BLOCKING OF HAEMOLYTIC COMPLEMENT BY HETEROGENEOUS FACTORS PRESENT IN LEPROMATOUS LEPROSY

Sir,

Patients with lepromatous leprosy have characteristically high levels of circulating immune complexes (CIC).[1] In another study, it has been reported that CIC vary in size when sera of systemic lupus erythematosus patients were separated on a gel column.[2] Of special interest is the study of whether or not the anticomplement activity (ACA) formed in sera of patients with lepromatous leprosy are homogeneous or heterogeneous substances which can be separated from other sera proteins. The present investigation has studied the possible use of gel filtration for removing ACA of lepromatous leprosy sera.

Fresh serum was obtained from patients presenting Hansen's bacillus infection and used immediately. Nine pooled lepromatous leprosy sera (4 ml) were chromatographed on a G-200 Sephadex column measuring 4×90 cm and the fractions were tested for complement haemolytic activity consumption.[3] Briefly, the protocol for each one of the intervals of the three peaks filtered through the column consisted of incubation of 100 μl of triethanolamine buffer, 100 μl of optimal dilution (1:30–1:50) of guinea-pig sera (complement source) and 50–100 μl of elution fractions for 10 min at 37°C. The sensitized sheep erythrocytes (EA, 25 μl) were then added. After incubation, 20 ml of ice-cold buffer was added; the tubes were centrifuged and the haemoglobin was determined

and recorded with the absorbance at 420 nm. For controls, buffer or fractions from normal sera were used instead of sera fractions from patients. The outlined method indicates that ACA was more evident in certain eluted fractions. Indeed, ACA was excluded from G-200 Sephadex resin just before the first peak, with only a small amount retarded in its elution (Figure 1). Thus: (a) the material (only 10 μg in protein) in the void volume peak inhibited 100% of EA lysis. It was further observed that heating this active material at 56°C for 30 min caused removal of most of ACA from the chromatographic fraction; (b) no ACA was detected in the top of the second peak even at a higher protein concentration (300–500 μg) (this is contrary to the pattern of separation of ACA in

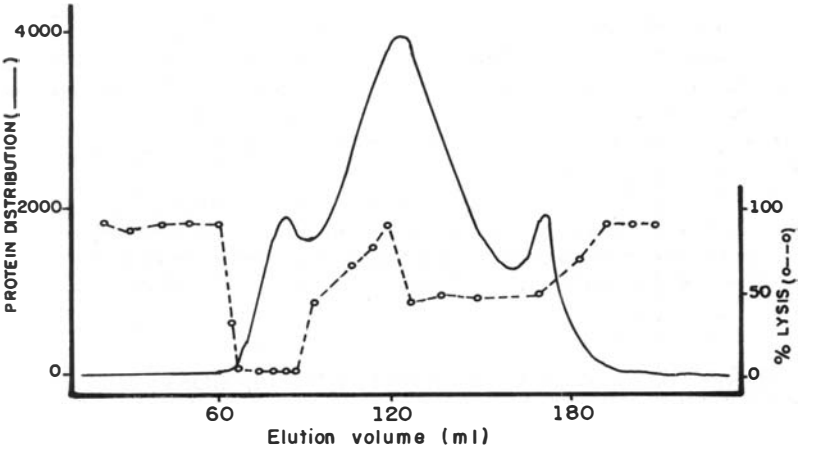

Figure 1. Protein distribution (280 nm) and haemolysis-inhibiting activity (420 nm) following G-200 Sephadex gel filtration of lepromatous leprosy serum.

patients with Chagas' disease or schistosomiasis mansoni)[3,4] and (c) the region between the descendent fraction of the second peak and the third peak showed 30–55% EA lysis inhibition at a higher protein concentration (200–300 μg). Chromatographic effluents from control individuals were negative for ACA (results not included). The relatively large size of the second peak compared to the other two peaks may be due to the hypergammaglobulinemia which is known to occur in the sera of lepers. However, this point was not pursued.

Although these experiments have not established the exact size of the proteins eluted from G-200 Sephadex and also have not defined the factors at the molecular level responsible for ACA, the anti-haemolytic action formed by lepromatous leprosy vary in size as can be seen by a heterogenous elution pattern of a G-200 Sephadex column.

Since ACA are often identified by the affinity for complement, a more widely used method for their demonstration is based on the decrease of complement haemolytic activity.[5] However, it has been reported that various inhibitors are not easily distinguished from circulating immune complexes[5,6] when one is using the haemolytic evaluation of the anticomplementary activity.[5] In this respect it is difficult to ascribe the ACA detected in the third fraction due to the presence of classical antigen antibody complexes. It is known that immune complexes can not be observed in the low molecular weight (third peak) of Sephadex G-200. Furthermore, the G-200 Sephadex column and the haemolytic techniques have made it possible to detect heterogenous material in fractions from sera of patients with lepromatous leprosy which inhibit the lytic effect of complement system, *in vitro*, so that their nature may be more precisely determined. The significance of the ACA in

lepromatous leprosy is uncertain. In fact, little is known about the role as yet played by the complement system in human parasitism.

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