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Immunological responses in late syphilis

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SUMMARY Thirty-one serum samples from 18 patients with clinically established late syphilis and 1319 from patients at other stages of the disease were fractionated by density gradient ultracentrifugation and examined for antilipoidal and antitreponemal antibodies of the IgM and IgG classes.

Sera from the patients with late syphilis always showed persistent concentrations of antilipoidal IgM and IgG and of antitreponemal IgG but never yielded detectable concentrations of antitreponemal IgM. When treated, these patients' antibody titres did not decline. Patients with secondary or latent syphilis also showed this serological picture after treatment but only transiently; their antibody titres continued to decline in a way which clearly distinguished them from the cases of late syphilis.

It is suggested that patients whose sera persist in showing the stable pattern described may develop late symptomatic syphilis.

Introduction

The immunological responses at various stages of syphilis have been described by several workers. Aho[1] examined sera from patients with primary, secondary, latent, late, and congenital syphilis for antilipoidal antibodies separated into 19S and 7S fractions. Some sera from primary cases contained detectable antibodies in the 19S fraction only whereas others reacted with both 19S and 7S fractions. Patients with secondary or early latent syphilis usually showed higher titres in the 7S fraction. Sera from patients with late syphilis had a predominance of 19S antibodies. Julian *et al*[2] examined sera from cases of early syphilis for lipoidal antibodies and found activity in both 19S and 7S fractions. Sometimes there was reactivity in only the 7S fraction, but in no instance did they find reactivity in the 19S fraction alone.

Studies of the presence of specific antitreponemal IgM showed that such antibodies may be present in latent and late syphilis even after treatment.[3-9] In contrast, Duncan and Kuhn,[10] in a similar study using chimpanzees, found that production of specific antitreponemal IgM ceased spontaneously in 10 out of 24 animals who had received no treatment, and Shannon and Booth[11] failed to demonstrate antitreponemal IgM reactivity in sera from patients with late syphilis.

In the present study, we examined sera from cases of late symptomatic syphilis for the presence of IgM and IgG class antibodies against both lipoidal (A-L) and treponemal (A-T) antigens. These data, together with Venereal Disease Research Laboratory (VDRL) slide test results, were correlated with the clinical diagnosis to define the course of the immune response in late syphilis.

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Patients and methods

SYPHILITIC SERA

One thousand three hundred and fifty specimens of sera from patients at various stages of the disease were selected from specimens taken at routine examination at the Bristol Public Health Laboratory. All the sera gave positive results in both the fluorescent treponemal antibody absorption (FTA-ABS) IgG test and the *Treponema pallidum* haemagglutination assay (TPHA).

FRACTIONATION PROCEDURES

Density gradient ultracentrifugations were performed in a 12·5-37·5% discontinuous sucrose gradient[12] using 5-ml polycarbonate ultracentrifuge tubes. A sample of serum (0·4 ml) was placed on top of the sucrose gradient and the tubes were centrifuged at 100 000 × *g* for 18 hours. Ten equal fractions were collected and subjected to

TABLE I *The clinical histories of cases of late syphilis*

Case No.	Age (years)	Sex	No of serum samples	Time span of serology (months)	Time treatment was started: Before first specimen	Time treatment was started: After first specimen	Clinical diagnosis
1	51	M	8	50	28 years	1 month	Cardiovascular syphilis
2	51	M	8	18		2 months	Meningovascular syphilis
3	57	M	7	24		1 month	Tabes dorsalis
4	68	M	6	18		1 month	Cardiovascular syphilis
5	42	M	2	12	4 years		Cardiovascular and neurosyphilis
6	65	M	3	11	*	*	Cardiovascular syphilis
7	57	M	3	1	*	*	Meningovascular syphilis
8	51	M	3	5	*	*	Neurosyphilis
9	59	M	1		*	*	Neurosyphilis
10	66	F	2	3	*	*	Meningovascular syphilis
11	54	M	2	1	*	*	Neurosyphilis
12	52	M	3	9	*	*	Cardiovascular syphilis
13		M	2	1	*	*	Tabes dorsalis
14	62	M	7	46	†	†	Neurosyphilis
15	79	M	1		†	†	Tabes dorsalis
16	55	M	7	30	†	†	Neurosyphilis
17	85	F	1		†	†	Gummatous syphilis
18	79	F	2	4	†	†	Cardiovascular syphilis

* Untreated
† History of treatment not known

complement-fixation tests (CFT) and fluorescent treponemal antibody absorption (FTA-ABS) tests.

Anti-lipoidal antibodies were detected by a Maltaner cardiolipin antigen CFT (Wellcome Reagents). Antitreponemal antibodies were detected by both Reiter protein antigen CFT (B-D Mérieux Antigène Trépońemique Souche Reiter) and FTA-ABS tests,[13] using specific antihuman IgM and IgG conjugates (Wellcome Reagents).

Results

LATE SYPHILIS

Fractionation procedures carried out on 31 serum samples from 18 patients presenting with symptoms of late syphilis (table I) showed that in all cases these sera contained A-L IgM and IgG and A-T IgG but no detectable concentrations of A-T IgM. The extended serological histories, available on nine patients with late symptomatic syphilis (cases 1-6, 12, 14, and 16), showed that there was little or no decrease in antibody titres whether they were treated or not. An example of the serological history of a case of late syphilis (case 1) is given in table II. From this, it can be seen that 15 months after treatment the titre of the A-T IgG had not fallen, the titres of the A-L IgM and IgG had fallen only moderately, and the VDRL titre remained constant within experimental variation.

The results of fractionation studies on a further 1319 serum samples were examined to determine whether the presence of A-L IgM and IgG and A-T IgG, in the absence of A-T IgM, was unique to patients with late clinical syphilis. This pattern was found at some stage of their immunological history in sera from 11 patients with treated secondary, from six with latent, and from three with adult congenital syphilis.

TABLE II *Immunological history of a patient (case 1) with late symptomatic syphilis**

Time (months)	VDRL titre¶	Cardiolipin CFT titres: IgM	Cardiolipin CFT titres: IgG	RPCFT titres: IgM	RPCFT titres: IgG	FTA-ABS-IgM test
0†	8	49	22	0	28	0
2‡	4	49	21	0	23	0
4§	8					
6§	16					
10	8	51	15	0	22	0
14	8	22	13	0	24	0
17	16	12	12	0	26	0
50§	8					

* This patient had a history of syphilis in 1946, when he was treated with arsenic and bismuth. In 1974 he developed an aortic aneurysm. Eight serum samples were received from this patient.
† Time 0 represents serology on presentation.
‡ Treatment was started immediately after this sample had been taken.
§ Insufficient serum for fractionation procedure.
¶ Reciprocal of the dilution.
CFT = Complement-fixation test
RPCFT = Reiter protein complement-fixation test

SECONDARY SYPHILIS

Twenty-seven serum samples from 11 patients who had been treated for secondary syphilis showed the pattern A-L IgM and IgG and A-T IgG but no A-T IgM. Sera from all these patients contained A-T IgM and the other three types of antibody at the time of diagnosis before treatment was given.

TABLE III *Immunological history of a patient with secondary syphilis*

Time (months)	VDRL titre‡	Cardiolipin CFT titres IgM	Cardiolipin CFT titres IgG	RPCFT titres IgM	RPCFT titres IgG	FTA-ABS-IgM test
0*†	32	94	222	26	65	+
2	8	17	19	0	18	0
3	4	13	19	0	8	0
5	2	6	2	0	5	0
9	0	4	0	0	4	0

* Time 0 represents serology on presentation.
† Treatment was started immediately after this sample had been taken.
‡ Reciprocal of the dilution.
+ Positive

After treatment, the A-T IgM titre fell below detectable levels, thus giving the serological pattern found in the 18 cases of late syphilis. Thereafter, however, the antibody titres declined and A-L IgG was usually the next to fall below detectable levels. This led to the appearance of the serological pattern (presence of A-L IgM and A-T IgG) that was observed in patients treated successfully.[11] The serological response of a typical case of secondary syphilis is given in table III. In secondary syphilis antibody concentrations fall rapidly after treatment unlike in late syphilis, where they show little or no reduction (table II). Secondary syphilis may therefore resemble late syphilis serologically for a time (usually not for more than three months) during this period; however, the titres continue to fall.

LATENT SYPHILIS

Examination of the immune response of patients diagnosed as having latent syphilis showed that only six, at some stage, had the A-L IgM and IgG and A-T IgG pattern. These patients fell into two groups: three had detectable concentrations of antitreponemal IgM at some stage but the other three had none. Two of the patients who presented with detectable concentrations of A-T IgM showed the serological pattern of treated secondary syphilis—that is, as their immune response declined after treatment, it transiently resembled that of late syphilis (table IV). The other patient, in whom concentrations of A-T IgM soon became undetectable, however, showed minimal decrease in the titres of the other three types of antibody despite treatment (table V). This patient may be considered a treatment failure and so liable to develop late symptomatic syphilis.

The other three cases of latent syphilis with no A-T IgM antibodies (table VI) serologically resembled cases of late syphilis. As in the example, however, their antibody concentrations fell markedly after treatment in contrast to cases of late syphilis, but the fall in titre was considerably slower than in the cases of secondary syphilis. A fourfold decrease in VDRL test titre took approximately two months in cases of treated secondary syphilis and eight months in these cases.

TABLE IV *Immunological history of a patient with latent syphilis presenting with a positive FTA-ABS-IgM test result*

Time (months)	VDRL titre‡	Cardiolipin CFT titres IgM	Cardiolipin CFT titres IgG	RPCFT titres IgM	RPCFT titres IgG	FTA-ABS-IgM test
0*†	16	225	21	13	50	+
1†	8	267	21	10	38	+
6†	2	119	9	0	2	±
16	0	8	0	0	2	0
23	0	0	0	0	0	0

* Time 0 represents serology on presentation.
† Treatment was started immediately after these samples had been taken.
‡ Reciprocal of the dilution.
+ Positive; ± weakly positive

TABLE V *Immunological history of a patient with latent syphilis presenting with a positive FTA-ABS-IgM test result and possibly developing late syphilis*

Time (months)	VDRL titre‡	Cardiolipin CFT titres IgM	Cardiolipin CFT titres IgG	RPCFT titres IgM	RPCFT titres IgG	FTA-ABS-IgM test
0*	32	59	94	13	72	+
1†	32	79	129	0	90	+
3†	128	54	146	0	162	+
11	16	48	74	0	16	0
12	8	28	30	0	45	0
16	16	12	16	0	32	0
17	32	12	24	0	32	0

* Time 0 represents serology on presentation.
† Treatment was started immediately after these samples had been taken.
‡ Reciprocal of the dilution.
+ Positive

TABLE VI *Immunological history of a patient with syphilis presenting with a negative FTA-ABS-IgM test result*

Time (months)	VDRL titre‡	Cardiolipin CFT titres IgM	Cardiolipin CFT titres IgG	RPCFT titres IgM	RPCFT titres IgG	FTA-ABS-IgM test
0*†	16	28	46	0	93	0
4	8	25	45	0	24	0
7	4	16	21	0	25	0
26	+	3	0	0	6	0

* Time 0 represents serology on presentation.
† Treatment was started immediately after this sample had been taken.
‡ Reciprocal of the dilution.
+ Positive (undiluted)

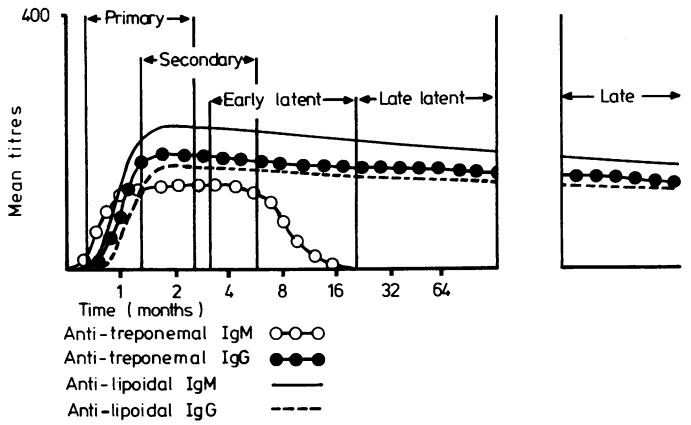

FIGURE *Immune responses in untreated syphilis*

CONGENITAL SYPHILIS

Adult congenital syphilis was diagnosed in three patients. The serological pattern of these patients was the same as that which we have associated with late syphilis. Clinical details were not available for one of the three cases, but the other two presented with signs and symptoms of late syphilis.

One patient presented with an unusual rash and could not therefore be included in any of the above categories. Serum from this patient was shown initially to contain both A-L IgM and IgG and A-T IgM and IgG antibodies, but after treatment the A-T IgM concentration fell below detectable levels. The A-L IgM and IgG and A-T IgG concentration thus remained unaltered 18 months after treatment. The serological pattern of this patient was identical to that seen in the case of latent syphilis described previously and illustrated in table V.

Discussion

Although examination of sera for A-T IgM antibodies (usually by the FTA-ABS-IgM test) may provide a useful indication of the active state of the disease in primary or secondary cases of syphilis, it would appear to be unreliable in patients at later stages.

We have shown above that 18 patients, diagnosed on clinical evidence as cases of late syphilis had no detectable concentrations of A-T IgM in their sera taken before or after treatment. Of six patients diagnosed as cases of latent syphilis three had A-T IgM in their sera taken before treatment but three did not. In contrast, patients presenting with untreated secondary syphilis invariably had A-T IgM present in their sera.

The results suggest that during the course of untreated syphilis A-T IgM production ceases, generally during the latent period between the end of secondary symptoms and the onset of late symptoms.

These findings are consistent with those of Wilkinson and Rodin,[8] who found that the number of positive FTA-ABS-IgM test results was considerably reduced by the time patients had progressed to the late latent stage of the disease. Unfortunately, no patients with untreated late symptomatic syphilis attended during their survey. O'Neill[9] also agreed that some cases of latent syphilis may present with a negative FTA-ABS-IgM test result.

Patients who have reached the late symptomatic stage of the disease, whether treated or not, lose the ability to produce A-T IgM while retaining high concentrations of A-L IgM and IgG and A-T IgG antibodies. O'Neill[9] suggested that cases of late syphilis, whether treated within the preceding five years or untreated, should be FTA-ABS-IgM-positive. Our findings do not support this opinion.

It is also apparent that successful treatment of secondary syphilis produces a cessation of A-T IgM production, and for a short period the serological pattern may mimic that of late syphilis (table III), but antibody concentrations continue to fall. The next immunoglobulin to become undetectable is A-L IgG, leaving A-L IgM and A-T IgG, a combination of immunoglobulins found to be typical of successfully treated syphilis.[11] Cases of treated latent syphilis showed a similar decline in antibody concentrations although at a lower rate than that seen in cases of secondary syphilis (tables IV and VI).

We therefore suggest that, because sera from treated cases of secondary and latent syphilis produce the serological pattern that we associate with late syphilis for only a short period, this is unlikely to be confused with the persistent response that is typical of late syphilis. The patients—one diagnosed as having latent syphilis (table V) and the other presenting with an unusual rash—who showed no such fall in antibody concentrations after treatment may be liable to develop late symptomatic syphilis in the future.

The patterns of the immune response in untreated syphilis are illustrated in the figure, where it can be seen that if an infection with *Treponema pallidum* is allowed to run its course untreated then some time after secondary signs have disappeared and the disease becomes latent the A-T IgM response declines. The latent stage can therefore be redefined as early latent—when A-T IgM antibody is still present—and late latent—when A-T IgM antibody has disappeared. Treatment of patients with latent syphilis usually produces a reduction in antibody concentrations to give the A-L IgM and A-T IgG picture that is typical of successful treatment. When the patient enters the late symptomatic stage of the disease the A-L IgM and IgG and A-T IgG responses remain typically raised and are unaffected by treatment. The persistence of the immune response in late syphilis may be due either to a reservoir of treponemes in locations inaccessible to antibiotics or to their presence in a non-dividing vegetative state,[14] and thus late syphilis may not respond serologically to treatment. Thus, a patient whose serum persistently produces the serological pattern of A-L IgM and IgG, and A-T IgG reactivity, may be considered a candidate for the development of late symptomatic syphilis.

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