INFECTION AND IMMUNITY, June 1970, p. 555-558

Vol. 1, No. 6
Printed in U.S.A.

# Immunoglobulin Class of Natural Human Antibodies Reactive with *Treponema pallidum*

A. J. JULIAN, L. C. LOGAN, L. C. NORINS, AND I. R. COHEN[1]

*Venereal Disease Research Laboratory, Venereal Disease Branch, State and Community Services Division, National Communicable Disease Center, Atlanta, Georgia 30333*

Received for publication 9 February 1970

To aid the development of serological tests for incubating syphilis, a characterization was made of the background of natural anti-*Treponema pallidum* antibodies against which the initial immune response to syphilis takes place. Sera from presumed normal persons were studied with monospecific antisera in an indirect fluorescent-antibody procedure. Of 36 sera tested at a 1:5 dilution, all showed IgG reactivity with *T. pallidum* (Nichols strain), 58% showed IgM reactivity, and 20% showed IgA reactivity. The titer of IgG reactivity was considerably higher than that of the other two immunoglobulins. Heating the sera for 1 hr at 65 C abolished IgA and IgM anti-*T. pallidum* reactivity, but one-third of the sera retained IgG reactivity. Human Cohn fractions II and $III_1$ from three commercial sources contained mostly IgG antibodies reactive with *T. pallidum*, but IgM and IgA antibodies were also present. IgG reactivity was found in a pool of presumably normal maternal sera from 20 mothers and in a corresponding pool of umbilical cord serum from their infants. IgM and IgA reactivities were present only in the maternal serum pool.

Little is known about the background of natural anti-*Treponema pallidum* antibodies against which the initial serological response to syphilis takes place. Yet, knowledge concerning these natural antibodies could aid development of an "early-warning" test for detection of the disease during the incubation period. Such a procedure could lead to improved control of syphilis, in that patients could be found before they developed primary chancres capable of transmitting the disease to others. We report here the immunoglobulin classes of natural human antibodies reactive with *T. pallidum*, characterized by means of an indirect fluorescent-antibody (FA) technique in which monospecific reagents are used.

## MATERIALS AND METHODS

**Sera.** Adult sera were obtained from 14 laboratory staff members and from 22 other presumed normal adults who by history had never been infected with *T. pallidum* or other pathogenic treponemes. These sera were all nonreactive in the VDRL slide test (14), the fluorescent treponemal antibody-absorption (FTA-ABS) test (14), and the *T. pallidum* immobilization (TPI) test (13).

In addition, a serum pool composed of maternal sera from 20 normal mothers and another pool of the corresponding umbilical cord sera of their infants were made available for testing from a previous study (2).

**Cohn fractions.** Human Cohn fractions II and $III_1$ were obtained in powdered form from each of three different sources. The American Red Cross, Washington, D.C., generously made fractions available through E. R. Squibb & Sons. Lederle Laboratories, Division of American Cyanamid Co., Pearl River, N.Y., kindly furnished another set of fractions. The third set was obtained from Hyland, Division Travenol Laboratories, Inc., Los Angeles, Calif. The Squibb and Hyland fractions had been prepared from peripheral plasma obtained from adult donors. According to information furnished us by the company, the Lederle fractions were processed from extracts of human placentas. The placentas were saved postpartum by various cooperating hospitals, and were frozen until and during shipment to the processing plant. All plasma or placenta donors were nonreactive in routine (nontreponemal) serological tests for syphilis.

For gel filtration, 1,000 mg of human Cohn fractions II and $III_1$ from each of the three companies was allowed to dissolve in 15 ml of tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer plus 1 M NaCl (*p*H 8.0) at 4 C for 24 hr. The samples were then centrifuged at 3,000 × *g*. Insoluble material was washed five times in distilled water, dehydrated for 1 week in a desiccator jar, and weighed on an analytical balance. The weight of the undissolved material was subtracted from the 1,000 mg of originally weighed-out powder to calculate the

[1] Present address: Weizmann Institute of Science, P.O. Box 26, Rehovoth, Israel.

TABLE 1. *Immunoglobulin classes of antibodies reactive with Treponema pallidum in sera from 36 presumed normal nonsyphilitic individuals*[a]

| Immunoglobulin | Per cent reactive | Reciprocal titer | |
|---|---|---|---|
| | | Range | Mean of those reactive |
| IgG | 100 | 5-2,560 | 640 |
| IgM | 58 | 5-20 | 5 |
| IgA | 20 | 5 | 5 |

[a] Sera were diluted 1:5 in phosphate-buffered saline (*p*H 7.2) and tested unheated by means of an indirect fluorescent-antibody procedure in which monospecific reagents were used.

weight of Cohn fraction actually dissolved in the 15 ml of buffer. For quantitation and immunofluorescence testing of immunoglobulins, 400 mg of each Cohn fraction was allowed to dissolve in 10 ml of phosphate-buffered saline (PBS), *p*H 7.2. Any undissolved material was removed by centrifugation before testing.

**Gel filtration.** Sera or Cohn fractions were fractionated by gel filtration through a bed of Sephadex G-200, 90 × 2.5 cm, as previously described (9).

**Quantitation of immunoglobulin.** The concentrations of IgG, IgM, and IgA were determined by using a radial-diffusion precipitin assay (Immunoplates, Hyland).

**Indirect FA assay.** This procedure was modeled on the FTA 1:5 technique (4). In brief, the unheated serum or fraction to be tested was overlaid on *T. pallidum* (Nichols strain) extracted from rabbit testicular syphilomas, and was fixed to a slide. It is emphasized that no sorbent (14) or other material related to Reiter treponemes was added to the serum or fractions unless specified. After incubation and rinsing, attachment of immunoglobulins to the organism was revealed by "staining" with fluorescein-labeled goat antisera (Hyland) specific for human IgG, IgM, or IgA, and the slide was examined with a fluorescence microscope. The detailed evidence for the monospecificity of each reagent, used at its working dilution, has been presented elsewhere (9).

## RESULTS

**Immunoglobulin activities of normal serum and fractions.** Sera from 14 laboratory personnel and 22 other individuals were tested in the indirect FA assay. No significant difference in the reactivities was found between the two groups; therefore, all 36 sera will be considered together. Table 1 shows the reactivities of IgG, IgA, and IgM. All sera showed IgG reactivity, 58% showed IgM reactivity, and 20% showed IgA reactivity. The mean titer of IgG reactivity was higher than that of the other two immunoglobulins.

To further confirm the immunoglobulin reactivities, and to minimize the possibility of competition between IgG and IgM (3), the 14 sera from laboratory personnel were fractionated on Sephadex G-200, and the fractions and sera were assayed in the indirect FA procedure. We found that IgG antibodies comprised the bulk of the reactivity; IgG antibody activity was detected in all five serum fractions, but the bulk was found in the 7*S* peak and 11*S* "shoulder." The IgM reactivity was present in lesser amounts than IgG reactivity, and appeared mainly in the first fraction (19*S*) of the filtration curve. Only two of the sera had detectable IgA reactivity; in those, it was present in low titer and was located in the 19*S* fraction.

Tests of the maternal and cord serum pools revealed that the titer of IgG antibody in the maternal serum pool was not significantly different from the IgG titer in the cord serum pool (Table 2). Only the maternal serum pool contained IgM and IgA antibodies. Testing 19*S*, 7*S*, and 3.5*S* gel filtration fractions of both pools confirmed the presence and distribution of these immunoglobulins. Antibodies of the IgG class were found mainly in the 7*S* fraction, and IgM antibodies were in the 19*S* fraction.

Treatment of these specimens with sorbent, as is done in the FTA-ABS test (14), eliminated all immunoglobulin reactivities with *T. pallidum*. All sera and fractions were nonreactive in the VDRL slide and TPI tests.

**Effect of heating on serum reactivities in the direct FA assay.** The 36 sera were diluted 1:5 in PBS and divided into two samples. One sample was heated at 56 C for 1 hr; the other, at 65 C for 1 hr. As shown in Table 3, heating at 65 C for 1 hr removed all IgA and IgM anti-*T. pallidum* indirect FA activity of normal serum, but IgG remained active in about one-third of the sera.

**Immunoglobulin content and indirect FA reactivities in human Cohn fractions II and $III_1$.**

TABLE 2. *Maternal and umbilical cord serum immunoglobulins reactive with Treponema pallidum*[a]

| Serum | Immunoglobulins reactive with *T. pallidum* | | |
|---|---|---|---|
| | IgG | IgM | IgA |
| Maternal serum pool | 40[b] | 20 | 5 |
| Umbilical cord serum pool | 20 | 0 | 0 |

[a] Unheated sera were diluted 1:5 in phosphate-buffered saline (*p*H 7.2) and tested by means of an indirect fluorescent-antibody procedure in which monospecific reagents were used.

[b] Reciprocal titer.

TABLE 3. *Effect of heating on the natural reactivity of normal sera with Treponema pallidum*

| Temp[a] | Per cent reactive[b] | | |
|---|---|---|---|
| | IgG | IgM | IgA |
| *C* | | | |
| 56 | 100 | 58 | 20 |
| 65 | 31 | 0 | 0 |

[a] After dilution 1:5 in phosphate-buffered saline (*p*H 7.2), 36 sera were heated at each temperature for 1 hr before being tested.

[b] Classes of immunoglobulins reactive with *T. pallidum* were determined by means of an indirect fluorescent-antibody procedure in which monospecific reagents were used.

To extend our observation beyond the limited number of individual sera, IgG, IgA, and IgM antibodies were assayed in Cohn fractions from three different companies. These pools were made up from a large number of normal donors. For gel filtration, preparations of Cohn fraction II readily went into solution, whereas those of Cohn fraction $III_1$ did not. The Squibb Cohn fraction $III_1$ was especially difficult to dissolve. Of 1,000 mg placed in 15 ml of Tris buffer, 245 mg remained undissolved. The fractions from the other two companies were less difficult to dissolve. With Cohn fraction $III_1$ from Hyland and Lederle, only 110 and 125 mg, respectively, remained undissolved. Placing 500 mg of each of these fractions in 10 ml of PBS yielded comparable results. Concentration and immunofluorescent reactivities of the immunoglobulins were determined in these solutions.

Table 4 shows the immunoglobulin contents and indirect FA reactivities in Cohn fractions II and $III_1$ obtained from three commercial sources. There were large variations in individual immunoglobulin concentration, especially with IgG. The predominant immunoglobulin in each fraction was IgG, and fraction $III_1$ contained more IgM than did fraction II. Both fractions contained significant IgG antibody reactive with *T. pallidum*. IgM antibodies reactive at greater than 1:5 dilution were found only in fraction $III_1$; IgA reactivity was present in only one preparation of fraction II. Squibb fraction II contained the highest titer of IgG antibody. The anti-*T. pallidum* reactivities found in Cohn fractions from two sources were not seen if the fractions were diluted 1:5 with sorbent (14) before testing. But with the fractions prepared by Squibb, after they had been diluted 1:5 with sorbent, IgG reactivity of 1+ intensity was still detectable in fraction $III_1$.

In all cases, indirect FA testing of 19*S* and 7*S* fractions of whole serum and Cohn fractions fractionated on Sephadex G-200 confirmed that IgG and IgM reactivities were present in the appropriate fractions.

## DISCUSSION

This study helps to characterize the background of natural treponemal antibodies against which the primary immune response to syphilis infection takes place. We have described elsewhere (9) the immunoglobulin classes of anti-*T. pallidum* reactivity present when syphilis reaches the primary and secondary clinical stages.

There have been few reports describing natural human antibodies reactive with *T. pallidum*. Deacon et al. (5) reported that, at a 1:5 dilution, sera from about 30% of apparently normal people reacted with the organism in an indirect FA procedure. These investigators used a fluorescein-labeled antiserum to human "globulin," and, at least theoretically, it could have bound to all three major immunoglobulin classes.

TABLE 4. *Immunoglobulins and Treponema pallidum reactivity present in Cohn fractions II and $III_1$ prepared from human plasma and placentas*

| Cohn fraction | Commercial manufacturer | IgG | | IgM | | IgA | |
|---|---|---|---|---|---|---|---|
| | | Amt[a] | Titer[b] | Amt | Titer | Amt | Titer |
| | | *mg/ml* | | *mg/ml* | | *mg/ml* | |
| II | Squibb (lot 2126) | 28.4 | 640 | 0.7 | 0 | 0.9 | 0 |
| | Hyland (lot 44904) | 32.3 | 40 | 0.6 | 0 | 0.8 | 0 |
| | Lederle (lot C-878) | 50.0 | 20 | 0.8 | 5 | 1.0 | 5 |
| $III_1$ | Squibb (lot 1986) | 10.3 | 80 | 2.0 | 20 | 1.2 | 0 |
| | Hyland (lot 71-807) | 26.8 | 40 | 1.9 | 20 | 1.2 | 0 |
| | Lederle (lot C-923) | 30.0 | 20 | 2.0 | 0 | 3.0 | 0 |

[a] Determined by radial immunodiffusion.

[b] Reactivity titers (reciprocal) were determined by means of an indirect fluorescent-antibody procedure in which reagents monospecfic for each immunoglobulin class were used.

Our experiments show that the natural reactivity removed from nonsyphilitic sera by treatment with sorbent, as is done in the FTA-ABS procedure (14), is mostly of the IgG class. The dominant contribution of IgG antibodies to "natural" anti-*T. pallidum* reactivity is comparable to the situation recently described by Cohen and Norins (1, 2) in regard to natural human antibodies reactive with gram-negative bacteria. The distribution of antibodies between maternal and umbilical cord serum is also in accord with their findings.

We believe that the reactivities we have demonstrated are natural antibodies, i.e., antibodies produced in the absence of artificial immunization or natural infection (11). Deacon and Hunter (6) postulated that natural antibodies reactive with *T. pallidum* might be stimulated by contact with saprophytic treponemes, such as those in the mouth, which have antigens in common with *T. pallidum*.

Because the *T. pallidum* routinely used as antigen in the present and other immunofluorescence tests had been harvested from rabbit testicular syphilomas, substances of rabbit origin may have coated or been absorbed onto the organisms (10, 12). Thus, it is conceivable that some of the natural reactivities we detected were directed at rabbit antigens in close association with the treponemes and not at the organisms themselves. However, this seems an unlikely possibility. Sera having strong "natural" reactivity with *T. pallidum* were obtained from five normal individuals. All sera were absorbed with finely minced normal rabbit testes, and then were tested with the three fluorescent antisera. No reduction was seen in the reactivity of the sera with *T. pallidum*.

The large variations in immunoglobulin concentrations that we found in various preparations of Cohn fraction II is, to a certain extent, in accordance with recent findings. Heiner and Evans demonstrated variable amounts of immunoglobulins and other serum proteins in 27 lots of gamma globulin preparations for 10 commercial drug companies (8).

Using a complement-dependent assay, Hederstedt (7) recently found that sera from apparently normal people can immobilize *T. pallidum*. But he suggested that this "natural" immobilizing activity against *T. pallidum* has not been noticed in the TPI test because, during the incubation period of the test, the sulfhydryl reducing agents or other reactants in the suspending medium suppress or inactivate the natural reactivity. He also found that the natural antibody was destroyed in many patients when the sera were heated at 56 C for 30 min, as is done for the TPI test.

In the light of Hederstedt's work, our previous immunofluorescence studies of antibodies in early syphilis (9), and the current study, we can assume a uniform concept of action wherein the FTA-ABS and TPI tests gain their apparent specificity. Both assays incorporate factors which remove, suppress, or inactivate natural anti-*T. pallidum* reactivity in the sera of normal people, while allowing the recognition of immune antibodies in infected individuals. In the FTA-ABS test, the suppression of natural reactivity is brought about by sorbent. In the TPI test, natural reactivity is suppressed by the heating of the serum before testing, by the medium in which the treponemes and serum are incubated, or by both. Both of these "specific" tests for syphilis, therefore, would seem to represent a biological balance between natural and immune antibody.

## LITERATURE CITED


1. Cohen, I. R., and L. C. Norins. 1966. Natural human antibodies to gram-negative bacteria: immunoglobulins G, A, and M. Science (Washington) **153**:1257–1259.
2. Cohen, I. R., and L. C. Norins. 1968. Antibodies of the IgG, IgM, and IgA classes in newborn and adult sera reactive with gram-negative bacteria. J. Clin. Invest. **47**:1053–1062.
3. Cohen, I. R., L. C. Norins, and A. J. Julian. 1967. Competition between, and effectiveness of, IgG and IgM antibodies in indirect fluorescent antibody and other tests. J. Immunol. **98**:143–149.
4. Deacon, W. E., V. H. Falcone, and A. Harris. 1957. A fluorescent test for treponemal antibodies. Proc. Soc. Exp. Biol. Med. **96**:477–480.
5. Deacon, W. E., E. M. Freeman, and A. Harris. 1960. Fluorescent treponemal antibody test. Modification based on quantitation (FTA-200). Proc. Soc. Exp. Biol. Med. **103**:827–829.
6. Deacon, W. E., and E. F. Hunter. 1962. Treponemal antigens as related to identification and syphilis serology. Proc. Soc. Exp. Biol. Med. **110**:352–356.
7. Hederstedt, B. 1968. Studies on the *Treponema pallidum* immobilizing activity in normal human serum. I. A method. WHO/VDT/RES/68.147:1–22.
8. Heiner, D. C., and L. Evans. 1967. Immunoglobulins and other proteins in commercial preparations of gamma globulin. J. Pediat. **70**:820–827.
9. Julian, A. J., L. C. Logan, and L. C. Norins. 1969. Early syphilis: immunoglobulins reactive in immunofluorescence and other serologic tests. J. Immunol. **102**:1250–1259.
10. Julian, A. J., J. Portnoy, and H. N. Bossak. 1963. False positive reactions in treponemal tests. Brit. J. Vener. Dis. **39**: 30–32.
11. Landy, M., and W. P. Weidanz. 1963. Natural antibodies against gram-negative bacteria, p. 275. *In* M. Landy and W. Braun (ed.), Bacterial endotoxins. Rutgers Univ. Press, New Brunswick, N.J.
12. Miller, J. N., J. H. Bekker, J. H. De Bruijn, and P. C. Onvlee 1966. The immunologic response of goats to normal and syphilitic rabbit testicular tissue. J. Immunol. **97**:184–188.
13. U.S. Department of Health, Education, and Welfare. 1964. Manual of serologic tests for syphilis. U.S. Public Health Serv. Publ. 411.
14. U.S. Department of Health, Education, and Welfare. 1969. Manual of tests for syphilis. U.S. Public Health Serv. Publ. 411.