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Antineutrophil cytoplasmic antibodies in myelodysplasia

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SUMMARY

Antibodies to neutrophil cytoplasmic antigens (ANCA) are good serological markers for patients with mainly vasculitic conditions. Two main types of ANCAs have been detected, the first termed cytoplasmic antineutrophil cytoplasmic antibody (cANCA) are mainly associated with patients with Wegener's granulomatosis, the other termed perinuclear antineutrophil cytoplasmic antibody (pANCA) are mainly associated with patients with renal vasculitis, rheumatic and collagen disorders. These antibodies are against various constituents of neutrophil granules. In patients with myelodysplasia, defects in normal granulocyte development are seen. We report a series of twelve patients with myelodysplasia of whom at least four showed a low titre and one a high titre of pANCA. Two of these patients also had demonstrable activity against myeloperoxidase (MPO). None of these patients had any evidence of systemic or cutaneous vasculitis or of any autoimmune disorder. There was no pANCA positivity in an age matched control group.

INTRODUCTION

The myelodysplastic conditions are a heterogenous group of haematological disorders characterised by refractory cytopenias. They include quantitative and qualitative abnormalities of the myeloid cell lineage resulting in reduced chemotaxis, phagocytosis and enzyme content.[1,2] In the 1980s autoantibodies directed against the cytoplasmic constituents of neutrophils (ANCA) were discovered. Many of the antigens were found to be enzymes normally contained within the neutrophil granules, these include myeloperoxidase, proteinage 3, elastase, lactoferrin and cathepsin G.[3]

At least two types of ANCAs can be distinguished by indirect immunofluorescence on ethanol fixed neutrophils: cytoplasmic (cANCA) and perinuclear (pANCA). Associations have been shown to exist between the occurrence of these antibodies and various vasculitic disorders such as cANCA with Wegener's granulomatosis and microscopic polyarteritis, and pANCA with renal vasculitis as well as various rheumatic and collagen vascular disorders.[4]

This study was undertaken to look into the occurrence of antineutrophil cytoplasmic antibody activity in myelodysplasia.

SUBJECTS AND METHODS

Twelve patients with an established diagnosis of myelodysplasia according to the French - American - British (FAB) classification,[5] had their sera tested for ANCA by indirect immunofluorescence according to the method described by Wiik[6] at the 1st International Workshop on ANCA as did twelve healthy controls. Briefly, serum samples were screened for ANCA at dilutions of 1 in 10 and 1 in 20, and if positive titrated until endpoint. 80µl of appropriate dilution was incubated at room temperature for 30 minutes on ethanol fixed neutrophil cytospins and dryed before use. After washing in PBS (Phosphate Buffered Saline) antihuman globulin conjugate (Dako UK, Ltd.) was added and incubated for a further 30 minutes

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TABLE

Details of patient group

Case	*Diagnosis*	*Age in years*	*pANCA*[1]	*MPO*[1]	*pANCA*[2]	*MPO*[2]
1.	RA	83	–	<15%	–	20%
2.	RA	90	20	<15%	10	<15%
3.	RAEB	81	20	<15%	20	<15%
4.	RAEB	72	–	<15%	–	<15%
5.	RAEB	78	–	<15%	20	<15%
6.	RAEB	79	–	<15%	–	<15%
7.	RAEB	78	20	16%	10	16%
8.	RA	71	160	<15%	NOT AVAILABLE	
9.	RAEB	78	20	<15%	20	<15%
10.	RAEB	70	–	<15%	10	<15%
11.	RA	54	–	<15%	–	<15%
12.	RARS	64	–	<15%	–	<15%

ABBREVIATIONS:

RA	Refractory anaemia
RAEB	Refractory anaemia with excess blasts
RARS	Refractory anaemia with ring sideroblasts

at room temperature. If required reincubation was performed with antihuman IgG and IgM (Dako UK, Ltd.) to determine immunoglobulin class specificity. The slides were then washed in PBS, mounted and viewed using a fluorescence microscope.

Serum containing pANCA was recognised as a non-granular perinuclear or nuclear staining of the neutrophils. A cANCA pattern was recognised by a granular cytoplasmic staining. If positive for either p or cANCA the serum was titrated until an end point was obtained. Sera from patients with antinuclear antibody (ANA) also exhibit a pANCA pattern therefore all positive sera were tested for ANA by indirect immunofluorescence. All serum samples were also tested for the presence of antimyeloperoxidase antibodies (MPO) by ELISA (Biodiagnostics Ltd England), Positivity was taken as greater than 15% of positive standard, and for the presence of anti-nucleolar, anti-mitochondrial, anti-smooth muscle and anti-gastric parietal cell antibodies by the indirect immunofluorescence technique.

Patients were retested after an interval of 2-6 months where possible.

A control group of healthy people from a similar age group was also tested.

RESULTS

Sera from 5 out of the 12 patients studied showed titres for pANCA on initial testing; a further patient negative on initial testing was positive on repeat testing. No patients showed positivity for cANCA, antinuclear antibody or other autoantibodies. One patient had borderline activity against myeloperoxidase initially, two patients were positive on repeat testing. The details are given in the table. In the control group no cases showed positivity for pANCA though two had demonstrable cANCA activity; all were negative for antimyeloperoxidase activity.

DISCUSSION

We have shown positive titres for pANCA in at least 5 out of 12 patients with myelodysplasia and

none in a control group of similair age. There is a strong association between ANCA in high titre (80 or greater) and many vasculitic diseases. There have been a few reports of an association between myelodysplasia and various vasculitides,[7,8] but none of our patients showed any clinical or laboratory evidence of systemic or cutaneous vasculitis or of any autoimmune disorder.

The pathophysiological significance of ANCA, especially in low titre, is uncertain. A recent study has shown that a wide range of conditions can be associated with low ANCA titre, especially pANCA. Many of these cases did not show any evidence of vasculitis.[9] Certain experimental data suggest that ANCAs do play a role in the pathogenesis of vasculitis.[10] Stimulated neutrophils express a number of molecules, including neutrophil granule enzymes at their surface. It has been suggested that ANCAs react with these surface enzymes to further activate neutrophils into producing reactive oxygen species with the further release of lysosomal enzymes leading to vasculitis.

Patients with myelodysplasia have reduced numbers of abnormal neutrophils and are at an increased tendency to infection.[11] In short term culture granulocyte-macrophage colony forming units (CFU-GM) are reduced and abnormal,[12] so indicating that there is a defect in neutrophil development. We have discovered that in this group low titres of pANCA are present, this in contrast with the control group where pANCA was not detected.

We postulate that the abnormal neutrophils in myelodysplasia may lead to exposure of abnormal constituents to the host immune system thereby stimulating antibody formation.

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