SHORT COMMUNICATION

Structural Variations in Pili Expressed during Gonococcal Infection

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Gonococci were cultured from the urethra of male patients and from the cervix and urethra of their female partners. SDS-PAGE of cell lysates from within each group of consorts showed that outer membrane protein I remained constant but considerable variations were seen in the apparent molecular weight of protein II. Pili were purified from the isolates of some groups of consorts. In each case the pili expressed by the isolates from the female cervix and urethra differed in subunit molecular weight and were usually also distinct from the pili expressed by the isolate from the male partner.

INTRODUCTION

Recent studies have demonstrated that multiple antigenic shifts may occur in the surface proteins of pathogens which prevent the host from mounting an effective immune response (Doyle *et al.*, 1980; Barbour *et al.*, 1982). In laboratory culture of a single gonococcal isolate two major surface components responsible for virulence, pili and outer membrane protein II show comparable antigenic variation.

Variants selected by differences in colony opacity when grown on conventional media (Swanson, 1978; Lambden & Heckels, 1979), or following the application of an *in vivo* selection pressure by growth in animals (Lambden *et al.*, 1981), show differences in subunit molecular weight of both pili and outer membrane protein II. In a single strain up to four pilus and six protein II types have been identified. Although peptide mapping reveals considerable structural similarities between these variant pili there is only limited antigenic cross-reactivity (Lambden, 1982). Similarly the protein II species appear to form a family of structurally related but antigenically distinct molecules (Diaz & Heckels, 1982). Such intra-strain antigenic variations have been associated with alterations in the adhesive properties of the organisms and their resistance to host defences (Lambden *et al.*, 1979).

In this study the surface protein profile of gonococcal isolates of the same strain found at different sites in the same patient and of isolates obtained from their consorts were compared.

METHODS

Gonococcal isolates. Patients attending the Genito-Urinary Medicine Clinic at Basingstoke District Hospital, who were suspected of having gonorrhoea were invited to take part in this study. Samples were taken with a cotton wool swab from the urethra of males and from the urethra and cervix of females. Samples were plated directly on to a non-selective colony typing medium as described previously (Lambden *et al.*, 1979) but with the addition of uracil (8 µg ml⁻¹) and hypoxanthine (3.2 µg ml⁻¹) to the medium. Other sites were not sampled since they were unlikely to yield sufficiently pure growth of gonococci on non-selective media.

Fig. 1. SDS-PAGE of whole cell lysates of *N. gonorrhoeae* obtained from one group of consorts. M, isolate from male urethra; F, isolate from female; CX, cervix; UR, urethra. Samples were derivatized at 100 °C (a) and 37 °C (b) and protein II identified by change in mobility is shown by the stars. Numbers denote molecular weight ($\times 10^{-3}$) of standard proteins.

Plates were incubated for 18–24 h in 5% (v/v) CO₂ at 37 °C. Colonies were examined with a stereomicroscope adjusted to differentiate between transparent and opaque colonial types (Lambden & Heckels, 1979). An almost pure growth of gonococci was obtained frequently and colonies were then scraped from the plate and stored in liquid nitrogen. In some specimens, usually from the female urethra, contaminating colonies were present. In this case 10–20 gonococcal colonies were randomly picked off and sub-cultured once on the same medium before storage in liquid N₂. All isolates were subsequently confirmed as gonococci by Gram stain, oxidase test and sugar fermentation tests and the auxotype was determined by growth on a defined medium lacking individual nutrients.

Pili preparation and SDS-PAGE. Pili were purified by blending gonococci in 0.15 M-ethanolamine/HCl, pH 10.5, followed by repeated cycles of precipitation with (NH₄)₂SO₄ and disaggregation in ethanolamine (Lambden *et al.*, 1981). Whole cell lysates, purified pili and outer membranes prepared as described by Lambden & Heckels (1979) were subjected to SDS-PAGE on linear gradients of 10–25% (w/v) acrylamide (Heckels, 1981).

Protein I serotyping. Protein I serotyping was performed by coagglutination as described by Tam *et al.* (1982) with reagents kindly supplied by Dr E. Sandstrom, Karolinska Institute, Stockholm.

Fig. 2. SDS-PAGE of purified pili from three groups of consort (a, b and c). M, isolate from male urethra; F, isolate from female; CX, cervix; UR, urethra. Numbers denote molecular weight ($\times 10^{-3}$) of polypeptides observed.

RESULTS AND DISCUSSION

Complete sets of isolates were obtained from seven groups of consort (each containing two to four individuals) and from the cervix and urethra of two additional female patients for whom no consort isolate was available. Three criteria were used to attempt to eliminate the possibility of separate infections with two or more strains. Within each group studied the isolates showed identical auxotype, outer membrane protein I serotype and molecular weight.

SDS-PAGE of whole cell extracts (Fig. 1) showed an almost identical pattern of protein bands within each group but variations were seen with bands in the region 27 000–30 000 molecular weight and 17 000–21 000 molecular weight. The former showed the molecular weight and heat modification characteristics of outer membrane protein II (Lambden & Heckels, 1979). The second variation was seen in the region 17 000–21 000 molecular weight characteristic of pili, although pili were difficult to unequivocally identify in whole cell extracts. Pili were therefore purified from three groups of isolates. SDS-PAGE of the pili is shown in Fig. 2. Although no consistent pattern was apparent, in most cases a single band predominated in each isolate but considerable variations were seen in the molecular weight of isolates from different sites within a group. In each group the molecular weight of the predominant pilus band differed between isolates from the cervix and urethra of the same female (Fig. 2) and this was a general phenomena in isolates from several other female patients (data not shown). Certain male isolates showed some similarities with pili from their female consort but there was no consistent relationship. Three equally intense bands were seen in the isolate from one female cervix (Fig. 2c) and it is not known whether this is due to one variant with pili made from different subunits, expression of different pili on a single variant or concurrent infection with three variants of the same strain bearing different homogeneous pili. However, the isolate from this site did appear homogeneous on the basis of colonial morphology.

Although many studies have shown gonococci to be capable of considerable antigenic variation during laboratory growth, the evidence for similar changes during human infection is limited. James & Swanson (1978) demonstrated a decrease in the occurrence of outer membrane protein II in isolates taken from women during the luteal phase of the menstrual cycle. Novotny and Cownley (1980) used immune electron microscopy to detect serological differences between pili expressed on isolates grown from different anatomical sites in some groups of consorts. Tramont *et al.* (1979) observed a change in pilus molecular weight on re-infection of a patient from the same primary source.

The results reported above suggest that variation of pili during growth *in vivo* is common and perhaps the norm. The variation could not be correlated with the phase of the menstrual cycle since most of the females were receiving oral contraceptives. Also the consistent difference in pili on isolates cultured at the same time from cervix and urethra shows that additional factors must influence pilus expression. Since variations *in vitro* have been associated with alterations in the antigenic and adhesive properties of the organisms the occurrence of such variations during the natural infection suggests an important role in adaptation to *in vivo* growth and in the pathogenesis of gonorrhoea.

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