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Autoantibodies against cytoplasmic structures of neutrophil granulocytes in Wegener's granulomatosis

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SUMMARY

Autoantibodies against cytoplasmic components of neutrophil granulocytes (ACPA) were detected in 18 of 32 patients with Wegener's granulomatosis (WG), but in none of the controls ($n=900$), including patients with glomerulonephritis, sarcoidosis, tuberculosis, polyarteritis nodosa, and connective tissue diseases, and healthy blood donors. The presence and, to a lesser extent, the titre of ACPA correlated with the severity and activity of the disease. ACPA could be detected in only three of 11 patients with the limited form of the disease and in none in complete remission. In contrast, in all patients with active extensive disease, ACPA were present in a higher titre, and in most of the patients in partial remission (eight of 12) antibodies were demonstrable, especially in those with frequent relapses. Furthermore, the antibody titre correlated significantly with the C-reactive protein concentration ($P<0{\cdot}05$), but with none of the other laboratory parameters. In conclusion, ACPA have proven to be a highly specific disease marker of great clinical significance that provides us with a useful tool to confirm, or even establish, the diagnosis of WG.

Keywords Wegener's granulomatosis autoantibodies anticytoplasmic antibodies

INTRODUCTION

Wegener's granulomatosis (WG) is a systemic necrotizing granulomatous vasculitis that in full-blown disease affects the eyes, the upper and lower respiratory tract and the kidneys; until now the diagnosis was based on typical clinical findings and characteristic histological lesions (Fauci, Haynes & Katz, 1978). No disease specific laboratory marker has been described until recently, when van der Woude *et al.* (1985) reported IgG autoantibodies against cytoplasmic components of neutrophil granulocytes and monocytes (ACPA) as a disease specific characteristic in WG. However, because other autoantibodies have also been described to be strongly associated with WG (Andrassy *et al.*, 1983) and pathognomonic autoantibodies are rare events, we studied the prevalence of ACPA and other autoantibodies (e.g. against nuclear antigen, double-stranded DNA, extractable nuclear antigens, rheumatoid factor) in 32 patients with WG and relevant controls.

With a severe disease such as WG it is useful to determine the specificity of a disease-associated marker but it is more important to ascertain the reliability with which its measurement detects patients who have the disease. Unfortunately there is no indication of the sensitivity of the ACPA assay in van der Woude *et al.* (1985), so the clinical features in our group of patients were characterized more fully to evaluate the significance of these autoantibodies. We have previously described the two main groups in WG (Gross *et al.*, 1986): in the beginning most patients present

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with a limited form of disease characterized by localized inflammation and later develop, sometimes after years, the well-known extensive form with generalized vasculitis and a marked clinical deterioration.

Van der Woude *et al.* (1985) reported that the titre of ACPA correlated with the activity of disease. However, other authors (Hind & Pepys, 1985) have questioned the distinction between their active and inactive groups because there was no apparent difference in the laboratory inflammation markers in the two groups. We therefore applied a more defined grading system combining clinical and laboratory findings (according to Fauci *et al.*, 1983) to correlate the activity of disease with the presence and titre of antibodies. We also investigated the association between ACPA and other laboratory parameters, such as erythrocyte sedimentation rate and C-reactive protein concentration which are usually used to define disease activity in WG.

MATERIALS AND METHODS

Patients. We studied 32 patients (21 female, mean age 50·6 years, range 23–78; 11 male, mean age 54·7, range 29–78) with Wegener's granulomatosis. The diagnosis was established on the basis of the classical clinical symptoms described by Fauci *et al.* (1983) or histopathological findings. Biopsy specimens showed, in 23 cases, typical granulomatous vasculitis (histological diagnosis: 'WG typical'); in five cases they revealed nonspecific changes of acute and chronic inflammation with necrosis (histological diagnosis: 'WG possible'). In four cases the biopsy material obtained was inconclusive. The patients were investigated with standard and clinical and laboratory techniques and categorized into groups with limited and extensive disease (Gross *et al.*, 1987). In the beginning most patients present with a limited form of disease characterized by localized inflammation, e.g. sinusitis, otitis, haemorrhagic rhinitis and focal lesions in lungs and kidneys. This stage is frequently followed by the well-defined extensive form characterized by generalized vasculitis with a marked clinical deterioration with fever, weight loss, skin rash, episcleritis, massive infiltrations of the lungs and a rapidly progressive glomerulonephritis. Table 1 shows the main clinical symptoms of both groups. Criteria for disease activity were fever without infection, malaise, weight loss, an acute exacerbation of inflammation in various organ systems, e.g. glomerulonephritis resulting in a decrease in renal function, pulmonary infiltration, episcleritis, skin rash, arthralgia; and an elevation of the erythrocyte sedimentation rate (ESR) and C-reactive protein concentration (CRP). Patients who suffered an acute exacerbation of inflammation combined with an elevation of ESR and CRP and various other symptoms were considered to have active disease. Partial remission was defined as clearcut suppression of the progress of disease activity with stabilization of organ abnormalities and progression towards improvement. Complete remission was defined as the complete absence of evidence of active disease with a normal ESR and CRP serum concentration. The control groups consisted of 300 healthy blood donors, 11 patients with other forms of vasculitis, seven with sarcoidosis, six with tuberculosis, 62 with glomerulonephritis, 52 with various connective tissue diseases, 80 with uveitis, and 382 with various other internal diseases.

Methods. Anticytoplasmic antibody (ACPA) titres were obtained by an indirect immunofluorescence technique, using an FITC conjugated sheep anti-human IgG antiserum (1:40) (Wellcome, Dartford, England) and as antigen, freshly prepared granulocytes from control individuals. Granulocytes were prepared from heparinized peripheral blood by dextran sedimentation and lysis of the red blood cells with distilled water. Cytocentrifuge slides were fixed with 95% ethanol at 4°C according to van der Woude *et al.* (1985). The slides were incubated for 30 min with the sera, washed twice in phosphate-buffered saline (PBS) and incubated with the sheep anti-human antiserum for 30 min. After two washings, glycerol buffer (90 ml glycerol (Merck, Darmstadt, FRG) plus 10 ml PBS) was added and the slides were evaluated under the fluorescence microscope. The presence of ACPA was easy to recognize by a strong characteristic fluorescence of the whole cytoplasm excluding the nucleus (Fig. 1). Only occasionally negative sera caused a very weak staining of the whole cells. The presence of antibodies to nuclear antigens, gastric cells, double-stranded (ds) DNA, thyroid microsomal antigen, heart muscle and mitochondria was tested by indirect immunofluorescence staining of tissue cryostat sections. Antibodies to RNP, SSA, SSB and SM were determined by

Table 1. Clinical findings in 11 patients with limited and 21 patients with extensive Wegener's granulomatosis

	Upper respiratory tract	Eyes	Lungs	Kidneys	Musculo skeletal system	Other organ systems
Limited disease						
1	R T					
2	R S T					
3	R S			P H		
4	R S O		N			
5	R					
6	R O	Epi				
7	S T		N TS			
8	S T					
9*	R O	Scl			A Ais	
10	R S O			P H		
11	R	Cj				
Extensive disease						
12†	R	Epi		P H ER	A My	F WL PN
13	R	Scl	HP N Ab	P H RI	A Ais	F
14†	R		N If		A	F PN
15	R S O U	Cj		P H	Ais	F WL SR
16	R S O		N Ef	P H ER	A	F
17	R T U	Epi	N If	P H RI	A My	F
18	R U			P H ER	Ais	F SR
19	R		N If Ef	P H RI		F
20†	U	Ret	HP N	P H		F SR
21	R U	Epi	HP If TS	P H	A Ais	F WL
22	O S		If	P H RI		F PC
23	R	Epi	N TS	P H	A	F PC
24*		I	HP N	P H		F
25*			If Ef	P H RI	A	F WL
26	R T		N	P H	My	F
27	R T		N	P H ER		F
28†		Epi	HP Ab	P H RI		F EC
29	S Ab		If	P H RI		F PN
30	R O	Cj		P H RI		F SR
31*	R T U		If	P H	A	F SR
32†	R O U		If		A	F

A, arthralgia; Ab, abscess; Ais, arthritis; Cj, conjunctivitis; EC, endocarditis; Ef, effusion; Epi, episcleritis; ER, end stage renal failure; F, fever; H, haematuria; HP, haematopnoe; I, iritis; If, infiltrate; My, myalgia; N, noduli; O, otitis; P, proteinuria; PC, pericarditis; PN, polyneuropathy; Ret, retinitis; R, rhinitis; RI, renal impairment; S, sinusitis; Scl, scleritis; SR, skin rash; T, tumour; TS, tracheal stenosis; U, ulcera; WL, weight loss.

* Without histological confirmation.

† Histological lesions consistent with WG.

counter immunoelectrophoresis. Rheumatoid factor (RF) was determined by the latex agglutination and a modified Rose-Waaler test (Boehringer, Mannheim, FRG). IgG, IgM, IgA, C-reactive protein and complement concentrations were routinely measured in an 'Autoanalyzer'. The serum level of IgE was investigated by an radioimmunoassay (Phadebas IgE Prist, Pharmacia, Uppsala, Sweden).

Statistical analyses. Differences in the various objective measurements between groups were sought in the Mann-Whitney *U*-test. For correlation studies Spearman's rank correlation test was applied. Probabilities below 0·05 were considered as significant.

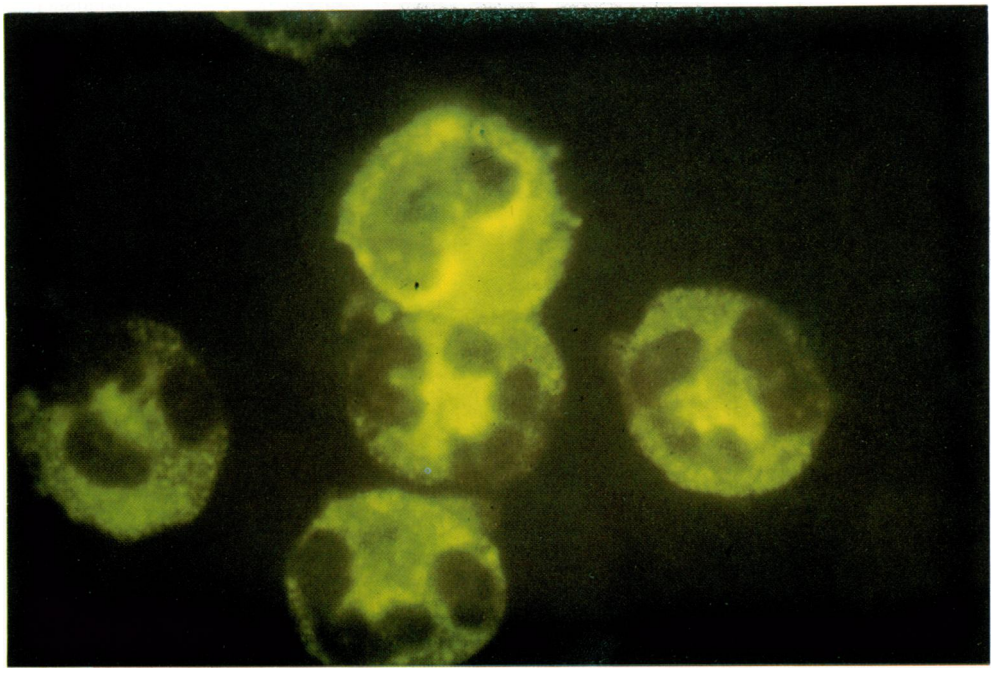

Fig. 1. Neutrophil granulocytes and one monocyte displaying the characteristic fluorescence pattern of anticytoplasmic antibodies.

RESULTS

Anticytoplasmic antibodies were found in 18 of 32 patients with Wegener's granulomatosis (WG), but in none of the 900 controls, including patients with tuberculosis, sarcoidosis, glomerulonephritis, uveitis, other forms of vasculitis, connective tissue diseases and healthy blood donors. These results reveal a high specificity (98·5%) of ACPA for WG. The sensitivity reached a value of 56% positive reactions in sera from patients with WG. The antibodies' titres ranged from 1:1 to 1:128.

In order to correlate the presence and titre of ACPA with the severity and activity of disease the patients were divided into two groups of limited and extensive disease. All patients with limited disease presented with a smouldering disease activity, whereas in the group of patients with extensive disease great variations of disease activity occurred. They were therefore subdivided into three groups of active disease, complete remission, and partial remission. This grading system revealed a high correlation between the presence and titre of ACPA and the severity and activity of disease. ACPA were only detectable in three of 11 sera from patients with limited disease (Fig. 2), and both patients with extensive disease in complete remission were negative for ACPA. In contrast ACPA were present in most of the sera (eight of 12) from patients in partial remission, especially in those with frequent relapses, and in all patients with active disease. Furthermore the highest titres were found in patients with active disease, and the ACPA values from the four groups differed significantly from each other ($P < 0{\cdot}01$).

Though ACPA presence is strongly correlated with disease activity the titre did not always exactly parallel the clinical improvement after induction of remission. In a follow-up study in six patients over a period of 9 months we observed in three patients no remarkable variation of ACPA titre, whereas in three patients the titre decreased significantly. Three of the patients were in partial remission with normal CRP values. In two of them the ACPA titre remained on the same level and one presented a decrease of the titre from 1:8 to 1:1. The other three patients experienced an acute exacerbation of disease with CRP values of 111 ± 15 mg/l (mean $\pm$ s.e.m.) that returned to normal after clinical improvement. Again, in one patient no remarkable ACPA titre reduction occured after

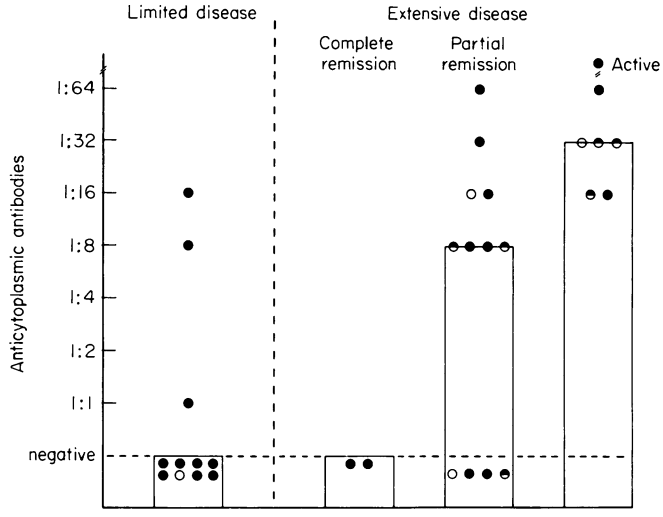

Fig. 2. Anticytoplasmic antibodies and disease activity. Individual values are plotted together with a histogram of the median. Histology: (●) typical; (◓) possible; (○) none.

Table 2. Mean laboratory findings in patients with Wegener's granulomatosis

		Extensive disease		
	Limited disease ($n=11$)	Complete remission ($n=2$)	Partial remission ($n=12$)	Active ($n=7$)
ESR (mm/h)	19·3±6·2	6·0±4·0	45·1±11·5	115·5±14·6
CRP (mg/l)	1·6±0·4	1·5±0·5	20·5±9·3	125·2±27·9
IgG (mg/l)	1263·2±171·2	1520·5±20·3	917·6±89·4	1019·8±257·2
IgM (mg/l)	102·1±28·5	127·9±15·7	70·8±17·1	73·2±12·7
IgA (mg/l)	204·2±50·3	432·4±71·1	228·6±51·3	255·4±43·8
IgE (U/l)	94·7±27·3	14·5±4·6	60·3±25·3	151·0±66·6
C3 (mg/dl)	122·4±18·7	166·7±12·5	157·6±31·5	144·7±43·6
C4 (mg/dl)	22·4±4·5	24·3±3·7	28·5±3·6	17·5±6·7
ACPA*	<1	<1	8	32

* Median of the reciprocal titre, all other parameters are expressed as mean±s.e.m.

induction of remission, whereas in two cases the titre fell from 1:16 to 1:1 and 1:32 to 1:2 respectively.

The four groups of patients with limited disease, extensive disease in complete or partial remission or in an active period showed marked differences in ESR and CRP values (Table 2). In patients with active disease the ESR (115·5±14·6) (mean±s.e.m.) and CRP (125·2±27·9) values were significantly higher ($P<0·01$) than those of the other groups. Patients with partial remission displayed a medium elevation of ESR (45·1±11·5) and CRP concentration (20·5±9·3), which also differed significantly from those of all other groups ($P<0·01$). In contrast, in patients with limited disease or in complete remission the mean values for ESR and CRP were normal (Table 2). A good

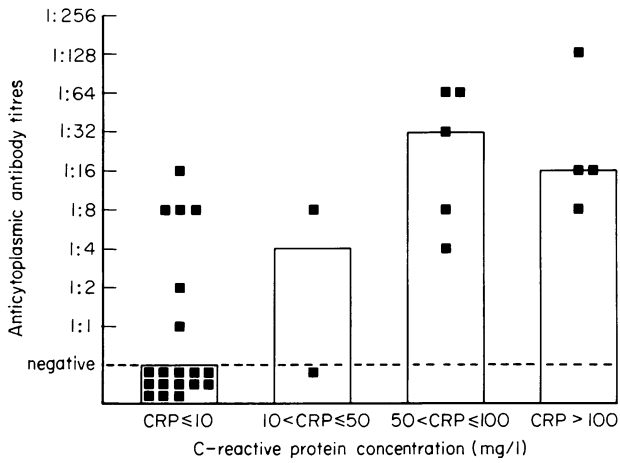

Fig. 3. Anticytoplasmic antibody titres correlated ($P < 0{\cdot}01$) with C-reactive protein concentrations (CRP).

correlation between CRP values and ACPA titres ($P < 0{\cdot}05$) could be observed (Fig. 3); the correlation between ESR values and ACPA titres was not significant.

IgG, IgM, IgA and C3 concentrations were within the normal range in all patients with WG and no significant differences could be found between the four groups (Table 2); C4 concentration was reduced in two of the patients with active extensive disease. IgE levels were elevated in eight of 32 patients, but again there were no differences between the groups and no significant correlation with ACPA titre. The autoantibody testing revealed a variety of other autoantibodies in small numbers: 4/30 for rheumatoid factor, 4/32 for nuclear antigen, 3/30 for heart muscle and 2/30 thyroid microsomal antigen. None of the other autoantibodies could be detected in any of the patients. There was no correlation between ACPA presence and other autoantibodies.

DISCUSSION

Anticytoplasmic antibodies against neutrophils (ACPA) were first performed by Allan Wiik in 1973 (Rasmussen *et al.*, 1987) and later by van der Woude *et al.* (1985) who reported them to be a disease specific characteristic in Wegener's granulomatosis (WG). In this study we could confirm these results. ACPA were demonstrable in 18 of 32 patients with WG but in none of the 900 control sera, which included differential diagnostic important diseases such as tuberculosis, sarcoidosis, glomerulonephritis, uveitis and various rheumatic diseases including vasculitis. Unfortunately the latter group (primary vasculitis) is too small to confirm that ACPA are not found in this closely related group of diseases. However, these findings demonstrate an extraordinarily high specificity of ACPA for WG. Apart from anti-acetylcholine receptor antibodies in myasthenia gravis and anti-double-stranded DNA antibodies in systemic lupus erythematosus, no other autoantibodies with such a high specificity have been described. The 56% sensitivity of ACPA was less striking, but, regarding the different stages of disease severity, we found a sensitivity of 71% in patients with extensive disease compared to only 27% in patients with limited disease. Furthermore, our results show that ACPA titre and presence correlated strongly with the activity of disease. A positive result for ACPA can therefore be considered as highly suggestive for WG. However, a negative result, especially in a patient with typical symptoms of the limited form of the disease (e.g. sinusitis, otitis, haemorrhageus rhinitis) does not exclude WG, as ACPA were not demonstrable in most of these patients.

Whether the finding of ACPA in some patients with the limited form of the disease is due to a smouldering disease activity or to an underlying pathogenetic mechanism that results in the

autoantibody production is at the moment unclear. The relatively low sensitivity of ACPA for limited disease is comparable to findings in patients with ocular myasthenia gravis. Anti-acetylcholine receptor antibodies are present in 90% of patients with generalized myasthenia gravis, but in only 30% of patients with ocular myasthenia gravis, and in lower titres (Nickolson, McLeod & Griffiths, 1983). It is also possible that the presence of ACPA in the three patients with limited disease indicates the threat of a switch from limited to extensive disease. In the two of these patients with higher ACPA titres (1:8 and 1:16) the diagnosis was only established within the last year and our experience has shown that the switch often occurs after a period of 1 to 2 years of limited symptoms. However, despite the strong correlation of ACPA with disease activity the titre did not exactly parallel the clinical improvements after inductions of remission. In some patients ACPA were still present when the disease was controlled, in terms of its clinical effects and objective measurements of target organ function. Presumably it takes a longer time for these autoantibodies to be cleared off and a longer persistence might indicate a smouldering disease activity not detectable with clinical and laboratory markers. Further longitudinal studies of ACPA titre would be necessary to reveal whether these autoantibodies mark an acute exacerbation of the disease, or the switch from limited to extensive disease.

ACPA correlated significantly ($P < 0{\cdot}05$) with C-reactive protein concentration but not with the erythrocyte sedimentation rate. This underlines their significance as a disease activity marker, agreeing with Hind *et al.* (1984) who found a good correlation between serum CRP levels, but not ESR, and overall clinical assessment of disease activity in WG. All other laboratory markers were found to be unremarkable in the patients studied, despite an IgE concentration elevation in eight of 32 patients which proved not to be significantly correlated with disease activity or ACPA titre. And other authors have also reported occasional elevation of IgE in WG (Conn *et al.*, 1976). Small numbers of autoantibodies other than ACPA were found in this study. This is in accordance with other reports (Shillitoe *et al.*, 1974) and probably represents a secondary reaction to tissue breakdown. We could not confirm the findings of Andrassy *et al.* (1983) of an increased presence of antibodies to SSA and SSB in patients with WG. None of the extractable nuclear antigens were demonstrable in any of the patients.

Despite the fact that WG is generally assumed to be caused by hypersensitivity or allergic reaction to an unknown antigen (Fauci *et al.*, 1983), the pathogenesis is still unclear. The specificity and correlation of ACPA with disease activity suggests a pathogenic significance of these autoantibodies. As yet the nature of the antigen is unknown but preliminary data from our group (unpublished results) show that the antigen is released during the degranulation of neutrophils. This way the antigen can come into contact with the antibodies and immune complex formation might occur. The pathological relevance of this event needs to be further investigated.

In conclusion, this study has shown that ACPA provide us with a very useful diagnostic tool for a disease that could hitherto only be diagnosed by clinical and histological criteria, often confusing and time consuming. ACPA are especially valuable in cases of patients who are first seen with fulminant disease, where prompt and adequate treatment, without the delay of a histological diagnosis, is required because of their high mortality rate. Further follow-up studies are planned to investigate the relation between ACPA titre and disease activity in more detail. For this purpose, the development of a specific enzyme-linked immunosorbent assay is in progress. This technique is not only less labour intensive, but also allows us to measure ACPA more precisely.

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