Neutrophil chemotaxis in sickle cell anaemia, sickle cell $\beta^o$ thalassaemia, and after splenectomy

E A DONADI, R P FALCÃO

From the Department of Clinical Medicine, School of Medicine, Ribeirão Preto, Brazil

SUMMARY Neutrophil chemotaxis was evaluated in 28 patients with sickle cell anaemia, 10 patients with sickle cell $\beta^o$ thalassaemia, 25 patients who had undergone splenectomy, and 38 controls. The mean distance migrated by patients’ neutrophils was not significantly different from that of neutrophils from controls. Although several immunological variables have been reported to be changed after loss of splenic function, we were unable to show a defect in neutrophil chemotaxis that could account for the increased susceptibility to infection.

Patients with sickle cell anaemia and those who have undergone splenectomy share an increased susceptibility to infection by encapsulated bacteria and also have defects of host defence mechanisms. These defects include impaired antibody response to intravenous immunisation, reduced serum opsonic activity against Streptococcus pneumoniae, abnormalities of the alternative complement system, decreased immunoglobulin synthesis by peripheral blood mononuclear cells, diminished capacity to reduce nitroblue tetrazolium to formazan, and deficiency of tuftsin, all of which have been ascribed to splenic hypofunction. In contrast, there are few reports on immunological function in patients with sickle cell $\beta^o$ thalassaemia, who also have an increased incidence of bacterial infection together with splenic hypofunction, usually associated with enlargement of the spleen. We measured neutrophil chemotaxis in patients from these three groups in order to evaluate its role in the increased susceptibility to infection shared by these patients.

Patients and methods

The following patients were studied: 28 patients with sickle cell anaemia (mean age 21.7 years, range 10–39) with a mean haemoglobin concentration of 8.3 g/dl (range 6.2–10.7); 10 patients with sickle cell $\beta^o$ thalassaemia (mean age 19.8 years, range 9–46) with a mean haemoglobin concentration of 8.4 g/dl (range 7.2–10.6), of whom seven had enlarged spleens and three had undergone splenectomy; and 25 subjects who had undergone splenectomy (mean age 26.2 years, range 10–46), with a mean haemoglobin concentration of 14.3 g/dl (range 11–15.9). Thirty-six normal subjects, including medical students and blood donors (mean age 32.6 years, range 19–56) with normal haemoglobin concentration were also studied. Splenectomy in patients without sickle cell disease had been performed between one and 24 years (mean 7.7) before the study. The indications for the operation were traumatic rupture in 12 cases and hereditary spherocytosis in 14. Absence of effective splenic function was confirmed in all cases by raised counts of pitted red cells. The diagnosis of sickle cell disease was established in each case by clinical, laboratory, and family studies. None of the patients or controls had received blood transfusions for at least two months before the study, and none had had signs of infection or had had vaso-occlusive painful crisis in the eight week period preceding blood collection.

ISOLATION OF LEUCOCYTES

Five millilitres of venous blood was collected into heparin 20 U/ml and the plasma separated after centrifugation at 500 g for 10 minutes. The cell sediment was resuspended in TC 199, mixed with 1 ml of dextran 6% (molecular weight 153 000 Sigma), and allowed to stand at 37°C for 25 minutes. Leucocyte rich supernatant was then collected and washed three times with TC 199. The cells were resuspended in 2 ml of TC 199, and the final concentration was adjusted to $2 \times 10^9$ cells/l.

CHEMOTAXIS

Neutrophil chemotaxis was measured by the leading front method in modified Boyden migration chambers using Millipore membranes with a pore diameter of 3 $\mu$m. Chemotactic factor was prepared by adding 0.1 ml of endotoxin solution (2.5 mg/ml of
Neutrophil chemotaxis in sickle cell disease and after splenectomy

lipopolysaccharide B from Escherichia coli 0127:D8 Difco in phosphate buffered saline, pH 7.5) to 0.5 ml of autologous plasma and 4.4 ml of TC 199. To evaluate non-stimulated migration the chemotactic factor was replaced by TC 199. The chambers were kept at 37°C for 30 minutes and then washed, stained with Harris’s haematoxylin, cleared in isopropyl alcohol and xylene, and mounted on a microscope slide with Canada balsam. The distance that cells migrated through the membrane was measured with a micrometer eye piece by focusing on the top of the membrane with an oil immersion objective at a magnification 100 x 100, and then focusing through the membrane until only five cells were in view. The reported values are the mean of five different fields. Results of chemotaxis were expressed in micrometres as the difference between the migration of neutrophils with and without endotoxin.

STATISTICAL ANALYSIS
The significance of the difference between the four groups was tested by one way analysis of variance. A probability of p < 0.05 was considered to be significant.

Results
The mean (SD) values for neutrophil chemotaxis were as follows: patients with sickle cell anaemia 33.4 µm (14.6), patients with sickle cell β0 thalassaemia 41.3 µm (15.0), patients who had undergone splenectomy 31.2 µm (12.6), and normal controls 38.4 µm (10.9). These values did not differ significantly (figure).

Discussion
These results show that neutrophils from patients with sickle cell anaemia or sickle cell β0 thalassaemia and patients who had undergone splenectomy had normal chemotaxis. They are consistent with the work of Akenzua and Amiengheme,15 who studied children with sickle cell anaemia using the same method. They reported normal values during the steady state of the disease, but found decreased chemotaxis in patients during painful crises. In vivo studies using the skin window method showed slightly impaired migration of neutrophils in patients with sickle cell anaemia or sickle cell β0 thalassaemia.16 Studies of chemotaxis in patients who had undergone splenectomy, however, have shown both normal values17 and diminished chemotaxis of normal neutrophils in the presence of serum from patients who had undergone splenectomy.18 Although other defects of neutrophil function have been described in patients with sickle cell disease10,16,19-22 and in patients who had undergone splenectomy,9,10,23,24 we were unable to show any abnormality of neutrophil chemotaxis which could account for the increased susceptibility to infection in these patients. Thus the absence of a functioning spleen does not seem to affect this particular part of phagocytic function.

References


Requests for reprints to: Dr Eduardo A Donadi, Department of Clinical Medicine, School of Medicine, 14.048 — Ribeirão Preto, SP, Brazil.