Relative positions of some epitopes on carcinoembryonic antigen

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Summary. To investigate whether anti-(carcinoembryonic antigen) monoclonal antibodies (mAb) react with single or repeated epitopes, sandwich radioimmunoassays in homologous and heterologous combinations were performed. Four mAb (I-27, I-47, II-17 and to some degree II-16) gave homologous binding while two mAb (I-38S1 and II-10) did not. Taken together with previous immunoprecipitation studies we conclude that all these mAb except II-10 react with repeated epitopes. The relative positions of the epitopes recognized by these mAb and of three additional mAb (II-6, II-7 and CB-CEA-1) were investigated using a plate antibody competition test with enzyme-labelled carcinoembryonic antigen (CEA). mAb I-38S1, II-6, II-7, II-10, II-16 and CB-CEA-1 were mutually cross-reactive, and were classified as belonging to one epitope group. mAb I-27 and I-47 fell outside this group and did not interfere with the binding of CEA conjugate to mAb II-17 either. They therefore represent a second epitope group. mAb II-17 showed no interference with the binding of CEA to any of the other mAb and must therefore represent a third epitope group. The slopes of the plate antibody competition curves were used for calculation of a correlation matrix, which in turn was used to depict the relative positions of the epitopes recognized by the mAb in the large group.

Introduction

Carcinoembryonic antigen (CEA), a glycoprotein with a molecular mass of 180 kDa was first described in 1965 by Gold and Freedman [6]. The protein part consists of a single polypeptide chain, containing a 107 amino acid NH-terminal domain and three highly homologous domains of 178 amino acids [2, 15]. Carbohydrate side-chains constitute approximately half of the molecular mass. CEA is associated with carcinomas, especially those from the gastrointestinal tract [16]. CEA immunooassays are now in use as a diagnostic adjunct in the diagnosis of colon cancer, as a prognostic tool and in the long-term monitoring of patients after colorectal tumour resection. Recent investigations indicate that CEA can be used for immunolocalization of certain carcinomas [1, 7, 13, 17].

A series of anti-CEA monoclonal antibodies (mAb) have been established by our group [10, 11]. All mAb bind to the polypeptide part of the molecule. In this work we investigated some of these mAb for their capacity to bind to more than one epitope on the CEA molecule. In addition insights were gained about the relative positions of the epitopes. The techniques used were sandwich radioimmunoassay and a plate antibody competition test (PACT) [11].

Materials and methods

Antigen. CEA was purified from liver metastases of colorectal cancer as previously described [8]. Briefly, the following sequence of purification steps was performed: extraction with 1 M perchloric acid, Sepharose 4B gel filtration, concanavalin-A-Sepharose affinity chromatography, and Sephadex G-200 gel filtration. One preparation of CEA (CEA-71) was used throughout this study.

Antibodies. Eight mouse monoclonal antibodies against CEA (mAb I-27, I-38S1, I-47, II-6, II-7, II-16 and II-17) from our laboratory and one anti-CEA mAb (CB-CEA-1) from Dr A. Gavilando, Center of Genetic Engineering, Havana, Cuba were included in the study. The properties of our mAb have been reported [10, 11]. They are all IgG1, and recognize conformation-dependent epitopes of a peptide nature. mAb II-7, II-10, II-16 and II-17 do not cross-react with isolated CEA-related antigens in enzyme-linked immunosorbant assay (ELISA) (e.g. NCA-55, NCA-95, NCA-160 or BGPI). The other mAb show variable degrees of cross-reactivity with CEA-related antigens. The IgG fraction of a polyclonal calf anti-CEA serum was used as a positive control.

mAb-coupled discs. mAb were coupled to CNBr-activated paper discs as described previously [12]. Reference discs were prepared using an unrelated mAb directed against thyroid-stimulating hormone (anti-TSH, IgG1 subclass).

Radiolabelling of mAb. mAb were iodinated with Na¹²⁵I using Iodo-beads (Pearce, Rockford, Illinois, USA) as the oxidizing agent [14]; 0.5 mCi Na¹²⁵I (Amersham) was used/mg mAb.

Sandwich radioimmunoassay. Anti-CEA or reference discs were incubated with 100 µl CEA at a concentration of 50 µg/ml in 0.067 M phosphate buffer, pH 7.2, containing...
Tween 20, for 18 h at 20°C in micro-ELISA plates. The plates were gently agitated. As a control, buffer without CEA was added to anti-CEA-coupled discs. After one washing the discs were incubated with different concentrations of $^{125}$I-labelled anti-CEA mAb or $^{125}$I-labelled polyclonal calf anti-CEA IgG (150 µg) for 6 h at 20°C. Aliquots of the supernatant (100 µl) were transferred in duplicate to the coated micro-ELISA plates, giving a final concentration of 0.1, 1.0 or 10 µg/ml. Three mAb (I-38S1, II-10 and II-16) gave low binding in homologous and heterologous combinations within this group. The binding of mAb II-17, I-27 and I-47, in contrast, was high both in homologous and heterologous combinations.

Homologous binding was studied in three additional experiments. Table 2 summarizes the results of all experiments of this type. As can be seen, clear-cut homologous binding was obtained with mAb I-27, I-47 and II-17 while mAb I-38S1 and II-10 did not show homologous binding. mAb II-16 probably also gives homologous binding but the uptake of conjugate is fairly low.

Plate antibody competition test (PACT). The method previously employed by our group was used [11]. Dynatech micro-ELISA plates were coated at 2 µg/ml with the mAb in 0.05 M carbonate buffer, pH 9.6, and incubated overnight at 4°C. After incubation and three washings with PBS containing 0.05% Tween 20 and 0.5 mg bovine serum albumin/ml (incubation buffer), serial tenfold dilutions of the different mAb to be tested were made in incubation buffer in test-tubes. Aliquots of 50 µl from each dilution were transferred in duplicate to the coated micro-ELISA plates, giving a final concentration of 0.1, 1.0 or 10 µg/ml. A 50-µl aliquot of conjugate (CEA-ALP) at a dilution of 1/1000 was added to each well. The plates were then incubated overnight at 4°C with gentle agitation. After three washings the plates were developed by adding 100 µl/well $p$-nitrophenyl phosphate dissolved in diethanolamine buffer, pH 9.8. The absorbance at 405 nm ($A_{405}$) was then measured in an ELISA spectrophotometer. We noticed that the inhibition curves, when drawn semilogarithmically, most often had a shape similar to radioactivity decay curves:

$$n = n_0 e^{-\lambda t}$$

(3)

After logarithmic transformations and substitutions: $A_{405} = n$, $a = \log(n_0)$ and $\log(c) = t$ ($c$ = concentration of inhibiting mAb), Eq. (3) assumes the form:

$$\log(A_{405}) = a - \lambda \log(c)$$

(4)

$\lambda$ was taken as an estimator of the slope of the inhibition curves. It was calculated by linear regression.

Results

Repetitive epitopes

A sandwich radioimmunoassay was used to study the reactivity of six anti-CEA mAb in homologous (the same mAb in the fluid as on the disc) and heterologous (a different mAb in the fluid from that on the disc) combinations. Radiolabelled mAb (= conjugate) were added at three different concentrations (2, 10 and 50 µg/ml). Figure 1 shows a typical experiment in which paper discs coupled with mAb II-16 were utilized. The concentration of conjugate bound to mAb-coupled discs for all combinations of mAb and at two different concentrations of added mAb is shown in Table 1. The numerical values given in the table are adjusted for differences in catching capacity between batches of coupled discs using the positive control. Generally, the concentration of bound conjugate increased with increasing concentrations of added conjugate. Three mAb (I-38S1, II-10 and II-16) gave low binding in homologous and heterologous combinations within this group. The binding of mAb II-17, I-27 and I-47, in contrast, was high both in homologous and heterologous combinations.

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![Fig. 1. Sandwich radioimmunoassay with mAb II-16 coupled to paper discs. The discs were first incubated with CEA at 50 µg/ml and then, after washing, with the respective mAb or polyclonal calf anti-CEA IgG](image_url)