Antineutrophil Cytoplasmic Antibodies of IgA Class in Neutrophilic Dermatoses With Emphasis on Erythema Elevatum Diutinum

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Objective: To evaluate the prevalence of IgA and IgG antineutrophil cytoplasmic antibodies (ANCAs) in erythema elevatum diutinum in comparison with 2 other groups of neutrophilic dermatoses: Sweet syndrome and pyoderma gangrenosum.

Design: Detection of IgA and IgG ANCAs in the serum of patients with neutrophilic dermatoses and characterization of the previously known antigenic targets.

Setting: All serum was analyzed without knowledge of diagnosis in the Immunology Department, Pitie-Salpêtrière Hospital, Paris, France.

Patients: Ten patients with erythema elevatum diutinum, 10 with Sweet syndrome, 10 with pyoderma gangrenosum, and 10 healthy volunteers.

Main Outcome Measures: IgA and IgG ANCAs were sought by indirect immunofluorescence with ethanol and formaldehyde-fixed human neutrophil preparations as the substrate. Enzyme-linked immunosorbent assays were further performed for antigen characterization.

Results: IgA ANCAs were observed in 60% and IgG ANCAs in 10 (33%) of the patients. All patients with erythema elevatum diutinum had IgA ANCAs. IgA fluorescence in formaldehyde-fixed neutrophils was restricted to those from patients with erythema elevatum diutinum. Enzyme-linked immunosorbent assays disclosed no single predominant target, and antigens remained largely undetermined in erythema elevatum diutinum.

Conclusions: The ANCAs, particularly of IgA class, may prove to be a helpful paraclinical marker in erythema elevatum diutinum and an interesting perspective for understanding the pathophysiology of the disease. The nature of the unidentified targets and the pathogenicity of ANCAs, however, remain to be assessed.

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Erythema elevatum diutinum (EED) is a rare leukocytoclastic vasculitis assigned to the group of neutrophilic dermatoses. Clinical features typically consist of persistent or recurrent papulonodular lesions symmetrically distributed over extensor surfaces with occasional systemic symptoms.1 Histologic examination shows a constellation of nonspecific signs: leukocytoclastic dense dermal neutrophilic infiltrate, parietal fibrinoid vascular necrosis, collagen fiber necrosis, and, late in the course of the disease, granulation or fibrosis.2,3 Although functional polymorphonuclear impairment seems likely,4 the pathogenesis remains elusive. Of significance is the association of EED with monoclonal or polyclonal IgA gammopathies.1 No serologic marker has been identified to date, but intriguing IgA antineutrophil cytoplasmic antibody (ANCA) activity has been observed in serum of patients with EED.5,6 Similarly, ANCAs have been reported in sporadic cases of pyoderma gangrenosum (PG)7 and in the setting of Sweet syndrome (SS),8 but these results remain equivocal. Given this controversy, we decided to investigate the presence of IgA ANCAs and IgG ANCAs in serum of patients with EED and to compare the results with those from 2 other groups of patients with neutrophilic dermatoses: PG and SS.

Methods

Patients with EED

Ten patients (6 males and 4 females; median age, 49 years; range, 15-81 years) were included. The medical records of patients coded with the diagnosis of EED were re-
patients with EED. Salpêtrière Hospital who were sex and age matched to the patients with SS, and PG. The IgA and IgG ANCAs were sought by indirect immunofluorescence using human neutrophil preparations as the substrate (Inova Diagnostics, Inc, San Diego, Calif), as previously described10-12 for IgG. Ethanol- and formaldehyde-fixed neutrophil preparations were incubated for 30 minutes with patient serum diluted 1:10 for IgA and 1:20 for IgG in phosphate-buffered saline. After washing, IgA and IgG were identified by means of a fluorescein isothiocyanate-conjugated anti-human IgA (Inova Diagnostics Inc) and IgG. Slides were examined under ultraviolet radiation by 2 different investigators blinded to the clinical diagnosis (J.-L.C. and M.-C.D.). Immunofluorescence labeling in ethanol-fixed neutrophils yielded 2 relatively distinct patterns, cytoplasmic (c) and perinuclear (p) (Figure), and diffuse cytoplasmic in formaldehyde-fixed neutrophils. The IgG- and IgA-negative and -positive serum controls were tested for all performed assays. For IgG ANCAs, positive and negative serum controls were obtained (Inova Diagnostics Inc). For IgA ANCA assays, we selected positive and negative serum controls. Positive serum was obtained from a patient with inflammatory bowel disease without skin lesions. This serum was strongly positive on indirect immunofluorescence immunoassay with a p-ANCA pattern.

Enzyme-linked immunosorbent assays (ELISAs) were further performed for myeloperoxidase (MPO), bactericidal/permeability-increasing protein (BPI), proteinase 3 (PR3), lactoferrin (LF), cathepsin G (CAT), and elastase (EL) detection by both IgA and IgG ANCAs (Euroimmun GmbH, Lübeck, Germany). The absorbance was automatically read on a spectrophotometer (Dynatech MR7000; Thermo Labsystems, Cergy-Pointoise, France) at 405 nm at the end of the linear phase of reaction. Positivity threshold for ELISA was defined according to a cutoff value calculated for each antigen with the mean value of the results obtained from the serum of patients and healthy controls that was negative on indirect immunofluorescence. Values were considered positive when they exceeded the mean value ± 2 SDs. The antigenic target of the selected positive IgA ANCA serum control was BPI.

RESULTS

CLINICAL DATA

The main clinical features of the patients in the study are summarized in Table 1. Most of the patients had associated conditions, mainly hematologic disorders (4 with EED, 3 with SS, and 1 with PG), relapsing polychondritis (2 with EED and 1 with SS), and inflammatory bowel disease (1 with SS and 5 with PG). Only 2 patients with EED, 1 with SS, and 2 with PG did not have any other associated manifestation or disease. Patient 2 with EED had previously had PG, which antedated EED by 2 years. He was included in the EED series because serum samples were drawn during the active phase of EED without evolutive PG.

SERUM IgA AND IgG LEVELS

Fourteen patients (7 with EED, 5 with SS, and 2 with PG) had elevated IgA levels (Table 2). Six of these patients had concurrent elevated IgG levels. As shown in Table 1, 3 patients with EED had monoclonal IgA gammopathy, while others had polyclonal high blood IgA levels (4 patients).
IgA-CLASS ANCA

In ethanol-fixed neutrophils, IgA ANCAs were present in all 10 patients with EDD (c-ANCA and p-ANCA patterns were observed in 5 patients each), 4 of 10 patients with SS (c-ANCAs in 3 patients and p-ANCAs in 1 patient), 4 of 10 patients with PG (all p-ANCAs), and none of the 10 healthy controls (Table 2). In formaldehyde-fixed neutrophils, IgA ANCAs were present in 6 of 10 patients with EED, and in none of the patients with SS or PG or the controls.

On ELISA testing, 1 patient with EED had anti-PR3 antibodies, 1 had anti-MPO antibodies, and 1 had anti-MPO and anti-CAT antibodies. In the remaining 7 patients with EED, no antigenic target could be identified within the panel of ELISA. Serum IgA recognized BPI in 2 patients with SS, MPO, and CAT in 1 patient with SS. No targets were identified in the remaining patients with SS, including 1 who had IgA ANCAs. Anti-BPI and anti-MPO were each observed in 2 patients with PG. No targets were found in the remaining 6 patients with PG, who were IgA ANCA negative.

IgG-CLASS ANCA

In ethanol-fixed neutrophils, IgG ANCAs were present in 4 of 10 patients with EDD (all p-ANCAs), 2 of 10 patients with SS (both p-ANCAs), 4 of 10 patients with PG (3 p-ANCAs and 1 c-ANCAs), and 0 of 10 healthy controls (Table 2). In formaldehyde-fixed neutrophils, IgG

Table 1. Clinical Data

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y*</th>
<th>Diagnosis</th>
<th>Distribution of Lesions</th>
<th>Extracutaneous Manifestations</th>
<th>Associated Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/81</td>
<td>EED</td>
<td>Dorsal hands, elbows, ankles, penis</td>
<td>None</td>
<td>Paget disease, IgA-MMGUS</td>
</tr>
<tr>
<td>2/M/33</td>
<td>EED</td>
<td>Dorsal hands, elbows, knees</td>
<td>None</td>
<td>PG</td>
</tr>
<tr>
<td>3/M/49</td>
<td>EED</td>
<td>Face, arms, forearms, fingers, buttoks</td>
<td>Arthralgia</td>
<td>IgA-MM</td>
</tr>
<tr>
<td>4/F/81</td>
<td>EED</td>
<td>Forearms, dorsal hands, thighs, legs</td>
<td>Conjunctivitis</td>
<td>CLL, neutrophilic eccrine hidradenitis</td>
</tr>
<tr>
<td>5/F/35</td>
<td>EED</td>
<td>Thighs, feet, toes</td>
<td>None</td>
<td>RP, seronegative polyarthritis</td>
</tr>
<tr>
<td>6/F/15</td>
<td>EED</td>
<td>Dorsal hands, elbows, knees, buttoks, feet</td>
<td>Fever, arthralgia</td>
<td>None</td>
</tr>
<tr>
<td>7/M/60</td>
<td>EED</td>
<td>Arms, elbows, legs, feet</td>
<td>None</td>
<td>RP</td>
</tr>
<tr>
<td>8/M/56</td>
<td>EED</td>
<td>Face, knees, fourth left finger, right foot</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>9/F/19</td>
<td>EED</td>
<td>Elbows, dorsal hands</td>
<td>Arthralgias, keratoconjunctivitis</td>
<td>Raynaud phenomenon, oral aphthosis</td>
</tr>
<tr>
<td>10/M/58</td>
<td>EED</td>
<td>Scalp, elbows, dorsal hands, palms, back, thighs</td>
<td>Fever, arthralgia</td>
<td>IgA-MM</td>
</tr>
<tr>
<td>11/M/56</td>
<td>SS</td>
<td>Chest, abdomen, thighs</td>
<td>None</td>
<td>APLS, cold urticaria</td>
</tr>
<tr>
<td>12/M/72</td>
<td>SS</td>
<td>Scalp, face, abdomen, back, limbs, dorsal hands</td>
<td>Episcleritis</td>
<td>RP</td>
</tr>
<tr>
<td>13/F/60</td>
<td>SS</td>
<td>Shoulders, arms, thighs</td>
<td>None</td>
<td>MALT lymphoma</td>
</tr>
<tr>
<td>14/F/61</td>
<td>SS</td>
<td>Right leg</td>
<td>Arthralgia</td>
<td>Large-cell lymphoma</td>
</tr>
<tr>
<td>15/F/41</td>
<td>SS</td>
<td>Palms, chest</td>
<td>Conjunctivitis, episcleritis, iritis</td>
<td>Crohn disease, Takayasu arteritis</td>
</tr>
<tr>
<td>16/M/67</td>
<td>SS</td>
<td>Face, chest, back</td>
<td>None</td>
<td>Polyarteritisnodosa, chronic active hepatitis B</td>
</tr>
<tr>
<td>17/M/72</td>
<td>SS</td>
<td>Scalp, face, ears, back, palms, thighs</td>
<td>Fever</td>
<td>None</td>
</tr>
<tr>
<td>18/M/54</td>
<td>SS</td>
<td>Scalp, ears, trunk, limbs</td>
<td>None</td>
<td>IgG-MMGUS, lymphocytic meningitis</td>
</tr>
<tr>
<td>19/F/32</td>
<td>SS</td>
<td>Face, forearms, dorsal hands</td>
<td>Fever</td>
<td>Oral and vulvar aphthosis</td>
</tr>
<tr>
<td>20/M/56</td>
<td>SS</td>
<td>Face, forearms, dorsal hands, thighs, legs</td>
<td>Conjunctivitis, arthralgia</td>
<td>HIV infection</td>
</tr>
<tr>
<td>21/F/58</td>
<td>PG</td>
<td>Buttoks, left breast</td>
<td>None</td>
<td>Ulcerative colitis</td>
</tr>
<tr>
<td>22/M/37</td>
<td>PG</td>
<td>Right leg, right ankle</td>
<td>None</td>
<td>Crohn disease, aseptic subcutaneous abscesses</td>
</tr>
<tr>
<td>23/F/67</td>
<td>PG</td>
<td>Legs</td>
<td>Arthralgia</td>
<td>Ulcerative colitis</td>
</tr>
<tr>
<td>24/M/36</td>
<td>PG</td>
<td>Legs, knees</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>25/M/71</td>
<td>PG</td>
<td>Left arm, back</td>
<td>None</td>
<td>Ulcerative colitis, aseptic liver abscesses</td>
</tr>
<tr>
<td>26/M/70</td>
<td>PG</td>
<td>Neck, abdomen, thighs</td>
<td>None</td>
<td>Leukocytoclastic vasculitis, renal failure</td>
</tr>
<tr>
<td>27/F/40</td>
<td>PG</td>
<td>Right hand, left leg</td>
<td>None</td>
<td>Crohn disease, pustular vasculitis</td>
</tr>
<tr>
<td>28/F/67</td>
<td>PG</td>
<td>Left forearm</td>
<td>Fever</td>
<td>AML</td>
</tr>
<tr>
<td>29/F/65</td>
<td>PG</td>
<td>Right leg</td>
<td>Fever, conjunctivitis</td>
<td>None</td>
</tr>
<tr>
<td>30/M/56</td>
<td>PG</td>
<td>Left arm, abdomen, thighs</td>
<td>Fever</td>
<td>Rheumatoid arthritis</td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute myelogenous leukemia; APLS, antiphospholipid antibody syndrome; CLL, chronic lymphocytic leukemia; EED, erythema elevatum diutinum; HIV, human immunodeficiency virus; MALT, mucosa-associated lymphoid tissue; MGUS, monoclonal gammopathy of unknown significance; MM, multiple myeloma; PG, pyoderma gangrenosum; RP, relapsing polychondritis; SS, Sweet syndrome.

*Age is expressed at the time of serum withdrawal and not disease onset.
ANCAs were present in 1 of 10 patients with PG and none of the other patients or controls. The ELISAs were negative except in 1 patient with EED who had IgG anti-MPO.

To test the hypothesis that ANCA detection was not related to elevated immunoglobulin levels, we performed a 2-tailed Fisher test comparing the prevalence of ANCAs in the subgroup of patients with normal serum immunoglobulins levels (10 of 16 patients) with the prevalence of ANCAs in the subgroup of patients who had elevated IgA and/or IgG levels (10 of 14 patients). Detection of ANCAs was not statistically linked to elevation of immunoglobulin levels ($P = .07$).

**DIRECT IMMUNOFLORESCEENCE ON SKIN SPECIMENS**

Direct immunofluorescence results on involved skin biopsy specimens were available for 7 patients with EED, 4 with SS, and 2 with PG. One patient with EED had isolated irregular IgA basal deposits at the dermoepidermal junction; 1 had coarse basal membrane and dermal IgA, IgM, and complement deposits; 1 had a coarse dermal pattern; and 1 had vascular deposits of IgA, IgG, IgM, and complement. One patient with PG had dermal IgA, IgG, and complement deposits. Results of direct immunofluorescence were negative in the remaining patients. Except for 1 patient with EED, IgA was not predominant with respect to IgG, IgM, or complement deposits.

**COMMENT**

Since the first report in 1964, clinical experience with ANCAs has considerably expanded and ANCA assay has become a routine laboratory test. Initial studies addressed mainly the IgG class of ANCAs; however, IgM and IgA subtypes emerged in later reports in variable clinical settings.

The gold standard for ANCA detection in patient serum is the indirect immunofluorescence method, using ethanol-fixed human polymorphonuclear neutrophils. The 2 major fluorescent staining patterns with ethanol are c-ANCA (cytoplasmic) and p-ANCA (perinuclear), related to artefactual redistribution of intracellular antigens. Formaldehyde fixation of neutrophils immobilizes neutrophilic cytoplasmic antigens, usually showing c-ANCAs. Characterization of ANCA intracellular antigenic targets relies on Western blot analysis or ELISAs for PR3, MPO, and other neutrophilic granule antigens.
The ANCA(抗体)ls of IgG isotype may be observed in a wide range of conditions, including Wegener granulomatosis, crescentic glomerulonephritis, microscopic polyangiitis, periarteritis nodosa, Churg-Strauss disease, inflammatory bowel diseases, and connective-tissue diseases. They can also occur in the setting of infections or be drug induced. However, the clinical relevance and diagnostic value of IgG ANCA are mainly restricted to Wegener granulomatosis, microscopic polyangiitis, and periarteritis nodosa. Their potential pathophysiologic significance in these vasculitides has been extensively investigated, and results suggest ANCA activation of neutrophils and subsequent endothelial cell damage. In addition, evidence of a direct role for the autoantigens PR3 and MPO in enhancing cellular immunity activation has recently been pointed out. The IgM ANCAls are rarely sought in laboratory assays. When positive, they often occur in association with the more frequent IgG ANCA. They likely represent early markers of ANCA seroconversion. The ANCAls of the IgA class may be detected in several conditions, including Henoch-Schönlein purpura, inflammatory bowel disease, and cystic fibrosis, but their clinical value remains unclear.

The ANCA activity has been sporadically reported in the setting of neutrophilic dermatoses; however, to our knowledge, IgA and IgG ANCAls have never been evaluated in a series of patients with neutrophilic dermatoses. In the present study, IgA ANCAls were observed in 60% and IgG ANCAls in 33% of patients with neutrophilic dermatoses. The sensitivity of ANCAls in neutrophilic dermatoses increased to 67% when IgA and IgG ANCA testing was combined. The ANCAls were observed in 100% of patients with EED, and fluorescence in formaldehyde-fixed cells appeared specific for EED, being observed only in this group (60%). Insofar as the high prevalence of IgA ANCAls in EED seems distinct from both other neutrophilic dermatoses and other cutaneous vasculitides, this finding may be pertinent to the pathophysiology of this peculiar entity, included in both groups of leukocytoclastic vasculitides and neutrophilic dermatoses. Furthermore, we hypothesize that IgA ANCAls may prove to be a helpful diagnostic adjunct for EED. Indeed, several reports emphasize potentially misleading clinical and pathological features of EED.

An obvious discrepancy was observed between ANCA and ELISA results in the present series, since antigenic targets were identified in a minority of ANCA-positive patients. This discrepancy could be related to a relatively low affinity of ANCAls for their antigenic targets or the nature of yet-unknown intracellular antigens. Indeed, while neutrophils contain numerous proteins and enzymes, commercially available ELISAs are restricted to a few recognizable targets, mainly MPO and PR3. The ELISA panel in this study additionally included BPI, CAT, LF, and EL but failed to disclose any predominant single target. Anti-MPO and anti-PR3 ANCAls, as observed in the serum of 3 patients with EED, could be related to the vasculitic component of this entity. Indeed, MPO and PR3 are the major recognized autoantigens in vasculitides. In 1 patient with PG exhibiting anti-MPO, a diagnosis of cutaneous leukocytoclastic vasculitis was made concurrently (Table 1). In 2 patients with PG and 1 with SS in whom ELISA elicited antineutrophilic cytoplasmic antibody, clinical association with an inflammatory bowel disease was observed. Given that BPI is one of the major antigens recognized by ANCAls in the setting of inflammatory bowel diseases, this finding may be of clinical relevance, indicating neutrophilic dermatoses-associated inflammatory bowel disease. Kemmet and coworkers' first report of ANCAls in patients with SS was later challenged by divergent results in other series. Of note is that Kemmet and colleagues' series included patients with inflammatory bowel disease, Churg-Strauss disease, and rheumatoid arthritis, whereas ANCA-negative series consisted mainly of patients who had isolated SS. With future development and standardization of IgA ANCA assays, it would be interesting to determine in larger series whether IgA and/or IgG ANCAls are helpful in distinguishing idiopathic from systemic disease-associated SS or PG.

Hints as to a possible pathogenic role of immunoglobulins of A class in EED are provided by the significance of ANCAls in EED. Indeed, several pieces of the pathophysiologic puzzle remain elusive. Given the high prevalence of IgA ANCAls in the present series of patients with EED, an attractive pathophysiologic hypothesis would involve neutrophil activation through IgA ANCA. The dramatic efficacy of dapsone in EED also suggests the involvement of neutrophil activation in the pathogenesis of EED. Indeed, dapsone interferes with various leucocyte functions. However, among all patients with neutrophilic dermatoses, no correlation was evident between the presence of IgA ANCA and efficacy of dapsone. Several pieces of the pathophysiologic puzzle are, however, still missing. Indeed, in contrast to IgG ANCAls, IgA ANCA interaction with neutrophils has not been fully addressed in experimental studies, and it remains to be established whether they represent a simple epiphenomenon of neutrophil activation and denaturation or pathogenic triggering factors.

A particularly important question concerns the possibility of false ANCA results linked to increased immunoglobulin levels and their nonspecific binding to autoantigens. This issue is particularly relevant to neutrophilic dermatoses, which are significantly associated with monoclonal or polyclonal IgA gammopathies and inflammatory bowel diseases. Our results showed that ANCA prevalence did not significantly differ between patients with normal and elevated immunoglobulin level. Moreover, the particular profile of ANCA immunofluorescence in EED compared with the other neutrophilic dermatoses renders it unlikely to be nonspecific. Another controversial issue is related to possible interaction of antinuclear antibodies with MPO, resulting in false-positive ANCA results. Of 4 serum samples that yielded IgA anti-MPO activity in our se-
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