Glycoconjugate Journal (1994) **11**:414–417

PA-I and PA-II lectin interactions with the ABO(H) and P blood group glycosphingolipid antigens may contribute to the broad spectrum adherence of *Pseudomonas aeruginosa* to human tissues in secondary infections

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Received 11 April 1994, revised 9 June 1994

Pseudomonas aeruginosa may cause serious infections in most human tissues/organs. Its adherence to them is mediated by a battery of adhesins including the PA-I and PA-II lectins, which are produced in this bacterium in high quantities. PA-I binds to the D-galactose of the erythrocyte glycosphingolipids exhibiting highest affinities for B and P^k (followed by P_1) antigens, while PA-II preferentially binds to the L-fucose of H, A and B antigens. Intact *P. aeruginosa* cells also exhibit a clear P^k and P_1 over p preference. Such affinities for the most common human ABH and P system antigens may underlie the widespread tissue infectivity and pathogenicity of this bacterium.

Keywords: *Pseudomonas aeruginosa* adherence; lectin interactions; glycosphingolipid antigens, ABO blood group; P system antigens.

Introduction

Pseudomonas aeruginosa is known as an opportunistic pathogenic bacterium [1, 2] which may cause infections and serious damage in most human tissues and organs including ear, eye, brain, skin, urinary and gastrointestinal tracts, lungs, kidneys, bones and joints [1, 2]. The damage is mainly caused by the bacterium extracellular toxic enzymes and factors (including proteases, phospholipases, haemolysins, exotoxin A, exoenzyme S, lipase, pyocyanin, etc.) [2–4] excreted following adherence of the bacterium to the host cells. The adherence which determines the tissue specificity of infections is mainly based on interactions between the bacterium adhesins and components of the host cell surface [5–9], although host adhesins may also react with the bacterium surface components. *Pseudomonas aeruginosa* was reported to produce a number of adhesive molecules including: sialic acid-binding, ganglioside-binding and hydrophobic adhesins [9–12] as well as the PA-I and PA-II lectins [9, 10, 13]. The PA-I and PA-II lectins, which were found to be mainly intracellular, were also shown to be on the bacterium cell surface [14]. In the present communication we show that these two lectins interact with the ABO(H) and P system blood group antigens of the human erythrocytes which are also common on most human tissue cells. These findings may contribute to the understanding of the role of *P. aeruginosa* lectins in the wide range of tissues infected by this bacterium and to the planning of strategies for the prevention of infection.

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Materials and methods

Intact P. aeruginosa *cell suspensions*

The *P. aeruginosa* cells were grown at 28 °C for 3 days in TSB (tryptic soybean broth, Difco) supplemented daily with 0.2% choline chloride and shaken. The cells were harvested by centrifugation (10 400 × **g** at 4 °C for 10 min), washed three times with 0.15 M NaCl solution, and suspended in the same solution to obtain 20% (wet w/v) suspension.

PA-I and PA-II lectins

The *P. aeruginosa* lectins (PA-I and PA-II) were purified in our laboratory from the extracts of the bacterium (ATCC 3347) as previously described [13]: heating at 70 °C, fractionation by ammonium sulphate and affinity chromatography (on sepharose 4B for PA-I and on D-mannose-bearing sepharose 4B for PA-II).

Human erythrocytes

The erythrocytes were obtained from healthy adult individuals differing in ABO(H) and P system blood types. P_1 type erythrocytes bear P_1 and P antigens, P_2 type bear only P antigen and p type lack both these antigens as well as P^k antigen which is present on P^k cells [15]. The P^k and p type cells are very rare. We examined approximately three to four specimens (depending on the ABO blood groups) of each, however, the numbers of P_1 and P_2 specimens were five to eight and three to 14, respectively, in each group. Several special rare specimens were kindly supplied by Drs A. Neil and P. Moores from the National Blood Transfusion Service in Durban (Bombay Oh type), by Mrs A. Pirkola from the Finnish Red Cross BTS, Helsinki, and Dr B. Cedergren Umea, Sweden (some of the p and P^k bloods). The red blood cells were stored in Alsever's solution. Prior to use they were washed three times and suspended to a 5% concentration in 0.15 M NaCl.

Papain-treated erythrocyte suspensions

Papain treatment of human erythrocytes was performed by incubating 5% washed cell suspension in 0.15 M NaCl with an equal volume of 0.2% papain (Sigma) and 0.02% cysteine in 0.15 M NaCl at 37 °C for 30 min. The treated cells were washed again three times in 0.15 M NaCl and the packed red blood cells were resuspended to a 5% concentration in 0.15 M NaCl.

Haemagglutination test

0.05 ml of the suspension of the papain-treated human erythrocytes was added to a series of tubes containing 0.05 ml two-fold dilutions of the lectin preparations in 0.15 M NaCl. After 30 min at room temperature and a short (30 s) centrifugation (3000 rpm), the agglutination of the cells was examined as previously described [13]. The starting concentration of PA-I and PA-II in the haemagglutination test was around 30 $\mu g\,ml^{-1}$. One haemagglutinating unit is the highest dilution leading to erythrocyte agglutination.

Inhibition of haemagglutination by specific sugars

The specificity of the haemagglutination reaction was shown in each test by parallel dilutions of the lectin preparations in a 0.15 M solution of the respective sugar (D-galactose for PA-I and L-fucose for PA-II) instead of saline.

Results

The purified PA-I and PA-II preparations which exhibited low agglutination activity with untreated human erythrocytes strongly agglutinated sialidase or papain-treated cells. Therefore, examination of the agglutination by them of the diverse blood cell samples were undertaken following papain treatment of the cells.

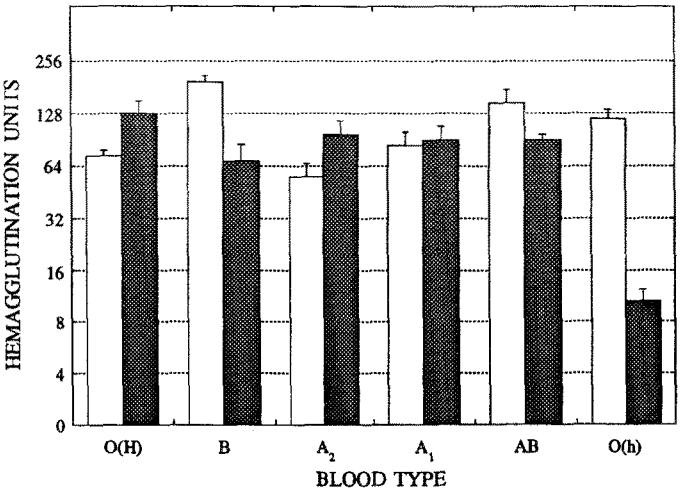

Figure 1. Agglutination of papain-treated human erythrocytes differing in ABO(H) types by *P. aeruginosa* PA-I (empty boxes) and PA-II (black boxes) lectins (means ±SEM). The number of samples examined were: O(H)-40, B-17, A_2-5, A_1-11, AB-12 and O(h)-5. One unit is the lowest lectin concentration required for haemagglutination.

Using hundreds of blood samples differing in their ABO(H) type antigens, it was shown that PA-I strongly agglutinated all of them, exhibiting some preference for the B blood type over A or O(H). The results obtained with 90 blood samples are presented in Fig. 1. The agglutination of Bombay type erythrocytes (Oh) was similar to the B cell agglutination. Most of the examined cells were also strongly agglutinated by PA-II. In this case, O(H) cells were agglutinated slightly more strongly than the A, B and AB blood types, and the most significant finding was the very weak agglutination of the Bombay (Fig. 1) as compared to the other ABO(H) types. With all the samples of the erythrocytes examined, full inhibitions of the haemagglutination by PA-I or PA-II were obtained in the presence of D-galactose or L-fucose, respectively.

With blood cells differing in P system antigens a marked difference in the red cell agglutinability by PA-I (but not with PA-II) was observed with the highest affinity for the P^k-bearing cells and lowest for p type cells (Fig. 2). This selectivity was most pronounced when comparing different O(H) cells (Fig. 2). The statistical significance of the differences between the O(H) cells of the types P^k and either P_2 or p was very high ($p < 0.0005$). Lower, but still significant differences were found between these types in A cells ($p < 0.01$) but the differences in B cells were not significant. The very strong affinity for the P^k cells as compared to the others was expressed not only by the agglutination intensity but also in the faster agglutination of the P^k cells of all the ABO cell types.

Highest agglutination activity on O(H)P^k cells as compared to those of the other O(H) cell types was also

Table 1. Simplified structures of the ABH and P system blood group antigens.

Antigen	*Structure*
P^k	Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer
P	GalNAcβ1 → 3Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer
P_1	Galα1 → 4Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer
H (type 2)	Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer Fucα1 → 2
B	Galα1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer Fucα1 → 2
A	GalNAcα1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer Fucα1 → 2

Cer, ceramide; Gal, D-galactose; GalNAc, *N*-acetyl-D-galactosamine; Glc, D-glucose; GlcNAc, *N*-acetyl-D-glucosamine; Fuc, L-fucose. The relation between P and P_1 antigens described here and the P_1, P_2 and p blood types described in Fig. 2 is noted in the description of human erythrocytes in Materials and methods.

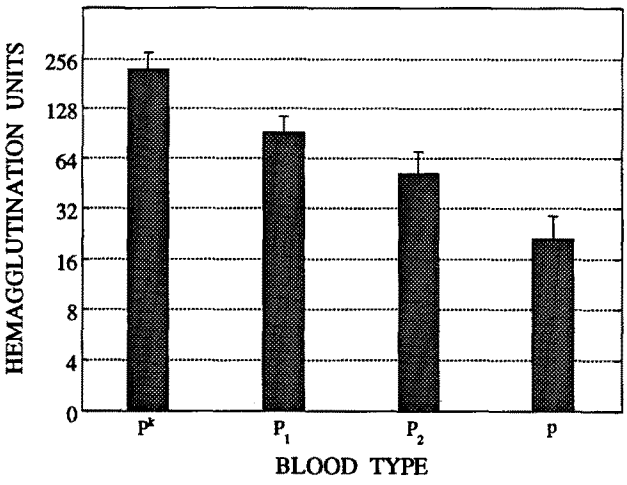

Figure 2. Agglutination of papain-treated O(H) human erythrocytes differing in P system antigens by *P. aeruginosa* PA-I lectin.

observed with the intact *P. aeruginosa* cells: P^k-100; P_1-50; P_2-32 and p-20 haemagglutinating units.

Discussion

Bacterial adherence to host cells and tissues is an important step in infection [5–9]. It can lead to colonization of the host tissues and sometimes to internalization of the microorganisms into the cells, where they produce their virulence exoproducts which damage the host cells, tissues and organs. The results presented in this paper show that both PA-I and PA-II lectins of *P. aeruginosa* agglutinate human erythrocytes much more strongly following papain treatment [10, 13]. PA-I interacts with both the ABO(H) and P system blood group antigens (Figs 1 and 2) since most of them contain terminal or subterminal galactose residues [16, 17] (Table 1). PA-II mainly interacts with the fucose of the H antigen which is absent only in the very rare Bombay (Oh) type cells (which are agglutinated by PA-I, Fig. 1). The higher affinity of PA-I (and intact *P. aeruginosa* cells) for B, P^k and P_1 antigens, resembles that described for the fish egg lectins [18, 19]. P system antigens as receptors for bacterial adherence have also been described in the case of the P-pili of uropathogenic *E. coli* strains [20, 21]. Several groups of investigators have reported that *P. aeruginosa* specifically interacts with pulmonary and other tissue glycolipids [12, 22–24] recognizing their GalNAcβ1 → 4Gal [7, 22] or Galβ1 → 4/3 GlcNAc disaccharide units [23]. Baker *et al.* [24] also showed that the exoenzyme S adhesin interacts with glycosphingolipids of buccal cells. Since the antigens of the ABO and P system are of general occurrence in human blood and most other tissues [17, 25], the interactions of PA-I and PA-II with them ensures broad spectrum adhesion of the bacterium to most human tissues. The fact that glycolipid molecules bearing these antigens are better exposed following enzyme degradation of the cell glycoproteins may underlie the observations that *Pseudomonas* infections are generally secondary to viral or other bacterial infections which are accompanied to cell surface deteriorations, unmasking the glycolipids.

Acknowledgments

This research was supported by grant no. 89-00454 from the United States–Israel Bi-National Science Foundation (BSF), by the Health Sciences Research Center and Mitzi-Dobrin Cancer Research Fund, Bar-Ilan University. The authors thank Ms S. Victor for the preparation of the manuscript.

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