thighs. Furthermore, a computed tomogram verified subependymal calcifications. He had no central nervous system symptoms. The following clinical investigations were normal: ophthalmic examination, roentgenograms of the skeleton and chest, and ultrasonographic examination of upper abdomen, including kidneys and heart. Biochemically, no abnormalities apart from those associated with hyperparathyroidism were found, including thyroid hormone and androgen activities.

Discussion

The coexistence of tuberous sclerosis (prevalence one in 100 000) and primary hyperparathyroidism (population prevalence in adults about one in 1000, much lower in juveniles), was unexpected. Only one previous case of tuberous sclerosis and parathyroid adenoma has been described. Over the past 10 years different symptoms, mostly adenomas, have been included in the syndrome. Parathyroid adenoma might be another adenomatous abnormality that is associated with tuberous sclerosis.

In hyperparathyroidism decalcification of bones with defects in metacarpal bones occur. The calvarium is thickened with lacunar

changes and cysts. In secondary hyperparathyroidism osteosclerosis is a feature.

In tuberous sclerosis intracranial calcification, cortical thickening of bones, osteosclerosis and bone cysts are common. The association between skeletal changes in tuberous sclerosis and hyperparathyroidism is not obvious, but, on the other hand, cannot be excluded. We therefore suggest that disturbances of calcium metabolism may occur in patients with tuberous sclerosis, and suggest that plasma calcium should be measured in these patients.

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Distinction between antinuclear antibody and P-ANCA

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Abstract

To differentiate between perinuclear immunofluorescence staining of antinuclear antibody (ANA) and the perinuclear form of anti-neutrophil cytoplasmic antibody (P-ANCA), the pattern after formaldehyde vapour fixation of normal human neutrophils was compared with that of standard ethanol fixation. Fifteen out of 17 myeloperoxidase antibody positive sera showed cytoplasmic staining on formaldehyde vapour fixed cells; 30 of the 32 ANA positive samples became negative or gave weak nuclear staining on the same substrate.

Formaldehyde vapour fixation is a simple, useful technique for differentiating between ANA and P-ANCA.

Anti-neutrophil cytoplasmic antibody (ANCA) is emerging as an important class of autoantibody, useful in the diagnosis of vasculitic diseases. The correlation between the cytoplasmic form (c-ANCA) and Wegener's granulomatosis is well established.¹ Perinuclear staining on neutrophils fixed in eth-

anol is gradually receiving more attention due to its reported association with myeloperoxidase antibody and necrotising and crescentic glomerulonephritis.² This artefactual perinuclear immunofluorescence, however, can closely resemble the perinuclear staining pattern of antinuclear antibody (ANA). In other cases P-ANCA may be masked by strong homogeneous staining due to the presence of associated ANA. The differentiation between P-ANCA and perinuclear ANA is of clinical importance, especially in populations with a high incidence of systemic lupus erythematosus (SLE). We describe a simple method of distinguishing between the two entities in routine clinical laboratory practice.

Methods

Two groups of serum samples were analysed. Group 1 comprised 32 patients with SLE with positive ANA demonstrable on Hep-2 cells but who were negative for myeloperoxidase antibodies. Group 2 comprised 17 serum samples from 11 patients with the antibodies. The presence of myeloperoxidase antibodies was determined by enzyme linked immunosorbent

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assay (ELISA), as described by Falk.² Unknowns were read from a standard curve created with a positive internal reference serum. The degree of binding was then expressed as a percentage of the reference. The normal range was defined as the percentage binding up to 3 SD above the mean of 52 normal healthy adults.

For indirect immunofluorescence, normal human leucocytes were sedimented with 2% methylcellulose, washed, and deposited on glass slides by cytocentrifugation. Serum samples diluted one in 20 were tested by immunofluorescence on neutrophil cytopsin slides fixed by two methods: (i) 95% ethanol at 4°C for five minutes, in accordance with the method recommended at the first International Workshop on ANCA;³ and (ii) formaldehyde vapour at room temperature for four minutes.

Results

Of the 17 myeloperoxidase antibody positive samples tested, all showed typical perinuclear immunofluorescence on neutrophils fixed in ethanol and 15 showed granular cytoplasmic staining on cells fixed in formaldehyde vapour (figure 1). The remaining two had low myeloperoxidase antibody activity and showed weak perinuclear staining on cells fixed by both methods. Cytoplasmic staining for the latter, however, could still be shown on formaldehyde vapour fixed substrate if a 1 in 5 dilution of sera was used.

Of the 32 SLE samples tested, 10 showed perinuclear staining on ethanol fixed cells that was indistinguishable from myeloperoxidase

antibody positive sera: the remaining 22 produced various amounts of nuclear fluorescence. With formaldehyde vapour fixation, SLE sera either gave the same or weaker nuclear staining (10/32) or became negative (20/32). Only two showed very weak cytoplasmic fluorescence which could well have been due to the increased background staining associated with this method.

Discussion

Our observations confirmed that myeloperoxidase antibodies produced an artefactual perinuclear immunofluorescence staining on ethanol fixed neutrophils. This pattern was indistinguishable from that of perinuclear ANA, sometimes seen when neutrophils were used as substrate for ANA determination. Wiik termed the latter granulocyte specific antinuclear antibody (GS-ANA). He reported its association with autoimmune diseases such as rheumatoid arthritis and Felty's syndrome.⁴

Our findings also suggested that the perinuclear immunofluorescence produced by myeloperoxidase antibodies could be "converted" to the cytoplasmic pattern if formaldehyde vapour was substituted as the fixative. The fixation time was, however, critical: prolonged fixation removed both cytoplasmic and nuclear staining; insufficient fixation, on the other hand, failed to show the "conversion". Other fixatives used, including liquid formalin, formol-acetone, and acetone, gave inconsistent results and sometimes produced unacceptable background staining. The reason why formaldehyde vapour was superior to liquid formalin is not entirely understood. Our speculation is that it is a milder form of fixation which can be more closely controlled, allowing cellular structure to be preserved and avoiding the redistribution of proteins in vitro.

In conclusion, formaldehyde vapour fixation can provide additional information on the specificity of perinuclear staining on ethanol fixed neutrophils. Conversion to the cytoplasmic pattern usually indicates the presence of myeloperoxidase antibodies. This simple method of fixation may prove invaluable as a screening test for myeloperoxidase antibodies, and for the differentiation of P-ANCA from ANA, which may occur together, particularly in cases of SLE.

Immunofluorescence pattern of myeloperoxidase antibody positive serum sample on a neutrophil cytopsin slide using (A) ethanol fixation at 4°C for five minutes; and (B) formaldehyde vapour fixation at room temperature for four minutes.

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