bacteria were trapped by this substance. The biological significance of pili as a virulence factor was also demonstrated in mouse infection models where the LD_{50} of strain PAO (=10^7 bacteria/mouse).

Structural studies on P. aeruginosa pili have revealed that they are cylindrical rods of 52 A diameter consisting of a 15,000 dalton subunit protein arranged in a helical array of 5 units/turn with a pitch of 41 A. Moreover, experiments with the PAK-specific single-stranded DNA tip binding bacteriophage Pf1 and various constructs of P. aeruginosa containing the PAK pilin gene in a PAO background suggest that P. aeruginosa pili do not contain a tip protein as a minor component of the pilus. Processing of P. aeruginosa propilin involves the removal of a 6-7 residue leader and N-methylation of the resulting pilin to generate a characteristic N-methylphenylalanine amino terminus.

Cloning and nucleotide sequencing of pilin genes from several clinical isolates of P. aeruginosa have shown that the pilin sequences are characterized by a highly conserved N-terminal region and a central hypervariable domain flanked by regions of semi-conserved amino acid sequence. The unprocessed pilus protein (propilin) contains a short leader sequence of 6-7 residues which is removed during processing and followed by methylation of the resulting N-terminal pheylalanine residue to generate a characteristic NMePhe amino terminus. The leader sequence as well as approximately 28 residues at the N-terminus of the mature pilin are highly conserved and seen in pilins from such diverse genera as Neisseria gonorrhoeae, Moraxella bovis, and Bacterioides nodosus.

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S3.3.3 Role of Outer Membrane Components in Pseudomonas aeruginosa Colonization of the Cystic Fibrosis Lung

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There has been no formal proof that outer membrane components play a role in colonization of the lungs of CF patients. However, the frequent isolation of P. aeruginosa cells with unusual outer membrane properties, and the vigorous antibody response mounted against outer membrane components, provides strong circumstantial evidence for such a role.

Unusual outer membrane phenotypes of the P. aeruginosa isolates from the cystic fibrosis lung. It has been well documented that the P. aeruginosa strains isolated from the colonized lungs of CF patients often produce copious

quantities of mucoid exopolysaccharide. However, only recently certain unusual outer membrane phenotypes have also been documented. It had previously been shown that many P. aeruginosa isolates from CF lungs were difficult to type using conventional typing sera. Up to 50% or more of strains were non-typable or were polyagglutinable (i.e., agglutinated by more than one typing sera). We were able to link these unusual typing reactions to a deficiency in the presence of smooth (O-antigen-contining) lipopolysaccharide (a major outer membrane surface component of clinical isolates of P. aeruginosa from all other infections and infection sites investigated)[1]. As a consequence of the "rough" phenotype, CF isolates were frequently serum sensitive. The serum sensitivity of these isolates, as well as the high serum concentrations of antipseudomonal antibodies (see below were proposed to explain why P. aeruginosa infections are located in the lungs and virtually never become bacteremic[1].

In general the outer membrane protein patterns of CF lung isolates of P. aeruginosa are quite similar to those of non-CF lung isolates[1]. However, as pointed out by Brown and colleagues[2], study of outer membrane protein patterns of isolates after in vitro growth, can be misleading. Consequently it is worth mentioning two studies in which such objections were overcome. By direct isolation of the outer membranes of P. aeruginosa obtained from the sputum of a heavily colonized patient, Brown et al. demonstrated the presence of a number of normally-observed major outer membrane proteins[2]. In addition, they observed a series of high molecular weight iron-starvation-derepressed outer membrane proteins which are not normally seen in bacteria grown on most (iron-rich) laboratory media but can be observed in bacteria taken from a variety of infection sites[3]. Our own studies[4] have shown that certain outer membrane proteins are present in vivo by using monoclonal antibodies to perform indirect immunofluorescent staining of outer membrane proteins F and H2 in lung sections from autopsied CF patients who died with Pseudomonas lung disease.

Outer membrane components of P. aeruginosa are recognized by the immune system of CF patients. Using crossed immunoelectrophoresis as a tool, Hoiby and colleagues demonstrated that in CF patients with P. aeruginosa lung colonization, the serum contained high titres of antibodies that reacted with a broad range of undefined antigens[5]. The number of precipitins and amounts of antibodies were generally associated with the length of colonization and prognosis of the patient We confirmed these observations for lipopolysaccharide-specific antibodies[6]. In addition, we observed that patients colonized for more than 1.5 years with P. aeruginosa had anti-outer membrane antibody titres that were, on average, three orders of magnitude higher than briefly colonized or uncolonized patients. By Western blotting, antibodies to a variety of specific outer membrane protein antigens were detected in the patients with high antibody titres. All patients examined by Western blotting[6] or crossed immunoelectrophoresis[7] produced antibodies to the major outer membrane protein F. Given the well developed serum immune response to surface antigens, it is worth considering why these antibodies fail to result in clearance of P. aeruginosa from the lungs of CF patients. The reason does not seem to be the exclusion of antibodies from the lung, since both IgG and IgM can be detected in sputa[8] and by immunofluorescient staining of these antibody types in autopsied CF lung sections[4]. However, the discovery that Pseudomonas elastase may be an IgG protease[9], calls to question whether all of these antibodies might be capable of opsonizing P. aeruginosa in vivo. Other potential mechanisms for the ineffectiveness of opsonophagocytic clearance of P. aeruginosa isolates, include inactivation of phagocytic cells by Pseudomonas leukocidin[10] and masking of the bacterial cell surface by mucoid exopolysaccharide[11]. It will be of great interest to determine the actual mechanism(s) of resistance to opsonophagocytic clearance, since this may provide new targets for therapeutic intervention.

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S3.3.4 THE ROLE of EXCRETED PRODUCTS in PSEUDOMONAS COLONIZATION

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Pseudomonas aeruginosa produces a large number of extracellular products. The question of which of these Pseudomonas products contribute to colonization of the airways of patients with cystic fibrosis can be approached in several ways. One approach is to compare the incidence and level of production of a product by strains isolated from cystic fibrosis patients to that of strains isolated from other sources. Such studies indicate that the environment does, in fact, influence the production of some extracellular products such as exoenzyme S, toxin A, phospholipase C and proteases. This appears to be a quantitative difference rather than a qualitative difference. Thus, CF isolates as a group, produce less toxin A and exoenzyme S than do strains from other sources. What is as yet unclear is whether the amount of a given product produced varies with progression of the disease. Furthermore, there is as yet little known about how production of these extracellular factors is regulated. In vitro studies indicate that toxin A is under both positive and negative regulation. The positive regulation occurs through a gene called reg A. Toxin A synthesis is negatively regulated by elevated iron in the environment. In the presence of excess iron, transcription of the reg A gene is impaired which in turn leads to a decrease in transcription of the toxin A