Update
Le point

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Development of a gonorrhoea vaccine: prospects, strategies and tactics*

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The sexually transmitted diseases constitute an enormous public health problem in most parts of the world. The present article concentrates on gonorrhoea and summarizes the magnitude of the problem, reviews the current knowledge of the molecular mechanisms employed by the gonococcus to establish infection, and describes the immunochemistry and genetics of the gonococcal surface antigens. It is pointed out that pili and protein II show marked heterogeneity, and that this probably represents a response by the organism to the selective pressure imposed by the human immune system. This evidence for the effectiveness of the immune system augurs well for the eventual success of a gonorrhoea vaccine. The prospects for the antigens currently under study in a number of laboratories and the strategies being employed to circumvent the problems posed by antigenic heterogeneity are summarized.

The extraordinary effectiveness of penicillin in the treatment of gonorrhoea led to a profound decline in interest in the study of the microbiology of the gonococcus for about 15 years following the universal availability of this antibiotic. However, interest was reawakened in the 1960s as it became evident that gonorrhoea had reached pandemic proportions in many parts of the world. During the last decade, there has been intensive research on various aspects of the gonococcus, in laboratories in many parts of the world. The interested reader is referred to several review articles discussing the physiology,[a] immunochemistry,[b, c] and genetics[d] of this organism.

THE PUBLIC HEALTH IMPACT OF GONORRHOEA

Gonorrhoea is a sexually transmitted disease found in all parts of the world. In men, the disease is usually symptomatic (90% of cases), prompting the patient to seek treatment for

* A French translation of this article will appear in a later issue of the *Bulletin*.

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[a] MORSE, S. I. The biology of the gonococcus. *CRC critical reviews in microbiology*, 7: 93-136 (1980).

[b] GOTSCHLICH, E. C. Gonorrhea. In: Germanier, R., ed., *Bacterial vaccines*, New York, Academic Press, 1984.

[c] SWANSON, J. L. & MAYER, L. W. Biology of *Neisseria gonorrhoeae*. In: Holmes, K. K. et al., ed., *Sexually transmitted diseases*, New York, McGraw Hill, 1984.

[d] CANNON, J. G. & SPARLING, P. F. The genetics of the gonococcus. *Annual review of microbiology*, 38: (1984) (in press).

the infection. Without treatment, males may suffer local extension of the infection to the prostate, epididymis, and accessory glands, which may be an important cause of male infertility. In areas of the world where prompt treatment is readily available, gonorrhoea in men is generally a relatively innocent disease of the mucosa of the anterior urethra. In contrast, in nearly 50% of recently infected women, symptoms are absent or not sufficiently severe for the patient to seek medical attention. In 10–15% of women, usually at the time of the first menses following infection, there is extension of the infection to the fallopian tubes, and development of pelvic inflammatory disease (PID). The importance of this complication is that it appears to be a major cause of infertility and tubal pregnancy, and can lead to chronic PID. This is true in affluent countries as well as in those with limited medical resources. For instance, in the USA, coincident with an explosive increase in the incidence of gonorrhoea, the number of ectopic pregnancies tripled from 13 000 cases in 1967 to 41 000 cases in 1977. Pelvic inflammatory disease is a known cause of sterility, with a 13% infertility rate following a single attack, 36% after two attacks, and 75% after three or more attacks. While it is important to recognize that gonorrhoea is not the only cause of PID, it is undoubtedly a major contributor to this problem. In 1979, the annual direct and indirect costs of PID and PID-related ectopic pregnancies in the USA (excluding the cost of fetal deaths as a result of ectopic pregnancy) were estimated to be US1.25×10^9. When a cost was assigned to fetal deaths caused by ectopic pregnancies, the annual cost of pelvic inflammatory disease was estimated to be greater than US2.7×10^9. Systemic invasion by the organism, causing arthritis, endocarditis, perihepatitis and disseminated infection, occurs in only 1–2% of men or women, and in most patients given adequate treatment, no permanent damage results.

In countries lacking the resources for treatment and contact-tracing, the prevalence of the disease can be alarming. For instance, one study found that 17.5% of women presenting for prenatal care at a clinic in Uganda were infected with gonococci. The epidemic infertility seen in some areas of sub-Saharan Africa, where as many as 50% of women never conceive, may be mainly due to prior episodes of PID and epididymitis. The fear of sterility leads to early attempts at childbearing and this undoubtedly further increases the risk of sexually transmitted disease in very young women and hence, the risk of sterility.

In developed countries, neonatal morbidity due to gonorrhoea is no longer great, partly because of routine prenatal screening for gonorrhoea and partly because of the routine use of prophylaxis for gonococcal ophthalmia neonatorum. However, in developing countries, this infection remains an important cause of blindness, as was the case in the United States in the 19th century when it was the leading cause of blindness in children. It should also be emphasized that, in developing countries, gonococcal infections may have unusual complications that have not yet been adequately identified.

The control of the infection has been complicated by the appearance in the last 7 years of gonococci showing plasmid-mediated resistance to penicillin. These ß-lactamase-producing strains have become increasingly common and in many parts of the world represent a substantial proportion of gonococcal isolates. An alarming aspect of this problem is that meningococci carrying this plasmid have also been isolated recently.

It should be recognized that gonorrhoea is only one facet of the much larger problem of sexually transmitted diseases. In the long term, health planners attempting to control these diseases will get the most cost-effective results if they recognize the unitary nature of the problem. Resources should be allocated to cover all such diseases rather than for piecemeal control of each of the diseases separately. Progress in the biomedical sciences has already provided an effective vaccine against one sexually transmitted disease, namely viral hepatitis B, and the promise of this approach for use against other such diseases is bright. It

is the purpose of this paper to review the prospects for immunoprophylaxis of gonorrhoea.

PATHOGENETIC MECHANISMS

Gonorrhoea vaccines based on the use of whole cells have been employed a number of times, but were not effective. In order to develop a vaccine based on purified components, it is necessary to understand the molecular interactions occurring in the various stages of the infection by the gonococcus. Until recently, very little information existed on this subject because of the lack of an animal model. In the last decade, however, a great deal has been learned about the early steps in the infection, primarily through studies of the interaction of gonococci with human fallopian tube organ cultures. These investigations have been pioneered by Watt and colleagues[e] in England and McGee and colleagues[f] in the USA.

When human fallopian tube explants are brought into contact with living gonococci, the first recognizable interaction is that the gonococci attach to the luminal surface of the columnar epithelial cells, and not to the ciliated cells. This distant attachment is clearly mediated by pili, which are long filamentous organelles on the surface of the gonococci. The next step is a close attachment phase in which gonococci come to lie directly on the surface of the epithelial cells, and very extensive and intimate contact is established between the outer membrane of the gonococcus and the cell's membrane. Thereafter, the epithelial cells phagocytose the adherent gonococci and rapidly transport them in phagosomes to the basal surface of the cell; there the living gonococci are egested onto the lamina propria. It is apparent that the gonococci provide a signal to the epithelial cell which causes it to provide the bacteria free access to the basement membrane. This step is in effect the first breach of the host's surface integrity. This unusual ability to subvert the function of an epithelial cell is not unique. Very similar events occur when meningococci are exposed to nasopharyngeal mucosa, or when *Shigella*, *Yersinia*, enteroinvasive *Escherichia coli*, or *Salmonella typhimurium* are placed on intestinal mucosal cells.

The steps outlined above occur in all cases of uncomplicated gonococcal genitourinary disease. The extension of infection to other parts of the genital tract simply represents seeding of the organism along the lumen of the tract and repetition of the mucosal invasion process. Invasion of the bloodstream and infection of distant organs are seen in about 1% of cases and can occur if the organisms present in the subepithelial space are innately resistant to the bactericidal action of the blood. Studies are needed to determine the causal relationship between these steps of the infection and the surface molecules present on gonococci, and to develop vaccine candidates that will interrupt one or more of these steps. It is evident that the most important complication, namely extension of the infection to the fallopian tubes, can only be prevented by a vaccine that is able to block the ability of the gonococcus to infect epithelial cells.

SURFACE COMPONENTS OF THE GONOCOCCUS

The definition of the surface structures of the gonococcus has been an extraordinarily effective effort of a large number of laboratories in the last decade. In retrospect, two early

[e] WARD, M. E. ET AL. The human fallopian tube: a laboratory model for gonococcal infection. *Journal of infectious diseases*, **129**: 650-659 (1974).

[f] JOHNSON, A. P. ET AL. Species specificity of attachment and damage to oviduct mucosa by *Neisseria gonorrhoeae*. *Infection and immunity*, **18**: 833-839 (1977).

contributions have had a substantial impact on developments in this field. The first was the recognition by Kellogg and his associates[g] that the colonial forms of gonococci freshly isolated from patients could be characterized; these were named T1 and T2. Upon further passes on agar, other colonial forms arise with high frequency, and these were called T3 and T4. Most important, it was demonstrated that, if the T1 or T2 colonial phenotype is maintained by selectively passing colonies with these characteristics, these gonococci are able to cause disease when inoculated into volunteers; the other colonial forms, T3 and T4, do not produce typical disease. This finding was further elaborated by Swanson and his associates,[h] who described additional characteristics of the colony morphology of gonococci, and developed a method of maintaining populations of gonococci of defined phenotypes *in vitro*. Another contribution, which is gaining increasing importance with the recent genetic manipulations of gonococci, is the development by Catlin[i] of chemically defined media for the growth of gonococci; she also recognized that strains of gonococci differ in their nutritional requirements and that these metabolic characteristics can be used to classify gonococcal strains into what are referred to as auxotypes.

Pili

In 1971, it was noted that the colonial types T1 and T2 carried pili on their surface, while the non-virulent types, T3 and T4, were devoid of these structures. It has been shown that these pili mediate adhesion to a number of human cells and that this property is essential for virulence. The central importance of pilus-mediated adhesion has been recognized in a large number of human and animal diseases caused by many different species of Gram-negative organisms. There is also evidence that gonococci bearing pili are not readily ingested by human polymorphonuclear leukocytes.

Pili are long hair-like structures, consisting of highly ordered aggregations of thousands of identical protein subunits which form a filament of about 4 μm in length. The subunits constituting the pili vary in relative molecular mass from about 17 000 to 21 000 in different gonococcal strains. This structural variation can also be demonstrated serologically. Generally, if an antiserum is prepared to pili of one strain of gonococcus it will show 10% or less cross-reactivity with pili prepared from other gonococcal strains. The number of serotypes of pili is not yet known, but is probably in excess of 50. It has been observed that a particular strain of gonococcus will, at different times, express serologically different pili. This variation is observed *in vivo* when identical strains, as judged by auxotype and other criteria, are isolated from more than one infected site of the same patient, or from his/her immediate sexual contact. This phenomenon can also be reproduced in the laboratory. The genetic basis of this variability is incompletely understood, but it is clear that the gonococcal genome contains two or more genes coding for the pilus structure.

However, structural and functional studies indicate that there is also substantial homogeneity among different pili. Intuitively this can be inferred from the ability of the different subunits to aggregate in an exquisitely accurate manner to form the pili filaments. The amino acid sequence of the pili of one strain has been completely identified, and comparison of this sequence with partial sequences available for a number of other pili indicates that at least the first 60 amino acids of the subunits are identical. Using selective

[g] KELLOGG, D. S. JR ET AL. *Neisseria gonorrhoeae*. I. Virulence genetically linked to colonial variation. *Journal of bacteriology*, **85**: 1274–1279 (1963).

[h] SWANSON, J. Studies on gonococcus infection. XII Colony color and opacity variants of gonococci. *Infection and immunity*, **19**: 320–331 (1978).

[i] CATLIN, B. W. Nutritional profiles of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Neisseria lactamica*, in chemically defined media and the use of growth requirements for gonococcal typing. *Journal of infectious diseases*, **128**: 178–193 (1973).

cleavage of the molecule at methionine residues or at arginine residues, it has been possible to prepare large fragments of the subunit. For instance, the subunit of pili from strain MS11, which consists of 159 amino acids, can be cleaved with cyanogen bromide to produce two major fragments, CNBr-2 and CNBr-3, representing respectively amino acids 7–92, and 93–159 of the sequence. The CNBr-2 fragments prepared from pili isolated from different strains are chemically quite similar, while CNBr-3 peptides differ substantially, both in peptide maps and serologically. When purified CNBr-3 fragments are used to immunize rabbits the antisera react only with the same CNBr-3 peptide and with the pili from which the fragment was isolated, and do not cross-react with other pili. Similarly, antisera raised after immunization with intact pili are generally not cross-reactive. In marked contrast, if isolated CNBr-2 is used to immunize animals, the antisera are markedly cross-reactive with pili of all strains that have been tested. This indicates that in intact pili the variable domain, located in the carboxy-terminal part of the molecule, is strongly immunodominant and preempts a significant response to the common portions of the molecule, even though these are exposed on the surface.

From the point of view of preventing the disease, the important consideration is whether antibodies to pili are able to inhibit adherence. Results from a number of laboratories indicate that they are. That this is also applicable to the human disease has been demonstrated by Brinton and his colleagues[j] in volunteer studies. Immunization of men with purified pili produced demonstrable immunity to challenge with the strain of gonococcus from which the pili were isolated, but not to other strains. These studies established that pili are immunogenic, giving rise to high levels of serum antibodies and readily detectable secretory antibodies, and that they are non-toxic and readily tolerated.

It has been shown that the binding function of the pili is mediated by a receptor site contained within the CNBr-2 peptide. This has been confirmed with another peptide (TC-2), obtained by arginine-specific cleavage and spanning positions 31–111 of the amino acid sequence. A number of laboratories are now examining the fine structure of the antigenic epitopes on pili using monoclonal antibodies and synthetic peptides.

The observed role of gonococcal pili in infection, the effectiveness of pilus-based vaccines in a number of animal diseases, and the demonstration in humans that immunization with pili has a protective effect suggest that further research on development of a gonococcal vaccine based on pili would be worthwhile. The main obstacle to an effective vaccine is the antigenic diversity of the pili. At this time, two approaches to circumventing this problem are being actively pursued. The first approach is to determine the extent of diversity of gonococcal pili in nature and to prepare a polyvalent vaccine containing a sufficient number of different pilus types to provide effective immunity. In principle, this is the same approach as that used in the development of the pneumococcal polysaccharide vaccine where the types causing the majority of disease were identified and included in the vaccine; this vaccine currently contains 14 different capsular polysaccharides and will soon be expanded to include 22 components, in order to achieve even better protection.

The second approach is to determine precisely which part of the pilus subunit molecule is responsible for adhesive activity; if it is found that this structure is universal among pili, antigens that concentrate the immune response on this area can be prepared. Candidate peptides for this purpose are the fragments CNBr-2 and TC-2. The promise of other peptides representing various areas of the pilus structure has not yet been evaluated. The possibility that this approach can best be exploited by using synthetic peptides should be fully investigated.

[j] BRINTON, C. C. ET AL. The development of a neisserial pilus vaccine for gonorrhea and meningococcal meningitis, In: Weinstein, L & Fields, B., ed., *Seminars in infectious diseases. IV: Bacterial vaccines*. New York, Thieme-Stratton, 1981, pp. 140–159.

Outer membrane proteins

To seek an understanding of the events that occur at the molecular level in the close attachment phase of the infection and perhaps identify the signalling mechanism that permits entry into the epithelial cells, one needs to examine the chemistry of the gonococcal surface. Like most Gram-negative organisms, the gonococcus is enclosed by two membranes, an inner cytoplasmic membrane, and an outer membrane. Since it is the outer membrane that comes into contact with the host, it has attracted the most attention. In many ways this membrane is typical of those seen in other Gram-negative organisms. Its characteristic features are that it contains endotoxic lipopolysaccharide and large amounts of certain proteins, which have been named protein I, II, and III.

Protein I

Usually one protein is found in greatest abundance in the outer membrane; this has been named protein I. The subunit relative molecular mass of this protein in the various strains can vary between 32 000 and 36 000. It has been shown that these proteins vary serologically and they have been separated into three major groups using a coagglutination method. More refined serological analysis indicates that about 10 serotypes are sufficient to characterize the vast majority of organisms. It is important to note that the kind of protein I expressed by a strain appears to be a stable characteristic and, together with auxotyping, is becoming an increasingly important method for typing gonococci. In addition, it has been shown that the isolates obtained from blood or joint fluids tend to be strains with characteristic auxotypes and to belong to a particular subtype of the coagglutination type 1. These strains are also resistant to the bactericidal action of normal serum. The mechanism of this resistance is not clear; although serum-resistant and serum-sensitive strains bind equal amounts of the late complement components, which constitute the membrane attack complex, yet the serum-resistant strains are not lysed.

The protein has been purified to homogeneity in a native state. The proteins of the different coagglutination types are different in primary structure and in their disposition within the gonococcal outer membrane. Nevertheless, parts of the amino acid sequence are homologous. It has been demonstrated that when the protein is inserted into a membrane, it acts as an aqueous channel, allowing small molecules to diffuse through the membrane. This is also a property of the predominant outer membrane proteins of other bacterial species and these proteins have been named porins. The gonococcal protein I channel allows anions to diffuse more readily than cations, which is unusual; porins of other species are cation-selective. Conductance studies have shown that if living gonococci, rather than the purified protein, are placed in contact with artificial membranes, the protein I translocates from the outer membrane of the gonococcus to the artificial membrane. This translocation is favoured if the acceptor membrane is relatively fluid compared with the donor membrane. The gonococcal outer membrane, like that of other Gram-negative organisms, is much more rigid than most eukaryotic cell membranes. If human red cells are exposed to gonococci, the transfer of protein I occurs very rapidly. This behaviour is also observed with meningococci, but not with the non-pathogenic *Neisseria sicca*.

Many independent lines of investigation have demonstrated that the earliest recognizable step in phagocytosis by professional phagocytic cells, such as polymorphonuclear leukocytes or monocytes, is a membrane depolarization event. The events occurring in antibody-mediated stimulation of phagocytosis by human macrophages have been elucidated in some detail. The aggregated immunoglobulin on the surface of the opsonized particle binds by its Fc region to a receptor located on the macrophage membrane. As a result of this binding, the receptor acts as an ion channel and triggers the series of events resulting in phagocytosis. It is possible that the translocation of the porin of the

gonococcus to an adjacent membrane may be the signal engaging the unusual phagocytic activity of the epithelial cells. This hypothesis is under intensive investigation.

There are several reasons why protein I is an attractive vaccine candidate. The protein is exposed at the surface of the organism; antibodies to the protein are bactericidal; and the number of serotypes is not very large, so that a polyvalent vaccine is feasible. In addition there are epidemiological data indicating that antibodies to protein I may confer immunity. Second attacks of PID are significantly less likely to be caused by the same serotype that caused the first episode. Partially purified protein I has been tested in human beings and was found to be well tolerated and a good immunogen. Protection studies have not yet been carried out.

Protein II

It has been noted that, when gonococcal colonies are viewed through a dissecting microscope using light directly reflected from a mirror, some colonial forms appear to be transparent and others opaque, i.e., they refract the light within the colony to give it an appearance akin to ground glass. By electron microscopy, it can be shown that this opacity is due to the fact that the outer membranes of these gonococci are sticky, causing the organisms to adhere to each other. Organisms of the opaque phenotype invariably contain a large amount of an outer-membrane protein that can vary in relative molecular mass between 26 000 and 30 000. These proteins have been named protein II and have a number of characteristics. They are heat-modifiable, which means that the apparent relative molecular mass, as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), will be higher if the sample is boiled prior to the electrophoretic separation. The proteins have been purified to homogeneity and have an isoelectric point in excess of pH 8.0. Their amino acid composition also indicates their basic nature.

Since these proteins clearly mediate the sticking of gonococci to each other, it is believed that they may be involved in the close attachment phase of adherence to the cells of the host; several lines of evidence suggest that this may be so, but definitive evidence has not yet been obtained. One of the most interesting aspects of protein II is that its presence or absence is related to the site of isolation of the organism. Gonococci obtained from males with uncomplicated anterior urethritis are most often of the opaque phenotype. Isolates obtained from blood or from PID are always of the transparent phenotype. Isolates from the pharynx or the rectum of homosexual males are as a rule intensely opaque, frequently carrying more than one form of protein II in their outer membrane. Even more remarkably, cervical isolates vary in concert with the menstrual cycle, so that at the time of the menses the gonococcal population consists primarily of transparent organisms, while at the time of ovulation the predominant phenotype is opaque.

This variation in phenotype has been observed to occur at random *in vitro* approximately once in every 1000 cell divisions. The same frequency of change is found in either direction and is much too high to be a mutational event; hence the change is classified as a phase variation. Not only can a strain of gonococcus express or not express protein II, but it can express different forms of protein II at different times, and at times more than one. The number of serologically different types of protein II is not known; however, peptide mapping studies have shown that the different forms share many peptides. Structural genes for one or another protein II have been cloned in several laboratories, and are being used to determine if the turning off and on of the expression of protein II, as well as the switching to another kind of protein II, result from physical rearrangement of the DNA. It is not known whether the association of the opaque phenotype with particular clinical situations represents the random variation seen *in vitro* with subsequent selection of a particular phenotype, or whether there exist chemical mechanisms in the gonococcus that sense the environment and change the phenotype accordingly.

Thus far, little direct effort has been devoted to protein II as a vaccine candidate. The variability of the protein and its absence in the transparent phenotype are dissuasive. However, its biology is quite complex, strongly suggesting that it is of major importance to the survival of the organism, and the protein is exposed on the surface. Thus its potential as a vaccine candidate remains an open question until its role in pathogenesis is much more clearly delineated.

Protein III

All gonococcal strains examined contain in their outer membrane a protein that by SDS-PAGE has an apparent relative molecular mass of 30 000 in the absence of reducing agent and of 31 000 following reduction. This protein has been named protein III. Monoclonal antibodies that are able to bind to protein III in living organisms have been prepared. This indicates that at least part of the protein is exposed at the surface. Curiously, all of the monoclonal antibodies isolated thus far belong to a non-complement-binding isotype, so that it has not been possible to determine whether antibody-mediated biological effects may be expected. Cross-linking studies have shown that this protein is non-covalently associated with protein I in the gonococcal outer membrane, but the functional significance of this is unknown. It is noteworthy that all meningococci contain a similar protein. Thus protein III appears to be a very interesting and promising candidate for inclusion in a vaccine but, as yet, its potential remains completely unexplored.

Lipopolysaccharides and capsules

Under light microscopy and using Indian ink, a clear halo is evident around the gonococcus, a property usually indicative of the presence of a capsule. However, no one has yet been able to isolate a capsular polysaccharide. Gonococci produce a substantial amount of extracellular polyphosphate, which may exclude the Indian ink and thus mimic a capsule. However, polyphosphate is not antigenic, and it is thus likely that earlier reports of the isolation of capsular polysaccharides were, in fact, due to the isolation of lipopolysaccharide (LPS).

LPS has considerable interest as a vaccine candidate. Antibodies to LPS are bactericidal. With regard to mucosal infection, there is evidence that LPS may cause the marked toxic effects which result in the expulsion of the ciliated cells from the mucosa. If this toxicity could be neutralized, it might be expected that the cleansing action of the mucosa would remain unimpaired, making it much more difficult for the gonococcus to establish an infection. The oligosaccharide chains are relatively short, as are those seen in the meningococcal LPS. The sugars that have been detected include ketodeoxyoctonate, glucose, galactose, heptose, glucosamine, and galactosamine. Serological studies indicate that there are antigenic determinants common to the LPS from all strains of gonococci, and these can be at least partially inhibited with lactose. In addition there are other determinants that have allowed the classification of gonococci into 6 types. Despite the obvious importance of the LPS there is, so far, very limited information about its chemical structure. However, with modern methods and the limited size of the saccharide portion, structural analysis is a feasible task.

Since LPS is very toxic, it will be necessary to remove the toxic lipid moiety from the oligosaccharide portion and to link the latter to a carrier protein (for instance pili) to enhance its immunogenicity.

Other outer membrane components

Thus far this review has considered only the components present in large amounts. However the outer membrane is complex and many proteins that are vital to the bacterium

are present in small amounts. For instance, the environment within the host is markedly deficient in iron and successful competition for this essential nutrient depends on outer membrane proteins; consequently, these are very attractive vaccine candidates. Additional outer membrane proteins have been defined by application of monoclonal antibody techniques and the analysis of gonococcal gene banks. At least one of these antigens has been shown to be common to all gonococci, to be exposed at the surface of the cell, and to induce antibodies that have bactericidal effects.

IgA protease

All gonococci examined secrete a protease that specifically cleaves human IgA1 immunoglobulin in the hinge region and inactivates the IgA1 antibodies. Similar proteases are also found in a number of other pathogenic organisms such as the meningococcus, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Streptococcus sanguis*. Conversely, non-pathogenic *Neisseria* and *Haemophilus influenzae* of serotypes other than b lack the ability to make the enzyme. The specificity of these enzymes is exquisite and they have not been found to act on any other substrate. There must be a selective pressure that causes the gonococcus (and the other organisms) to maintain this protease; if this pressure is related to the ability to inactivate antibodies by cleaving IgA1, it follows that the antibodies produced by the host are effectively antibacterial, when not neutralized by this enzyme. It is probable that any vaccine should include this protease (or a portion of the protein containing the enzymatically active site) in order to raise antibodies capable of neutralizing the activity of the enzyme and hence allowing the antibodies to the other components of the vaccine to have maximal protective effect.

The IgA protease has been purified and partially characterized. The structural gene for the protease of the gonococcus has been cloned in plasmid vectors and inserted into *Escherichia coli* cells to produce small quantities of active protease; *E. coli* strains producing large amounts of the protease could easily be constructed. There is evidence that some strains of gonococci may elaborate a slightly different IgA protease, but the extent of dissimilarity has not been established. It is of great interest to localize the active site of this enzyme and to determine whether an immune response can be focused against this site, such that the antibodies not only bind to, but inactivate, the enzyme.

IMMUNE MECHANISMS

Natural immunity to gonorrhoea is not readily demonstrable. It is a common observation that patients can acquire the infection many times even from the same consort. With the demonstration that both protein II and pili serotypes can change *in vivo*, the lack of demonstrable immunity is not surprising. This variability obviously presents a major hurdle and has caused much pessimism about the prospects for a vaccine. However, if one views this antigenic polymorphism from an ecological point of view and keeps in mind what is known about other organisms, such as trypanosomes, where variability is particularly prominent, it is evident that the only selective pressure that could account for this variability is immune selection. This clearly implies that the gonococcus is not insensitive to the human response, but rather has developed elaborate mechanisms to deal with it.

It is likely that over the next few years a number of vaccines based on one or another of the components discussed above will be tested for immunogenicity, toxicity, and efficacy. Each of these may by itself have perceptible, but perhaps limited, effectiveness, since it is probable that to prevent fully the mucosal invasion several sequential molecular steps will

need to be interrupted. The second generation vaccines will probably contain various components that interfere with several or all of the processes such as adhesion, inactivation of antibodies by IgA protease, the toxic effects on cilia, the epithelial phagocytic step, and gonococcal iron metabolism.

It is essential to remember that the development of an effective gonorrhoea vaccine is not a panacea, but only a powerful tool in the public health effort required to control gonorrhoea specifically and sexually transmitted disease generally. Information on the prevalence and epidemiology of these diseases must be obtained in many parts of the world, in order to define accurately the magnitude of the problem, to identify the subgroups of the population who should receive the vaccine so that it may be used in a cost-effective manner, and to be able to predict the likely effects of immunization.