

Serum immunoglobulin response in uncomplicated gonorrhoea

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SUMMARY Sera from 225 men and 140 women were examined by an indirect immunofluorescent antibody technique for antibody reactive with *Neisseria gonorrhoeae*. Antigonococcal IgM was demonstrated at a titre of ≥ 16 in about 45% of infected, but in only 3% of non-infected, patients. Most of this antibody occurred in sera of patients who had been infected for less than 14 days. Antibody of the IgA class was found at a titre of ≥ 16 in over half the infected, but in none of the non-infected, patients. IgG antibody reactive with the gonococcus was found in each infected patient at a titre of ≥ 16 but in only 8% of controls. The mean log titre of this antibody was significantly higher in patients who had been infected for more than seven days than in those whose infection was of shorter duration.

Introduction

Although gonorrhoea is an infection usually confined to the mucosal surfaces of the body, a systemic antibody response may be demonstrated by various methods such as complement-fixation tests, haemagglutination reactions, and indirect immunofluorescent antibody techniques (Ratnalinga, 1971; Ward and Glynn, 1972; Welch and O'Reilly, 1973). The classes of antibody involved in this response have not, however, been clearly defined other than under experimental conditions (Cohen *et al.*, 1969).

It is the purpose of this paper to record the classes of immunoglobulins reactive with *Neisseria gonorrhoeae* which were found in the serum of patients with naturally acquired uncomplicated infection and to examine the effect of treatment.

Material and method

PATIENTS

Sera were obtained from patients (225 men and 140 women) attending the department of venereology at the Black Street Clinic, Glasgow.

Urethral gonorrhoea in men was diagnosed by microscopical examination of a Gram-stained smear

and confirmed by culture on selective medium. The identity of suspected colonies was confirmed by direct immunofluorescence and sugar fermentation reactions. Material from the urethra was taken for culture from all the male patients and where there had been homosexual contact specimens from the oropharynx and anorectal area were also taken for culture.

Gram-stained smears of urethral and cervical secretions were examined and culture specimens taken from these sites and from the oropharynx and anorectum in all female patients. Gonococcal infection was excluded only if three sets of cultures taken at weekly intervals from these sites gave negative results.

Neither those patients who had had antimicrobial treatment within the six weeks preceding attendance at the clinic nor those with complicated infection were included in the study.

ANTIGEN PREPARATIONS

As gonococcal antigen, strain 9 of *N. gonorrhoeae* as described by O'Reilly *et al.* (1973) was used (kindly supplied by Dr D. S. Kellog, US Center for Disease Control, Atlanta), as it had been shown to contain antigenic characteristics which were common to a variety of gonococcal strains but not shared by other neisseriae. The organism was cultured on a selective medium (Young, 1978). Cultures of *Neisseria meningitidis*, *Neisseria perflava*, *Neisseria lactamica*, and *Neisseria catarrhalis* for use in

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control studies were identified by fermentation reactions. They were obtained from stock cultures held in the Department of Microbiology at the University of Edinburgh.

Colonies were suspended in phosphate-buffered saline (PBS), pH 7.4, and the suspensions diluted until the fluid was faintly turbid. Aliquots were stored at -20°C until required.

A drop of suspension was placed in each well of a Multidot slide (Hendley, Essex, UK) and dried by incubation at 37°C for 10 minutes.

INDIRECT IMMUNOFLUORESCENT TEST

A standard indirect immunofluorescent technique (IF test) was used (Johnson *et al.*, 1978). Fluorescein-conjugated sheep anti-human IgA, IgG, and IgM sera were obtained from commercial sources (Wellcome Reagents, UK) and their specificity confirmed by gel immunodiffusion.

Doubling dilutions of sera, previously inactivated by heating at 56°C for 30 minutes, were prepared and layered on to the prepared slides. After the slides had been incubated at 37°C for 30 minutes and washed in PBS, conjugated antiserum at a dilution of 1/16 was added, and the slides maintained at 37°C for 30 minutes. The slides were then thoroughly washed in PBS and mounted in buffered glycerol (Difco).

Preparations were examined with a Zeiss microscope (Large Universal). After being scanned with

a low power objective detail was examined with a $\times 100$ oil immersion lens.

Fluorescence was graded according to the system used by Welch and O'Reilly (1973) as follows: 4+ indicated brilliant fluorescence of all organisms; 3+, well-defined fluorescence of all organisms in the field; 2+, low density fluorescence of at least 75% of organisms; and 1+, occasional fluorescing organisms. Only a 2+ fluorescence or higher reading was recorded as a positive result.

Statistical comparisons were made by the method of binomial probabilities and Student's *t* test.

Results

UNTREATED PATIENTS

Tables 1 and 2 show the results obtained in the indirect immunofluorescent antibody test applied to the serum from infected and non-infected men and women.

IgM

A titre of ≥ 16 of IgM reactive with *N. gonorrhoeae* was found in 44.8% (56/125) of men with untreated gonorrhoea but in only 3% (3/100) of non-infected men ($P < 0.001$ by the method of binomial probabilities). Similarly in 45.7% (32/70) of infected women and in 2.9% (2/70) of non-infected women antigenococcal antibody of this class was found at a titre of ≥ 16 ($P < 0.001$).

Table 1 *Immunoglobulin classes of antibody against Neisseria gonorrhoeae in sera from men with untreated gonorrhoea and from non-infected patients*

Patients	No. of sera	IgM titre					IgA titre					IgG titre				
		≤ 8	16	32	64	128	≤ 8	16	32	64	128	≤ 8	16	32	64	128
Infected																
For 1-7 days	52	19	19	9	5	0	19	14	12	6	1	0	4	13	18	17
8-14 days	45	25	11	8	1	0	17	12	13	3	0	0	0	0	10	35
15-21 days	14	11	3	0	0	0	12	1	1	0	0	0	0	0	4	10
22-28 days	2	2	0	0	0	0	1	1	0	0	0	0	0	0	1	1
≥ 29 days	12	12	0	0	0	0	12	0	0	0	0	0	0	0	4	8
Non-infected	100	97	3	0	0	0	100	0	0	0	0	92	8	0	0	0

Table 2 *Immunoglobulin classes of antibody against Neisseria gonorrhoeae in sera from women with untreated gonorrhoea and from non-infected patients*

Patients	No. of sera	IgM titre					IgA titre					IgG titre				
		≤ 8	16	32	64	128	≤ 8	16	32	64	128	≤ 8	16	32	64	128
Infected																
For 1-7 days	15	4	5	6	0	0	7	3	4	1	0	1	4	8	2	0
8-14 days	24	7	7	8	2	0	3	9	8	3	1	0	0	0	6	18
15-21 days	19	15	4	0	0	0	10	7	1	1	0	0	0	0	4	15
22-28 days	6	6	0	0	0	0	5	1	0	0	0	0	0	0	2	4
≥ 29 days	6	6	0	0	0	0	6	0	0	0	0	0	0	0	2	4
Non-infected	70	68	2	0	0	0	70	0	0	0	0	66	4	0	0	0

In 54.6% (53/97) of men who had been infected for 14 days or less but in 10.7% (3/28) who had been infected for longer the titre of antigenococcal IgM exceeded 8 ($P < 0.05$). The titre of IgM antibody was ≥ 16 in 71.8% (28/39) of women infected for less than 14 days but in only 12.9% (4/31) of women whose infection was of a longer duration.

IgA

Antibody of the IgA class reactive with the gonococcus was found at a titre of ≥ 16 in 51.2% (64/125) of men and in 55.7% (39/70) of women. Patients who were not infected (100 men and 70 women) had serum titres of ≤ 8 ($P < 0.001$).

When the infection had been present for 14 days or less antigenococcal IgA was found (at a titre of ≥ 16) in the serum of 62.9% (61/97) of men but in only 10.7% (3/28) of men who had been infected for longer ($P < 0.05$). Similarly, IgA antibody was found at a titre of ≥ 16 ($P < 0.05$) in 74.3% (29/39) of women who had had gonorrhoea for 14 days or less but in only 32.3% (10/31) of women whose infection was of longer duration.

IgG

Antibody of the IgG class reactive with the gonococcus was found at a titre of ≥ 16 in the serum of all the 125 (100%) men with untreated gonorrhoea but in only 8% (8/100) of men who were not infected ($P < 0.001$). In women, this antibody was detected at a titre of ≥ 16 in the serum of 98.6% (69/70) of infected patients and in 5.7% (4/70) of non-infected patients ($P < 0.001$).

The arithmetic mean of the log titre of IgG reactive with *N. gonorrhoeae* in the serum of men infected for seven days or less was 1.7830 and in patients who had been infected longer it was 2.0289. This is a statistically significant difference ($P < 0.001$ by Student's *t* test). Similarly, the arithmetic mean log titre of IgG antibody in women was 1.4249 and 2.4903 in the serum of women infected for seven days or less and for eight days or more respectively ($P < 0.001$).

Naturally occurring IgA reactive with other neisseriae

Table 3 shows the results obtained in the indirect immunofluorescent antibody test with monospecific IgA when applied to sera diluted to 1/16 from patients with gonorrhoea and from non-infected controls.

EFFECT OF TREATMENT ON CLASSES OF ANTIBODY

Table 4 shows the arithmetic mean log titre of antigenococcal antibodies before and at intervals after treatment. The decline in titre of antibodies of the IgM and IgA classes is shown as well as the much more gradual fall in IgG titre.

Discussion

Although indirect immunofluorescent antibody techniques using various antigen preparations have been evaluated as diagnostic tests (Welch and O'Reilly, 1973; Rodas and Ronald, 1974), there have been few studies correlating the distribution

Table 3 Results of the indirect immunofluorescent antibody test with monospecific anti-human IgA on sera of patients with gonorrhoea and of non-infected controls

Antigen	Patients' case no. (and sex)											
	Infected						Non-infected					
	1 (M)	2(M)	3(M)	4(F)	5(F)	6(F)	7(M)	8(M)	9(M)	10(F)	11(F)	12(F)
<i>N. gonorrhoeae</i>	4+	4+	4+	4+	4+	4+	—	—	—	—	—	—
<i>N. meningitidis</i>												
group A	—	—	—	—	—	—	—	—	—	—	—	—
group B	—	—	—	—	—	—	—	—	—	—	—	—
group C	—	—	—	—	—	—	—	—	—	—	—	—
group D	2+	2+	2+	—	2+	1+	—	—	2+	1+	—	—
group E	—	—	—	—	—	—	—	—	—	—	—	—
group 29E	—	—	—	—	—	—	—	—	—	—	—	—
group W135	—	—	—	—	—	—	2+	—	—	—	1+	2+
group X	—	1+	1+	1+	1+	—	1+	1+	1+	2+	—	2+
group Z	—	—	—	—	—	—	—	—	1+	—	—	—
<i>N. lactamica</i>	2+	—	2+	—	—	1+	1+	—	2+	1+	1+	1+
<i>N. perflava</i>	2+	1+	1+	1+	2+	3+	2+	2+	2+	2+	2+	2+
<i>N. catarrhalis</i>	1+	1+	1+	1+	2+	2+	1+	1+	1+	2+	1+	2+

- 1+ Occasional fluorescing organisms
 2+ Low intensity fluorescence of all organisms
 3+ Well-defined fluorescence of all organisms
 4+ Brilliant fluorescence of all organisms

Table 4 Arithmetic mean of log titres of antibodies reactive with *Neisseria gonorrhoeae* in the serum of infected patients before and after successful treatment

	No. of patients	Immunoglobulin class		
		IgM	IgA	IgG
Before treatment				
Men	125	1.108	1.170	1.927
Women	70	1.118	1.183	1.901
7 days after treatment				
Men	62	1.039	1.131	1.869
Women	43	1.120	1.071	1.904
14 days after treatment				
Men	48	0.947	1.029	2.157
Women	41	0.977	1.057	1.894
28 days after treatment				
Men	45	0.903	0.917	1.772
Women	39	0.903	0.911	1.860

of antigenococcal antibodies within the immunoglobulin classes with the duration of infection.

In the present study antibody of the IgM class reactive with *N. gonorrhoeae* was detected at a titre of ≥ 16 in 45% of infected men and in 46% of infected women before treatment. The results obtained in women were similar to those recorded by Wilkinson (1975), who found this class of antigenococcal antibody at a titre of ≥ 16 in 43% of infected patients. He found similar antibody in only 32% of infected men; the duration of infection was not recorded.

IgM antibody was detectable in the serum of more than half of the men and of two-thirds of the women with untreated gonorrhoea who had been infected for less than two weeks. When infection was of longer duration this antibody was less commonly found at the lowest dilution of serum examined. This early IgM response was observed under experimental conditions by Cohen *et al.* (1969), who also showed that even without treatment those subjects who developed antigenococcal IgM had titres of this antibody which gradually fell over a period of about four months. Few patients in the present study who had been infected for more than 14 days had titres of antibody of ≥ 16 . Cohen *et al.* (1969), however, used inocula of organisms which were much larger than those which would be acquired in natural infection, and this may partly explain the more gradual decline in IgM activity which they had observed.

The antigenococcal IgA response to infection was similar to that of IgM. Antibody of this class reactive with *N. gonorrhoeae* was found in the serum of about two-thirds of men and of three-quarters of women who had been infected for less than 14 days. When the infection was of longer duration only about 11% of men and 32% of women had this antibody in the serum at a dilution of 1/16 or

greater. Similar findings of a rapid decline in serum IgA antibody activity within two to three weeks of acquisition of infection were reported by Cohen *et al.* (1969). The relationship between production of antigenococcal secretory IgA in the mucous membranes and the detection of serum IgA antibody will be dealt with elsewhere.

No naturally occurring IgA reactive with *N. gonorrhoeae* was detected, although natural antibody against *N. perflava* and *N. catarrhalis* was found in infected and non-infected patients as was, less commonly, antibody against *N. lactamica* and *N. meningitidis*, groups D, W135, and X.

Antigenococcal antibody of the IgG class was found in the serum at a dilution of 1/16 or greater of all infected men and of almost all infected women. The presumed false-positive rates were 8% and 6% respectively for men and women. In one study, where heat-labile antigen and antihuman IgG were used in a fluorescent antibody test, the false-positive rate was lower as was the sensitivity (Gaafar and D'Arcangelis, 1976).

IgG antibody activity increased throughout the first week of infection, so that by the second week most patients had antibody at high titres (≥ 64). No obvious decline in antibody activity occurred in the subsequent two to three weeks. These results agree with those obtained by Cohen *et al.* (1969).

Successful treatment of infection in both sexes resulted in a rapid decline in antigenococcal IgM and IgA activity. A much more gradual decline in IgG antibody activity occurred. This presumably reflects differences in the half-life of the antibody classes (IgM, five days; IgA, six days; and IgG, 22 days [Tomasi, 1976]). This gradual decline in IgG antibody activity makes interpretation of diagnostic immunofluorescent test results difficult if the patient has recently been treated for gonorrhoea.

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