Occurrence of autoantibodies to human leucocyte elastase in Wegener's granulomatosis and other inflammatory disorders

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Abstract

Antineutrophil cytoplasmic antibodies (ANCAs) constitute a new class of autoantibodies that seem to recognise myeloid lysosomal enzymes. The occurrence of ANCAs with specificity for human leucocyte elastase (HLE) was assessed in serum samples that were routinely submitted for ANCA determination. During a study period of more than six years anti-HLE was found in only six out of 1102 serum samples that produced a perinuclear or an atypical cytoplasmic staining pattern on ethanol fixed granulocytes. These six serum samples were from four patients with a clinical diagnosis of Wegener's granulomatosis but without a definite histological diagnosis, one patient with systemic vasculitis, and one patient with Cogan's syndrome. To further evaluate the prevalence of anti-HLE we tested 315 serum samples from patients with different forms of vasculitis and related disorders. Anti-HLE was detected in two patients only. Thus autoantibodies to HLE are rarely found in serum samples from patients with vasculitic or related disorders.

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The demonstration of autoantibodies to neutrophil cytoplasmic antigens (ANCAs) in systemic vasculitides such as Wegener's granulomatosis, classic polyarteritis nodosa, and the Churg-Strauss syndrome, and in idiopathic crescentic glomerulonephritis has placed these disorders within the class of autoimmune diseases.[1] Two major types of ANCAs have been recognised.[2] The first type is called c-ANCA ('classic' or 'cytoplasmic' ANCA) and is strongly associated with Wegener's granulomatosis.[3–5] These antibodies produce a characteristic granular cytoplasmic staining pattern on ethanol fixed granulocytes when detected by a standard indirect immunofluorescence technique.[6] Recently, it has been shown that the antigen recognised by c-ANCA is identical to proteinase 3, a 29 kilodalton glycoprotein from azurophilic granules with serine protease, antibiotic, and myeloblastic activity.[7–11] The second type of ANCA is called p-ANCA ('perinuclear' ANCA). A substantial number of p-ANCA positive serum samples were shown to contain autoantibodies to myeloperoxidase.[12 13] These antibodies produce a perinuclear pattern on ethanol fixed cytocentrifuged granulocytes, but a cytoplasmic staining pattern when granulocytes are treated with cross linking fixatives,[12] or when freshly prepared thin blood smears are used as a substrate.[14] Myeloperoxidase antibodies are found in patients with idiopathic crescentic glomerulonephritis (that is, without systemic vasculitis), crescentic glomerulonephritis associated with vasculitis, or alveolar haemorrhage, or both, hydralazine induced glomerulonephritis, and in patients with Churg-Strauss syndrome or classic polyarteritis nodosa.[12 13 15–17]

Recently, it became apparent that a substantial number of p-ANCA positive serum samples do not react with myeloperoxidase.[16 18 19] Serum samples positive for p-ANCA but negative for myeloperoxidase antibodies may be directed to other myeloid enzymes, such as human leucocyte elastase (HLE)[7 13] or lactoferrin.[19 20] Antibodies to HLE have been described in some patients with vasculitis,[17 21] in patients with hydralazine induced glomerulonephritis,[15] and occasionally in patients with systemic lupus erythematosus.[15 22] Because the clinical significance, that is, the prevalence and possible clinical associations of autoantibodies specific for HLE is not well delineated, we further investigated the clinical associations of these antibodies by analysing clinical data of all consecutive patients whose serum samples were routinely submitted for determination of ANCAs and were positive for HLE antibodies. We also tested for the prevalence of this autoantibody in a large group of clinically well characterised patients.

Patients and methods

SERUM SAMPLES

About 9000 serum samples were sent to our laboratory between June 1984 and April 1991 to be tested for ANCAs. These samples were, in general, derived from patients with a suspected diagnosis of vasculitis or glomerulonephritis from different Dutch hospitals. A total of 913 produced a perinuclear or nuclear staining pattern and 189 produced an atypical cytoplasmic staining pattern on ethanol fixed granulocytes. These 1102 serum samples were selected for testing for HLE antibodies by enzyme linked immunosorbent assay (ELISA). Also, 50 consecutive serum samples that produced a cytoplasmic staining pattern (c-ANCA) on ethanol fixed granulocytes and were simultaneously positive for anti-proteinase 3 by ELISA, and 218 serum samples from normal controls were tested for the presence of HLE antibodies by ELISA.

COLLECTION OF CLINICAL DATA

Clinical details of the patients with HLE

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antibodies were collected. Charts were reviewed according to a protocol with emphasis on histological findings and signs and symptoms associated with vasculitic syndromes.[16]

PATIENTS

To further define the significance of HLE antibodies in relation to proteinase 3 antibodies and myeloperoxidase antibodies we tested serum samples from all consecutive patients with classic polyarteritis nodosa (n=9), polyangiitis overlap syndrome (n=12). Churg-Strauss syndrome (n=10), temporal arteritis (n=12), and Takayasu's arteritis (n=4) who were seen at the department of internal medicine between June 1984 and April 1991. Diagnoses were based on clinical, histological, and/or angiographic criteria.[17 23] Serum samples were also tested from consecutive patients with chronic midline destructive disease (n=14) diagnosed by the presence of a progressive and locally destructive disease involving the nose, paranasal sinuses, or palate.[24] Nasal biopsies showed granulomatous inflammation with or without vasculitis—compatible with Wegener's granulomatosis—in 13 of 14 patients. None of these patients, however, had systemic disease with kidney involvement as seen in generalised Wegener's granulomatosis.

Serum samples were also tested from all consecutive patients with pauci-immune crescentic glomerulonephritis (n=45),[13] subclassified as Wegener's granulomatosis when granulomatous inflammation of the respiratory tract or respiratory tract symptoms compatible with Wegener's granulomatosis were present, or as idiopathic glomerulonephritis when systemic or respiratory tract manifestations were absent.

Finally, serum samples were tested from patients with cutaneous vasculitis (n=14), systemic lupus erythematosus with or without central nervous system involvement[25] (n=38), rheumatoid arthritis (n=38), sarcoidosis (n=16), mycobacterial infection (n=14), inflammatory bowel disease (n=59), autoimmune liver disease (n=30, comprising primary sclerosing cholangitis (n=8), primary biliary cirrhosis (n=15), and autoimmune chronic active hepatitis (n=7).

Serum samples were obtained when the disease in question was in an active phase.

DETECTION OF ANTIBODIES BY INDIRECT IMMUNOFLUORESCENCE

Detection of ANCAs was performed as previously described[3 5] with minor modifications.[26] Briefly, granulocytes from a healthy donor were obtained by Isopaque-Ficoll and dextran sedimentation of heparinised blood. After washing the cells, 25 µl of a suspension of 1×10^6 granulocytes/ml were put into each well of 12 well Cooke slides at 37°C for 30 minutes. After attachment to the wells, cells were ethanol fixed for 10 minutes at 4°C. Test or control serum samples were overlaid in a dilution of 1:16 on the slides and incubated for one hour. After washing thrice with phosphate buffered saline (PBS) for five minutes, bound antibody was detected with fluorescein isothiocyanate conjugated 1:400 diluted goat antihuman IgG (Kallestad, Chaska, Minnesota, USA). Finally, the slides were washed again in PBS and mounted in glycerin PBS for immunofluorescence microscopy.

Samples were scored as positive if most neutrophils showed positive fluorescence. Staining patterns were described as 'cytoplasmic' when granular staining of the cytoplasm with accentuation between the nuclear lobes was present, as 'perinuclear' when a (peri)nuclear pattern was seen, and as 'atypical cytoplasmic' when cytoplasmic staining was non-granular or when the accentuation between nuclear lobes was lacking.[27] To investigate whether the perinuclear staining pattern seen on ethanol fixed granulocytes was due to redistribution of the antigen(s) involved, cytocentrifuge preparations of neutrophils were also fixed with phosphate buffered paraformaldehyde (0·5%) at pH 8·5.[28]

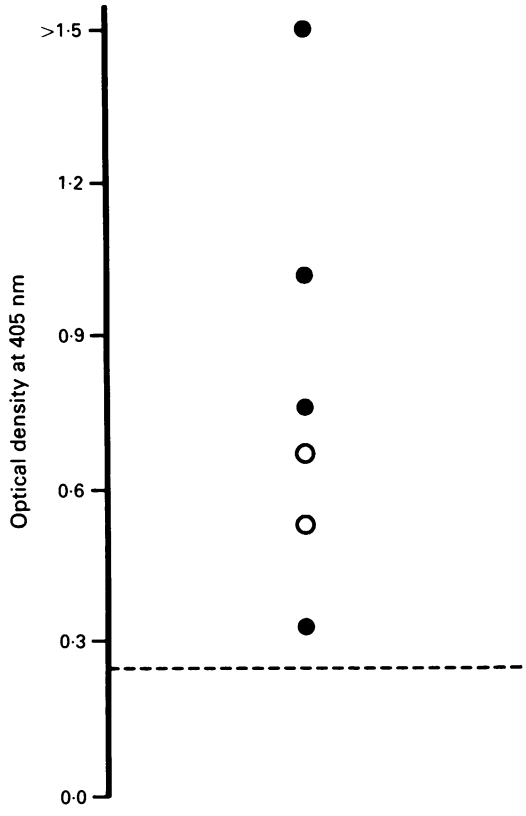
DETECTION OF AUTOANTIBODIES TO HLE, MYELOPEROXIDASE, AND PROTEINASE 3 by ELISA

Detection of autoantibodies to HLE, myeloperoxidase, and proteinase 3 by ELISA was performed as previously described.[13]

Results

OCCURRENCE AND CLINICAL ASSOCIATION OF HLE ANTIBODIES IN SERUM SAMPLES SUBMITTED ROUTINELY FOR ANCA DETECTION

Five of 913 serum samples with a p-ANCA pattern, one of 189 with an atypical cytoplasmic staining pattern, and none of 50 with a c-ANCA pattern were positive for antibodies to HLE by ELISA (figure). Also, none of 218 serum samples from normal controls with negative immunofluorescence on ethanol fixed granulocytes were positive for anti-HLE. Two of the six samples positive for anti-HLE were simultaneously positive for myeloperoxidase antibodies. Proteinase 3 antibodies were not detected in these six samples. The six serum samples positive for anti-HLE produced a granular cytoplasmic staining pattern on paraformaldehyde fixed granulocytes. These six samples were obtained from five women and one man, mean age 68 (range 55–84) years. Tables 1, 2, and 3 list the clinical, histopathological, and laboratory features of these patients and samples. Four patients had a clinical diagnosis of (limited) Wegener's granulomatosis. Three of them presented with chronic nasal inflammation, persistent sinusitis, constitutional symptoms, fever, and arthralgia and myalgia. In all three patients radiographs of the sinuses showed opacification or fluid levels, whereas chest radiographs disclosed a dense localised infiltrate in two of them. Multiple cultures did not grow bacteria, mycobacteria, or fungi, and antibiotic treatment did not result in clinical improvement. Serum creatinine concentrations were normal in these patients, but urine analysis disclosed a trace of albumin accompanied by haematuria without cellular casts in one of them. According to histopathological criteria a definite diagnosis of Wegener's granulomatosis



Autoantibodies to human leucocyte elastase (HLE) as detected by ELISA in six of 1152 serum samples that were routinely submitted for the detection of antineutrophil cytoplasmic antibodies and produced a (peri)nuclear or cytoplasmic staining pattern by indirect immunofluorescence on ethanol fixed granulocytes. Open circles represent serum samples that are positive both for myeloperoxidase antibodies and for anti-HLE, and solid circles represent serum samples that are positive for anti-HLE only. Dashed line denotes the upper limit of control samples (mean+2SD).

could not be made in these patients, although extravascular granulomatous inflammation was found in a nasal biopsy specimen in one patient (patient 1) and vasculitis of the skin in another (patient 3) (table 2). Open lung biopsies and renal biopsies were, however, not performed. Treatment with high dose prednisolone (accompanied by oral cyclophosphamide in one patient (patient 1)) resulted in prompt recovery in all three patients.

Patient 4 presented with cough and severe dyspnoea. A chest radiograph disclosed multiple interstitial infiltrates, and a transbronchial biopsy showed extravascular granulomatous inflammation without vasculitis (table 2). No upper respiratory tract or renal manifestations were present. Treatment with high dose corticosteroids resulted in partial remission. Because of progressive dyspnoea while the dose of steroids was generally reduced (four months after diagnosis) an open lung biopsy was performed that disclosed nodular interstitial fibrosis with cicatricial vascular changes without signs of active vasculitis. Bronchoalveolar lavage performed at that time, however, showed a pronounced increase in neutrophils and eosinophils compatible with active Wegener's granulomatosis.[29] Dosage of steroids was increased and treatment with oral cyclophosphamide started, resulting in a remarkable clinical improvement.

Patient 5 had Cogan's syndrome. She had a three year history of audiovestibulatory abnormalities consisting of vertigo, bilateral tinnitus, nausea, vomiting, nystagmus, and bilateral hearing loss. Interstitial keratitis in combination with uveitis was also present, but other manifestations of systemic vasculitis were not seen.

Patient 6 had systemic vasculitis and was classified as having polyangiitis overlap syndrome. This patient has already been described.[17]

OCCURRENCE OF HLE ANTIBODIES IN GROUPS OF PATIENTS WITH SELECTED DISEASES

To further test for the prevalence of anti-HLE and its relation to anti-myeloperoxidase and anti-proteinase 3 we analysed serum samples from consecutive patients with different forms of vasculitis (n=61), necrotising crescentic glomerulonephritis (n=45), and chronic midline destructive disease (n=14) who were seen at the department of internal medicine, University Hospital, Groningen, The Netherlands, during a period of more than six years (table 4). Antibodies to HLE were only found in one patient with chronic midline destructive disease (patient 1) and in one patient with polyangiitis overlap syndrome (patient 6). To assess the specificity of anti-HLE for systemic vasculitis we tested serum samples from patients with systemic lupus erythematosus (n=38), rheumatoid arthritis (n=38), granulomatous disorders

Table 1 Clinical features of six patients with human leucocyte elastase antibodies

Patient No	*Upper respiratory tract findings*	*Pulmonary disease findings*	*Other manifestations*
1	Rhinitis, sinusitis, destructive midline disease	Haemoptysis Chest x ray film: localised consolidation	Fever, arthralgia, pyoderma gangrenosum
2	Rhinitis, sinusitis	Cough, dyspnoea Chest x ray film: localised consolidation	Fever, myalgia
3	Rhinitis, sinusitis	Cough, dyspnoea Chest x ray film: no abnormalities	Fever, arthralgia, purpura, proteinuria, haematuria
4	—	Cough, dyspnoea Chest x ray film: diffuse bilateral interstitial infiltrates	Fever
5	—	—	Interstitial keratitis, audiovestibular symptoms, alopecia
6	—	—	Fever, myalgia, arthralgia, polyneuropathy, skin nodules

Table 2 Histopathological findings for six patients with antibodies to human leucocyte elastase (HLE)

Patient No	Histological findings
1	Nasal biopsy: acute and chronic inflammation with necrosis, mucosal ulceration, eosinophilic granulocytes, histiocytes, and giant cells
2	Nasal biopsy: acute and chronic inflammation with mucosal ulceration
3	Nasal biopsy: acute and chronic inflammation with mucosal ulceration. Skin biopsy: leucocytoclastic vasculitis
4	Transbronchial biopsy: chronic inflammation with a mixed infiltrate of neutrophils, eosinophilic granulocytes, and scattered giant cells Open lung biopsy*: nodular interstitial fibrosis with cicatricial vascular changes. Muscle biopsy*: mild non-specific perivascular inflammation
5	No biopsy
6	Subcutaneous nodule: granulomatous arteritis

*Performed after beginning treatment with corticosteroids.

Table 3 Laboratory results for six patients with antibodies to human leucocyte elastase at the time of active disease and before the start of treatment

Patient No	ESR (mm/h)	Hb (g/l)	WBC (10^9/l)	Hyper-eosinophilia (>0·5×10^9/l)	Thrombocytes (10^9/l)	α_1 anti-trypsin (g/l)*	Antineutrophil cytoplasmic antibodies	
							IF	ELISA
1	32	113	10	–	633	1·9	p	Anti-HLE
2	118	125	11	–	471	6·0	p	Anti-HLE
3	85	106	7	–	238	2·9	p	Anti-HLE
4	76	140	18	+	378	3·3	c	Anti-HLE and anti-MPO
5	81	128	13	+	382	2·4	p	Anti-HLE and anti-MPO
6	75	126	22	–	836	3·5	p	Anti-HLE

*Reference range for α_1 antitrypsin 1·5–3·0 g/l.
ESR=Erythrocyte sedimentation rate; Hb=haemoglobin; WBC=white blood cell count; IF=immunofluorescence; ELISA=enzyme linked immunosorbent assay; p=perinuclear staining pattern; c=atypical cytoplasmic staining pattern; HLE=human leucocyte elastase; MPO=myeloperoxidase.

Table 4 Prevalence of antineutrophil cytoplasmic autoantibodies with specificity for human leucocyte elastase (HLE), proteinase 3 or myeloperoxidase in serum samples from patients with systemic necrotising vasculitis and other closely related diseases, and in serum samples from normal control subjects

Disease entity (No of serum samples tested in parentheses)	Antibody to HLE	Antibody to proteinase 3	Antibody to myeloperoxidase	None
Churg-Strauss syndrome (10)	–	–	8	2
Classic polyarteritis nodosa (9)	–	–	2	7
Polyangiitis overlap syndrome (12)	1	6	2	3
Temporal arteritis (12)	–	–	–	12
Takayasu's arteritis (4)	–	–	–	4
Cutaneous vasculitis (14)	–	–	–	14
Necrotising crescentic glomerulonephritis:				
Associated with Wegener's granulomatosis (40)	–	30	10	–
Idiopathic (5)	–	1	4	–
Chronic midline destructive disease (14)	1	11	–	2
Systemic lupus erythematosus				
With CNS involvement (9)	–	–	–	9
Without CNS involvement (29)	–	–	–	29
Rheumatoid arthritis (38)	–	–	–	38
Sarcoidosis (16)	–	–	1	15
Mycobacterial infections (14)	–	–	1	13
Crohn's disease (24)	–	–	–	24
Ulcerative colitis (35)	–	2	1	32
Autoimmune liver diseases (30)	–	–	1	29
Normal controls (218)	–	–	–	218

CNS=Central nervous system.

(n=30), inflammatory bowel disease (n=59), and autoimmune liver disease (n=30) (table 4). None of these contained anti-HLE.

Discussion

Autoantibodies specific for HLE are not often found in serum samples from patients with vasculitic disorders. As a reference laboratory we detected HLE antibodies in only six patients during a period of more than six years. For comparison, myeloperoxidase antibodies were detected in 53 patients during a period of 4·5 years,[16] whereas 20 new cases with proteinase 3 antibodies are detected in our laboratory every year. These data show that in a population of patients with suspected vasculitis and glomerulonephritis anti-proteinase 3 antibodies occur 20 times more often than anti-HLE and anti-myeloperoxidase 12 times more often. Recently, the fact that the occurrence of anti-HLE is rare has also been reported by others.[15 22 30]

Four of six patients with HLE antibodies had a clinical diagnosis of (limited) Wegener's granulomatosis, one patient had Cogan's syndrome, and one patient had polyangiitis overlap syndrome. None of the four patients with a clinical diagnosis of Wegener's granulomatosis had a definite histological proof of that diagnosis. Histological findings of biopsy specimens of the respiratory tract, however, showed an inflammatory reaction with necrosis and granulomatous changes in two of four patients. Vasculitis was not found in these specimens. Because vasculitis can easily be missed in small biopsy specimens, larger biopsies of the nose and sinus or open lung biopsies might have been diagnostic. Vasculitis may also be missed in these larger biopsy specimens,[31] however, and it has been emphasised that a diagnosis of Wegener's

granulomatosis sometimes has to be made in the absence of vasculitis.[32–34] Also, one patient had leucocytoclastic vasculitis of the skin. Although granulomatous inflammation was not detected in respiratory tract biopsy specimens, this patient fulfilled the American College of Rheumatology 1990 criteria for the classification of Wegener's granulomatosis.[35]

Antibodies to HLE produced a perinuclear staining pattern on ethanol fixed normal human granulocytes in five of six patients. This staining pattern proved an artefact of fixation as a granular staining pattern of the cytoplasm was seen when neutrophils were treated with cross linking fixatives such as paraformaldehyde; this has also been shown for anti-myeloperoxidase.[12]

In the present study we detected HLE antibodies and myeloperoxidase antibodies simultaneously in two patients. The combined presence of these has already been described, and it has been suggested that this combination of antibodies might be diagnostic of hydralazine induced glomerulonephritis.[15] Their simultaneous occurrence has recently been reported, however, in some patients with connective tissue diseases.[36] As we did not have the opportunity to study cases of hydralazine induced glomerulonephritis we were not able to verify the hypothesis. Our two patients with both anti-HLE and anti-myeloperoxidase, however, did not use hydralazine, nor did they have clinical evidence of glomerulonephritis. Antibodies to HLE have also occasionally been found in patients with idiopathic systemic lupus erythematosus. Nässberger *et al* [15] found anti-HLE in four of 96 patients with systemic lupus erythematosus, and three of them had involvement of the central nervous system. In the present study we could not confirm this report as we did not find HLE antibodies in patients with active systemic lupus erythematosus (with or without central nervous system disease). During the third international workshop on ANCAs (November 1990, Washington DC) the occurrence of anti-HLE in patients with systemic lupus erythematosus was debated, as at least some positive results may, in fact, be due to interaction of HLE with DNA.[30]

The rare occurrence of HLE antibodies in patients with Wegener's granulomatosis, which is strongly associated with anti-proteinase 3,[3–5] has not been described before. Both proteinase 3 and HLE are serine proteinases with elastinolytic activity and both enzymes are involved in tissue destruction. This may suggest a common background for the autoimmune responses.

In conclusion, autoantibodies to HLE are rarely found in serum samples from patients with vasculitis. Testing for HLE antibodies seems to be useful only in patients with active Wegener's granulomatosis or systemic vasculitis who lack antibodies to proteinase 3 and myeloperoxidase.

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