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CEA-Like Activity in Normal Colon Tissue

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CEA, ORIGINALLY described by Gold and Freedman [1], is an immunoreactive glycoprotein found in various tumour tissues [2]. Although high levels of CEA occur in the serum of patients with entodermally derived tumours, CEA has also been detected in patients with other cancers and in patients with a variety of non-neoplastic diseases [3]. Several laboratories have also reported the presence of small amounts of CEA in various adult normal tissues including colon [4], plasma [5], saliva [6] and faeces [7] as well as in foetal gut and meconium. Moreover, similarity between tumour CEA and CEA from various normal tissues has been demonstrated by immunological and chemical techniques [4–6, 8]. Recent investigations in this laboratory, reported here in preliminary form, have now shown that important chemical and immunological differences appear to exist between the CEA active components of normal colon and those of tumour CEA although these differences have not yet been resolved by existing CEA assays using conventional antisera.

Both our routine double antibody assay and the commercial Abbott CEA–EIA diagnostic kit have been used to show that the amount of CEA in perchloric acid extracts of normal colon tissues ranges from 25–840 ng/mg of extract. The wide variation has also been shown by rocket electrophoresis using several anti-CEA antisera including our assay serum, absorbed 227 (Fig. 1C) and antisera "ace 36" and absorbed G61 donated by Drs. Todd and Gold respectively. The assay values for similar extracts of normal liver and spleen are 8.5 and 23 ng/mg, respectively, indicating that our assay is not sensitive to other normal tissue proteins or NCA.

Rocket electrophoresis using antiserum 227 has confirmed the established immunological similarity between tumour CEA and normal colon CEA by showing rockets which fuse in a reaction of identity. In addition, this technique has resolved an overlapping rocket with tumour CEA showing a second antigenic component which appears not to be present in significant amounts in the crude normal colon extracts so far tested (Fig. 1A). We have designated the normal and tumour CEA-like components CEA-L1 and CEA-L2 respectively. Normal colon CEA-L1 has been purified from a CEA-rich normal colon extract by immunoadsorption using a Sepharose 4B-linked IgG fraction of a goat anti-CEA (PK-1G) previously absorbed (5 mg/ml of serum) with extracts of normal liver, normal spleen, normal plasma and a normal colon extract which had a very low content of CEA. The IgG-bound fraction containing CEA-L1 was eluted with glycine–HCl buffer at pH 2.8, dialysed against distilled water and finally concentrated by Amicon ultrafiltration.

Purified normal colon CEA-L1 showed unusual concanavalin A binding properties. After fractionation on a column of Con A–Sepharose [9] most of the CEA activity measured by radioimmunoassay appeared in the unbound fraction (Table 1). The Con A binding profile of normal colon CEA is therefore quite different from that of colonic tumour CEA which has been shown by several workers [9–11] to bind strongly to Con A–Sepharose. Since the Con A binding is de-

Table 1. *Fractionation of purified CEA-L1 on Con A–Sepharose**

CEA applied	μg CEA 40.3	% CEA applied
Fraction 1 (unbound)	19.9	49
2A	3.2	8
2B	8.6	21
3	5.4	13
4	6.3	16

*For details see reference [9].

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pendent on the structure of the carbohydrate chains in CEA, this result strongly suggests that CEA, which is a normally occurring colonic glycoprotein, is modified in tumour tissue either by altered glycosyltransferase activity or by degradative processes, the result of which is to increase its binding to Con A. Our previous studies on foetal colon and meconium [9] have indicated that most of the CEA in these tissues, like that of normal colon, is not bound to Con A–Sepharose.

Further studies on normal colon extracts have shown that heat treatment at 85°C for 30 min led to a marked decrease in the CEA value as measured by our routine CEA assay and the Abbott kit (Table 2). This effect was also demonstrated by rocket electrophoresis using antiserum 227 where a considerable decrease in the intensity of the rocket of normal colon extract was obtained after heating (Figs. 1A and 1B). The residual activity in rocket electrophoresis, since the peak height is similar after heat treatment, indicates that normal colon contains a different, heat stable, antigen in addition to CEA-L1. The tumour component CEA-L2 appeared to be heat stable as judged by the intensity of the rockets before and after heat treatment (Fig. 1B).

Antisera which reacted poorly with the heat labile CEA and normal colon CEA-L1 but still retained precipitin activity with heat stable tumour CEA-L2 were produced by immunising rabbits with CEA which had been heat treated in phosphate buffer at pH 6.8 at 85°C for 30 min. One hundred μg of heat treated CEA in complete Freund's adjuvant was used to immunise each rabbit and a similar booster given after 40 days. The antiserum designated 241, absorbed (20 mg/ml of serum) with normal liver, spleen, plasma and normal colon which was low in CEA, produced a reaction of identity in immunodiffusion with various conventional anti-CEA antisera when tested against tumour CEA (Fig. 2A). However, immunodiffusion and rocket electrophoresis showed antiserum 241 to have a more restricted specificity than many conventional antisera which we have tested. Although antiserum 241 retained activity with tumour CEA, a single weaker rocket was produced and only a very weak reaction was observed between antiserum 241 and the crude extracts of normal colon (Figs. 2B and 2C). This latter reaction however, may be due to the presence of small amounts of heat stable CEA-L2 in normal colon or to the presence of low titre anti-CEA-L1 in antiserum 241. The use of antiserum 241 for CEA radioimmunoassay is currently being investigated in this laboratory.

In conclusion it appears that normal colon CEA-active glycoprotein is largely a heat labile material which does not bind to concanavalin A, but which shares an antigenic determinant with colonic tumour CEA. Colon tumour CEA on the other hand contains in addition to this determinant, an antigen which is heat stable. Certain conventional anti-CEA antisera are clearly able to react with both antigenic components, however whether CEA immunoassays in general are able to measure both components remains to be established.

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*Table 2. Heat lability of CEA as measured by radioimmunoassay**

Extract (freeze-dried)	Double antibody RIA		Abbott EIA kit	
	Control	Heat-treated	Control	Heat-treated
Normal colon crude extract (0.3 mg/ml)	206†	139	236	148
Normal colon Con A unbound fraction	39	14	30	8
Normal colon immunopurified	17	5	36	13
Normal spleen (10.0 mg/ml)	9	8	24	15

*Full details of this study to be reported.
†CEA (ng/ml of extract).

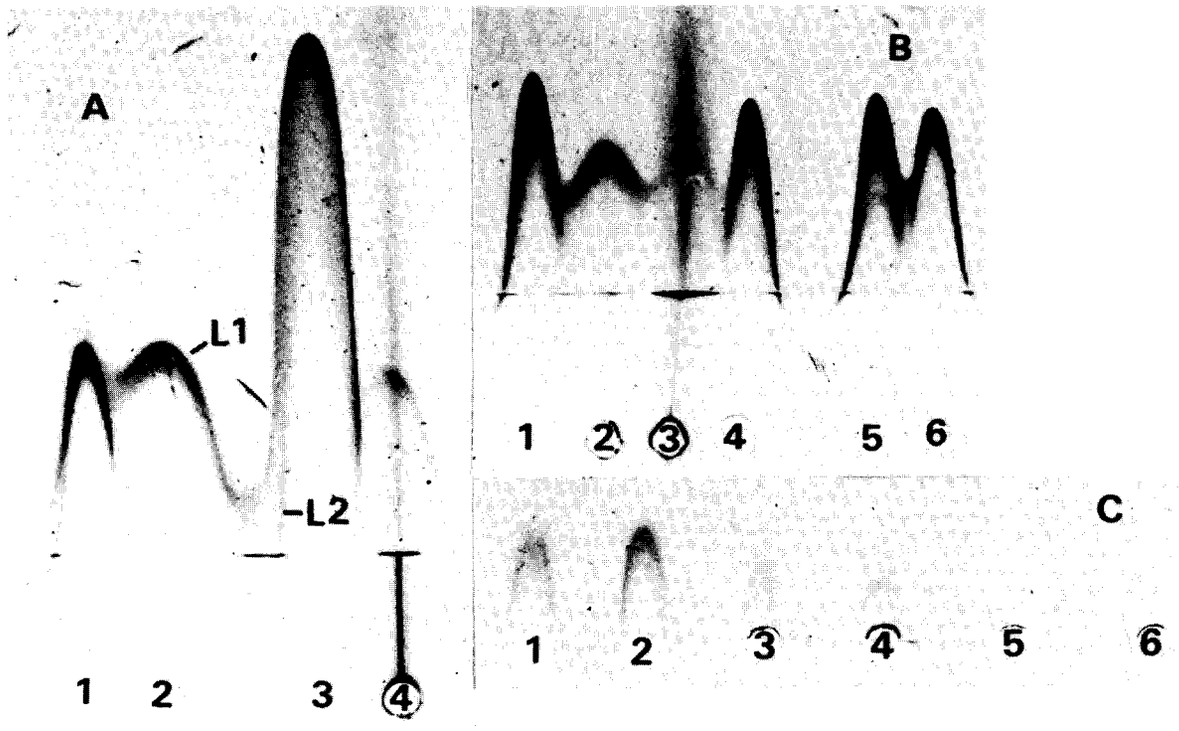

Fig. 1. *Rocket electrophoresis in agarose gel containing anti-CEA antiserum* 227 (1%). (*A*) *shows the two CEA-like components L*1 *and L*2 *in tumour CEA* (*wells* 1 *and* 3) *and component L*1 *in the normal colon extract* (*well* 2). *Well* 4 *shows a residual precipitin reaction given by the normal colon extract after heat-treatment.* (*B*) *shows the rockets of tumour CEA before* (*wells* 1 *and* 5) *and after heat-treatment* (*wells* 4 *and* 6) *and rockets of normal colon extract before* (*well* 2) *and after heat-treatment* (*well* 3). (*C*) *demonstrates the quantitative variation of CEA active glycoprotein in* 6 *different extracts of normal colon.*

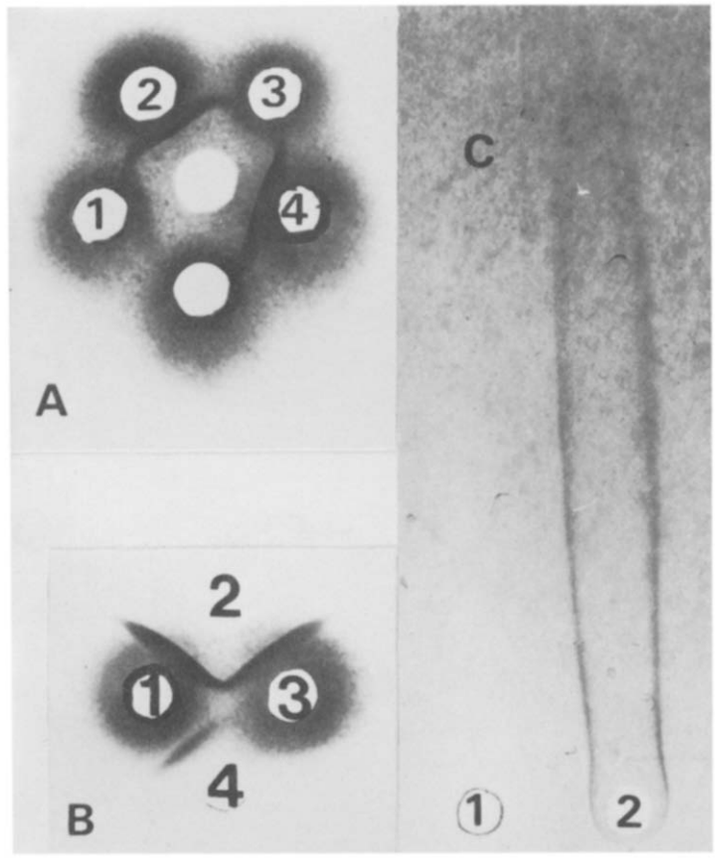

Fig. 2. (*A*) *Identity on immunodiffusion between tumour CEA* (*centre well*) *and the following anti-CEA antisera: "ace"* 36 (*well* 1), *G61* (*well* 2), 241 (*well* 3) *and* 227 (*well* 4). (*B*) *Immunodiffusion showing identity of reaction between anti-CEA G61* (*well* 1) *and antiserum* 241 (*well* 3) *against tumour CEA* (*well* 2). *Note the restricted reaction given between antiserum* 241 *and normal colon extract* (*well* 4). (*C*) *Rocket electrophoresis in agarose gel containing antiserum* 241 (1%) *demonstrating a single rocket with tumour CEA* (*well* 2) *and failure to react with normal colon extract* (*well* 1).

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