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SEROLOGIC INVESTIGATION OF THE IMMUNE RESPONSE IN VARIOUS TYPES OF GONOCOCCAL INFECTION

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Abstract. Serum specimens from patients with or without complications to genito-urinary gonorrhoea were tested with the use of the gonococcal complement fixation test (GCFT) and immunoelectrophoresis (IE) tests. Positive results with the GCFT were obtained in 20–25% of males and 35–40% of females with uncomplicated gonorrhoea, in 75–80% of women with acute salpingitis and concomitant genito-urinary gonorrhoea, and in 95% of patients with septic gonococcal dermatitis. An active immune response, as measured by increasing serum titres, was recorded in all patients with septicemia and a positive GCFT, in half of the women with acute salpingitis and a positive GCFT but very seldom in patients with uncomplicated genito-urinary gonorrhoea and a positive GCFT. The use of a polyvalent antigen was found superior to the use of a monovalent one but the former gave positive results in 5% of controls compared with 2% with the monovalent antigen. With the use of IE tests, precipitins were demonstrated in 67% of patients with gonococcal septicemia, in 30% women with acute salpingitis and concomitant genito-urinary gonorrhoea, and in 24% of the patients with an uncomplicated genito-urinary gonorrhoea. However, precipitins were also demonstrated with IE tests in 14% of blood donors' sera, serving as controls. A fast-moving gonococcal antigen with negative charge, called the *a-antigen*, was responsible for most of the reactions in the IE tests. Another antigen, termed the *b-antigen*, often gave reactions in IE-tests with serum specimens from patients with gonococcal septicemia. The significance of the findings with the GCFT and IE tests and their relationships to the immune response in gonococcal infections are discussed.

The existence of an immune response in some gonococcal infections has been known since the beginning of this century. The gonococcal complementfixation test, abbreviated GCFT, also called the "Gonoreaction", was early found useful for the detection of gonococcal antibodies. In Scandinavian countries and Great Britain it was used to a great extent during the 1920s and the 1930s (16, 20), but the interest in serodiagnosis of gonorrhoea soon diminished. This was partly due to the advent of chemotherapeutic and antibiotic agents which allowed an efficient and rapid cure of the disease and which mostly meant that an immune response did not develop. Also, at this time, more efficient techniques for the culture of GC organisms were introduced. These factors, in combination with a general opinion that the Gonoreaction had a low sensitivity combined with a lack of specificity, all contributed to a diminished interest in serodiagnosis of gonorrhoea.

The rapid increase of gonorrhoea during the last 10 years, with many asymptomatic carriers, especially among females but also among males (13) and the relatively high frequency of complications such as septicemia and pelvic inflammatory disease (1, 2, 3, 11) has created a new interest in serological tests for this disease. In various parts of the world in general (4, 19, 21, 29, 30) and at the Venereal Disease Research Laboratory of the Center for Disease Control especially (5, 8, 17, 18, 22, 23, 24, 26) efforts have been made during recent years to develop a simple serologic test that could be routinely used for the diagnosis of gonorrhoea. However, despite all efforts no ideal serodiagnostic test for gonorrhoea is yet available. This may be due partly to our lack of knowledge about factors involved in the immune response, for example in uncomplicated versus complicated gonococcal infections. Today we therefore need to increase our knowledge on the following points:

1. The immune response in various types of gonococcal infections, in other words a serologic test profile with available techniques for use in

Table I. *Summary of results obtained in CF and IE tests with serum from blood donors*

IE tests performed with SupMSC. PWC = Polyvalent whole cells. MWC = Monovalent whole cells. MSC = Monovalent ultrasonic treated cells. SupMSC = Supernatant of MSC

No. of blood donors	Type of GC-Ag	Number of blood donors' sera with CF titres				Per cent of blood donors' sera positive in		
							IE tests	
		≤1:4	1:4	1:8	1:16	GCFT (%)	1 Prec. line (%)	2 Prec. lines (%)
100	PWC	95	3	2	0	5		
	MWC	99	1	0	0	1		
	MSC	98	2	0	0	2		
	Sup. MSC	99	1	0	0	1	12	2

uncomplicated versus complicated gonococcal infections.

2. Are there one or more gonococcal antigens that take part in a possible immune response and which of these antigens is of greatest importance?

3. To what extent are these antigens specific for Neisseria gonorrhoea?

Two years ago a collaborative work was started between Karolinska Sjukhuset in Stockholm and the Central County Hospital in Örebro, Sweden, in order to elucidate some of these questions. We started our investigations with the purpose of elaborating a serologic test profile in patients with uncomplicated versus complicated gonococcal infections with the use of the GCFT, which would provide us with information about the immune response in quantitative terms and with the use of immunoelectrophoresis (IE) tests that would give us information in qualitative terms concerning gonococcal antigens involved in the immune response. Preliminary results from these investigations will be presented in a subsequent report.

MATERIALS AND METHODS

Patients' Sera

Serum specimens were obtained from 183 patients with gonorrhoea, and 100 healthy blood donors. The patients were classified as follows:

1. One hundred patients, 45 males and 55 females, with genito-urinary gonorrhoea without clinical signs of complications. They had no history of previous gonorrhoea. Serum specimens were taken as soon as the diagnosis of gonorrhoea was bacteriologically confirmed. The patients were then treated with penicillin as recommended by the Royal Swedish Medical Board, i.e. 1.0 mega-unit of aqueous benzylpenicillin and 1.2 mega-units of aqueous procain penicillin in a single intramuscular injection, the dose being repeated once or twice on successive days in patients with gonococcal strains with decreased sensitivity to penicillin. Additional serum specimens were obtained from 42 of the patients, 20 males and 22 females, 1 week after the first blood specimen was drawn, and from 36 of the patients, 17 males and 19 females, 3 to 4 weeks after blood sampling.

2. Thirty-eight female patients hospitalized for acute salpingitis and with *Neisseria gonorrhoeae* isolated from the genito-urinary tract. Serum specimens were obtained from the patients within 2 days of their admission to the hospital. The patients were then treated for 10–14 days with 1.65 mega-units of aqueous benzylpenicillin and 0.6 mega-units of aqueous procain penicillin intramuscularly twice daily, and for 2–4 weeks with Demethylchlortetracycline 300 mg *per os* twice daily. Additional serum specimens were obtained from 37 patients 2–3 weeks, and from 25 patients 4–16 weeks after the first blood specimen was drawn.

3. Forty-five patients, 11 males and 34 females, with septic gonococcal dermatitis (gonococcal septicemia), the diagnosis of which was based on their typical clinical picture (1, 2, 3) and the demonstration of *Neisseria gonorrhoeae* in specimens from the genito-urinary tract and/or from the blood and/or cutaneous manifestations (3). The epidemiologic, clinical and laboratory observations in 20 of these patients were reported elsewhere (3). Serum specimens for serologic examinations were obtained from 34 of the patients within 1–4 days of their admission to the hospital. The patients were treated as described (3) and additional serum specimens were obtained from 42 of the patients 10–28 weeks after the first blood specimen was drawn.

4. Serum specimens, obtained from 100 healthy blood donors without previous history of veneral disease, served as controls in the present study. They were subjected to the same test procedures as the sera from the gonorrhoea patients.

Serologic Methods and Test Procedures

A. *Preparation of gonococcal antigens*

Seven strains of *Neisseria gonorrhoeae* were selected for the production of antigens. One of these strains, isolated from a patient with septic gonococcal dermatitis with a

strong immune response against this gonococcal strain, was used for the production of a *Monovalent antigen* which was used in complement fixation (CF) tests, and immunoelectrophoresis (IE) tests. The other six strains, isolated from three males and three females with uncomplicated genito-urinary gonorrhoea, were used for the production of a *Polyvalent antigen* which was used in CF tests only. The production and preparation of antigens were as follows:

Colony morphology types T1 or T2 and T3 or T4 were selected from each of the strains with the use of the medium described by White & Kellogg (32). They were then inoculated on a sufficient number of plates with GC medium prepared from Difco GC Agar Base enriched with Bacto haemoglobin and BBL defined supplement. The plates were incubated for 18–22 hours at 35°C in an atmosphere of 4–6% CO_2. The gonococcal cells were harvested in saline, washed once and then centrifuged at 3 500 rpm for 15 min after which the wet weight of the cells was estimated.

1. *Preparation of Monovalent Antigens.* The GC cells were divided into three portions and prepared as follows:

(*a*) The first portion was dissolved at a concentration of 25 mg/ml in Veronal buffer (VB), pH 7.2, used in the CF test. The suspension was heated for 30 min at 60°C. This antigen preparation was designated monovalent whole cells (abbreviated MWC). It was divided into small portions and kept frozen at −20°C until use. Chessboard titrations were performed in the usual way to ascertain the optimal antigen dilution for the CF tests. It was found that 0.5 mg/ml of MWC, as compared with the original wet weight, contained 1 antigen unit. Two units of antigen were used in the CF test (see below). No anticomplementary effect of the antigen was noted at this concentration.

(*b*) The second portion was dissolved at a concentration of 100 mg/ml in VB and then subjected to ultrasonic (S) treatment with the use of a 100 watt MSE ultrasonic apparatus. This was operated at maximal efficiency and after 180 sec all the cells were disintegrated. This antigen was designated *m*onovalent *S*-treated *c*ells (abbreviated MSC). It was used in CF tests at a concentration of 1 mg/ml = 2 antigen units. The supernatant of the MSC, obtained by centrifugation at 40 000 *g* for 30 min, was also used in complement fixation tests. This antigen was abbreviated Sup MSC and is equivalent to crude gonococcal protoplasm used by Danielsson et al. (8), Schmale et al. (26) and Reising et al. (23).

(*c*) The third portion was dissolved in distilled water at a concentration of 100 mg/ml and then subjected to ultrasonic treatment as described above. The supernatant of the disintegrated cells, obtained as described above, was lyophilized and the dry weight estimated. It was then dissolved in 0.025 M barbital buffer and used in IE tests. Various concentrations were tested in IE tests and a concentration of 50–60 mg/ml was found to give optimal results. Corresponding results were arrived at by concentrating the supernatant of the US-treated cells 10 times by negative pressure dialysis instead of lyophilizing it. This corresponded to 1 000 mg/ml as compared with the original wet weight.

2. *Preparation of Polyvalent Antigens.* The gonococcal cells for each of the six strains (see above) were handled in the same way as the MWC antigen. They were tested separately in chessboard titrations to ascertain the optimal antigen dilution. Two units of antigen varied from 4 mg/ml to 1 mg/ml as compared with the wet weight. Equal portions of the cells were pooled and the antigen was designated *p*olyvalent *w*hole *c*ells (abbreviated PWC). The CF tests with this antigen were then carried out in the same way as with the MWC.

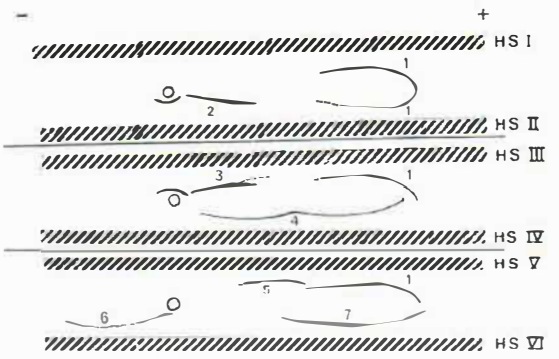

Fig. 1. A schematic drawing of precipitin lines obtained in immunoelectrophoretic tests (IE tests) between gonococcal antigen and various patients' sera, HS I–VI (see text). Precipitin lines: 1 = aA, 2 = bB, 3 = b_1B_1, 4 = cC, 5 = dD, 6 = eE, 7 = fF.

B. *Serologic assay techniques*

The following serologic assay techniques were used:

1. The micro-modification of the *Laboratory Branch Complement Fixation* (*LBCF*) test (28) was used, with one exception: Before the addition of the haemolytic system, the plates were incubated for 45 min at 37°C instead of 18 hours at 3–4°C. In our experience the test technique gave more clear-cut reactions in this way. Serum specimens, heat inactivated at 56°C for 30 min, were twofold diluted in VB buffer, pH 7.2, starting at an initial dilution of 1 : 4. Two units of the various antigen preparations (see above) as well as the other reagents, guinea pig complement and the haemolytic system, were added as recommended in the LBCF test procedure (28). The dilution of a serum specimen that gave an inhibition of the haemolysis of 70% or more was regarded as the final titre. Each serum specimen was tested twice, and appropriate controls were included in each test. One and the same positive control serum with an original titre of 1 : 64 was used throughout the present investigation to check the quality of the antigen preparations. As soon as the titre of this control serum dropped more than one dilution step a new batch of the test antigen in question was included.

2. *Immunoelectrophoresis* (*IE*) tests were performed as described by Grabar & Burtin (12). One per cent agarose in 0.025 M barbital buffer (LKB), pH 8.6, was used. The corresponding buffer was used to dissolve the lyophilized supernatant of S-treated gonococcal cells at concentrations of 50–60 mg/ml. Holes and troughs were cut with LKB equipment. The gonococcal antigen was subjected to electrophoresis for 50 min at a voltage of 7 volts/cm. Serum

Table II. *Summary of results obtained in CF and IE tests with serum specimens from patients with uncomplicated genito-urinary gonorrhoea*

No. of patients	Days after diagnosis	Type of GC–Ag	Number of patients' sera with CF titres						Per cent of patients' sera positive in		
										IE tests	
			<1:4	1:4	1:8	1:16	1:32	1:64	GCFT (%)	1 Prec. line (%)	2 Prec. lines (%)
100 (55 ♀, 45 ♂)	0 days	PWC	71	5	16	4	1	3	29		
		MWC	81	7	4	4	1	3	19		
		MSC	82	8	3	3	2	2	18		
		SupMSC	84	6	3	4	1	2	16	21	3
42 (22 ♀, 20 ♂)	6–9 days	PWC	28	4	6	2	1	1	33		
		MWC	37	2	2	0	1	0	12		
		MSC	37	2	1	1	0	0	12		
		SupMSC	38	3	0	1	0	0	9.5	16.6	2.4
36 (20 ♀, 16 ♂)	15–24 days	PWC	25	5	4	1	0	1	30.5		
		MWC	32	2	1	1	0	0	11		
		MSC	33	2	1	0	0	0	8.3		
		SupMSC	34	2	0	0	0	0	5.5	13.8	2.8

specimens were applied in throughs, 3 mm wide, and at a distance of 3 mm from the holes. After a reaction time of 2 days, the plates were washed, dried, stained with Amido black B and then recorded.

RESULTS

1. *Reactivity of serum specimens from blood donors*

Serum specimens for 100 blood donors without previous history of gonorrhoea served as controls in the present study. The overall reactivity in CF tests and IE tests is summarized in Table I.

It will be seen from the table that 5% of the blood donors gave a positive CF test with PWC antigen with titres ranging between 1 : 4 and 1 : 8. With the use of various preparations of monovalent gonococcal antigen only 1 to 2% of the blood donors gave a positive reaction with titres not exceeding 1 : 4.

Table I also shows that sera of blood donors gave precipitin lines in IE tests and it was found that 12 serum specimens formed one precipitin line and that two sera gave two lines. The position and the pattern of the precipitin lines in the IE test are schematically drawn in Fig. 1. According to this figure the aA precipitin line (*a* stands for antigen and *A* for its corresponding antibody) was formed by 12 sera and the lines aA + dD and eE + fF respectively by two sera. The antigen used in IE tests corresponded to Sup MSC. Therefore it is of interest to note that only one of the 14 sera that gave precipitin lines was positive in CF tests with this antigen while the other 13 were negative. It should also be mentioned that two out of the five serum specimens positive in the GCFT with PWC antigen formed preciptin lines.

2. *Reactivity of serum specimens from patients with uncomplicated genito-urinary gonorrhoea*

The overall reactivity in CF tests and IE tests with serum specimens from this group of patients is summarized in Table II.

It will be seen from the table that on the day of diagnosis of gonorrhoea 29% of the patients (20% males and 36% females) were positive in CF tests performed with PWC antigen. The serum titres ranged between 1 : 4 and 1 : 64 with the majority of them on 1 : 8. With the use of various preparations of monovalent gonococcal antigen (see Methods) in the CF tests, the yield was only 16 to 19%. All those positive with the monovalent antigens were also positive with PWC. The table also shows that serum specimens from 24 patients formed precipitin lines in IE tests. The majority of them gave only one precipitin line and according to Fig. 1 the aA precipitin line was formed by 20 sera and the cC line by one serum specimen. Eleven of these sera were negative in CF tests with SupMSC. Three patients' sera formed 2 precipitin bands, the aA line in combination with the

Table III. *Summary of results obatined in CF and IE tests with serum specimens from patients with acute salpingitis and genito-urinary gonorrhoea*

No. of patients	Days after admission to hospital	Type of GC–Ag	Number of patients' sera with CF titres						Number and per cent of patients' sera positive in		
									GCFT	IE tests	
			<1:4	1:4	1:8	1:16	1:32	1:64	(%)	1 Prec. lines (%)	2 prec. lines. (%)
38	0–2	PWC	18	8	6	5	1	0	20 (52.6)		
		MWC	24	7	3	4	0	0	14 (36.8)		
		MSC	26	7	5	0	0	0	12 (31.6)		
		SupMSC	31	5	1	1	0	0	7 (18.4)	9 (23.7)	0
37	14–21	PWC	9	11	7	7	2	1	28 (75.7)		
		MWC	19	7	6	4	0	1	18 (48.6)		
		MSC	22	7	5	2	1	0	15 (40.5)		
		SupMSC	24	6	4	2	1	0	13 (35.1)	11 (30)	0
25	28–112	PWC	10	7	7	1	0	0	15 (60.0)		
		MWC	15	5	4	0	1	0	10 (40.0)		
		MSC	15	6	3	1	0	0	10 (40.0)		
		SupMSC	18	4	3	0	0	0	7 (28.0)	6 (24)	0

bB line, and these sera were positive in CF tests with SupMSC.

A second serum specimen was obtained from 42 patients 6–9 days after the first one and the results of the serologic investigation will be found in Table II. It will be seen that the highest yeild was obtained in the CF test with the PWC antigen and 14 patients in all were positive (25% males, and 40% females). Twelve of these were positive at the first examination. A significant fourfold rise of the serum titre was observed in only 3 patients.

It will be seen from Table 2 that the yield with various preparations of monovalent GC antigen was only 9.5 to 12%. This might possibly be explained by the fact that the GC strain used for monovalent antigen was a local strain from Örebro while the 42 serum specimens were obtained from patients living in Stockholm. An analysis of the serum specimens obtained on the day of diagnosis of gonorrhoea from 100 patients of whom 58 were from Örebro, gave support to this view.

Table II also shows that precipitin lines were obtained in IE tests in 8, i.e. 19%, of the patients from whom a second serum specimen was obtained. These patients' sera were also positive in IE test on the first occasion. It should be mentioned that 6 of them were negative in CF tests with SupMSC.

A third serum specimen was obtained from 36 patients 15–24 days after the first one. The results did not vary significantly from those of the second serum specimens.

3. *Reactivity of serum specimens from patients with genito-urinary gonorrhoea and acute salpingitis*

Serum specimens from women, hospitalized because of acute salpingitis and in whom genito-urinary gonorrhoea was established by culture, were examined with CF and IE tests. The results are summarized in Table III.

It will be seen from the table that nearly 53% of the serum specimens, obtained from 38 patients within 2 days of their admission to the hospital, were positive in CF tests with PWC antigen, with titres ranging between 1 : 4 and 1 : 32. The yields were, however, much lower with the various preparations of the monovalent antigen, especially with the SupMSC antigen. Two serum specimens that were positive in CF tests with monovalent antigen were negative in tests with PWC antigen. The table also shows that on this occasion 9 sera, i.e. 24%, gave a precipitin line in IE-tests. According to Fig. 1 the aA precipitin line was formed by eight sera and the bB line by one serum. It should be mentioned that only three out of the nine sera positive in IE tests were positive in CF tests with the SupMSC while five of them were positive with PWC.

Table IV. *Summary of results obtained in CF and IE test with serum specimens from 45 patients with septic gonococcal dermatitis*

No. of patients	Days after admission to hospital	Type of GC-Ag	Number of patients' sera with CF titres								Number and per cent of patients' sera positive in		
			1:4<	1:4	1:8	1:16	1:32	1:64	1:128	1:256	GCFT (%)	IE tests 1 Prec. line (%)	IE tests 2 or 3 Prec. lines (%)
34	1–4	PWC	18	10	5	1	0	0	0	0	16 (44.1)		
		MWC	25	5	4	0	0	0	0	0	9 (26.5)		
		MSC	25	5	4	0	0	0	0	0	9 (26.5)		
		SupMSC	27	4	3	0	0	0	0	0	7 (20.5)	8 (23.5)	4 (11.8)
42	10–28	PWC	2	2	8	12	3	5	9	1	40 (95.2)		
		MWC	4	3	10	9	4	7	4	1	38 (90.5)		
		MSC	6	4	13	4	6	6	2	1	36 (85.7)		
		SupMSC	8	10	9	4	6	4	0	1	34 (80.9)	13 (30.9)	15 (35.7)

A second serum specimen was obtained from 37 out of the 38 patients 14–21 days after their admission to hospital. The results obtained in CF and IE tests will be found in Table III. As can be seen, 75 % of the serum specimens were positive in the CF tests with PWC antigen with titres ranging between 1 : 4 and 1 : 64. Again, the yields were lower with the various preparations of the monovalent antigen; 35 % of the specimens were positive with SupMSC and 40 to 48 % with MSC and MWC respectively. One serum specimen that was positive with MWC and MSC with a titre of 1 : 4 was negative with PWC antigen. This means that the total yield in CF tests with monovalent and polyvalent antigens was nearly 80 %. It should also be mentioned that fourfold or eightfold rises of the serum titres were obtained in 8 of the patients and a twofold rise in a further 7. The table also shows that on this occasion 11 sera, i.e. 30 %, gave a precipitin line in IE tests. Two of these sera were negative in the IE tests on the first occasion. According to Fig. 1 the aA line was formed by 10 sera and the bB line by one serum. Only three out of the 11 serum specimens positive in IE tests were positive in CF tests with SupMSC while eight of them were positive in CF tests with PWC.

A third serum specimen was obtained from 25 of the 38 patients, 28 to 112 days after the first one. It will be seen from Table 3 that 60 % of these specimens were reactive in CF tests with PWC antigen with titres ranging from 1 : 4 to 1 : 16, and that 28 to 40 % were reactive with various preparations of the monovalent antigen with titres ranging from 1 : 4 to 1 : 32. Fourfold or eightfold falls of the serum titres were noted in 4 patients as measured with the PWC antigen, and a twofold fall in a further 6. Five patients showed no change or only a twofold rise of the serum titres. The table also shows that on this occasion serum specimens from 6 of the patients, i.e. 24 %, gave precipitin lines in IE tests. According to Fig. 1 the aA line was formed by five sera and the bB line by one serum. All these patients' sera also formed precipitin lines on the first and second occasions specimens were obtained. Only one of those positive on the two earlier occasions was negative this time.

4. *Reactivity of serum specimens from patients with septic gonococcal dermatitis*

Serum specimens were obtained from a total of 45 patients with the diagnosis of septic gonococcal dermatitis (see Materials and Methods). The overall reactivity obtained with CF tests and IE tests is summarized in Table IV.

It will be seen from the table that serum specimens were obtained from 34 out of 45 patients 1–4 days after their admission to hospital and that 16 of them, i.e. 44 %, were positive in CF tests with PWC and that 7 to 9 of them, i.e. 20 to 26 %, were positive with the various preparations of the monovalent antigen. The titres were low with the majority of them between 1 : 4 and 1 : 8. Serum specimens positive with the monovalent antigen were also positive with the PWC antigen.

The table also shows that 12 serum specimens, i.e. 35 %, formed precipitin lines in IE tests. Eight sera gave one precipitin line and according to Fig. 1 the aA line was formed by three sera and the bB line by the other five. Four sera gave two precipitin lines and according to Fig. 1 the aA line and the bB or b_1B_1 lines were formed. The bB line was characterized by a somewhat fogged appearance and was always accompanied by a precipitin line situated on the level with the antigen well. The b_1B_1 line was similar to the bB line with the addition of a hook, as illustrated in Fig. 1. It should also be mentioned that only one of the serum specimens positive in the IE tests was negative in CF tests with the SupMSC and the PWC antigens.

A second serum specimens was obtained from 42 of the 45 patients 10–28 days after their admission. It will be seen from the table that 40 patients, i.e. 95 %, were positive in CF tests with PWC antigen, and that 34 to 38 patients, i.e. 81 to 90 %, were positive with the various preparations of the monovalent antigen. The serum titres ranged between 1 : 4 and 1 : 256 with the majority of them on 1 : 8 to 1 : 128. Most of the serum specimens showed fourfold up to 32-fold and even 64-fold increases of the titres. The table also shows that serum specimens from 28 patients, i.e. nearly 67 %, formed precipitin lines in IE tests. Thirteen sera gave one precipitin line and according to Fig. 1 the aA line was formed by four and the bB line by the other nine. All these sera were positive in CF tests, except one which, forming the aA line, was negative in the CF test with SupMSC but positive in PWC.

Fifteen sera formed 2 or 3 precipitin lines. According to Fig. 1 the aA line in combination with the bB or b_1B_1 line was formed by 12 sera, the aA, bB and cC line by one and the aA and bB in combination with the dD line by two. All these sera were positive in the CF tests with the PWC antigen as well as with the various preparations of the monovalent antigen.

DISCUSSION

Various figures of the seroreactivity in patients with complications to gonorrhoea are reported in the literature. Kristjansen in 1930 (16) gave figures between 68.5 % and 100 %, Magnusson & Kjellander in 1965 gave a figure of 65 % (19), and very recently Ratnatunga gave a figure of 41 % (21). In the present paper gonococcal antibodies were demonstrated with the GCFT in 75–80 % of women with acute salpingitis and concomitant genitopurinary gonorrhoea, and in as many as 95 % of the patients with gonococcal septicemia. In this connection it is of interest to note the importance of examining at least two blood samples taken 10–14 days apart. If this had not been done 25 % of the females with gonorrhoea and acute salpingitis would have been missed and only 44 % of the patients with septicemia would have been regarded seroreactive.

The findings also showed that a strong immune response, as measured with the increase in serum titre, occurred in most of the patients with septicemia. This is to be expected as a gonococcal septicemia undoubtedly is of clinical significance. The results of the present investigation also clearly show that a GCFT is of great diagnostic value in these patients since many authors have reported that this disease is easily mis-diagnosed (1, 2, 3).

The immune response was less pronounced in women with acute salpingitis but twofold to eightfold increases of the serum titres were recorded during the convalescent phase of the disease in half of the patients with a positive GCFT on admission to hospital. These observations point out the pathogenic significance of a coexisting genitourinary gonorrhoea for the development of acute salpingitis.

The findings with the GCFT in patients with uncomplicated gonorrhoea confirmed recent observations by Magnusson & Kjellander (19) and by Ratnatunga (21). The females were nearly twice as often positive, i.e. in 35–40 %, as the males. The GCFT in its present state is therefore of little diagnostic value in these cases but the findings of an immune response may reflect a clinically undetected complication to a genitourinary gonorrhoea considered as uncomplicated. This is supported by the fact that at least 15–20 % of females with gonorrhoea develop acute salpingitis (11) and that gonococci may invade the accessory genital glands in as many as 20 to 40 % of the males with a genito-urinary gonorrhoea considered as uncomplicated (10, 15).

The importance of selecting antigens for use in the GCFT are illustrated by the results of this investigation. By using an antigen from a single

gonococcal strain a high specificity is achieved but the sensitivity is low except on those occasions where a true systemic infection has occurred, i.e. in patients with septicemia. The use of whole gonococcal cells also seems superior to the use of crude protoplasm of ultrasonically treated cells. The sensitivity of the GCFT is increased with the use of antigens from several gonococcal strains but at the same time there is an increase of probable non-specific reactions. Similar results have been reported by others (9, 19, 21, 30) which point out the dilemma of gonococcal serology today, namely the lack of known serotypes of gonococcal strains, antigens in common as well as strain-specific antigens. A careful study of the immunochemistry of gonococcal strains is therefore urgently needed.

Agar gel diffusion technique are well established as valuable tools in qualitative studies of antigen-antibody reactions. In a previous work Danielsson et al. (8) used the Ouchterlony technique to identify an antigen of importance in the immune response to gonococcal infections. In the present investigation the immunoelectrophoretic technique was used. The results obtained in these IE tests merit some comments.

It was noteworthy that precipitins reacting with soluble gonococcal antigens were demonstrated in as many as 14% of the blood donors' serum specimens. The reaction of the positive sera occurred with a fast-moving antigen with negative charge, which antigen was called *antigen a*. However, only one of the sera, positive in the IE tests, reacted with the corresponding antigenic preparation in the GCFT. It should be investigated if this is due to antibodies with poor complement fixing activity or if it is due to different concentrations of the antigen preparations used in the GCFT versus the IE test. However, the percentage of control sera reacting in the latter test is very close to the figures obtained with such sera in haemagglutination test by Logan et al. (18), in flocculation tests by Lee & Schmale (17), Reising (24), Wallace et al. (29) and by Watt et al. (30). The antibodies reacting with these antigens may reflect present or previous infections with meningococci, or a carrier state with these organisms, as it is well known that gonococci share antigens with meningococci (7, 33).

Work is now in progress to isolate the antigens and antibodies responsible for the reactions in these tests, and also their relationship to infections and/or carrier state with gonococci and meningococci or possibly other *Neisseria* species. Information about these conditions is of primary importance for the development of a reliable serodiagnostic test for gonorrhoea.

The pattern of the positive IE test was more complex in patients with septic gonococcal dermatitis than in the other patients. Many of the serum specimens from the patients with septicemia reacted with the fast-moving *antigen a*, but besides this many of them also reacted with a slow-moving complex antigen, called *antigen b*. The appearance of the reaction with this antigen was correlated to the immune response and the appearance of a positive GCFT.

The complex composition of antigens in gonococcal organisms has been shown by several authors (6, 25, 31). In the present investigation we were able to demonstrate with the IE tests reactions with six different antigenic factors. Work is in progress for an immunochemical characterization of these antigens and their occurrence in different gonococcal strains and strains of other *Neisseria* organisms. It will also be of interest to see if the antigenic factors demonstrated in the present work do have any relationship with the occurrence of pili in virulent gonococcal organisms recently described by Jephcott et al. (14) and by Swanson et al. (27).

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