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THE ROLE OF IMMUNE COMPLEXES IN EARLY SYPHILIS AND IN THE JARISCH-HERXHEIMER REACTION

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Abstract. Circulating immune complexes (CIC) were detected in one of 11 patients with primary syphilis and in 5 of 12 patients with secondary syphilis. The level of CIC was significantly increased in patients with secondary syphilis. Four weeks later a significant decline in CIC was found. No relationship was demonstrated between CIC and affection of the skin, lymph nodes, or kidneys. An increased albumin excretion rate was demonstrated before treatment. No differences were found in the excretion rate of β-2-microglobulin before or after treatment. Nineteen patients had a Jarisch-Herxheimer reaction during treatment. An increase in CIC was found in 5 patients and a decrease in 7 patients. No correlation could be demonstrated between the level of or changes in CIC and the severity of the Jarisch-Herxheimer reaction.

Key words: Albuminuria; β-2-microglobulin; Immune complexes; Proteinuria; Syphilis

The presence of circulating immune complexes (CIC) in patients with early syphilis has been reported previously (2, 13, 15). CIC have been detected most frequently in patients with secondary syphilis, with frequencies varying from 44 to 65% (2, 13). It is likely that CIC are of importance for the development of the syphilitic nephropathy (6, 14, 18). The role of CIC in other manifestations in the varied clinical picture of syphilis is unknown.

In the present study we have investigated skin and renal affection in patients with early syphilis, regarding differences between patients with CIC and patients without, and also studied the possible relationship of CIC to the Jarisch-Herxheimer reaction.

MATERIAL AND METHODS

Patients. A total of 23 patients (17 men and 6 women) were investigated. Eleven patients had primary and 12 secondary syphilis. Range of age: 23 to 47 years (mean 33 years).

The diagnosis of syphilis was based on the demonstration of *T. pallidum* on darkfield microscopy and/or positive reagin tests: The Wassermann reaction (WR), the Kahn test (KR), the Meinicke flocculation test (MR), and the specific serological tests: *Treponema pallidum* immobilization test (TPI) and/or absorbed fluorescent treponemal antibody test (FTA-ABS). In the case of negative serological tests, the diagnosis was based only on the demonstration of *T. pallidum* on darkfield microscopy.

The patients were treated with aqueous procain penicillin G 600000 units daily, in reagin-negative patients for 8 days and in reagin-positive patients for 14 days. All patients were hospitalized on the first day of treatment. Before and every hour after starting treatment, the rectal temperature was measured. An increase in temperature above 38°C 4 to 8 hours after treatment was regarded as diagnostic for the Jarisch-Herxheimer reaction.

Blood and urine samples. From all patients a blood sample was obtained before treatment, 6 hours after first injection of penicillin and from 22 of the 23 patients also 4 weeks after treatment. The serum samples were stored at −70°C until analysis.

From all patients a 120-min urine sample was obtained before treatment, and from 22 of the 23 patients another 120-min urine sample was obtained 4 weeks later. The samples were kept frozen at −20°C until analysis.

Detection of circulating immune complexes (CIC). Two methods were used for the detection of CIC; both had to be positive before CIC were assumed to be present. The immune complexes were quantified as equivalent amounts of heat-aggregated human gammaglobulin per ml serum (Cohn fraction II, 63°C 12 min).

Clq-BA test (Clq-binding activity). A radioimmunoassay was used, modified after Nydegger (11, 16, 19).

PP-Lc (PEG-precipitable light chain determinants). A radioimmunoassay was used to detect immunoglobulins by their light chain determinants after precipitation of CIC by polyethylene glycol 6000 (PEG) (16).

Albumin and beta-2-microglobulin were measured by means of sensitive radioimmunoassays (3, 10).

"In vitro" Jarisch-Herxheimer reaction. Sera collected before treatment from the 23 patients were divided in two and mixed with either an equal volume of phosphate-buffered saline, 1 g/l albumin, or an equal volume of antigen material from destroyed *T. Reiter* (Behringwerke). The CIC were measured by the C1q-BA test.

Molecular weight (MW) of CIC. The MW of CIC was measured by gel filtration on a calibrated column of

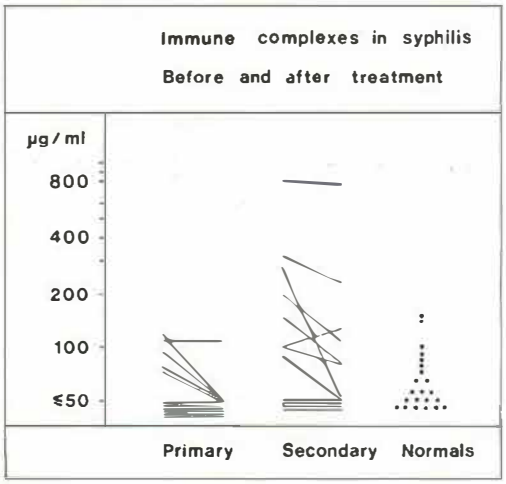

Fig. 1. CIC measured by the C1q-BA test in patients with early syphilis, and quantitated as equivalent amounts of heat-aggregated human gammaglobulin per ml serum. The values before and 4 weeks after treatment are shown.

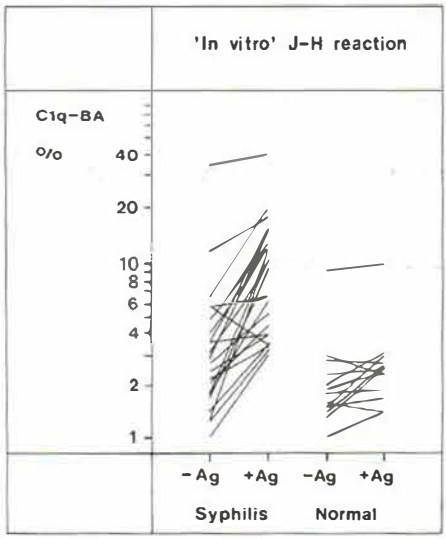

Fig. 2. A significant increase in C1q-BA was found when Treponema antigen was added to 23 sera from patients with early syphilis, but not in normal sera. *J-H*, Jarisch-Herxheimer reaction; *+Ag*, Treponema antigen; *−Ag*, buffer control.

Sepharose CL-6B, range of separation 10^4–4×10^6. All eluted 1 ml fractions were tested by the C1q-BA test (17).

Immunofluorescence studies of the skin were performed as previously described (4).

RESULTS

Overall CIC were found in 6 of the 23 patients with early syphilis. Four weeks after treatment a significant decline in CIC was found (Fig. 1) ($2p<0.01$, Wilcoxon signed rank test). The C1q-BA and the PP-Lc tests were significantly correlated (Spearman test, Rho $=0.84$, $n=23$, $2p<0.001$).

Primary syphilis. The level of CIC did not differ significantly from normals (Mann-Whitney test). Only one of the 11 patients had CIC before treatment. Another patient had CIC during the Jarisch-Herxheimer reaction.

Secondary syphilis. The level of CIC was significantly increased compared with normals ($2p<0.05$, Mann-Whitney test). Five of the 12 patients had CIC before treatment and another patient had CIC during the Jarisch-Herxheimer reaction. These 6 patients all had an exanthema. Skin eruptions were also seen in 4 of the 6 patients without CIC. All 6 patients with CIC had widespread lymphadenitis, but this was also found in 4 patients without CIC.

A skin biopsy was performed in 9 of the 10 patients with skin lesions. The biopsy specimens showed unspecific inflammation compatible with syphilis. By direct immunofluorescence, deposits of IgG, IgA and IgM were demonstrated in only one of the 9 specimens. This biopsy was from a patient without CIC.

Overall, a weak relationship was found between CIC measured as C1q-BA and the serological tests (WR: Rho $=0.46$, $2p<0.05$). Regarding only patients with secondary syphilis, however, no relationship could be demonstrated.

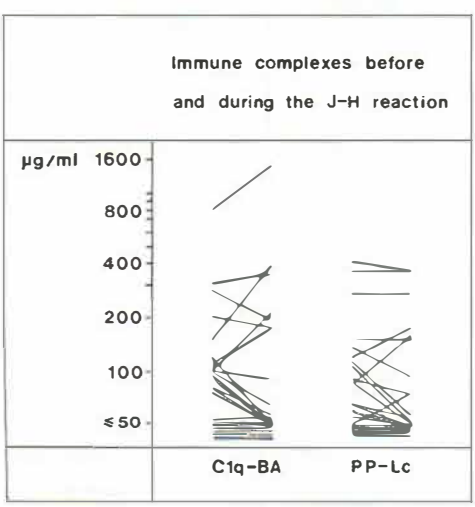

Fig. 3. CIC measured by the C1q-BA and the PP-Lc test before and 6 hours after initiation of treatment.

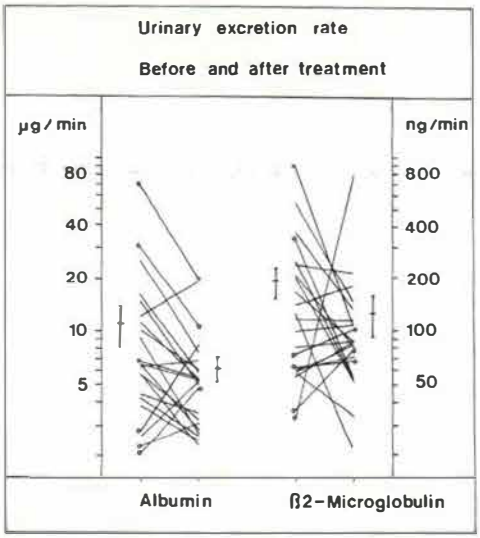

Fig. 4. Urinary albumin and β-2-microglobulin excretion rates before and 4 weeks after treatment. 0 signifies that the patient had CIC.

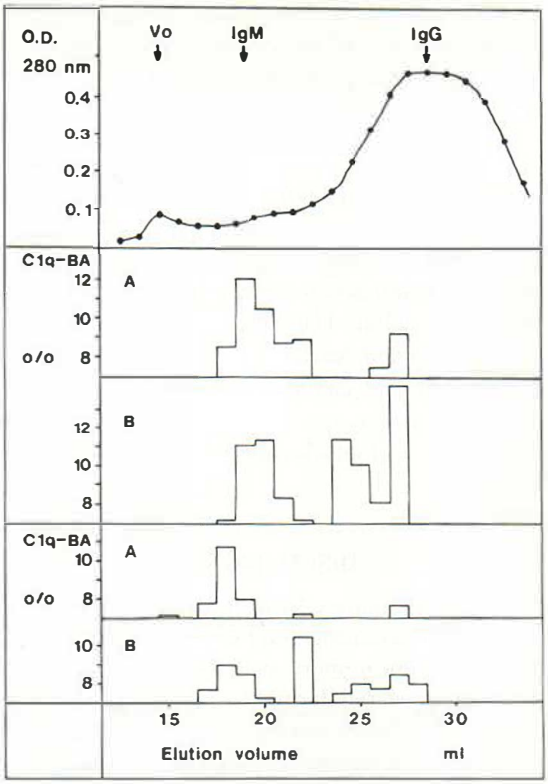

Fig. 5. Optical density (O.D. 280 nm) and peak values of C1q-BA after gel filtration of sera from 2 patients with CIC before and during the Jarisch-Herxheimer reaction. An increased amount of low MW CIC was found in these 2 patients during the J-H reaction. A = before and B = during J-H reaction.

The Jarisch-Herxheimer reaction. The applicability of the C1q-BA tests for detection of an increased amount of antigen load was tested in vitro. An increase in C1q-BA was observed when *T. Reiter* antigen was added to syphilitic sera, but not to normal sera ($2p<0.01$, Wilcoxon signed rank test) (Fig. 2).

Eight of the 11 patients with primary and 11 of the 12 patients with secondary syphilis showed the Jarisch-Herxheimer reaction within 8 hours after treatment. All 6 patients with CIC before treatment had a Jarisch-Herxheimer reaction. No difference was observed between patients with CIC and patients without, regarding the severity of the Jarisch-Herxheimer reaction estimated by the maximal temperature (Mann-Whitney test).

Of all 23 patients, 5 had an increase in C1q-BA during the Jarisch-Herxheimer reaction. Seven patients had a decrease in C1q-BA and in 11 patients the change in C1q-BA was less than 10% of the pretreatment value (Fig. 3). With the PP-Lc test an increase was observed in only 3 patients. Six patients had a decrease in PP-Lc and in 14 patients the change was less than 10% of the pretreatment value (Fig. 3). The change in C1q-BA or PP-Lc was not related to the severity of the Jarisch-Herxheimer reaction.

Renal impairment. All patients had serum creatinine concentrations within normal limits, and none of the patients had proteinuria by "Albustix". The albumin excretion before treatment was 11.1±2.9 (SEM, standard error of mean) µg/min, and after treatment 6.2±1.0 (SEM) µg/min. The difference is significant ($2p<0.01$, Wilcoxon signed rank test) (Fig. 4) (normal range: 1.6–14.2 µg/min). No difference was observed in albumin excretion rate between patients with primary vs. secondary syphilis or between patients with CIC and patients without.

The β-2-microglobulin excretion rate before treatment was 188±42 (SEM) ng/min and after treatment 124±33 (SEM) ng/min. The difference is insignificant (normal range: 12.5–232 ng/min). No difference was found between patients with primary vs. secondary syphilis or between patients with vs. without CIC.

The excretion rates of albumin and β-2-micro-

globulin were not correlated to the titres of the serological tests for syphilis or to the increase in temperature during the Jarisch-Herxheimer reaction (Spearman test). None of the patients had a temperature above 37.5°C when the urine sample was collected.

Molecular weight (MW) of CIC. The MW was measured by gel filtration in 5 patients with CIC before and during the Jarisch-Herxheimer reaction. CIC were heterogeneous, with MW varying from 200000 to 1 million. During the Jarisch-Herxheimer reaction 2 patients had an increased amount of "low MW CIC" (about 200 000 to 500 000) (Fig. 5), one patient had an increased amount of "high MW CIC" (about 1 million) and 2 patients had no change in MW of CIC.

DISCUSSION

In the present study CIC were detected in 5 of 12 patients with secondary syphilis and in only one of 11 patients with primary syphilis. In another 2 patients CIC appeared during the Jarisch-Herxheimer reaction. The pathogenic role of these CIC for syphilitic organ lesions is unknown. We have not been able to demonstrate any significant relationship between CIC and affection of the skin, lymph nodes, or kidneys. "Pathogenic" complexes responsible for such manifestations may however already have been deposited and apparently "non-pathogenic" CIC may be present in the circulation. Factors such as size, antigen and antibody type may be of importance for CIC to become pathogenic. The methods used does not permit any distinction between different types of CIC.

None of the methods at present available for detection of CIC is entirely specific. Bacterial lipopolysaccharides and ribonucleic acids released during the Jarisch-Herxheimer reaction may be a source of error (11, 19). In the present study a combination of two tests was used to make the detection of CIC more specific: one method detecting C1q-binding CIC and the second, immunoglobulins in precipitated CIC. The two tests were significantly correlated.

Immune deposits were detected in skin biopsy specimens from one patient only, and this patient had no CIC. The absence of immune deposits in the vasculitic lesions of patient with secondary syphilis has been reported previously (12). This does not necessarily exclude an immune complex mechanism, since deposited immunoglobulin and complement may disappear within hours (1).

The Jarisch-Herxheimer reaction may be triggered by release of antigen material from spirochetes destroyed by treatment (7). Fulford et al. (5) have reported that the Jarisch-Herxheimer reaction was preceded by a reduction in complement factors, and an associated antibody consumption was demonstrated, suggesting that complement utilization and perhaps immune complex formation may be involved in the Jarisch-Herxheimer reaction.

By in vitro studies we have shown an increase in C1q-BA when Treponema antigen was added to sera from patients with early syphilis, although the antigens were form *T. Reiter* and not *T. Pallidum*. The participation in vivo of a Treponema antigen/antibody system in the measured CIC is suggested by the presence of antitreponema antibodies in isolated and dissociated CIC (2), and by the demonstration of treponema antigen and antibodies in glomeruli from patients with syphilitic nephropathy (6, 18).

Nineteen of the 23 patients in this study showed a Jarisch-Herxheimer reaction. An increase in CIC similar to the "in vitro studies" was seen in only 5 patients. No relationship was demonstrated between the level of CIC and the increase in temperature during the Jarisch-Herxheimer reaction. One explanation for the absence of CIC during the Jarisch-Herxheimer reaction in spite of complement and antibody consumption may be that Treponemes are largely present in the tissues (lymph nodes, skin) and immune complex formation and complement consumption may occur mainly in the extravascular body compartment (5).

The MW of CIC in 5 patients varied widely, between 200 000 and 1 million. During the Jarisch-Herxheimer reaction 2 patients showed increased amounts of "low MW CIC" and one patient an increased amount of "high MW CIC". The explanation may be that antigens liberated during the Jarisch-Herxheimer reaction have reacted with circulating antibodies, resulting in CIC of differing size.

The renal function estimated by serum creatinine was normal in all patients. None of the patients had evidence of proteinuria by "Albustix". Proteinuria in early syphilis is an uncommon event. Herman & Marr (8), using the Esbach albuminometer, did not detect albuminuria in 31 patients with primary

syphilis and only in 5 of 61 patients with secondary syphilis. The lack of proteinuria does not, however, exclude the possibility of an increased albumin excretion, since the excretion rate can be ten to twenty times higher than normal without being detected by "Albustix". Using a radioimmunoassay an increased urinary albumin excretion was demonstrated before treatment. No difference was found between patients with primary and secondary syphilis. A "febrile proteinuria" (9) was excluded, since all patients had normal temperatures when the urine samples were collected.

Increased albuminuria is often regarded as evidence of a glomerular lesion. Such a lesion could be brought about by CIC, but no relationship was demonstrated between CIC and the albuminuria. The glomerular lesion may be caused by already deposited immune complexes. The participation of CIC in the syphilitic nephropathy with subepithelial dense deposits has previously been considered probable (6, 14, 18).

An increased excretion of β-2-microglobulin is considered to indicate a renal tubular lesion. No difference was observed on comparing the excretion before and after treatment, and no correlation was demonstrated between CIC and β-2-microglobulin excretion.

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