

Antineutrophil cytoplasm antibodies in Kawasaki disease

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SUMMARY Autoantibodies against components of neutrophil cytoplasm develop during adult vasculitic diseases such as Wegener's granulomatosis and microscopic polyarteritis, and they are predominantly of the IgG class. Similar but distinct antibodies have been described in children with Kawasaki disease and both IgM and IgG class antibodies are represented. This adds another clinically distinct childhood form of vasculitis to the adult forms in which autoantibodies to neutrophil cytoplasmic antigens have been detected.

Kawasaki syndrome is an acute febrile vasculitic illness of childhood which may be associated with the development of coronary artery aneurysms in 30% of cases.^{1 2} The aetiology and pathogenesis are unknown, although retroviral infections³ and immune mechanisms of tissue injury may be implicated. Both T cell and B cell abnormalities have been described by Leung *et al* including increased numbers of activated T4+ and Ia+ helper cells, reduced T8+ cells, and increased numbers of circulating B cells that spontaneously secrete IgG and IgM.^{4 5} More recently they have described IgG and IgM antiendothelial cell cytotoxic autoantibodies that seem to be directed towards monokine inducible determinants on the endothelial cell surface.^{6 7}

Autoantibodies have recently been described in adult vasculitic diseases including Wegener's granulomatosis and microscopic polyarteritis.^{8 9} We have developed a solid phase radioimmunoassay and indirect immunofluorescence assay that detect autoantibodies to neutrophil cytoplasm components with high sensitivity and specificity in patients with Wegener's granulomatosis and microscopic polyarteritis.¹⁰ Using these assays we screened 78 coded serum samples from 31 children, both normal control subjects and children with various illnesses (pneumonia, diabetes mellitus, IgA nephropathy, antiglomerular basement membrane antibody mediated nephritis, haemolytic uraemic syndrome, cutaneous vasculitis, Henoch-Schönlein purpura, Kawasaki disease, and microscopic polyarteritis). Serum samples from three children with Kawasaki disease contained antibodies with antineutrophil reactivity, as did four from five children with

polyarteritis. This finding was therefore examined in more detail using a larger number of children with Kawasaki disease from whom serum was available.

Patients and methods

Twelve children with Kawasaki disease were investigated, 11 with the acute disease (five of these also provided serum samples when they were convalescent, from two to 12 months after the acute illness). In one patient only serum taken during the convalescent period was tested. Serial samples were available from three patients during the first month, all of whom had been treated with human immunoglobulin (Sandoglobulin). All patients fulfilled the criteria for the diagnosis of Kawasaki disease with fever and at least four of five primary features (conjunctivitis, redness of the lips or oropharynx, desquamation of the skin of the extremities, cervical lymphadenopathy, or a polymorphous rash on the trunk).¹¹ Clinical details are shown in the table. Control samples were taken from eight children with vasculitis that was not Kawasaki disease, Wegener's granulomatosis, or polyarteritis and from six children with other febrile illnesses.

INDIRECT IMMUNOFLOURESCENCE FOR ANTINEUTROPHIL CYTOPLASM ANTIBODIES

Autoantibodies directed towards components of normal neutrophils were sought using alcohol fixed cells, as previously described.⁹ Antibody binding was detected using a fluorescein isothiocyanate rabbit antihuman IgG light chain antibody that also recognises IgM and IgA classes (Dako).

Table Clinical details of 12 patients with Kawasaki disease

Case No	Age (months)	Sex	No of primary diagnostic features	Coronary aneurysms present	Platelets ($\times 10^9/l$)	IgG (% normal)
1	33	Male	5	Not known	620	47
2	27	Female	5	No	653	136
3	27	Female	4	No	804	96
4	57	Male	5	Yes	1100	70
5	26	Male	5	No	598	66
6	64	Female	4	No	418	124
7	42	Female	4	Yes	861	Not known
8	13	Female	4	Yes	1340	Not known
9	12	Male	5	No	794	Not known
10	12	Female	5	Yes	1124	Not known
11	24	Female	4	Yes	790	212
12	39	Male	5	No	957	98

RADIOIMMUNOASSAYS FOR ANTINEUTROPHIL CYTOPLASM ANTIBODIES

IgG class antineutrophil cytoplasm antibodies were measured using a solid phase radioimmunoassay with a specific inhibition step, as previously described.¹⁰ IgM class antineutrophil cytoplasm antibodies were detected using a modification of the IgG assay whereby the specific antibody binding to the solid phase ligand was detected using a 1:200 dilution of rabbit antihuman IgM in phosphate buffered saline incubated at 37°C for one hour. After washing the plate three times with phosphate buffered saline, ¹²⁵I labelled goat antirabbit IgG was applied as the final layer in the manner described for IgG class antibodies.

MEASUREMENT OF IgG CONCENTRATIONS AND OTHER AUTOANTIBODIES

IgG concentrations in the serum samples from patients with Kawasaki disease were measured using a standard Mancini technique.¹² Antinuclear antibodies were sought using indirect immunofluorescence techniques on HEp2 cells and using rat or monkey tissue for antigastric parietal cell antibodies, antimitochondrial antibodies, antireticulin antibodies, antiskeletal muscle antibodies, and anti-smooth muscle antibodies.¹³

ADSORPTION STUDIES AND BINDING TO HL60 CELLS

In adsorption studies, all serum samples were diluted to 1:8 in phosphate buffered saline, and 100 μ l of diluted serum was incubated for one hour at room temperature with 10^5 fresh normal neutrophils or lymphocytes isolated with Ficoll Hypaque. After centrifugation at 120 g for five minutes, serum was aspirated off the cells and then binding by indirect immunofluorescence was compared with preadsorption serum.

Serum samples from patients with Kawasaki

disease were also tested by indirect immunofluorescence for binding to cultured HL60 cells, a human promyelocyte cell line.

Results

INDIRECT IMMUNOFUORESCENCE STUDIES

By indirect immunofluorescence, 10 of the 11 serum samples from patients with Kawasaki disease and none of those from control subjects showed binding to the cytoplasm of immobilised normal human neutrophils. Fig 1 shows the binding of antibodies in serum from patients with Kawasaki disease compared with the binding of antibodies in serum from an adult with Wegener's granulomatosis. Differences in the binding pattern between serum sample from patients with these two diseases were observed: in Kawasaki disease the cytoplasmic immunofluorescence is diffuse with linear accentuations, while in Wegener's granulomatosis the pattern is coarsely granular. Cytoplasmic staining was readily detected with all samples from patients with Kawasaki disease, although the binding pattern was most easily seen in samples that had high titres of antibody.

Preadsorption with normal polymorphonuclear leucocytes almost completely eliminated fluorescence activity compared with a minimal reduction using equal numbers of normal peripheral blood lymphocytes. Studies using HL60 cells showed that antibodies present in the serum of patients with Kawasaki disease recognised cytoplasmic determinants, although the pattern of binding was different from that seen with antibodies in Wegener's granulomatosis and microscopic polyarteritis.

RADIOIMMUNOASSAY STUDIES

Results of the radioimmunoassays are shown in fig 2. Raised titres of specific IgG or IgM antineutrophil

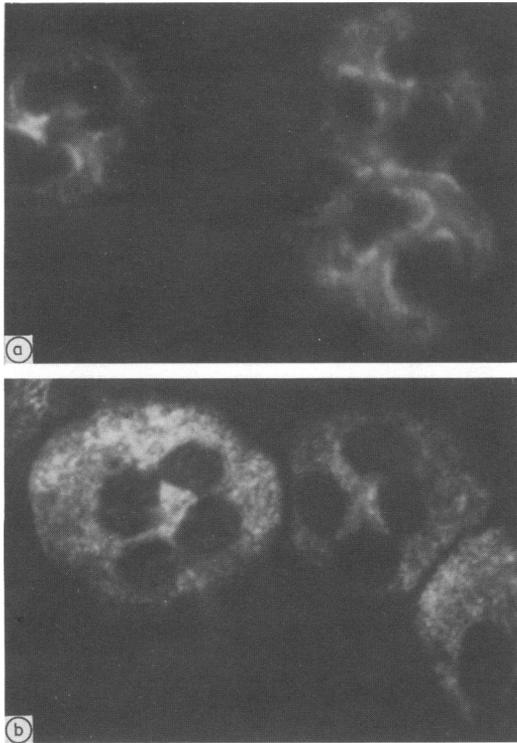


Fig 1 Antineutrophil cytoplasm antibody staining detected by indirect immunofluorescence in a patient with (a) Kawasaki disease and (b) Wegener's granulomatosis.

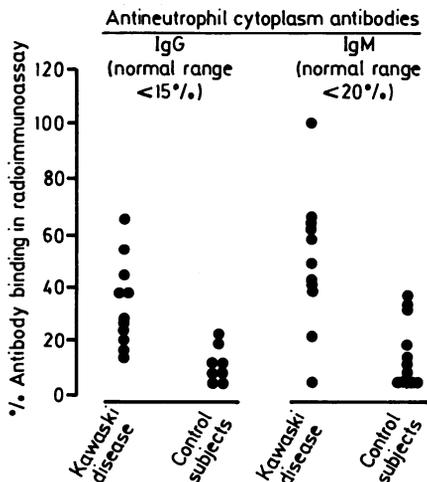


Fig 2 Titres of IgG and IgM antineutrophil cytoplasm antibodies in serum samples from 11 patients with acute Kawasaki disease and 14 control subjects.

cytoplasm antibodies were found in serum samples from 10 of the 11 patients with acute Kawasaki disease, but not in any of the samples taken when the patients were convalescent, nor in those from the control subjects. Some samples from the control subjects showed increased binding (two in the IgG assay and three in the IgM assay) but this was found to be non-specific.

Sequential studies of IgG class antibodies in three patients from whom serum samples were available showed disappearance of the antibodies by the third and 10th days after the start of treatment with human normal immunoglobulin in two patients; in the third patient, antibody titres could still be measured after one month, despite similar treatment.

IgG CONCENTRATIONS AND OTHER AUTOANTIBODY STUDIES

The total IgG antibody concentrations ranged from 47 to 212% normal human serum (table). Antibodies to nuclear protein, mitochondria, gastric parietal cell, reticulin, and skeletal muscle were not detected. Low titres of antismooth muscle antibodies were found in seven patients 1/20 (n=2), 1/40 (n=4), and 1/80 (n=1).

Discussion

Adult vasculitic diseases such as Wegener's granulomatosis and microscopic polyarteritis are associated with autoantibodies against determinants in neutrophil cytoplasm that we have suggested are associated with the enzyme alkaline phosphatase.⁹ Routine detection of these antibodies has proved useful for diagnosis.¹⁰ Both our experience and that of other workers, therefore, suggest that these antibodies are highly specific to particular forms of vasculitis, particularly Wegener's granulomatosis and microscopic polyarteritis. We have screened more than 3000 samples over the last 2½ years and others have screened similar large numbers of patients.^{8 14 15} There have been occasional reports of antineutrophil cytoplasm antibodies associated with the Churg-Strauss syndrome.¹⁶ We have failed to detect them in Henoch-Schönlein purpura¹⁷ although others have detected antibodies of the IgA class in a single patient with this disease.¹⁸ We have, however, detected them in children with rapidly progressive glomerulonephritis both idiopathic (that we regard as a limited form of microscopic polyarteritis) and associated with Wegener's granulomatosis.¹⁹ In addition, antibodies against neutrophil cytoplasm components have now been detected in acute Kawasaki disease, although they may not necessarily be directed against the

same determinants as the antineutrophil cytoplasm antibodies that are present in, for example, Wegener's granulomatosis or microscopic polyarteritis.

The pathogenetic role of antineutrophil cytoplasm antibodies in the development of vasculitis remains to be established. In other studies a mouse monoclonal antibody (W8), was raised to neutrophil cytoplasm antigen⁹; subsequently it was also found to recognise determinants on vascular endothelial cells, suggesting that such cross reactivity might initiate the development of vasculitis in patients with autoantibodies to neutrophil cytoplasm antigen (unpublished observations). Antiendothelial cell antibodies have been described in Kawasaki disease. Initially IgM class antibodies to interferon gamma inducible antigenic determinants were found, and then both IgM and IgG class antibodies to tumour necrosis factor and interleukin 1 inducible endothelial cell antigens were described that differed from those induced by interferon gamma. Both varieties are cytotoxic to endothelial cells in culture. We have now found that serum from patients with acute Kawasaki disease also contains IgM and IgG antineutrophil cytoplasm antibodies that can be detected using conventional indirect immunofluorescence and radioimmunoassay techniques. Adsorption studies suggested that the antigenic determinants were peculiar to neutrophils rather than lymphocytes, and binding did not seem to be mediated through the crystallisable fragment of immunoglobulin receptors, because binding was observed with HL60 cells that either lack or have reduced expression of such receptors.²⁰ The target of antineutrophil cytoplasm antibodies in Kawasaki disease is unknown, and may differ from the target of antibodies in adult vasculitic disease, particularly as differences in the pattern of antibody binding to neutrophils are discernible. These antibodies may develop as part of a polyclonal activation of B cells that occurs in Kawasaki disease, such that autoantibodies develop that are directed towards both monokine inducible endothelial cell determinants and normal neutrophil cytoplasm components, as well as to smooth muscle and to antigenic components of the Hanganutziu-Deicher heterophile system where antibodies are of IgM and IgE class.²¹

The inter-relations and implications of these findings require further study but it is likely that these antibodies develop in association with immune activation that leads to vascular injury.

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