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Antineutrophil cytoplasm antibodies (ANCA) of IgA isotype in adult Henoch–Schönlein purpura

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SUMMARY

ANCA are associated with certain forms of systemic vasculitis, and have been reported previously to be of the IgG and IgM isotype. We examined the possible association between IgA ANCA and the IgA-related diseases Henoch–Schönlein purpura (HSP) and IgA nephropathy (IgAN). IgA and IgG ANCA were detected by isotype-specific solid-phase assays with a crude neutrophil extract, and their presence was confirmed by antigen-specific fluid-phase competitive inhibition tests and by indirect immunofluorescence. The possible interference by IgA rheumatoid factor was excluded. IgA ANCA were detected in sera from 11/14 HSP patients (79%), from 1/30 IgAN patients (3%), from 1/40 patients with vasculitides classically associated with IgG ANCA (2·5%), and in none of 60 sera from healthy blood donors. IgG ANCA were present with IgA ANCA in three patients with HSP. Only one HSP serum had anti-myeloperoxidase (MPO) activity by both IgA and IgG isotype-specific ELISA, and none was positive for proteinase 3 (PR3). Western blot analysis performed with neutrophil extract showed that the four strongest IgA ANCA-positive HSP sera reacted with a 51-kD protein; Western blot performed on cellular fractions showed that this protein is primarily membrane-associated, and different from fibronectin. Our study suggests that adult HSP is closely associated with circulating IgA ANCA, which may be directed against a different autoantigen than that recognized by IgG ANCA.

Keywords antineutrophil cytoplasmic antibodies autoimmunity IgA Henoch–Schönlein purpura systemic vasculitis

INTRODUCTION

The vasculitis syndromes comprise a spectrum of diseases as yet still defined by clinical and histological criteria [1,2]. The association of ANCA with Wegener's granulomatosis [3], microscopic polyarteritis [4] and renal limited vasculitides [5] has indicated that autoimmune mechanisms may underlie the development of these diseases, and increasing evidence points to their importance in pathogenesis [6]. Studies so far indicate that ANCA are predominantly of the IgG isotype. However, ANCA of IgM isotype (without IgG) have been found in patients with a pulmonary/renal, anti-glomerular basement membrane (GBM) negative syndrome [7], as well as in association with IgG ANCA in patients presenting with pulmonary haemorrhage [8].

Henoch–Schönlein purpura (HSP) is a systemic vasculitis, more common in children, but well documented in adults [9], which is associated with IgA abnormalities. These include raised circulating IgA levels [10,11], the presence in serum of IgA-containing immune complexes [10,12,13] and IgA deposits in skin [14,15], as well as renal mesangium [14,16]. The same renal immunopathologic and serologic IgA abnormalities are typically found in IgA nephropathy (IgAN), and the two diseases can be distinguished only by the extrarenal vasculitic involvement in HSP [15,17,18].

IgA ANCA have been reported to occur in HSP [19], but as yet this finding has not been confirmed by others [20], and it has been pointed out that patient IgA–fibronectin complexes may account, at least in part, for some IgA ANCA activity [21]. We examined the possible association of IgA ANCA with HSP and IgAN, excluding IgA rheumatoid factor interference, and investigated autoantibody specificity by means of antigen-specific solid-phase immunoassays and Western blotting.

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PATIENTS AND METHODS

Patients

HSP ($n = 14$, mean age 45 years, age range 17–76 years, 12 male and two female). Patients included in this group fulfilled the criteria of the American Rheumatism Association. Renal biopsy was performed in 12 patients and showed mesangial

deposition of IgA in all cases. Sera were stored from all patients at presentation, or at times of clinical relapse. None of the patients was receiving treatment at the time serum was obtained.

IgAN ($n=30$, mean age 36 years, age range 16–60, 24 male and six female). These patients had impairment of renal function (or haematuria/proteinuria) and diffuse mesangial IgA deposition, without extrarenal involvement. Sera from all IgAN patients were taken at onset of the disease, and in six patients during remission, as well as during episodes of macroscopic haematuria.

IgG ANCA-positive systemic vasculitis ($n=40$). Wegener's granulomatosis ($n=9$) or microscopic polyarteritis ($n=31$).

Healthy donors ($n=60$). These were studied as negative controls (age range 17–64 years, 38 male and 22 female).

ANCA solid-phase immunoassay

Screening on the crude neutrophil extract for IgG ANCA was performed as described [4]. Detection of IgA ANCA was by ELISA using the same neutrophil preparation, blocking with 2% bovine serum albumin (BSA); gelatin was used as a blocking agent in a subsequent ELISA performed with the sera which showed high reactivity, to exclude non-specific binding of IgA to BSA. For this purpose, controls also included antigen-free wells blocked with BSA or gelatin and incubated with patient and control sera. Serum dilutions were calculated for each sample from the total IgA concentrations in order to obtain a final IgA concentration of 20 mg/dl in both patient and control sera. Binding was detected by adding alkaline phosphatase-conjugated polyclonal goat anti-human IgA (Sigma, A9669) diluted 1:500, and alkaline phosphatase substrate buffer. After 40 min absorbance was measured at 402 nm. All volumes were 100 ml/well and all incubations were for 1 h at 37°C, with triple washes of PBS containing 0·1% Tween 20 (PBS-T) between stages. ELISA positivity was defined as absorbance > 2 s.d. over the mean of values obtained from a panel of normal sera. IgA ANCA-positive and normal control sera were also tested in a modified ANCA solid-phase immunoassay (SPIA), using mouse anti-human IgA1 and IgA2 MoAbs (Unipath, M12011 and M27010) and a rabbit anti-mouse peroxidase-conjugated antibody (Dako, P260) as revealing system. In this case bound IgA was revealed with orthophenylenediamine and H_2O_2 in citric acid buffer, and reaction blocked after 20 min with 2 M sulphuric acid. Absorbance was read at 492 nm.

Antigen-specific assays were performed: IgG anti-myeloperoxidase (MPO) as described [22] and IgG anti-proteinase 3 (PR3) using a commercially available kit (Bio-Carb, Sweden) according to the manufacturer's instructions. These assays were adapted to detect IgA anti-MPO and IgA anti-PR3 by using the same detection system as for the total IgA ANCA SPIA.

Inhibition studies

The specificity of IgA ANCA binding in the ANCA SPIA was tested by means of a fluid-phase inhibition test [4]. Aliquots of sera were retested in the ANCA SPIA after incubation for 1 h at 37°C with respectively PBS, haemoglobin and neutrophil extract at protein concentrations ranging from 0·005 to 4 mg/ml. The test was considered to be positive when the reduction in ANCA binding was greater than 20% following incubation with neutrophil extract, but not with the irrelevant protein, and when a dose-response related to the neutrophil extract concentration was observed.

Indirect immunofluorescence

Indirect immunofluorescence (IIF) was performed on ethanol-fixed human neutrophils, according to the method previously described [4], modified for detection of IgA antibodies by using an anti-IgA fluorescein-conjugated antibody (Dako, F204) diluted 1:5 in PBS. Serum dilutions used were 1:2, 1:4 and 1:8 for each patient and control sample. The fluorescence was assessed independently by three investigators.

IgA and IgG rheumatoid factor ELISA

A specific ELISA kit for the detection of IgA rheumatoid factor (RF) was used on all IgA ANCA-positive sera according to the manufacturer's instructions (SELISA; Walker Laboratories Ltd., Ely, UK). The standard curve of IgA RF activity was obtained using IgA RF-positive serum, provided by the manufacturer, from a patient with rheumatoid arthritis (fulfilling the criteria of the American Rheumatism and Arthritis Council for Rheumatoid Arthritis); the serum had been previously calibrated against the International Standard of IgA RF in order to detect from 3·0 to 600 IgA RF U/ml. The presence of IgG anti-IgA RF was tested by ELISA on IgA-coated plates.

Two HSP patients' sera and a serum positive for IgA RF were chromatographed on a Sepharose column coupled with Protein A at dilutions of 1:10, 1:20 and 1:40. The loaded and the effluent fractions were tested for IgA ANCA by ELISA as described.

Criteria for IgA ANCA positivity

Positivity was defined by the association of all the following criteria: (i) positive IgA ELISA and antigen-specific dose-dependent fluid-phase inhibition; (ii) positive IgA IIF; and (iii) absence of IgA RF activity.

Western blot for IgA ANCA

Neutrophils were isolated on a Hypaque gradient [4], resuspended in PBS containing 0·5 mM $MgCl_2$, 0·9 mM $CaCl_2$ and protease inhibitor (phenylmethylsulfonylfluoride 1 mM) and incubated at 37°C for 30 min with 1 mg/ml phorbol myristate acetate (PMA) to induce the release of secondary granules [23]. After centrifugation the supernatant was removed and stored at −80°C until use in Western blot, and the cells washed three times with PBS. The cells were then incubated in PBS with 1% Triton X100, phenylmethylsulfonylfluoride 1 mM and EDTA 2 mM at 4°C for 40 min. Following centrifugation at 500 *g* for 5 min at 4°C, the supernatant was frozen at −80°C until use in Western blot. SDS–PAGE was performed with: (i) the stored supernatant after PMA stimulation; and (ii) the neutrophil extract in reducing or non-reducing buffer. Western blot was performed according to the method described by Laemmli [24]. IgA ANCA-positive and control sera were diluted 1:20 in Tris-buffered saline containing 1% Tween 20 and 2% dried skimmed milk (TBSTM) and incubated with the nitrocellulose strips for 3 h at room temperature. The strips were then washed with TBSTM and incubated for 1 h with alkaline phosphatase-conjugated anti-human IgA (Sigma, A9669), diluted 1:1000 in TBSTM. Binding was detected by adding alkaline phosphatase substrate.

In order to study the antigen localization in the neutrophils, cellular fractions were prepared from a total extract, i.e. without PMA stimulation. Neutrophils were isolated on a Hypaque gradient, washed twice in PBS and incubated in PBS containing

Table 1. Occurrence of IgA and IgG ANCA in Henoch–Schönlein purpura (HSP), IgA nephropathy (IgAN), IgG ANCA-positive vasculitis (SV) patients and controls

	HSP (%)	IgAN (%)	SV (%)	Controls (%)
IgA ANCA	11/14 (79)	1/30 (3)	1/40 (2·5)	0/60 (0)
IgG ANCA	3/14 (21)	0/30 (0)	40/40 (100)	0/60 (0)

1% Triton X100, phenylmethylsulfonylfluoride 1 mM and EDTA 2 mM at 4°C for 40 min. Cellular extract was then centrifuged at 10 000 *g* for 15 min at 4°C. The pellet was passed several times through a 22 G needle and stored until use. The supernatant, containing solubilized membranes and cytosolic material, was divided into two aliquots. One aliquot was stored until use and the other was centrifuged at 105 000 *g* for 40 min at 4°C. Both the pellet and the supernatant obtained with this last centrifugation were saved for use, the pellet being resuspended in a volume equivalent to the supernatant. SDS–PAGE was performed in reducing conditions with: (i) pellet and supernatant after centrifugation at 10 000 *g* (containing respectively nuclei + coarse fragments of membrane and solubilized membranes + cytosolic material); (ii) pellet and supernatant after centrifugation at 105 000 *g* (representing respectively solubilized membrane-enriched material and soluble cytoplasm). After Western blot, IgA reactivity against each fraction was tested using an IgA ANCA-positive serum (which had previously been shown to react against the 51-kD protein) and a control serum.

In order to investigate whether IgA–fibronectin interactions could be responsible for the 51-kD band of reactivity observed in the Western blot of HSP IgA, Western blot experiments with a total neutrophil extract and the membrane-rich fraction were also performed to test the reactivity of three commercially available anti-fibronectin antibodies (rabbit polyclonal anti-human fibronectin, mouse monoclonal anti-human fibronectin and mouse monoclonal anti-cellular fibronectin; Sigma, St. Louis, MO). Two IgA ANCA-positive HSP sera were used as positive controls. Revealing antibodies were alkaline phosphatase-conjugated anti-rabbit IgG, anti-mouse IgG, anti-mouse IgM and anti-human IgA.

RESULTS

IgA ANCA were detected in 11/14 (79%) of HSP patients, in 1/30 (3%) of IgAN, and in 1/40 (2·5%) systemic vasculitis (SV) patients. None of the sera from the healthy controls was found to have IgA ANCA activity (Table 1). IgG ANCA was found in association with IgA ANCA in three of the HSP patients, but not in any of the patients with IgAN, nor in healthy controls.

ANCA SPIA with inhibition study

The result of the IgA ANCA SPIA is shown in Fig. 1. High total IgA levels are a common finding in HSP and IgAN patients; we therefore used for each sample a different serum dilution (from 1:10 to 1:33) to obtain 20 mg/dl of total IgA to exclude the possibility of non-specific binding; this theoretical possibility was also excluded by testing each serum on both antigen-free wells blocked with BSA and antigen-free wells blocked with gelatin on the same microtitre plate. None of the positive sera

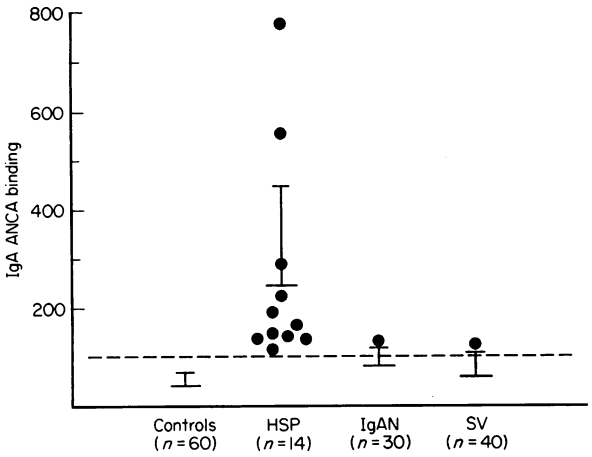

Fig. 1. IgA ANCA ELISA results relative to patients with Henoch–Schönlein purpura (HSP), IgA nephropathy (IgAN), IgG ANCA-positive systemic vasculitis (SV), and controls. On the *y*-axis is the optical density (OD). Bars show mean ± s.d. for each population. The dotted line represents the upper normal value (mean + 2 s.d. of values from controls). Individual values within the normal range are not plotted.

Table 2. Maximal per cent inhibition of IgA ANCA activity in ELISA, obtained by co-incubation of IgA ANCA-positive sera with neutrophil extract and with haemoglobin

	Neutrophil extract	Haemoglobin
HSP		
1	89	5
2	52	0
3	40	0
4	24	2
5	30	0
6	36	0
7	26	0
8	61	0
9	62	2
10	21	0
11	33	3
IgAN*	49	8
SV*	45	0

* One serum out of 30 IgA nephropathy (IgAN) and one out of 40 systemic vasculitis (SV) sera with IgA ANCA positivity. See text. HSP, Henoch–Schönlein purpura.

Sera were diluted 1:40 and competitor protein concentration was 2 mg/ml. The inhibition was dose-dependent in all cases.

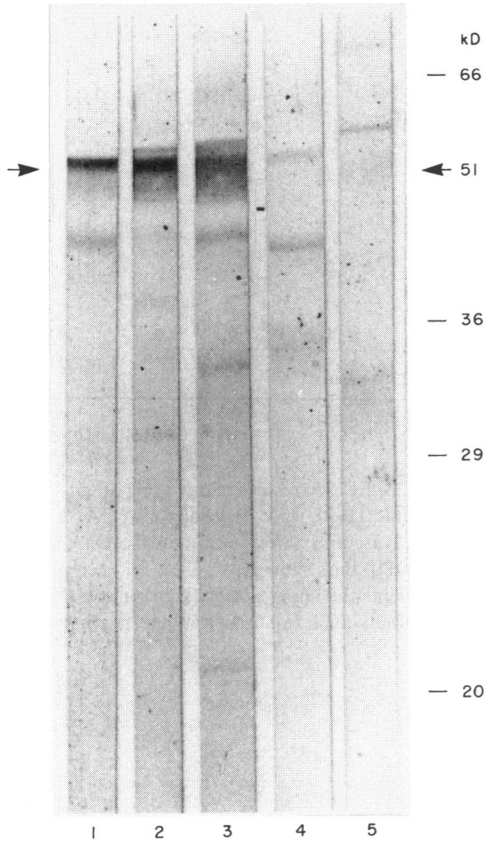

Fig. 2. IgA ANCA Western blot obtained with Triton X100 neutrophil extract after phorbol myristate acetate (PMA) stimulation, run in SDS–PAGE under reducing conditions. Lanes 1–3 were blotted with three IgA ANCA Henoch–Schönlein purpura (HSP) sera, and show IgA reactivity with a 51-kD protein. Lanes 4 and 5 were blotted with normal sera. The other 12 normal and five IgA nephropathy control sera produced no band (same appearance as in lane 5). The faint band of lane 4 is discussed in the text.

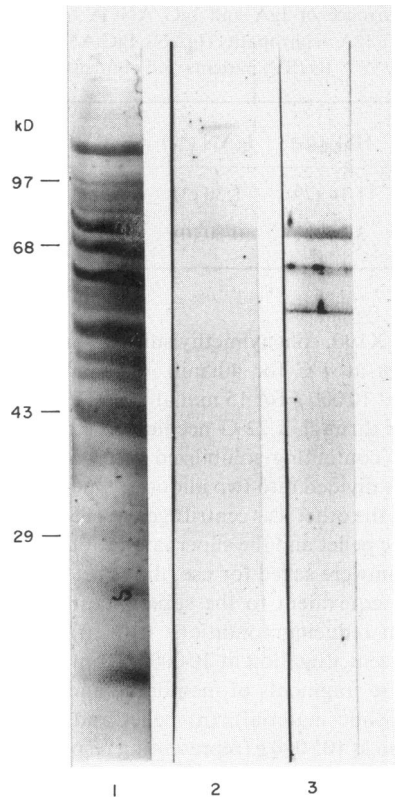

Fig. 3. IgA ANCA Western blot obtained with fractions of the neutrophil extract. The neutrophil fraction containing solubilized membranes and cytosolic material, obtained as supernatant after a centrifugation at 10000 *g*, was found to contain the 51-kD protein. Lane 1 is the coomassie blue-stained nitrocellulose after the protein transfer. Lane 2 was blotted with a normal serum. Lane 3 was blotted with an IgA ANCA-positive Henoch–Schönlein purpura (HSP) serum. IgA in this serum reacted with the 51-kD protein and also with a 57-kD protein. The band at 68 kD in lanes 2 and 3 is non-specific and is due to direct binding of the alkaline phosphatase-conjugated anti-human IgA.

showed significant IgA binding to the antigen-free wells. IgA ANCA-positive HSP sera retained positivity with serum dilutions ranging from 1:40 to 1:80. In all but one IgAN patients IgA ANCA were not detectable, regardless of the disease phase.

All IgA ANCA-positive sera were also positive in the IgA1 ANCA SPIA; of these three HSP, one IgAN and one SV sera also showed raised levels of IgA2 ANCA.

The inhibition of IgA ANCA activity obtained for each positive patient by preincubation of serum with neutrophil extract and with haemoglobin is shown in Table 2.

Indirect immunofluorescence

Positive IgA IIF was obtained with all the positive sera described above, and produced a diffuse cytoplasmic staining, which did not have the granular appearance typically observed with IgG ANCA IIF. Serum from one HSP patient produced both cytoplasmic and perinuclear (PN) staining; this serum was also IgG ANCA-positive and produced a PN pattern in IgG IIF. Cytoplasmic staining was obtained by testing the two other IgG ANCA-positive HSP sera in IgG-specific IIF.

IgA RF ELISA

None of the HSP and IgAN sera showed IgA RF or IgG anti-IgA RF activity. IgA RF was found in one SV patient, positive for both IgA and IgG ANCA; this patient was not considered as being positive for IgA ANCA.

To further exclude IgA RF interference in IgA ANCA measurements, sera from two IgA ANCA-positive HSP patients and from the IgA RF-positive patient used as control were diluted 1:10, 1:20 and 1:40 and chromatographed on a Sepharose column coupled to Protein A, which binds IgG, does not bind IgA1, and binds very weakly IgA2. IgA ANCA activity in the loaded and in the IgG-depleted material was then assessed by ELISA. In the case of the positive IgA RF serum, a reduction in IgA ANCA activity of 29%, 55% and 85%, compared with the loaded material, was obtained by testing the effluent respectively at 1:10, 1:20 and 1:40. No difference was observed in IgA ANCA activity between the IgG-depleted and the loaded HSP sera at any of the dilutions.

Antigen-specific SPIA

Two patients were found to have IgA and IgG anti-MPO

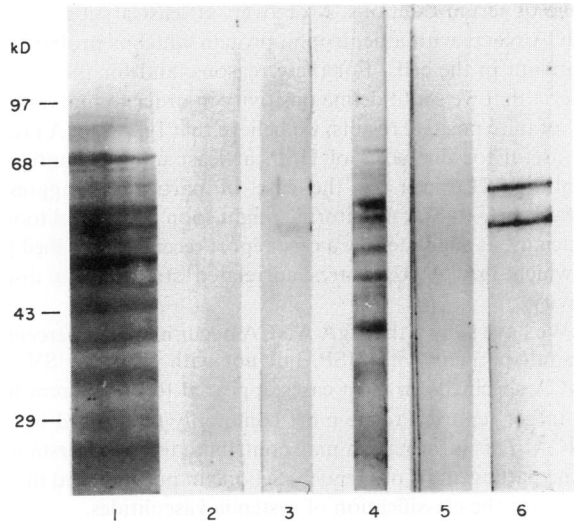

Fig. 4. IgA ANCA Western blot obtained with fractions of the neutrophil extract. The neutrophil fraction used in the experiment shown in Fig. 3 was further fractionated by centrifugation at 105 000 ***g*** and the same Henoch–Schönlein purpura (HSP) and control sera as in Fig. 3 were used for the immunoblot. Results obtained with the supernatant, that is soluble cytoplasmic material, are shown in lanes 1, 2 and 3; results relative to the pellet, that is the membrane-rich fraction, are shown in lanes 4, 5 and 6. Lanes 1 and 4 show the coomassie blue-stained nitrocellulose after protein transfer: the membrane-rich fraction (lane 4) is relatively depleted in protein content. Lanes 2 and 5 were incubated with the healthy control serum and lanes 3 and 6 with the HSP patient serum. The almost complete lack of reactivity of patient's IgA against the soluble cytoplasmic fraction of the neutrophil extract and the conserved reactivity against the membrane-rich fraction, relatively depleted in protein content, indicate that the followed procedure provides a partially purified antigen preparation and that the 51-kD protein recognized by the four strongest IgA ANCA-positive HSP sera is probably membrane-associated.

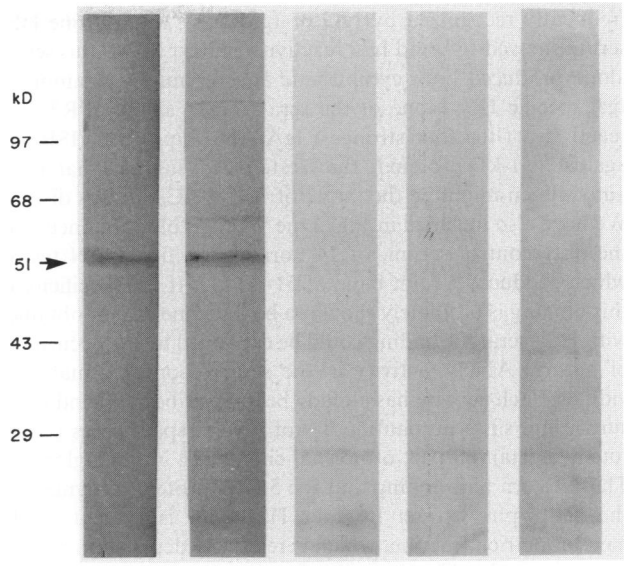

Fig. 5. Western blot on total neutrophil extract to test reactivity of anti-fibronectin antibodies. Lanes 1 and 2 were blotted with IgA ANCA-positive Henoch–Schönlein purpura (HSP) sera as positive controls. Lanes 3, 4 and 5 were blotted respectively with rabbit polyclonal anti-human fibronectin, mouse monoclonal anti-human fibronectin and mouse monoclonal anti-cellular fibronectin. None of the anti-fibronectin antibodies recognized the 51-kD protein which is recognized by HSP IgA. The three antibodies reacted with a 25-kD protein in the neutrophil extract and two of them with a 40-kD protein. IgA from one of the patients produced a faint band of reactivity at 25 kD. Anti-fibronectin antibodies reacted with the same molecular species in experiments (not shown) performed using the membrane-rich fraction of neutrophil extract, but the reactivity was much weaker. In this case HSP IgA which had weakly reacted with the 25-kD protein did not produce any detectable band at that molecular weight, whilst both HSP IgA strongly reacted with the 51-kD protein.

antibodies (one HSP and one SV). Neither IgG nor IgA anti-PR3 activity was detected in the sera with IgA ANCA positivity.

Western blot for IgA ANCA

The three strongest IgA ANCA-positive HSP sera reacted with a 51-kD protein in the Triton X100 neutrophil extract after PMA stimulation, under reducing conditions (Fig. 2). The same reaction was observed at a lesser degree of intensity by testing the sera on the supernatant obtained after PMA stimulation. Another IgA ANCA-positive HSP serum reacted against the 51-kD and against another protein of 57 kD (Fig. 3). A very faint band at 51–52 kD was produced by one normal serum. None of the other 13 normal and five IgAN control sera showed any reactivity. Western blot performed with the various cellular fractions showed that the membrane-rich fraction, that is the pellet obtained after centrifugation at 105 000 ***g***, is strongly enriched in the 51-kD protein recognized by IgA ANCA-positive HSP sera (Fig. 4). The three different anti-fibronectin antibodies tested by Western blot on a total neutrophil extract and on the membrane-rich fraction did not show any reactivity at 51 kD (Fig. 5). All three produced a band of reactivity at 25 kD and two of them a second band at 40 kD. IgA from one of the HSP sera tested produced a very faint band at 25 kD in Western blot performed with total neutrophil extract (Fig. 5).

DISCUSSION

The high prevalence of IgA ANCA in HSP (79%) but not in IgAN (3%) nor in other systemic vasculitides (2·5%) suggests that IgA ANCA are possible serologic markers for HSP. However, as the HSP population in this study did not include any child below 17 years of age [25], it is still to be determined whether juvenile HSP also shows such a strong association with IgA ANCA.

The question of whether or not HSP and IgAN are one disease has always been difficult to answer: the finding of a laboratory as well as a clinical difference suggests that these are distinct entities. However, in both categories of our patients the renal biopsy findings were indistinguishable. The presence of IgA ANCA in one of our patients with IgAN may reflect the difficulty of detecting extrarenal disease in HSP; for example, there are patients who have only transient scanty purpura [9]. Alternatively, it is possible that in a few cases the renal manifestations of HSP might precede the wider expression of disease in a fashion analogous to the occasional late evolution of 'idiopathic' rapidly progressive glomerulonephritis (RPGN) towards a multisystem vasculitis [26], both the latter also being diseases associated with ANCA [5].

The autoantigen recognized by IgA ANCA in HSP appears in most cases to be a different molecule from the targets most

frequently recognized by IgG or IgM ANCA: only one HSP serum showed IgA and IgG reactivity against MPO; this serum alone produced both cytoplasmic and perinuclear staining in IgA-specific IIF. None of the sera reacted against PR3. The reactivity of the four strongest IgA ANCA-positive HSP sera against a 51-kD protein in the Western blot suggests that a new autoantigen might be the target of IgA ANCA in this disease. We have also included in Fig. 2 the Western blot obtained with the only control serum, of 14 normal and five IgAN tested, which produced a faint band at 51–52 kD. If the specificity of this binding is ultimately shown to be the same as that obtained with HSP sera, the finding could be explained by the occurrence of natural ANCA activity in the sera of some normal individuals. Such activity has already been described [27] and is not surprising, since autoantibodies of several specificities constitute a substantial part of normal circulating immunoglobulin. The Western blot finding that the 51-kD protein recognized in the neutrophil extract by four HSP sera is present in the membrane-rich fraction, which is relatively depleted in protein content, and is almost completely absent from the cytoplasmic soluble compartment, indicates that the IgA ANCA target is probably a membrane-associated protein. Characterization of this antigen by amino acid sequence analysis is currently the objective of a separate study.

One of the most controversial issues about IgA ANCA in IgA-related renal diseases is the possibility of obtaining false positive results in ELISA or immunofluorescence tests, due to the increased levels of circulating IgA, or to physicochemical changes of circulating IgA in these patients, which could increase the ability of IgA to bind to several molecules through 'non specific' interactions [28]. In particular it has been pointed out that, because fibronectin is found in small amounts in neutrophil extracts [21,29] and because IgA–fibronectin complexes are increased in IgAN [30], IgA–fibronectin complexes could account, at least partly, for the IgA ANCA reactivity detected in IgAN and HSP [21]. We have performed Western blot experiments to test whether IgA reactivity towards fibronectin could be responsible for the IgA ANCA activity that we detected in HSP sera. Three different anti-fibronectin antibodies did not react with the 51-kD protein in the neutrophil extract that appears to be the major target for HSP IgA. All anti-fibronectin antibodies reacted with a 25-kD protein and two of them with a 40-kD protein, supporting previous studies indicating that fibronectin fragments could be present in the neutrophil extract. We also found that one of the HSP sera tested produced a weak band of reactivity at 25 kD. Taken together, these findings support the view that some antibody species within the IgA circulating pool in HSP patients may be able to interact with neutrophil-associated fibronectin [21], but also confirm that within such pool other antibodies specifically associated with HSP are able to react with a neutrophil antigen, and deserve to be further studied. A matter of debate could now be whether all these antibodies should be called IgA ANCA. Until we have more information about fibronectin-reactive antibodies, at present we call IgA ANCA the antibody activity which we detected specifically in HSP patients and which appears to be directed against a 51-kD protein.

IgA ANCA are probably autoantibodies with a relatively low affinity for the antigen or/and present in small amounts in the circulation, as suggested by the fact that they can be detected in ELISA and in immunofluorescence within a relatively narrow range of serum dilutions. Moreover, at least a subset of IgA ANCA reacts with a neutrophil protein which is probably not abundant in the cells. For these reasons, and for the multiple criteria that we set to define positivity in order to minimize the risk of false positive results, we believe that IgA ANCA are not yet useful for diagnosis of HSP, at least until the antigen is identified. However, in the case of particularly aggressive disease, IgA ANCA monitoring might soon be a useful tool for clinicians, as suggested by a case report recently published [31], in which IgA ANCA titres correlated strictly with disease activity.

We have shown that IgA ANCA occur in a high percentage of adult patients with HSP, but not with IgAN or SV. IgA ANCA specificity in most cases appeared to be different from the target reported to be most commonly recognized by IgG ANCA. This information may contribute to an understanding of the pathogenesis of Henoch–Schönlein purpura and may be useful for the classification of systemic vasculitides.

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REFERENCES

1 Fauci AS, Haynes BF, Katz P. The spectrum of vasculitis. Ann Int Med 1978; **89**:660–78.
2 Lie JT, Members and Consultants of the American College of Rheumatology. Illustrated histopathologic classification criteria for selected vasculitis syndromes. Arthritis Rheum 1990; **33**:1074–87.
3 Van der Woude FJ, Rasmussen N, Lobatto S *et al.* Autoantibodies against neutrophils and monocytes; tool for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet 1985; **i**:425–9.
4 Savage COS, Winearls CG, Jones S *et al.* Prospective study of radioimmunoassay for antibodies against neutrophil cytoplasm in diagnosis of systemic vasculitis. Lancet 1987; **i**:1389–93.
5 Falk RJ, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. N Engl J Med 1988; **318**:1651–7.
6 Ewert BH, Jennette JC, Falk RJ. Anti-myeloperoxidase antibodies stimulate neutrophils to damage human endothelial cells. Kidney Int 1992; **41**:375–83.
7 Jayne DRW, Jones SJ, Severn A *et al.* Severe pulmonary hemorrhage and systemic vasculitis in association with circulating anti-neutrophil cytoplasm antibodies of IgM class only. Clin Nephrol 1989; **32**:101–6.
8 Esnault VLM, Soleimani B, Keogan MT *et al.* Association of IgM with IgG ANCA in patients presenting with pulmonary hemorrhage. Kidney Int 1992; **41**:1304–10.
9 Cream JJ, Gumpel JM, Peachy RDG. Schönlein–Henoch purpura in adults: a study of 77 adults with anaphylactoid or Schönlein–Henoch purpura. Q J Med 1970; **39**:461–84.
10 Levinsky RJ, Barratt TM. IgA immune complexes in Henoch–Schönlein purpura. Lancet 1979; **ii**:1100–3.
11 Trygstad CW, Stiehn ER. Elevated serum IgA globulin in anaphylactoid purpura. Paediatrics 1971; **47**:1023–8.
12 Coppo R, Basolo B, Martina G *et al.* Circulating immune complexes containing IgA, IgG and IgM in patients with primary IgA nephropathy and with Henoch–Schönlein nephritis. Correlation with clinical and histologic signs of activity. J Clin Nephrol 1982; **18**:230–9.
13 Kauffmann RH, Herrmann WA, Meyer CJLM *et al.* Circulating IgA immunocomplexes in Henoch–Schönlein purpura. Am J Med 1980; **69**:859–65.

14 Giangiacomo J, Tsai CC. Dermal and glomerular deposition of IgA in anaphylactoid purpura. Am J Dis Child 1977; **131**:981–3.
15 Baart de la Faille-Kuyper EH, Kater L, Kuijten RH *et al.* Occurrence of vascular IgA deposits in clinically normal skin of patients with renal disease. Kidney Int 1976; **9**:424–9.
16 Sinniah R, Fend PH, Chen BTM *et al.* A clinical and morphological study of renal biopsies. Clin Nephrol 1978; **9**:219–28.
17 Nakomoto Y, Asamo Y, Dohi K *et al.* Primary IgA glomerulonephritis and Schönlein–Henoch purpura nephritis: clinicopathological and immunohistological characteristics. Q J Med 1978; **47**:495–516.
18 Whitworth JA, Leibowits S, Kennedy MC *et al.* IgA and glomerular disease. Clin Nephrol 1976; **5**:33–36.
19 Van der Wall-Bake AWL, Lobatto S, Joges L *et al.* IgA antibodies against cytoplasmic antigens of polymorphonuclear leukocytes in patients with Henoch–Schönlein purpura. Adv Exp Med Biol 1987; **216**:1593–8.
20 O'Donoghue DJ, Nusbaum P, Noel LH *et al.* Antineutrophil cytoplasmic antibodies in IgA nephropathy and Henoch–Schönlein purpura. Nephrol Dial Transplant 1992; **7**:534–8.
21 Tadros M, Radice A, Pozzi C *et al.* IgA-ANCA in patients with Henoch-Schönlein purpura (HSP). JASN 1992; **3**:666.
22 Esnaut VLM, Jayne DRW, Weetman AP *et al.* IgG subclass distribution and relative functional affinity of antimyeloperoxidase antibodies in systemic vaculitis at presentation and during follow-up. Immunology 1991; **74**:714–8.
23 Wright DG, Bralove DA, Gallinn JI. The differential mobilization of human neutrophil granules: effects of phorbol myristate acetate and ionophore A23187. Am J Pathol 1977; **87**:273–83.
24 Laemnli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; **227**:680–5.
25 Mills JA, Michel BA, Bloch DA *et al.* The American College of Rheumatology 1990 criteria for the classification of Henoch–Schönlein purpura. Arthritis Rheum 1990; **33**:1114–21.
26 Woodworth TG, Abvelo JG, Austin HA *et al.* Severe glomerulonephritis with late emergence of classic Wegener's granulomatosis. Medicine 1987; **66**:181–91.
27 Harrison DJ, Kharabanda R. Autoantibodies to neutrophil cytoplasm antigens in systemic vasculitis have the same target specificity. J Pathol 1989; **158**:233–238.
28 Coppo R, Amore A, Gianoglio B *et al.* IgA-ANCA in children with Henoch-Schönlein (HSP) and primary IgA nephropathy (pIgAN). JASN 1992; **3**:652.
29 Weissmann G, Pearlstein E, Perez HD *et al.* Neutrophils synthesize and deposit fibronectin on surfaces to which they attach. Trans Assoc Am Phys 1986; **93**:72–84.
30 Cederholm B, Wieslander J, Bygren P *et al.* Circulating complexes containing IgA and fibronectin in patients with primary IgA nephropathy. Proc Natl Acad Sci USA 1988; **85**:4865–8.
31 Shaw G, Ronda N, Bevan JS *et al.* Antineutrophil cytoplasmic antibodies (ANCA) of IgA class correlate with disease activity in adult Henoch–Schönlein purpura. Nephrol Dialysis Transpl 1992; **7**:1238–41.