Lymphocyte changes in β -thalassaemia major

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SUMMARY Lymphocyte subpopulations were studied in 20 hypertransfused patients with β -thalassaemia major, some of whom had been splenectomised. B-lymphocytes were normal but T-lymphocytes were decreased in all patients. The T-cell count was lower in the splenectomised patients than in the nonsplenectomised ones. In the former, the active rosette-forming lymphocytes were also diminished, but the difference was not significant. In all patients the percentage of null cells was greater and the activity of K-cells increased compared with controls.

Many patients with β -thalassaemia develop chronic liver disease (Ellis *et al.*, 1954; Panizon and Vullo, 1957; Beltrami and Nenci, 1969; Masera and Jean, 1973). This complication has been attributed to hepatitis virus infection in a liver already stressed by the iron overload (Masera and Jean, 1973; Masera *et al.*, 1976). As chronic hepatitis may be related to an immunological dysfunction, we studied the lymphocyte subpopulation in a group of patients with thalassaemia major. A decreased proportion of T-cells associated with greater K-cell activity is reported.

Methods

20 patients, aged between 3 and 22 years, with thalassaemia major were studied. Eight had undergone splenectomy. Each was regularly transfused with packed red cells every 20-30 days in order to maintain Hb > 9 g/dl. 12 normal subjects, aged between 3 and 12 years, served as controls. Serum transaminases were determined; Hb_sAg and Hb_sAb by the solid radioimmunoassay method (AUSRIA II and AUSAB); immunoglobulin levels and C3 and C4 components of serum complement by single radial immunodiffusion, using specific antisera (Kallestad). Purified lymphocytes were obtained by fractionation of heparinised blood on a Ficoll-Hypaque density gradient (MSL Eurobio). In some

experiments residual phagocytic cells were eliminated by incubating the cells with iron powder and removing the phagocytic ones with a magnet. T-lymphocytes were identified by sheep red cell rosetting (SRCR) technique (Jondal *et al.*, 1972) and active rosette-forming lymphocytes were identified by the method of Wybran *et al.* (1972), modified by Horowitz *et al.* (1975). B-lymphocytes were identified by the presence of fluorescence on their surfaces when treated with antiwhole immunoglobulin conjugate (Kappel Laboratories), using the technique of Permis *et al.*, 1970. K-cell activity was measured by ⁵¹Cr-labelled chicken red blood cell (CRBC) technique. Antibodies against chicken red cells were obtained in rabbits by injecting them with 1 ml 10% CRBC suspension twice weekly for 3 weeks. For the assay, CRBC (0.2 ml) were labelled with 100 μ Ci of ⁵¹Cr sodium chromate for 30 min at 37°C. After incubation, the red cells were washed 3 times with RPMI 1640 and diluted to a concentration of 2.5×10^6 /ml. Then 0.1 ml of the lymphocytes at a concentration of 5.0×10^6 /ml was placed in a plastic tube containing 0.5 ml inactivated antibody at a dilution of 1:1000, 0.2 ml ⁵¹Cr-labelled red cells, and 1.7 ml tissue culture medium. All the dilutions were made in RPMI 1640, supplemented with 10% fetal calf serum previously absorbed with CRBC. Control incubations were carried out in the absence of antibodies or lymphocytes, and a second control tube containing labelled red cells and distilled water was included in the experiment. After incubation overnight at 37°C, the tubes were gently centrifuged, the supernatant collected, and radioactivity of the supernatant measured.

Cytotoxicity was evaluated according to the formula:

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$$\frac{(^{51}\text{Cr sample tube}) - (^{51}\text{Cr control tube})}{(^{51}\text{Cr distilled water}) - (^{51}\text{Cr control tube})} \times 100$$

The spontaneous release of ^{51}Cr was always $<10\%$ in the control tube.

Results

The serological data for 20 hypertransfused patients are listed in Table 1. An increase in serum transaminases was observed in both groups, particularly in the splenectomised patients. IgA and IgG immunoglobulins were also increased in the thalassaemic patients, again particularly in the splenectomised ones. C3 and C4 in thalassaemic patients were normal, but C3 was slightly low in splenectomised patients. The Hb_sAg and Hb_sAb tests gave one Hb_sAg positive and 11 Hb_sAb positive, the remaining 8 patients had negative results. Eight patients developed a typical icteric hepatitis B. B- and T-lymphocyte assay and K-cell activity in the thalassaemic patients and controls are shown in Table 2 and the Figure. Regularly transfused patients had normal B-lymphocytes but fewer T-lymphocytes than controls. The T-cell count was lower in the splenectomised than the nonsplenectomised patients ($P<0.005$). The active rosette-forming lymphocytes were also diminished, but this difference was not significant.

Figure Antibody-mediated cytotoxicity to CRBC of lymphocytes from 12 normal controls and 20 patients with β -thalassaemia major. Normal controls 10.35 ± 4.34 ; splenectomised patients 32.88 ± 17.95 ; nonsplenectomised patients 25.89 ± 18.81 .

Table 1 Serological data (means $\pm$ SD) for 20 patients with β -thalassaemia major

	Sex		Age (years)	Hb (g/dl)	Transaminases		Immunoglobulins			Complement	
	Male	Female			AST (mU)	ALT (mU)	IgA (IU/ml)	IgM (IU/ml)	IgG (IU/ml)	C3 (g/l)	C4 (g/l)
Normal values				13.84 ± 1.05	0-12	0-12	83.30 ± 42.84	116.15 ± 48.30	11.46 ± 3.75	1.13 ± 0.32	0.27 ± 0.07
β -Thalassaemia, splenectomised (n = 8)	4	4	13.75 ± 6.31	9.81 ± 1.16	64.75 ± 27.42	41.25 ± 19.91	219.15 ± 114.18	198.51 ± 123.42	21.33 ± 6.73	1.01 ± 0.14	0.18 ± 0.07
β -Thalassaemia, nonsplenectomised (n = 12)	4	8	5.50 ± 1.93	9.00 ± 1.88	32.00 ± 12.68	24.16 ± 20.60	76.30 ± 40.74	178.88 ± 128.05	14.94 ± 5.69	0.94 ± 0.18	0.22 ± 0.05
Total β -thalassaemia (n = 20)	8	12	8.80 ± 5.84	9.33 ± 1.65	45.10 ± 25.32	31.00 ± 21.60	133.44 ± 104.50	206.65 ± 124.43	17.49 ± 6.77	0.97 ± 0.17	0.20 ± 0.06
P (Student's <i>t</i> test)	1	2	<0.0025	<0.15	<0.0025	<0.05	<0.0005	<0.40	<0.025	<0.20	<0.15

Table 2 Lymphocytes (means $\pm$ SD) in 12 normal controls and in 20 patients with β -thalassaemia major

	Lymphocytes (%)				Lymphocytes ($10^9/l$)			
	B-	T-	Null	Active RF*	B-	T-	Null	Active RF*
Controls (n = 12)	18.66 ± 5.06	60.41 ± 4.36	20.00 ± 6.86	46.88 ± 10.68	764.75 ± 400.02	2447.16 ± 962.45	896.00 ± 482.01	1833.72 ± 762.87
β -Thalassaemia, splenectomised (n = 8)	19.75 ± 9.28	39.00 ± 9.35	41.25 ± 13.04	38.00 ± 9.84	577.37 ± 383.63	1106.37 ± 407.69	1197.37 ± 552.91	1012.37 ± 298.91
β -Thalassaemia, nonsplenectomised (n = 12)	19.83 ± 9.24	47.66 ± 9.63	33.83 ± 14.70	39.25 ± 6.15	787.58 ± 634.50	1948.25 ± 750.91	1462.16 ± 1022.90	1622.91 ± 715.88
Total β -thalassaemia (n = 20)	20.00 ± 9.16	44.20 ± 10.24	36.80 ± 14.20	38.25 ± 8.49	703.50 ± 377.23	1611.50 ± 752.82	1356.25 ± 857.97	1378.70 ± 650.99
P (Student's <i>t</i> test)	1 $\rightarrow$ 4 2 $\rightarrow$ 3				<0.35 <0.15	<0.01 <0.005	<0.05 <0.30	<0.05 <0.025

*RF = Rosette-forming.

The percentage of null cells was significantly higher in the thalassaemic patients compared with controls, but the splenectomised did not differ from nonsplenectomised patients in this respect.

The activity of K-cells in the 20 hypertransfused patients was increased ($P < 0.0025$) compared with controls, but there was no difference between splenectomised and nonsplenectomised patients ($P < 0.25$). This increased K-cell activity was unaffected when phagocytes were removed with a magnet so that the greater activity did not appear to be caused by contamination with monocytes and polymorphs.

Discussion

Our study shows that a decrease of T-lymphocytes takes place in patients with β -thalassaemia major. This decrease is also reflected in the number of active rosette-forming lymphocytes. Wyler (1976) observed lower T-cell counts in children with splenomegaly due to malaria; he suggested that the T-cell lymphopenia was due to selective sequestration of T-lymphocytes in the spleen. However, our splenectomised patients had a lower mean T-cell count than nonsplenectomised ones. These results seem to exclude the spleen from a role in the depletion of T-lymphocytes. As our results give no evidence that the presence of a serum factor interferes with rosette formation, the mechanism of T-lymphocyte depletion in β -thalassaemia remains unknown.

The selective depletion of T-lymphocytes in β -thalassaemia seems to be unassociated with impairment of cell-mediated immunity (Kanakoudi-Tsakalidis *et al.*, 1977). It could be due to a depletion of particular T-cells, perhaps suppressor T-cells. Such a depletion of suppressor T-cells may contribute to the increased production of immunoglobulin observed in hypertransfused patients (a normal percentage of B-circulating lymphocytes was noted).

Casali *et al.* (1978) observed an increase in circulating soluble immunocomplexes in thalassaemics treated with multiple transfusions, related to the age of the patient; complement levels were also decreased. This increase of immunocomplexes seems to be a consequence of the development of chronic hepatitis due to HB virus or to iron overload, or both (Masera and Jean, 1973; Masera *et al.*, 1976). The immunocomplexes in β -thalassaemia could also occur through the isoantigen antibodies complexes due to blood transfusion (Pearson and O'Brien, 1975). Such immunocomplexes may stimulate K-cell activity, the greater number of null cells being then due to the increased number of K-cells.

It is not known whether the increased activity of

K-cells is due to an increase in cytotoxic activity of individual cells or to an increase in cell number. Increased K-cell activity has been observed only in hypertransfused patients and is directly related to the age of the patient. Whether or not the patients had been splenectomised seemed to be immaterial, despite the fact that the splenectomised patients were significantly older than the nonsplenectomised ones. Increase of K-antibody-dependent cells was observed in chronic active hepatitis by Cochrane *et al.* (1976) and also in many acute and chronic liver diseases of infancy (Smith *et al.*, 1977). The presence of increased K-cell activity in β -thalassaemia suggests that these cells may play a key role in the cirrhosis affecting thalassaemic children.

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¹ International Steering Committee of Medical Editors. Uniform requirements for manuscripts submitted to biomedical journals. *Br Med J* 1979; **1**: 533–535.

² Lancet. The Vancouver style. Uniform requirements for manuscripts submitted to biomedical journals. *Lancet* 1979; **1**: 429–430.