THE SIXTH C. L. OAKLEY LECTURE*

MOLECULAR STUDIES ON THE PATHOGENESIS OF GONORRHOEA

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In this review I shall describe work carried out, in collaboration with my colleagues in Southampton, correlating knowledge of gonococcal structure with an understanding of the molecular basis of the pathogenesis of gonorrhoea. We have concentrated our studies on the major gonococcal surface antigens, because they are involved in host-bacterial interactions such as colonisation of mucosal surfaces of the genital tract, invasion of epithelial cells and resistance to host defences, any of which may determine the course of infection.

I shall first outline the stages in the pathogenesis of gonococcal infection which we should like to understand in molecular terms. Symptomatic gonorrhoea is characterised by destruction of the urethral mucosa with accumulation of gonococci and polymorphonuclear leukocytes in the sub-epithelial connective tissue (Harkness, 1948). Electronmicroscopy of human fallopian tubes challenged with gonococci *in vitro* has suggested the sequence of events leading to infection (Ward, Watt and Robertson, 1974). Gonococci first adhere to non-ciliated columnar epithelial cells of the genital tract, allowing the bacteria to become established despite the flow of mucus and other secretions. Microvillous projections of the host cell make initial contact with the gonococci which then become engulfed by the cell membrane and lie within a phagocytic vacuole inside the cell. Intracellular multiplication then occurs and bacteria may subsequently penetrate the subepithelial connective tissue, giving rise to the marked local inflammatory response that is responsible for the typical symptoms of gonorrhoea, or may avoid normal host defences leading to the complications of disseminated infection. The ability to attach to and, subsequently, invade the mucosal surface of the genital tract distinguishes gonococci from non-invasive commensal neisseriae. The fallopian-tube model also reveals that gonococci attach to cells of the human genital tract but not to those from other animals (Johnson, Taylor-Robinson and McGee, 1977). This suggests that the virulence properties of gonococci may be a consequence of specific interaction between epithelial cell receptors and virulence determinants on the bacterial surface.

The first surface antigens to be identified as important virulence factors in gonococci were pili. These are filamentous protein appendages extending several micrometres from the surface of gonococci; they can be detected in cultures of fresh clinical isolates. The importance of pili was indicated by the observations that loss of

Received 19 Apr. 1984; accepted 11 Jun. 1984.

* Given at the 148th meeting of the Pathological Society of Great Britain and Ireland, January 1984.

virulence on laboratory subculture (Kellogg *et al.*, 1963) was accompanied by a concomitant loss of pilus expression (P) (Swanson, Kraus and Gotschlich, 1971). Subsequent studies in several laboratories have shown that pili facilitate the adhesion of gonococci to a range of human cells including erythrocytes, vaginal epithelial cells, fallopian-tube epithelium and buccal epithelial cells (reviewed by Buchanan, 1977). We investigated the possible role of pili in attachment by experiments in which gonococci were chemically modified to alter their surface charge (Heckels *et al.*, 1976). Like other bacteria and human cells, gonococci possess an overall net negative charge, and blocking of free amino and carboxyl groups abolished this. The modified bacteria showed increased attachment to human cells growing in tissue culture but P⁺ organisms no longer had any advantage in attachment. The implication of these experiments is that the role of pili in adhesion is to overcome the repulsive electrostatic barrier to attachment which exists between two negatively charged surfaces. Thus attachment can be envisaged as a two-stage process. Initial contact made by pili would increase the probability of a closer approach, favouring adhesion between the bacterium and host cell, because attractive forces act over a much shorter distance than the electrostatic repulsive force. Electronmicrographs show that this stable adhesion involves direct interaction between the host cell membrane and the gonococcal outer membrane and leads to penetration of the epithelial cell (Watt *et al.*, 1978).

Components present in the outer membrane must also play an important role in gonococcal virulence; therefore we have studied their structure and immunochemistry in some detail.

Outer-membrane proteins (OMP)

Surface labelling of gonococci with ¹²⁵I and lactoperoxidase revealed that two major classes of OMP (proteins I and II) are exposed on the gonococcal surface (Heckels, 1978). Following initial observations by Swanson, Sparling and their co-workers it became apparent that the OMP profiles were subject to considerable variations within individual strains, and were associated with changes in colonial morphology (Walstad, Guymon and Sparling, 1977; Swanson 1978). Using one strain P9, we were able to select a range of colonial variants that differed in their OMP content (Lambden and Heckels, 1979). A variant with transparent colonial phenotype (O⁻) contained a protein I of mol. wt 36×10^3 as the major surface protein. A series of colonial variants with opaque colonial phenotypes (O⁺) was also isolated which possessed one or two additional proteins of mol. wt $(27-29) \times 10^3$ (protein II) (fig. 1). Protein-II species were purified from outer membranes by detergent extraction followed by chromatography on Sephadex gels (James and Heckels, 1981) and subjected to further analysis. Tryptic peptide maps of proteins which had been extensively labelled with ¹²⁵I and chloramine-T, in the presence of sodium dodecyl sulphate (SDS) to unfold the molecule, showed considerable similarity with only a few peptides unique to any one protein (Heckels, 1981). In contrast, autoradiographs of peptide maps of proteins II from cells which had been specifically surface labelled with ¹²⁵I and lactoperoxidase showed fewer spots and considerable differences, with the major labelled peptides being those unique to that particular protein-II type. Thus the different proteins II produced by a single strain apparently form a family of

FIG. 1.—Variation in OMP II in colonial opacity variants of *N. gonorrhoeae* strain P9. Outer membranes were prepared from a series of different colonial types and subjected to SDS-PAGE. (1) Transparent (O^-) colonial variant lacking protein II; (2)–(7) opaque (O^+) variants with either one or two different molecular species of protein II. (Lambden *et al.*, 1979).

structurally related proteins with a common region embedded in the membrane and a surface-exposed region capable of considerable variations.

The alterations in protein II structure can be correlated with important differences in surface properties of the variants. Antiserum specific for a particular protein II species was produced by immunisation of rabbits with outer membranes from O^+ variants and absorption of the resulting sera with outer membrane from an O^- variant that lacked protein II. The serum was highly specific for the homologous protein II and lacked reactivity with the other protein-II species produced by the same strain, confirming that variation occurs in that part of the antigen which is exposed on the surface (Diaz and Heckels, 1982). These antigenic variations are also associated with changes in virulence properties of the organisms. Variants with protein II showed reduced sensitivity to the bactericidal effect of normal human serum and increased association with polymorphonuclear leukocytes (Lambden *et al.*, 1979). When a series of variants of strain P9 was examined for their ability to adhere to human buccal epithelial cells, those with protein II showed greater attachment than the variant that lacked protein II (Lambden *et al.*, 1979). The protein-II profile also alters the specificity of adhesion. Comparison of two variants of strain P9 with proteins II of mol. wt 28.5×10^3 (IIa) or 28.0×10^3 (IIb) revealed that although both variants attach

Fig. 2.—Effect of OMP II on adhesion of outer membranes to buccal epithelial cells. (a) Variation in adhesion of ^{125}I -labelled outer membranes with pH showing the advantage conferred at neutral pH values by possession of protein II; ●—● membranes from O⁻ variant P9-1 lacking protein II; ■—■ membranes from O⁺ variant P9-13 with 28.5×10^3 mol.wt protein IIa. (b) Langmuir isotherm analysis of adhesion at pH 7.5 showing, from the gradients, equivalent binding sites per cell for each variant, but from the intercepts, that possession of protein IIa increases the avidity of attachment five-fold.

more avidly to epithelial cells than does the transparent variant, the variant with protein IIa is more effective in attachment to buccal cells and that with protein IIb attaches more effectively to the Chang conjunctival cell line (Heckels, 1982); this suggests that structural variation of protein II may influence the ability of gonococci to colonise different anatomic sites.

The contribution of protein II to adhesion has been investigated further by studying the attachment of ^{125}I labelled outer membranes to buccal epithelial cells as a model system (Heckels and James, 1980). At neutral pH values, membranes containing protein IIa showed greater attachment than those containing only protein I although at lower pH values this advantage was lost (fig. 2a). The data were subjected to Langmuir isotherm analysis which showed that, although there were approximately equivalent numbers of binding sites for each membrane type, those membranes that contained protein IIa had five-fold greater avidity of attachment (fig. 2b). Various sugars were tested in the system and did not inhibit attachment, but modification of the host cell surface by glycosidases reduced the attachment of the membranes containing protein IIa to that of membranes lacking protein II. Furthermore, when membranes were treated with trypsin the attachment of those that contained protein IIa was reduced to the level of that from the O⁻ variant. Because trypsin treatment degrades protein II with concomitant cleavage of the surface-exposed portion of protein I, these observations confirm the contribution of protein II in adhesion to epithelial cells and suggest that protein I does not play a significant role. The evidence also suggests that host cell surface carbohydrates are involved in the adhesive process.

Pili

When two P⁺ variants of strain P9 were examined by electronmicroscopy differences in pilus morphology were seen which suggested differences in pilus structure. Pili were purified from the two variants and those from the O⁻ variant (α pili)

were found to have an apparent subunit mol. wt of 19.5×10^3 on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) whereas those from the O^+ variant had pili with subunits of mol. wt 20.5×10^3 (β pili). Experiments with purified pili showed that α pili attached well to buccal epithelial cells whereas β pili did not, which suggests that this pilus variant lacks the receptor involved in adhesion to that cell type (Lambden, Robertson and Watt, 1981*b*). The nature of the receptor was further investigated by comparing the adhesive properties of the α -pilate variant with those of the P^- type (Trust, Lambden and Watt, 1980). Although none of the sugars tested in the system inhibited adhesion, treatment of the buccal cells with a mixture of glycosidases reduced the binding of the α -pilate variant to that of the P^- variant. Thus, adhesion of α pili to epithelial cells appears to involve the presence of a carbohydrate group on the host-cell surface but β pili lack the receptor and so fail to adhere.

Antigenic variation in vivo

As described above, we have been able to select, in laboratory conditions, a series of variants of a single strain of gonococcus with alterations in protein II and pili which are responsible for considerable differences in their antigenic and virulence properties. The potential for variation in surface antigens during growth *in vitro* suggests that similar variations are likely to occur *in vivo* and might endow particular antigenic types with a selective advantage. We have, therefore, used the guinea pig subcutaneous chamber model of gonococcal infection (Veale *et al.*, 1975) to investigate the occurrence of antigenic variation *in vivo*. Each of the chambers was infected with a mixture of variants of strain P9 with different protein-II and pilus composition (McBride *et al.*, 1981). In each case P^- and $O^+ P^+$ organisms were rapidly eliminated from the chambers but $O^- P^-$ organisms survived relatively well. P^- organisms disappeared permanently and were totally replaced by P^+ variants. Subsequently, $O^- P^+$ variants began to be eliminated and were replaced by $O^+ P^+$ variants. The final isolate from each chamber was analysed for protein-II and pilus composition. In the majority of cases the survivors expressed new protein-II types that were not present in the original inoculum (table I). Furthermore, the survivors expressed pili with subunits of mol. wt 21×10^3 and 18.5×10^3 (γ and δ pili respectively) that were distinct from the original α and β pili (fig. 3) (Lambden *et al.*, 1981*a*). Thus in each case, antigenic shift occurred during the course of the chamber infection and variant gonococci were selected with proteins that were not present initially; this suggested that similar variations were likely to occur during the course of natural infection. The lack of correlation between pilus and protein-II type also suggested that antigenic shift in the two proteins occurred independently and that the two were not genetically linked. This was confirmed by selection of a series of O^- variants lacking protein II but each expressing one of the pilus types (Lambden, 1982).

The variant pili obtained have been purified and subjected to further analysis (Lambden, 1982). The pili differed in subunit mol. wt on SDS-PAGE and in subunit isoelectric point on agarose gels. Amino acid analysis showed close homology between the different types but a reduction in the content of aspartate and serine was notable in the low-mol. wt δ pili, whereas the β and γ pili contained more alanine residues. Structural homology was also demonstrated in peptide maps of tryptic plus chymotryptic digests of pili labelled with ^{125}I -iodohydroxybenzimidate. Several major

TABLE I

Effect of guinea pig in-vivo selection pressure on expression of pili and protein II

Antigens		Antigens expressed in										
		initial challenge	survivors in chambers									
			1	2	3	4	5	6	7	8	9	10
Protein II	a	+	—	—	—	—	+	+	—	—	—	—
	b	+	—	+	—	—	—	—	—	—	—	—
	c	+	—	—	—	—	—	—	—	—	—	—
	d	—	—	+	—	—	+	—	—	—	—	—
	e	—	+	—	+	—	+	+	—	—	—	—
	f	—	—	—	—	—	—	—	—	—	—	+
Pili	α	+	+	—	—	—	—	—	—	—	—	—
	β	+	—	—	—	—	—	—	—	—	—	—
	γ	—	—	+	—	—	+	+	—	—	+	—
	δ	—	—	+	+	+	—	—	+	+	—	+

Plastic chambers subcutaneously implanted into guinea pigs were infected with a mixture of variants of strain P9 expressing the antigens shown in the first column. Survivors were cultured from each chamber and the proteins expressed were identified by SDS-PAGE. In most cases the antigens originally present were eliminated and replaced by variant proteins not present initially (Lambden *et al.*, 1981a; McBride *et al.*, 1981).

peptides were detected which were common to all four pilus proteins, some were shared between δ and α pili or between β and γ pili and a few were unique to a particular pilus type. Antisera to each type of purified pili were raised in rabbits and showed only about 10% cross-reactivity with the heterologous pilus types (Virji, Everson and Lambden, 1982). Thus, like protein II, the variant pili produced by a single strain of gonococcus are closely related in structure but differ significantly in immunodominant domains

FIG. 3.—Variation in pilus subunits from colonial variants of *N. gonorrhoeae* strain P9. Pili were purified from four P^+O^- colonial variants and subjected to SDS-PAGE; (1) γ pili mol. wt 21×10^3 ; (2) β pili mol. wt 20.5×10^3 ; (3) α pili mol. wt 19.5×10^3 ; (4) δ pili mol. wt 18.5×10^3 .

and are, thus, antigenically quite distinct. Furthermore, antigenic shift in pilus production is associated with alterations in adhesive properties. Variants of strain P9, differing only in the pilus type expressed, show differences in attachment to epithelial cells. The variant with α pili showed greater adhesion to buccal epithelial cells than that with β pili, whereas in tests with Chang conjunctival cells grown in tissue culture, the position was reversed (Heckels, 1982). These differences in adhesion can be correlated with differences in the virulence of the variants for the Chang cell line (Virji and Everson, 1981).

Antigenic variation during natural infection

The antigenic shifts in pili and protein II described above, which can be seen in laboratory cultures, readily occur during gonococcal growth in laboratory animals and are associated with alterations in antigenic and virulence properties. These associations suggest that similar variations occurring during the course of the natural infection could explain the ability of gonococci to colonise anatomically distinct sites such as the urethra, cervix, pharynx and rectum and to persist at these sites despite the host immune response. However, little information has been available on the occurrence of antigenic variation during gonococcal infection. Because it is impossible, for ethical reasons, to follow variation in antigen expression during the course of infection in a single patient, we have investigated antigenic variation by examining isolates from different sites in groups of sexual partners. Gonococci were isolated from the urethra of male patients and from the cervix and urethra of females (Zak *et al.*, 1984). To rule out the possibility that variation between isolates from different sites was due to concurrent infection with more than one strain, we compared isolates from each group of patients for auxotype and for mol. wt and serotype of OMP I. All isolates from a given group of patients were confirmed as belonging to the same strain by these criteria. However, SDS-PAGE of surface antigen preparations showed considerable differences in two regions of the gel, one corresponding to protein II and the other to pili. Protein-II species could be identified by their characteristic change in mobility when treated with SDS at different temperatures and pili were identified following our standard purification procedure. Thus, for example, whereas all three isolates from one group expressed a protein II of mol. wt 28×10^3 , the isolate from the male urethra had an additional protein II of mol. wt 32×10^3 and that from the female cervix one of mol. wt 30×10^3 (fig. 4). Moreover, each variant produced pili of distinct subunit mol. wt. Similar variations in pili and protein II were seen in each of the groups so that in all cases the pili differed between isolates from the cervix and urethra of female patients (Duckworth *et al.*, 1983). Thus antigenic variation of pili and protein II occurs commonly during natural infection and indeed is probably normal.

The precise nature of the selective pressures that operate on gonococci during natural disease, and produce such a diversity of antigens, is unknown. No correlation could be made between the expression of proteins of particular mol. wt and their site of isolation. Nevertheless, by analogy with in-vitro studies, one possibility is that variant proteins confer particular tissue tropisms, allowing colonisation of the different mucosal surfaces encountered. Another potential influence is the host immune response. Therefore, we measured the antibody levels to each of the proteins present in a group of isolates by radioimmune precipitation assay. The isolates from within a

FIG. 4.—Variation in surface proteins between gonococci isolated from the urethra (U) of one male (M) and from the urethra and cervix (C) of two female (F) consorts; surface-antigen preparations containing outer membrane and pili were subjected to SDS-PAGE. Protein-II species (arrowed) were identified by changes in mobility from a gel run with samples dissociated at 37°C (Zak *et al.*, 1984).

given patient group were surface labelled with ^{125}I then dissolved in detergent. The detergent extracts were mixed with serum from the patients to allow antibody-antigen interaction to occur and immune complexes were removed with solid phase protein A. After dissociation, the precipitated material was subjected to SDS-PAGE and the antigens reacting with antibodies present in the serum were identified by autoradiography. Antibodies to pili, when present, gave cross-reactions among isolates from a given group of patients, although to a variable extent. Antibodies to protein II, however, showed much greater specificity as can be seen from the results with one group consisting of one male and two female contacts (fig. 5). Serum from one of the women reacted with a 31×10^3 -mol. wt protein but not a 29.5×10^3 -mol. wt protein present in her urethral isolate. No activity was detected in this woman's serum against the 29.5×10^3 -mol. wt protein in her cervical isolate or against a 32×10^3 -mol. wt protein in the isolate from her male partner. The isolate from the cervix of the other female contact contained the 31×10^3 -mol. wt protein II in addition to a 29×10^3 -mol. wt protein. The serum of the first woman reacted with both these proteins despite the absence of the 29×10^3 -mol. wt protein in either isolate of this patient. Thus, when present in a patient's serum, antibodies directed against one molecular species of

FIG. 5.—Radioimmune precipitation assay showing specificity of immune response to protein II. Reactions are shown between serum from a female patient (F1) and extracts of isolates from her own cervix (C) and urethra (U), from her male partner (M) and from a second female contact (F2). (a) Diagrammatic representation of protein-II species present in each isolate; (b) autoradiograph showing proteins which react with antibodies present in the serum.

protein II show little cross-reactivity with other protein species of the same strain. Indeed the occurrence of antibodies that react with protein II in isolates from other individuals within a group of contacts but not with the patient's own isolates is of particular interest.

In the light of the above results our model of antigenic shift is that an invading gonococcus originally produces one of several possible protein-II variants inducing a specific host immune response which results in the elimination of variants bearing that protein. However, the high rate of transition associated with protein-II production would ensure the presence of a significant minority population, with an antigenically distinct protein-II profile, which would then overgrow the original variant to establish a new majority population. Similar selection pressures presumably operate on pilus expression.

The use of monoclonal antibodies to study antigenic shift

The widespread occurrence of variants in clinical cultures shows that antigenic shift

TABLE II

Cross-reactivities of monoclonal antibodies directed against gonococcal pili in tests with strains showing inter- and intra-strain antigenic variation

Antibody	Reaction of given antibody with pili from								
	Strain P9				Group 1			Group 2	
					M	F		M	F
						SU51U*	SU51C*		
	α	β	γ	δ	SU50	SU51U*	SU51C*	SU72	SU73U* SU73C*
SM1	+	+	+	+	—	—	—	—	—
SM2	+	+	+	+	—	—	—	—	—
SM3	+	—	—	—	—	—	—	—	—
SM4	+	—	—	—	—	—	—	—	—
SM5	+	—	—	—	—	—	—	—	—
SM6	—	—	+	+	—	—	—	—	—
SM7	—	—	+	+	—	—	—	—	—
SM8	—	—	+	—	—	—	—	—	—
SM10	+	+	±	+	+	—	—	+	—
SM11	+	—	—	—	—	—	—	—	—
SM13	+	—	+	—	...	...	...	...	...

Pili purified from colonial variants of strain P9 and from gonococci isolated from two groups of consorts were used in ELISA to determine the extent of cross-reactivity of a panel of monoclonal antibodies. M=fresh clinical isolates were from the urethra of male patients; F=isolates from their female partners. (Virji and Heckels, 1983).

* U=urethra; C=cervix.

plays an important role in the pathogenesis of gonorrhoea providing a powerful mechanism which allows the gonococcus to adapt to the changing environment during the course of an infection. The genetic mechanisms responsible for control of antigen production are not clear although the switches from P⁺ to P[−] and from O⁺ to O[−] types appear to involve chromosomal rearrangement (Meyer, Mlawer and So, 1982). The antigenic variability of the gonococcal surface also poses considerable problems in terms of the development of an effective vaccine. We have, therefore, begun to investigate the immunochemistry of antigenic shift by the use of monoclonal antibodies and initially chose to study pilus variation.

A panel of monoclonal antibodies was obtained after fusion of spleen cells from mice which had been immunised with purified pili from strain P9 and then given booster doses of live gonococci (Virji, Heckels and Watt, 1983). Antibodies with two types of reactivity were observed when clones were tested against α , β , γ and δ pili from strain P9 (table II). Two antibodies (SM1 and SM2) reacted with all pilus types whereas the remainder showed specificity for one or sometimes two pilus types. When the antibodies were tested against variant pili from a number of strains, SM1 and SM2 reacted with all pili tested but the type specific antibodies showed very limited reaction (table II) (Virji and Heckels, 1983). The distribution of epitopes on pili was investigated by competitive radioimmunoassay with ¹²⁵I-labelled monoclonal antibody and increasing concentrations of unlabelled antibody. The rationale of these experiments is that inhibition of binding of one antibody by another indicates that they recognise overlapping or closely positioned epitopes whereas lack of inhibition indicates distinct epitopes. The results obtained with α pili are shown in table III. Two major points of interest emerge. First, the two cross-reacting antibodies SM1 and SM2 are not

FIG. 7.—Biological effect of monoclonal antibodies directed against gonococcal pili. (a) Effect of antibodies on the attachment of ^{125}I -labelled pili to human buccal epithelial cells. Pili were incubated with buccal cells in the presence of antibodies; bound pili were detected by gamma counting. Final dilutions of ascitic fluid were: ■, 1 in 10; ▨, 1 in 100; ▩, 1 in 1000. (b) Protection of Chang conjunctival epithelial cells by monoclonal antibodies. Epithelial cells were exposed to gonococci in the presence or absence of antibodies. Final dilutions of ascitic fluid were: ■, 1 in 10; ▨, 1 in 50; ▩, 1 in 250. (Virji and Heckels, 1984).

independently shared among other strains. Thus, only one of the epitopes detected in α pili by monoclonal antibodies is present in pili expressed on the isolate from the urethra of a male patient but is absent from either of the variants obtained from his female partner (fig. 6).

Use of monoclonal antibodies to probe pilus-epithelial cell interaction

Although antigenic variation poses problems for vaccine development one attractive strategy is to use peptide fragments containing only the common determinants as immunogens (Buchanan *et al.*, 1982; Schoolnik *et al.*, 1982). However, the role of the conserved and variable regions in adhesion to epithelial cells has not yet been defined and, therefore, the inhibitory effect of antibodies directed against each is unknown. The monoclonal antibodies provide specific tools to probe the role of variable and conserved regions in pilus binding and in virulence of gonococci for human epithelial cells (Virji and Heckels, 1984). The effect of antibodies on the interaction of purified pili with human buccal cells is shown in fig. 7a. With α pili, the three type-specific antibodies show inhibition of attachment at a range of concentrations. In contrast cross-reacting antibody SM1 showed no significant inhibition and SM2 showed slight inhibition only at the highest concentration tested. Similar results were obtained with purified γ pili. When the antibodies were tested for their ability to protect tissue culture cells against the cytotoxic effect of live gonococci, the results closely paralleled those seen in the pilus-attachment system (fig. 7b). With both variants, the type-specific antibodies protected the cells against damage but the cross-reacting antibodies were largely ineffective. The close similarity between the two systems suggests that gonococcal virulence is reduced by inhibition of initial pilus adhesion to the epithelial cells and occurs only with type-specific antibodies. Although these results show the importance of antibodies directed against the variable region, we cannot yet eliminate some protective role for antibodies against the common region. Clearly a larger panel of cross-reacting monoclonal antibodies directed against different epitopes in the common region would be required to unequivocally establish this point. Nevertheless, these data combined with differences in adhesion seen with the variant pilus types strongly suggest an important role for the variable region in pilus-epithelial cell interaction.

Conclusions

The occurrence of extensive antigenic variation in the two major surface antigens known to be involved in gonococcal virulence poses problems for formulation of a gonococcal vaccine. Two approaches to the problem appear useful. The first, identification and isolation of conserved functional domains on the variable antigens, is already receiving attention. We must await developments in this area. An alternative strategy is the identification of antigens which are widely shared between strains. One such candidate is OMP III which appears immunologically identical in all strains tested (Swanson, Mayer and Tam, 1982). Our studies on the immune response in patients with gonorrhoea have identified another candidate, a surface protein of mol. wt 43×10^3 , which is widely distributed among strains (Zak *et al.*, 1984). However, we will have to gain knowledge of the possible protective effect of antibodies directed against these antigens before they can be assessed as future vaccine candidates.

I am indebted to all my colleagues in Southampton who have been involved in the work described in this review and in particular to Professor P. J. Watt, Dr P. R. Lambden and Dr M. Virji. These studies have been supported by a Medical Research Council Programme Grant and by the World Health Organization.

REFERENCES

- Buchanan T M 1977 Surface antigens: Pili. In: Roberts R B (ed) *The Gonococcus*, J Wiley, New York, pp. 255-272.
- Buchanan T M, Siegel M S, Chen K C S, Pearce W A 1982 Development of a vaccine to prevent gonorrhoea. In: Weinstein L *et al.* (eds) *Seminars in infectious disease*, vol IV: Bacterial vaccines, Thieme-Stratton, New York, pp. 160-164.
- Diaz J-L, Heckels J E 1982 Antigenic variation of outer membrane protein II in colonial variants of *Neisseria gonorrhoeae* P9. *Journal of General Microbiology* 128:585-591.
- Duckworth M, Jackson D, Zak K, Heckels J E 1983 Structural variations in pili expressed during gonococcal infection. *Journal of General Microbiology* 129:1593-1596.
- Harkness A H 1948 Pathology of gonorrhoea. *British Journal of Venereal Diseases* 24:137-147.
- Heckels J E 1978 The surface properties of *Neisseria gonorrhoeae*: topographical distribution of the outer membrane protein antigens. *Journal of General Microbiology* 108:213-219.
- Heckels J E 1981 Structural comparison of *Neisseria gonorrhoeae* outer membrane proteins. *Journal of Bacteriology* 145:736-742.
- Heckels J E 1982 Role of surface proteins in the adhesion of *Neisseria gonorrhoeae*. In: Schlessinger D (ed) *Microbiology 1982*, American Society for Microbiology, Washington, D.C., pp 301-304.
- Heckels J E, Blackett B, Everson J S, Ward M E 1976 The influence of surface charge on the attachment of *Neisseria gonorrhoeae* to human cells. *Journal of General Microbiology* 96:359-364.
- Heckels J E, James L T 1980 The structural organisation of the gonococcal cell envelope and its influence on pathogenesis. In: Danielsson D, Normark S (eds) *Genetics and immunobiology of pathogenic neisseria*, University of Umea, Sweden, p 25.
- Heckels J E, Virji M Monoclonal antibodies against gonococcal pili; uses in the analysis of gonococcal immunochemistry and virulence. In: Macario A J L, Macario E C (eds) *Monoclonal antibodies against bacteria*, Academic Press, New York, (in press).
- James L T, Heckels J E 1981 An improved method for the isolation of the major protein of the gonococcal outer membrane in an antigenically reactive form. *Journal of Immunological Methods* 42:223-228.
- Johnson A P, Taylor-Robinson D, McGee Z A 1977 Species specificity of attachment and damage to oviduct mucosa by *Neisseria gonorrhoeae*. *Infection and Immunity* 18:833-839.
- Kellogg D S, Peacock W L, Deacon W E, Pirkle C I 1963 *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. *Journal of Bacteriology* 85:1274-1279.
- Lambden P R 1982 Biochemical comparison of pili from variants of *Neisseria gonorrhoeae* P9. *Journal of General Microbiology* 128:2105-2111.
- Lambden P R, Heckels J E 1979 Outer membrane protein composition and colonial morphology of *Neisseria gonorrhoeae* strain P9. *FEMS Microbiology Letters* 5:262-265.
- Lambden P R, Heckels J E, James L T, Watt P J 1979 Variations in surface protein composition associated with virulence properties in opacity types of *Neisseria gonorrhoeae*. *Journal of General Microbiology* 114:305-312.
- Lambden P R, Heckels J E, McBride H, Watt P J 1981a The identification and isolation of novel pilus types produced by variants of *N. gonorrhoeae* P9 following selection *in vivo*. *FEMS Microbiology Letters* 10:339-341.
- Lambden P R, Robertson J N, Watt P J 1981b The preparation and properties of α and β pili from variants of *Neisseria gonorrhoeae* P9. *Journal of General Microbiology* 124:109-117.
- McBride H M, Lambden P R, Heckels J E, Watt P J 1981 The role of outer membrane proteins in the survival of *Neisseria gonorrhoeae* P9 within guinea pig subcutaneous chambers. *Journal of General Microbiology* 126:63-67.

- Meyer T F, Mlawer N, So M, 1982 Pilus expression in *Neisseria gonorrhoeae* involves chromosomal rearrangement. *Cell* 30:45-52.
- Schoolnik G K, Tai J-Y, Gotschlich E C 1982 The human erythrocyte binding domain of gonococcal pili. In: Weinstein L. *et al.* (eds) *Seminars in infectious disease*, vol. IV: *Bacterial vaccines*, Thieme-Stratton, New York, pp 172-180.
- Swanson J 1978 Studies on gonococcus infection. XIV. Cell wall protein differences among color/opacity colony variants of *Neisseria gonorrhoeae*. *Infection and Immunity* 21: 292-302.
- Swanson J, Kraus S J, Gotschlich E C 1971 Studies on gonococcus infection. I. Pili and zones of adhesion: their relation to gonococcal growth patterns. *Journal of Experimental Medicine* 134:886-906.
- Swanson J, Mayer L W, Tam M R 1982 Antigenicity of *Neisseria gonorrhoeae* outer membrane protein(s) III detected by immunoprecipitation and western blot transfer with a monoclonal antibody. *Infection and Immunity* 38:668-672.
- Trust T J, Lambden P R, Watt P J 1980 The cohesive properties of variants of *Neisseria gonorrhoeae* strain P9: specific pilus-mediated and non-specific interactions. *Journal of General Microbiology* 119:179-187.
- Veale D R, Smith H, Witt K A, Marshall R B 1975 Differential ability of colonial types of *Neisseria gonorrhoeae* to produce infection and an inflammatory response in subcutaneous perforated plastic chambers in guinea-pigs and rabbits. *Journal of Medical Microbiology* 8:325-335.
- Virji M, Everson J S 1981 Comparative virulence of opacity variants of *Neisseria gonorrhoeae* strain P9. *Infection and Immunity* 31:965-970.
- Virji M, Everson J S, Lambden P R 1982 Effect of anti-pilus antisera on virulence of variants of *Neisseria gonorrhoeae* for cultured epithelial cells. *Journal of General Microbiology* 128:1095-1100.
- Virji M, Heckels J E 1983 Antigenic cross-reactivity of *Neisseria* pili: investigations with type- and species-specific monoclonal antibodies. *Journal of General Microbiology* 129:2761-2768.
- Virji M, Heckels J E 1984 The role of common and type-specific pilus antigenic domains in adhesion and virulence of gonococci for human epithelial cells. *Journal of General Microbiology* 130:1089-1095.
- Virji M, Heckels J E, Watt P J 1983 Monoclonal antibodies to gonococcal pili: studies on antigenic determinants on pili from variants of strain P9. *Journal of General Microbiology* 129:1965-1973.
- Ward M E, Watt P J, Robertson J N 1974 The human fallopian tube: a laboratory model for gonococcal infection. *Journal of Infectious Diseases* 129:650-659.
- Watt P J, Ward M E, Heckels J E, Trust T J 1978 Surface properties of *Neisseria gonorrhoeae*: attachment to and invasion of mucosal surfaces. In: Brooks G F *et al.* (eds) *Immuno-biology of Neisseria gonorrhoeae*, American Society for Microbiology, Washington D.C., pp 253-257.
- Walstad D L, Guymon L F, Sparling P F 1977 Altered outer membrane protein in different colonial types of *Neisseria gonorrhoeae*. *Journal of Bacteriology* 129:1623-1627.
- Zak K, Diaz J-L, Jackson D, Heckels J E 1984 Antigenic variation during infection with *Neisseria gonorrhoeae*: detection of antibodies to surface proteins in sera of patients with gonorrhoea. *Journal of Infectious Diseases* 149:166-174.