

Reactivity of Two Gonococcal Antigens in an Automated Microhemagglutination Procedure

LESLIE C. LOGAN, PATRICIA M. COX, AND LESLIE C. NORINS

Venereal Disease Research Laboratory, Center for Disease Control, U.S. Department of Health, Education, and Welfare, Atlanta, Georgia 30333

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In studies of several hundred sera, a passive-hemagglutination technique with soluble antigen of sonically treated gonococci as the sensitizing material for tanned erythrocytes and *Neisseria sicca* sonically treated material as an absorbent detected gonococcal antibodies in 77% of males and 88% of females infected with uncomplicated gonorrhea. However, 6% of the sera from individuals in celibate religious orders and 18% of the sera from a group of females having cervical cultures negative for gonococci were also reactive with this procedure. Erythrocytes sensitized with an alkaline extract of gonococci reacted with 23% of the sera from infected males, 49% of the sera from infected females, and 2% of the sera from celibate females.

Published reports describing the detection of gonococcal antibody by means of passive-hemagglutination assays have not given much information on the reactivity of sera from patients with uncomplicated gonorrhea. Both untanned and tanned sheep erythrocytes have been sensitized with various extracts of gonococci. Maeland (5) sensitized untanned cells with an alkaline extract of gonococci and found hemagglutination titers ranging from 1:2 to 1:32 in the 25 human sera tested. However, it was not indicated whether the serum donors were infected or uninfected. Hirschberg and Harper (3) used tanned sheep erythrocytes sensitized with a saline extract of gonococci (prepared by extracting the organisms for 2 weeks at 4 C) and found only one reactor among 16 males with urethral smears positive for gonococci and no reactors among 13 culture-positive females.

In the present study, several hundred sera from patients with uncomplicated gonorrhea and from presumably noninfected individuals were tested against untanned erythrocytes sensitized with an alkaline extract of *Neisseria gonorrhoeae* and tanned erythrocytes sensitized with sonically treated material of the organism. Since the asymptomatic female patient is believed to comprise a reservoir of gonorrhea, particular attention was given to this category. Performance of the quantitative microhemagglutination test was facilitated by use of an automatic serial-dilution instrument (2).

MATERIALS AND METHODS

Sera. Sera from 153 patients (126 females, 27 males) in celibate religious orders and 355 females who had at least one negative gonococcal culture were included in the presumed nongonococcal group. Also available were sera from 379 patients who had uncomplicated gonorrhea (263 females with positive cervical cultures and 116 males with positive cultures or positive Gram-stained smears of urethral exudate). Before testing, the sera were thawed, heated at 56 C for 30 min, and absorbed with sheep cells. For this absorption, three parts of serum were added to one part of washed, packed sheep erythrocytes, and the mixture was incubated for 15 min at 37 C, for 30 min at room temperature, and for 30 min at 4 to 6 C. The erythrocytes were then removed by centrifugation.

N. gonorrhoeae organisms. *N. gonorrhoeae* F62 type 1 (4) was grown on GC agar base enriched with IsoVitalex (BBL). The plates were streaked with seed cultures, placed in a candle jar, and incubated overnight at 37 C.

Alkaline extract of gonococci. The growth from 50 plates was harvested in 10 ml of physiological saline (0.85%) and extracted by the method of Chanarin (1).

Soluble antigen of gonococcal sonically treated material. The growth from 50 plates was harvested in 20 ml of distilled water, disrupted in a Raytheon sonic oscillator (250 w, 10 kc/sec, 0 to 5 C) for 30 min, and centrifuged at $27,138 \times g$ for 1 hr at 4 C. Then the supernatant fluid was lyophilized and stored at -20 C. To prepare stock solutions, 20 ml of the lyophilized material was dissolved in 20 ml of phosphate-buffered saline (PBS, pH 6.4), dispensed in 1-ml samples, and stored at -20 C.

TABLE 1. Reactivity of an automated microhemagglutination procedure with tanned erythrocytes sensitized with a sonically treated antigen of *Neisseria gonorrhoeae*

Serum category	No. tested	Unabsorbed		Absorbed with <i>Neisseria sicca</i>		
		Reactive 1:1,536 or greater		Reactive 1:12 or greater		Reactive 1:24 or greater (%)
		Per cent	Median titer	Per cent	Median titer	
Gonococcal						
Males (anterior urethritis)	116	35	1:1,536	77	1:96	62
Females (cervical culture positive)	263	47	1:3,072	88	1:192	76
Presumed nongonococcal						
Males (celibate)	27	11	1:3,072	4	1:12	0
Females (celibate)	126	19	1:3,072	6	1:48	6
Females (cervical culture negative)	355	10	1:1,536	18	1:24	12

Sensitization of sheep erythrocytes. The antigens were used at their optimal dilution, i.e., the dilution of antigen giving the highest agglutination titer with a rabbit antiserum to the gonococcus. The alkaline extract of the gonococcus was adsorbed onto the sheep cells by the method of Chanarin (1) and adjusted to a 0.75% suspension for use. Tanned erythrocytes were prepared by adding equal volumes of 2.5% cells and 1:20,000 dilution of tannic acid (Fisher Scientific Co., Pittsburgh, Pa.), incubating the mixture at 37 C for 15 min, washing the tanned cells once in PBS (pH 7.2), and adjusting them to 2.5% in PBS (pH 6.4). Soluble antigen was adsorbed to the tanned cells by mixing equal volumes of the cells and the optimal dilution of the antigen [or an equal volume of PBS (pH 6.4) for preparation of control cells], incubating at 37 C for 15 min, washing twice in diluent [PBS (pH 7.2) containing 1% nonreactive rabbit serum and 0.01% polyoxyethylene sorbitan monoleate (Tween 80)], and adjusting to 0.75% suspension in diluent.

***N. sicca* and *N. flava* antigens for absorption.** Soluble antigens of *N. sicca* and *N. flava* used for absorption were prepared from organisms grown on Blood Agar Base (BBL) with 5% normal sheep cells. The plates were heavily seeded, placed in a candle jar, and incubated overnight at 37 C. The cells from 100 plates were harvested in 20 ml of physiological saline. This suspension was adjusted to a 40 × McFarland 10 density standard, disrupted for 30 min in a Raytheon sonic oscillator (250 w, 10 kc/sec, 0 to 5 C), and stored in 3-ml samples at -20 C. Prior to use, a sample was thawed and centrifuged at 27,138 × *g* for 1 hr at 4 C and the supernatant fluid was retained for use. All sera were absorbed for 30 min with three parts of the soluble supernatant antigen to one part serum; as a control, one part serum was mixed with three parts of diluent.

Automated microhemagglutination procedure. A 0.05-ml sample of each absorbed serum to be tested was pipetted into the first row of cups in the plastic tray, 0.025 ml was pipetted into the last row of cups, and the tray was then placed on the Autotiter (Cernalco, Inc.; 2). The machine automatically added 0.025-ml volumes of the diluent, made 13 twofold

TABLE 2. Comparison of *Neisseria sicca*, *N. flava*, and a combination of *N. sicca* and *N. flava* as absorbents for selected sera in an indirect hemagglutination procedure^a

Serum category	No. reactive 1:12 or greater when absorbed with			
	No. tested	<i>N. sicca</i>	<i>N. flava</i>	Equal parts <i>N. sicca-N. flava</i>
Presumed non-gonococcal	12	12	5	4
Gonococcal	10	10	10	10

^a These sera were selected on the basis that a sample of each had been reactive after an absorption with *N. sicca*. The procedure involved the use of erythrocytes sensitized with a sonically treated antigen of *N. gonorrhoeae*.

serial dilutions, and added 0.05-ml volumes of the 0.75% sensitized erythrocytes. As a control, 0.05 ml of unsensitized erythrocytes was added to the sera in the last row of cups. Reactive, nonreactive, and diluent controls were included in the testing for each day. The final serum dilution in each cup was determined after the addition of diluent and sensitized sheep cells. After a 2-hr incubation period at room temperature, the settling patterns were read according to published criteria (6).

RESULTS

Erythrocytes sensitized with an alkaline extract of *N. gonorrhoeae*. Erythrocytes sensitized with an alkaline extract of *N. gonorrhoeae* reacted with 23% of the sera from 150 infected males (median titer 1:3), with 49% of the sera from 249 infected females (median titer 1:6), and with 2% of the sera from 50 celibate females (median titer 1:3).

Tanned erythrocytes sensitized with soluble antigen of sonically treated *N. gonorrhoeae*. With

tanned erythrocytes sensitized with soluble antigen of sonically treated *N. gonorrhoeae*, 887 sera were examined by using an unabsorbed and an absorbed technique (Table 1). Preliminary data from testing a small number of sera in the unabsorbed procedure suggested that a titer of 1:1,536 was the border between the gonococcal and presumed nongonococcal groups, although there was some overlap of titers between the two groups. However, when the results of testing the 887 sera were examined, considering as positive a titer of 1:1,536 or greater allowed detection of only 47% of the infected females and 35% of the infected males. Inspection of the data revealed that it would be useless to increase the apparent sensitivity by considering a titer of less than 1:1,536 as the minimum significant titer, since such a criterion would cause a large increase of reactions in presumed nongonococcal specimens.

In an attempt to improve both the sensitivity and specificity of the procedure, it was decided to absorb sera with soluble antigens of the saprophyte *N. sicca* and to consider a titer of 1:12 or greater as significant. By using this approach, the sensitized erythrocytes reacted with 88% of the sera in the infected female category and only 6% of the sera in the group of celibate females; reactivity in the group of culture-negative females was 18%. The latter percentage could be reduced to 12% if the minimum significant titer was considered to be 1:24, but the reactivity in the infected female group would in turn decrease to 76%.

Additional absorption experiments were performed to determine whether the presumed nongonococcal sera which contained reactivity that persisted after the described *N. sicca* absorption might contain antibodies removable by a greater concentration of *N. sicca* or by antigens from another member of the *Neisseria* group, *N. flava*. Doubling the concentration of *N. sicca* used for absorption seemed to remove no additional reactivity from the few selected sera in which reactivity had persisted after the usual *N. sicca* absorption. However, the data in Table 2 show that the *N. flava* absorption removed reactivity from 7 of 12 presumed nongonococcal sera which were reactive after absorption with *N. sicca*, whereas none of the 10 gonococcal sera were reduced to nonreactivity. Similar results were obtained if *N. sicca* and *N. flava* absorptions

were carried out concurrently by using a mixture of these antigens.

DISCUSSION

The main objective of this study of several hundred sera is to document for the first time that passive-hemagglutination procedures can detect gonococcal antibody in a significant percentage of patients having uncomplicated gonorrhea.

As compared to the unabsorbed technique, the use of soluble antigens of *N. sicca* and *N. flava* as absorbents reduced the reactivity of the procedure with presumed nongonococcal sera, suggesting that some of the reactivity found in nongonococcal sera is caused by cross-reactive antibodies stimulated by saprophytic *Neisseria*.

Before the passive-hemagglutination procedure can be considered ready for widespread use as a serological screening test for gonorrhea, further modifications are needed to reduce or eliminate reactivity found in the presumed nongonococcal group. Hopefully, this could be accomplished either by refining the absorption technique or by using a highly purified gonococcal antigen, lacking cross-reactive antigens, to sensitize the sheep erythrocytes.

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