In: Advances in Experimental Medicine and Biology. Vol 185
Immunology of proteins and peptides - III
Viral and bacterial antigens.
(Eds M.Z. Atassi and H.L. Bachrach)
Plenum Press. New York USA. 1985 p 215-222

MONOCLONAL ANTIBODIES AGAINST BACTERIAL OUTER MEMBRANE ANTIGENS

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ABSTRACT

Monoclonal antibodies have proved to be highly specific tools for defining the antigenic epitopes of Pseudomonas aeruginosa outer membrane macromolecules. In this article we have highlighted the use of monoclonal antibodies in the study of lipopolysaccharide heterogeneity and in particular have demonstrated that single monoclonal antibodies can recognize epitopes on lipid A which are conserved in all Gram negative bacteria tested. Monoclonal antibodies against P. aeruginosa outer membrane proteins have been used to demonstrate the strong conservation of specific antigenic sites in all P. aeruginosa strains tested. In the case of one monoclonal antibody, specific for outer membrane lipoprotein H2, the antigenic site recognized by the antibody was also found to be conserved in all group 1 Pseudomonads. The implications of these monoclonal antibodies to bacterial taxonomy is discussed. Monoclonal antibodies against two separate conserved surface epitopes on outer membrane protein F were isolated and differentiated according to their reactions with 2 mercaptoethanol-reduced protein F and with proteolytic and cyanogen bromide peptide fragments of protein F. . One of these protein F-specific monoclonal antibodies has been demonstrated to have immunotherapeutic potential.

INTRODUCTION

Monoclonal antibodies provide several advantages over conventional polyclonal sera derived from immunization of animals with antigenic fractions. For example, they have defined specificity for a single epitope (i.e. antigenic site) on one species of macromolecule. This allows one to accurately quantify and identify macromolecules under all circumstances, regardless of the presence of contaminating antigens. In addition, monoclonal antibodies interact with their target antigens in a highly reproducible fashion, whereas polyclonal sera are notorious for their batch to batch variation. Also hybrid cells producing these monoclonal antibodies can be grown in large amounts in tissue culture or in ascites and thus provide potentially inexhaustable supplies of these reagents.

We have applied hybridoma technology to the production of monoclonal antibodies against the outer membrane antigens of Pseudomonas aeruginosa. In this article we will attempt to highlight the potential uses of the monoclonal antibodies in a variety of areas of study.

LIPOPOLYSACCHARIDE HETEROGENEITY

LPS is a major surface component in gram negative bacterial cells (Nikaido and Nakae, 1979). It consists of a tripartite structure with lipid A (adjacent to and inserted into the outer monolayer of the outer membrane) covalently bonded to the rough core which is itself covalently attached to the O antigenic side chain. The lipid A usually consists of diglucosamine phosphate substituted with five or six fatty acids, including around four moles of hydroxy fatty acids. The rough core region contains a variety of saccharides often including octoses, heptoses and hexoses. The most distal (to the membrane) portion of LPS is the O antigen which usually consists of repeated tri-to pentasaccharide units. It is now recognized that the LPS can be quite heterogeneous both in the substitution of various groups (including phosphates, ethanolamines, aminoacids and acetyl groups) at various places along the saccharide chain and in the length of individual O antigenic side chains (reviewed in Darveau and Hancock, 1983). LPS has two properties of major importance to the pathogenesis of gram negative bacterial infections. Firstly the O antigen is usually the immunodominant portion of the bacterial surface and in many bacterial species differences in O antigen composition are the basis of serotyping. Secondly the lipid A, also called endotoxin, confers many interesting properties on the LPS including pyrogenicity, toxicity, mitogenicity, adjuvanticity, tumor necrotising ability, etc. (Bradley, 1979).

We have used monoclonal antibodies to probe the antigenic conservation of different regions of the LPS (Hancock et al., 1982; Mutharia et al., 1984). A monoclonal antibody specific for the O antigen of our wild type P. aeruginosa strain interacted with strains of equivalent serotype (O-5 in the International Antigen Typing Scheme, IATS) and weakly with strains of a related serotype (O-17) (Table 1).

In contrast monoclonal antibodies specific for rough LPS (lacking O side chains) reacted with a limited subset of strains from the 17 serotypes of the IATS (Table 1), demonstrating greater conservation of the rough core. Monoclonal antibodies against lipid A interacted with all of the strains of P. aeruginosa tested as well as many other genera of the Gram negative bacteria (Table 2). Thus, as a general rule it appears that LPS heterogeneity is increased for substituents that are further from the outer membrane surface.

CONSERVATION OF PROTEIN ANTIGENS IN P. AERUGINOSA

The Pseudomonas aeruginosa outer membrane contains a 6-9 species of polypeptides present in high copy number (10^4-10^5 copies per cell) and thus called major outer membrane proteins

Table 1: Reaction of LPS specific monoclonal antibodies with P. aeruginosa strains

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Outer Membranes		Reaction using monoclonal antibodies to LPS			
		O Antigen type 5 (MA1-8)	Rough Core (MA3-5)	(MA3-6)	Lipid A (5E4)
P. aeruginosa	PA01	+	+	+	+
P. aeruginosa	rough	-	+	+	+
P. aeruginosa	serotype 5	+	+	+	+
P. aeruginosa	serotype 17	+[a]	-	-	+
P. aeruginosa	serotypes 7,8,10,14,16	-	+	+	+
P. aeruginosa	serotype 11	-	-	+	+
P. aeruginosa	serotypes 1,2,3,4,6,9 12,13,15	-	-	-	+
P. aeruginosa	clinical strains	+/- (2/34)[b]	+/-(1/20)[b]	+/-(1/20)[b]	+(20/20)[b]

[a] weaker reaction due to a shared determinant between serotype 17 and serotype 5.

[b] postive reactions out of the number of strains tested.

(Mutharia et al., 1982). We have demonstrated that these proteinsare immunogenic in animals and in humans, in that the sera of cystic fibrosis patients with P. aeruginosa pulmonary infections (Hancock et al., 1984), of convalescent patients recovered from P. aeruginosa bacteremia (Lam et al., 1983) and of rats with induced chronic P. aeruginosa lung infections (Lam et al., 1983), all have antibodies that interact with the major outer membrane proteins of our laboratory wild type strain PA01. The simplest explanation for this data is the outer membrane protein antigens are antigenically conserved in P. aeruginosa. We have now confirmed this suggestion for outer membrane proteins F and H2 using monoclonal antibodies (Table 2).

POTENTIAL USE OF MONOCLONAL ANTIBODIES IN THE TAXONOMY OF BACTERIA

At present the major tools in the taxonomic grouping of bacterial cells are determination of growth characteristics , DNA:DNA hybridization (Palleroni et al., 1973), rRNA homology studies (Palleroni et al., 1973; DeVos and DeLey, 1983) and fatty acid analysis (Moss et al., 1972). Each of these methods is time consuming and in the latter three cases involves expensive reagents or equipment. Thus there is some justification for the development of new taxonomic aids for the identification of groups of gram negative bacteria. We have considered monoclonal antibodies directed against outer membrane antigens as potential taxonomic tools.

To date we have identified three monoclonal antibodies (Table 2) which may have taxanomic value. Monoclonal antibody 5E4 was produced by Centocor, PA and is directed against the lipid A portion of the lipopolysaccharide (LPS) of E. coli strain J5 (Mutharia et al., 1984). This antibody interacts with almost all (97%) Gram negative outer membranes (i.e. lipid A), including those from representatives of 4 Families, 16 species and up to 30 strains of a single species. Unfortunately this is a moderately low affinity antibody (thus giving weak ELISA reactions) and the delineation of bacterial cells into Gram negative and Gram positive is usually more simply accomplished by the Gram stain. However, this or a similar antibody may be of interest in those cases in which Gram staining provides a Gram indefinite phenotype.

A second monoclonal antibody MA1-6, specific for the outer membrane peptidoglycan-associated lipoprotein H2 (Hancock et al., 1982), may have greater taxanomic value. This antibody interacts with all known species of the group ("true") Pseudomonads as classified by Palleroni et al. (1973) and by DeVos and DeLey (1983), but not with those strains of Pseudomonadaceae shown by nucleic acid homology to be distantly related to these strains. The group 1 Pseudomonads (and the related organism Azotobacter vinelandii) share one or more of the following characteristics, DNA homology (Palleroni et al., 1973), rRNA homology (Palleroni et al., 1973; deVos and deLey, 1983), and the presence of the hydroxy fatty acids α-hydroxy decanoic acid, α-hydroxydodecanoic acid (occasionally)and ß-hydroxydodecanoic acid attached to their LPSs (Moss et al., 1972). In contrast they differ substantially in morphology, nutritional requirements and production of fluorescent pigments - all of which are characteristics previously used to differentiate the Pseudomonadaceae.

Table 2: Potential taxonomic va of monoclonal antibodies

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Bacterial outer membrane antigen	Reaction using monoclonal antibodies to Outer membrane proteins H2 (MA1-6)	F (MA5-8)	F (MA4-4)	LPS Lipid A (5E4)	Relationship to Pseudomonas aeruginosa established by a rRNA homology	DNA homology	Fatty acid homology
P. aeruginosa (33 strains)	+	+	+	+	+	+	+
P. putida	+	-	+	+	+	+	+
P. syringae	+	-	+	+	+	+	+
P. chloraphis	+	-	-	+	+		
P. aurofaciens	+	-	-	+	+		
P. stutzeri	+	-	-	+	+	+	+
P. fluorescens	+	-	-	+	+	+	+
P. anguilliseptica	+	-	-	+			+
Azotobacter vinelandii	+	-	-	+	+		
P. maltophilia[b]	-	-	-	+	-	-	-
P. acidovorans[b]	-	-	-	+	-	-	-
P. solanacearum[b]	-	-	-	+	-	-	-
P. cepacia[b]	-	-	-	+	-	-	-
Enterobacteriaceae (4 Genera)	-	-	-	+	-	-	-
Vibrionaceae (4 Genera)	-	-	-	+	-	-	-
Rhizobiaceae	ND[c]	ND	ND	+	-	-	-

a. + relationship established; - no relationship; blank - no available data.
b. These strains were established as taxonomically distinct from the P. fluorescens group of the Family Pseudomonadaceae and fit into 3 distinct rRNA and DNA homology groups.
c. ND - not determined.

A third antibody of interest is MA5-8 specific for porin protein F of Pseudomonas aeruginosa (Mutharia and Hancock, 1983). This antibody cross-reacts with all 33 strains of P. aeruginosa we have examined, but no other Gram negative bacterial strains. Interestingly, other monoclonal antibodies against porin protein F have lesser specificity, since they recognize P. putida and P. syringae strains. Nevertheless, even these antibodies may turn out to be of taxonomic interest.

Thus monoclonal antibodies can distinguish both major subgroupings and more minor subgroupings of bacterial cells. However, due to the potential for removal of given antigens by mutation it seems unlikely that they will become more than an adjunct technique to assist classical taxonomic methods.

CHARACTERIZATION OF TWO ANTIGENIC EPITOPES ON PROTEIN F

In our studies on the cross reactivity of monoclonal antibodies against outer membrane protein F of Pseudomonas aeruginosa we demonstrated monoclonal antibodies with two distinct specificities. One antibody, MA5-8, interacted only with P. aeruginosa strains whereas three other monoclonal antibodies, MA2-10, MA4-4 and MA4-10, cross reacted with P. syringae and P. putida strains. These two antibodies were distinguishable by a variety of other tests (Table 3).

Table 3: Differentiation of two classes of monoclonal antibodies against protein F

	Monoclonal Antibody	
	MA5-8	MA2-10, MA4-4, MA4-10
Reactivity with purified protein F	+	+
Surface labelling of intact P. aeruginosa	+	+
Reactivity with P. putida and P. syringae protein F	–	+
Reactivity with a 31 kD trypsin or 29 kD papain proteolytic fragment of protein F	–	+
Reactivity with cyanogen bromide fragments of protein F	+ (23,28 kD)	–
Antigenic reactivity stable to 2-mercaptoethanol	+	–
Binding to oligomers of protein F on SDS polyacrylamide gels	+	–

Both classes of antibodies recognized a surface epitope as judged by indirect immunofluorescent labelling (Mutharia and Hancock, 1983) and colony immunoblotting (Mutharia and Hancock, submitted) of intact P. aeruginosa cells. The data favour the existence of two separate highly conserved surface epitopes on outer membrane protein F in P. aeruginosa. While these studies have not as yet allowed us to propose a realistic molecular model for protein F, it is anticipated that the isolation and characterization of a greater variety of monoclonal antibodies may permit structural predictions to be made.

USE OF MONOCLONAL ANTIBODIES IN PASSIVE IMMUNOTHERAPY

Monoclonal antibodies are a good candidate for passive vaccines due to their high specificity. We considered the possibility that one of our monoclonal antibodies against protein F would provide protection against a subsequent lethal challenge with P. aeruginosa. The results shown in part in Table 4 strongly suggest that this antibody, which recognizes a conserved surface epitope and can protect mice against P. aeruginosa of a serotype different to the strain used to originally isolate the monoclonal antibody, has good potential as an immunotherapeutic agent.

Table 4. Protective effect of protein F-specific monoclonal antibody MA4-4 against challenge of B6/D2 mice with P. aeruginosa strain M2

Group Protection	Survival	Significance of Difference (Fisher exact test)
Control	1/10	
MA4-4 injected	9/10	$p < 0.01$

Antibody MA4-4 (0.1 mg) of the IgG_{2a} class was injected into the tail vein of 25 Gram mice two hours prior to intraperitoneal challenge with 4×10^6 strain M2 organisms suspended in physiological saline. Survival was recorded after 3 days. No mice died after this time. Results are taken from two separate experiments. A control monoclonal antibody MA1-6 specific for a non surface located epitope of protein H2 did not afford protection.

CONCLUSIONS

This short review has attempted to highlight the potential uses of monoclonal antibodies directed against bacterial surface antigens. It is our feeling that monoclonal antibodies will have a major impact on serology, immunochemistry, taxonomy and immunotherapy. This is emphasized by numerous studies in addition to our own studies reported here. Their continued use should open up many areas of research by providing specific reagents for defining bacterial antigens.

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