V. DISCUSSION

Tuberculosis (TB) is an enormous global health problem, responsible for three million deaths per year. The health burden of TB is increasing, due to the dissemination of antibiotic-resistant strains of *Mycobacterium tuberculosis* and the synergistic pathology of coinfection with HIV (Dye et al., 2002). *M. tuberculosis* infections are acquired through inhalation of infective bacilli. Once in the lung, the mycobacteria are internalized by alveolar macrophages in which they survive and replicate and set up infection foci in the tissue of the alveolar wall. At this site, recruitment of macrophages and lymphocytes leads to the granuloma formation.

A variety of mechanisms are proposed to explain the survival of *M. tuberculosis* within the macrophage, including inhibition of phagosome-lysosome fusion, inhibition of the acidification of phagosome and resistance to killing by oxygenated metabolites. However, macrophage activation and macrophage production of proinflammatory cytokines are also crucial for a successful immune response against intracellular pathogens such as *M. tuberculosis*. The cytokines which involve in the control of *M. tuberculosis* infection including IFN-γ, IL-12 and TNF-α. More recently, evidence has emerged that host defense against *M. tuberculosis* infection may also occur by apoptosis of mycobacteria-infected macrophages with little or no tissue damage. However, *M. tuberculosis* has developed mechanisms to modulate inflammatory and apoptotic responses in order to survive in humans, its unique biotope.

In recent year, intense investigations have been conducted in order to determine the molecular basis of the mycobacteria virulence and pathogenicity. The recent work showed that lipoarabinomannan (LAM), a lipoglycans which found in the enveloped of all mycobacteria species, has the capacity to inhibit IL-12 production by human dendritic cells and modulate *M. tuberculosis*-induced macrophage apoptosis (Dao et al., 2004). LAM is classified into two classes: ManLAMs, characterized by the presence of mannosyl caps and the PILAMs, containing phosphoinositide caps. Thus far, ManLAMs have been found in the slow-growing mycobacteria, *M. tuberculosis*,

M. laprae and *M. bovis* BCG, while PILAMs have been identified in the fast-growing mycobacteria, *M. smegmatis* and *Mycobacterium* sp. There is now an emerging consensus that PILAMs are proinflammatory molecules stimulating the production of TNF-α and IL-12 while ManLAMs are antiinflammatory molecules inhibiting the production of TNF-α and IL-12 by human macrophages/dendritic cells. This paradigm provides an interesting correlation between LAM structure and their immunomodulatory effect, on one hand, and the intramacrophagic fate of the corresponding mycobacteria, on the other. Indeed, the inability of *M. smegmatis* to survive inside activated macropages correlates with the proinflammatory effect of PILAMs. Likewise, the capacity of *M. tuberculosis* and *M. bovis* BCG to survive and multiply inside macrophages is in agreement with the antiinflammatory effect of ManLAMs. ManLAMs clearly emerges as a major virulence factor that contributes via an immunosupressive effect, to the persistence of *M. tuberculosis* and *M. bovis* BCG within phagocytic cells (Nigou et al., 2002). Additionally, there is an evidence indicates that ManLAMs is largely responsible for the inhibition of apoptosis in *M. tuberculosis*-infected macrpohages through mitochondrial-dependent and -independent mechanisms. Among the events triggered by ManLAMs are: (1) preferential induction of IL-10 production, which negatively regulate the production of NO and caspase activation, even in the presence of TNF-α ; (2) stabilization of Bcl-2 expression; (3) inhibition of the caspase activation cascade and partial inhibition of p53 expression (Rojas et al., 1999).

In this study, we determined the apoptosis of *M. tuberculosis*-infected macrophages in normal persons and tuberculosis patients. There has an evidence that human peripheral blood monocytes have plasma membrane receptor for the plasma fibronectin, and these receptors are not expressed on other leukocytes (Bevilacqua et al., 1981). The efficient technique to purify human peripheral blood monocytes was developed. This technique based on the fact that monocytes have high affinity for fibronectin immobilized on a gelatin-coated surface and human peripheral blood monocytes reversibly adhere to fibronectin coated gelatin treated surfaces via a magnesium dependent mechanism. Cell preparations obtained by this method were characterized with monoclonal antibodies showed that they are more than 90% monocytes (Freundlich and Avdalovic, 1983). The study compared four monocyte

separation methods: (1) rosetting with sheep erythrocytes pretreated with 2-aminoethylisothiouronium bromide hydromide followed by monoclonal antibody and complement treatment; (2) adherence to gelatin/plasma-coated flasks; (3) adherence to plastic dishes; and (4) separation by Sepracell technique. The results indicated that of the four methods compared, adherence to gelatin/plasma-coated flasks produced the highest purity and recovery (Jones et al., 1989). Therefore, we used this method for the isolation of monocytes in our experiments. Our results showed that the percent recovery of monocytes in adherent cells when compared with the number of the monocytes in whole blood in normal persons and tuberculosis patients were 115.53±19.21% and 36.79±20.2%, respectively. The percent recovery of monocytes in adherent cells when compared with the number of the non-specific esterase positive cells in PBMC from normal persons and tuberculosis patients were 77.29±4.95% and 101.36±9.98%, respectively.

Many healthy persons have been shown to have serum antibodies to mycobacteria. Uptake of *M.avium* complex and *M. tuberculosis* by human peripheral blood monocytes (PBM) was enhanced when serum was present during the uptake period. This effect of serum is mediated by substances opsonizing onto the mycobacterial surface rather than by the serum directly activating the PBM, as has been shown to occur in other systems. The results obtained after heating the serum to 56°C for one hour indicated that complement is a major constituent of the substances opsonizing on to the mycobacterial surface (Swartz et al., 1988). Likewise, our result showed that phagocytosis of *M. tuberculosis*-ingested macrophages was increased significantly in the present of 10% pooled human AB serum during the uptake period in both normal persons and tuberculosis patients.

Alveolar macrophage was promoted apoptosis by infecting with *M. tuberculosis* (Keane et al., 1997). Alveolar macrophage cytotoxicity was significantly greater with H37Ra infection than with H37Rv infection. However, there was an evidence showed that intracellular replication of H37Rv was greater than that of H37Ra in human macrophages. It is therefore unlikely that the greater cytoxicity with H37Ra infection was due to simply to excessive intracellular proliferation of H37Ra compared with that of H37Rv (McDonough et al., 1993; Silver et al., 1998). Alveolar macrophage infection with either H37Rv or H37Ra induces comparable levels of TNF-α but that

TNF- α bioactivity is reduced in supernatants of H37Rv-infected macrophage because of the releasing of soluble TNF receptor 2 (sTNFR2), leading to formation of TNF- α -TNFR2 complex accounted for the difference in the TNF- α -bioactivity in these cultures (Balcewicz-Sablinska et al., 1998). The later study found a consistent pattern of reduced alveolar macrophage apoptosis and cytotoxicity after infection by virulent *M. tuberculosis* complex bacilli as compared with attenuated or avirulent isogenic strains. Virulent bacilli also consistently demonstrated faster intracellular growth than the attenuated strains despite their association with enhanced host macrophage viability. However, they were unable to establish a consistent relationship between the level of TNF- α and TNFR2. It appears that other mechanisms may also be involved in the modulation of alveolar macrophage apoptosis by virulent *M. tuberculosis* (Keane et al., 2000). Infection of peripheral blood monocytes with *M. bovis* BCG or induction with heat-killed *M. tuberculosis* H37Ra resulting in downregulation of the Bcl-2 protein, an inhibitor of apoptosis. At the same time points, there was no change in the expression of Bax or Bcl_{xs}, inducers of apoptosis. Moreover, they observed significantly more apoptosis in involved segments of five tuberculosis patients (14.89±1.9%) than in those in normal controls (<1%) or in uninvolved segments (4.3±0.9%).

In this study, we found that when phagocytosis in the absent of 10% pooled human AB serum, the percent apoptosis of *M. tuberculosis* H37Ra-infected macrophages was significantly higher than in *M. tuberculosis* H37Rv-infected macrophages in both normal persons and tuberculosis patients. When 10% pooled human AB serum was presented during phagocytosis, the percent apoptosis of macrophages from tuberculosis patients which infected with *M. tuberculosis* H37Ra- was significantly higher than infected with *M. tuberculosis* H37Rv. In normal persons, the percent apoptosis of *M. tuberculosis* H37Ra-infected macrophages was higher than in *M. tuberculosis* H37Rv-infected macrophages. One of the normal subjects (N2) showed lower percentage of macrophages apoptosis (4.47%) than the others when infected with *M. tuberculosis* H37Ra in the present of 10% pooled human AB serum. The difference in the percentage of phagocytosis between the absent or present of 10% pooled human AB serum during the uptake period was not effect apoptosis. Additionally, there was no significant different in the percentage of

apoptosis of both H37Ra or H37Rv-infected macrophages between the normal persons and tuberculosis patients. However, the monocyte-derived macrophages from tuberculosis patients in this experiment were isolated from peripheral blood but not alveolar macrophages from the involved sites or from the lobe with infection therefore it is possible to reason that we are not able to find a significantly higher macrophage apoptosis in patients compared with in normal persons. Our tuberculosis patients in this study are all multidrug-resistant tuberculosis (MDR-TB). One of them is diabetes mellitus with tuberculosis (P3). Percentage of phagocytosis of P3 showed similar results with other patients but percentage of apoptosis of *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv-infected macrophages from P3 were less than other patients. Diabetes mellitus is an important predisposing factor for tuberculosis. Diabetics with TB infection are three times increase the risk of progression become to the disease. Mice with streptozotocin-induced diabetes mellitus were prone to *M. tuberculosis* infection, as indicated by increased numbers of live bacteria in lung, liver and spleen. In diabetic mice, the levels of IL-12 and IFN-gamma in the lung, liver and spleen were lower than those in control animals on day 14 postinfection. In addition, peritoneal exudate cells obtained from diabetic mice produced lower amounts of IL-12 and NO than those from control mice, when stimulated in vitro with *M. bovis* BCG. Spleen cells from diabetic mice infected with *M. tuberculosis* produced a significantly lower amount of IFN-gamma upon restimulation with purified protein derivatives (PPD) than those from infected nondiabetic mice. Finally, control of blood glucose levels by insulin therapy resulted in improvement of the impaired host protection and Th1-related cytokine synthesis. Their results suggest that the reduced production of Th1-related cytokines and NO account for the hampered host defense against *M. tuberculosis* infection under diabetic conditions (Yamashiro et al., 2005).

The elucidation of the mechanisms that governs macrophage cell death during *M. tuberculosis* infection will open up new possibilities for understanding host mycobacteria interactions and manipulating host immune and inflammatory responses. A better understanding of host response to *M. tuberculosis* may contribute to improvements in the treatment and prevention of tuberculosis.