA or of radiolabeled leucine into the newly synthesized proteins in the proliferating cells provide most commonly used and convenient means of assessing lymphocyte transformation. The test reflects the sensitization or previous exposure to that antigen.

The cellular immune response may be localized at the site of the antigenic stimulus. Tuberculous pleurisy in man represents an example of such a localized immune response. The predominance of T-cells in pleural fluid over peripheral blood in patients with tuberculous effusion and in patients with malignant effusion may

represent some localized cellular immune responses (61-68). Previous reports showed that tuberculous pleural fluid lymphocytes responded to tuberculin with blastic transformation and DNA synthesis (43,63,70,74,75). The tuberculin-induced reactivity of tuberculous effusion lymphocytes is usually greater than such reactivity of peripheral blood lymphocytes (43,70,74). Similary, the sequestrated tuberculin specific lymphocytes in lymph nodes of patients with tuberculosis (82) or in cerebrospinal fluid of patients with tuberculous meningitis (89) responded to PPD stimulation more than twice as intensely that of the peripheral blood lymphocytes.

2. Leukocyte migration inhibition test (LIF test).

Leukocyte migration inhibition factor (LIF) is one of a variety of lymphokines released from lymphocytes stimulation with mitogens, alloantigens or specific antigens (27). It can inhibit the migration of polymorphonuclear cells (27). LIF test can be performed as a direct, or one step reaction, in which the same human leukocyte preparation is used as the source of reactive cells and as the indicator cells. It can also be performs as an indirect or two-step technique, in which patients leukocytes or mononuclear cells are incubated with antigens and the culture supernatants are tested for LIF activity on other appropiate indicator cells (23). The original human LIF

tecnique described by Bendixon and Soborg (44), was the direct LIF assay by placing the leukocyte-filled capillary tube in media and antigen chambers. Clausen (57) modified the direct capillary tube method by placing mixture of leukocyte and antigen in wells made in the semisolid agarose medium contained in the petri dishes, and then measuring the areas of leukocyte migration under the agarose medium. Fuerthermore, the agarose microdroplet technique, originally described by Harrington et al (45) in guinea pigs, was modified to human cells by McCoy et al (46). More recently, this direct micromethod has been further miniaturized as a two stage indirect test and adapted for a rapid photoelectric device(33).

PPD-induced LIF production of tuberculous pleural fluid lymphocytes has been studied by two groups of investigators with different techniques. Jakubusek et al (61) using the capillary technique showed that 8 in 12 specimens of tuberculous effusion lymphocytes stimulated with PPD exhibited LIF activity. The second group (75) demonstrated LIF activity in 3 out of 5 cases but by the use of induction of autologous clotted plasma technique.

3. Other lymphokines assays.

In the lymphocyte mitogenic factor (LMF) assay, Fujiwara et al (74) showed that PPD-stimulated

tuberculous pleural fluid lymphocytes in all of 12 cases clearly produced more LMF than their peripheral blood lymphocytes as measured by induced tritiated thymidine incorporation of non-specifically responsive purified B-lymphocytes. In addition, Shimokata et al (67) showed that PPD induced interferon production of tuberculous pleural fluid lymphocytes from 17 out of 18 patients was much higher than that of peripheral blood lymphocytes.

4. Serological tests

The serologic diagnosis of tuberculosis has been a subject of interest to many investigators since 1918. Many serologic procedures have been used to detect antibodies directed against a variety of antigenic constituents of tubercle bacilli (6). Most of the studies are faced with the problems of sensitivity and specificity. Besides tuberculosis, the tests were also positive in other mycobacterial infections, in sacoidosis, in lung cancers, as well as in healthy individuals (6). In addition, the test generally cannot differentiate active from inactive tuberculous infections and may at times, give false-negative results. The reasons for discrepancies include generalized non-specific or specific suppression of the antibody response, immune complex formation with deposition of complexes in tissues or removal of complexes by phagocytic cells or highly effective degradation of antigens by macrophages

(71). Finally, as alternatives to tests for specific antibodies, detection of antigens or specific chemical components of tubercle bacilli such as phosphatide, a plasma membrane antigen of Mycobacterium tuberculosis, have been developed (32). The validity of the antigen testing needs to be further investigated.

The objectives of the research proposal.

1. To phenotype the cells from the pleural fluids of tuberculous and non-tuberculous patients.

2. To study the proliferative response and LIF production of these cells when stimulated with PPD in vitro.

3. To study the above mentioned quantitative and qualitative relationships between the pleural fluid and peripheral blood mononuclear cells.

4. To assay free LIF-like activity in the pleural fluids.

5. To evaluate the values of the above mentioned tests in differentiating tuberculous effusion from pleural effusion of other causes when compared to other conventional diagnostic tests.