

Latent Syphilis: Immunoglobulins Reactive in Immunofluorescence and Other Serological Tests

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Received for publication 7 October 1970

Study of sera from 69 patients with untreated or inadequately treated latent syphilis revealed that immunoglobulin (Ig)G antibodies made up the bulk of the fluorescent treponemal antibody-absorption (FTA-ABS)-test reactivity found in the sera. IgM and IgA antibodies also contributed in some cases. Venereal Disease Research Laboratory slide-test reactivity was found in both 19 and 7S serum fractions, whereas *Treponema pallidum* immobilization-test reactivity was found mainly in the 7S fraction.

Serologic tests for syphilis are an important, and often the only, means for the detection of latent syphilis. It therefore seemed of interest to determine the immunoglobulin classes of the antibodies responsible for the serologic reactivity seen at this stage of the disease.

MATERIALS AND METHODS

Sera. Sixty-nine sera from latent syphilitics were studied. Forty-three sera were obtained from male syphilitics older than 55 years (5). The duration of infection in these patients was at least 37 years. Another 26 sera were supplied by health officers in the public health clinics of Houston, Tex.; Detroit, Mich.; and St. Louis, Mo. All 26 sera were from untreated subjects less than 45 years of age. In 10 of the subjects, the duration of infection was less than 2 years, and in 8 the duration of infection was 4 to 12 years.

Serological tests. The *Treponema pallidum* immobilization (TPI) and Venereal Disease Research Laboratory (VDRL) slide tests were performed by standard methods (7). To identify the immunoglobulin classes of *T. pallidum* antibodies reactive in the fluorescent treponemal antibody-absorption (FTA-ABS) test, fluorescent-antibody conjugates monospecific for immunoglobulin (Ig)G, IgM, and IgA were used in the FTA-ABS procedure (8). The technique for the use of these conjugates and the evidence for their specificity have been described in detail elsewhere (3).

Preparation of 19 and 7S serum fractions. The 19 and 7S fractions of sera were prepared by filtration on Sephadex G-200, as previously described (3). The 46 sera fractionated comprised (i) 10 of the 43 sera from patients having syphilis 37 years or longer (these sera were selected on the basis that they had high titers of reactivity in the VDRL slide test); (ii) all 26 sera from patients having syphilis 12 years or

less; and (iii) 10 sera from presumably normal individuals (fractionated for control purposes).

2-Mercaptoethanol treatment. The procedure was performed as previously described (6).

RESULTS

Immunofluorescence tests on whole sera. Table 1 illustrates that IgG, IgM, and IgA antibodies reactive with *T. pallidum* can be found in sera of latent syphilitics. IgG antibodies were reactive most frequently and showed the greatest intensity of fluorescence. Only IgG antibodies were reactive at titers above 1:160 (range 1:5 to 1:1,280), and the reactivity found was present only at a titer of 1:5, which was the lowest serum dilution tested. No reactivity was found in sera from 10 presumably normal individuals, nor in sera from 29 nonsyphilitic individuals of the same age group, sex, race, geographic area, and socioeconomic group as the patients with infections of at least 37 years in duration.

Studies on 19 and 7S serum fractions. Results of testing the 19 and 7S fractions with the monospecific fluorescent-antibody reagents in the FTA-ABS test confirmed the presence of the immunoglobulin classes of anti-*T. pallidum* reactivity found in the whole sera.

The results of VDRL slide tests are shown in Table 2. It may be seen that both 7 and 19S reactivities were present. Five VDRL-reactive 19S fractions from each of the two groups were treated with 2-mercaptoethanol; after this treatment, VDRL slide-test reactivity disappeared.

Table 3 indicates the results of TPI tests. The

TABLE 1. Immunoglobulin classes of serum antibodies reactive with *Treponema pallidum* in an FTA-ABS procedure with monospecific fluorescent antisera

Antiserum	Duration of syphilis infection (yr)	No. of patients tested	Intensity of fluorescence ^a			
			3, 4+	2+	1+	N, ±
IgG	<4	18	18			
	≥4	51	51			
IgM	<4	18	3	4	7	4
	≥4	51	10	8	15	18
IgA	<4	18			3	15
	≥4	51			5	46

^a Fluorescence was subjectively scored on an arbitrary scale ranging from nonreactive (N) to 4+.

TABLE 2. VDRL slide test reactivity in whole serum and 19 and 7S serum fractions from 36 patients with latent syphilis

Material tested	Duration of syphilis infection (yr)	Per cent reactive	1/ Median titer	Range of 1/titer
Whole serum	<4 ^a	92	32	8-128
	4->39 ^b	62	4	1-32
19S fraction	<4	78	16	4-128
	4->39	32	2	1-16
7S fraction	<4	100	32	2-64
	4->39	66	4	1-16

^a Eighteen patients: 10 had syphilis <2 years, and eight had syphilis 2 to >4 years.

^b Eighteen patients: eight had syphilis 4 to 12 years and 10 had syphilis >39 years.

TABLE 3. TPI-test reactivity in whole serum and 19 and 7S serum fractions from 36 patients with latent syphilis

Material tested	Duration of syphilis infection (yr)	Test results			
		Reactive	Nonreactive	Inconclusive ^a	Anticomplementary
Whole serum	<4 ^b	12	0	6	0
	4->39 ^c	14	3	1	0
19S fraction	<4	3	0	7	8
	4->39	1	5	4	8
7S fraction	<4	18	0	0	0
	4->39	12	6	0	0

^a Result reported when less than 70% of the treponemes in the control tube were motile.

^b Ten patients had syphilis <2 years and eight had syphilis 2 to >4 years.

^c Eight patients had syphilis 4 to 12 years and 10 had syphilis >39 years.

7S fraction was reactive in 30 of 36 instances, whereas the 19S fraction was reactive in only 4 of 36 cases. On the other hand, the 19S fraction frequently gave anticomplementary or "inconclusive" results. Inconclusive is reported when less than 70% of the treponemes in the control tube are motile (7).

None of the 7 and 19S fractions of the 10 presumably normal sera was reactive in the VDRL slide, TPI, or immunofluorescence tests.

DISCUSSION

The present study indicates that, at the latent stage, IgG antibodies make the major contribution to FTA-ABS-test reactivity. This pattern is similar to the pattern we have reported previously for the FTA-ABS test in untreated early syphilis (3) and in syphilis 13 or more years after treatment at the late latent or late stage (4). Our finding that in latent syphilis the 7S serum fraction is the main contributor to TPI test reactivity and our findings that VDRL slide-test reactivity is present in both 19 and 7S fractions are essentially the same pattern of serologic reactivity as we found in early syphilis (3).

In the light of the above findings, it does not appear that the stages of syphilis can be serologically differentiated one from the other simply by using monospecific fluorescent antisera in the present FTA-ABS test or by focusing on either the 19 or 7S serum fraction in the VDRL slide or TPI tests.

The finding that some syphilitics with infections of many years duration still have 19S VDRL slide-test reactivity and IgM FTA-ABS-test reactivity is immunologically intriguing. According to classic experiments in which relatively simple, nonreplicating antigens were injected into animals, one might have expected the late response, whether late primary response or late booster response, to be entirely IgG in character.

The classification of the patients whose sera were studied as "latent" syphilitics rested primarily on the judgment of the examining physician in balancing the results of history, physical examination, serologic tests, and epidemiologic interviews. Practical conditions did not permit a reappraisal of these patients, and the possibility of an occasional erroneous classification must therefore be kept in mind. Although the elderly group of patients with syphilis of greater than 37 years duration had never been specifically treated for syphilis, there is the possibility that small amounts of antibiotics may have been received over the years by some of the individuals. Therefore, strictly speaking, it might be prudent to consider this group as "untreated or inadequately treated."

ACKNOWLEDGMENTS

We thank M. Lantz and W. Halbert for performing the cardiolipin antigen tests and E. Kennedy for performing the TPI tests.

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