

Tumour-associated products

Nature and significance of the antigens associated with human gastrointestinal tumours

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The problem of tumour-associated antigens is of importance, and many attempts are being made to prove their presence in experimental and human neoplasms. As far as the latter are concerned, there has been much work in recent years on gastrointestinal adenocarcinomas in which several antigens have been identified by either immunochemical studies or by techniques which detect cell-mediated immune reactions. This paper summarizes our present knowledge of these antigens.

Immunochemical Studies

Immunochemical studies have led to the demonstration of several tumour-associated antigens, two of which will be described here.

The carcinoembryonic antigen (CEA) of gastrointestinal tumours was discovered in the extracts of colonic cancers by Gold and Freedman (1965) and later by von Kleist and myself (1969a). Although it is only one of the carcinoembryonic antigens most information is available about it, hence the current name of carcinoembryonic antigen which is given to it, and the generally used abbreviation 'CEA'. Due to a special feature, which was of great help in its discovery, namely, its solubility in perchloric acid, and to a few preparative steps including mainly filtration on Sephadex G-200 and Sepharose 6B, CEA was obtained in a pure state. Its physicochemical properties were determined (Krupey, Gold, and Freedman, 1968; Banjo, Gold, Freedman, and Krupey, 1972): it is a glycoprotein having a molecular weight around 200 000 and an estimated sedimentation constant of 7-8 S. It contains about 50% carbohydrates: sialic acid, mannose, galactose, acetyl N-glucosamine, and fucose. The sequence and the linkage of these sugars is still unknown. The electrophoretic mobility of CEA is similar to that of a beta globulin, but it is heterogeneous. It was

possible to separate by chromatography on DEAE cellulose the fast moving molecules (alpha CEA) from those of slowest mobility (beta CEA) (Newman, Petras, Hamilton, Hager, and Hansen, 1972). Both have the same sugars, but in different amounts; they react identically with anti-CEA antisera.

The high percentage of carbohydrates in CEA molecules led some authors to suggest that this antigen might be related to the blood group substances, especially group A. However, the immunodominant sugar of A substance, acetylgalactosamine, is generally considered to be absent from CEA. This substance is a frequent contaminant of CEA, and since this fact has been ignored by some workers, it may be the explanation of some later unconfirmed theories.

Carcinoembryonic antigen was found in all the adenocarcinomas of the colon and the rectum, of the pancreas, the stomach (with a few exceptions), and in some hepatomas. It was also present in the metastases of these neoplasms. Since the yield of CEA is very low, it is necessary to extract hundreds of grams of neoplastic tissue to obtain a few milligrams of purified CEA; this amount can be obtained almost only from metastases.

Carcinoembryonic antigen is also found in the mucosa of the fetal colon, and in some glands of the fetal gastric mucosa. We have never detected it in the small intestine of the fetus.

CELLULAR LOCALIZATION OF CEA

With immunofluorescence techniques, we showed that CEA is localized at the apical pole of malignant epithelial cells—the usual pattern was that of a fluorescent line bordering the lumen of the glands (von Kleist and Burtin, 1969b). Intraluminal deposits were often stained also. When the neoplasm lost its glandular organization, which appeared to be a frequent event in gastric tumours, some invasive

cells were surrounded by a fluorescent ring: CEA seemed to be associated with the cell membrane. In cases of linitis plastica, different results were obtained: CEA was mainly in the cytoplasm, occupying the whole cell or part of it, giving either diffuse, or more frequently granular, staining. Very bright fluorescence—sometimes reminiscent of that of immunoglobulins in plasma cells—was obtained by either immunofluorescence or the immunoperoxidase method. The cells containing such large amounts of CEA stained strongly with the periodic acid Schiff (PAS) technique (Burtin *et al.*, 1973).

In fetal colonic mucosa, CEA has the same localization as in colonic tumours, ie, on the luminal border of the epithelial cells.

SITE OF SYNTHESIS OF CEA

Some investigators have questioned the ability of cancer cells to synthesize CEA. A clear answer was given by the following evidence: (1) the presence of CEA in gastrointestinal tumours maintained *in vitro*, these cultures consisting of epithelial cells, without any connective or lymphoid tissue (Burtin, Buffe, von Kleist, Wolff, and Wolff, 1970); (2) the presence of CEA in human colonic tumours serially propagated in hamsters (Goldenberg, Pavia, Hansen, and Vandevoorde, 1972); (3) the secretion of CEA by some permanent lines of colonic tumour cells (Laing, Heppner, Kopp, and Calabresi, 1972).

It is likely that CEA is synthesized in the cytoplasm of cancerous cells and is then secreted through the cell membrane at the apical pole of the cell. Although CEA is generally not detectable in the cytoplasm of cancer cells, there are several possible explanations of this: some of them, such as the lack of penetration of antibody into the cell or the masking of the antigen, appear to be excluded by the observations on cells of linitis plastica. It is possible that, due to its rapid secretion, CEA passes undetected through the cytoplasm. It may be that in the case of linitis plastica the secretion of CEA is hindered, hence the cytoplasmic accumulation of the antigen.

CEA AND THE CELL MEMBRANE

Is CEA a membrane-associated antigen? It is apparently so in some cases, especially when cancer cells are scattered or arranged in clumps. Gold, Krupey, and Ansari (1970) localized CEA by an electron-immunoferritin technique to the cell coat (glycocalix) of cultured cancerous cells. However, in the great majority of neoplasms, CEA was found only on the membrane at the apical pole of the cells: this is probably a temporary location before secretion, and does not require a true association with the membrane. It is worthwhile to add that, immuno-

logically, CEA has not the properties of a membrane-associated tumour antigen; it does not induce antibodies (Collatz, von Kleist, and Burtin, 1971) nor lymphocyte transformation (Lejtenyi, Freedman, and Gold, 1971).

CANCER SPECIFICITY OF CEA

Cancer specificity of CEA, which was claimed by the early workers, was not questioned for several years. Then, Martin and Martin (1970) reported that they had been able to absorb out an anti-CEA antiserum using large amounts of the perchloric extracts of non-cancerous colonic mucosa. These results were corroborated by experiments in our laboratory: we showed by immunofluorescence the presence of CEA in histologically normal colonic mucosa adjacent to tumour tissue (von Kleist and Burtin, 1969b). Later, we found CEA regularly with the same technique in a series of 25 polyps of the colon and rectum. These were of different histological types, some of them histologically benign, others frankly malignant (Burtin, Martin, Sabine, and von Kleist, 1972b), and, therefore, the biological behaviour of the polyps cannot be deduced from CEA studies. We confirmed this result with an immunochemical investigation: the perchloric extract of nine pooled benign polyps contained CEA identical to that of malignant adenocarcinomas. Carcinoembryonic antigen was also found in ulcerative colitis and very often in 'haemorrhoidal' mucosa—which is obviously neither cancerous nor precancerous. Finally, we regularly demonstrated CEA with immunofluorescence in juvenile colonic mucosa and this result was confirmed immunochemically (Burtin, Sabine, and Chavanel, 1972c). In all these non-cancerous mucosae, CEA had exactly the same cellular localization as in colonic adenocarcinomas, although the intensity of the fluorescence was often weaker. It is thus clear that CEA is not a cancer-specific antigen, since it is found in colonic mucosae which show only inflammatory lesions. The presence of CEA in truly normal adult colonic mucosa is still doubtful.

We recently studied non-cancerous gastric mucosae for carcinoembryonic antigen. Histologically normal glands situated even in the close vicinity of gastric tumours did not contain CEA, and this is in striking contrast to the patterns found in colonic mucosae. However, anti-CEA antiserum stained strongly gastric glands showing intestinal metaplasia in stomachs resected either for peptic ulceration or a carcinoma (Burtin *et al.*, to be published).

Traces of CEA were isolated from normal liver (Kupchik and Zamcheck, 1972), and this antigen has also been detected in carcinomas of breast and

lung (Mach *et al*, to be published). Its presence in normal lung is still doubtful. These results show that CEA is not organ-specific.

FACTORS INFLUENCING PRESENCE AND AMOUNT OF CEA

The role of CEA is as yet unknown, but several factors are known to influence the amount present in tissues.

Is there a direct relationship between the amount of CEA and the rate of cell multiplication in a given tissue or tumour? The presence of CEA in fetal intestine would point to this being the case, but anaplastic carcinomas of the colon contain less CEA than the better differentiated adenocarcinomas, in which cell multiplication seems to be less rapid (Denk, Tappeiner, Eckerstorfer, and Holzner, 1972). Tissue differentiation seems to play an important role, as evidenced by several findings: in our study of the polyps of the colon we observed that well differentiated glands were much richer in CEA than the undifferentiated ones, and the same holds for carcinomas of the colon. The only glands in gastric mucosa which contained CEA showed intestinal metaplasia (*vide supra*). There is possibly a correlation between the content of CEA and the number of goblet cells.

Inflammation also apparently increases the amount of CEA, possibly by influencing tissue proliferation and differentiation.

The greater content of CEA in cancerous tissues—compared with normal tissue—is easily explained by the larger number of CEA-producing cells. It is not certain whether the amount of CEA produced per cell is greater if the cell is cancerous. Another possible factor is the lack of excretion of secretory material, evidenced by the intraglandular deposits, which are often found in cancerous glands but absent from normal glands.

NON-SPECIFIC CROSS-REACTING ANTIGEN (NCA)

Another antigen present in the perchloric extracts of colonic carcinomas is also a beta globulin, the precipitin line of which is always closer to the antibody trough than that of CEA (von Kleist and Burtin, 1969b). We noticed this some years ago, without being able to explain it at that time. This second antigen was present in noticeable amounts in normal colonic mucosa, at least in the perchloric extract. More recently, this globulin was isolated from colonic tumours (Mach and Pusztaszeri, 1972; von Kleist, Chavanel, and Burtin, 1972) and from the normal lung, in which it is relatively abundant. It is a glycoprotein having a molecular weight around 60 000, a sedimentation constant about 3.5 S (it is thus easy to separate it from CEA

by filtration on Sephadex G-200). It contains nearly 40% of carbohydrates the constituents are the same as those of carcinoembryonic antigen but in different proportions.

A very important fact, first noticed by Mach (Mach and Pusztaszeri, 1972), is that this antigen crossreacts with anti-CEA antiserum. That is why we proposed that it should be called nonspecific cross-reacting antigen (NCA) since it crossreacts with CEA and is neither cancer- nor organ-specific (von Kleist *et al*, 1972). This cross reaction explains why NCA, which reacts with only a fraction of the anti-CEA antibody, gives a precipitin line different to that of CEA: it is nearer the antibody trough. When we used some antisera against normal colonic mucosa, or more recently anti-NCA antisera, there was a 'spurring' of NCA over carcinoembryonic antigen. This means that CEA and NCA have an overlapping antigenic structure and that each of them has a specific part: NCA is not a fragment or a degradation product of carcinoembryonic antigen. It is tempting to form a hypothesis that the structure common to both antigens is, or lies in, their glucidic moiety, as they contain the same sugars.

Since antisera against CEA generally react with NCA, and are thus able to reveal this latter antigen in the tissues, our results showing the presence of CEA in non-cancerous tissues by immunofluorescence could have been questioned. Fortunately, they were corroborated by immunochemical studies: the antigen detected in intestinal polyps and juvenile colonic mucosa was identical to the CEA of colonic tumours. It is clear that for immunofluorescence (and eventually for radioimmunoassay) studies an anti-CEA antiserum previously absorbed with NCA should be used.

Non-specific cross-reacting antigen is found in many normal organs (lung, spleen, and colonic mucosa) and in cancerous tissues, especially those of the gastrointestinal tract. Traces of it can be detected in the plasma (Burtin, Chavanel, and von Kleist, 1972a). We have studied its localization in the tissues, by immunofluorescence and immunoenzymology, using anti-NCA antisera absorbed with CEA, and have shown that it is very similar to that of CEA: NCA is situated at the excretory pole of the glandular cells and in intraluminal deposits, but it can be found much more frequently than CEA on cellular walls (Burtin *et al.*, 1973).

Autoimmunity and Cellular Immunology Studies

Antibodies able to react with the cells or extracts of colonic carcinomas were looked for in the sera of cancerous patients by several groups. Gold (1967) described autoantibodies against CEA in such

patients, but we were unable to reproduce his findings. In our laboratory, the antibodies found in colonic cancer sera did not react with CEA but with normal organ antigens (Collatz *et al*, 1971). Lo Gerfo, Herter, and Bennett (1972), using the very sensitive radioimmunological method, did not find anti-CEA antibodies.

Studies of cell-mediated immunity look more promising. They were undertaken first by Hellström, Hellström, Pierce, and Yang (1970a), who showed by their colony inhibition test, then by microcytotoxicity, that the lymphocytes of cancer patients were often able to kill the tumour cells obtained from the same patient, or from other patients with a neoplasm of the same organ. Moreover, the lymphocytes of patients suffering from a colonic carcinoma could also kill cells of fetal intestine (Hellström, Hellström, and Shepard, 1970b). It follows that one or several carcinoembryonic antigens could behave as tumour-specific antigens. In other studies, skin tests were made with extracts of autologous colonic tumours, these extracts being the membrane-rich pellet of the tumour homogenate (Hollinshead, Glew, Bunnag, Gold, and Herberman, 1970) or prepared by perchloric acid (Hollinshead, McWright, Alford, Glew, Gold, and Herberman, 1972). Some positive delayed reactions were obtained. The antigen(s) responsible for these reactions did not seem to be the CEA.

Other laboratories are exploring the same problem with other techniques, such as cytotoxicity or leucocyte migration inhibition. The aim is to characterize the antigen(s) involved in specific cell-mediated immunity in patients afflicted with a carcinoma of the colon.

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