Archives of Disease in Childhood, 1978, **53**, 141–143

Occurrence of circulating immune complexes in β-thalassaemia major

P. CASALI, P. BORZINI, D. VERGANI, G. MIELI-VERGANI, G. MASERA, AND C. ZANUSSI

From Istituto di Clinica Medica IV, and Clinica Pediatrica, University of Milan, Italy

SUMMARY The presence of circulating soluble immune complexes and the level of complement were investigated in sera from 21 patients with β-thalassaemia major, including both splenectomised and nonsplenectomised patients. A high level of immune complexes was found in half of these cases. Reduced complement levels were seen less frequently. There was no correlation between the presence of circulating immune complexes, decreased complement levels, and the presence or absence of the spleen. The level of immune complexes increased with the age of the individual, i.e. with the duration of the disease.

Many patients with β-thalassaemia major develop glomerulonephritis, pericarditis, or arthritis (Wasi, 1971), and nearly all develop chronic liver disease (Masera *et al.*, 1976). In this last condition, circulating immune complexes are often present and they are thought to play a significant part in the genesis of these immunopathological disorders. Until now there has been no investigation of immune complexes in β-thalassaemia major, or of their possible association with the secondary diseases developing in thalassaemic patients. We have therefore studied a group of patients with β-thalassaemia major to find whether circulating immune complexes occur and, if so, whether any relation can be shown between the presence of complexes and other features of the disease.

Materials and methods

Patients. Sera from 21 patients with β-thalassaemia major were studied. There were 13 males and 8 females, aged from 3½ to 8½ years. 10 had had splenectomy. All patients were treated by periodic blood transfusion. Blood samples for testing were taken immediately before transfusion. Sera from 10 healthy children of the same age range were used as controls. The blood was allowed to clot for 2 hours at room temperature and then centrifuged at 4°C. The collected serum was stored at −70°C until used.

Detection of circulating immune complexes. These were detected in native serum using radiolabelled C1q as described by Zubler *et al.* (1976). The ^{125}I-C1q binding test is based on the following principle. In a first step, the tested native serum is incubated for 30 minutes at 37°C with EDTA in order to prevent the integration of the ^{125}I-C1q into intrinsic macromolecular C1qrs complex. In a second step, ^{125}I-C1q and 3% polyethylene glycol are added to this mixture, which is further incubated at 4°C for 1 hour. Under these conditions, free C1q remains soluble while C1q bound to the complexes is precipitated. After centrifugation and discarding of the supernatant the radioactivity of the precipitate is counted. Results are expressed as per cent ^{125}I-C1q precipitated in a 'TCA control tube', in which all protein is precipitated by addition of 20% trichloroacetic acid. Heat-aggregated gamma globulins serially diluted in fresh normal human serum (NHS) are used as positive control. For our purposes, C1q was purified from fresh NHS according to Volanakis and Stroud (1972). 2 mg C1q were obtained from 40 ml of serum. Purity of separated material was assessed by immunoelectrophoresis (1% agarose) with rabbit antihuman C1q and rabbit antiwhole human serum antisera (Behringwerke, Marburg-Lahn). C1q was labelled with ^{125}iodine (Radiochemical Centre, Amersham) by use of the lactoperoxidase (LP-B grade, Calbiochem, San Diego) method (Heusser *et al.*, 1973). The ^{125}I-C1q with a specific activity of 1 μCi/μg was stored at −70°C until used.

Complement studies. Total haemolytic activity (CH50) was titrated according to Lachmann *et al.* (1973). Concentrations of complement factors C1q,

Received 3 June 1977

C4, and C3 were estimated by single radial immunodiffusion in 1% agarose. The specific antisera were purchased from Behringwerke (Marburg-Lahn). All results were expressed as percentage of values obtained with a pool of 20 NHS stored in liquid nitrogen.

Statistical analysis. Fisher's exact method, Student's *t* test, and linear regression analysis by the method of the least squares were used.

Results

The results are summarised in the Table, which also shows the age, sex, and the presence or absence of the spleen in 21 patients with β-thalassaemia major. Using the ^{125}I-C1q binding activity test, immune complexes were detected in 12 patients, 7 of whom had had splenectomy. The ^{125}I-C1q binding mean value of the patients was significantly higher than for the control group ($t = 3{\cdot}23$, $P<0{\cdot}01$). There was no significant difference between the splenectomised and nonsplenectomised groups ($t = 0{\cdot}09$, $P>0{\cdot}4$).

The level of circulating immune complexes correlated positively with the age of the individual ($r = 0{\cdot}46$, $P<0{\cdot}05$). The presence of complexes did not correlate with whether or not the patient had been splenectomised ($\chi^2 = 0{\cdot}48$, $P>0{\cdot}25$). The mean age of splenectomised patients was higher ($t = 3{\cdot}04$, $P<0{\cdot}01$) than that of the nonsplenectomised ones.

The complement values of the thalassaemic patients were within the normal range, except for the values of CH50 and C3, which were, respectively, slightly reduced and slightly increased. By linear regression analysis no correlation was found between the complement levels and the values of the ^{125}I-C1q binding activity.

Discussion

Our results indicate that circulating immune complexes occur in β-thalassaemia major and that the level increases progressively with the age of the individual. The level of circulating immune complexes did not correlate with whether or not the patient had been splenectomised, despite the fact that the mean age of the splenectomised patients was significantly higher than in the nonsplenectomised group. The presence of a considerable amount of immune complexes appears to be a common feature occurring during evolution of Cooley's anaemia. Interference with spleen function may be related to other factors including extramedullary haematopoiesis, abnormal red cell destruction, and iron overload (Pearson and O'Brien, 1975). Immune complexes may also play an important collateral role in the splenic disorder.

Nearly all β-thalassaemic patients develop chronic liver disease (Masera *et al.*, 1976). In this hepatic impairment, circulating immune complexes involving HBs antigen have been demonstrated (Theofilopoulos *et al.*, 1976); in our cases HBs antigen could have been acquired as a result of repeated blood transfusion. However, other types of immune complexes could account for the high degree of ^{125}I-C1q binding activity found in β-thalassaemia major: anti-isoantigen antibodies, secondary to the blood transfusion therapy, have been demonstrated (Pearson and O'Brien, 1975) and isoantigen anti-isoantigen complexes could be a normal feature in such cases. Immune complexes occurring in β-thalassaemia major require a characterisation to determine the nature of the antigen(s) involved.

If circulating soluble immune complexes are formed in Cooley's anaemia, they may play a role in the evolution of the disease. There is an increased incidence of infections during the disease which are often severe and lethal (Weatherall and Clegg, 1972), although evidence of deficiency of circulating antibodies specific for the micro-organisms most frequently involved has not been shown. The presence of immune complexes may alter the immunological competence of the patients, either by a direct

Table *^{125}I-C1q binding activity and complement level in patients with β-thalassaemia major and controls*

	Sex			*Immune complexes*		*Complement† (mean±SD)*			
	M	*F*	*Age (yr) (mean±SD)*	*^{125}I-C1qBA* (mean±SD)*	*% positive*	*CH50*	*C1q*	*C4*	*C3*
Controls (n = 10)	6	4	6·9±1·2	4·6±1·0	0	100±15	100±20	100±25	100±20
β-thalassaemia nonsplenectomised (n=11)	7	4	6·1±1·8 } §	22·2±28·6‡	45	73±23·8‡	87·4±31·6	86·7±28	103±27·8
β-thalassaemia splenectomised (n=10)	6	4	7·9±0·8 } §	23·2±23·5‡	70	89·5±17·4	107±37·8	90·3±14	115±28·9
β-thalassaemia total (n=21)	13	8	7·0±1·7	22·7±25·6‡	57	81·0±22·1‡	96·7±35·2	87·9±22·1	129±24·4§

*% of ^{125}I-Clq precipitated.
†% of a pool of normal human serum.

The differences between the groups were analysed by Student's *t* test (for populations with unequal variances): ‡$P<0{\cdot}05$, §$P<0{\cdot}01$. Except for age, where there was a significant difference between the two thalassaemic subgroups, for the other parameters the discrimination is in relation o the control group.

blocking of the reticuloendothelial system (Mannik *et al.*, 1974) or by consumption of complement. Immune-adherence is promoted by C3, and C5a is a potent phagocytic cell stimulator (Goldstein and Weissman, 1974). Complement impairment may therefore involve a concomitant reduction of phagocytic efficiency. Our results may be consistent with the hypothesis of a direct blocking of the reticuloendothelial system and did not indicate a severe impairment of complement functions, although there was some decrease in CH50 activity. Preliminary investigations (Auderset *et al.*, 1976) suggest that a functional deficiency of properdin factor B of the alternative pathway of complement activation occurs in β-thalassaemia major. Thus there may be two independent factors which contribute towards the diminution of immunological competence in thalassaemic patients.

We thank Dr L. Mackey for help with the manuscript. This work was partly supported by HOECHST-Italia, Milan, Italy. P. C. is the recipient of an Assegno Biennale di Formazione Scientifica e Didattica from University of Milan.

References

Auderset, M. J., Casali, P., and Lambert, P. H. (1976). Decreased factor B activity in β-thalassemia major. *Pathologie et Biologie*, **26**, 391.

Goldstein, I. M., and Weissman, G. (1974). Cellular and humoral mechanisms in immune complexes injury. *Progress in Immunology, II*, Vol. 5, p. 81. Ed. by L. Brent and J. Holborow. North-Holland, Amsterdam.

Heusser, C., Boesman, M., Nordin, J. H., and Isliker, H. (1973). Effect of chemical and enzymatic radioiodination on *in vitro* human C1q activities. *Journal of Immunology*, **110**, 820–828.

Lachmann, P. J., Hobart, M. J., and Aston, W. P. (1973). Complement technology. *Handbook of Experimental Immunology*, Chapter 5. Ed. by D. M. Weir. Blackwell, Oxford.

Mannik, M., Haakenstad, A. O., and Arend, W. P. (1974). The fate and the detection of circulating immune complexes *Progress in Immunology, II*, Vol. 5, p. 91. Ed. by L. Brent and J. Holborow. North-Holland, Amsterdam.

Masera, G., Jean, G., Gazzola, G., and Novakova, M. (1976). The role of chronic hepatitis in the development of thalassaemic liver disease. *Archives of Disease in Childhood*, **51**, 680–685.

Pearson, H. A., and O'Brien, R. T. (1975). The management of thalassemia major. *Seminars in Hematology*, **12**, 255–265.

Theofilopoulos, A. N., Wilson, C. B., and Dixon, F. J. (1976). The Raji cell radioimmunoassay for detecting immune complexes in human sera. *Journal of Clinical Investigation*, **57**, 169–182.

Volanakis, J. E., and Stroud, R. M. (1972). Rabbit C1q: purification, functional and structural studies. *Journal of Immunological Methods*, **2**, 25–34.

Wasi, P. (1971). Streptococcal infection leading to cardiac and renal involvement in thalassaemia. *Lancet*, **1**, 949–950.

Weatherall, D. J., and Clegg, J. B. (1972). *The Thalassaemia Syndromes*, p. 272. Blackwell, Oxford.

Zubler, R. H., Lange, G., Lambert, P. H., and Miescher, P. A. (1976). Detection of immune complexes in unheated sera by modified ^{125}I-C1q binding test: effect of the heating on the binding of C1q by immune complexes and application of the test to systemic lupus erythematosus. *Journal of Immunology*, **116**, 232–235.

Correspondence to Dr P. Casali, WHO Immunology Research and Training Centre, Hôpital Cantonal, 1211 Geneva 4, Switzerland.