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SHORT COMMUNICATIONS

EVIDENCE FOR CIRCULATING IMMUNE COMPLEXES IN SARCOIDOSIS

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SUMMARY

Immune complexes were detected by the platelet aggregation technique in sera of six out of twenty-six patients with sarcoidosis. Five of these patients had acute bilateral hilar lymphoma syndrome, four of them with concomitant erythema nodosum. The size of the immune complexes was 19S or larger.

INTRODUCTION

The clinical picture of acute sarcoidosis often includes, besides bilateral hilar lymphomas (BHL), erythema nodosum (EN), arthritis, and occasionally uveitis and parotitis. The aetiology of sarcoidosis is still unknown and although there has been much speculation no specific causative agent has so far been identified. It seems, however, fairly well established that immune mechanisms are involved in the pathogenicity of sarcoidosis (Siltzbach, 1971). Moreover, the type of clinical symptoms of acute sarcoidosis may indicate immune complexes being involved.

Recently, sera from patients with lepromatous leprosy were shown to react with the C1q component of complement (Moran *et al.*, 1972). The incidence of sera that reacted was higher in patients with active erythema nodosum leprosum (ENL). This was taken, although circumstantial, as evidence for the immune complex aetiology of ENL. Thus, it seemed worthwhile to examine whether, in sera from patients with active sarcoidosis, circulating immune complexes could be detected.

The platelet aggregation (Pl.a.) test developed by Penttinen and co-workers (Penttinen *et al.*, 1969) was used to detect the presence of circulating immune complexes. By this technique immune complexes have been demonstrated in sera from patients with hepatitis (Penttinen, 1972), measles virus infection (Myllylä, Vaheri & Penttinen, 1971), rheumatoid disease (Norberg, 1973), and *Mycoplasma pneumoniae* infection (Biberfeld & Norberg, unpublished).

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MATERIALS AND METHODS

Materials

Altogether twenty-six patient sera were analysed. Ten sera were from patients with acute BHL syndrome. Seven of the patients had EN going on, one patient had joint symptoms but no EN, one had parotitis and one iritis, the two last mentioned patients had both diminishing symptoms. Sixteen sera were taken from patients with a history of sarcoidosis of more than 6 months, four had BHL and twelve had pulmonary lesions of varying severity. None was on steroid therapy. In all patients the diagnosis of sarcoidosis was supported by histopathology of lymph nodes and/or by a positive Kveim reaction.

Sera from a hundred blood donors served as controls. All sera were stored at $-20°$ until tested.

Methods

Platelet aggregation test. Pl.a. was performed as described by Penttinen & Myllylä (1968). Only unheated sera were used. Pl.a. was performed both at pH 7·6 and at pH 6·5. Sera with a Pl.a. titre of 8 were regarded as positive.

Sedimentation analysis. 0·5 ml of serum diluted 1/2 was layered over a gradient of sucrose, ranging from 10–37%, with a final volume of 5 ml. The gradient was centrifuged in a Spinco SW 50 rotor at 3,5000 rpm for 18 hr at 4°C. Thirty to thirty-five serial fractions were collected drop-wise from the bottom of the tube. IgM and IgG levels of the fractions were determined by single radial immunodiffusion (Mancini, Carbonara & Heremans, 1965). Pl.a. was performed on each fraction.

RESULTS

Pl.a. at pH 7·6 was demonstrated with sera from six out of twenty-six patients with sarcoid-

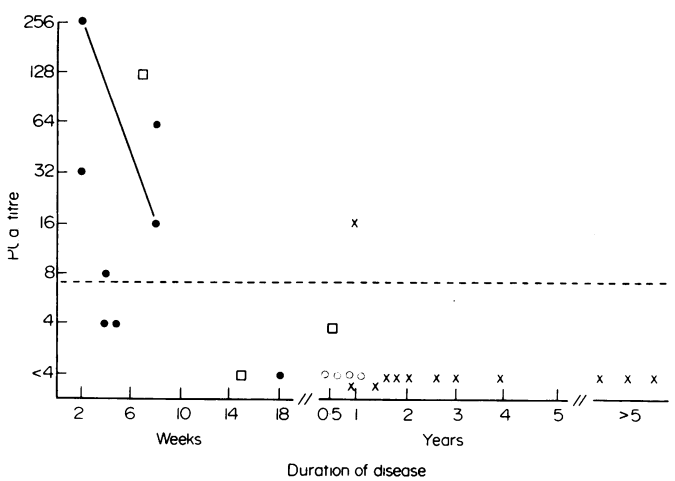

FIG. 1. Pl.a. titres of twenty-six sera in relation to time after onset of illness. (○) Patients with BHL syndrome. (●) Patients with acute BHL syndrome and concomitant EN. (□) Patients with acute BHL syndrome and concomitant symptoms other than EN. (×) Patients with parenchymatous lesions.

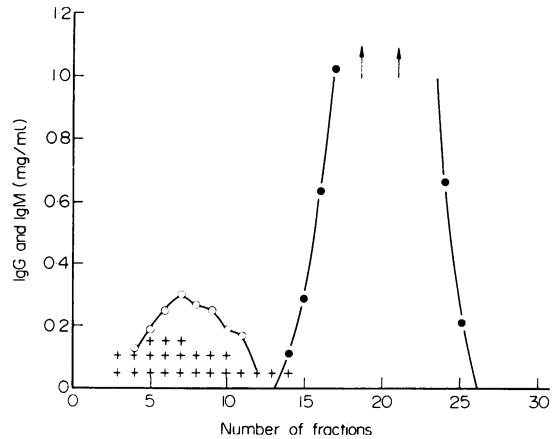

FIG. 2. Sucrose gradient fractionation of a serum showing direct Pl.a. titre of 256. Besides (+) direct platelet aggregation, (○) determination of IgM and (●) IgG was performed on each fraction.

osis. Five of these patients had acute BHL syndrome, four of them with concomitant EN and one with joint symptoms. The serum titres ranged from 16 to 256. Only one out of a hundred sera from healthy blood donors gave a positive Pl.a. test (titre 8). All sera were negative when the test was performed at pH 6·5.

The results of the Pl.a. test in relation to time after onset of symptoms or after the diagnosis of sarcoidosis was established, are illustrated in Fig. 1. With one exception Pl.a. was only demonstrable during the first months after the onset of disease.

After fractionation of sera by sucrose density gradient centrifugation the direct Pl.a. activity was found mainly in the fractions sedimenting faster than the 19S marker (Fig. 2).

DISCUSSION

According to Penttinen *et al.* (1973) direct Pl.a. by sera can be due to: (1) immune complexes; (2) antibody to platelets; and (3) aggregated IgG. It is unlikely that Pl.a. by these sarcoidosis sera was caused by aggregated IgG due to ageing in sera, since the sera were stored for only a short period of time and further, the reactivity of the sera was not related to the length of time for which they were stored. In order to avoid aggregation only unheated sera were used. Performance of the Pl.a. test at both pH 7·6 and pH 6·5 permits differentiation between type 1 and type 2 reactions, since at pH 7·6 both types aggregate platelets, but at pH 6·5 antibodies but not immune complexes cause aggregation (Myllylä, 1973). In the present study the reactivity of all positive sera disappeared when tested at a low pH. Thus, the Pl.a. observed might be taken as evidence for the presence of immune complexes in the sera.

The size of the complexes in the sarcoidosis sera was mainly 19S or larger. It is well known from experimental studies in animals that only complexes larger than 19S are pathogenic (Cochrane & Hawkins, 1968). Such complexes are primarily deposited in kidneys, joint tissue and skin.

The significance of the finding is as yet difficult to evaluate. It has been shown that the

active papules of ENL contain deposits of immunoglobulins and complement, suggesting that these were the sites of immune complex deposition (Wemambu *et al.*, 1969). To our knowledge no such investigations exist in EN due to sarcoidosis, but it might be reasonable to assume deposits of immune complexes also in these efflorescences. It is not known whether the immune complexes found would contribute to the pathological reactions seen in sarcoidosis. It is noteworthy, however, that the immune complexes were demonstrable during the early time period when the clinical symptoms were most pronounced. It would not be possible to establish whether sera from all patients with sarcoidosis in initial phase demonstrate circulating immune complexes, since patients without obvious clinical signs such as EN or joint symptoms, usually have had their disease for a long time period before discovery.

By Pl.a. applied on serum fractions obtained by various preparative techniques it might be possible to isolate the fractions containing immune complexes and to determine their nature more precisely.

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