

SERUM ANTIBODY RESPONSE IN EXPERIMENTAL HUMAN GONORRHOEA IMMUNOGLOBULINS G, A, AND M*†

BY

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This report concerns the sequential development of the immunoglobulin response to *Neisseria gonorrhoeae* in ten human male volunteers who were experimentally infected with virulent organisms during a study on the correlation of virulence with colonial type (Kellogg, Cohen, Norins, Schroeter, and Reising, 1968). An indirect fluorescent antibody (IFA) procedure, using fluorescent antisera specific for human IgG, IgA, and IgM (Ceppellini and others, 1964) antibodies, was used to detect the immunoglobulin class of the patients' antibodies reactive with heat-labile surface or heat-stable somatic antigens.

Material and Methods

Inoculation Procedure Ten individuals were selected for inoculation from among a large number of volunteers in Atlanta, Georgia. The patients were admitted to a special ward in two groups of three, and one group of four. Particulars regarding the selection of volunteers, the method of intraurethral inoculation, the clinical response, and the criteria for assessing infection and cure, as well as the methods used in preparing the bacteria have been presented in detail elsewhere (Kellogg and others, 1968).

All patients were treated on the fifth day of infection except Patient 3 who, at his request, was treated on the third day. Eight patients received one intramuscular injection of 2.4 million units aqueous procaine penicillin G. Patients 4 and 6 were allergic to penicillin and were treated orally with 1.5 g. oxytetracycline. These patients had a culture-proven relapse on Day 7 and were re-

treated with 3.0 g. oxytetracycline on Days 7 and 8, after which they were judged to be cured.

Sera The patients were bled before inoculation and on Days 1, 3, 5, 7, 10, 14, 21, 60, and 120 after infection. The sera on Day 5 were taken before treatment given that day. The bloods were allowed to clot overnight at 4°C., and the sera were removed and stored in aliquots at -20°C. and -50°C. Sera were diluted in phosphate buffered saline (PBS), pH 7.2, on the day of testing and were used unheated, unless specified.

Indirect Fluorescent Antibody (IFA) Procedure The IFA procedure was done as previously described (Cohen, 1967). Briefly, 3 per cent. formalin in PBS was used to preserve the heat-labile surface antigens (F) of the *N. gonorrhoeae* clone T1, strain F62, which was used to infect the patients. The heat-stable somatic antigens (121°C.) were exposed by heating the bacteria at 121°C. for 2 hrs. Standard concentrations of these antigens were fixed to circumscribed areas of glass slides. One drop (0.04 ml.) of the diluted test serum was placed over the appropriate area of the slide, and the slide was incubated at 37°C. for 30 min. and then washed in fresh PBS for three 5-min. periods. The classes of antibodies binding to *N. gonorrhoeae* antigens were detected by incubating the slide-fixed antibody-bacterium complexes with 1 drop of the appropriate fluorescent antiserum specific for human IgG, IgA, or IgM. The slide was then incubated and washed as before, and examined, after mounting and coding, with a fluorescence microscope. Throughout this study, all of the coded slides were scored by the same observer. The highest dilution of test serum scored as producing a 2+ fluorescent reaction (on a 1+ to 4+ scale) was considered to be the titre of the measured antibody.

Fluorescein-conjugated goat antisera specific for human IgG, IgA, and IgM were obtained from Hyland Division Travenol Laboratories, Inc., Los Angeles, California. The evidence for specificity of these reagents at the 1:40 dilution at which they were used has been given in detail elsewhere (Cohen and Norins, 1968).

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Results

The Table and Fig. 1 summarize the development of IgG, IgM, and IgA antibodies to F and 121°C antigens. In no serum was there found a 4-fold rise in IgG or IgM antibody titres to 121°C. antigens; however, in seven patients there was a 4-fold increase in IgA reactivity. Nine of the ten patients demonstrated 4-fold increases in titre of IgG antibodies to F antigens. Seven of these nine sera reached their peak IgG titres within 10 days of inoculation. On the other hand, only four of the ten patients demonstrated 4-fold increases in IgM antibody titres to F antigens, and only three patients showed a 4-fold increase in IgA antibodies to these antigens. Fig. 2 shows the antibody changes for a typical patient.

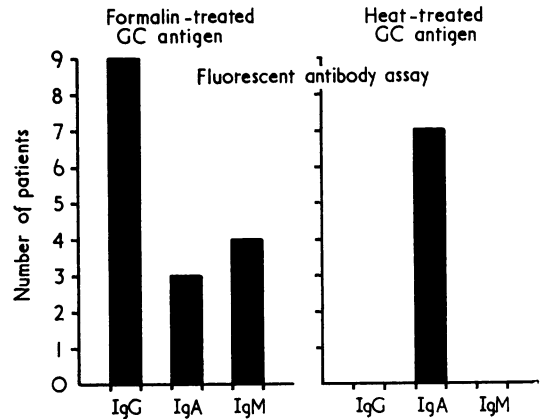


FIG. 1 Patients showing 4-fold increase in immunoglobulin titres after infection with *Neisseria gonorrhoeae* (GC).

TABLE

TEMPORAL APPEARANCE OF A 4-FOLD INCREASE IN ANTIBODY TITRE

Patient No.	History of Previous Infection	Immunoglobulin Categories and Gonococcal Antigens Examined					
		IgG		IgM		IgA	
		F	121°C.	F	121°C.	F	121°C.‡
1	No	5*	N†	N	N	5	N
2	Yes	N	N	N	N	3	10
3	No	10	N	10	N	3	5
4	No	5	N	10	N	N	10
5	No	5	N	5	N	N	5
6	Yes	5	N	21	N	N	3
7	Yes	5	N	N	N	N	10
8	Yes	21	N	N	N	N	5
9	No	60	N	N	N	N	N
10	Yes	10	N	N	N	N	N

*The numbers indicate the day after inoculation with *Neisseria gonorrhoeae* by which a 4-fold increase in antibody titres occurred. †N=indicates a lack of a 4-fold increase in antibody titre. ‡F=formalin-fixed "heat-labile" antigens (see Material and Methods). §121°C.=autoclaved antigens (see Material and Methods).

Discussion

The paucity of previous data on antibody response to gonococcal infection prompts us to record these findings. However, we emphasize that there are several important limitations and cautions to be recognized in interpreting these data. The small number of subjects and the practical conditions of the study precluded various ancillary studies that might have been desirable. Although the selected Type 1 clones of the *N. gonorrhoeae* strain were virulent (Kellogg and others, 1968), the inoculum was far larger than that likely to be experienced in natural transmission. Also, it was not possible to carry out certain additional studies which would have more firmly established the specificity of the serological effects observed. For example, no volunteers were inoculated intra-urethraly with the culture medium alone, nor were

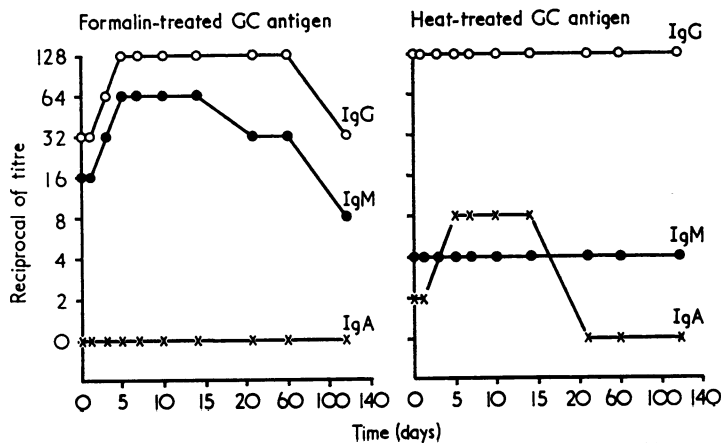


FIG. 2. Sequential changes in titre of immunoglobulins reactive with antigens of *Neisseria gonorrhoeae* (GC), as illustrated by sera from Patient 5. An indirect fluorescent antibody assay was used. Inoculation with virulent gonococcal cells was performed on Day 0 after serum was obtained. Therapy was administered on Day 5.

we able to compare in our assay pre-infection and post-infection sera from patients with various forms of non-gonococcal urethritis, to observe if any of these stimulated a rise in immunoglobulins reactive with *N. gonorrhoeae*.

We did not consider a change in titre significant unless it was 4-fold in magnitude. All subjects showed a 4-fold increase in one or more of the immunoglobulins reactive with either the F or the 121°C. antigens or both, but not one of the titre increases was greater than 4-fold.

While admittedly these 4-fold changes in titre are relatively small, we nevertheless believe them to be real, rather than subjective or intrinsic variations in test results. All slides were coded before they were read, and, throughout the study, the same observer scored all the coded slides. Also, over the time a particular subject was developing a 4-fold increase in titre of a particular immunoglobulin against one of the two (F or 121°C.) antigens, the same subject showed virtually no fluctuation in titre of the same immunoglobulin against the other of the two antigens. Further, the titre changes over time were consistent with the induction and treatment of the experimental infection. These points, and a small additional study showing complete or extremely close reproducibility on coded replicate specimens, suggest to us the validity of the observed 4-fold-titre increases.

These findings in the experimental infection appear to confirm those IFA immunoglobulin reactivities previously reported in sera from males with naturally acquired gonococcal urethritis (Cohen, 1967). The heat-labile antigens against which the predominant IFA-detectable experimental response is directed may also be similar to the phenol-extract antigens of the microprecipitin assay, in which the same experimental subjects were found to develop reactivity (Kellogg and others, 1968).

Summary

Sequential serum samples were obtained from ten human male volunteers who developed an anterior urethritis following infection with virulent clones (T1) of *Neisseria gonorrhoeae*. An indirect fluorescent antibody procedure, using reagents

specific for human IgG, IgM, and IgA antibodies, was used to measure the development of antibodies reactive with heat-labile surface antigens and heat-stable somatic antigens of *Neisseria gonorrhoeae*. It was found that nine of ten patients developed a 4-fold increase of IgG antibody reactivity to heat-labile surface antigens. Fewer patients showed increased IgM or IgA reactivities to heat-labile antigens. Heat-stable somatic antigens, in contrast, did not appear to stimulate any increased IgG or IgM activity. However, seven of ten patients showed increased IgA antibody titres to somatic antigens.

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Constatation d'anticorps sériques dans la gonococcie expérimentale humaine Immunoglobulines G, A, et M

SOMMAIRE

Des échantillons successifs de sérum furent recueillis chez 10 volontaires masculins qui présentèrent une uréthrite antérieure après inoculation de *Neisseria gonorrhoeae* issus d'une souche virulente particulière (T1). Une technique d'immunofluorescence indirecte, utilisant des réactifs spécifiques pour les anticorps humains IgG, IgM, et IgA servit à mesurer l'apparition d'anticorps réagissant avec des antigènes de surface thermolabiles, et avec des antigènes somatiques thermostables, de *Neisseria gonorrhoeae*. Il a été constaté que 9 des 10 malades multipliaient par 4 leur réactivité IgG vis à vis des antigènes de surface thermolabiles. Il y eut peu de malades chez lesquels la réactivité IgM ou IgA augmentât vis à vis des antigènes thermolabiles.

Les antigènes somatiques thermostables, par contre, n'ont paru stimuler aucun accroissement de l'activité IgG ou IgM. Pourtant, 7 des 10 malades présentèrent une augmentation des titres d'anticorps IgA vis à vis des antigènes somatiques.