# Evidence for possible emergence of immunochemically distinct nucleotide-peptides in malignant transformation[1]

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*Summary.* Antibodies against nucleotide-peptides of beef heart do crossreact with nucleotide-peptides of other beef organs, but not with nucleotide-peptides of Ehrlich tumor cells. Antibodies against the latter do not crossreact with nucleotide-peptides of normal organs, but do so with nucleotide-peptides of a rat hepatoma.

The presence of nucleotide-peptide compounds in cells of the Ehrlich-Lettré ascites carcinoma and their possible metabolic role has been reported in the previous paper[3]. The presence of these compounds and their biological significance in various mammalian tissues has not been investigated extendively yet.

Heterogeneity both in qualitative and quantitative terms among nucleotide-peptides isolated from cells of various organisms does exist though some similarity with respect to the predominant presence of glutamic acid as well as of adenylic acid, has been revealed particularly among nucleotide-peptides of various mammalian liver tissues[4–11]. Whether this heterogeneity is reflected in their immunochemical behaviour and whether tissue specificity does exist in regard to these compounds, was the purpose of the present study.

*Methods.* Nucleotide-peptides were prepared from the following tissues as reported in the previous paper[3]. Beef: Brain, heart, kidney, liver, lung and spleen. Rat: Normal rat liver and dimethyl-aminoazobenzene (DAB)-induced hepatoma. Mouse: Ehrlich-Lettré ascites carcinoma.

Preparation of anti-nucleotide-peptides antibody. 5 mg of nucleotide-peptides in 0.5 ml of normal saline mixed with Difco's complete adjuvant was injected in equal amount into the 4 footpads of New Zealand white rabbits. At biweekly intervals the same amount of antigen was injected but without the adjuvant. 1 week after following the fourth injection blood was withdrawn by bleeding the ear. After allowing the blood to stand overnight in the cold the serum was prepared by centrifugation. The serum was initially tested for antibody by the Lancefield capillary tube test[12] and the immunological behaviour of the antigen was studied by double immunodiffusion in agarose, on microscope slides and in tubes.

Double immunodiffusion on microscope slides. Double immunodiffusion was carried out in 1% agarose (Mann Chemicals) in 60 mM (with respect to glycine) Trisglycine buffer pH 8.5 containing 0.9% NaCl on microscope slides. Peripheral wells were filled up with 2.5 mg/ml nucleotide-peptides solution in 0.9% NaCl and the central well with the antisera. Diffusion was carried out at room temperature for 72 h. After removing the unreacted proteins with several changes of normal saline the slides were stained with Coomassie blue for 16 h. The unreacted dye was removed by washing with several changes of solution containing 50% methanol and 7.5% acetic acid.

Double immunodiffusion in tubes. 1% agarose solution as prepared for double immunodiffusion on microscope slide was layered on 50 µl immunoglobulin solution prepared by salt precipitation into tubes (i.d. 5 mm) to a height of 1.8 cm. After the gel was formed 50 µl of anitgen solution (same concentration as used for double immunodiffusion on microscope slides) was applied on top of each gel column. The tubes were sealed with parafilm and the diffusion was carried out for 7 days. The unreacted proteins were removed, gels were stained and destained using the same procedure as used for slides.

*Results.* Antibody made against nucleotide-peptides of cells of the Ehrlich-Lettré ascites carcinoma did not cross-react with the nucleotide-peptides of various beef organs, as shown in figures 1 and 2. In another experiment where the same antibody was tested against nucleotide-peptides of DAB-induced rat hepatoma and of normal rat liver it was found to cross-react only with the former. When the diffusion was carried out in tubes

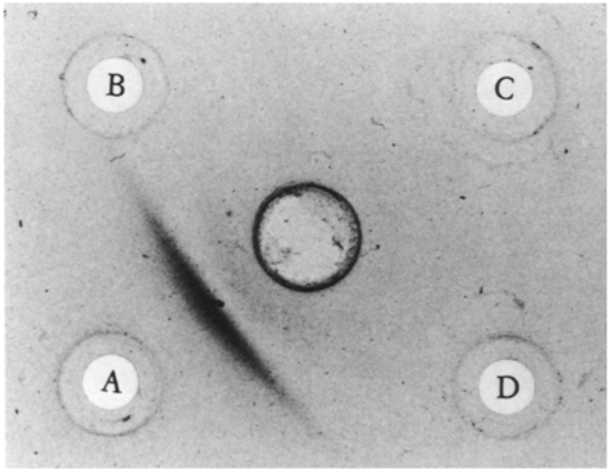


Fig. 1. The central well contained antiserum against Ehrlich-Lettré ascites carcinoma nucleotide-peptides. The antigen wells contained nucleotide-peptides of *A* Ehrlich-Lettré ascites carcinoma, *B* beef heart, *C* beef lung and *D* beef kidney.

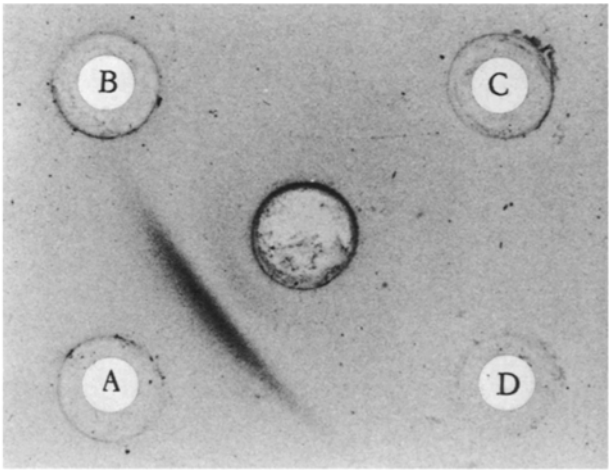


Fig. 2. The central well contained antiserum against Ehrlich-Lettré ascites carcinoma nucleotide-peptides. The antigen well contained nucleotide-peptides of *A* Ehrlich-Lettré ascites carcinoma, *B* beef spleen, *C* beef liver and *D* beef brain.

the nucleotide-peptides of Ehrlich-Lettré ascites carcinoma gave 2 precipitin bands whereas nucleotide-peptides of DAB-induced hepatoma gave a single band, figure 3. It appeared that the precipitin band due to nucleotide-peptides of DAB-induced hepatoma corresponded to the pricipitin band representing the fast moving nucleotide-peptide band of Ehrlich-Lettré ascites carcinoma.

Antibody made against nucleotide-peptides of beef heart seemed to cross-react with nucleotide-peptides of beef organs but not toward that of the Ehrlich-Lettré ascites carcinoma cells. It should be noted here that unlike the nucleotide-peptides of Ehrlich-Lettré carcinoma cells the nucleotide-peptides of beef heart showed very poor antigenicity in rabbit. Antibody titer obtained against beef heart nucleotide-peptides was too faint for photographic recording of the precipitin line though these were clearly visible with the naked eye.

*Discussion.* From these observations it appears that immunochemically there is no tissue specificity in regard to isolated nucleotide-peptides in the same animal. The most significant fact that emerges from these observations is that one of the nucleotide-peptides of Ehrlich-Lettré ascites carcinoma is antigenically similar to that of the DAB-induced hepatoma and also that the normal liver tissue differs from the hepatoma tissue at least in regard to this particular nucleotide-peptide. The additional nucleotide-peptide of the Ehrlich-Lettré ascites carcinoma which represents the slow moving band may be regarded as specific for this particular tumor.

It is premature to predict that immunochemically distinct nucleotide-peptides emerge in malignant transformation unless many other tumor lines are examined, but nevertheless in DAB-induced hepatoma it appears to be so. As it appeared[3] that the nucleotide-peptides may have a regulatory role in cell metabolism it would undoubtedly be of great interest to examine many other tumor lines to see of there is indeed a difference between the normal and malignant cells at the regulatory level and nucleotide-peptides will no doubt serve as materials of choice in this type of experiments.

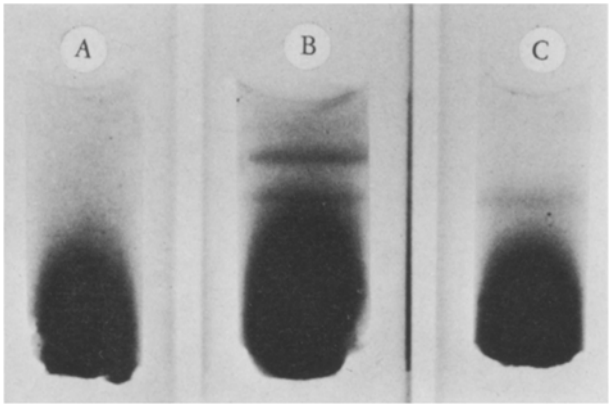


Fig. 3. The gel columns were prepared by layering 1% agarose solution on top of anti-Ehrlich-Lettré ascites carcinoma nucleotide-peptides immunoglobulin solution. Antigens were applied on top of the gel column. *A* Nucleotide-peptide of normal rat liver, *B* nucleotide-peptides of Ehrlich-Lettré ascites carcinoma, *C* nucleotide-peptides of DAB-induced rat hepatoma.

1 Supported by a research grant from the Government of Québec.
2 Acknowledgments. I am very grateful to Dr C. Godin of Laval University for providing materials and space in his laboratory and Dr D. Dufour of the same University for his kind gift of DAB-induced rat hepatoma tissue. I also wish to thank Drs E. R. M. Kay and B. Cinader of the University of Toronto for correcting this manuscript and for their expressed interest in this piece of work.
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## New lectin receptors in carcinoembryonic antigen (CEA)[1]

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*Summary.* The glycoprotein CEA (carcinoembryonic antigen) carries carbohydrate groups, which react with the plant lectins from *Agaricus bisporus*, *Arachis hypogaea* (peanut), with Tridacnin from invertebrate clams and with the anti-A lectins from snails. Accordingly, it has cryptantigenic structures, which correspond to the T or T-like antigen, the Tridacnin receptor and to the so called A-like antigen.

The biochemistry of the carcinoembryonic antigen (CEA), its immunological properties and its role as a tumor marker substance has been extensively reviewed recently[2]. The purpose of this communication is, however, to describe additional lectin receptors on CEA, which have been detected by newly discovered lectins from plant and invertebrate sources. Those heterophile receptors represent also additional markers for CEA; they may help to clarify its heterogeneity and distribution, its origin and variation, and may serve to facilitate its isolation and purification.

CEA was prepared according to the method of Newman et al.[3]: Liver metastases of colorectal carcinomas were homogenized and centrifuged. To the supernatant 1.2 M perchloric acid (PCA) was added, and after centrifugation the supernatant neutralized and then dialyzed against

1 Acknowledgment. This work has been supported by Landesamt für Forschung NRW.
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