

Lymphocyte subpopulations in anaphylactoid purpura

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SUMMARY The immunoglobulins, complement components C3 and C4, lymphocyte subpopulations, and K-cell activity were studied in 13 children with anaphylactoid purpura and in 12 children of the same ages who acted as controls. The children with anaphylactoid purpura had significantly lower T-cell counts, greater K-cell activity and IgM values, and lower C3 levels than the controls.

Patients with anaphylactoid purpura (AP) often develop renal involvement after the acute phase of the disease.¹⁻² Nephropathy in AP patients is probably due to immunocomplexes³⁻⁴ which precipitate in the glomeruli, as shown by immunofluorescence.⁵ In fact, circulating antigen-antibody complexes are known to be important factors in the pathogenesis of many glomerular lesions.⁶

Lymphocytes have an important role in the pathogenesis of immunocomplex diseases. Therefore, it seemed worthwhile studying the lymphocyte subpopulations in AP patients.

Patients and methods

13 patients (6 girls and 7 boys) aged between 2 and 10 (5.0 ± 2.3) years and affected by AP were studied within 24 hours of admission to our paediatric clinic. All patients had skin lesions and joint pain, and 4 of them also had abdominal pain; occult blood was present in the stools of 2 of them. Two children had severe renal involvement with proteinuria and haematuria, and one also had increased blood urea nitrogen. Symptoms generally disappeared spontaneously in 2 weeks without treatment. As normal controls, 12 children aged between 3 and 12 (4.5 ± 2.5) years and admitted for nonimmunological diseases were studied. The investigation had been approved previously by our ethical committee, and each study was made with the full informed consent of the mother, often in her presence.

Immunoglobulins, C3 and C4 components of complement were estimated by single radial immunodiffusion using the method of Mancini *et al.*,⁷ with antisera purchased from Behringwerke A G,

Marburg-Lahn. Lymphocyte subpopulations were studied after separation from heparinised blood by Ficoll-Hypaque density gradient (Lymphoprep).

T-lymphocytes were identified using the sheep red cell rosetting technique.⁸ B-lymphocytes were identified by the presence of fluorescence on their surfaces when treated with antiwhole immunoglobulins conjugated with fluorescein.⁹ K-cell activity was measured by ⁵¹Cr-labelled chicken red blood cells (CRBC) technique: antibodies against CRBC were obtained in rabbits by injecting 1 ml 10% cell suspension twice weekly for 3 weeks. For the assay, CRBC were labelled with 100 μ Ci ⁵¹Cr-sodium chromate for 30 minutes at 37°C, washed 3 times with *RPMI 1640, and diluted to a concentration of 2.5×10^8 /ml. Then 0.1 ml lymphocytes at a concentration of 5.0×10^6 /ml were placed in a plastic tube containing 0.5 ml inactivated antibody diluted 1:1000, 0.2 ml ⁵¹Cr-labelled red cells, and 1.7 ml tissue culture medium. All the dilutions were made in RPMI, supplemented with 10% fetal calf serum previously adsorbed with CRBC. Control tubes without antibodies or lymphocytes, and a tube containing labelled red cells and distilled water were included in the experiment.

After incubation at 37°C overnight, the tubes were gently centrifuged, the supernatant was collected and the radioactivity measured. Cytotoxicity was evaluated according to the formula: (⁵¹Cr sample tube - ⁵¹Cr control tube) / ⁵¹Cr distilled water - ⁵¹Cr control tube) \times 100. The spontaneous release of ⁵¹Cr was always less than 10% in the control tube.

Statistical comparison of AP and control groups was by Student's *t* test.

*RPMI medium 1640, Eurobio, Paris.

Results

AP patients had normal levels of IgG and IgA but higher levels of IgM compared with the controls. The C3 values were decreased in the patients, but C4 values were normal (Table 1).

B-lymphocytes (expressed both in percentages and in absolute numbers) were similar in the two groups, whereas T-cell counts were significantly lower in the AP patients than in controls. The null cells were

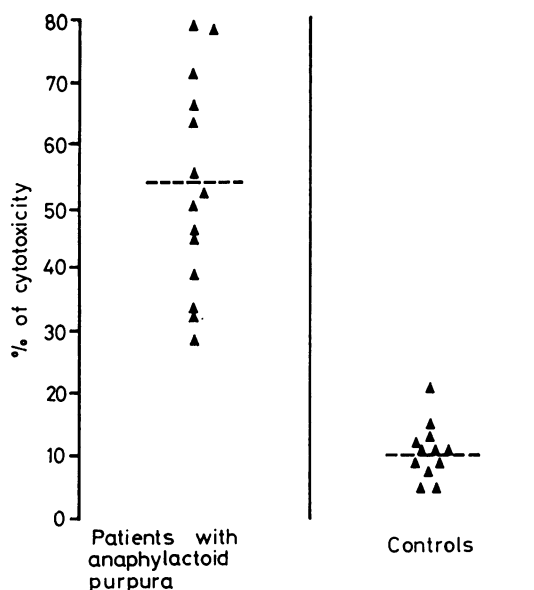


Figure Antibody-mediated cytotoxicity to CRBC of lymphocyte from children with anaphylactoid purpura (mean 54.4 ± 16.3) and from controls (mean 10.45 ± 4.3). Cytotoxicity was measured as described in the text.

increased in AP patients (both in percentages and in absolute numbers) compared with the controls, but only the increase in percentage was significant by the Student's *t* test ($P < 0.005$) (Table 2). K-cell activity was significantly greater in AP patients (Figure).

One patient developed severe nephropathy and was found to have high levels of IgA and low values of C3 and C4, while another patient (who also had renal involvement) was found to have a low level of C3 and normal values of immunoglobulins. In both patients as well as in those without nephropathy, K-cell activity was greatly increased, 50 and 46% respectively.

Discussion

We found increased IgM, normal IgA and IgG, and decreased C3 levels in our patients. These results are slightly different from those of Similä *et al.*,¹⁰ who found increased IgA and IgM. They are different also from those of Garcia-Fuentes *et al.*,¹¹ who obtained normal C3 and C4 levels in patients with AP.

The presence of immunocomplexes in AP patients has been shown on the basis of low C3 levels which confirm complement activation, and by the finding of immunoglobulins—mainly IgA, C3, and fibrinogen deposits—within the glomerular mesangium and capillary cells.¹² Antigen-antibody reaction with the associated action of complement is conceivably the main factor leading to nephropathy in AP patients.

According to the stage of differentiation and the nature of their surface receptors, immunocomplexes can modulate the lymphocyte function,¹³ and changes in lymphocyte subpopulations probably play an important role in the pathogenesis of tissue damage by immunocomplexes. Their importance has been suspected in the nephrotic syndrome, and it has been attributed to imbalance between helper and suppressor T-cells.¹⁴ Renal involvement occurs with impaired antibody formation.¹⁵

Table 1 Immunoglobulins and complement components C3 and C4 in children with anaphylactoid purpura compared with controls

	Age (years)	IgG (IU/ml)	IgA (IU/ml)	IgM (IU/ml)	C3 (g/l)	C4 (g/l)
Patients (n = 13)	5.0 ± 2.3	10.1 ± 3.7	109.0 ± 54.7	168.2 ± 62.5	0.49 ± 0.18	0.20 ± 0.01
Controls (n = 12)	4.5 ± 2.5	11.5 ± 3.7	83.3 ± 42.8	116.2 ± 48.3	1.13 ± 0.32	0.27 ± 0.07
P	NS	NS	NS	<0.005	<0.001	NS

Table 2 Lymphocyte subpopulations in children with anaphylactoid purpura compared with controls

	B-lymphocytes		T-lymphocytes		Null cells	
	%	$\times 10^9/l$	%	$\times 10^9/l$	%	$\times 10^9/l$
Patients (n = 13)	19.4 ± 10.1	0.633 ± 0.338	49.7 ± 10.1	1.555 ± 0.431	34.9 ± 15.3	1.326 ± 0.904
Controls (n = 12)	18.7 ± 5.1	0.765 ± 0.400	60.4 ± 5.0	2.447 ± 0.362	20.2 ± 6.2	0.896 ± 0.482
P	NS	NS	<0.005	<0.001	<0.005	NS

The changes in Ig levels, complement components, and lymphocyte subpopulations in our AP patients give additional evidence of the immune nature of this disease. In particular our lymphocyte data—low proportions of T-cells and high proportions of null cells, with raised K-cell activity—suggest there is a disturbance of lymphocyte function, which may be primary or secondary.

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