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CIRCULATING IMMUNE COMPLEXES IN SYPHILIS

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Abstract. 13 patients with syphilis were investigated regarding the presence of circulating immune complexes by the methods of C1q-binding-activity and anticomplementarity. Elevated C1q-binding-activity was demonstrated in 6 of 7 patients with secondary syphilis, a significantly greater incidence than among patients with primary syphilis and neurosyphilis. Anticomplementarity was demonstrated in five of seven patients with secondary syphilis and in two patients with neurosyphilis. Anticomplementarity was found in only one of four patients with primary syphilis. The presence of immune complexes may be of importance in the aetiology of some of the lesions of secondary and perhaps tertiary syphilis.

Key words: Immune complexes; Syphilis; Complement; C1q-binding-activity; Anticomplementarity

Circulating immune complexes are thought to constitute a pathogenetic factor in human diseases such as lupus erythematosus, acute post-streptococcal glomerulonephritis, polyarteritis nodosa (13). The clinical picture of syphilis suggests that immune complexes also are a pathogenetic factor in the syphilitic lesions (2-4, 6, 8, 24), but circulating immune complexes have not hitherto been reported in syphilitic patients. In the present study, serum from patients with primary and secondary syphilis was studied in order to demonstrate the presence of circulating immune complexes, by employing a sensitive radioimmunoassay using ^{125}I-labelled C1q as devised by Nydegger et al. (19) and an anticomplementary assay (17, 21).

MATERIALS AND METHODS

Materials

13 patients were investigated, 6 men and 7 women. 4 patients had primary syphilis, 7 patients had secondary syphilis and 2 patients had tabes dorsalis. One patient with secondary syphilis (no. 11) had nephrotic syndrome and a mild affection of the liver with elevated alkaline phosphatase (22). Renal function as estimated by serum creatinine was normal in all patients. One patient with primary syphilis (no 3) showed treponema pallidum on darkfield microscopy, but negative serologic tests for syphilis, i.e. the Wassermann reaction (WR), the Kahn test (KR), the Meinicke flocculation test (MR) and the treponema pallidum immobilization test (TPI). All other patients had positive serologic tests.

As soon as the diagnosis of syphilis had been established, samples of blood were collected for the determination of immune complexes prior to treatment with procaine penicillin retard (PAM), of which 600000 units per day was given for 10 days. Blood for estimation of immune complexes was collected 1 to 3 months after treatment with PAM in 7 of 11 patients. The 2 patients with tabes dorsalis had received penicillin treatment prior to examination.

Methods

Blood samples were allowed to coagulate for 2 hours at 20°C, centrifugated twice at 1500 *g* for 15 min at 4°C, and the serum stored in sterile tubes at −70°C until tested. Samples once thawed were not used again.

C1q-binding activity (C1q-BA) was estimated as devised by U. Nydegger et al. (19).

Preparation of C1q. C1q was prepared as described by V. Agnello et al. (1). The C1q was precipitated in EDTA-serum by DNA, and after centrifugation redissolved by DNAase. Then the C1q was purified by gelfiltration using G-200 Sephadex SF and radio-iodinated with ^{125}I, using lactoperoxidase according to Heusser et al. (10). The solution was diluted to a concentration of C1q of 1 μg/ml in phosphate-buffered saline, pH 7.4 containing 1 per thousand bovine serum albumin. Before use the solution was centrifugated 30 min at 3000 *g* to remove aggregated C1q.

Aggregated gammaglobulin was prepared by heating human gammaglobulin (Statens Seruminstitut) at 63°C in 12 min. This solution was used as reference.

Performance of the assay. 0.4 ml serum was mixed with 0.1 ml 125 I-C1q and incubated 60 min at 20°C. Polyethylene glycol (PEG, MW 6000) dissolved in 0.1 M boric acid, pH 8.4, was added to a final concentration of 2.5% W/V in the incubate, and a second incubation followed at 4°C for 24 hours. The mixture was then centrifugated for 30 min at 1000 *g* and the precipitated ^{125}I-C1q was measured. Results were expressed as a percentage of ^{125}I-C1q precipitated in relationship to the radioactivity precipitated by trichloracetic acid (TCA): 1 ml ^{125}I-C1q mixed with 1 ml 20% TCA. The mean value of 24 sera from healthy blood donors was 6% ± 5% (S.D.). A precipitation of more than 16% was considered abnormal. This value

Table I. *C1q-binding activity and anticomplementarity in 13 patients with syphilis before and/or after treatment*

ND=not done

	C1q-binding activity		Anticomplementarity		WR	
Patient no.	Before	After	Before	After	Before	After
Primary syphilis						
1	4	4	Pos	Neg	12	ND
2	9		Neg		4	ND
3	4		Neg		0	ND
4	6	0	Neg	Neg	12	ND
Secondary syphilis						
5	18		Neg		6	ND
6	29		Pos		14	ND
7	52	19	Pos	Neg	11	Neg
8	28	1	Pos	Neg	14	4
9	17	4	Neg	Pos	12	Neg
10	13	18	Neg	Neg	12	ND
11	16	6	Pos	Pos	14	8
Tertiary syphilis						
12		2		Pos		
13		3		Pos		

corresponded to the precipitation found in a serum containing 60 μg aggregated gammaglobulin per ml serum. The test was carried out in duplicate and all sera collected from one patient were analysed on the same day.

Anticomplementary assay modified after Mowbray & Svejgård (19, 21)

Materials. Veronal buffer. Stock solution. NaCl 85 g, 5.5-diethyl barbituric acid 5.75 g, sodium salt of 5.5-diethyl barbituric acid 3.75 g, $MgCl_2$ 5 ml of a 1 M solution, $CaCl_2$ 1.5 ml of a 1 M solution, demineralized water to 2000 ml. Before use 100 ml of the stock solution was mixed with 400 ml sterile distilled water.

Sensitized sheep red blood cells (SSRBC). An equal volume of a haemolysin dilution in Veronal buffer containing 4 maximal haemolytic doses was added to a 6% suspension of sheep red blood cells under continuous swirling. The mixture was then incubated for 30 min at 37°C and stored at 4°C. The solution was used on the day of preparation.

Complement titrations. 40 μl of serial dilutions of human complement in Veronal buffer were added to 0.1 ml SSRBC in 0.5 ml veronal buffer. The dilution giving 85% haemolysis was used.

Performance of the assay. Serum was diluted with an equal volume of Veronal buffer and incubated at 56°C for 60 min. To 40 μl of this solution, 40 μl Veronal buffer, 40 μl 0.9% NaCl containing 0.1% Na-azide and 40 μl of the complement dilution was added. The mixture was incubated for 1 hour at 37°C with remixing after 30 min. 0.1 ml SSRBC and 0.5 ml Veronal buffer were then added, the contents mixed and incubated for 40 min at 37°C with remixing after 20 min. Non-haemolysed cells were sedimented at 1400 *g* for 4 min and 0.5 ml of the supernatant was mixed with 3 ml Drabkin's solution ($NaHCO_3$ 1 g, $K_3Fe(CN)_6$ 250 mg, KCN 50 mg, demineralized water to 1000 ml). OD_{341} was read in a Beckman spectrophotometer. 23 of 24 healthy blood donors showed haemolysis of the SSRBC between 72% and 100%. Haemolysis below 72% was regarded as anticomplementary, corresponding to a concentration of more than 10 μg/ml serum of aggregated gammaglobulin.

Serum complement. C1q, C4, C3 and C3PA were determinated by single radial immunodiffusion (15). Antibodies and protein standards containing C4, C3 and C3PA were from Behringwerke. C1q was quantitated relative to a normal serum stored at −70°C.

RESULTS

The results of the investigation for C1q-binding activity (C1q-BA) and anticomplementarity (AC) are shown in Table I. Six of the 7 patients with secondary syphilis showed elevated C1q-BA. One patient (no. 10) was borderline at the first investigation but positive 1 month later. All the patients with primary syphilis and tabes dorsalis showed normal values. The difference between patients with secondary and primary syphilis is significant ($p<0.013$), (method of paired comparisons). Four patients, 2 with primary and 2 with secondary syphilis, were not reinvestigated after treatment. Among the remaining patients with secondary sy-

Table II. *Serum complement in 13 patients with syphilis before and/or after treatment*

Patient no.	C1q, % normal		C4, mg %		C3, mg %		C3PA, mg %	
	Before	After	Before	After	Before	After	Before	After
Primary syphilis								
1	132	113	8	21	67	61	28	16
2	103		14		90		23	
3	105		40		110		18	
4	147	100	40	17	80	64	35	21
Secondary syphilis								
5	108		18		77		24	
6	125		34		88		30	
7	87	108	26	15	108	75	26	23
8	85	113	17	26	62	106	13	28
9	100	103	33	22	62	67	20	21
10	117	118	17	24	75	95	27	30
11	150	125	37	29	98	87	20	23
Tertiary syphilis								
12		114		35		68		25
13		82		4		33		13

philis a significant decrease in C1q-BA was found after treatment ($p<0.05$).

Anticomplementarity (AC) was demonstrated among 4 of 7 patients with secondary syphilis but not in any of the patients with primary syphilis. Both patients with tabes dorsalis had positive AC.

The results of the determinations of serum complement are shown in Table II. It was not possible to demonstrate a decline in serum complement in association with elevated C1q-BA and AC.

As an example, Fig. 1 shows the levels of C1q-BA and AC during treatment in patient 7. A high concentration of C1q-BA was found before and during treatment. After treatment a rapid decline in the concentration of C1q-BA to a near-normal value is seen. AC was positive during treatment, but negative after treatment.

DISCUSSION

The detection of circulating immune complexes has been undertaken using a variety of methods (1, 14, 17–20, 23, 25). In this study we used two methods based on the higher affinity of complement for complexes as compared with monomeric immunoglobulins. Nydegger et al. (19) have shown that the C1q-BA is useful for the detection of immune complexes in diseases such as systemic lupus erythematosus (SLE), hepatitis B, and rheumatoid arthritis. The AC method has been used by Mowbray et al. (11, 17) for the detection of immune complexes in dermatitis herpetiformis, SLE and polyarteritis nodosa. Using these methods we have demonstrated increased C1q-BA in 6 of 7 patients with secondary syphilis and positive AC in 4 of the 7 patients. The elevation of C1q-BA differed significantly from patients with primary syphilis ($p<0.013$). Penicillin treatment restored the C1q-BA towards normal ($p<0.05$) (Fig. 1 and Table I). AC was demonstrated in both patients with tabes

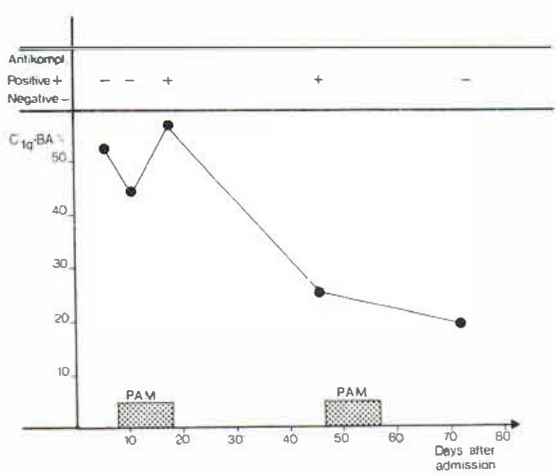

Fig. 1. Levels of C1q-binding activity and anticomplementarity in a patient with secondary syphilis before, during, and after treatment.

dorsalis. Lassus et al. (12) have demonstrated that AC can be found among patients with syphilis, and more often in patients with advanced syphilitic infection, frequently persisting after treatment. While the C1q-BA seems restricted to secondary syphilis, AC can be demonstrated later, even though both methods rely on complement fixation. Discrepancies between complement fixing methods have also been described by Johnson et al. (11) who distinguished between C1q-solubilizing complexes not associated with disease and anticomplementary complexes associated with vasculitis. Mohammed et al. (16) have proposed the presence of two kinds of immune complexes in dermatitis herpetiformis: primary complexes responsible for the tissue destruction, and secondary complexes caused by tissue destruction. This may in part be the explanation for the difference in results between C1q-BA and AC. The AC method is the most sensitive, detecting 10 μg/ml serum of aggregated gammaglobulin. This test, however, requires heat inactivation at 56°C for 60 min with subsequent risk of aggregating gammaglobulin or dissociating immune complexes with heat-labile antigens or low-avidity antibodies, as demonstrated by Zubler (25). This could also explain differences in results obtained with the two methods.

Fulford et al. (5) were not able to demonstrate circulating immune complexes in secondary syphilis by C1q-gel precipitation using the method of Agnello (1). Their negative results could be due to a lower test sensitivity, their method detecting only 250 μg/ml serum of aggregated gammaglobulin. Only one patient in the present study (no. 7) would possibly be positive when tested by this method. Some serum samples from Fulford and co-workers, however, showed anticomplementary activity.

Concentrations of complement proteins measured in our study (C1q, C3PA, C3 and C4) were largely found to be normal, with only small variations during treatment. Similar results have been reported by Wicher et al. (27) when studying rabbits infected with *Treponema pallidum*. One patient (no. 11) had nephrotic syndrome. A renal biopsy was performed, revealing subepithelial hump-like electron-dense deposits on the capillary basement membrane and fusion of epithelial foot-processes (22). The syphilitic nephropathy has been investigated in recently published studies (2–4, 6, 8, 24). In antibody elution studies performed on kidney biopsy material, Gamble et al. (6) have demonstrated the presence of antitreponemal antibody within the glomerular deposits. Using an indirect fluorescent antibody technique, Tourville et al. (24) demonstrated the presence of Treponemal antigen in the glomerular deposits. This strongly suggests that circulating immune complexes are of significance in the immunopathogenesis of syphilitic nephropathy.

Renal disease was found in only one of our patients with secondary syphilis, whereas increased C1q-BA values were registered in 6 of the patients. All patients had normal renal function, as estimated by serum creatinine levels. The examinations for proteinuria were not carried out systematically, but the frequency of proteinuria has earlier been determinated to be 7% (9). Wicher et al. (27) who infected 34 rabbits with Treponema pallidum found normal kidney function and normal histopathology of the kidneys 5 to 6 months after infection. Consequently circulating immune complexes can be present in the blood without affecting the kidneys. Similar results have been reported in rheumatoid arthritis, dermatitis herpetiformis, and Crohn's disease (16, 26). This may be due to the size of the immune complexes which may be cleared by the reticulo-endothelial system (7).

A negative C1q-BA and AC does not preclude the presence of immune complexes, since IgA complexes bind complement poorly. The presence of C1q-BA or AC suggests that immune complexes may in part be responsible for some of the lesions of secondary syphilis.

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