PAPERS AND ORIGINALS

Smooth Muscle Antibody in Malignant Disease

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British Medical Journal, 1971, 4, 511-513

Summary

Smooth muscle antibody at titres of 1/10 or more was found in 54 (67·5%) out of 80 patients with malignant disease and in 9 (20%) out of 46 controls. The incidence of S.M. antibody ranged from 18/30 (60%) in malignant melanoma to 7/8 (83%) in carcinoma of the ovary. The presence of this antibody is possibly related to changes in the malignant cell membrane.

A new antibody directed at an antigen presumed to be located in bile canaliculi among other sites is described.

Introduction

Smooth muscle (S.M.) autoantibody, demonstrated by indirect immunofluorescence on cryostat sections of tissues containing smooth muscle fibres, is a recognized serological marker of autoimmune forms of chronic hepatitis (Doniach and Walker, 1969), and is usually present also in the sera of patients with acute infectious hepatitis (Farrow *et al.*, 1970). S.M.-antibody-positive sera have been shown to react with a normal constituent of the membranes of certain cell types (Farrow *et al.*, 1971). Since the malignant transformation of cells is often associated with changes in the cell membrane we report here the results of a preliminary survey for this antibody in a variety of malignant disorders.

Materials and Methods

Patients' Sera.—To avoid effects resulting from therapy serum was taken before any form of treatment and stored at −20°C. Sera were examined from 80 patients with histologically confirmed malignant tumours (30 malignant melanoma, 8 hypernephroma, 16 neuroblastoma, 6 carcinoma of the bronchus, 8 carcinoma of the ovary, and 12 carcinoma of the breast). The ages of the patients ranged from 1 to 76 years. Sera from 46 normal subjects aged 17-79 years were also examined.

Immunofluorescence.—Fluorescent staining was carried out by the indirect "sandwich" method. Serum diluted 1/10 was applied to 6-μ cryostat sections from a composite block of rat stomach, kidney, and liver for half an hour, and, after washing, the sections were treated with fluorescein-labelled monospecific antihuman globulin reagents directed at IgM or IgG. These were prepared from specific rabbit antihuman IgG and sheep antihuman IgM with fluorescein isothiocyanate (isomer 1) (B.D.H.) according to the procedure described by Holborow and Johnson (1967). For both conjugates the ratio $O.D._{495}$: $O.D._{280}$ was 1·1, and both were used at a dilution of 1/60. The sections were then examined for staining of smooth muscle fibres between the gastric glands, in the muscularis mucosae, and in the walls of arteries. Sera giving positive staining at these sites gave a polygonal pattern of staining around liver cells (Johnson *et al.*, 1966) and also stained renal glomeruli (Whittingham *et al.*, 1966). All these five sites in rat tissue contain antigenic determinants in common with human smooth muscle (myometrium) (Farrow *et al.*, 1971), and we have shown that sera which stain rat smooth muscle also stain human myometrium. Positivity was assessed on a points system which has produced comparable results in the hands of different observers. Each of the five features described above was scored as follows: 0 = no staining, 1 = probable staining, 2 = definite staining, and 3 = brilliant staining, the total score for each serum being assessed out of a possible 15; 0-5 was regarded as negative, 6-9 as weak positive, and 10-15 as strong positive. All positive sera were then titrated.

Microscopy.—Stained sections were examined with a Reichert Zetopan microscope equipped with an IQ light source, FITC-3 (Balzer) interference filter, a Wratten 12 secondary filter, and a cardioid dark ground condenser.

Absorption Tests.—Sera were diluted with an equal volume of phosphate-buffered saline (pH 7·2). One volume of this diluted serum was then mixed with four volumes of an extract of human myometrium in 50% glycerol (Jones *et al.*, 1970) kindly supplied by Dr. U. Groschel-Stewart. The mixture was then incubated at 37°C for one hour. The supernatant after centrifugation was tested as described above.

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Results and Discussion

Smooth muscle antibody at titres of 1/10 or more was found in 54 (67·5%) of the 80 patients and in 9 (20%) of 46 controls. It is of note that all the controls positive for S.M. antibody were under the age of 31; almost half the controls tested were over this age. The titres of S.M. antibody in the controls and the cancer patients are compared in Table I, and Fig. 1 shows the distribution of IgG and IgM antibody titres. The incidence of S.M. antibody ranged from 18/30 (60%) in malignant melanoma to 7/8 (83%) in carcinoma of the ovary. In most positive sera the antibody was predominantly IgG, though IgM antibody was often also present in lower titre. In a few patients IgM antibody alone was found. With one exception titres did not exceed 1/40.

TABLE I—*Comparison of Titres (IgG or IgM) of S.M. Antibody in Control and Cancer Patients*

	S.M. Antibody Titre				
	0	1/10	1/20	1/40	1/80
Cancer patients ..	32·5%	25%	27·5%	14%	1%
Controls	80%	11%	9%		

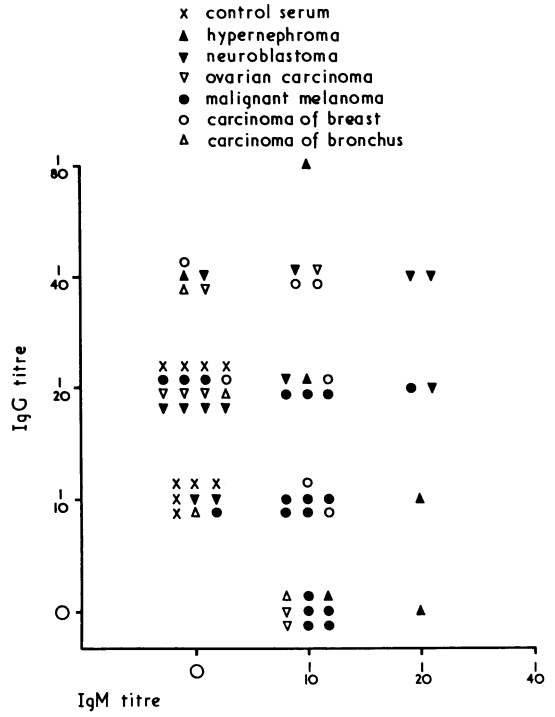

FIG. 1—Distribution of IgG and IgM S.M. antibody titres of 1/10 or more in control sera and sera from the different cancer groups.

The S.M. antibody present in the sera of patients with malignant disease seemed to be identical with that occurring in the sera of patients with chronc active and acute infectious hepatitis. Identical patterns of staining were produced on the composite tissue sections and irrespective of the diagnosis the antibody was completely removed by absorption with an extract of human myometrium. This was shown for five sera from patients with malignant disease, and for eight sera from patients with acute or chronic hepatitis. Antinuclear antibodies and gastric parietal cell antibody were not removed by the absorption procedure.

The overall incidence of antinuclear factor was 24% among the cancer patients, being highest in patients with carcinoma of the breast (50%) and absent in patients with neuroblastoma and in the controls. This absence of antinuclear antibody in neuroblastoma may well reflect the resistance of the immune system in childhood to those autoantigenic stimuli that require impairment of thymus cell control for their humoral expression. Table II shows the distribution of antinuclear factor titres.

TABLE II—*Incidence of Antinuclear Antibodies in Cancer Patients*

	No. Positive/No. Tested	Titres of Positive Sera	
		IgG	IgM
Malignant melanoma ..	6/30	—	1/10
		—	1/20
		1/20	1/40
		1/10	—
		—	1/10
		1/10	1/20
Hypernephroma ..	4/8	1/20	—
		—	1/20
		1/10	—
		1/10	1/40
Neuroblastoma	0/16	—	—
Carcinoma of bronchus ..	1/6	1/20	—
Carcinoma of ovary ..	2/8	1/20	—
		1/40	—
		—	1/10
		—	1/10
Carcinoma of breast ..	6/12	—	1/10
		1/20	1/20
		1/10	1/10
		—	1/10

In seven sera (one control, one malignant melanoma, five neuroblastoma) a new IgG antibody was found. This gave brilliant and distinct staining of sections of liver (Fig. 2A) in a pattern mimicking the polygonal pattern produced by true S.M. antibody, but differing from it in showing segments of sharply defined double-line staining in relation to most of the parenchymal cells, suggesting that this new antibody may be directed at an antigen present in bile canaliculi. Sera giving this pattern stained with equal brilliance the renal glomeruli (Fig. 2B); a constituent of arterial walls in close relation to but distinct from the fibres of the smooth muscle coat; and the free luminal surfaces of the epithelial cells lining the gastric glands (Fig. 2C) and certain renal tubules (probably the collecting tubules). This new antibody was distinguishable from S.M. antibody though the two occurred together in the five positive sera from neuroblastoma. The pattern of staining produced by both antibodies was anatomically closely related in the liver, glomeruli, and arterial walls, but the new antibody did not stain the gastric muscularis mucosae nor the smooth muscle fibres between the gastric glands (Fig. 2D). The distinction between the two antibodies was especially apparent on titration; S.M. antibody titred out at a maximum dilution of 1/40, while the new antibody still showed strong staining of the sites described up to dilutions of 1/320. Furthermore, the ability of sera containing the new antibody to produce the characteristic staining was unaffected by absorption with human uterine extract.

We have assumed that these new staining patterns result from a single antibody, since the separate features dilute out simultaneously and belong to one immunoglobulin class, IgG. Though we have not yet defined the new antigen chemically, it too is evidently a component of normal human tissue since the antibody produces the same staining pattern on human fetal liver as on rat liver. This antibody provides further evidence that autoantibodies against normal tissue components may be produced in malignant disease.

Smooth muscle antibody seems to be present in most of the sera examined from patients with malignant disease. Since it has been shown that this antibody also reacts with a normal constituent of the cell membrane of some cell types (Farrow *et al.*, 1971) the interaction of this antibody with tumour cell mem-

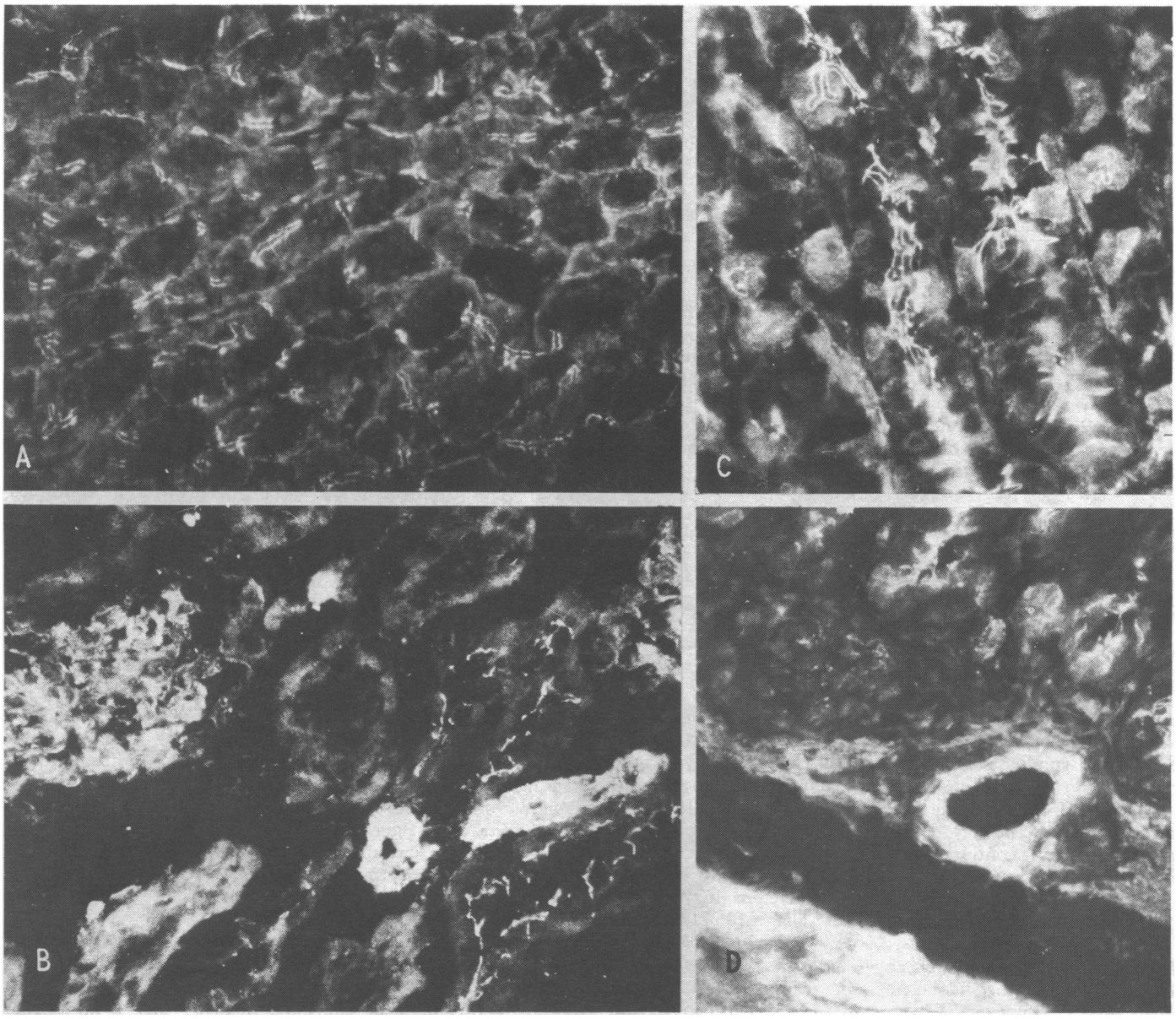

FIG. 2—Cryostat sections of rat tissues stained by indirect immunofluorescence (anti-IgG) to show reactions of the new autoantibody. A-C, neuroblastoma serum. D, melanoma serum. (A) liver: outlining of parenchymal cells (polygonal pattern) due to smooth muscle antibody, with segments of double-line staining due to the new antibody, probably reacting with bile canaliculi. (B) kidney: staining of glomeruli and segments of small arteries; also linear staining of free (luminal) surfaces of cells lining three tubules (right). (C) Stomach: linear staining of free (luminal) surfaces of cells lining the gastric glands. (D) stomach: serum containing the new antibody but no smooth muscle antibody stains arterial wall but not adjacent muscularis mucosae. The thick muscle coat (bottom left) contains the same antigen. Gastric glands also stained as in (C).

branes must obviously be excluded before the existence of tumour-specific antibodies can be recognized.

It is not clear at the present time how production of S.M. antibody is related to the presence of a tumour. Hitherto S.M. antibody has been reported chiefly in hepatic disease, and this might suggest that its demonstration in cancer patients is merely a manifestation of metastatic involvement of the liver. Preliminary examination of the clinical data relating to the patients with malignant melanoma does not support this explanation since S.M. antibody was found in six out of nine patients in whom the lesion was considered to be in the primary stage. The presence of S.M. antibody in this group of patients may also argue against necrosis as a significant factor in its production. Among other possibilities under investigation by us is that a change in the membrane of the malignant cell results in the exposure of an actomyosin-like antigen, or leads to its production in a more immunogenic form.

This investigation was supported by a grant to the Chester Beatty Research Institute by the Medical Research Council and the Cancer Research Campaign, and also by a special grant to one of us (J.M.A.W.) by the Wellcome Foundation. We are grateful to Dr. Iris Hamlin, Dr. Graham Currie, and Dr. P. Seah for help in providing sera, and to Miss Lydia Bertrand and Miss Nean Ferguson for technical help.

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