Antibody response to outer-membrane antigens of *Pseudomonas aeruginosa* in human burn wound infection

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Summary. There is little information about the local and systemic antibody response to surface antigens of bacteria growing *in situ* in infected lesions in man. In this study, *Pseudomonas aeruginosa* was obtained directly from the infected wounds of two patients with burns and studied without subculture. Outer-membrane proteins (OMPs) were investigated and compared with those of cells cultivated in the laboratory, with the aim of selecting defined growth conditions to give surface antigens more closely resembling those found *in vivo*. Several high-mol. wt (77 000–101 000) proteins were expressed in the outer membranes of the bacteria from the patients and could be phenotypically induced by cultivating the same isolate in iron-depleted conditions *in vitro*. Other major OMPs (D, E, F, G and H) were also observed in cells taken from the lesions. Immunoblotting demonstrated that proteins D and E were recognised by different classes of immunoglobulins in the sera of both patients as was flagellar antigen present in the outer-membrane preparation of the *P. aeruginosa* from patient 1. Iron-regulated membrane proteins (IRMPs) were similarly detected, but more strongly by IgM from patient 1. Furthermore, a marked antibody response to IRMPs was noted at the site of infection. Bands of a similar intensity were seen after absorption of the sera with lipopolysaccharide (LPS) purified from the infecting strain. This indicated that the response observed was directed against OMPs (including IRMPs) and not against contaminating LPS.

Introduction

Infection is the most frequent cause of morbidity and mortality in the severely burned patient (Pruitt *et al.*, 1983). The burn site remains relatively sterile during the first 24 h; thereafter, colonisation of the wound by gram-negative bacteria is common (Pruitt *et al.*, 1983). *Pseudomonas aeruginosa* has emerged as a predominant member of the burn-wound flora and, in the USA, in the absence of topical therapy, is cultured from the burn injuries of 70% of patients by the third week (Pruitt, 1974).

For invasive infection to occur, the micro-organisms must multiply to a population sufficient to mount a successful attack on the host. Severe thermal injury destroys the barrier function of the skin and this enables bacteria to gain easy access to the injured tissue. The denatured protein of the

burn eschar provides a good environment for microbial growth and the avascularity of the burn wound partly shields the micro-organisms from host defence mechanisms (Order *et al.*, 1965).

The surface composition of bacteria plays an important role in pathogenicity (Smith, 1977; Costerton *et al.*, 1979) and the expression of bacterial surface components is influenced by the environment in which they grow (Holme, 1972; Brown and Williams, 1985*a, b*). Growth rate and specific nutrient deprivation have gross effects on sensitivity to host defences (Finch and Brown, 1978; Brown and Williams, 1985*a, b*). Restricting the availability of iron is an important non-specific defence mechanism against bacterial infection (Weinberg, 1978, 1984; Bullen, 1981; Griffiths, 1983). The freely available iron concentration in body fluids is extremely low ($c.10^{-18}$ M) because of the presence of biological iron-chelating molecules such as transferrin and lactoferrin which have a high affinity for iron (Weinberg, 1978; Griffiths,

1983). The bacteria respond to these conditions by synthesising their own iron-scavenging compounds (siderophores) which have an even higher affinity for iron (Neilands, 1974), and also by expression of high-mol. wt iron-regulated membrane proteins (IRMPs) in the outer membrane (OM) which function as receptors for iron-siderophore complexes (Neilands, 1974; Griffiths, 1987).

Preliminary investigations (Anwar *et al.*, 1985) of a burn patient during the acute phase of *P. aeruginosa* wound infection indicated the presence of serum IgG antibodies to outer-membrane proteins (OMPs) including the IRMPs. In the present study, we investigated the OMP antigens of *P. aeruginosa* taken directly from infected wound tissue and studied the patients' immune responses to these antigens as they occurred naturally in infection.

Materials and methods

Collection and identification of bacteria

Skin samples (c. 100 g containing c. 10^9 bacteria) were collected from two patients with severe burn injuries. The samples were collected after wound excision about 1 week after signs of colonisation. Microbiological analysis indicated that *P. aeruginosa* was the sole infecting organism in the burn wounds of patient 1 and the predominant isolate from patient 2. *Klebsiella pneumoniae* was also isolated from the second patient but only as a minor contaminant. *P. aeruginosa* strains were O-serotyped by Dr T. Pitt (Central Public Health Laboratory, Colindale, London). Serotype O4 was isolated from patient 1 and serotype O6 from patient 2. Saline (NaCl 0.85% w/v, 100 ml) was added to the skin sample which was vigorously blended for 60 s. This was followed by coarse filtration to remove cell debris. Material retained by the filter was washed twice with saline and the filtered washings added to the bulked filtrate. The bacteria were harvested by centrifugation at 5000g for 10 min and washed twice with saline. The pellet which contained the bacteria was resuspended in distilled water and OMs then prepared. The filtrate supernate, designated as infected tissue fluid, was lysophilised and resuspended in 100 ml of 10 mM Tris-HCl in NaCl 0.85% w/v, pH 7.4 (TBS) containing Tween 20 0.075% v/v (TBS-Tween) and stored at -20°C for immunoblotting.

Clinical strains of *Serratia marcescens*, *Escherichia coli*, *K. pneumoniae* and *Proteus mirabilis*, as described by Shand *et al.* (1985), were also included in this study.

Cultivation of bacteria

Bacteria were cultivated in iron-depleted tryptone soy broth (TSB - Fe) and iron-sufficient tryptone soy broth (TSB + Fe) as described by Kadurugamuwa *et al.* (1987).

Bacteria were grown overnight in an orbital shaking incubator, harvested by centrifugation at 5000g for 10 min and washed once with saline.

Outer-membrane preparation

The bacterial pellet was resuspended in 20 ml of distilled water and broken by 10×60 -s pulses of sonication in an ice bath, with 60-s intervals for cooling. Unbroken cells were removed by centrifugation at 5000g for 10 min. Sarkosyl (N-lauryl sarcosinate, sodium salt; Sigma) was added to the supernate to a final concentration of 2% w/v. The mixture was incubated for 30 min at room temperature and then centrifuged at 38 000g for 1 h (Filip *et al.*, 1973). The outer-membrane pellets were washed twice with distilled water and stored at -20°C .

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Membrane preparations were subjected to SDS-PAGE by the system described by Lugtenberg *et al.* (1975) as modified by Anwar *et al.* (1983) with 14% w/v acrylamide gels and specially purified SDS (BDH, Poole). Each lane was loaded with c. 50 μg of protein. Separated OMPs were either stained with Coomassie blue R-250 0.1% w/v or used for immunoblotting. OMPs were labelled according to the convention of Mizuno and Kageyama (1978) as modified by Hancock and Carey (1979).

Isolation of flagella

Flagellar preparations were isolated by a modification of the method of Montie *et al.* (1982). Bacterial culture (2 L), grown for 48 h in TSB + Fe in a slowly rotating orbital incubator at 37°C , was harvested by centrifugation at 5000g for 10 min. The cells were resuspended in 20 ml of saline and blended vigorously for 10 s to shear the flagella. Flagella were isolated by a process of differential centrifugation as follows: after blending, the suspension was centrifuged at 5000g for 10 min to remove whole cells and the supernate centrifuged at 100 000g for 4 h to precipitate the flagella. This procedure was repeated until a clear pellet was obtained. SDS-PAGE was used to determine the purity of the preparation and this gave a single band after staining with Coomassie blue.

Extraction of LPS

Lipopolysaccharide (LPS) was extracted by the hot phenol-water method of Westphal and Jann (1965).

Immunoblotting

OMPs separated on polyacrylamide gels were transferred to nitrocellulose (NC) paper and antigenic sites visualised by a modification of the method of Towbin *et al.* (1979). The NC paper was first incubated in TBS-

Tween for 30 min to saturate non-specific binding sites. This was followed by incubation with patient's serum diluted 1 in 20 in TBS-Tween, or with infected tissue fluid, for 4 h at 37°C and overnight at 4°C. The NC paper was washed thoroughly with TBS and incubated for a further 2 h at 37°C with horseradish peroxidase-goat anti-human IgG, IgM or IgA conjugate (Miles-Yeda, Rehovot, Israel) diluted 1 in 2000 in TBS-Tween. After incubation, the NC paper was again washed thoroughly and the bands were visualised with a solution containing 4-chloro-1-naphthol 25 µg/ml and H₂O₂ 0.01% v/v in TBS. As a control, replicate NC transfers were stained with amido black 1% w/v in methanol 10% v/v and acetic acid 7% v/v to show qualitative transfer of all proteins from the acrylamide gel to the NC paper; these NC replicates were used to identify the protein bands.

Serum

Patient's serum was separated from blood obtained by venepuncture during the course of clinical investigation. A small portion of blood was allowed to clot at 37°C for 2 h then centrifuged at 2000g for 10 min and the serum collected and stored at -20°C.

Absorption of serum with LPS

Undiluted serum (1 ml) was mixed with LPS 2 mg extracted from the infecting strain of *P. aeruginosa* and incubated for 1 h at 37°C followed by 18 h at 4°C. Precipitated immune complexes were pelleted by centrifugation at 5000g for 15 min. The supernatant serum was removed and re-absorbed with an additional 2 mg of LPS as above.

Results

Analysis of *P. aeruginosa* OMPs

Fig. 1 shows the SDS-PAGE OMP profiles of *P. aeruginosa* obtained directly from patient 1 (lane 3) and the same isolate grown in TSB-Fe (lane 2) and TSB+Fe (lane 1). Several high-mol. wt OMPs (77 000-101 000) known as iron-regulated membrane proteins (IRMPs) were strongly induced in the OM of cells grown in TSB-Fe (lane 2). The expression of these proteins was also observed in the OM of bacterial cells taken directly without subculture from the burn wound (lane 3) but they were barely detectable in the OM of the same strain cultivated in TSB+Fe (lane 1). Proteins D, E, F, G, H and I were apparent in all three lanes but minor differences between cells taken from the wounds and cells grown in TSB-Fe were noted. An additional protein band (mol. wt c. 55 000) just above band D was observed in the OM preparation of bacteria taken directly from the wound (lane 3)

Fig. 1. SDS-PAGE of OMPs of *P. aeruginosa* taken directly from the infected wound tissue of patient 1 (lane 3) and of the same isolate cultivated in TSB-Fe (lane 2) or TSB+Fe (lane 1). OMPs were stained with Coomassie blue and labelled according to the convention of Mizuno and Kageyama (1978). Numbers refer to mol. wts (10^3).

and, weakly, in OMs from bacteria grown *in vitro* (lanes 1 and 2). Closely similar results were obtained with *P. aeruginosa* from patient 2 (data not shown).

Antibody response to *P. aeruginosa* OMPs

The OMP antigens separated by SDS-PAGE (fig. 1) were electrophoretically transferred to NC paper and qualitative transfer of protein bands was confirmed by staining with amido black (not shown). Replicate immunoblots were allowed to react with antibodies present either in the serum or in wound tissue fluid.

Fig. 2a shows those antigens of *P. aeruginosa* recognised by IgM antibodies in the serum of the

Fig. 2. Immunoblots of the OMPs in fig. 1 (lanes 1–3 as described), electrophoretically transferred to NC paper and probed with serum from patient 1, obtained 1 week after onset of infection. Reactions were visualised with peroxidase-labelled anti-human (a) IgM (b) IgG and (c) IgA.

first patient, 1 week after initial signs of infection. IRMPs (predominantly the 77 000- and 85 000-mol. wt proteins) were detected in *P. aeruginosa* grown *in vitro* in conditions of iron-depletion (lane 2) or taken directly from the burn wound (lane 3), more strongly in the latter case. There was no recognition of such proteins in cells grown in iron-plentiful conditions (lane 1). Furthermore, a response to proteins D, E and H in all three lanes was noted along with the 55 000-mol. wt antigen in lane 3, but F and G were detected only faintly. Serum samples obtained in subsequent weeks gave similar patterns of response (data not shown). Serum IgG antibodies (fig. 2b) also detected IRMPs although to a lesser degree than did the IgM. Serum IgG antibodies additionally detected proteins D and E, but the response to porin protein F was again weak.

There was a stronger reaction with the 55 000-mol. wt antigen in all three lanes. Protein H was recognised in the OM of cells grown *in vitro* (lanes 1 and 2), particularly in the iron-depleted cells (lane 2) but there appeared to be no corresponding reaction to the protein in the OM preparation of cells taken from the burns (lane 3). The IgA response (fig. 2c) was similar to that seen with IgG (fig. 2b); antibodies were directed against IRMPs, the 55 000-mol. wt antigen and proteins D and E. Protein H was again detected only in the OMs of cells grown *in vitro* (lanes 1 and 2) and not in the OM of cells taken from the burns (lane 3).

Fig. 3 represents an immunoblot of flagellin protein purified from *P. aeruginosa* (lane 2); it migrated as a single band and was recognised strongly by serum antibodies. Furthermore, it was

Fig. 3. Immunoblot of purified flagella (lane 2) and OMPs of *P. aeruginosa* taken directly from burn wounds (lane 1) electrophoretically transferred to NC paper and probed with serum from patient 1. The reaction was visualised with peroxidase-labelled anti-human IgA.

seen to co-electrophorese with the 55 000-mol. wt protein present in the OM preparations, suggesting the possible identity of this antigen. The band in question was not as distinct in the Coomassie blue-stained gel (fig. 1), presumably because the amount of flagellin present was below the sensitivity of the protein stain.

Fig. 4a shows the OMP profiles of *S. marcescens* (lane 1), *E. coli* (lane 2), *K. pneumoniae* (lane 3), *P. mirabilis* (lane 4) and *P. aeruginosa* (lane 5) transferred to NC paper and stained with amido black. The organisms were grown in TSB-Fe and IRMPs were expressed in all except *S. marcescens* (lane 1). When the separated OM antigens of these five gram-negative bacteria were incubated with serum from patient 1, a very strong IgG response

to the surface antigens of the Enterobacteriaceae was observed, including the rough LPS of these organisms which ran at the front of the gel (fig. 4b, lanes 1 to 4). However, the reaction with *P. aeruginosa* OM antigens was much less intense, which probably reflects the acute stage of infection with this organism. Pre-infection serum was not available for us to investigate the immune status of this patient before colonisation with *P. aeruginosa*.

The presence of immunoglobulins at the site of infection was investigated by incubating the separated OMPs shown in fig. 1 with tissue fluid from the wound from which the bacteria were isolated (figs. 5a, b and c). The IgG response (fig. 5b) reflected the results obtained with serum (fig. 3b). IgM (fig. 5a) and IgA (fig. 5c) antibodies present in the tissue fluid were predominantly directed against the IRMPs of *P. aeruginosa* with only a faint recognition of proteins D and E.

The serum antibody response of patient 2 was investigated with the separated OMPs of the bacteria isolated from this patient—*K. pneumoniae* and *P. aeruginosa*. Each isolate was cultivated *in vitro* and OMs were prepared but the OMP profile obtained with the *K. pneumoniae* isolate bore no resemblance to the OM preparation from organisms taken directly from the wound. Therefore, it was considered that the low concentration of the contaminating organisms did not contribute significantly to the *P. aeruginosa* profile observed. However, it is not known whether the co-existence of other pathogens at the site of infection affects the expression of surface antigens of *P. aeruginosa*.

The OMP profile of the *P. aeruginosa* strain from patient 2 was identical to those observed with the strain from patient 1 (data not shown) and again demonstrated the expression of IRMPs by bacteria taken directly from the burn wounds. Fig. 6a (i) and (ii) shows the IgG response of patient 2 to *P. aeruginosa* antigens at 1 and 3 weeks after the first signs of infection. IRMPs, again especially the 77 000- and 85 000-mol. wt proteins, were recognised by IgG (fig. 6a, i and ii, lanes 2 and 3). There was also a reaction with proteins D and E and a significant increase in the recognition of protein F by the second week after onset of infection (fig. 6a, ii). The latter may indicate the reaction of antibody with non-conformational epitopes of protein F (Mutharia and Hancock, 1985). A band below protein F is believed to be partly undenatured form of this protein as described by Hancock and Carey (1979). As with patient 1, IgG antibodies recognised protein H only in OM preparations from cells grown *in vitro* and not in those for cells taken directly from the burn wound (lanes 2 and 3). A

Fig. 4. OMPs of *S. marcescens* (lane 1), *E. coli* (lane 2), *K. pneumoniae* (lane 3), *P. mirabilis* (lane 4) and *P. aeruginosa* (lane 5) cultivated in TSB-Fe. OMPs were electrophoretically transferred to NC paper and (a) stained with amido black 1% w/v, or (b) probed with serum from patient 1 and the reaction visualised with peroxidase-labelled anti-human IgG.

protein above D was observed in the OM of cells directly taken from the burn wound tissue (lane 3) but was barely detectable in the OM of cells grown *in vitro* (lanes 1 and 2).

IRMPs and proteins D and E elicited IgA antibody formation (fig. 6b) and, similarly, a band above protein D was recognised in OM preparations of cells taken directly from the burn wound (lane 3). This band appeared to have an electrophoretic mobility different from that of the flagella purified from the isolate. A marked response was noted to protein H in OM preparations from cells cultivated *in vitro* (lanes 1 and 2). Indeed these represented the most intense bands on the immunoblot. No major difference in response was observed in immunoblots with subsequent serum samples (data not shown).

A very weak IgM response was obtained with the serum of this patient; it was too faint to be photographed. The IgG response locally at the wound site (data not shown) was indistinguishable from that found with serum (fig. 6a).

To confirm that the immunoblotting results represented binding of antibodies to the OMPs, immunoglobulins against LPS were removed from patients' serum samples by immunoprecipitation after addition of LPS purified from the appropriate strain cultivated *in vitro* (Westphal and Jann, 1965). Probing immunoblots of separated LPS with untreated serum revealed a faint ladder pattern (fig. 7, lane 1) which was not observed on probing with the LPS-absorbed serum (fig. 7, lane 2). However, when the same two sera were allowed to react with

Fig. 5. Immunoblot of the OMPs in fig. 1 (lanes 1–3 as described) electrophoretically transferred to NC paper and probed with infected tissue fluid from patient 1. Reactions were visualised with peroxidase-labelled anti-human (a) IgM (b) IgG and (c) IgA.

replicate OMP immunoblots, identical patterns and intensities of banding were seen (fig. 7, lanes 3 and 4 respectively).

Discussion

Information concerning properties of bacteria *in situ* in infection sites, especially in burn wounds, is generally lacking. Hypotheses about the behaviour of micro-organisms in human infections have been extrapolated from studies with bacteria cultivated *in vitro*. Such results may be misleading because the surface characteristics of bacteria grown *in vitro* may not represent those infecting burn wounds (Brown and Williams, 1985a, b). The results of this investigation clearly indicated that *P. aeruginosa* was growing under iron-restricted conditions in the

infected burn wounds of two patients, as judged by the expression of IRMPs in the OM of bacteria obtained directly from the wound. Similar observations have been reported in infections in cystic fibrosis patients (Brown *et al.*, 1984), in urinary tract infections in man (Lam *et al.*, 1984; Shand *et al.*, 1985) and in experimentally induced infections in animals (Griffiths *et al.*, 1983; Sciortino and Finkelstein, 1983; Cochrane *et al.*, 1987). Preliminary data also indicated that, for patient 1, bacteria taken directly from the wounds displayed altered LPS profiles when compared to their counterparts grown *in vitro* (results not shown), as has been shown by Cochrane *et al.* (1988).

The IRMP antigens expressed *in vivo* and *in vitro* in iron-depleted conditions were recognised by antibodies present in serum taken early in the

Fig. 6. OMPs of *P. aeruginosa* isolated directly from the infected wound tissue of patient 2 (lane 3) and of the same isolate cultivated in TSB + Fe (lane 2) and TSB + Fe (lane 1). OMPs were electrophoretically transferred to NC paper and probed with serum from patient 2 obtained (i) 1 week and (ii) 3 weeks after onset of infection. Reactions were visualised with peroxidase-labelled anti-human (a) IgG or (b) IgA. With IgA, there was no difference between the reactions with serum collected 1 week or 3 weeks after onset of infection.

infection and also locally in wound tissue. *P. aeruginosa* produces iron-chelating compounds such as pyochelin and pyoverdine in iron-depleted conditions *in vitro* (Cox *et al.*, 1981; Cox and Adams, 1985). However, it is not known which of the IRMPs function as receptors for iron-siderophore complexes. The production of antibodies against these antigens may hinder the uptake of iron and, therefore, reduce the growth rate of the organism *in vivo*. Antibodies of all three classes of immunoglobulins were shown to be directed against the IRMPs, indicating their importance as targets of host defences. The iron-restricted nature of the wound site may have further implications because there is a significant rise in the production of toxin

A by *P. aeruginosa* in iron-depleted conditions (Bjorn *et al.*, 1978).

Furthermore, immunoblotting demonstrated recognition of proteins D, E and H, as well as flagellar protein, by serum antibodies. The latter antigen has been shown to be highly immunogenic in other studies (Mutharia *et al.*, 1982; Anwar *et al.*, 1984). Motility is thought to be an important virulence factor in burn-wound sepsis (Holder, 1985) and antibodies which inhibit the functioning flagellum are likely to reduce the invasive capacity of the organism. The efficacy of a divalent flagellar preparation as a vaccine has been tested in a burned mouse model (Holder and Naglich, 1986). Protection was afforded against live *P. aeruginosa* in the

Fig. 7. Immunoblot of LPS (lanes 1 and 2) and OMPs (lanes 3 and 4) of *P. aeruginosa* cultivated in TSB – Fe, probed with untreated patient's serum (lanes 1 and 3) and LPS-absorbed serum (lanes 2 and 4). The reaction was visualised with peroxidase-labelled anti-human IgG.

burn area and was independent of both the flagellar and somatic antigen of the challenge strain.

The OMPs of *P. aeruginosa* used in these immunoblotting studies had been treated with SDS and 2-mercaptoethanol. Conformational epitopes in the antigens may have been affected by this treatment which could explain the low level of recognition of protein F (Mutharia and Hancock, 1985). This should be explored further with monospecific monoclonal antibodies.

The lack of immunological recognition of protein H in OMs of cells taken directly from burns may suggest that the method of preparation caused loss of antigenicity of protein H in these OMs. Possibly there is competition for the antigenic binding sites between other classes of immunoglobulin such as IgM (fig. 2a, lane 3). Further work is needed to study the binding of immunoglobulins to protein H molecules. In addition, it is not clear whether the response observed to the protein of cells grown *in vitro* is to H₁ or H₂ as described by Hancock and

Carey (1979) because the individual proteins were not separated in the system used here (Anwar *et al.*, 1983).

The strong IgG response to Enterobacteriaceae surface antigens (fig. 4) is to be expected since these bacteria are all common members of the gut flora. Furthermore, despite possible post-burn immunosuppression, there were still sufficient circulatory immunoglobulins present to react with the OM antigens of the Enterobacteriaceae. In a previous study we showed that antibodies in serum from volunteers with no previous history of pseudomonas infection reacted strongly with enterobacterial antigens but not with pseudomonal antigens (Anwar *et al.*, 1985). This suggests that there is little cross-reactivity between the two bacterial families and that the level of antibodies to *P. aeruginosa* in the population is low except following infection with the organism.

The band above protein D recognised in OM preparations of cells taken directly from the burn

wound (fig. 2) is interesting. Its expression may be related to surface growth of the organisms in the burn wound or to the influence of other environmental factors. The identity of this band remains to be investigated by monoclonal antibody. The *P. aeruginosa* strains isolated from the two patients possessed different flagellar types and absence of flagella in the OM preparation obtained from bacteria grown *in vivo* in patient 2 may reflect shearing and loss of this antigen during sonication.

The humoral immune response to OMPs of *P. aeruginosa* has been investigated following subcutaneous infection in mice (Hedstrom *et al.*, 1984) and the results showed production of antibodies to proteins F, H₂ and I. The different picture observed in our investigation probably reflects variation in the immunological response of man and mouse. Furthermore, bacteria cultivated under iron-sufficient conditions were used in their study so the response to IRMPs could not be determined and serum was not obtained until 14 days after onset of infection.

Sera from cystic fibrosis patients with pseudomonas lung infection has also been used in immunoblotting studies (Anwar *et al.*, 1984). Such sera detected IRMPs and proteins D and H₂ and showed a particularly strong reaction with proteins F and G. In addition, a band below H₂ which was postulated as representing rough LPS had stimulated a marked response. Strong recognition of this antigen or of proteins F and G was not observed with the burn patients in this investigation. In cystic fibrosis, *P. aeruginosa* causes a chronic infection which may persist for upwards of 20 years. The bacteria grow in glyocalyx-enclosed micro-colonies embedded in exopolysaccharide and mobile swarmer cells are frequently released resulting in exacerbation of pneumonia (Costerton *et al.*, 1983). Over the years, *P. aeruginosa* exposes many of its antigens to the immune system, thus stimulating the production of high titres of antibodies to numerous bacterial components (Høiby and Axelssen, 1973). This is in contrast to *P. aeruginosa* in burn wounds which essentially produces an acute and possibly life-threatening infection, and thus a different immune response.

It has been reported that OMPs separated by SDS-PAGE and transferred to NC paper may be contaminated with co-migrating LPS (Poxton *et al.*, 1985; Lam *et al.*, 1987). Probing of replicate immunoblots of OMPs with patient's serum before and after absorption with purified LPS resulted in identical patterns and intensities of bands. The LPS was purified from cells cultivated *in vitro* because insufficient bacteria could be obtained

from the burn wound for the extraction procedure. It is possible that if the LPS from bacteria taken directly from the burn wound and those grown *in vitro* differs, absorption of sera with the latter may not remove all the antibodies to the former. Nonetheless, the results suggested that the presence of LPS on the immunoblot did not contribute significantly to the bands observed.

Several papers have reported the induction of a protein of mol. wt 14 000 in *P. aeruginosa* under conditions of iron-deprivation (Sokol and Woods, 1983, 1984, 1986; Sokol, 1984). The initial study (Sokol and Woods, 1983) relied on incubation of OM preparations with ⁵⁹Fe-pyochelin followed by SDS-PAGE analysis. However, it is unlikely that the binding of ⁵⁹Fe to pyochelin or of ⁵⁹Fe-pyochelin to receptor protein would withstand SDS denaturation unless the links between molecules were covalent. There is no evidence to suggest that this is so, and no SDS-PAGE gels of ⁵⁹Fe-pyochelin running alone were shown. A 14 000-mol. wt protein has been reported in periplasmic fractions of iron-deprived *P. aeruginosa* (Cox, 1985) and it is possible that this protein was demonstrated in the membrane fractions investigated. Alternatively, the protein in question may represent a fragment of a larger protein which binds ⁵⁹Fe-pyochelin. We have investigated wild type and laboratory strains of *P. aeruginosa* of many serotypes and have never observed induction of a 14 000-mol. wt protein under iron-deprived conditions (Anwar *et al.*, 1984, 1985; Brown *et al.*, 1984; Kadurugamuwa *et al.*, 1987).

Commercial vaccines against *P. aeruginosa* based largely on LPS are available but are rarely used in clinical practice. This may be due partly to problems associated with their toxicity and serotype specificity (Pennington, 1979). In an attempt to overcome these problems some researchers have considered OMPs as immunotherapeutic agents, since they are non-toxic and are antigenically conserved among serotypes (Mutharia *et al.*, 1982; Anwar *et al.*, 1985). Gilleland *et al.* (1984) reported that antibodies to protein F offered protection against intraperitoneal challenge with laboratory strains of *P. aeruginosa* and, more recently, Matthews-Greer and Gilleland (1987) demonstrated protection with purified protein F against challenge with six heterologous LPS Fisher-Devlin immunotypes in a burned mouse model. The ability of IRMPs and other major OMPs, individually or in combination, to protect animals against challenge with different serotypes of *P. aeruginosa* remains to be investigated. The information gained in this study will be useful in the rational design of vaccines so that

protection of individuals with burns against infections caused by *P. aeruginosa* may be achieved.

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