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PSEUDOMONAS AERUGINOSA: INFECTION AND IMMUNITY

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Pseudomonas aeruginosa is a major cause of infections in Western society, being the causative agent of about 200 000 nosocomial infections per year in North America. Such infections tend to be rapidly progressing and pose serious risks to patients. However, *P. aeruginosa* can also cause long-term (up to 10 years or more) chronic infections in the lungs of patients with cystic fibrosis, and the onset of *P. aeruginosa* infection signals the eventual deterioration of lung function in such patients, leading to death from respiratory failure. *P. aeruginosa* shows high intrinsic antibiotic resistance. While there are antibiotics that show initial success against *Pseudomonas* infections, even modest mutations lead to clinically significant resistance, making development of antibiotic resistance common and successful treatment difficult. For this reason considerable effort has been expended in seeking vaccine or immunotherapeutic solutions to such infections. At the same time, diagnosis and epidemiology, each of which use specific antigens, has become important in monitoring the onset and course of infection and can influence the eventual success of therapy.

Process of infection

P. aeruginosa causes rapidly progressing infections in animals and humans. Generally speaking, these infections only occur in those animals and people which have a reduced capacity to defend against infection (or at body sites that have intrinsically weaker defenses). Thus, in animal models, mild alcohol burns or neutropenia induced by cyclophosphamide lead to a reduction in the LD_{50} (the concentration of bacteria that kill 50% of animals) by up to 100 000-fold. Similarly, the types of human conditions which tend to predispose towards *P. aeruginosa* infections include neutropenia induced by anticancer drugs, burns, major surgery, steroid therapy, etc. In such patients the likelihood of *P. aeruginosa* infection increases with length of hospital stay.

P. aeruginosa produces a multitude of virulence

Table 1 Immunogenicity and antibody response to major antigens of *Pseudomonas aeruginosa*

Antigen	*Characteristics/function*	*Specific antibodies/use*	*Immunogenicity*
Cell associated:			
Outer membrane protein OprF	Major pore-forming protein; structural role	Opsonic and protective; mAbs used in structural studies, diagnostics; antigenically conserved	Strong Ab response
OprL (= H2)	Peptidoglycan-associated lipoprotein (PAL); structural role	mAb reveals conserved antigen in type I Pseudomonadaceae	Strong Ab response
OprI	Braun lipoprotein	Protective; mAbs reveal antigenic conservation	Strong Ab response
Iron-regulated outer membrane proteins	Transport of iron–siderophore complexes		Ab response accompanies chronic infection
Variant lipopolysaccharide (LPS) A-band (D-rhamnan)	Common antigen, induces influx of PMN	Not neutralizing or opsonic	Ab response appears late in patients with chronic infections
LPS B-band (O antigen of LPS)	O-polysaccharide of conventional LPS; forms the basis for serotype-specificity	Serotype-specifically opsonic and protective; mAb/polyclonals against each serotype produced; useful in serotyping and epidemiology	Strong Ab response
LPS core polysaccharide	Conserved region which links O antigen polymers to lipid A	Cross-reactive; inner core epitope exposed on cell surface	Strong Ab response
LPS lipid A	Anchors LPS in the outer membrane; endotoxic moiety – causes fever and septic shock	Ab broadly cross-reactive even with other species; reacts poorly with intact cells	Weak immunogen, includes *P. aeruginosa*-specific response
Mucoid exopolysaccharide/alginate	Exopolysaccharide in the form of loose slime; associated with biofilm/microcolony formation	Both conserved and selective Abs available	Weak unless conjugated to protein
Flagella	Motility; chemotaxis and invasion	Two major serogroups (type a with 4 subgroups and type b); opsonic; inhibit motility; protective; mAbs available	Strong Ab response
Pili	Adherence and initial colonization	About 14 types; antigenically variable; Neutralizing and passively protective; many mAbs available	Weak to intermediate Ab response
Ribosomes	Protein synthesis	Protective property not confirmed; may be due to contamination	
Extracellular proteins:			
Elastase	Degrades connective tissues. Tissue invasion		Observed in chronic infections
Exotoxin A	Inhibition of host cell protein synthesis	Cross-reactive neutralizing activity	Strong Ab response
Exoenzyme S	Inhibition of host cell protein synthesis	Neutralizing activity	

factors including the extracellular toxins exotoxin A and exoenzyme S, a periplasmic cytotoxin, two secreted proteases elastase and alkaline protease, secreted lipase, phospholipase and heat-stable hemolysin (also called rhamnolipid), a neurominidase, slime glycolipoprotein, mucoid exopolysaccharide, and endotoxin. Each of these has been shown in animal models to have a role in pathogenesis, making

Pseudomonas pathogenesis truly multideterminant, and in many cases in those same animal models, immune intervention directed against the virulence factor in question will reduce the impact of, or even prevent infection. In rapidly progressing infections, which can be common in nosocomial situations, perhaps the most important characteristic of *P. aeruginosa* is its ability to grow rapidly *in vivo*, with a doubling time equivalent to that observed on a nutrient medium *in vitro*. Thus in a relatively short time (24–36 h) such infections can become overwhelming.

In the more complex situation posed by the chronic infections observed with cystic fibrosis patients, it is still not entirely clear why this disease predisposes a patient towards a long-term, localized lung infection by a bacterium which normally causes rapidly developing invasive infections. The suggestion is that localized defects lead to establishment of infection. One newer concept is that the high salt concentration induced by the mutation of the cystic fibrosis transmembrane regulator (CFTR) protein, renders ineffective the natural antimicrobial defense peptides in the lung.

Antigenicity

As indicated in Table 1, there are a wide range of antigens that have been studied in detail in *P. aeruginosa*. Specific antisera and in many cases monoclonal antibodies (mAbs) have been raised against these (and other) antigens and have been useful in demonstrating the varied levels of serological conservation in these antigens. Thus, there are 20 lipopolysaccharide (LPS) O-serotypes, about 14 pilin antigenic variants and about five flagellin variants (representative of two major serotypes) in *P. aeruginosa*. This makes these antigens ideal as targets for epidemiological studies. On the other hand, the outer membrane proteins OprF, OprL and OprI, LPS rough core and lipid A, exoantigens and mucosal exoplysaccharide have only one or two variant antigenic types. Such antigens therefore have substantial potential as targets for diagnosing *P. aeruginosa* infections and most of these are or have been considered as vaccines. The mAbs raised against *P. aeruginosa* antigens have also proven to be excellent tools for studying these antigens and have assisted in the study of the structure, function, molecular genetics and immunology of these proteins.

Immunogenicity and immunotherapy

All of the antigens listed in Table 1 have been shown to elicit antibodies in animal models, cystic fibrosis patients with *P. aeruginosa*-infected lungs, and/or humans with burn wound infections. This has provided the rationale for pursuit of vaccine and passive immunotherapy studies (Table 2). In many cases,

Table 2 Recent immunotherapeutic or immunoprophylactic preparations against *Pseudomonas aeruginosa*

Vaccine	*Efficacy assessment and other remarks*
Octavalent LPS O-polysaccharide–toxin A conjugate vaccine	Engendered IgG response that was protective. However human intravenous immunoglobulins versus this vaccine did not show statistically significant protection in human trials
Alginate–toxin A conjugate vaccine	Serum antibodies from rabbits were opsonic and promoted killing of *Pseudomonas aeruginosa*, and had neutralizing activities towards toxin A
Anti-idiotype antibodies	Induced LPS-specific response in mice
OprF–OprI hybrid vaccine	Significant antibody titers in human volunteers after immunization; protective in immunosuppressed mouse model study
Alginate vaccine	Different sizes of alginate in preparation had variable effect in eliciting opsonic antibody response; large molecular size antigen preparation was tolerated well in volunteers; human trials apparently abandoned
Synthetic peptides of OprF epitopes	Linear B cell epitopes of OprF-conjugated with carrier protein as vaccine. Elicited protective response
ScFv of OprF-specific antibody	Modern technology; single-chain antibody specific against an OprF domain. Applicable for epitope mapping and further antibody engineering
Killed whole bacteria delivered mucosally	Demonstrated heterologous protection in mice
Pilin peptides	Limited cross-reactivity observed but attempts are being made to improve this by peptide engineering; protective in mice
Human mAbs	Five serotype-specific anti-LPS O-antigen human mAbs were opsonophagocytic and protective in mice; three flagella specific human mAbs were protective in mice
Human–Murine heterohybridomas	Human antibody specific against *Pseudomonas aeruginosa*, scale-up production successful, but efficacy is not defined at present

injection of the antigen into animals leads to a sufficient production of antibodies that promote opsonophagocytosis and protection against subsequent infections. Since *P. aeruginosa* is generally considered to be an extracellular infection, there is a general consensus that an antibody response is required for protection. However, this is not necessarily sufficient for protection since the lungs of patients with cystic fibrosis continue to remain chronically colonized despite exuberant serum antibody responses to several antigens. Furthermore, there are few circumstances in which *P. aeruginosa* infections are truly predictable, making active vaccination a less attractive prospect. For this reason passive protections with hyperimmune intravenous immunoglobulins from volunteers immunized with a *Pseudomonas* antigen has been pursued. Unfortunately, the most recent of these studies using an intravenous immunoglobulin with strong titers to the most predominant LPS serotypes and to exotoxin A, was abandoned due to its inability to show statistically significant protection in humans at the dosage used combined with a less favorable adverse reaction profile. While it is too early to discard the possibility for success with one of the other vaccine strategies being pursued, it may require a novel approach to yield success.

See also: **Bacteria, immunity to; Bacterial cell walls.**

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