Neisseria gonorrhoeae: a versatile pathogen

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SUMMARY *Neisseria gonorrhoeae* is one of the most important causes of sexually transmitted disease. We do not fully understand the pathogenesis of infection with this organism, although recent improvements in immunological and molecular techniques have brought us closer to an answer. These techniques are now also being used to detect and identify *N gonorrhoeae* and to analyse the epidemiology of gonorrhoea. Plasmid and chromosomal mediated antibiotic resistance increases the difficulty of controlling gonorrhoea. Resistant strains occur all over the world and new patterns of resistance are still emerging.

A better understanding of gonococcal pathogenicity is necessary for the development of an effective vaccine. Despite work on pili and outer membrane proteins no vaccine yet exists. The control of gonorrhoea still depends on diagnosis, treatment, and epidemiological control, facilities that are not widely available in many of those parts of the world where gonorrhoea is a major problem.

Diagnosis of *Neisseria gonorrhoeae*

Culture of the causative organism *Neisseria gonorrhoeae* remains the gold standard for the diagnosis of gonorrhoea for two reasons: the sensitivity of culture, although probably not 100%, is higher with other techniques; and the development of antibiotic resistance requires that in vitro susceptibility tests be performed.

Sensitivity of culture depends on the quality of the specimen. The ideal procedure is to take the specimen from the patient and culture directly on to selective medium. If incubated immediately at 36°C in 5–7% carbon dioxide this produces the highest isolation rate. When this is not possible, a transport medium such as the buffered media of Stuart and Amies is adequate, if the swab transferred to selective media within six to eight hours.

The design of a suitable medium for the growth of *N gonorrhoeae* from clinical specimens must include sufficient nutrients for this fastidious organism and antibiotics to suppress the growth of normal flora particularly from the rectum and pharynx which will also be present.

Gonococci have an absolute requirement for cysteine and iron and the only carbohydrate they use is glucose. In some media these requirements are met by the addition of blood, often chocolatised, or haemoglobin to a GC agar base containing peptones and starch, but in others supplements such as Kellogg's¹

or IsoVitallex (BBL) without any blood products are used. The choice of an antibiotic cocktail to suppress normal flora is difficult because in some instances gonococci will also be inhibited. The most popular combination includes vancomycin (3 mg/l), which inhibits Gram positive bacteria; colistin (7.5 mg/l), which is active against Gram negative rods; and trimethoprim (5 mg/l), which prevents the swarming of *Proteus* sp. Nystatin (12 500 µ/l) or amphotericin (1.5 mg/l) is also added to inhibit yeasts.

Colistin and trimethoprim are only slightly toxic to gonococci at the concentrations used, but some strains of *N gonorrhoeae* (*env mutants*) are sensitive to vancomycin. The prevalence of these strains varies but has been reported to be as high as 30%.² Lincomycin has been suggested as an alternative to vancomycin,³ but in our experience results in overgrowth of other flora, which prevents the detection of more, strains of gonococci than would have been missed due to the addition of vancomycin.

In many laboratories both selective and non-selective media are used, and this overcomes the problem particularly with male urethral samples that are not heavily contaminated with normal flora. The two selective media commonly used are a modified Thayer-Martin medium⁴ and New York City medium.⁵ For research studies it is often necessary to distinguish between colonial types, and for this a clear medium is required: GC agar base with the addition of Kellogg's defined supplement or IsoVitallex is used.

We have added antibiotics to this medium and use it to isolate gonococci from clinical specimens. We found over a 10 year period that it gives an equal or higher number of positive results. Gonococci grow quickly on this medium and culture on it gives a faster result for patients heavily infected with this organism and a higher isolation rate for patients infected with relatively few organisms. Media for the determination of nutritional requirements⁶ and opaque and transparent colonies⁷ have also been used, but rarely in clinical laboratories.

The correct identification of gonococci can be essential for legal reasons. Presumptive identification can be made by the presence of typical colonies (that may change on different media) that are oxidase positive Gram negative diplococci. These colonies can be confirmed to be *N gonorrhoeae* by various methods, but carbohydrate utilisation tests are still the most commonly accepted. Either solid media, which require overnight incubation, or liquid media, which can be read within four hours, can be used. Serum sugar slopes have largely been replaced by either cystine tryptophan agar (CTA), or GC agar base⁸ as the solid medium with glucose, maltose, lactose and sucrose added (1–2% final concentration) and phenol red as a pH indicator. It is important to use highly purified maltose not contaminated with glucose, or erroneous results may be obtained. These media can be used as stabs, slopes, or in Petri dishes, but all require at least 18–24 hours' incubation before reading. The presence of carbon dioxide changes the pH of the medium and therefore they should be incubated without carbon dioxide or left on the bench for 30 minutes before reading.

All rapid methods for carbohydrate degradation are modifications of the principle developed by Kellogg and Turner in 1973.⁹ Small volumes of buffered carbohydrates containing phenol red indicator are incubated with a heavy inoculum of the culture and can give a result in as little as 15–30 minutes, although incubation for up to four hours is recommended.

Carbohydrate utilisation tests are the accepted method for identification, but differentiation between the species of *Neisseria* in many cases depends only on the use of a single carbohydrate—maltose distinguishes between *N gonorrhoeae* and *N meningitidis*. Several methods have been developed recently to produce either a more rapid or more accurate identification. Fluorescence antibody tests can be used but commercially available reagents using polyclonal antibodies have not been found to be fully specific; false positive fluorescence being shown by some strains of *N lactamica* and *N meningitidis*. The inclusion of monoclonal antibodies¹⁰ in such reagents (GC Microtrak; Syva, USA) has resulted in greater specificity and sensitivity. This rapid and simple tech-

nique may therefore be used more widely in the future.

Coagglutination reagents using both polyclonal and monoclonal antibodies for the identification of gonococci are available.¹¹ The principle on which they work is that staphylococci rich in protein A bind immunoglobulin G subclasses 1, 2, and 4 non-specifically by the Fc portion. In the test, staphylococci coated with specific monoclonal gonococcal antibodies are mixed with a boiled suspension of the gonococci under test, and if positive, give visible agglutination. Polyclonal antibodies raised in rabbits gave similar specificity problems as with the immunofluorescence test, but the monoclonal reagent has both a high sensitivity (97%) and a high specificity (97%).¹¹ The commercial reagents (Phadebact, Pharmacia; Gonogen, New Horizons) have become popular, particularly in laboratories dealing with only occasional isolates, because of their ease of use. Attempts to overcome the necessity for boiling the gonococcal suspension have been unsuccessful.

Biochemical tests have also been regarded as either alternatives or adjuncts to carbohydrate tests. Amino-peptidase activity was used by D'Amato *et al*¹² to distinguish between *Neisseria* sp, and forms the basis of the Gonocheck (E-Y Laboratories, USA: Tissue Culture Services, England) system. The commercial reagent performs well in differentiating *N gonorrhoeae* from *N meningitidis*,^{13 14} but we have found it to be less reliable for the identification of non-pathogenic strains. The presence of glutamyl aminopeptidase activity in meningococci has been found useful^{15 16} and can aid the identification of maltose negative meningococci. Recently, kits detecting preformed enzymes have become available, some of which also identify *Haemophilus*. Results are obtained within four hours and are compared with identification profiles stored in a database. The final choice of a gonococcal identification system will be influenced not only by the efficiency of the test but also by the cost and workload of isolates.

The methods for diagnosis of gonorrhoea described above needs two to three days to achieve. In many clinics, however, particularly in large cities with transient populations, it would be advantageous to diagnose and treat gonorrhoea at the patient's first visit. In men with urethral discharge the Gram stain has been used to diagnose gonorrhoea for many years and gives a sensitivity of >98% compared with that of culture.^{17–19} Other antigen detection systems such as enzyme linked assays (Abbott Laboratories),^{18–22} immunofluorescence (Syva),²³ and DNA probes²⁴ can equal the sensitivity in this population but are more complex, time consuming, and expensive.

The Gram stain, however, rarely gives a higher sensitivity than 40–50% in women,^{17–19} in whom

Table *Gonococcal plasmids*

Plasmid size (megadaltons)	Function
2.6	Unknown
3.2	Penicillinase production
4.4	Penicillinase production
24.5	Conjugative
25.2	Conjugative with high level tetracycline resistance

diagnosis is usually made after culture of the organism. Attempts to increase the sensitivity by staining smears with fluorescence labelled polyclonal antibody have been unsuccessful. A monoclonal antibody fluorescence test has been shown to be highly specific with a sensitivity in women of 80%.²³ Although it is a considerable improvement on the Gram stain, this reagent is not yet commercially available.

The evaluation of the Gonozyne ELISA method in women has also shown a noticeable improvement over Gram staining in the sensitivity (78–100%), but the specificity has been found to be variable (70–100%).^{18–22} The number of false positive results that this would produce, particularly in low prevalence populations,²² has led to concern and consequently limited the use of the test.

The potential for monoclonal antibodies to provide the sensitivity and specificity needed in a direct rapid test for gonorrhoea should encourage the evolution of antigen detection systems in the next few years. Cost will, however, remain an important factor, particularly where levels of antibiotic resistance make culture of the organism necessary for sensitivity testing.

Typing and epidemiology

Good reproducible typing systems with sufficient discrimination are essential for effective epidemiological work on bacterial infections. In this, as in so many other aspects, the gonococcus has provided a formidable challenge. Standard typing methods such as phage typing, simple serotyping with polyclonal reagents, or bacteriocine typing, are of no value. Antibiotic sensitivity patterns can be of some small value in particular situations, but are of less use generally.

The following techniques have been used widely for typing gonococci: auxotyping; plasmid characterisation; and serology, using polyclonal and monoclonal antibodies directed against gonococcal outer membrane protein I.

Auxotyping was the first method used extensively to type gonococci. Introduced by Catlin in 1973,⁶ it entails the determination of stable nutritional requirements of gonococci grown on a chemically defined medium. The most common and important auxotypes are wild type (prototrophic or requiring no

additives); proline requiring (Pro⁻); arginine requiring (Arg⁻); and arginine, hypoxanthine, and uracil requiring (AHU).

Auxotypes correlate well with certain patterns of gonococcal infection, geographical origin of isolates, and antibiotic sensitivity.²⁵ AHU strains are associated with disseminated gonococcal infection, serum resistance, asymptomatic genital infection and sensitivity to antibiotics.²⁶ Gonococci carrying the 4.4 megadalton (MD) penicillinase coding plasmid tend to be wild type or Pro⁻, while those carrying the 3.2 MD penicillinase plasmid tended to be Arg⁻.²⁷ The spread of these plasmids all over the world has been accompanied by a blurring of these clear auxotype patterns. Occasionally, unusual auxotypes have appeared and spread among gonococcal isolates in a particular region.²⁵

While auxotyping is reproducible, it is technically demanding and time consuming and therefore impractical for most diagnostic laboratories. By itself the discrimination provided by auxotyping is limited because only four of 35 types described are common. It is therefore not suitable for fine epidemiological work.

Plasmid analysis came to the fore with the appearance of penicillinase producing *Neisseria gonorrhoeae* (PPNG) in 1976. The table shows the main gonococcal plasmids and their characteristics. The nature of the 2.6 MD plasmid is unknown, but it is in most gonococcal isolates. The 3.2 and 4.4 MD plasmids, which only occur in PPNG, are closely related genetically, but appeared simultaneously in different parts of the plasmid. The 24.5 MD conjugate plasmid occurs in both PPNG and non-PPNG. As it uses simple extraction techniques, plasmid analysis is not difficult but again is not suitable for routine diagnostic use. Plasmids are not stable gonococcal characteristics, however, discrimination is poor, and this limits their value as epidemiological tools.

Serology is the third main typing system. A major gonococcal cell wall antigen is the outer membrane protein I, a porin. Wang *et al*²⁸ originally used a microimmunofluorescence system with antibodies against protein I to divide gonococci into three groups (A, B, and C). Sandstrom and Danielsson²⁹ then used coagglutination to divide gonococci into serological groups WI, II, and III (which corresponded to Wang's groups A, B, and C).

Sandstrom, Chen, and Buchanan³⁰ showed that protein I could be split by peptide mapping into two main antigenic types, IA and IB (corresponding to WI and WII/III, respectively). Tam *et al*¹⁰ have raised a series of monoclonal antibodies to epitopes on protein IA and IB. A standard panel of 12 such antibodies was developed by Knapp *et al*³¹ to provide a system of 18 IA and 28 IB gonococcal serovars. This

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system can be used in conjunction with auxotyping, giving further division into auxotype/serovar (A/S) classes. The use of two stable and genetically distinct characteristics enhances this discrimination.

Thus over seven years gonococcal serology has progressed from a simple polyclonal system with poor discrimination to a highly developed monoclonal system, which for the first time has the potential for detailed epidemiological use. Despite the limited discrimination of the polyclonal WI WII/III system Bygdeman *et al* were able to use it to examine epidemiological patterns of gonococcal disease and associations between serology and antibiotic resistance.^{32 33} Until recently, the monoclonal reagents needed for this typing system have not been generally available. Arrangements have now been made for their distribution to interested laboratories. The coagglutination system used is simple and sensitive, protein I types are stable, and the system is reproducible and provides good discrimination. Likely applications are the study of collections of strains from different parts of the world, temporal changes in strains from a particular area, the distinction between treatment failures and reinfection, and the use in forensic cases of sexual abuse.

Although auxotyping and serotyping, backed up in some cases by plasmid analysis, are likely to be the main methods of gonococcal typing in the future, another approach has been tried. Plant and animal lectins recognise specific carbohydrate structures on the bacterial surface. Patterns of lectin agglutination can be used to differentiate between bacterial species and bacterial strains within a species. Such patterns have been used to type gonococci.³⁴ There seems to be a good correlation between this method and gonococcal serology, though whether it offers anything more than serology remains to be seen.

Antibiotic resistance

Very early in the development of penicillin during the Second World War, its efficacy against gonorrhoea was exploited. Venereal diseases such as gonorrhoea were considered to be a major problem by army authorities, and penicillin was soon being used to treat gonorrhoea, as well as more serious wound infections. Since then the widespread use and abuse of β lactam producing agents and other antibiotics, combined with increasing ease of global travel, have stimulated and facilitated the development and dissemination of antibiotic resistant gonococci.

Gonococci are naturally sensitive not only to penicillin (minimum inhibitory concentration (MIC) of <0.06 mg/l), but also to tetracycline. By 1958 Reyn, Horner, and Bentzon³⁵ were able to show an increase in resistance to penicillin in some strains of

over 20 times that of 1944 levels. In some parts of the world, notably the Far East and Africa, this increase in resistance to penicillin developed rapidly. Keys, Halveson, and Clarke³⁶ found 74% of Far Eastern gonococci to have reduced sensitivity to penicillin (MIC >0.06 mg/l), while Arya and Phillips³⁷ reported similar levels of penicillin resistance in gonococci from Uganda. In contrast, few strains isolated in Europe or North America at this time showed this trend.

CHROMOSOMALLY MEDIATED GONOCOCCAL RESISTANCE

The rate at which penicillin MICs increased in this form of resistance was slow and could be countered by increasing the dose of penicillin or ampicillin and by adding probenecid. Nevertheless, by the early 1970s gonococcal strains with MICs of >1.0 mg/l that were resistant to penicillin were being found in the Far East. Antibiotic misuse, prostitution, and local wars with large numbers of servicemen in the area were all responsible. Resistant strains were exported to Europe and the United States by servicemen and business travellers. Until recently, however, these did not constitute a major problem outside the Far East.

Up to 1976 gonococcal resistance to antibiotics was exclusively chromosomally controlled (chromosomally mediated resistant *Neisseria gonorrhoeae* or CMRNG). The mechanism of resistance in CMRNG has been studied mainly by Cannon *et al*.³⁸ Mutations at various loci on the gonococcal chromosome each produce small increments in resistance to agents such as penicillin, tetracycline, and chloramphenicol. Mutations at some loci are specific for resistance to a particular antibiotic (*pen A* for penicillin). At other loci (*pen B*, *mtr*) mutations result in non-specific increases in resistance to a range of antibiotics (penicillin, tetracycline, erythromycin, chloramphenicol) and hydrophobic molecules. Strains with clinically important levels of penicillin resistance (>1.0 mg/l) contain multiple mutations and almost invariably show reduced sensitivity to other antibiotics. This whole area has recently been well reviewed by Sparling and Cannon.³⁸

Spectinomycin is an antibiotic with excellent activity against penicillin sensitive and resistant gonococci. In common with streptomycin, resistance to spectinomycin in the gonococcus is controlled by the chromosome. Mutations giving rise to spectinomycin resistance have been described in strains isolated from the Philippines, the United Kingdom, and Korea.^{39 40} At present spectinomycin resistance does not seem to pose a major clinical problem. Given the value of spectinomycin for treating penicillin resistant gonococci, however, it is important to monitor this

where spectinomycin is being used extensively. The mechanisms of chromosomal resistance to some antibiotics are known. With penicillins, resistance is associated with reduced affinity for penicillin binding proteins 1 and 2 and outer membrane permeability barriers.⁴¹⁻⁴² With streptomycin and spectinomycin, resistance results from changes to the 30S ribosome. For others, such as tetracycline, the mechanism remains unclear.

The appearance of penicillinase producing *Ngonorrhoeae* (PPNG) in 1976 overshadowed CMRNG, particularly in the United States and Europe. Recently there has been a resurgence of interest in CMRNG: several outbreaks of infection have been reported in the United States.⁴³ In London between 1981 and 1985, we saw a noticeable increase in CMRNG, although this may not be the case in the United Kingdom generally.⁴⁴

An increase in infections caused by CMRNG presents several practical problems. First, there is no simple rapid way of detecting CMRNG, short of quantitative sensitivity tests. Second, antibiotics active against PPNG may not necessarily be effective against CMRNG. With these strains, there does seem to be some cross resistance between penicillin and cephalosporins and cephamycins such as cefuroxime and cefoxitin. It remains to be seen whether this will affect the more active β lactam producing agents such as cefotaxime and ceftriaxone. Similarly, antibiotic combinations containing amoxycillin or ampicillin with a β lactamase inhibitor such as clavulanic acid or sulbactam will be ineffective against CMRNG.

CMRNG do seem to have certain characteristic biological features. Serologically their outer membrane protein I is of the IB (WII/III) group, while the proline requiring (Pro⁻) auxotype is also predominant.⁴³⁻⁴⁵ Characterisation of CMRNG from various parts of the world using the serovar test with the more specific and discriminating single monoclonal reagents now available needs to be done. The precise clinical importance of these associations is still not clear, but it is an important area for future work.

In considering chromosomal control of antibiotic resistance in the gonococcus, there is one peculiarity that could be of clinical importance. Mutations at the *mtr* locus can result in non-specific antibiotic resistance. A further mutation at the *env* locus (which normally occurs in *mtr* containing strains) results in hypersensitivity to a range of antibiotics.⁴⁶ These include vancomycin. *Env* containing strains, which may comprise up to 10–30% of all gonococci in some areas, may not grow on selective gonococcal media that contain vancomycin.²⁻⁴⁷

PLASMID MEDIATED GONOCOCCAL RESISTANCE
The whole position of gonococcal resistance to peni-

cillin was changed by the first reports of PPNG in England and the United States in 1976.⁴⁸⁻⁵⁰

The enzyme in PPNG is a TEM-1 type β lactamase. Two small plasmids have a role: a 3.2 MD plasmid and a 4.4 MD plasmid. The former was first described in strains originating from West Africa, the latter in strains from the Philippines. DNA hybridisation has shown that these R plasmids have a non-transposable region homologous to part of the transposon TnA coding for β lactamase. TnA sequences are also found in plasmids coding for TEM-1 in *Haemophilus*. The 3.2 MD gonococcal plasmid represents a deletion of non-TnA elements from the larger plasmid.⁵¹

PPNG with the 3.2 MD plasmid are characteristically arginine requiring (Arg⁻) and sensitive to unrelated antibiotics like tetracycline. In contrast, strains with the 4.4 MD plasmid are either prototrophic or Pro⁻ and relatively resistant to these drugs. Both these gonococcal plasmids are incapable of conjugate transfer. A 24.5 MD plasmid, however, found in gonococci as early as the 1940s is capable of mobilising the β lactamase plasmids, not only into other strains of *Ngonorrhoeae* but also to other *Neisseria*.⁵¹

Beta lactamase production in PPNG can be detected rapidly and simply by iodometric tests or by the nitrocefin method. PPNG are highly resistant to penicillin and ampicillin. Second and third generation cephalosporins and cephamycins, such as cefotaxime, cefoxitin, and ceftriaxone, are effective for PPNG infections, as is spectinomycin. Newer agents such as the monobactams and 4 quinolones show promise. Combinations of ampicillin or amoxycillin with β lactamase inhibitors such as clavulanic acid and sulbactam are effective against PPNG, but their limitations against CMRNG have already been stated.

Between 1976 and 1980 most infections with PPNG in both Britain and the United States were imported. There was little evidence of indigenous spread. The number of PPNG infections reported rose slowly but steadily. After 1980, however, this situation changed dramatically. In Britain the prevalence of PPNG infections rose rapidly between 1980 and 1982, with evidence of indigenous spread.⁵² Strains carrying the 4.4 MD plasmid were mainly responsible. This rise reached a peak in 1983, followed by a reversal in 1984.⁴⁴⁻⁵³ It is still not certain whether this check is a temporary phenomenon or whether the rise in the prevalence of PPNG infections in Britain is over. In other European countries, notably the Netherlands, there were similar sharp rises in prevalence of PPNG. There the predominant strains carried the 3.2 MD plasmid.

In the United States prevalence of PPNG also rose sharply, with endemic foci in many large cities. In

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Miami during three months in 1986, PPNG accounted for half of all reported cases of gonorrhoea.⁵⁴ In the Far East such levels were already common. PPNG has not been a serious problem in homosexual men.⁵⁵

The speed with which this type of gonococcal resistance to penicillin has spread illustrates the tremendous potential for spread of plasmid mediated, as opposed to chromosomally mediated, resistance. In 1985 a new phenomenon of high level plasmid mediated resistance to tetracycline was described; strains had MICs of >16 mg/l. These strains contain a 25.2 MD plasmid coding for tetracycline resistance, which probably arose as a recombinant between the 24.5 MD conjugal plasmid and the *tet M* determinant from streptococci. This determinant has been found in other genital tract species.⁵⁴ The 25.2 MD plasmid can be transferred to sensitive strains either by transformation or conjugation. The true prevalence of these strains in the United States is unknown. There are as yet no reports of similar strains elsewhere in the world. We have screened several hundred strains of gonococci for high level resistance to tetracycline but have found none. Although tetracycline has been used extensively for the treatment of gonorrhoea, increasing low level resistance, particularly among the serogroup 1B strains common in the Far East, has limited its usefulness. It remains, however, the antibiotic of choice for other common genital tract pathogens such as *Chlamydia trachomatis*. Even if not used to treat gonorrhoea, tetracycline clearly can still exert a strong influence over a wide range of genital organisms.

The gonococcus presents a continuous challenge to the continued use of β lactam producing antibiotics including the new cephalosporins, to tetracycline, erythromycin, and chloramphenicol, and to a lesser degree, spectinomycin. CMRNG is difficult to monitor. Tetracycline resistance and spectinomycin resistance of the high level type present similar difficulties in screening and detection. Even with new highly promising agents such as the quinolones there is already at least one report of gonococcal resistance. As far as chemotherapy is concerned the gonococcus continues to present a substantial challenge.

Pathogenicity

N. gonorrhoeae primarily infects the mucosa of the lower genital tract—the urethra in men, and the endocervix in women. Infection of the rectum and pharynx, however, also occurs, particularly in homosexual men, while conjunctival infections occur mainly in neonates. In a few patients complicated or disseminated gonococcal infection can develop, and present

with salpingitis, bacteraemia, or arthritis. Sterility and ectopic pregnancy are long term sequelae of salpingitis, particularly in those parts of the world where treatment is inadequate or unavailable.

Gonorrhoea remains a major cause of sexually transmitted infections, even in those countries that can provide good chemotherapy and have resources for contact tracing. The gonococcus is a highly adapted pathogen that has acquired or developed antibiotic resistance and which has surface structures that can undergo phase and antigenic variation.⁵⁶ Both mechanisms ensure the continuing high prevalence of gonorrhoea. The failure of current control measures has resulted in considerable interest and research into a gonococcal vaccine as an alternative to or supplement for chemotherapy, to be used mainly in high risk populations. It was recommended by a National Institutes of Health panel⁵⁷ that such a vaccine should be directed at the prevention of pelvic inflammatory disease, with mucosal immunity in men a secondary aim. An understanding of the pathogenesis and immunology of gonococcal infection is an essential step in choosing a candidate for a vaccine. Over the past decade this need has stimulated a considerable amount of study into the basic mechanisms of gonococcal host interactions at mucosal surfaces.

Infection by gonococci has been shown to occur in two phases. First, attachment to the mucosa, and second, invasion of the epithelial cell. The initial step of attachment is that the gonococcus and epithelial cell are brought into close proximity. Both bacterial and host cell are negatively charged, and non-specific factors such as pH, surface charge, and hydrophobic interactions may be important in achieving the association. These factors have been reviewed extensively by Watt and Ward.⁵⁸ Specific gonococcal surface structures, primarily pili, and the outer membrane protein PII then mediate attachment to the epithelial cell. In human fallopian tube culture the gonococci attach to non-ciliated epithelial cells.⁵⁹ The ciliated cells are damaged, which may be due to the toxic effects of gonococcal lipopolysaccharide⁶⁰ and slough off. After attachment the gonococci appear to be taken into the cell, where they multiply and are eventually excreted into the lamina propria, setting up infection. This apparent endocytosis of gonococci may be mediated by the outer membrane protein PI.

The outer membrane of the gonococcus is exposed to the environment and the host's immune response and carries the major virulence factors. Its structure is similar to many other Gram negative organisms in that it consists of a phospholipid bilayer that is attached on the inside to a thin layer of peptidoglycan. Embedded in the membrane are proteins (of which there are three major proteins) and lipopolysaccharide. Pili extend from the outer membrane.

PILI

Pili, the primary mediators of attachment, have received the most attention as a potential vaccine candidate. Pili are proteinaceous surface appendages, 6 nm in diameter and 1–4 μ m in length, comprising up to 10 000 identical protein subunits termed pilin. Piliated gonococci have been shown to adhere to sperm,⁶¹ red blood cells,⁶² and a variety of human epithelial cells,^{58–63} including endocervical and fallopian tube mucosal cells.⁶⁴

Pili are associated with the distinctive colonial types (1 and 2), new clinical isolates, and the ability to infect male volunteers.^{65–66} Non-piliated colonies occur with repeated subculture and produce colony types (3 and 4), which are less infective. The switch from piliated (P^+) to non-piliated (P^-) occurs with high frequency, but the reverse, P^- to P^+ , occurs less frequently (phase variation). Pili also exhibit antigenic variation; up to eight antigenically different pili have been isolated from a single strain.^{67–68} Expression of different types of pili may be forced *in vivo* to enable the gonococcus to evade the host's immune response, but there is also some *in vitro* evidence that they confer specificity for different mucosal surfaces.⁶⁹

Functional and antigenic domains of gonococcal pili have been determined and show that the fragment containing the amino acid sequence which mediates adherence is highly conserved and common between different isolates.⁷⁰ The sequence responsible for antigenic variation, however, is normally immunodominant. Despite a detailed knowledge of pilus structure and function a successful vaccine has yet to be produced. A clinical trial in which purified pili from a single strain was given parenterally failed to protect a large number of men.⁷¹ Antibody was produced in these men but presumably was not specific for the conserved part of the molecule. Recent work has shown that synthetic peptides spanning common, normally immunorecessive regions of the pilus, will produce polyclonal antibody that can inhibit attachment of both homologous and heterologous gonococci to epithelial cells *in vitro*.⁷² This finding suggests that a vaccine to a common pilin peptide might be effective.

OUTER MEMBRANE PROTEINS (OMP)

Protein I is the most dominant of the outer membrane proteins and is present in all strains. For this reason it has been the second candidate for a vaccine and has been used in a small number of studies. It remains of interest because Buchanan *et al*⁷³ showed that women with salpingitis were not reinfected with strains with the same protein I serotype. The function of this protein in gonococcal infection is now thought to be two-fold. It has been shown to act as a porin, probably

when complexed with protein III, and hence allows water soluble nutrients essential to gonococcal metabolism to enter the cell. More recently it has been shown that protein I has the ability to transfer from the gonococcal membrane into erythrocyte ghosts and lipid bilayers.⁷⁴ It is possible that this insertion into eukaryotic membrane is important in the internalisation of gonococci by epithelial cells.

Protein I remains an important vaccine candidate because it is a major constituent of the outer membrane, about 60% of the total weight. There has also been considerable research, into the development of a serological classification of gonococci, using a series of monoclonal antibodies raised to two epitopes of the protein I molecule PrIA and PrIB.¹⁰ It is hoped that this technique will give better discrimination than previous typing schemes and that international dissemination of the technique will provide useful information regarding the distribution and prevalence of different serotypes and their association with clinical syndromes and antibiotic resistance. Such information may prove essential in the choice of strains to provide vaccines.

The expression of the outer membrane protein PII has been shown to enhance the attachment of gonococci to epithelial cells *in vitro* and for this reason has stimulated much interest in recent years. Protein II is a family of heat modifiable proteins with apparent subunit molecular weight between 24–32 000 daltons.⁷⁵ A single strain of gonococci can express none to six different PIIs, each of which is antigenically distinct. Expression of PII is subject to both phase and antigenic variation.⁵⁶ In contrast to pili, PII shows high frequency switching between PII^+ and PII^- in both directions. The presence of PII has been associated with opaque colonies, and its absence with transparent ones. Colonies isolated from uncomplicated infection in men and from the endocervix at certain times of the menstrual cycle are opaque, whereas colonies isolated from disseminated infection and the endocervix during menstruation are transparent.^{76–77} The inference from these observations is that the lack of expression of PII in some way facilitates invasion to cause systemic disease. The antigenic diversity shown by this family of proteins could help the gonococcus to evade the immune response. Different PIIs were detected in serial isolates of contact pairs⁷⁸ and from an epidemic caused by a single strain.⁷⁹ This suggests that again the gonococcus is adapted to a hostile environment by producing different antigenic variants. Another possibility is that individual PII species exhibit the ability to adhere to different mucosal surfaces⁸⁰ and hence may enable gonococci to survive in a number of environments within the host. Although PII has been associated with adherence, susceptibility to killing by normal

human serum, and interaction with polymorphs, its antigenic diversity has limited its potential as a candidate for vaccine. The unique peptides of PII are surface exposed and immunogenic, but although PIIs from different strains share many common peptides, they are believed to be buried in the gonococcal membrane.⁸¹ If this region of the molecule could be shown to produce antibody in the host which will interfere with part of the infective process it might be given more serious consideration as a candidate for a vaccine.

IgA1 protease

IgA protease is an enzyme produced by the pathogenic neisseriae *N gonorrhoeae* and *N meningitidis*, but not commensal neisseriae. Its function is to split antibodies of the IgA1 subclass, and is therefore believed to be a virulence determinant. Although its role in the pathogenesis of gonorrhoea is still unconfirmed, it remains a potential candidate, to be used probably in conjunction with a pilus or protein I vaccine.

Despite the failure of current clinical trials the search for a vaccine continues. The ideal candidate would have conserved antigenic components common to all gonococci. Protein III is an outer membrane protein found in all strains tested, and peptide mapping has shown that its structure is conserved.⁸² Cannon *et al*⁸³ also detected an antigen, H8, which is found in pathogenic but not in commensal neisseriae. Interest now focuses on proteins that are expressed by phenotypes influenced by the growth environment, some of which may be present *in vivo*. Proteins expressed under iron limiting^{84,85} and anaerobic conditions⁸⁶ have been shown to be expressed immunogenically in gonococcal infections.

Conclusions

In the past 10 years we have seen the appearance of PPNG and the establishment of CMRNG in new parts of the world. Much progress has been made in understanding the biology and pathogenicity of the organism, but we still have no vaccine, but the new antibiotics that can treat and help control gonorrhoea are expensive and may therefore not be widely available in many parts of Asia and Africa where infection is rife.

Against this, new antibiotics have been developed with good activity against gonococci. A potentially good typing system is now available, which may allow epidemiological studies to be done with a good microbiological base. Controlling the spread of resistant organisms requires good epidemiology as well as effective antibiotics. Rapid identification systems, particularly for detecting gonorrhoea in women, are

now a real possibility, and we have a range of identification systems.

There has been real progress, but the versatility of the gonococcus is likely to prove a continuing challenge for both basic and clinical research workers.

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