

Antitreponemal IgE in early syphilis

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SUMMARY Using a solid-phase radioimmunoassay technique, mean serum IgE concentrations were found to be raised in patients with early syphilis. Antitreponemal specificity of the IgE response was investigated by the fluorescent treponemal antibody absorption test using a fluorescein-isothiocyanate-labelled antiserum against the Fc-fragment of human IgE. Validity of this test procedure was assessed by blocking experiments.

The results provide evidence of the antitreponemal specificity of the IgE response in syphilis and indicate a possible role for antitreponemal IgE in the pathogenesis of the Jarisch-Herxheimer reaction and in the immune-complex origin of some of the lesions of secondary syphilis.

Introduction

Antibodies of the different immunoglobulin classes can be separately detected by the use of monospecific antihuman fluorescein-isothiocyanate-(FITC)-labelled immunoglobulins. The use of monospecific antisera against human IgG, IgA, and IgM to detect antibodies using the fluorescent treponemal antibody (FTA) test have been reported by several authors.¹⁻⁵ These reports have indicated specific antitreponemal IgG, IgA, and IgM in sera from patients with syphilis. Using the FTA-200 test, Matuhasi *et al*⁶ could not demonstrate IgD antibodies.

No reports on the existence of an IgE antitreponemal antibody have been published. Green *et al*⁷ however found that the mean serum IgE concentrations for groups of healthy controls was significantly lower than that for a group of patients with primary syphilis.

In this study IgE concentrations in syphilis were investigated and the antitreponemal specificity of the IgE response evaluated.

Methods

The FTA-ABS test was performed according to the standard method of Kawamura *et al*⁸ with the modifications described below.

ANTIGEN PREPARATIONS

Marked slides (see below) containing eight circles of 0.6 cm diameter were stored in 96% alcohol. Fresh *Treponema pallidum* suspension (see Materials) was thoroughly mixed and centrifuged at 200 × g for 10 minutes to free the suspension of cellular (rabbit) debris. The suspension was then diluted with distilled water until 30-40 micro-organisms per field were visible at × 400 magnification by darkground microscopy and allowed to mix for 10 minutes. Before use, these slides were wiped with absorbent paper. On each circle, 0.01 ml of the diluted antigen suspension was applied and allowed to dry at 37°C for 60-90 minutes. After drying the preparations were fixed in acetone for 10 minutes and stored in polyethylene sealed phials at -70°C until required.

TEST SERA

Ten millilitres of peripheral blood was collected aseptically and allowed to clot for one hour at room temperature; it was then centrifuged for 10 minutes at 400 × g. The serum was then pipetted off and divided into 0.2-ml aliquots. The sera were stored at -70°C until required. No inactivation was carried out. Before use the test sera were diluted in sorbent (see Materials) and incubated for 30 minutes at room temperature.

INCUBATION OF TEST SAMPLES

From each test sample (patient serum diluted 1/5 in sorbent, control serum diluted 1/5 in sorbent, patient serum diluted in phosphate-buffered saline (PBS) for estimation of titre), 0.02 ml was applied to the antigen circle and incubated in a humid atmosphere at 37°C for 30 minutes. The slides were then gently

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washed with PBS and placed in dishes containing PBS for 10 minutes.

INCUBATION OF CONJUGATES

After the slides had dried, 0.005 ml of PBS-diluted conjugate (see Materials) was applied to each antigen smear and incubated in a humid atmosphere for 30 minutes at 37°C. The experiments were ordered in such a way that only one type of conjugate was used for each antigen slide. After incubation, washing and drying were carried out as for the test samples.

MICROSCOPY

Mounting fluid and cover slips were applied to the slides after they had dried. A Leitz Orthoplan microscope with a Xenon XBO-75 lamp and a Leitz vertical illuminator was used. FITC-fluorescence was detected with the filter combination BG 38 (4 mm), KP 490 (2 mm), GG 475 (2 mm), and a secondary filter OG 513 (3 mm). For routine use examination was performed with a ×10 ocular and a Leitz ×40 dry objective. The results were read and interpreted as follows: strong fluorescence, positive (+); weak fluorescence, borderline (+/-); and no fluorescence, negative (-).

CONTROLS

In each experiment, the following controls were always included for each of the applied monospecific antisera: (1) positive control serum (see Materials) diluted 1/5 in sorbent; (2) negative control serum (see Materials) diluted 1/5 in sorbent; (3) sorbent only; and (4) antigen and conjugate but no human serum to serve as a control for non-specific staining of the antigen. Since the sorbent used during this study was from one manufacturer's batch tests to evaluate its absorbing activity were done infrequently.

FITC-LABELLED ANTIHUMAN GLOBULIN

For research purposes antihuman FITC-labelled monospecific conjugates directed against human Ig, Fc-fragments of human IgG, IgA, IgM, IgD, and IgE were used. Specificity of the antisera was demonstrated in direct cytoplasmic immunofluorescent staining of monoclonal human bone marrow cells and in immunoelectrophoresis.

For each conjugate, a chess-board titration against the positive reference serum was carried out to indicate the working dilution (Tables I and II). As non-specific fluorescence occurred the conjugate was absorbed with a suspension of *T pallidum*.

TABLE II Results of limited chess-board titrations of GAHu/IgE(Fc)/FITC after previous absorption with *T pallidum* suspension and of two other unrelated antihuman IgE conjugates

Antiserum dilution	Control sera			
	Non-specific	Sorbent	Negative (1/5)	Positive (1/5)
GAHu/IgE(Fc)/FITC				
1/20	-	-	-	+
1/40	-	-	-	+
1/80	-	-	-	-
1/160	-	-	-	-
GAHu/IgE/FITC				
1/20	-	-	-	+
1/40	-	-	-	+/-
1/80	-	-	-	+/-
1/160	-	-	-	-
ShAHu/IgE/FITC				
1/20	-	-	-	-
1/40	-	-	-	-
1/80	-	-	-	-
1/160	-	-	-	-

GAHu = goat antihuman conjugate
 ShAHu = sheep antihuman conjugate
 + Positive, strong fluorescence; +/- borderline, weak fluorescence; - negative, no fluorescence

TABLE I Results of chess-board titrations of GAHu/IgE(Fc)/FITC without previous absorption with a *Treponema pallidum* suspension

Anti-serum dilutions	Control sera				Positive reference control serum dilutions							
	Non-specific	Sorbent only	Negative (1/5 dilution)	Positive (1/5 dilution)	1/5	1/20	1/50	1/100	1/200	1/400	1/800	1/1600
1/1					+	+	+	+	+	+	+	+
1/10					+	+	+	+	+	+	+	+
1/20	+/-	+	+	+	+	+	+	+	+	+	+/-	+/-
1/40	-	-	-	+	+	+	+	+	+/-	-	-	-
1/80	-	-	-	+/-	+	+/-	+/-	-	-	-	-	-
1/160	-	-	-	-	-	-	-	-	-	-	-	-
1/320					-	-	-	-	-	-	-	-
1/640					-	-	-	-	-	-	-	-

+ Positive, strong fluorescence; +/- borderline, weak fluorescence; - negative, no fluorescence

T PALLIDUM/CONJUGATE ABSORPTION PROCEDURE

Dilutions of a *T pallidum* suspension (Baltimore Biologic Laboratories (BBL), Cockeysville, Maryland) were made with PBS. The exact proportions of antiserum and *T pallidum* suspension were determined in previous titrations.

Five microlitres of a 1/100 dilution of *T pallidum* suspension were added to 500 μ l of goat antihuman (GAHu)/IgE(Fc)/FITC conjugate and mixed. The *T pallidum*/conjugate mixture was incubated for one hour at 37°C and centrifuged for one hour at 12 000 rev/min in a cold environment (4°C); supernatants were divided into small aliquots and stored at -20°C until required.

DETERMINATION OF IgE CONCENTRATIONS

Total IgE concentrations of a group of IgE-FTA-ABS-test-positive sera were determined by a non-competitive solid-phase radioimmunoassay⁹ at the Central Laboratory of the Red Cross Blood Transfusion Services (Amsterdam, The Netherlands). IgE concentrations of the control group were also made available by the Central Laboratory.

SERUM SPECIMENS

Consecutive serum specimens from the venereology outpatient department of our hospital were investigated by all monospecific FTA-ABS tests. Sera from patients with definite allergic symptoms or signs were excluded from the statistical analysis.

Determinations of antitreponemal IgE titres in the FTA test were performed in PBS dilutions without previous absorption.

STATISTICAL METHODS

The χ^2 test (with Yates's correction) and Student's *t* test were used for statistical analysis of IgE concentrations.

SPECIFICITY TESTS

Blocking experiments

To ascertain the specificity of the observed fluorescence in the IgE-FTA-ABS test, a blocking experiment was performed. In this procedure unlabelled and diluted antisera* against the different immunoglobulin classes were incubated on antigen slides after IgE-positive sera had been applied but before incubation with the FITC-labelled anti-IgE in the working dilution of 1/20 in PBS. Blocking—that is, preventing the occurrence of otherwise expected fluorescence—may only be obtained with unlabelled anti-IgE.

Three known IgE-FTA-ABS-test-positive sera, obtained from patients with primary, secondary, and early latent syphilis, were included in the assay.

Unlabelled swine antihuman antisera against IgG, IgA, IgM, and IgE were used as blocking agents.

Use of other FITC-labelled antihuman IgE conjugates

Another way of proving the specificity of the test procedure is to use other unrelated commercially available FITC-labelled antihuman IgE conjugates. Limited chess-board titrations were therefore carried out with FITC-labelled antihuman IgE antisera obtained from distinct sources.

Materials

ANTIGEN

T pallidum (Nichols strain) was used as antigen. A fresh *T pallidum* suspension was provided by the National Institute of Public Health, Bilthoven, The Netherlands, and transported in Nelson's medium. For absorption of non-specific fluorescence from the anti-IgD and IgE conjugates, a *T pallidum* suspension obtained from BBL (batch No L9ELVP) was used.

CONTROL SERA

A pooled positive control reference serum was also provided by the National Institute of Public Health. No decrease in fluorescence was observed when dilutions in sorbent were compared with dilutions in PBS. Non-specific and negative control sera were obtained from healthy, non-syphilitic volunteers.

SORBENT

A FTA-ABS test sorbent, prepared according to the standardised method described by Stout *et al*¹⁰ (obtained from BBL) (batch No L9ELSW), was used.

HUMAN SYPHILITIC REFERENCE SERUM

A lyophilised international reference preparation of human syphilitic serum was obtained from the Statens Seruminstitut, Copenhagen, Denmark.

PHOSPHATE-BUFFERED SALINE (pH 7.4)

PBS (8.2 g NaCl + 1.6 g Na₂HPO₄.2H₂O + 0.2 g NaH₂PO₄.2H₂O dissolved in 1 litre distilled water) was used.

SOLVENT

Reagent-grade acetone was used for fixation of slides.

OBJECT SLIDES

Toxoplasmosis slides (Bellco Glass Inc., Vineland, New Jersey) were used.

*Specifications and working dilutions of antisera used may be obtained from the authors.

MOUNTING FLUID

FA mounting fluid, pH 7.2, (Difco Laboratories, Detroit, Michigan) was used.

Results

MONOSPECIFIC FTA-ABS TESTS

From the results of chess-board titrations of all monospecific antihuman conjugates against the positive reference serum, it was concluded that syphilitic sera contained antitreponemal activity in the IgG, IgA, IgM, and, unexpectedly, IgE subclass of immunoglobulins (Tables I and II). No activity was noted in the IgD class.

SPECIFICITY TESTS

From the results of blocking experiments, unlabelled anti-IgE in a working dilution of 1/80 in PBS was able to prevent the occurrence of otherwise expected fluorescence (Table III). Unlabelled anti-IgG, anti-IgA, and anti-IgM were unable to prevent the occurrence of fluorescence at the same working dilution. From the results of limited chess-board titrations with other FITC-labelled anti-IgE sera (Table II) the GAHu/IgE(Fc)/FITC conjugate (obtained from Meloy Laboratories Inc) gave similar results whereas the sheep anti-IgE gave negative results. The latter observation is difficult to explain, but differences in staining properties between distinct conjugates is well-known in immunofluorescence.¹¹

Thus results of specificity tests strongly support the specificity of the test procedure and confirm the existence of an antitreponemal IgE in syphilis.

IgE-FTA-ABS TEST

A total of 116 sera from patients attending the out-patient department were studied, together with sera from 20 healthy volunteers (Table IV).

Although the numbers were small, the results of the IgE-FTA-ABS tests indicate an increase in antitreponemal IgE with longer duration of antigenic

TABLE IV Results of IgE-FTA-ABS tests in 44 patients with syphilis, 47 patients with other conditions, and 45 healthy persons (expressed in absolute numbers)

Diagnosis	IgE/FTA-ABS test result			Total
	+	+/-	-	
Primary syphilis	15	2	12	29
Secondary syphilis	6	0	3	9
Early latent syphilis	4	0	0	4
Tertiary syphilis	2	0	0	2
Total	27 (61%)	2 (5%)	15 (34%)	44
Ulcer molle	0	0	5	5
Herpes simplex (types 1 and 2)	0	0	13	13
Non-specific ulcer*	0	1	15	16
Gonorrhoea	0	0	5	5
Chlamydial urethritis	0	0	3	3
Acute biological false-positive reactor	0	0	5	5
Healthy controls	0	0	20	20
Healthy contacts†	0	0	25	25
Total	0	1	91	92
Total	27	3	106	136

*An ulcer which healed spontaneously and from which *T pallidum* could not be found by darkground microscopy and gave negative results to herpes virus culture, *H ducreyi* culture, and to serological tests and culture for lymphogranuloma venereum.

†Patients attending as contacts of syphilis but in whom there were no clinical or serological indications of infection after observations for six months.

+ Positive, strong fluorescence; +/- borderline, weak fluorescence; - negative, no fluorescence.

stimulation with *T pallidum*. When the results of the FTA-ABS test with anti-IgG, anti-IgM, and anti-IgE were compared in individual sera obtained at various intervals after infection it became evident that the already well-known primary IgM response against *T pallidum* was followed by an antitreponemal IgE and IgG response.

IgE CONCENTRATIONS IN POSITIVE TEST SERA

A total of 19 sera were obtained and investigated, all from patients in the early stages of syphilis. In the patients with early syphilis 13 (68%) of 19 sera contained more than 100 IU/ml of IgE whereas in the

TABLE III Blocking of FITC-labelled antihuman IgE with unlabelled antihuman IgE but not with unlabelled antihuman IgG, IgA, or IgM (1/80 PBS)

Serum No	Dilution (1/5)	Specificity and working dilution of unlabelled antisera							
		IgG		IgA		IgM		IgE	
		1/40	1/80	1/40	1/80	1/40	1/80	1/40	1/80
78-13	PBS	+	+	+	+	+	+	+/-	-
	Sorbent	+	+	-	+	+	+	+	-
78-27	PBS	-	+	+	+	+	+	+/-	-
	Sorbent	-	+	+	+	-	+	-	-
78-58	PBS	-	+	+	+	+	+	-	-
	Sorbent	-	+	+	+	+	+	-	-

PBS = phosphate-buffered saline

+ Positive, strong fluorescence; +/- borderline, weak fluorescence; - negative, no fluorescence

healthy control group only eight (32%) of 25 sera contained more than 100 IU/ml. This difference is statistically significant (χ^2 0.025 < P < 0.05). (Precise numbers were given when the results were above 100 IU/ml whereas in cases where the result was below 100 IU/ml no further specification was obtained.) IgE-FTA-ABS test positive sera from patients from tropical or subtropical countries were excluded from the statistical analysis.

Furthermore, the mean IgE concentration from patients with primary and secondary syphilis above 100 IU/ml (332 IU/ml; range 100-680 IU/ml) was significantly higher than that in the healthy control group (204 IU/ml; range 110-370 IU/ml) (t = 2.0, 0.025 < P < 0.05).

From these data infection with *T pallidum* appears to be associated with an increase in serum IgE concentration.

ESTIMATION OF IgE TITRES

End-point titrations of IgE-positive sera were carried out in PBS-dilutions without previous absorption of the sera with *Treponema phagedenis* (Reiter) sorbent. The range of antitreponemal IgE titres was 50-1600 with a mean value of 340 (reciprocal value). No correlation occurred with total IgE concentrations.

Discussion

The results of determinations of IgE concentrations support the findings of Green *et al.*,⁷ although in our study IgE concentrations in patients with primary and secondary syphilis were evaluated as a group without further differentiation of the stage of disease. Taken together, these studies indicate an IgE response in *T pallidum* infection.

The association between immunodeficiencies, especially T-lymphoid cell deficiency, and hyper-IgE production is well known,¹² and increased IgE production may be related to decreased cellular immunity in the early stages of the disease.³ In fact studies presently being carried out in our laboratory indicate decreased numbers of circulating T-lymphoid cells as determined by the rosette technique.

Performance of the IgE-FTA-ABS test and results of analysis of its specificity provide substantial evidence for the antitreponemal specificity of the IgE response. The separation of IgG and IgE in mono-specific FTA-ABS tests results indicate that on antigenic stimulation of the host with *T pallidum* firstly an IgM response is initiated followed by the simultaneous production of anti-treponemal IgG and IgE.

Possibly antitreponemal IgE plays a part in the pathogenesis of the Jarisch-Herxheimer reaction. In this reaction, a complex series of clinical and physiological events follows the start of treatment in patients with syphilis and in other spirochaetoses, such as yaws and louse-borne relapsing fever.¹⁴ In primary seronegative syphilis the incidence of the reaction was estimated to be 55% whereas in primary seropositive syphilis the incidence can rise to 95%.¹⁵ Degranulation of mast cells and basophil leucocytes on binding of treponemal antigens with membrane-bound IgE with a subsequent release of histamine, slow-reacting substance of anaphylaxis (SRS-A), chemotactic factor for eosinophil leucocytes, and platelet aggregating factor may account for at least some of the symptoms and signs of the Jarisch-Herxheimer reaction.

Another clinical aspect of antitreponemal IgE is its possible role in the development of immune-complex disease, which may be of importance in the aetiology of some of the lesions in secondary syphilis.¹⁶ Platelet aggregating factor may induce platelet accumulation at the endothelial site followed by release of platelet mediators; these increase vascular permeability, and circulating immune complexes may be deposited in the vessel wall damaging tissue.¹⁷

Finally it should be pointed out that fluorescence in the IgE-FTA-ABS test is considerably less than in the IgG-FTA-ABS test, thus making the procedure unsatisfactory for routine use. Its results however may be helpful in understanding the pathogenesis of syphilis.

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