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ORIGINAL ARTICLE

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The significance of IgA class of antineutrophil cytoplasmic antibodies (ANCA) in childhood Henoch–Schönlein purpura

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Abstract Antineutrophil cytoplasmic antibodies (ANCA) have been identified in a wide variety of vasculitic disorders, but it is controversial whether ANCA are present in the sera of patients with HSP. This prospective study was designed to assess the place of ANCA, particularly their IgA subclass, in HSP. Thirty-five patients (18 boys, 17 girls) aged 9.4 ± 4 (3–16) years with a clinical diagnosis of HSP based on American College of Rheumatology (ACR) criteria were enrolled. Thirteen patients (6 boys, 7 girls) aged 8.3 ± 5.5 (2–21) years with other vasculitides, consisting of classic polyarteritis nodosa (PAN) ($n = 2$); cutaneous polyarteritis nodosa ($n = 1$); acute infantile hemorrhagic edema ($n = 2$); acute urticarial vasculitis ($n = 2$); hypocomplementemic vasculitis ($n = 1$); and unclassified vasculitis ($n = 5$) served as disease controls and 10 healthy children served as normal controls. Twenty-five HSP patients and 7 disease controls were re-evaluated in the resolution phase that was described as 4–6 weeks after all symptoms subsided and all medications were stopped. Blood samples for ANCA and IgA rheumatoid factor (RF) were studied by indirect immunofluorescence (IIF) and ELISA, respectively. IgG ANCA was significantly lower in percentage in HSP patients (2.8%) than in disease controls (40%) ($p = 0.002$). In contrast, IgA ANCA in cytoplasmic pattern was detected in a significantly higher percentage of HSP patients (82.3%) in the acute phase compared to those in the disease controls (38%) ($p = 0.004$). In the resolution phase, IgA ANCA was negative in 88% of the patients ($p = 0.001$ for acute vs resolution phases). Neither IgG nor IgA ANCA were seen in normal controls. No relationship was found between disease severity of HSP and IgA ANCA. Positive IgA rheumatoid factor was present in only two patients with HSP. In conclusion, our results suggest that IgA ANCA may be useful to confirm the diagnosis of HSP in children.

Keywords Childhood · Henoch–Schönlein purpura · IgA antineutrophil cytoplasmic antibodies

Abbreviations ANCA: Antineutrophil cytoplasmic antibodies · EBV: Epstein–Barr virus · HSP: Henoch–Schönlein purpura · IIF: Indirect immunofluorescence · PAN: Polyarteritis nodosa

Introduction

Henoch–Schönlein purpura (HSP), which is associated with IgA abnormalities, is one of the most common vasculitides of childhood and mainly affects the vessels of the skin, gastrointestinal tract, and kidneys [1]. Sometimes, differentiating it clinically from other vasculitides is difficult, especially in severe cases.

Antineutrophil cytoplasmic antibodies (ANCA) have been identified in a wide variety of vasculitic disorders [2, 3, 4, 5]. ANCA have been demonstrated to be important, especially for the diagnosis and follow-up of microscopic polyangiitis and Wegener's granulomatosis [5,6]. It is controversial whether ANCA are present in the sera of patients with HSP. Whereas some authors have not been able to demonstrate either IgG or IgA classes of ANCA, others have shown IgA class of ANCA in HSP patients [7, 8, 9, 10, 11, 12, 13, 14, 15]. This prospective study was designed to assess the place of ANCA, particularly its IgA subclass, in HSP.

Patients and methods

Between November 2001 and July 2002, 35 patients (18 boys, 17 girls) aged 9.4 ± 4 years (3–16) with a clinical diagnosis of HSP were enrolled at Hacettepe University

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Medical School Pediatric Nephrology and Rheumatology Unit. All the patients fulfilled the criteria for the diagnosis of HSP established by the American College of Rheumatology (ACR) [1].

Thirteen patients (6 boys, 7 girls) aged 8.3 ± 5.5 (2–21) years with other vasculitides consisting of classic polyarteritis nodosa (PAN) ($n = 2$); cutaneous polyarteritis nodosa ($n = 1$); acute infantile hemorrhagic edema ($n = 2$); acute urticarial vasculitis ($n = 2$); hypocomplementemic vasculitis ($n = 1$); and unclassified vasculitis ($n = 5$) served as disease controls. Of these, two had hepatitis B-related vasculitis and one had Epstein–Barr virus (EBV) infection. One PAN patient was diagnosed with microaneurysms on renal angiography, and in the others the diagnosis was based on skin biopsy if indicated. Ten healthy children served as normal controls.

Twenty-five HSP patients and 7 disease controls could be re-evaluated in the resolution phase that was described as 4–6 weeks after all symptoms subsided and all medications were stopped. Two HSP patients with renal involvement had renal biopsies because of proteinuria exceeding 1 g/day on follow-up.

Biochemical and microbiologic investigation

In the acute phase, throat culture, automated complete blood counts, urinanalysis, erythrocyte sedimentation rate (ESR), antistreptolysin O (ASO) and antinuclear antibody tests were performed by standard methods in patients with HSP and in the disease controls. Serum immunoglobulin A (Turbiquant IgA, DADE-Behring, Germany) and C-reactive protein (CRP) (Turbiquant CRP, DADE-Behring, Germany) levels were measured by the turbidimeter method. Blood samples for IgG and IgA subclasses of ANCA as well as IgA rheumatoid factor were collected in the active phase of the disease before treatment and in the resolution phase of both patients and disease controls, and kept frozen at −20 °C until assayed.

Indirect immunofluorescence

Sera (1:20 dilution) for detection of ANCA were studied on ethanol-fixed human neutrophils, modified for the detection of IgA antibodies by using an anti IgA fluorescein-conjugated antibody by the indirect immunofluorescence (IIF) method (The Binding Site Ltd, UK). The immunofluorescence labeling pattern was designated as cytoplasmic or perinuclear. The results were expressed as negative for weak or lack of fluorescence, and positive for moderate or intense fluorescence.

IgG ANCA were detected by indirect immunofluorescence as previously described (The Binding Site Ltd, UK). IgA and IgG ANCA were tested in all consecutive patients.

IgA 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 instruction (IMTEC Immunodiagnostika GmbH, Germany). The standard curve of IgA RF activity was obtained using IgA RF-positive serum provided by the manufacturer. The serum had been previously calibrated against the International Standard of IgA RF in order to detect from 1.25 to 20 IgA RF U/ml. Results above 2.5 U/ml (cut-off value) were considered positive.

Statistical analysis

Results were given as mean ± SD and percentage. For statistical analysis, Wilcoxon, Mann–Whitney U and χ^2tests were used to compare data. P values less than 0.05 were considered significant. Sensitivity and specificity as well as positive and negative likelihood ratios were calculated for the diagnostic performance of the test of IgA ANCA.

Results

Age and sex distribution were comparable between study groups ($p > 0.05$). Typical purpuric lesions were present in all HSP patients. A history of upper respiratory tract infection preceding HSP was present in most of the patients (90%). Arthritis in 23 (65%), gastrointestinal symptoms in 13 (37%), hematuria and/or proteinuria in 8 (22%) and acute renal failure in 1 (0.02%) were noted. At least one relapse was seen in 2 patients within 3 weeks. Sixteen patients (45%) were treated with oral prednisone (1 mg/kg/day) because of gastrointestinal symptoms for approximately 10 days. In the disease control group 5 patients received oral steroid with the same dose and duration. β-Hemolytic streptococcus was grown in throat culture in 10 HSP patients (28%) and (7.6%) in 1 disease control patient, and was treated with penicillin. ASO titer was high in 16 (45%) HSP patients and in 3 (15%) disease controls. All acute-phase reactants as well as serum IgA levels were significantly higher in the acute phase of HSP patients than those in the resolution phase, in contrast to the disease controls (Table 1). None of the patients or the disease controls had antinuclear antibodies.

IgG ANCA were significantly lower in percentage in HSP patients (2.8%) than in the disease controls (40%) ($p = 0.002$). The diagnosis of patients with IgG ANCA was PAN in 2, HSP in 1, unclassified vasculitis in 2, acute infantile hemorrhagic edema in 1. In contrast, IgA ANCA in cytoplasmic pattern were significantly higher in percentage in HSP patients (82.3%) in the acute phase than in the disease controls (38%) ($p = 0.004$) (Table 2) (Fig. 1). In the resolution phase, IgA ANCA were negative in 22 (88%) HSP patients; only 3 patients (12%) showed weak positivity ($p = 0.001$ for acute vs resolu-

Table 1 Laboratory values of patients with HSP in the acute and resolution phases

	Acute (mean ± SD)	Resolution (mean ± SD)	Disease control (mean ± SD)
WBC (mm^3)	11750 ± 6577*	7404 ± 1472	9973 ± 4324**
ESR (mm/h)	32.5 ± 20.9*	16.7 ± 6.5	34.7 ± 20.5**
CRP (mg/dl)	1.8 ± 2.2*	0.3 ± 0.1	1.8 ± 2.6**
IgA (mg/dl)	265 ± 117*	171 ± 40	346 ± 218**

*$p < 0.05$ acute vs resolution
**$p > 0.05$ acute vs disease control

Table 2 Serum IgG and IgA classes of ANCA in the acute and resolution phases of patients and disease controls

ANCA	Patients		Disease controls	
	Acute	Resolution	Acute	Resolution
IgG ANCA	1 (2.8%)*	–	6 (40%)	–
IgA ANCA	30 (82.3%)**	3 (12%)***	5 (38%)	1 (10%)

*p: 0.002 patients with HSP vs disease controls in the acute phase
**p: 0.004 patients with HSP vs disease controls in the acute phase
***p: 0.001 acute vs resolution phase in patients with HSP

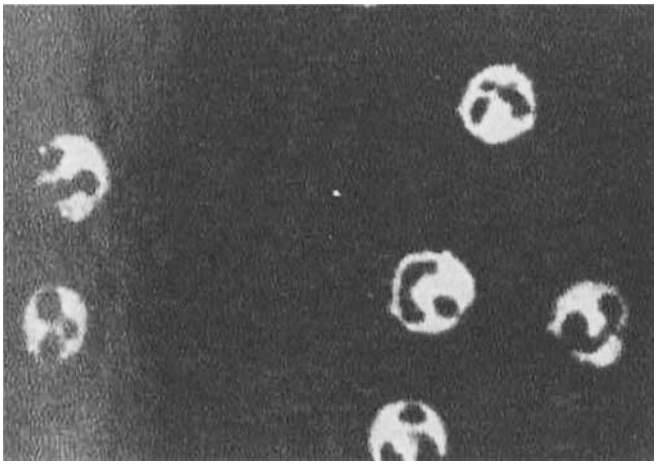

Fig. 1 Strong cytoplasmic staining pattern for IgA ANCA in children with HSP

tion phases) (Table 2). The sensitivity and specificity of IgA ANCA were 85.7% and 54%, respectively. The positive and negative likelihood ratios were 1.86 and 0.26, respectively. No relationship between disease severity of HSP and IgA ANCA was found. Positive IgA rheumatoid factor was present in only 2 patients with HSP. Neither IgG and IgA ANCA nor IgA RF was found in normal controls.

Discussion

The aim of this study was to assess the presence of IgA ANCA by IIF in HSP patients. However, the target antigen of ANCA was not sought for in this study. We found a marked IIF staining in a considerable number of patients (all but 3) with HSP. Furthermore, this positive IIF finding disappeared during remission. It was found in only 38% of the patients with other vasculitides. Furthermore, it was not found in any of the healthy controls. IgA ANCA has been reported in variable percentages of patients with HSP [7, 8, 9, 10, 11, 12, 13, 14, 15]. However, the specificity and sensitivity of IgA ANCA in the adult and childhood HSP population have not previously been referred to. In the present study, IgA ANCA positivity in children with HSP was detected in 82% in the acute phase, which introduced a high sensitivity; this suggests that it is a good marker for confirming the diagnosis. However, the specificity and the positive likelihood ratio were low. Therefore, in future it may need to be used in conjunction with a test yielding higher specificity, yet to be determined.

The presence of IgA ANCA in the sera of patients with Henoch–Schönlein purpura (HSP) is controversial. Whereas IgG ANCA was generally found to be negative in HSP [8], conflicting data have been reported on the presence of IgA ANCA [7, 8, 9, 10, 11, 12, 13, 14, 15]. This discrepancy in results may be due to low patient numbers in some series, possible differences in the inciting etiologic factors, and maybe technical differences.

Coppo et al. [14] suggested that IgA ANCA in HSP might be due to some atypical characteristics of circulating IgA, rather than to antigen–antibody recognition. Ronda et al. [8] speculated that the autoantigen recognized by IgA ANCA in HSP might be a different molecule from the targets most frequently recognized by IgG ANCA. They also showed in their 4 HSP patients with the strongest IgA ANCA positivity that a new autoantigen weighing 51 kDa might be the target of IgA ANCA [8].

One of our HSP patients (2.8%) exhibited both IgG and IgA ANCA in the acute phase. The coexistence of both classes of ANCA could be simply an epiphenomenon, secondary to increased IgA and IgG production. None of our patients with polyarteritis nodosa exhibited IgA ANCA in contrast to IgG ANCA.

Our study can not address some concerns. One of the most debated issues about IgA ANCA is the possibility of obtaining false positive results in IIF tests owing to elevated circulating IgA levels or to physicochemical changes of circulating IgA, which could increase the ability of IgA to bind several molecules through nonspecific interactions [14]. In particular, it has been pointed out that because fibronectin is found in small amounts in neutrophil extracts, IgA–fibronectin complexes could at least in part account for the IgA ANCA reactivity [13]. However, Ronda et al. [8] tested the IgA ANCA target antigen with antifibronectin antibodies and they did not interact. IgA RF might explain the false positive results as well. In our study, the detection of IgA RF in only 2 patients' serum led us to think that at least IgA RF was not a reason for IgA ANCA positivity.

Another concern is whether IgA ANCA have a putative pathogenic role. Evidence supporting a pathogenic role for IgG ANCA in other vasculitides has been derived from clinical observations and in vitro experiments [2, 3, 4, 5]. Although the diagnostic value of IgG ANCA has been well established for patients with Wegener's granulomatosis, microscopic polyangiitis and idiopathic rapidly progressive glomerulonephritis, the diagnostic importance of IgA ANCA for HSP patients remains unclear. In the present study 30 of 35 HSP patients were positive for IgA ANCA. In contrast, only 5 of 13 patients with other vasculitides were positive for IgA ANCA, suggesting that the search for IgA ANCA may be a useful tool for differential diagnosis of HSP.

IgA plays a critical role in the development of HSP [16]. In our study the elevated levels of IgA in the acute phase further supported an IgA-mediated pathogenesis. IgA is the principal antibody in the respiratory system for defense against microbial agents; serum IgA is increased in almost half of the patients and circulating IgA-containing immune complexes may be present [16]. HSP is often preceded by an upper respiratory tract infection. Several different organisms have been implicated as the initiating factors, among them streptococci, which have been associated with the disease [16]. In our study, the presence of a history of upper respiratory tract infection in almost all our patients (90%) and elevated ASO titers in approximately 50% of the patients confirmed the importance of microorganisms as an initiating factor once again.

In conclusion, we suggest that the presence of IgA ANCA with lack of IgG ANCA may help to confirm the diagnosis of HSP. Further studies are still needed to determine the pathogenetic and clinical significance of IgA ANCA positivity in HSP.

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