VI. SUMMARY

Tuberculosis, once believe to be approaching eradication in developed countries, has reemerged in recent year as a potentially serious public health problem. Particularly threatening is the spread of multidrug-resistant strains, raising the spectra of untreatable disease (Molloy et al., 1994). Despite this, little is known about the capacity of the human immune response to eliminate *M. tuberculosis* or about the virulence mechanisms used by the organisms to evade these defenses (Silver et al., 1998).

A variety of mycobacterial species have been shown to be capable of causing macrophages to undergo increased rates of apoptosis in vitro. Consequently, considerable interest has emerged recently in the potential role of macrophage apoptosis in host defense against mycobacterial infection. In vitro infection of both monocytes and alveolar macrophages with *M. tuberculosis* has been shown to result in apoptosis and increased frequency of apoptosis has been observed in alveolar macrophages recovered from patients with pulmonary tuberculosis. It has also been reported that in comparison to relatively avirulent strains, infection of macrophages with virulent *M. tuberculosis* results in less apoptosis (Lopez et al., 2003).

In order to study apoptosis of *M. tuberculosis*-infected macrophage in normal persons and tuberculosis patients, monocytes were isolated by adherence to gelatin/plasma-coated flasks. The average percentage of viability of the adherent cells in normal persons and tuberculosis patients were 98.40±0.06% and 97.80±0.42%, respectively. The average percentage of positive for non-specific esterase staining in normal persons was 92.83±0.39% and in tuberculosis patients was 89.68±1.20%.

Macrophages were infected with *M. tuberculosis* H37Ra (avirulent strain) or *M. tuberculosis* H37Rv (virulent strain) in the absent or present of 10% pooled human AB serum during the uptake period with the multiplicity of infection (MOI) at 10 mycobacteria per macrophage for 240 min. The average percentages of phagocytosis of *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv by macrophages from normal persons were 13.87±0.24% and 12.00±1.06%, respectively. The percent of 10%

pooled human AB serum, the percentages of phagocytosis of *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv were 33.74+1.62% and 32.57+1.22%, respectively. The average percentages of phagocytosis of *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv by macrophages from tuberculosis patients were 12.94±1.85% and 11.20±0.72%, respectively. The percentages of phagocytosis of *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv in case with 10% pooled human AB serum were 32.50±0.58% and 30.06±1.18%, respectively. However, there was no significant different in the percent phagocytosis of *M. tuberculosis* H37Ra or *M. tuberculosis* H37Rv between the normal persons and tuberculosis patients. Percentages of phagocytosis of *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv were significantly increased in both normal persons and tuberculosis patients when 10% pooled human AB serum was presented during the uptake period.

To determined apoptosis of *M. tuberculosis*-infected macrophages in normal persons and tuberculosis patients, macrophages were infected with *M. tuberculosis* H37Ra or *M. tuberculosis* H37Rv at the multiplicity of infection (MOI) 10 mycobacteria per macrophage for 240 min in the present or absent of 10% pooled human AB serum during the uptake period. The apoptotic cells were detected by staining with AnnexinV FLUOS staining kit (Roache) and analysed by fluorescence microscope.

In normal persons, the percentage of apoptosis of *M. tuberculosis* H37Ra-infected cells was significantly higher than *M. tuberculosis* H37Rv-infected cells. In the case of phagocytosis without 10% pooled human AB serum, the percentage of apoptosis of *M. tuberculosis* H37Ra-infected cells was 8.33±0.43% and *M. tuberculosis* H37Rv-infected cells was 4.74±0.20%, respectively. However, in the case of phagocytosis with 10% pooled human AB serum, there was no significant different in the percentage of apoptosis of *M. tuberculosis* H37Ra-infected cells (6.89±1.45%) and *M. tuberculosis* H37Rv-infected cells (4.46±0.15%).

In tuberculosis patients, there was significantly different in the number of the apoptotic cells when compared between *M. tuberculosis* H37Ra-infected cells and *M. tuberculosis* H37Rv-infected cells. In the case of phagocytosis without 10% pooled human AB serum, the percentage of apoptosis of *M. tuberculosis* H37Ra-infected cells was 12.94±3.12 and *M. tuberculosis* H37Rv-infected cells was

5.30±1.46 and the control uninfected cells was 1.12±0.39. Likewise, in the case of phagocytosis with 10% pooled human AB serum, there were significantly different in the number of the apoptotic cells when compared between *M. tuberculosis* H37Ra-infected cells and *M. tuberculosis* H37Rv-infected cells. The percentage of apoptosis of *M. tuberculosis* H37Ra-infected cells was 12.13±1.02% and *M. tuberculosis* H37Rv-infected cells was 5.49±0.98% and the control uninfected cells was 1.10±0.08..

However, when compared the percentages of apoptosis of *M. tuberculosis* H37Ra or *M. tuberculosis* H37Rv-infected cells between normal persons and tuberculosis patients, we are not able to find significantly different in the percentage of apoptosis between them. The control of both groups was also no significantly different in the percent of apoptotic cells.