Circulating immune complexes after splenectomy

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SUMMARY Circulating immune complexes were evaluated in 25 patients (age range 10 to 46 years) who had undergone splenectomy for non-malignant conditions by studying a polyethylene glycol insoluble serum fraction. Although the extent of binding to Clq was within normal limits, these patients had increased concentrations of factor B in the immune complex serum fraction. These findings indicate that an unusual type of circulating immune complex may be detected after splenectomy, suggesting a possible role for the spleen in the removal of circulating immune complexes.

Circulating immune complexes have been detected during the course of several diseases, although not always associated with their pathogenesis.¹ Once formed in the circulation they are cleared mainly by the mononuclear phagocyte system.² Experimental and clinical studies have shown that the liver is an important site for the removal of circulating immune complexes.^{3,4} Although there is experimental evidence of impaired removal after splenectomy,^{5,6} immune complexes have not been systematically evaluated in patients who have had their spleens removed.^{7,8} This paper describes a study of circulating immune complexes in patients who had undergone splenectomy for non-malignant conditions, to evaluate a possible role for the spleen in the clearance of circulating immune complexes.

Material and methods

Twenty five subjects who had had their spleen removed (mean age 27.4 years, range 10-46), with a haemoglobin concentration of 11.0-15.9 g/dl (mean 14.3) and 25 sex and age matched healthy controls were studied. Splenectomy was performed between one and 24 years (mean 7.9) before the study. The indications for surgery were traumatic rupture in 12 cases and hereditary spherocytosis in 13. Absence of effective splenic function was indicated in all cases by high pitted red cell counts.⁹ None of the patients or controls had received blood transfusions for at least two months before the study and none had had evidence of infection in the eight weeks preceding blood collection.

IMMUNE COMPLEX STUDIES

A serum fraction enriched in immune complexes was obtained by a polyethylene glycol (PEG) precipitation method,¹⁰ with minor modifications: one volume of serum was mixed with three volumes of isotonic borate-edetic acid buffer (25 mM disodium tetraborate, 20 mM edetic acid, 150 mM sodium chloride, pH 8.4, containing 4% PEG (molecular weight 6000). Immunoglobulins (IgG, A, and M) and complement components (C3, C4, and factor B) were assayed in the PEG-insoluble fraction and in serum by an automated nephelometric method (Beckman Immunochemistry Analyzer II, Fullerton, California, USA). The protein content of the PEG-insoluble fraction was measured by Hartree's method¹¹; while in serum it was measured by the standard biuret method.¹² The results of this PEG assay for circulating immune complex were expressed as the ratio [concentration in PEG-precipitate:concentration in serum] $\times 100$.

The determinations of Clq precipitins were carried out as described previously.¹³ Human gamma globulin purified by passage through DEAE-cellulose chromatography and heat-aggregated by incubation at 63°C for 20 minutes (HAGG) was used for the calibration of reference curves of absorption nephelometry in a double beam spectrophotometer (Beckman model 35). The results were reported as $\mu\text{g/dl}$ equivalents of HAGG.

The Mann-Whitney U-test was used for comparisons, with $p < 0.05$ taken as the level of significance.

Results

Determinations of Clq precipitins after removal of spleen (187.6 $\mu\text{g/dl}$ equivalents of HAGG, 135.7-262.2; median, range) were not significantly different

Table Serum concentrations (g/l) and relative values of proteins, immunoglobulins, and complement components in circulating immune complexes as expressed by ratio (concentration in PEG precipitate: concentration in serum) $\times 100$

	PEG precipitate				Serum		
	Controls	Patients with splenectomy	p Value		Controls	Patients with splenectomy	p Value
Protein	0.59 (0.13–1.05)	0.62 (0.13–0.91)	0.28		76.6 (65.9–98.0)	75.0 (66.9–120.0)	0.46
IgG	2.0 (0.6–4.0)	2.4 (1.1–5.2)	0.54		10.6 (7.3–13.6)	11.9 (8.1–17.0)	0.001
IgA	4.0 (1.3–9.5)	4.7 (0.3–17.3)	0.10		1.77 (1.09–3.42)	2.30 (1.04–3.76)	0.06
IgM	19.6 (7.3–38.8)	22.7 (7.0–69.5)	0.10		1.12 (0.61–2.28)	1.05 (0.47–2.46)	0.94
C3	7.0 (1.2–12.3)	9.1 (3.8–17.1)	0.12		1.08 (0.78–1.68)	0.98 (0.67–1.41)	0.23
C4	12.3 (2.1–25.0)	15.2 (2.5–29.7)	0.064		0.21 (0.13–0.38)	0.24 (0.12–0.71)	0.42
Factor B	14.4 (8.0–25.0)	19.6 (4.1–36.1)	0.012		0.34 (0.20–0.48)	0.31 (0.19–0.37)	0.15

The data are reported as median (range).

from those of controls (180.4 μ g/dl equivalents of HAGG, 35.5–216.1). Three (12%) of the patients who had had their spleens removed, however, showed values above the highest control level.

The amount of factor B of the alternative complement pathway in the PEG precipitate was also higher when compared with the equivalent value in controls. In contrast, the concentrations of protein, IgG, IgA, IgM, C3 and C4 did not differ significantly between groups (table). The serum concentration of these immunoglobulins and complement components were normal except for an increased IgG concentration found in patients after splenectomy (table).

Discussion

Circulating immune complexes in the serum of patients who had undergone splenectomy for non-malignant conditions were evaluated by studying the extent of the PEG-insoluble serum fraction to bind to Clq and by a partial characterisation of the material precipitated by PEG.

The Clq precipitin determinations in those who had had their spleen removed did not show differences when compared with controls. These results agree with the findings of Kragballe *et al*⁷ and Lanng Nielsen *et al*,⁸ who reported the presence of circulating immune complex in about 20% of their patients.

Study of the material insolubilised by PEG showed that only the concentration of factor B was increased after splenectomy. Although the importance of this finding is unclear, it has been previously reported that in sickle cell anaemia, which also occurs with hyposplenism, an increased synthesis of factor B associated with high catabolic rate of this component was shown, suggesting continuous complement activation.^{14,15}

How much the spleen participates in the removal of circulating immune complex and how much its absence causes immunological abnormalities has not been completely established. Previous reports have shown that the spleen is responsible for the clearance of particulate immune complexes^{3–5} and that several

immune complex mediated diseases have defective splenic clearance of these complexes, thus contributing to tissue deposition and damage.^{16–18} On the other hand, increased amounts of circulating immune complex, especially those containing IgA, have been found in coeliac disease and in dermatitis herpetiformis, both associated with splenic hypofunction and nephropathy.^{19,20} Patients with concomitant sickle cell anaemia and nephrotic syndrome also have several glomerular and mesangial abnormalities which have been ascribed to circulating immune complex deposition.^{21–25} Finally, it has been reported that children with primary nephrotic syndrome usually have decreased splenic hypofunction during the symptomatic period, which returns to normal after remission.²⁶

These findings would suggest that after the loss of splenic function circulating immune complexes could be increased, with deposition in tissues and damage—especially in the kidneys where they are trapped. When preformed immune complexes were administered to rats treated with cobra venom factor, they were initially removed from circulation by the liver and to a lesser extent by the spleen, subsequently accumulating in the kidneys.²⁸ To the best of our knowledge, however, there are no reports of an increased incidence of nephritis in people who have undergone splenectomy.

In conclusion, our findings have shown that patients who have had their spleens surgically removed for non-malignant conditions have a distinctive pattern of circulating immune complexes containing factor B. Whether this complexed factor B remains longer in the circulation, or whether it is deposited elsewhere producing tissue damage, or whether it is responsible for some of the immunological abnormalities seen in such patients remain to be elucidated.

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