CELLULAR IMMUNITY IN PSEUDOMONAS AERUGINOSA INFECTION

THESIS

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By

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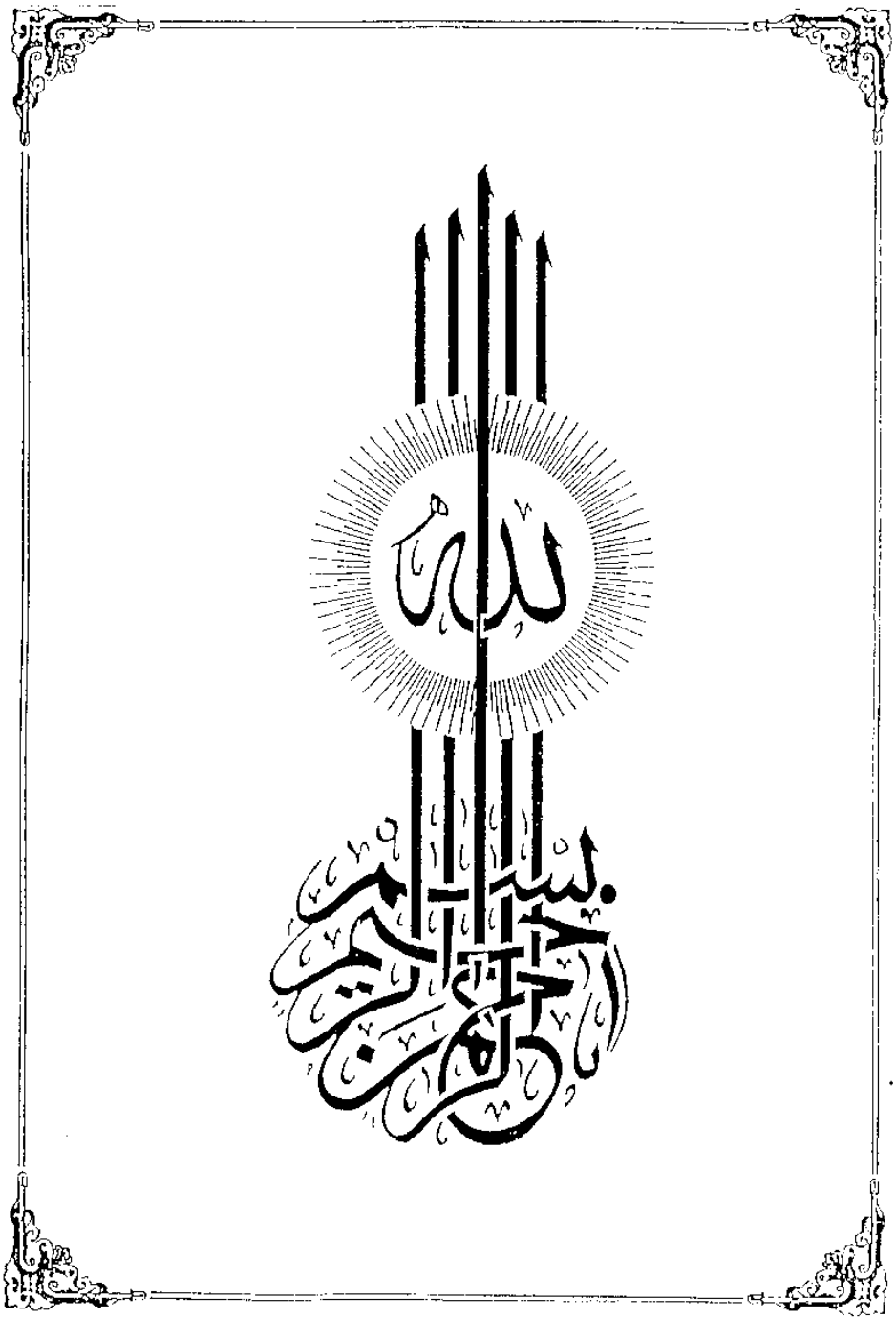
سُبْحَانَكَ

لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

صَدَقَ اللهُ الْعَظِيمُ

سورة البقرة آيه ٣٢



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INTRODUCTION

Pseudomonas aeruginosa (P. aeruginosa) is an extracellular Gram-negative bacterium that did not receive much clinical attention before the introduction of antimicrobial therapy. Due to its antibiotic resistance, this organism tends to proliferate in pathological process where antibiotic sensitive bacteria are eliminated and infections caused by this organism are difficult to eradicate ***(Liu et al., 1961).***

P. aeruginosa is one of the most frequent pathogens isolated from patients with nosocomial infections ***(Schaberg et al., 1991; and Widmer et al., 1993).***

Janda and Bottone (1981) mentioned that the spectrum of clinical diseases produced by *P. aeruginosa* ranges from urinary tract infections to septicaemia, pneumonia, meningitis and infections of drainage sites resulting from surgery or trauma.

It has been reported that *P. aeruginosa* inhibits cellular immunity, as evidenced by prolonged survival of skin homografts in human and laboratory animals ***(Stone et al., 1967; and Floersheim et al., 1971),*** suppression of tuberculin skin reaction in guinea pigs and depression of contact sensitivity to oxazolone in mice ***(Colizzi et al., 1978 & 1979).***

Woodruff et al., (1969) noticed that a patient with an excellently functioning renal graft displayed on three occasions a sharp decline of renal function when a *P. aeruginosa* infection was treated with polymyxin B. Therefore, the possibility may be that the infection in this patient was exerting an immunosuppressive action.

It has been demonstrated that extracellular substances of *P. aeruginosa* such as protease, elastase, collagenase, licithinase, haemolysin, exotoxin A, and enterotoxin play an important role in the pathogenicity of *P. aeruginosa* infection ***(Nonoyama et al., 1979).***

Infection with *P. aeruginosa* is an important clinical problem specially in compromised patients, e.g. those with burns, cystic fibrosis, or neoplastic disease, and often results in severe morbidity or even mortality ***(Ulmer et al., 1990).*** *P. aeruginosa* and its various products have been shown to affect the specific and non-specific defense mechanisms of the host. Heat-killed *P. aeruginosa* or a lyophilizate of this bacteria inhibits immune responses *in vivo* as well as *in vitro* ***(Floersheim et al., 1971; and Issekutz and Stoltz, 1985).*** The polymorphonuclear leucocyte "PMN" inhibitor is an extracellular substance produced by virulent strains of *P. aeruginosa* to inhibit the phagocytic and killing activities of PMN ***(Nonoyama et al., 1979).*** Exotoxin A is an extracellular product of *P. aeruginosa* with immunomodulating properties that is considered to play a significant role in pathogenicity ***(Holt and Misfeld, 1986).*** Alkaline

protease and elastase are exoproteins of the bacteria which inhibit T-lymphocyte and natural killer cell functions *in vitro* ***(Theander et al., 1988).***

P. aeruginosa secretes a number of toxins including phenazine pigments. The major phenazine pigment produced by approximately 50% of all *P. aeruginosa* clinical isolates is pyocyanin ***(Knight et al., 1979).*** It has been shown that pyocyanin inhibits specific as well as non-specific immune reactions *in vitro* ***(Miller et al., 1987).*** It inhibits reactions necessary for specific defense mechanisms of the host such as proliferation of T-lymphocytes, production of IL-2, expression of IL-2 receptors, development of cytotoxic T-lymphocytes, and secretion of antibodies. Thus, pyocyanin may contribute to the immunosuppressive action of *P. aeruginosa* ***(Ulmer et al., 1990).***

AIM OF THE WORK

The aim of this work is to test cell-mediated immunity and phagocytic activity of patients infected with *P. aeruginosa*, in order to study its role in the pathogenesis of infection and modulation of the patients immune responses.

CLASSIFICATION OF PSEUDOMONAS SPECIES

It is based on the internal division of Pseudomonas into various RNA homology groups which represent natural genetic arrangements. Within the RNA groups are subgeneric groups, e.g. the fluorescent group, which contains species that share many common phenotypic properties ***(Gilardi, 1985):***

RNA group I :

Fluorescent group.

Stutzeri group.

Alcaligenes group.

RNA group II :

Pseudomallei group.

RNA group III :

Acidovorans group.

RNA group IV :

Diminuta group.

RNA group V :

Pseudomonas maltophilia.

Pseudomonas mesophilica.

Pseudomonas paucimobilis.

Pseudomonas putrefaciens.

Pseudomonas sp. group Ve-1

Pseudomonas sp. group Ve-2

PSEUDOMONAS SPECIES

According to ***Gilardi (1985),*** several Pseudomonas species are recognized :

(1) Fluorescent Group *(Pseudomonas aeruginosa, Pseudomonas fluorescens,* and *Pseudomonas putida)* :

Most strains of the three species of the fluorescent group grow in mineral base medium containing ammonium ion as sole source of nitrogen, and glucose as sole source of carbon and energy. They can produce water-soluble pigments (pyoverdins) which fluoresce when exposed to UV light of short wave length. Fluorescent pigments may be yellow-green, yellow-brown. They are not soluble in chloroform. Production of pyoverdins is influenced by nutritional factors. Media which support growth of the organism may not promote synthesis of its pigment. Most strains produce indophenol oxidase and arginine dihydrolase. Those strains which lack these features may be more closely related to phytopathogenic fluorescent Pseudomonas such as *Pseudomonas syringae*.

(2) Stutzeri Group *(Pseudomonas stutzeri* and *Pseudomonas mendocina)* :

The strains are motile with polar monotrichous flagella. Lateral flagella are produced by some strains. These two species grow with ammonium ion as sole source of nitrogen and acetate as sole source of carbon and energy. Indophenol oxidase and oxidative acidity from glucose are produced. They are susceptible to polymyxins. The two species are salt

(6.5% NaCl) tolerant and non-halophilic but require sodium cation for growth. They grow under anaerobic conditions in nitrate-containing media, as do *P. aeruginosa*, *P. pseudomallei*, and other bacterial species which reduce nitrate to nitrogen gas ***(Gilardi, 1985).***

(3) Alcaligenes Group *(Pseudomonas alcaligenes* and *Pseudomonas pseudoalcaligenes)* :

The two species in the alcaligenes group have the characters of the genus Pseudomonas. The cells of most strains have no somatic curvature, but some few others are distinctly curved. They are polar monotrichous when flagellated. The flagellar morphology of the species in the alcaligenes group is similar to that of *P. aeruginosa*. Most strains grow in mineral base medium containing ammonium ion as sole source of nitrogen and acetate as sole source of carbon and energy. Alkali accumulates in unsealed oxidative-fermentative (OF) glucose medium and OF basal medium. Indophenol oxidase is produced. Pyocyanin and pyoverdins are not produced ***(Gilardi, 1985).***

(4) Pseudomallei Group *(Pseudomonas pseudomallei, Pseudomonas mallei, Pseudomonas cepacia* and *Pseudomonas pickettii)* :

The principal feature shared by the species in the pseudomallei group is nutritional versatility in the type and number of organic compounds utilized as sole sources of carbon and energy, which include carbohydrates, mono- and dicarboxylic acids, mono- and polyalcohols, aromatic compounds, amino acids, and amines. Growth occurs in mineral base

medium containing ammonium ion as sole source of nitrogen and glucose as sole source of carbon and energy. Few strains fail to produce indophenol oxidase, and others produce a slow and very weak indophenol oxidase reaction. The majority of strains are not susceptible to antibiotics of the polymyxin class. *P. pickettii,* the animal pathogen, *P. mallei* and *P. pseudomallei,* and the plant pathogens, *P. cepacia, P. gladioli (P. marginata, P. alliicola),* and *P. caryophylli* are enzymologically, phenotypically, and genotypically related ***(Ballard et al., 1970; and Whitaker et al., 1981).***

(5) Acidovorans Group *(Pseudomonas acidovorans* and *Pseudomonas testosteroni)* :

The species in the acidovorans group have the characters of the genus Pseudomonas, and they also share other attributes. Cells are motile with a polar tuft of flagella. Some strains produce cells with lateral flagella. Growth is not pigmented, but colonies of some strains are surrounded by a tan or brown zone of discolored agar medium. Most strains grow in mineral base medium containing ammonium ion as sole source of nitrogen and acetate as sole source of carbon and energy. Alkali accumulates at the surface of OF glucose medium. Acid is not produced from glucose in OF basal medium, and most carbohydrates are not oxidized. Indophenol oxidase is produced ***(Gilardi, 1985).***

(6) Diminuta Group *(Pseudomonas diminuta* and *Pseudomonas vesicularis)* :

The distinctive characteristic of the strains of these species is the very tightly coiled monotrichous flagellum, with a wave length that varies from 0.62 to 0.98 μm. The wave length of most polar monotrichous Pseudomonas is approximately 2 μm. Specific growth factors are mostly required; however, in most peptone media, they can grow without growth factor supplement. Indophenol oxidase is produced. Acid is produced from primary alcohols by all strains that can utilize alcohols, and some strains hydrolyze gelatin and starch ***(Whitaker et al., 1981; and De Vos and Ley, 1983).***

(7) ***Pseudomonas maltophilia (Xanthomonas maltophilia)*** :

The morphological and biochemical reaction pattern of *P. maltophilia* is remarkably uniform. Colonies develop a characteristic lavender-green color on blood agar media. Brown-colored by-products of metabolism may accumulate in certain agar media. The intensity of the brown color is enhanced by certain factors such as elevated incubation temperature. Some strains slowly produce a very faint yellow, intracellular pigment which does not diffuse out of the colony into the agar medium. This water-soluble yellow pigment may be due to flavins. Water-soluble melanin is not produced in tyrosine-containing media. Haemolysis does not occur around discrete colonies, but there is a greenish discoloration of erythrocytes around confluent growth. Growth on blood agar is accompanied by a strong odour of ammonia. Although a weak indophenol oxidase reaction is produced by a few *P. maltophilia* strains, the species is usually described as negative for this test ***(Hugh, 1981).***

Proposals have been made to transfer *P. maltophilia* to the genus Xanthomonas as a separate species, *Xanthomonas maltophilia.* This is based partly on the following common reactions : acid reactions from OF glucose, lactose, and maltose; alkaline reactions from rhamnose and mannitol; negative indophenol oxidase reaction; hydrolysis of esculin and O-nitrophenyl-β-D-galactopyranoside "ONPG"; and a requirement for amino acids as growth factors ***(De Vos and Ley, 1983; and Swings et al., 1983).***

(8) ***Pseudomonas mesophilica*** **:**

Colonies are shiny, smooth, round, raised, entire, and rarely visible before 3 days of incubation at 30°C. An intracellular, non-diffusible, water-insoluble, pink, oxocarotenoid pigment is produced. Pink pigmentation is a characteristic of most methanol-utilizing bacteria. Acid is produced slowly, within 2 to 4 days from fructose, xylose, and methanol in OF basal medium. Indophenol oxidase, urease, and amylase reactions are produced. Strains are motile, with polar monotrichous flagella ***(Austin and Goodfellow, 1979).***

(9) ***Pseudomonas paucimobilis*** **:**

Colonies are either butyrous, or mucoid and viscid and develop an intracellular, non-diffusible, carotenoid pigment on most media. No brown pigments are produced. The name "*paucimobilis*" indicates that only a few cells of a strain in a population may be motile. Motility is best demonstrated at room temperature. When motile, they are polar monotrichous. Indophenol oxidase is produced, and ONPG and esculin are hydrolyzed. Acid is produced in OF basal medium from a number of carbohydrates but