LOSS OF GASTRO-INTESTINAL-SPECIFIC ANTIGEN IN NEOPLASIA

BY

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Loss of organ-specific antigens has been shown to occur in experimental and human carcinomas (Weiler, 1952, 1956a, 1956b, 1956c, 1959; Nairn *et al.*, 1960; Piekovski and Witz, 1961; Goudie and McCallum, 1962) and may have an important bearing on their malignant behaviour (Burnet, 1957; Green, 1959, 1961; Vogt, 1960; Nairn, 1962a). The present investigation, by serological and immunofluorescence methods, of a gastro-intestinal-specific antigen (Nairn *et al.*, 1961; Nairn, 1962b) in human gastro-intestinal neoplasia demonstrates that loss of antigen is a feature of colonic and gastric carcinomas. Lesser degrees of depletion are found in colonic polyps, and lesions diagnosed by conventional methods as benign or premalignant have shown qualitative antigenic differences which are readily detected by fluorescent antibody-staining.

Methods

Preparation of the gastro-intestinal-specific antigen and antiserum, serological testing, immunofluorescence, and histochemical methods have been described in the preceding paper (Nairn *et al.*, 1962). The material examined comprised fresh operation specimens of 43 colonic carcinomas, including lymph-node metastases from nine of these, colonic polyps from 11 cases, 11 gastric carcinomas (one with lymph-node metastasis), and a single gastric polyp. Six of the colonic and two of the gastric carcinomas were classed as mucoid; one colonic polyp was from a patient with the Peutz-Jeghers syndrome. For immunofluorescence studies, snap-frozen blocks of the gastro-intestinal specimens were taken wherever possible from the centre of the tumour, from the adjacent normal bowel wall, and from the edge of the lesion to include both normal mucosa and tumour tissue.

Results

Depletion of the gastro-intestinal-specific antigen in a microsomal fraction from colon carcinoma has already been demonstrated by the results of the serum absorptions described in the preceding paper (Nairn *et al.*, 1962). The anti-gastro-intestinal activity of the kidney-absorbed anti-colon serum was neutralized by absorption with a microsomal fraction from normal colonic mucosa whereas absorption with the corresponding fraction from tumour tissue left complement-fixing and strong immunofluorescence staining activity. Table I of the preceding paper (see p. 1789) should be compared with the present results summarized in the Table printed on this page.

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Only two precipitin lines were produced in gel-diffusion tests of the unabsorbed antiserum against colon carcinoma microsomal material, and they showed the reaction of identity with two of the three lines against the colon mucosa microsomes. We cannot explain the absence of the third line: the antigen concerned was apparently present in the carcinoma microsomal material, as this could be used to neutralize the

Reactions of Unabsorbed and Absorbed Anti-human-colon Rabbit Serum with Gastro-intestinal Tumours

Absorptions with human microsomal material:	Anti-colon Serum				Normal (Pre-immunization) Serum	
	Nil	Kidney +			Nil	Kidney
		Kidney	Colon Cancer	Colon Mucosa		
Gel-diffusion lines at 48 hours against colon carcinoma microsomal material	2	1	0	0	0	0
Staining with fluorescent antibody:						
Benign polyps	++	+	+	-	(+)	-
Premalignant polyps	..	Scattered staining of individual cells of acini			-	-
Carcinomas	..	+	-*	-*	(+)	-

* An occasional carcinoma showed staining similar to the premalignant polyps, though the scattered cells were much sparser.
(+) = Weak unsystematic staining.

corresponding serum antibody in absorption tests; perhaps its concentration was too low for demonstration by gel diffusion. It is not the organ-specific antigen responsible for the immunofluorescence staining, because this was obtained with sera from which all three precipitins had been removed by absorption with carcinoma material.

Fluorescent Staining

Most carcinomas (45 out of the 54 cases) showed no specific fluorescence with absorbed antiserum though the adjacent normal mucosa stained brilliantly (Fig. 1). Nine adenocarcinomas, including one of the two mucoid gastric carcinomas and four of the six mucoid colonic lesions, showed occasional brightly fluorescent abnormal cells scattered among otherwise non-staining acini (Fig. 2). The mucoid carcinomas with such cell staining also

FIG. 1.—Section of human colon including normal mucosa above and edge of adenocarcinoma below. Treated first with rabbit anti-colon serum which had been absorbed with microsomal material from human kidney and colon carcinoma, and then with RB 200-conjugated goat anti-rabbit-globulin. Bright specific staining in normal colonic glands but no staining of the adenocarcinomatous acini, the form of which can be made out from their autofluorescence. The scanty fluorescent flecks in the carcinoma are due to non-specific staining of leucocytes. ($\times 130$)

contained a few extracellular pools of mucin which stained specifically but never with great intensity. Although all the pools of extracellular mucin reacted positively with alcian blue, only a small proportion combined with the fluorescent antibody. Thus mucoid carcinomas appear to be capable of producing some specific antigen which is apparently derived from a few scattered cells, but much of the mucin secreted is of different molecular structure. Poorly differentiated spheroidal-cell and signet-ring-cell carcinomas, which comprised 9 of the 11 gastric lesions, were less easily studied than the adenocarcinomas, chiefly because the reaction of normal gastric mucosa was itself inconstant; suitable tumour margins adjacent to well-stained mucosa were infrequent. However, no specific fluorescence was found in the tumour cells although they commonly contained mucin which reacted with alcian blue.

One gastric and three colonic polyps, which by conventional histological criteria would be regarded as pre-invasive or premalignant—that is, with glandular aberration, nuclear hyperchromatism, and cytoplasmic basophilia but no invasive growth—showed a fluorescence picture resembling that of the carcinoma illustrated in Fig. 2 but with many more brightly fluorescent acinar cells. As in the carcinomas, staining was either complete or absent, and never partial; the cells concerned contained abnormally shaped clumps of intensely fluorescent material quite different from the well-organized appearance of normal mucosal cells described in the preceding paper.

In contrast, the staining pattern found in the eight benign colonic polyps, including one from the patient with the Peutz-Jeghers syndrome, closely resembled that of normal mucosa (Fig. 3). It was fairly uniform in all cells of the acini, usually dimmer than normal mucosa and without any of the brilliantly fluorescent cells of the premalignant lesions. Cell staining was often confined to a narrow band adjacent to the gland lumen, while the unstained area corresponded to the conspicuous basophilic basal zone of conventional histological preparations.

FIG. 2.—Section of colon carcinoma, treated as in Fig. 1, showing mostly unstained acini but with a few scattered fully antigenic cells. This picture was found more commonly in mucoid lesions. ($\times 120$.)

Specific fluorescence has not been observed in the metastatic cells in lymph nodes from the ten cases studied; only two of these cases showed scattered fluorescent cells in the primary tumour.

FIG. 3.—Section of benign polyp of colon, treated as in Fig. 1, showing fairly uniform staining of the cell zone adjacent to the acinus lumen. The non-staining basal zone is more conspicuous than in normal mucosa. ($\times 120$.)

Discussion

This study provides further evidence that loss of organ-specific antigens is a characteristic feature of malignancy. It may be significant that another group of polysaccharide antigens, the blood-group substances, which are not organ-specific, persist in gastric carcinomas (Glynn and Holborow, 1959). Absence of the specific gastro-intestinal mucin from a large proportion of the gastric and colonic carcinomas is not an altogether unexpected observation, since it is well known that such tumours may fail to secrete detectable mucin. The striking finding is that mucin-secreting cells and glands of certain tumours or certain areas of tumours failed to react with the fluorescent antibody. In the few carcinomas, mainly mucoid, in which brightly stained cells were observed, these were sparse and scattered in non-fluorescent acini. It seems reasonable to suppose that the specific extracellular gastro-intestinal mucin occurring in some carcinomas is derived from such cells; it could accumulate in an amount disproportionate to the number of secreting cells because of lack of an organized secretory system with consequent failure of elimination. Preservation of better organization in the premalignant polyps, which also showed scattered stained cells, would tend to prevent such extracellular accumulation of mucin.

The qualitative difference in antigenic patterns between benign and premalignant polyps could hardly have been detected without immunofluorescence. *In vitro* serological procedures with antigen prepared from homogenates or eluates of these lesions could not have distinguished between the total loss of antigenic material in a proportion of cells of the premalignant lesions and the partial loss in all cells of the benign lesions. This difference and the retention of the scattered fully antigenic cells in a few of the carcinomas are difficult to explain but most probably indicate instability of the neoplastic cell phenotype—that is, the

failure of antigen production is more likely to be due to genetic change associated with incomplete penetrance than to genetic loss. An alternative or additional possibility cannot, however, be excluded—that exuberant cell division causes dilution of a particulate cytoplasmic factor responsible for the formation of the antigen.

Loss of organ-specific antigens could influence tumour metastasis by diminishing normal cell adhesion at the primary tumour site or by removing specific cell differences which might prevent secondary growth of tumour cells in another organ. A preliminary study has been made of the possible importance of the gastro-intestinal-specific antigen in this connexion: it could, in theory, behave as a "marker" antigen by coating the mucosal cell surface. However, the information so far available is too meagre for any useful discussion, though the absence of antigen in lymph-node metastases of all ten cases examined is a clear indication for further study. More investigation is also required to assess the possible diagnostic usefulness of immunofluorescence staining of gastro-intestinal tumours, especially for distinguishing between benign and premalignant polyps. Long-term follow-up studies of a large series of cases will be needed to confirm that the observed qualitative staining differences provide a reliable means of distinguishing benign from malignant states.

Summary

A gastro-intestinal-specific antigen demonstrated in mucoid cells by immunofluorescence staining and serological methods, probably an acid mucopolysaccharide, was totally absent from 45 out of 54 gastro-intestinal carcinomas. In the remaining nine carcinomas, five of which were mucoid, staining of antigen was obtained in a small proportion of cells scattered among unstained acini. The immunohistological appearance of four premalignant polyps was similar but the scattered fluorescent cells were more numerous. In eight benign polyps, however, staining was fairly uniform in all cells of the acini, and, though dimmer, showed much the same distribution as in normal mucosa. Lymph-node metastases from ten cases did not stain.

The staining differences, which appear to portray an intrinsic distinction between benign and malignant states, may prove to be of diagnostic value.

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LUMBAR SPINE DENSITOMETRY

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Spinal osteoporosis is characterized by a loss of trabecular bone which in turn causes a reduction in radiographic bone density. In severe cases the diagnosis is made easily by the subjective assessment of a radiologist, but in milder cases that is quite unreliable. There is therefore a need for a reliable and sensitive method of measuring the density of the vertebral bodies. The available methods applicable to the ulna (Keane, Spiegler, and Davis, 1959; Doyle, 1961), the metacarpal (Bywaters, 1948; Koch and Kaplan, 1961), and the os calcis (Mayo, 1961) cannot be applied to the spine, which is mainly affected by the osteoporotic process.

The present paper describes a method of assessing spinal density and presents some preliminary results.

Methods

The procedure depends on the comparison of the radiographic densities of the vertebral bodies and the intervertebral disks on a lateral tomogram of the lumbar spine. Variations in physical characteristics between exposures, such as line voltage, development, and film speed, are controlled by exposing a specimen of normal human lumbar spine simultaneously. The body:disk density ratio of the patient's spine can therefore be compared with that of the standard spine on the same film.

A dissection of the second, third, and fourth lumbar vertebrae from a normal human spine mounted in formol-saline in a "perspex" box is used as the standard. The depth of the fluid in the box is such that the x-ray density of the standard is similar to that of a normal subject of average size.

The patient is placed on the couch in the right lateral position with the standard spine placed against his back. The height of the standard is adjusted with strips of wood until it is in the same horizontal plane as the patient's spine (Fig. 1). A single tomographic cut is then made through the sagittal plane of the patient and the standard. A typical film is shown in Fig. 2.

The images of the spine and the standard are cut out to make two film strips about 2 in. (5 cm.) wide and