SHORT ARTICLE

ABSENCE OF BACTERICIDAL ANTIBODIES AGAINST GROUP-I LIPOPOLYSACCHARIDE DETERMINANTS OF *NEISSERIA GONORRHOEAE* DURING HUMAN INFECTION

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SUMMARY. None of 34 sera from patients with gonorrhoea contained antibodies bactericidal for strains of *Neisseria gonorrhoeae* with Group-I lipopolysaccharide (LPS). All contained antibodies against a strain with Group-II LPS, as do sera from uninfected controls. The absence of Group I-LPS antibodies in infected humans contrasts with previous findings that mice immunised with strains from either of the LPS groups produced bactericidal antibody to Group I. Our hope that detection of antibodies to Group-I strains would provide a screening test for gonorrhoea was, therefore, not realised.

INTRODUCTION

Gonococcal isolates from uncomplicated genital infections and disseminated infections have been divided into two broad groups based upon their pyocin-sensitivity spectrum. This classification has been shown to correlate with interactions of strains from localised genital infections with both humoral and phagocytic components of the human immune system. Briefly, gonococcal strains sensitive to pyocins of Kageyama's group R5, lipopolysaccharide (LPS) Group I, are resistant to normal human serum and they bind in lower numbers to polymorphonuclear leukocytes than strains of Group II (Blackwell *et al.*, 1983). This second group is sensitive to pyocins of Kageyama's group R1 in addition to R5 and strains of this group are killed by the majority of normal human sera tested (Kageyama, 1975; Blackwell, Young and Anderson, 1979; Winstanley *et al.*, 1983). Strains of Group II are thought to have extra LPS components, absent or cryptic in strains of Group I, to which there are bactericidal antibodies in the majority of normal human sera (Schneider *et al.*, 1982; Winstanley *et al.*, 1983).

Earlier studies revealed that specific antisera raised in mice against strains of Group I or II were bactericidal for other strains of the homologous group. They were also active against strains of the heterologous group (Winstanley *et al.*, 1983). In that study we also screened sera from a control population of women attending a postnatal clinic for bactericidal antibodies to both groups of gonococci. Antibodies against Group II only were found. This is a population in which there is a very low incidence of gonorrhoea. We have now screened sera from individuals with gonorrhoea for bactericidal activity against strains of LPS Groups I and II. Our hypothesis was that individuals exposed to or infected with *N. gonorrhoeae* would, like the mouse model, have antibodies to both groups and that detection of bactericidal activity for Group I might be used as a serological screening test for gonococcal infection.

MATERIALS AND METHODS

Bacteria. Strains of LPS Group I (E757 and M9131) and Group II (P280) were isolated from patients attending the Department of Genitourinary Medicine, Royal Infirmary, Edinburgh. All were from localised genital infections (Winstanley *et al.*, 1983). They were

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cultivated on Modified New York City Medium (MNYC) (Young, 1978) at 37°C in a humidified atmosphere enriched with 10% CO₂.

Assay for bactericidal antibodies. Bactericidal tests were performed as described by Winstanley *et al.* (1983) and as outlined below.

Sera taken for syphilis serology from patients attending the Department of Genitourinary Medicine were obtained from Dr H. Young, STD Diagnostic Laboratory, Department of Bacteriology, University of Edinburgh. Human AB serum from a donor with no history of gonococcal infection was used as the source of complement. It was absorbed for 24 h at 4°C with a live suspension of the gonococcal strains used in the study, in order to remove cross-reacting antibodies, and was distributed in 200- μ l portions and stored at -20°C before use. The haemolytic titre of this complement source was 32-64. An 18-h culture of each gonococcal strain was diluted in Dulbecco's phosphate buffered saline, supplemented with Mg⁺⁺ and Ca⁺⁺ ions (0.5mmol and 0.9mmol respectively), to give *c.* 10⁴ cfu/ml. Twofold dilutions (50 μ l) of the sera to be tested were made in microtitre plates and to each was added 40 μ l of the gonococcal suspension and 10 μ l of the human complement source. After incubation at 37°C for 30 min, the numbers of viable organisms in each well were determined by culturing three 20- μ l drops from each on MNYC. The bactericidal titre of a serum was the highest dilution that caused a reduction in viable count of 80%, compared with that of a control to which no complement was added.

RESULTS

The titres of bactericidal antibody in sera from 22 patients with gonorrhoea against strains E757 (Group I) and P280 (Group II), are shown in the table. Similar results were obtained with a second set of 37 sera from the STD Diagnostic Laboratory; the patients' diagnoses were not known at the time the sera were tested. They were screened for bactericidal activity against strain P280 and Group-I strain M9131. Amongst these sera, 12 were from patients with gonococcal infection and 25 from patients from whom no neisseriae were isolated. There were no differences in titres of bactericidal antibodies to strain M9131 between sera from the infected

TABLE. *Titres of bactericidal antibody against gonococcal strains E757 (Group I) and P280 (Group II) in sera from patients with gonorrhoea.*

Patient No.	Titre of antibody to strain	
	E757	P280
1	<4	>128
2	<4	>128
3	<4	64-128
4	4	64
5	<4	64
6	<4	32-64
7	<4	64-128
8	<4	32-64
9	<4	32-64
10	<4	64-128
11	4-8	64-128
12	<4	32-64
13	<4	>128
14	<4	>128
15	<4	64-128
16	<4	16
17	<4	64
18	<4	>128
19	<4	128
20	<4	>128
21	4	>128
22	<4	>128

and uninfected patients; titres were ≤ 4 . The titres to strain P280 were similar to those recorded in the table.

DISCUSSION

The absence or extremely low titres of bactericidal antibodies against strains M9131 and E757 in sera from patients with gonorrhoea refutes our hypothesis, based on results obtained with immune mouse sera (Winstanley *et al.*, 1983), that infection with *N. gonorrhoeae* in man would result in the production of antibodies bactericidal for strains of Group I. This failure may result from the stimulation by Group-I antigens of antibodies of classes that do not activate the classical complement pathway, e.g., classes IgA and IgG4, or by failure of the human immune system to respond to antigens of the gonococcal LPS associated with the binding site for R5 pyocins.

It has been suggested recently that susceptibility and resistance to gonorrhoea of a group of Chinese prostitutes was associated with differences in the major histocompatibility complex genes. The HLA "haplotype" A11, B15 appeared to be associated with resistance to the disease amongst these women who were at very high risk of exposure to infection (Chan and Rajan, 1982). Similarly, the ability to respond immunologically to LPS antigens associated with the binding site of R5 pyocins might depend upon the genetic constitution of the individual.

Bactericidal antibody is an important host defence that prevents the dissemination of gonococci from the mucosa (Brooks, Ingwer and Peterson, 1978). The majority (c. 85%) of gonococcal strains isolated from localised genital infection are of Group II (Winstanley *et al.*, 1983) and are thought to be prevented from dissemination by "natural" bactericidal antibodies and complement. Strains of Group I, however, being resistant to normal human serum, have a greater predisposition to cause bacteraemia; they are isolated from approximately 42% of disseminated infections (Winstanley *et al.*, 1983).

The apparent inability of man to produce antibodies bactericidal for gonococcal strains of Group I may be a host factor that retards recovery from disseminated gonococcal infection and it may also pose difficulties in the immunoprophylaxis of gonorrhoea. These results indicate that, like other attempts to identify antibodies against gonococcal LPS (Ward and Glynn, 1971 and 1972; Watt, Ward and Glynn, 1971; Maeland and Matre, 1975), screening of sera for bactericidal activity against LPS-group-I strains will not be of use as a diagnostic test.

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REFERENCES

- Blackwell C C, Kowolik M J, Winstanley F P, Kinane D F, Weir D M, Law J A, Chok Y L H W 1983 ABO blood group and susceptibility to gonococcal infection I. Factors affecting phagocytosis of *Neisseria gonorrhoeae*. *Journal of Clinical and Laboratory Immunology* 10:173-178.
- Blackwell C C, Young H, Anderson I 1979 Sensitivity of *Neisseria gonorrhoeae* to partially purified R-type pyocins and a possible approach to epidemiological typing. *Journal of Medical Microbiology* 12:321-335.
- Brooks G F, Ingwer I, Peterson B H 1978 Importance of serum bactericidal and opsonic activity and complement in *Neisseria gonorrhoeae* infections. In: Brooks G F, Gotschlich E C, Holmes K K, Sawyer W D, Young F E (eds) *Immunobiology of Neisseria gonorrhoeae*. American Society for Microbiology, Washington, DC, pp 199-203.
- Chan S H, Rajan V S 1982 HLA—resistance and susceptibility to venereal disease. In: Dawkins R L, Christiansen F T, Zilko P J (eds) *Immunogenetics in rheumatology*, Excerpta Medica, International Congress Series No 602, Amsterdam, p 20.
- Kageyama M 1975 Bacteriocins and bacteriophages in *Pseudomonas aeruginosa*. In: Mitsuhashi S

- Hashimoto H (eds) Microbial drug resistance. University of Tokyo Press, Tokyo, pp 291-305.
- Maeland J A, Matre R 1975 Specificity of human serum antibodies against endotoxin from *Neisseria gonorrhoeae*. British Journal of Venereal Diseases 51:176-178.
- Schneider H, Griffiss J M, Williams G D, Pier G B 1982 Immunological basis of serum resistance of *Neisseria gonorrhoeae*. Journal of General Microbiology 128:13-22.
- Ward M E, Glynn A A 1971 Antibodies to gonococcal lipopolysaccharides in patients with gonorrhoea. Journal of Medical Microbiology 4: p ii.
- Ward M E, Glynn A A 1972 Human antibody response to lipopolysaccharides from *Neisseria gonorrhoeae*. Journal of Clinical Pathology 25:56-59.
- Watt P J, Ward M E, Glynn A A 1971 A comparison of serological tests for the diagnosis of gonorrhoea. British Journal of Venereal Diseases 47:448-451.
- Winstanley F P, Blackwell C C, Weir D M, Kinane D F 1983 Blood groups and susceptibility to gonococcal infection. II. The relationship of lipopolysaccharide type to gonococcal sensitivity to the bactericidal activity of normal human serum. Journal of Clinical and Laboratory Immunology 11:27-32.
- Young H 1978 Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. British Journal of Venereal Diseases 54:36-40.