

Raised IgE levels in β -thalassaemia: correlation with splenectomy and hepatitis B virus infection

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SUMMARY

IgE values obtained in 117 β -thalassaemia patients were significantly higher than in age matched normal subjects. In 31 patients (26.5%) IgE levels were above 2 s.d. of normal values for age, but the frequency of IgE with reaginic activity was lower in patients (5.1%) than in controls (11.9%). The highest values were observed in splenectomized patients who were also positive for one or more serological markers of hepatitis B virus infection. The increase of IgE levels was directly correlated with the number of years after splenectomy, and patients with biopsy proven chronic liver disease had higher IgE levels than those without evidence of liver damage. On the other hand, IgE levels were not correlated with the number of transfusions, age, IgG, IgA, IgM levels or T cell subsets and mitogen responsiveness. These results show that β -thalassaemia patients develop elevated IgE levels to which splenectomy and hepatitis B virus infection contribute in a synergistic manner.

Keywords β -thalassaemia IgE splenectomy hepatitis B virus infection

INTRODUCTION

β -thalassaemia major is essentially a severe disturbance of β -globin synthesis with consequent poor erythropoiesis and reduced erythrocyte survival (Ohene-Frempong & Schwartz, 1980). Immunological changes have also been reported in this disease, consisting of reduced values of T lymphocytes (Musumeci *et al.*, 1979), increased IgG, IgA and IgM levels (Vierucci *et al.*, 1972), and modifications in neutrophil function (Khan *et al.*, 1977; Tovo, Miniero & Ponzone, 1977).

No extensive report has appeared about IgE levels in transfusion-dependent thalassaemics, even if transfused patients are known to be good producers of IgE against erythrocyte antigens (Ropars *et al.*, 1979). The study of IgE levels in thalassaemics can offer useful information also about IgE production in liver disease, since hepatitis B virus (HBV) infection and/or chronic liver disease are present in the majority of thalassaemics (Vierucci *et al.*, 1972; De Virgiliis *et al.*, 1980).

In the present study, IgE levels were evaluated in 117 β -thalassaemia patients, and the results were correlated with splenectomy, HBV markers in the serum, chronic liver disease, the number of transfusions, ferritin, IgG, IgA and IgM levels, as well as with T cell subsets and mitogen responsiveness. We observed an increase in IgE levels which was directly related to HBV infection, chronic liver disease and splenectomy.

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MATERIALS AND METHODS

Patients. One hundred and seventeen patients with β -thalassaemia major (64 males and 53 females, aged 1–26 years, mean 12.8 years) were examined in the Departments of Paediatrics of Florence and Ferrara. Fifty-six patients (30 males and 26 females, mean age 13.2 years) had undergone splenectomy between 1 and 20 years before the beginning of this study. All patients had received multiple transfusions (mean number: 266.7), with no significant difference between splenectomized and non-splenectomized patients. Repeated duodenal juice, stool examinations and peripheral blood eosinophil counts were carried out in all patients to prove absence of infection with parasites. None of the patients had taken drugs for at least 1 month prior to the immunological tests, except chelation treatment with desferrioxamine. Blood samples were taken at least 20 days after the last transfusion.

Immunoglobulin levels. IgG, IgA and IgM levels were assayed using radial immunodiffusion technique. Total IgE levels were measured by radioimmunoassay (PRIST, Pharmacia, Upsala, Sweden). Specific IgE for house dust, *Dermatophagoides pteronissinus*, skin derivatives of dog and cat, grass pollen (*Cynodon dactylon* and *Lolium perenne*), weeds (*Artemisia vulgaris* and *Plantago lanceolata*), trees (*Olea europea* and *Platanus acerifolia*), mould allergens (*Cladosporium herbarium* and *Alternaria tenuis*) or food allergens (milk, egg and fish) were determined in patients and controls using a radioimmunoassay technique (RAST, Pharmacia).

Serum HBV markers. Hepatitis B surface antigen (HBsAg) and antibodies to surface (anti-HBs) or core (anti-HBc) antigens were tested, using radioimmunoassay techniques (AUSRIA, AUSAB, and CORAB, respectively; Abbott Lab., Chicago, Illinois, USA). In HBsAg positive patients, hepatitis B e antigen (HBeAg) was also studied, using a radioimmunoassay technique (HBe, Abbott).

Liver disease characterization. Alanine aminotransferase (AAT) and aminoaspartate (AST) transaminases were determined in each patient using standard methods. In those patients in whom AAT and AST were above normal values for over a year, Menghini needle liver biopsy was carried out and the histology was evaluated according to the criteria of Bianchi *et al.* (1977).

Ferritin levels. Ferritin levels were evaluated in each patient using a radioimmunoassay (FERK-M, Sorin Biomedica, Saluggia, Italy), as previously described (Addison *et al.*, 1972).

Lymphocyte surface markers and mitogen responsiveness. Evaluation of T cell subsets and *in vitro*

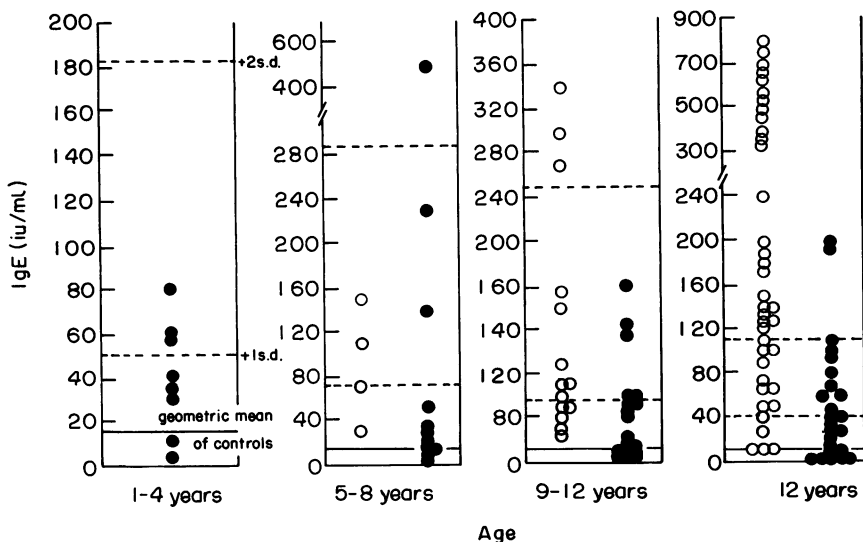


Fig. 1. IgE levels in splenectomized (O) and non-splenectomized (●) patients with β -thalassaemia.

response to phytohaemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM) was carried out in 63 patients and in their corresponding controls. OKT3, OKT4, OKT6, OKT8 and OKT11 monoclonal antibodies (MoAb) (Ortho Pharmaceutical Corp., Raritan, New Jersey, USA) were used, as previously described (Aiuti *et al.*, 1983), to recognize the total number of T lymphocytes and T cell subsets in which helper T cells, thymocytes, cytotoxic suppressor T cells and E rosette forming cells are thought to be included. In addition, the total number of T lymphocytes was estimated with the usual E rosette technique (Jondal, Holm & Wizgell, 1972). Mitogen responsiveness was evaluated according to a standard method (Romagnani, Biliotti & Ricci, 1975). PHA (DIFCO Laboratories, Detroit, Michigan, USA) was employed in three different dilutions (1:6, 1:20, 1:120), whereas the response to Con A (Pharmacia) and PWM (GIBCO New York, USA) was determined with 5 µg/ml and 10 µg/ml of mitogen, respectively. Control cultures were performed without adding mitogen.

Controls. A normal age and sex matched control subject was evaluated for each patient studied. Controls were patients admitted for minor surgery, or personel from our laboratory or medical students. None of the controls, who were all in apparently good health, had taken any medication for at least 1 month.

Statistical analysis. Data were analysed by Student's *t*-test for differences between means, by χ^2 for differences between frequencies in the groups studied, and Wilcoxon's test for differences between paired data. Correlation coefficient (*r*) between variables was calculated and linear regressions plotted.

RESULTS

IgE levels

Fig. 1 shows the IgE values obtained in the 117 patients studied (subdivided into groups on the basis of age and case history with or without splenectomy) and in controls (whose results are expressed as geometric mean + 1 and 2 s.d.). Comparing the age matched patient control pairs, significantly higher levels were found in patients ($P < 0.001$). No correlation was found between IgE levels and transfusion number.

IgE and splenectomy

Patients with abnormally increased IgE levels (i.e. above 2 s.d. of the geometric mean of normal values for age) were more frequently found among splenectomized (27 out of 56 cases, 48.2%) than non-splenectomized patients (four out of 61 cases, 6.5%) ($P < 0.001$). In addition, there was a significant correlation ($r = 0.427$) in splenectomized patients over 12 years of age between IgE levels and years since splenectomy (Fig. 2).

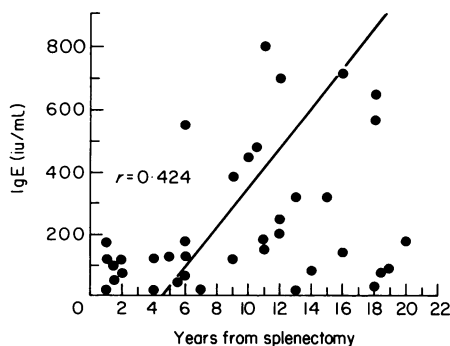


Fig. 2. Correlation between IgE levels and years since splenectomy in 38 patients with β -thalassaemia aged over 12 years.

Table 1. Frequency of patients with normal (within geometric mean +2 s.d. of age matched controls) or abnormally increased (above geometric mean +2 s.d. of age matched controls) IgE levels in relationship to HBV serological markers and splenectomy

HBV serological markers					Splenectomized patients with				Non-splenectomized patients with			
					Normal IgE levels		Abnormally increased IgE levels		Normal IgE levels		Abnormally increased IgE levels	
HBsAg	Anti-HBs	Anti-HBc	n	%†	n	%†	n	%†	n	%†	n	%†
-	-	-	21	17.9	4	19.0	0	—	17	81.0	0	—
-	-	+	5	4.2	1	20.0	1	20.0	3	60.0	0	—
-	+	+	64	54.7	17	26.6	13	20.3	30	46.9	4	6.3
+	-	+	14	11.9	1	7.1	7	50.0	6	42.8	0	—
-	+	-	13	11.1	6	46.1	6	46.1	1	7.6	0	—
Total			117	100	29	24.8	27	23.0	57	48.7	4	3.4

* Percentage of column and † of row.

IgE with reaginic activity

The study of IgE specific for allergens showed the presence of reagins in six patients (5.1%) and 14 controls (11.9%) ($P < 0.001$).

Serum HBV markers and IgE

As shown in Table 1, none of the 21 patients negative for HBV markers had abnormally increased IgE levels, while this happened in 31 of 96 (32.2%) patients positive for one or more markers ($P < 0.01$). In the 56 splenectomized patients none of the four cases negative for HBV markers had abnormally increased IgE levels, whereas they were present in 27 out of 52 (51.9%) patients positive for one or more markers ($P < 0.05$). Moreover, among the 52 splenectomized patients positive for HBV markers, abnormally increased IgE levels were more frequently observed in HBsAg positive (seven out of eight cases, 87.5%) than in HBsAg negative patients (20 out of 44 cases, 45.5%) ($P < 0.05$). No difference was found between patients positive or negative for HBV markers with respect to the mean number of transfusions (275.8 and 259.9, respectively).

IgG, IgA, IgM levels and IgE

Increased levels of IgG ($2,396 \pm 875$ mg/100 ml), IgA (537 ± 184 mg/100 ml) and IgM (147 ± 51 mg/100 ml) were found in the patients, and these are significantly different ($P < 0.01$) from those of controls ($1,221 \pm 327$, 243 ± 99 , 86 ± 27 mg/100 ml, respectively). No correlation was observed between IgG, IgA, and IgM levels and IgE levels.

Ferritin levels and IgE

Ferritin levels were increased in all patients (mean \pm s.e.: $4,624 \pm 341$ ng/ml), but no correlation was observed between ferritin and IgE levels.

Liver disease and IgE

AAT and AST levels were over the upper normal level (i.e. over 40 iu/l) in 105 patients (89.7%); normal transaminase levels were found in 12 cases. Comparing IgE values of patients with normal transaminase levels and those of corresponding controls no difference was found. Moreover, in none of the 12 patients with normal AAT and AST levels abnormally increased IgE levels were

Table 2. T cell subsets and mitogen responsiveness in patients with β -thalassaemia (mean \pm s.d.)

	Patients (n=63)	Controls (n=63)	P
Lymphocytes/mm ³	2,893 \pm 954	3,004 \pm 786	n.s.*
OKT3 ⁺ cells %	68.9 \pm 6.5	69.3 \pm 7.1	n.s.
OKT4 ⁺ cells %	41.9 \pm 8.4	42.1 \pm 7.0	n.s.
OKT8 ⁺ cells %	26.9 \pm 7.9	27.3 \pm 5.0	n.s.
OKT11 ⁺ cells %	64.9 \pm 7.2	65.0 \pm 7.1	n.s.
E rosettes %	62.1 \pm 6.4	63.9 \pm 5.2	n.s.
Mitogen responsiveness†			
Background	1,052 \pm 458	918 \pm 327	n.s.
PHA 1:6	16,078 \pm 5,415	32,081 \pm 6,031	<0.001
PHA 1:20	20,643 \pm 4,810	52,168 \pm 8,529	<0.001
PHA 1:120	34,216 \pm 8,761	10,757 \pm 4,721	<0.001
Con A	20,013 \pm 3,138	25,418 \pm 4,321	<0.001
PWM	14,381 \pm 2,311	20,613 \pm 3,593	<0.001

* Not significant. † Expressed as ct/min.

present. On the other hand, HBV markers were less frequently found in these patients (seven out of 12 cases, 58.4%) than in the remaining ones (89 out of 105 cases, 84.8%) ($P < 0.05$).

There was no difference in the frequency of splenectomized cases among the patients with (51 out of 105 patients, 48.5%) or without abnormal transaminases (five out of 12 patients, 41.6%).

Liver biopsy was performed in 40 patients. Twenty-two cases had chronic persistent hepatitis (CPH), 10 had chronic active hepatitis (CAH) and eight had cirrhosis. Comparing the IgE values of these 40 subjects with those of the corresponding controls, significantly higher values were found ($P < 0.01$) in the patients. Within each group of these patients, differences in the number of those with abnormally increased IgE levels were found between patients with cirrhosis (five out of eight cases) and those with CPH (five out of 22 cases) ($P < 0.05$). No differences were observed as to the frequency of splenectomy or HBV markers among these biopsied patients.

Lymphocyte surface markers and mitogen responsiveness

The number of E rosette forming cells as well as of lymphocytes positive with OKT3, OKT4, OKT8 and OKT11 MoAb did not differ in patients and controls (Table 2). In both patients and controls OKT6 positive cells were less than 1% of the total lymphocyte population.

The patients showed significantly depressed PWM and Con A responses as compared to those of controls (Table 2). Lymphocyte response to PHA of patients differed from that of controls as to reduced responsiveness at the higher concentrations (1:6 and 1:20) and also to increased responsiveness at the highest dilution (1:120) (Table 2).

T cell subsets and mitogen responsiveness did not differ in either splenectomized or HBV serological markers positive patients from non-splenectomized or HBV serological markers negative patients. No significant correlations were found between T cell subsets or mitogen responsiveness and age, number of transfusions, ferritin values, IgG, IgA, IgM and IgE levels, and (in splenectomized patients) with the years elapsed since splenectomy.

DISCUSSION

In this study we observed that IgE levels are higher in β -thalassaemia patients than in age matched normal subjects. In various diseases, including parasitosis, some immunodeficiencies, neoplasia and liver diseases, high IgE levels are present, even in the absence of a state of atopia (Buckley & Becker, 1978; Johansson & Foucard, 1978; Levo & Shalit, 1981). Our results indicate that two factors affect an increased production of IgE in β -thalassaemia, i.e. splenectomy and HBV infection.

The regulatory activity of the spleen on IgE synthesis has been demonstrated in animals (Okumura & Tada, 1971, 1980). If the results in animals can be extrapolated to humans, then splenectomy would cause a loss of humoral and cellular mechanisms able to regulate IgE synthesis. However, HBV infection is a further factor in β -thalassaemia patients in relation to IgE levels. In fact, (1) both splenectomized and non-splenectomized patients did not show any tendency to develop elevated IgE levels if serum HBV markers were absent; (2) HBsAg was associated to high IgE levels only in the splenectomized patients and (3) HBsAg-positive splenectomized patients showed a stronger tendency to develop high IgE levels than HBsAg negative splenectomized patients.

It is known that various viruses cause increased IgE production in the host, sometimes specific to viral antigens (Nordbring, Johanson & Espmark, 1972; Perellmutter, Potvin & Phipps, 1979; Wzilliver & Ogra, 1983). Therefore, the possibility should be examined that IgE antibodies against HBV antigens develop in β -thalassaemia. However this does not provide an explanation of the preferential increase in IgE levels in splenectomized patients.

The patients with β -thalassaemia we examined produced more IgE than normal, but they were unable to form IgE with reaginic activity. This pattern is similar to that observed in rats infected with *Nippostrongylus brasiliensis*; they had very high IgE levels, but were incapable of forming IgE specific for allergens (Turner, Fisher & Holt, 1982). On the other hand, the remarkable incidence in the general population of atopic subjects means that β -thalassaemia patients frequently receive IgE specific against the most common allergens during transfusion and could develop anti-idiotype antibodies with a mechanism which is already known (Katz, 1980).

A role of transfused antigens in provoking high IgE levels cannot be ruled out. An IgE response has been observed following transfusion (Ropars *et al.*, 1979) and in recipients of bone marrow transplants at the height of graft versus host reaction (Johansson & Benich, 1982). Moreover, factors which regulate IgE synthesis are produced during mixed lymphocyte cultures (Katz, 1980). While a mechanism of this type cannot be rejected, it does not seem likely that a graft versus host reaction is present in β -thalassaemia patients.

Transfusions may have an indirect effect on the regulation of IgE synthesis by virtue of iron overload. A modulatory activity of iron on lymphocyte function is known (Brock, 1981), but no correlation was found between IgE levels and ferritin values, a measure of iron overload (Addison *et al.*, 1972).

Patients with biopsy proven chronic liver disease had much higher IgE levels than controls, but this was not so in patients without clinical signs of liver damage. The behaviour of patients with an apparently normal liver compared with those with liver damage could be due to the lower prevalence of serological HBV markers, but the difference between the patients with CPH and CAH or cirrhosis is more difficult to explain, as the three groups were similar in frequency of HBV markers. The association of raised IgE levels with liver disease is at present a controversial point (Joske, Turner & Murphy, 1976; Charlesworth *et al.*, 1978; Levo & Shalit, 1981). However, increased IgE levels have been found in cirrhosis patients by Peters & Johnson (1972) and Heiner & Rose (1970). Also a defect in T suppressor cells has been described (Tomino *et al.*, 1982).

IgE synthesis is T-dependent (Katz, 1980) and modifications in T lymphocytes are often associated with an increase in IgE production (Buckley & Becker, 1978). Our results show that T cell numbers and T cell subsets distribution are normal. Modifications in mitogen responsiveness have been observed, but they did not correlate to IgE levels, splenectomy or HBV serological markers. Therefore it is likely that a more subtle immunological modification leads splenectomized HBV serological markers positive thalassaemics to develop high IgE levels.

The nature of this modification remains unknown. However, the observation that an immunological mutilation (splenectomy) and a viral infection (hepatitis B) play a synergistic role in causing high IgE levels provide further perspectives to our understanding of the mechanisms of IgE production and modulation.

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