Lymphocyte Transformation in Repeated Gonococcal Urethritis

STEPHEN J. KRAUS, GOLDIE H. PERKINS, AND RUTH C. GELLER

*Venereal Disease Research Laboratory, Venereal Disease Branch, Center for Disease Control,
Atlanta, Georgia 30333*

Received for publication 11 June 1970

In an attempt to ascertain whether cell-mediated hypersensitivity develops in the course of a naturally acquired gonorrhea infection, lymphocytes from men with gonococcal urethritis were cultured in vitro with a gonococcal antigen. The lymphocyte response was quantitated by a radioactive assay of deoxyribonucleic acid synthesis. Lymphocytes from subjects without gonorrhea were cultured in a similar manner to determine the specificity of the reaction. The recurrent nature of many gonococcal infections allowed a concurrent evaluation of whether the intensity of the in vitro lymphocyte response is related to the history of previous exposure to the antigen concerned. This study revealed a significantly greater lymphocyte response to the gonococcal antigen in those with gonorrhea infections than in the nongonorrhea subjects ($P < 0.01$). The intensity and timing of the response were also consistent with specific lymphocyte stimulation.

Although gonorrhea is the most frequently reported infectious disease (12), little is known about the host's response to gonococcal infections. The relationship of such a response to immunity and morbidity in gonorrhea is unknown. Morbidities frequently attributed to gonorrhea include urethral strictures, chronic salpingitis, and pelvic inflammatory disease. The protracted nature of these complications and their pathological manifestations of abscess cavities and fibrosis suggest that cell-mediated hypersensitivity to the gonococcus may exist.

As a first step in defining the existence of cell-mediated hypersensitivity in gonorrhea, we studied the lymphocyte response to the gonococcus. The technique of lymphocyte transformation allowed an in vitro evaluation of such a response (11, 14). The recurrent nature of male gonococcal urethritis facilitated a concurrent evaluation of a relationship between the intensity of the lymphocyte response and the number of naturally acquired infections.

MATERIALS AND METHODS

Human lymphocytes. Blood samples were obtained from 27 men at the time they were treated for gonococcal urethritis at a venereal disease clinic. The diagnosis of gonorrhea was confirmed by the demonstration of intracellular, gram-negative diplococci in smears of the urethral exudate and the growth of oxidase-positive gram-negative diplococci on Thayer-

Martin selective media (18). The number of prior gonorrhea infections was ascertained by history from the patients, as well as from the records of the venereal disease clinic. Blood samples were also obtained from 23 men who denied a history of gonorrhea. Twelve of these subjects were physicians, and the remainder were men presenting for premarital blood serologies. The gonorrhea and non-gonorrhea subjects were studied concurrently.

Fifty milliliters of blood was drawn into a syringe containing 5,000 units of sodium heparin (Upjohn). The blood was placed in tubes (16 by 125 mm) and allowed to sediment spontaneously for 1 hr at 37 C. The lymphocyte concentration in the supernatant was determined, and the cells were diluted so that the final suspension always contained 0.75×10^6 small lymphocytes per ml in a mixture of 80% Eagle medium and 20% autologous plasma. The final medium contained 80 μ g of streptomycin per ml.

Gonococcal antigen. The gonococcal antigenic material was obtained from a virulent colony type 1 of *Neisseria gonorrhoeae* strain F62 (7). The organisms were grown as previously described (10), disrupted by sonic treatment for 10 min in a refrigerated Raytheon 10-kc sonic oscillator operated at peak output, and the entire sonically treated material was lyophilized. A suspension (2.80 mg/ml) of the material was prepared in Eagle medium, and small portions were stored at -60 C. Each day a sample was thawed and the following dilutions were prepared in Eagle medium: 1:20, 1:50, 1:100, and 1:200.

Two milliliters of the final lymphocyte suspension and 0.05 ml of a gonococcal antigen dilution were added to tissue culture tubes (16 by 125 mm). The

tubes were gassed with 5% CO₂ in air and incubated at 37 C. Identical cultures, but without antigen (37 C control), were run concurrently. Cultures with and without antigen were run in quintuplicate.

Assay of lymphocyte transformation. The degree of lymphocyte transformation was measured by the amount of deoxyribonucleic acid (DNA) synthesis (9). A lack of DNA synthesis in the lymphocyte cultures could have been due to a lack of transformation or to cell death resulting from toxic conditions in the culture system. The latter was periodically monitored throughout the study by cultures identical to the 37 C control cultures but containing 0.05 ml of a 1:7 dilution of Difco Phytohemagglutinin P (PHA). An adequate response to PHA provided evidence that the culture conditions were suitable for lymphocyte survival and transformation if an appropriate stimulus was present. A variability in PHA response was observed during the study, but it could not be attributed to technical problems and, indeed, it was similar to the individual variability of the PHA response reported by others (17).

DNA synthesis was quantitated by the uptake of an ¹²⁵I-labeled thymidine analogue: 5-iodo-2'-deoxyuridine (IDU). The technique was that of Boylston et al. (1) with the following modifications: 0.1 ml of a 2.57×10^{-6} M ¹²⁵IDU solution prepared with a specific activity of 1.75 Ci/mmol was added to each culture, and the cells were washed twice with cold isotonic saline and twice with cold trichloroacetic

acid (10% w/v). The activity of the precipitate after the final trichloroacetic acid wash represented a summation of the ¹²⁵IDU in DNA and any unreacted ¹²⁵IDU not completely removed by the saline and trichloroacetic acid washes. An estimate of the activity due to unreacted ¹²⁵IDU was taken as the activity observed in the precipitate from cultures identical to the 37 C controls but incubated entirely at 2 C (2 C control). This control was included in the cultures of each experiment, and its activity, which was consistently less than 10% of the 37 C control, was subtracted from the activity of the 37 C cultures. For each experimental subject, the degree of DNA synthesis induced by the gonococcal antigen was expressed as a function of DNA synthesis in cultures without antigen (37 C control). The degree of lymphocyte transformation with the gonococcal antigen was therefore expressed by the following equation: transformation ratio = [(counts per minute with antigen cultures) - (counts per minute with 2 C control cultures)] / [(counts per minute with 37 C control cultures) - (counts per minute with 2 C control cultures)].

The optimal antigen concentration and duration of incubation to affect transformation were ascertained by preliminary studies. Five-day cultures containing the various dilutions of antigen revealed that a 1:20 dilution was optimal in most instances

TABLE 1. Degree of lymphocyte transformation observed with various dilutions of *Neisseria gonorrhoeae* sonicate antigen

Subject	Antigen dilution				
	Undiluted	1:20	1:50	1:100	1:200
A	8.0 ^a	8.7	NT ^b	7.3	2.3
B	4.2	4.0	4.5	2.0	1.5
C	6.0	6.8	NT	1.4	1.3
D	5.1	5.2	2.4	2.0	NT
E	5.8	5.9	2.7	2.3	1.9
F	6.2	6.2	4.9	4.4	3.2

^a Transformation ratio.

^b Not tested.

TABLE 2. Degree of lymphocyte transformation observed after various days of culture

Subject	Day of culture						
	1	2	3	4	5	6	7
G		1.1 ^a		1.6		3.4	
H	0.7		1.2	1.2	5.1		4.7
I		1.0				2.4	
J	0.6		1.1		4.6		4.4
K		1.3		1.2		3.0	

^a Transformation ratio.

TABLE 3. Lymphocyte transformation in nongonorrhea subjects with a gonococcal antigen

Subject	Max. transformation		
	Transformation ratio	Antigen dilution	Transformation ratio with 1:20 antigen dilution
1	0.5	1:20	0.5
2	0.6	1:100	0.5
3	0.8	1:200	0.7
4	0.9	1:20	0.9
5	0.9	1:50	0.6
6	1.0	1:100	0.6
7	1.0	1:50	0.6
8	1.0	1:20	1.0
9	1.0	1:20	1.0
10	1.2	1:20	1.2
11	1.3	1:20	1.3
12	1.4	1:100	1.3
13	1.5	1:100	1.0
14	1.6	1:20	1.6
15	1.6	1:20	1.6
16	1.6	1:20	1.6
17	1.6	1:20	1.6
18	1.7	1:50	1.0
19	1.9	1:20	1.9
20	2.1	1:20	2.1
21	2.1	1:20	2.1
22	6.8	1:100	6.5
23	12.0	1:20	12.0

(Table 1). A 1:20 dilution was then used in 1-, 3-, 5-, and 7-day, or 2-, 4-, and 6-day cultures; 5- and 6-day cultures effected maximal transformation (Table 2). All further experiments were carried out for five days with 1:20, 1:50, 1:100, and 1:200 antigen dilutions. For each subject, transformation ratios were calculated from the data obtained with each antigen dilution. Analyses of lymphocyte transformation data were based on either maximal transformation responses without regard to antigen concentrations (2) or transformation responses obtained with a single antigen concentration (5, 13). The study reported here was designed so that the results could be analyzed with both types of data. The response to a single antigen concentration was arbitrarily taken as the transformation induced by the 1:20 dilution.

RESULTS

The transformation ratios for subjects in the nongonorrhea and gonorrhea groups are presented in Tables 3 and 4, respectively. These tables outline each subject's maximal transformation ratio, the antigen concentration that affected this response, as well as the transformation ratio observed with the 1:20 antigen dilu-

tion. The number of infections is included for those subjects in the gonorrhea group.

Statistical analysis of the maximal transformation ratios (Student's *t* test) revealed a significantly greater degree of transformation in the total gonorrhea group than in the nongonorrhea controls ($P < 0.01$), but no significant differences between the control group and those gonorrhea subjects with only one infection. Within the gonorrhea group, greater transformation occurred in those with two or more infections than in those with their initial infection ($P < 0.01$), and greater transformation occurred in those with three or more infections than in those with one or two infections ($P < 0.01$). Statistical analysis of the transformation ratios observed with a single antigen concentration (1:20 dilution) resulted in identical conclusions and similar probability values.

DISCUSSION

Lymphocyte transformation induced by a gonococcal antigen was shown to exist in certain men with gonococcal urethritis. Although not always demonstrable at the time of an initial infection, it was usually present in those with two or more infections.

The degree of transformation with lymphocytes from patients during their initial gonococcal infection did not differ significantly from that observed in individuals without gonorrhea. The reason for this lack of transformation cannot be answered by our data, although a possibility may be that the estimated 9- to 10-day interval between infection and treatment (8) may be too short to develop a significant degree of delayed hypersensitivity as detected by lymphocyte transformation.

This study adds *N. gonorrhoeae* to the group of pyogenic bacteria capable of eliciting specific lymphocyte transformation. Such a response is known to exist in streptococci (4), pneumococci (15), tetanus toxoid (3), typhoid-paratyphoid vaccine (3), diphtheria toxoid (3), and endotoxins of gram-negative organisms (16).

The characteristics of gonococcal-induced lymphocyte transformation are those of specific stimulation in contrast to the nonspecific stimulation typified by PHA. PHA-stimulated transformation, with the technique outlined in this study, is maximal after four days of culture, results in transformation ratios in the range of 50 to 200, and occurs with lymphocytes from most subjects. The gonococcal antigen-induced transformation was maximal after 5 to 6 days of culture, resulted in transformation ratios up to 12.8, and was mostly limited to those known to have gonorrhea.

TABLE 4. *Lymphocyte transformation in gonorrhea subjects with a gonococcal antigen*

Subject	Gonorrhea infections	Max. transformation		Transformation ratio with 1:20 antigen dilution
		Transformation ratio	Antigen dilution	
24	1	0.6	1:200	0.4
25	1	0.8	1:20	0.8
26	2	0.8	1:50	0.5
27	1	1.0	1:20	1.0
28	1	1.2	1:20	1.2
29	2	1.3	1:20	1.3
30	2	1.6	1:100	0.7
31	3	1.6	1:20	1.6
32	1	1.8	1:20	1.8
33	3	2.0	1:100	1.1
34	1	2.3	1:20	2.3
35	3	2.5	1:20	2.5
36	1	3.0	1:20	3.0
37	1	3.6	1:20	3.6
38	6	3.7	1:20	3.7
39	4	4.0	1:20	4.0
40	5	4.6	1:50	3.9
41	13	5.2	1:20	5.2
42	10	5.7	1:100	5.1
43	7	5.9	1:20	5.9
44	2	6.2	1:20	6.2
45	7	6.3	1:20	6.3
46	8	6.8	1:20	6.8
47	15	7.3	1:20	7.3
48	3	8.7	1:20	8.7
49	2	8.8	1:50	4.1
50	12	12.8	1:20	12.8

The explanation for the high degree of transformation observed in two nongonorrhea subjects cannot be answered by our data. Although prior infection with gonorrhea cannot be absolutely excluded, this observation raises the possibility of cross-reactions with other antigens such as those of other *Neisseria*. The possibility is supported by reactive-delayed skin tests with gonococcal antigens in patients with meningococcal meningitis (6).

ACKNOWLEDGMENT

We thank Dennis D. Tolsma of the Venereal Disease Branch for statistical analysis.

LITERATURE CITED

1. Boylston, A. W., R. D. Guttman, and J. P. Merrill. 1968. A simplified method for the quantitation of *in vitro* leucocyte culture. *Int. Arch. Allergy Appl. Immunol.* 34:339-344.
2. Brand-Auraban, A., I. Gery, D. Benezra, A. Jacob, and A. M. Davies. 1969. Transformation of lymphocytes by streptolysin S as a test for rheumatic fever. *Is. J. Med. Sci.* 5:953-954.
3. Elves, M. W., S. Roath, and M. C. G. Israels. 1963. The response of lymphocytes to antigen challenge *in vitro*. *Lancet* 1:806-807.
4. Francis, T. C., J. J. Oppenheim, and M. F. Barile. 1969. Lymphocyte transformation by streptococcal antigen in guinea pigs and man, p. 501-518. *In* W. O. Rieke (ed.), *Proc. 3rd Annu. Leucocyte Conf.* Appleton-Century-Crofts, New York.
5. Heilman, D. H., and W. MacFarland. 1966. Inhibition of tuberculin-induced mitogenesis in cultures of lymphocytes from tuberculous donors. *Int. Arch. Allergy Appl. Immunol.* 30:58-66.
6. Irons, E. E. 1912. Cutaneous allergy in gonococcal infections. *J. Infect. Dis.* 11:77-93.
7. Kellogg, D. S., Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and C. I. Pirkle. 1963. *Neisseria gonorrhoeae* I. Virulence genetically linked to clonal variation. *J. Bacteriol.* 85:1274-1279.
8. Lodin, A. 1955. Has the incubation period of gonorrhoea undergone a change? *Acta Dermato-Venerol.* 35:457-462.
9. MacKinney, A. A., Jr., F. Stohlman, Jr., and G. Brecher. 1962. The kinetics of cell proliferation in cultures of human peripheral blood. *Blood* 19:349-358.
10. Martin, J. E., Jr., W. L. Peacock, Jr., G. Reising, D. S. Kellogg, Jr., E. Ribi, and J. D. Thayer. 1969. Preparation of cell walls and protoplasm of *Neisseria* with the Ribi cell fractionator. *J. Bacteriol.* 97:1009-1011.
11. Meuwissen, H. J., O. Stutman, and R. A. Good. 1969. Functions of lymphocytes. *Semin. Hemat.* 6:28-65.
12. National Communicable Disease Center. 1968. Annual supplement to the morbidity and mortality weekly report, fiscal year 1968, p. 1-59. U. S. Department of Health, Education, and Welfare, Public Health Service, Atlanta.
13. Newberry, W. M., J. W. Chandler, T. D. Y. Chin, and G. Kirkpatrick. 1968. Immunology of the mycosis. I. Depressed lymphocyte transformation in chronic histoplasmosis. *J. Immunol.* 100:436-443.
14. Oppenheim, J. J. 1969. Immunological relevance of antigen and antigen-antibody complex induced lymphocyte transformation. *Ann. Allergy* 27:305-315.
15. Oppenheim, J. J., R. M. Blaese, and T. A. Waldmann. 1969. The transformation of normal and Wiskott-Aldrich lymphocytes by nonspecific, leucocyte, bacterial, protein, and carbohydrate stimulants. *Clin. Res.* 17:357.
16. Oppenheim, J. J., and S. Perry. 1965. Effects of endotoxins on cultured leukocytes. *Proc. Soc. Exp. Biol. Med.* 118:1014-1019.
17. Parker, J. W., and R. J. Lukes. 1969. The variability of PHA-induced lymphocyte transformation in repetitive studies, p. 111-128. *In* W. O. Rieke (ed.), *Proc. 3rd Annu. Leucocyte Conf.* Appleton-Century-Crofts, New York.
18. Thayer, J. D., and J. E. Martin, Jr. 1966. Improved medium selective for cultivation of *N. gonorrhoeae* and *N. meningitidis*. *Pub. Health Rep.* 81:559-562.