Gonococcal outer-membrane protein PIB: comparative sequence analysis and localization of epitopes which are recognized by type-specific and cross-reacting monoclonal antibodies

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Comparison of the inferred amino acid sequence of outer-membrane protein PIB from gonococcal strain P9 with those from other serovars reveals that sequence variations occur in two discrete regions of the molecule centred on residues 196 (Var1) and 237 (Var2). A series of peptides spanning the amino acid sequence of the protein were synthesized on solid-phase supports and reacted with a panel of monoclonal antibodies (mAbs) which recognize either type-specific or conserved antigenic determinants on PIB. Four type-specific mAbs reacted with overlapping peptides in Var1 between residues 192–198. Analysis of the effect of amino acid substitutions revealed that the mAb specificity is generated by differences in the effect of single amino acid changes on mAb binding, so that antigenic differences between strains are revealed by different patterns of reactivity within a panel of antibodies. The variable epitopes in Var1 recognized by the type-specific mAbs lie in a hydrophilic region of the protein exposed on the gonococcal surface, and are accessible to complement-mediated bactericidal lysis. In contrast, the epitope recognized by mAb SM198 is highly conserved but is not exposed in the native protein and the antibody is non-bactericidal. However, the conserved epitope recognized by mAb SM24 is centred on residues 198–199, close to Var1, and is exposed for bactericidal killing.

Introduction

Protein I (PI) is the most abundant protein present on the surface of *Neisseria gonorrhoeae* and, unlike several other major surface antigens, does not undergo antigenic shift during infection (Zak *et al.*, 1984). PI appears to be essential for gonococcal viability, occurring in the outer membrane as a trimer which functions as an anion-selective porin and allows uptake of essential nutrients (Young *et al.*, 1983; Lynch *et al.*, 1983). In addition to its

porin activity, PI may play an important role in the pathogenesis of gonococcal infection, since *in vitro* studies have shown that, on contact, it may be transferred from the gonococcal outer membrane into the cell membrane of eukaryotic cells. Such a process may play a crucial role in gonococcal interaction with host cells *in vivo* (Blake, 1985). Antibodies directed against PI have been shown to be opsonic for phagocytosis by polymorphonuclear leucocytes, to promote complement-mediated bactericidal killing and to inhibit gonococcal damage to epithelial cells (Virji *et al.*, 1986, 1987). For these reasons PI is of considerable interest as a potential component of a gonococcal vaccine.

Although expression of PI is stable within a strain, differences occur between strains which are responsible for antigenic diversity. Thus, immunization with native PI results in the production of antibodies having limited reactivity with heterologous strains (Heckels *et al.*, 1989). Biochemical and immunological studies have revealed two major classes of PI (PIA and PIB); these can be further subdivided into a number of different serovars (Sandstrom *et al.*, 1982; Knapp *et al.*, 1984).

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Abbreviations: mAb, monoclonal antibody; PCR, polymerase chain reaction.

The complete nucleotide sequence of the *por* gene from strain P9 and the partial sequences of the other strains have been submitted to the GenBank/EMBL database and have been assigned the accession numbers X52823–X52827.

Antigenic analysis of PI has been aided by the use of monoclonal antibodies (mAbs). Differences in the pattern of reactivity of gonococcal strains with panels of mAbs have been utilized to define serovars of both PIA- and PIB-expressing strains (Knapp *et al.*, 1984; Sandstrom *et al.*, 1985). In our previous studies, a panel of mAbs was used to investigate the immunochemistry of PIB and the potential protective effect of antibodies directed against different PIB epitopes (Fletcher *et al.*, 1986; Virji *et al.*, 1986). Although the majority of mAbs obtained demonstrated restricted reactivity among the panel of strains tested, one antibody (SM24) was obtained which recognized 92% of PIB-expressing strains (Fletcher *et al.*, 1986). In *in vitro* studies mAb SM24 was opsonic, bactericidal and prevented cell damage, revealing the presence of a protective, but immunorecessive, conserved determinant on PIB. Such determinants represent attractive targets for vaccination.

Knowledge of the structure of the PIB molecule is therefore of considerable importance in attempting to identify both the conserved epitopes with vaccine potential and the variable epitopes responsible for serovar differences between strains. The nucleotide sequences of the genes (*por*) responsible for PIB expression in two strains have been determined (Gotschlich *et al.*, 1987; Carbonetti *et al.*, 1988). Despite antigenic differences between the strains R10 and MS11, the inferred amino acid sequences show extensive homology, suggesting that limited structural variations may be responsible for generating antigenic diversity. In this paper we report the inferred amino acid sequence of PIB from strain P9, the immunobiology of which has been extensively studied (Heckels, 1977; Lambden & Heckels, 1979; Virji *et al.*, 1986; Heckels *et al.*, 1989, 1990), and demonstrate that sequence variations occur between a number of other serovars in two discrete regions of the PIB molecule. In addition, we have used the sequence information to construct synthetic peptides and map the epitopes recognized by both the type-specific and cross-reacting, protective, anti-PIB mAbs.

Methods

Bacterial strains and growth conditions. Gonococci were grown on proteose peptone agar at 37 °C in 5% (v/v) CO₂. Strain P9-17 (Pil⁺ PII⁺) has been described previously (Lambden & Heckels, 1979); strains SU50, SU75, SU85 and SU97 were local isolates (Zak *et al.*, 1984). Strain R10 was obtained from Professor E. C. Gotschlich, Rockefeller University, New York, USA, and strain MS11 was from Professor F. Sparling, University of North Carolina, USA. Serovar determination was done by Dr C. Ison, St Mary's Hospital Medical School, UK.

mAbs. mAbs SM20, SM21, SM22 and SM24, which react with PIB from a number of gonococcal strains, were obtained following

sequential immunization of mice with outer membranes from a variety of strains as described in detail previously (Virji *et al.*, 1986; Fletcher *et al.*, 1986). mAbs SM198 and SM203 were obtained subsequently using similar protocols after immunization of mice with outer membranes of strain P9 which had first been extracted with sodium cholate (Heckels, 1977).

The reactivity of mAbs with gonococcal strains was determined by 'dot blotting' (Virji *et al.*, 1987); antibody binding was detected with ¹²⁵I-protein A or goat anti-mouse IgG-alkaline phosphatase conjugate (Zymed) using AP colour development reagents (Bio-Rad) as substrates.

Sequence analysis of PIB. The complete nucleotide sequence of the *por* gene encoding expression of PIB in strain P9 has been determined (Butt *et al.*, 1990). This information was used to obtain the sequences of variable regions from other strains of gonococci using material generated by the polymerase chain reaction (PCR). Crude chromosomal DNA preparations were amplified using two primers spanning the region of interest: primer 17, GAATTCAAGCTT-570AACTACCAAAACAGCGGCTTCTTCG⁵⁹⁴; and primer 18, GTCGACCTGCAG-832GCGGTAGCGGCAACTTCGGT⁸¹³ (underlined regions show additional bases carrying restriction sites used for subsequent cloning). Reactions were carried out in a Perkin-Elmer Cetus thermal cycler and subjected to 25 cycles of amplification. The cycle times were as follows: annealing, 2 min at 37 °C; primer extension, 3 min at 72 °C; denaturation, 1 min at 94 °C. On the last cycle the denaturation step was omitted and the extension step increased to 10 min. PCR-amplified material was digested with *Hind*III and *Pst*I and purified by agarose gel electrophoresis. The desired fragments were excised from the gel, purified by GeneClean (Bio 101) and cloned into M13mp18 or M13mp19. For each gonococcal variant templates were prepared from 10 recombinant phage, pooled and subjected to sequence analysis using dideoxy chain termination and deoxyadenosine 5'-α-[³⁵S]thiotriphosphate.

Computer analysis of the predicted protein secondary structure was done using the SERC, SEQNET node at Daresbury, UK, using the algorithms of Kyte & Doolittle (1982) and Hopp & Woods (1981). Multiple sequence alignment was done using the CLUSTAL algorithm (Higgins & Sharp, 1988).

Solid-phase peptide synthesis. This was done using a commercially available kit (Cambridge Research Biochemicals) in which peptides are synthesized onto polyethylene rods (Geysen *et al.*, 1987). Synthesis was done as described previously (Virji & Heckels, 1989) using pentafluorophenyl active esters of fluorenylmethoxycarbonyl-L-amino acids with t-butyl side-chain-protecting groups (Pfp-FMOC amino acids, Milligen), except in the case of methionine which had a trimethylsulphonyl side-chain-protecting group and serine and threonine in which the oxybenzotriazine active ester was used. After peptides of the desired length had been synthesized the terminal amino group was acetylated by reaction with acetic anhydride. Each synthesis was done in duplicate.

Detection of the immunological reactivities of the synthesized peptides on the rods was done by ELISA as described previously (Virji & Heckels, 1989). The rods were incubated with mAbs in phosphate-buffered saline (PBS; 0.15 M-sodium chloride, 0.15 M-sodium phosphate, pH 7.2) containing 1% BSA, 1% ovalbumin and 0.1% Tween 20. After washing and reaction with goat anti-mouse IgG conjugated to horseradish peroxidase (Zymed), colour was developed using 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) as substrate (Sigma). The solid-phase peptides were reused after bound antibody had been dissociated by sonication of the rods in 1% SDS, 0.1% 2-mercaptoethanol in 0.1 M-phosphate buffer, pH 7.2, at 60 °C for 30 min. Immunological reactivity was always observed in duplicate peptides and in assays repeated on at least two occasions.

Results

mAb reactivity. The mAbs were tested by dot blotting against a panel of 25 gonococcal strains expressing PIA and 25 strains expressing PIB (Table 1). Greatest reactivity was seen with mAb SM198, which reacted with all PIB-expressing strains tested, and SM24, which reacted with 92% of PIB strains. As expected from previous studies the remaining antibodies reacted, in differing patterns, with over 30% of PIB-expressing strains (Fletcher *et al.*, 1986). None of the antibodies reacted with PIA-expressing gonococci or with meningococci. Five PIB-expressing strains of different serotype and reactivity pattern were chosen for further study and included one of the only two PIB-expressing strains which failed to react with mAb SM24. The reactivity of these strains, together with strains R10 and MS11 for which the sequence of the PIB gene was available, is shown in Table 1.

Reactivity of cross-reacting mAbs with peptides from strain R10. The deduced amino acid sequence of PIB from strain R10 became available on the cloning of the PIB structural gene from that strain (Gotschlich *et al.*, 1987). To locate the epitopes recognized by the mAbs reacting with strain R10, a series of decapeptides spanning the entire molecule with adjacent peptides overlapping by five residues were synthesized and reacted with each of the mAbs. Antibody SM198 reacted with a single peptide corresponding to residues 106–115, whereas mAb SM24 reacted with two adjacent peptides corresponding to residues 191–200 and 196–205. No reaction was detected with the other mAbs used.

To define more precisely the epitope recognized by mAb SM198, a series of decapeptides corresponding to the region of interest, between residues 101 and 120, were synthesized with adjacent peptides differing by a single amino acid residue. Antibody SM198 reacted with six peptides all containing the common sequence ¹⁰⁷WESGK¹¹¹ (Fig. 1a), defining this as the minimum epitope recognized by the antibody.

To identify precisely the epitope recognized by mAb SM24, a series of hexapeptides were synthesized between residues 193 and 204, together with a series of smaller peptides centred on residue 198. Antibody SM24 reacted with five hexapeptides, all of which contained the sequence SI. Reactivity was approximately equal with the decapeptide ¹⁹⁶TYSIPSLFVE²⁰⁵, the hexapeptide ¹⁹⁶TYSIPS²⁰¹ and the pentapeptide ¹⁹⁶TYSIP²⁰⁰ defining this as the optimal minimum epitope (Fig. 1b). Nevertheless, significant reactivity was seen with all peptides containing SI, including the dipeptide itself (Fig. 1b). The specificity of the reaction was confirmed by synthesizing the inverted peptide 200–196, which showed no reactivity with mAb SM24.

Reaction of type-specific mAbs with peptides from strain P9. To determine the epitopes recognized by the type-specific mAbs which did not react with peptides corresponding to strain R10, the complete amino acid sequence of strain P9 was required. The nucleotide sequence of the PIB gene from strain P9 was determined (Butt *et al.*, 1990) and a comparison of the predicted amino acid sequences of PIB from strains P9, R10 and MS11 is shown in Fig. 2. The three sequences show considerable homology, with significant variations being

Table 1. *Properties and reactivity of anti-PIB mAbs*

mAb reactivity was determined after dot blotting of whole cell suspensions on to nitrocellulose sheets.

Characteristic			mAb					
			SM20	SM21	SM22	SM24	SM198	SM203
Isotype			γ2a	γ2a	γ3	γ2a	γ1	γ2a
Bactericidal effect			+	+	+	+	—	+
Reaction with:	{	PIA-expressing gonococci	0%	0%	0%	0%	0%	0%
		PIB-expressing gonococci	40%	35%	35%	92%	100%	56%
		meningococci	0%	0%	0%	0%	0%	0%
Reaction with individual gonococcal strains expressing PIB*	{	Strain Serovar						
		SU97 IB-21	—	+	—	+	+ ^a	+
		SU50 IB-2	—	—	—	+	+ ^a	±
		SU75 IB-8	—	—	—	—	+ ^a	—
		SU85 IB-3	+	—	—	+	+ ^a	±
		P9 IB-26	+	+	+	+	+ ^a	+
		MS11 IB-9	+	+	+	+	+ ^a	+
		R10 IB-3	+	—	—	+	+ ^a	—

* +, Strong reaction; ±, weak reaction; —, no reaction; +^a, weak reaction seen against whole cells of SM198 which increased significantly when cell suspensions were treated with 1% SDS before dot blotting.

Fig. 1. Localization of epitopes recognized by cross-reacting mAbs SM24 and SM198. (a) mAb SM198. A series of decapeptides spanning the region between residues 101 and 120 of strain R10, with adjacent peptides differing by a single amino acid residue, were reacted in ELISA with mAb 198. (b) mAb SM24. A series of hexapeptides spanning the region between residues 193 and 204 of strain R10, together with a series of smaller peptides centred on residue 198 were reacted in ELISA with mAb SM24. The optimal decapeptide was included to permit quantitative comparison of reactivity of the smaller peptides.

largely confined to two regions centred on residues 196 and 237. A series of solid-phase decapeptides were synthesized corresponding to the P9 sequence in the variable regions between amino acids 182–208 and 227–243. Each of the antibodies tested reacted with one or more peptides in the region 182–208; none of the antibodies reacted with peptides corresponding to sequences from the region 227–243. Although the degree of reactivity varied considerably between the antibodies, considerable overlap was seen in the individual peptides recognized (Fig. 3*a–d*). Thus, on the basis of the hexapeptides recognized, the minimum epitope recognized by both mAbs SM21 and SM203 was the hexapeptide ¹⁹²YEHQVY¹⁹⁷, while that recognized by SM22 was the heptapeptide ¹⁹⁰IEYEHQVY¹⁹⁷. The reactivity seen with mAb SM20 was too weak to allow confident prediction of the epitope recognized, but results implicated the heptapeptide ¹⁹²YEHQVYS¹⁹⁸.

Differences in amino acid sequences between strains and reaction of mAbs with variable peptides. The similarity in the epitopes recognized by the type-specific mAbs was somewhat surprising given their spectrum of reactivity with different strains. To investigate the effect of differences in amino acid sequences between strains on mAb binding, the sequence of the PIB gene between the positions coding for residues 166–250 was determined for each of the strains shown in Table 1. Two oligonucleotide primers were synthesized, one corresponding to the coding sequence for amino acids 166–173 and the other complementary to the coding sequence for amino acids 247–253. These were used as primers in the PCR, to amplify the chromosomal DNA between these positions. The amplified DNA was cloned into M13 phage and sequenced. The comparison of the deduced amino acid

Fig. 2. Deduced amino acid sequence of PIB from strain P9 (upper sequence) aligned with PIB sequences from strains R10 (centre; Gotschlich *et al.*, 1987) and MS11 (bottom; Carbonetti *et al.*, 1988). Dots denote identity with the sequence of PIB from strain P9.

Fig. 3. Localization of epitopes recognized by type-specific antibodies. A series of solid-phase decapeptides was synthesized corresponding to the P9 sequence in the variable regions Var1 and Var2 between amino acids 182–208 and 227–243. Adjacent peptides differed by a single amino acid residue. Peptides were reacted with the type specific antibodies SM20 (a), SM21 (b), SM22 (c) and SM203 (d).

	180	190	200	210	220	230	240				
P9 (PIB)	QYAGLFQRYGEGTKKIEYEHQVYSIPSLFVEKLQVHRLVGGYDNNALYVSVA	QQQDAKLYGAR	--RANSHNSQ								
SU97 (PIB)		F	Y	A		A	V	QNQLV	D		
SU50 (PIB)			DS	Y		A				TW--	
SU75 (PIB)			M	--GYT	N		A			QNQIV	D
SU85 (PIB)				DD						M--	SG
R10 (PIB)				DD	T					M--	SG
MS11										QNQLV	D

Fig. 4. Comparison of the deduced amino acid sequence between residues 166–252 of PIB from gonococcal strains with differing mAb reactivity. Dots denote identity with the sequence of PIB from strain P9.

sequences determined in this way is shown in Fig. 4. Significant differences between strains were found in the two regions previously noted.

Sequence comparisons directly revealed the strain specificity of two mAbs. The putative epitope for mAb SM22 (IEYEHQVY) was present only in strains P9 and MS11 and only these strains reacted with the antibody. In addition, all the strains recognized by the broadly cross-reacting SM24 with the putative epitope TYSIPS contained at least the sequence YSIPS, while in the only two non-reacting strains tested this was replaced by the sequence YNIPS, reinforcing the necessary presence of the dipeptide SI. In contrast, the putative epitope (YEHQVY) recognized by mAbs SM21 and SM203

could not be confirmed by sequence comparison since it was not present in strain SU97 which reacted strongly with both antibodies.

To examine the effect of any amino acid change on the reactivity of each mAb a series of peptides based on the decapeptide ¹⁹¹EYEHQVYSIP²⁰⁰ and the hexapeptide ¹⁹⁶VYSIPS²⁰¹ found in strain P9 were synthesized, in which individual amino acid residues were substituted, either singly or in combination, by each of the different residues found in the other strains. Reaction of these peptides with mAbs SM21, SM24 and SM203 revealed that some amino acid substitutions had little effect on mAb binding while others completely abolished reactivity (Fig. 5). Substitution of Y for ¹⁹⁴H or A for ¹⁹⁶V in

Fig. 5. Effect of amino acid replacements in the epitopes recognized by mAbs. A series of peptides based on the decapeptide $^{193}\text{EYEHQVYSIP}^{200}$ and the hexapeptide $^{196}\text{VYSIPS}^{201}$ found in strain P9 was synthesized, in which individual amino acid residues were substituted, either singly or in combination, by each of the different residues found in the other strains. Peptides were reacted with mAbs SM21 (a), SM203 (b), SM24 (c).

peptide $^{191}\text{EYEHQVYSIP}^{200}$ had no effect on the binding of mAbs SM21 or SM203, consistent with their reactivity with the sequence found in strain SU97. The two antibodies did show differences in reactivity when other substitutions were made. Antibody SM21 also reacted with peptides containing the substitutions ^{194}S , ^{196}Y or ^{196}T but not ^{194}D , while SM203 reacted with all substitutions at either position. The reactivity of SM21 was completely abolished by the substitution of D for ^{193}E , consistent with its failure to react with all three strains containing this substitution. Reaction of SM203 was also abolished by the ^{193}D substitution although it did show weak reactivity with one of these strains.

Antibody SM24 reacted equally well with all of the decapeptides, in accord with its reactivity with all six strains represented by the replacement peptides. SM24

also reacted with the hexapeptide $^{196}\text{VYSIPS}^{201}$ and with the replacement peptide containing the substitution T for ^{196}V . Reactivity of SM24 was however completely abolished by the substitution N for ^{198}S , again consistent with its failure to react with the two strains containing this substitution.

Discussion

Comparison of the deduced primary amino acid sequence of PIB from strain P9 with those previously reported for strains R10 and MS11 confirms the considerable homology between the antigenically distinct proteins, with less than 5% sequence difference between the strains. Such homology suggests a conserved three-dimensional structure of the proteins in accord with a highly conserved functional role as a porin (Young *et al.*, 1983; Lynch *et al.*, 1983) and also perhaps in interactions with host cells (Blake, 1985). The similarity between the deduced amino acid sequences suggests that antigenic differences between strains must largely result from sequence variations in localized regions. The sequence alignment of the three proteins indicates two such regions centred on residues 196 (Var1) and 237 (Var2), and the variability of these regions is confirmed by the partial sequences of a further five strains.

The localization of linear epitopes recognized by mAbs has been greatly facilitated by the development of techniques for the concurrent synthesis of large numbers of peptides for testing by ELISA (Geysen *et al.*, 1987; Virji & Heckels, 1989). Using this technique it was revealed that five of the six mAbs tested reacted with peptides corresponding to the residues between positions 190 and 201 with the four type-specific mAbs reacting with overlapping peptides in the region 192–198 and the broadly cross-reacting mAb SM24 recognizing the sequence $^{196}\text{TYSIP}^{200}$.

The close similarity and indeed apparent identity of the epitopes recognized by mAbs on strain P9 (Table 2) was initially surprising given the differing patterns of strain reactivity seen with the antibodies. However, analysis of the effect of amino acid substitutions reveals that additional mAb specificity is generated by differences in the influence of single amino acid changes on mAb binding. Indeed it seems likely that given the limited variation between the proteins, any scheme for serological differentiation of PI must rely on similar influences on mAb binding, so that antigenic differences between strains are recognized not simply by their reactivity with individual mAbs but rather by different patterns of reactivity with a panel of antibodies. This is precisely the situation with two schemes for the definition of serovars of PIB (Knapp *et al.*, 1984;

Table 2. Epitopes recognized by mAbs on PIB

X denotes position within epitope where all substitutions tested reacted with antibody.

	110	190	200
mAb	...NAWESGKFTG.....	TKKIEYEHQVYSIPSLFVE.....	
SM22		...IEYEHQVY.....	
SM21		H V ...YE/Q/Y..... Y A	
SM203		...YEXQX.....	
SM24		SIPS.....	
SM198	WESGK.....		

Type-specific

Cross-reacting

Sandstrom *et al.*, 1985). Although in the current study each of the type-specific antibodies reacted with sequences in Var1, it would appear likely that sequence differences in Var2 could also generate epitopes with potential for serological differentiation. Indeed, this appears to be the case, since strains P9 and MS11, which belong to different serovars, have identical sequences in Var1 but differ significantly in Var2. It therefore appears likely that the antigenic diversity of PIB is largely generated by sequence variation in only two discrete regions of the molecule. Despite antigenic differences between the strains, comparison of the sequences shows significant conservation of sequences in the variable regions of strains from different geographical locations. Thus strain MS11, isolated in the USA, shows homology with the British isolates strain P9 in Var1 but not in Var2 and, in contrast, with strain SU97 in Var2 but not Var1. Such mixing and matching between the two variable regions suggests that one mechanism by which genetic diversity may have arisen involved recombination events between the *por* genes of different strains.

In this context it is interesting to compare this situation with that of the closely related meningococcus. Meningococci express two rather than one porin proteins (Barlow *et al.*, 1989; West & Clarke, 1989), the class 1 and class 2/3 proteins, which are responsible for serotype and subtype specificity, respectively (Frasch *et al.*, 1985). PIB shows significant sequence homology with both proteins (Barlow *et al.*, 1989; Murakami *et al.*, 1989) suggesting similar organization within the outer membrane. Comparative sequence analysis and epitope mapping on the class 1 protein have also revealed two discrete variable regions, and that mixing and matching of these sequences in these regions generates two independent sero-subtype specificities in each strain

(McGuinness *et al.*, 1990). Interestingly these meningococcal variable domains occur at quite different regions of the molecule to the variable regions on PIB.

Immunization with outer membranes (Heckels *et al.*, 1989) or detergent micelles containing PI in its native conformation (Kersten *et al.*, 1988; Wetzler *et al.*, 1988) produces antibodies which are type-specific, demonstrating that the variable determinants are immunodominant. Secondary structural analysis of PIB from strain P9 shows alternating domains of hydrophilic and hydrophobic residues similar to those reported for PIA (Carbonetti & Sparling, 1987) and typical of other outer transmembrane proteins (Vogel & Jagnig, 1986). Thus, although several hydrophilic domains of the protein may be exposed on the bacterial surface it appears that those containing variable determinants are particularly accessible to the immune system and hence are particularly immunogenic. The surface exposure of Var1 is also consistent with the ability of all the antibodies recognizing sequences within it to react with the native protein and, in addition, to cause complement-mediated bactericidal killing. In contrast, the epitope recognized by mAb SM198 lies in a region of low hydrophilicity, the antibody reacts only weakly with intact cells and is not bactericidal, suggesting that it is not well exposed on the surface of the gonococcus.

Antibodies to PI may be particularly important in preventing the complications associated with gonococcal infection. Bactericidal antibodies directed against PI produced in response to gonococcal salpingitis appear to protect against reinfection by strains of the same serovar (Buchanan *et al.*, 1980). Clearly any potential vaccine should produce immunity to the widest possible range of strains. The location of the epitope, ¹⁹⁶TYSIP²⁰⁰ recognized by mAb SM24 is therefore of particular interest since this antibody reacts with, and is bactericidal for, 92% of PIB-expressing strains tested (Fletcher *et al.*, 1986). Comparison of the sequences reveals the reason for the failure of this broadly cross-reacting antibody to react with two of the panel of strains, since these both contain in Var1 the sequence YNIPS, which lacks the essential dipeptide at the centre of the epitope. In a previous study the SM24 epitope was shown to lie between residues 193–204 and synthetic peptide corresponding to this region 193–204 from strain R10 was used to immunize rabbits (Heckels *et al.*, 1990). The sera obtained reacted with native PI from the homologous strains and were bactericidal for it, but showed only limited reactivity with strain P9. The reason for the limited cross-reactivity of the antisera raised against the peptide containing the conserved SM24 epitope is now revealed since the sera obtained showed specificity for the N terminus of the peptide, containing the sequence differences found in Var1, rather than the C terminus

which contains the conserved epitope. Clearly, future studies with synthetic peptides will greatly benefit from the insight into the structure and immunochemistry of PIB provided by the current studies.

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