

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 precipitin 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<sup>3</sup> 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 if 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.

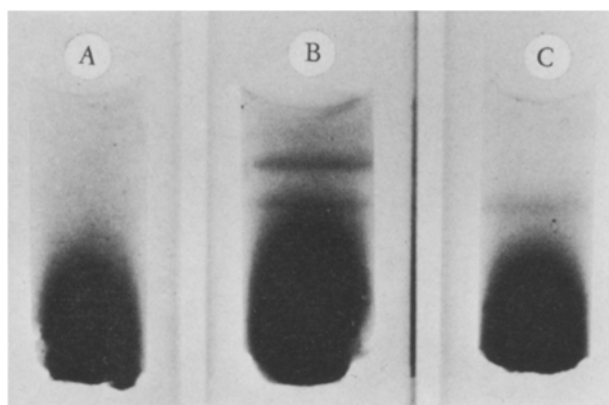


Fig. 3. The gel columns were prepared by layering 1% agarose solution on top of anti-Ehrlich-Lettré ascites carcinoma nucleotide-peptides immunoglobulin solution. Antigens were applied on top of the gel column. A Nucleotide-peptide of normal rat liver, B nucleotide-peptides of Ehrlich-Lettré ascites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

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## New lectin receptors in carcinoembryonic antigen (CEA)<sup>1</sup>

G. Wintzer, G. Uhlenbruck, G. Steinhausen and H. Carmann

*Chirurgische und 1. Medizinische Universitätsklinik, D-5 Köln 41 (Federal Republic of Germany), and Immunological Laboratory, F. Hoffmann-La Roche & Co AG, CH-4133 Schweizerhalle (Switzerland), 25 July 1977*

**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<sup>2</sup>. 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.<sup>3</sup>: 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

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Precipitin reactions in agar of purified CEA with different lectins from plant and invertebrate origin

Lectin	Specificity	Reaction with CEA
I Anti-T <i>Arachis hypogaea</i>	$\beta$ -Galactosyl 1-3 N-acetyl-galactosamine →	+
<i>Agaricus bisporus</i>	$\beta$ -Galactosyl → (N-acetyl-galactosamine?)	+
II Tridacnins* <i>Tridacna maxima</i>	$\beta$ -Galactosyl 1-6 (galactose) →	+
<i>Tridacna gigas</i>	$\beta$ -Galactosyl 1-4 (N-acetyl-glucosamine?) →	+
III Anti-A-like* <i>Helix pomatia</i>	$\alpha, \beta$ -N-Acetyl-galactosaminyl →	+
<i>Helix aspersa</i>	$\alpha$ -N-Acetyl-galactosaminyl →	+
<i>Cepaea nemoralis</i>	$\alpha$ -N-Acetyl-galactosaminyl →	+
IV Anti-H-like <i>Ulex europaeus</i>	$\alpha$ -L-Fucosyl →	+
V Anti-galactose-lectins <i>Axinella polyoides</i> *	$\beta$ -D-Galactosyl (1-6) →	-
<i>Rutilus rutilus</i> **	R → Galactosyl → R ( $\beta$ )	-
<i>Ononis spinosa</i>	$\beta$ -D-Galactosyl →	-
<i>Robinia pseudoacacia</i>	$\beta$ -D-Galactosyl →	-
<i>Ricinus communis</i>	$\beta$ -D-Galactosyl →	-
<i>Glycine soja</i>	$\beta$ -D-Galactosyl →	-
<i>Abrus precatorius</i>	$\beta$ -D-Galactosyl (1-4?) →	-
Anti-Pneumococcus } Type XIV Serum** }	$\beta$ -D-Galactosyl 1-4 (N-acetyl-D-glucosamine, D-glucose) →	-
VI Anti-glucosaminyl-lectins <i>Triticum vulgare</i>	$\beta$ -N-Acetyl-D-glucosaminyl →	-
<i>Datura stramonium</i>	(N-Acetyl-D-glucosaminyl) <sub>n</sub>	-

+, precipitin line; -, no visible reaction; \* invertebrate lectins; \*\* vertebrate lectins.

distilled water. Concentration was done by ultrafiltration. From this extract, CEA was prepared by ion resin exchange chromatography, Sepharose gel filtration and Sephadex G 200 filtration.

The purity of our CEA preparation (LEW 1) was checked immunologically by gel diffusion and immunoelectrophoresis with monospecific antisera. In both systems, only 1 precipitation line was detected. In addition, rabbits were immunized with CEA-LEW 1. The antibody obtained by this procedure showed also only 1 precipitin arc with the following antigens: the crude PCA-extract, CEA-LEW 1 and an internal CEA-standard BP 160.

Lectins were prepared as described in a previous paper<sup>4</sup>, where also the immunodiffusion technique is demonstrated. All precipitin lines give identity reactions with standard test substances, for instance the anti-A lectins with blood group A substance, the tridacnins with galactans etc., so that unspecific reactions could be excluded. The results of our experiments are given in the table. As can be deduced from this list, our CEA preparation has the following lectin receptors: T or a T-like (*Arachis*) antigen (I), receptors for tridacnins (II), a blood group A-like antigen (III) and a blood group H-like antigen (IV), whereas all other anti-galactosyl lectins (V) and anti-glucosaminyl reagents proved negative in this precipitating test system. Surprising are especially the non-reactivity of the anti-pneumococcus tape XIV antiserum, of the *Abrus* lectin and of *Triticum vulgare* (wheat germ) lectin, substances which could be expected to react<sup>2</sup>.

In summary, a  $\beta$ -(1-3?)galactosyl-N-acetyl-galactosaminyl structure, probably linked alkali-labile to threonine or serine, an  $\alpha$ -N-acetyl-galactosaminyl group, linked in a similar way, and  $\beta$ -(1-4 or 1-6)galactosyl groups linked to N-acetyl-glucosamine or galactose may be assumed on

the carbohydrate moiety of CEA. The latter one and the H-like receptor could be arranged on an alkali-stable carbohydrate chains.

Our results are consistent with the concept of the occurrence of cryptantigenic structures in tumors<sup>5</sup>, as has been demonstrated by Springer for the T antigen<sup>6,7</sup>, for the *Tridacna* receptor<sup>8</sup> and the A-like antigen<sup>8,9</sup>, 3 receptors which can be identified in many glycosubstances from membranes after treatment with neuraminidase<sup>10</sup>. They are also in agreement with the carbohydrate analysis of colon CEA<sup>2,11</sup>.

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