CHAPTER 4

DISCUSSION

Pseudomonas aeruginosa, particularly, is a frequently encountered gram negative organism in hospital acquired infection (42). Inspite of low virulence in healthy individuals, it may cause serious and lethal infections in debilitated or immunosuppressed hosts such as cancer, burn and cystic fibrosis patients, etc. *Pseudomonas aeruginosa* usually produces a variety of extracellular products that may contribute to its pathogenicity including hemolysins, proteases, enterotoxin and exotoxin. Exotoxin, which was originally designated as exotoxin A by Liu et al (3, 25, 44), was a potentially important virulent factor (3, 30, 45, 46, 47) which in the experiment, induced necrosis and death of mice. Numerous methods of production, concentration and purification of this exotoxin were developed. Most of them based on modification of combined techniques such as precipitation, ion exchange chromatography, gel filtration, and electrophoresis (19, 25, 36, 48).

With this study, we provided a simple method in preparation of partially purified exotoxin by using 60% final concentration of ammonium sulfate for precipitation and concentration, and employing column chromatography with Sephadex G-200 for purification. Applying of spectrophotometry for the detection of exotoxin during the purification was more convenient and accurate than the animal model procedure.

The exotoxin obtained as previously described was almost pure. The preparation showed one main peak of protein following with a trace protein peak (Fig. 2). Immunodiffusion test confirmed the two components contained in this exotoxin preparation by revealing a heavy and a faint precipitin lines (Figure 7). It meant that our procedure for purification was not efficient enough in order to isolate the completely pure exotoxin. it might need further isolation of these two components apart such as by ion-exchanged chromatography. However, it might be interpreted at this step that the exotoxin might contain either one or two of protein components, because the pathogenic effect of the toxin still remained in our preparation. If the toxin itself consisted of a single component, our results supported the mouse LD_{50} reported by Liu et al (19, 29), since our preparation was not pure enough and our mouse LD_{50} was greater than other reports.

Concerning the role of exotoxin in the pathogenesis of mice, our microscopic observations are resembled with those made by Liu (3) who briefly reported the liver necrosis, edematous and hemorrhagic lung and necrotic and hemorrhagic kidney in mice after giving exotoxin intraperitoneally. But our microscopic observations were somewhat differed from those made by Olgerts et al (47) who reported that when exotoxin was injected into mice intravenously the lesions were found only in the liver, neither spleen nor kidney contained the lesion. Our histopathologic and immunofluorescent studies revealed that the main site of tissue injury and exotoxin localization was the liver but minor effects were also observed in kidney and spleen. It would be emphasized that the mice

of our study were subjected to a single administration of large dose of partially purified exotoxin. On the other hand, concerning human infections, the host will be exposed to the continuous release of small amount of many toxic substances, each of which induces different biochemical and pharmacological effects. Thus, it is not at all a surprise that in Pseudomonas aeruginosa infection in humans, the response of the host to the toxin is difference. Since the exotoxin was the most lethal component produced by Pseudomonas aeruginosa, it is hardly believed that it does not play a role in pathogenesis. The work presented here may well portray the clinical effects of this lethal toxin while other variable factors were almost ignored.

The obtained immune sera had a highly specific antitoxin, as judged by double immunodiffusion reaction performed with concentrated Pseudomonas aeruginosa exotoxin, and had neutralizing activity against exotoxin in mice as reported in this study.

Pollack et al reported that patients with Pseudomonas infections formed antibodies to Pseudomonas toxin which led to understand that the toxin was sufficient to elicit detectable quantity of circulating antibody (49).

Liu, Hsieh and Lynn demonstrated the protective value of antitoxin for the protection of mice challenged by either crude toxin or viable organisms (26, 48).

Several types of whole cell and subcellular Pseudomonas vaccines have been tested in experimental and clinical situation to control such infections with varying degrees of success (24, 50, 51, 52, 53).

These findings suggested that the feasibility of producing a toxoid in studying of immunoprophylaxis and/or treatment of Pseudomonas diseases may be valuable for future investigation.