Volume 261, number 2, 405–409 FEBS 08179 February 1990

Escherichia coli of human origin binds to carcinoembryonic antigen (CEA) and non-specific crossreacting antigen (NCA)

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Received 10 December 1989; revised version received 11 January 1990

Immobilized carcinoembryonic antigen (CEA) and non-specific crossreacting antigen (NCA) bound 3 strains of *E. coli* of human origin. The binding was dose dependent, saturable, and of high avidity. Binding of the bacteria to CEA and NCA was completely abolished in the presence of 10 mM α-methyl D-mannopyranoside. Bacteria did not bind to concanavalin A. In addition, binding to deglycosylated CEA was either absent or significantly reduced. These findings indicate that the *E. coli* strains bind to D-mannosyl residues in CEA and NCA. Considering the tissue distribution of CEA (brush border of colonic epithelium) and NCA (granulocytes), these glycoproteins may be involved in the recognition of bacteria.

Carcinoembryonic antigen; Non-specific crossreacting antigen; α-Methyl D-mannopyranoside; Bacterial binding

1. INTRODUCTION

Carcinoembryonic antigen (CEA), a marker for human colorectal carcinomas, is a major glycoprotein of the glycocalyx of human colonic carcinoma cells [1]. In normal colonic mucosa, the highest amounts of CEA have been identified on the cells lining the luminal surface [2,3]. CEA has been isolated from normal colonic mucosa [4], from colon washings of healthy persons [5], and from the supernatant of organ cultures of normal colon mucosa [6]. Recently, several genes which are highly homologous to the CEA gene have been described [7,8]. One of the genes codes for the 'non-specific crossreacting antigen', a glycoprotein which is actively produced by normal granulocytes [9–11]. The CEA gene family is a member of the immunoglobulin supergene family [12].

Here we present first data that *E. coli* of human origin binds to immobilized CEA and NCA. Since both the colonic epithelium and granulocytes are involved in the recognition of bacteria, bacterial recognition may be one of the biological functions of the family of CEA and related glycoproteins.

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Abbreviations: CEA, carcinoembryonic antigen; ConA, concanavalin A; *E. coli*, *Escherichia coli*; NCA, non-specific crossreacting antigen

2. MATERIALS AND METHODS

2.1. *Strains and culture methods*

The *E. coli* strains were isolated from human feces (Ty 7717) and from tracheal secretions (Va 068/1, Va 084) and passaged on DST agar (Oxoid, Wesel, FRG) for 18 h at 37°C under aerobic conditions. Subsequently, binding and inhibition studies to CEA and NCA as well as first hemagglutination studies were performed. Two strains (Va 084 and Va 68/1) were further passaged in Luria broth for an additional 48 h at 37°C. Subsequently, hemagglutination assays were repeated.

2.2. *Hemagglutination assay*

A hemagglutination test routinely used for the determination of mannose-sensitive or mannose-resistant hemagglutination was applied using guinea pig erythrocytes. Agglutination of erythrocytes in the absence but not in the presence of 25 mM α-methyl D-mannopyranoside was defined as mannose-sensitive hemagglutination [13].

2.3. *Adhesion and inhibition assays*

Microtiter plates (Type Maxisorp, diameter per well: 7 mm; Nunc, Wiesbaden, FRG) were coated with 50 μl of solutions of either CEA, NCA, deglycosylated CEA (amounts indicated in figure legends and table 1), or concanavalin A (10 mg/l) in 0.2 M carbonate buffer, pH 8.3, overnight at RT. CEA was purified as described [14]. N-terminal and internal amino acid sequences of the preparation have been published [12]. NCA was purified from human spleen by perchloric acid extraction and immunoabsorbent purification over the monoclonal anti-CEA antibody T84.1 [15,16]. The sequence of the 24 N-terminal amino acids matches the sequence published for NCA [12]. The concentrations of CEA and NCA were determined by amino acid analysis. CEA was deglycosylated as described [12]. The concentration of deglycosylated CEA used for coating was chosen to give signal intensities in enzyme immunoassays with monoclonal antibody T84.1 [15] similar to native CEA. The concentrations refer to

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00145793/90/$3.50

the protein moieties of the antigens. Unspecific binding sites were blocked by a solution of 1% BSA in PBS (2 h, RT). Wells were washed 3 times with PBS containing 0.05% Tween-20 (v/v). This solution was also used for subsequent washing steps. Bacteria were suspended in PBS and adjusted to a turbidity corresponding to 6×10^8–2×10^9 bacteria/ml. 150 μl of the suspension was added to the wells and incubated overnight at 4°C. For inhibition experiments with carbohydrates (all from commercial sources), the bacterial suspension was centrifuged, the supernatant was discarded, and the bacteria were resuspended in PBS containing the appropriate concentrations of carbohydrates.

2.4. *Detection of attached bacteria*

Binding of attached bacteria was revealed by enzyme immunoassays. For the detection of *E. coli* by antisera, a rabbit pool serum against *Klebsiella pneumoniae* crossreacting with *E. coli* (1:100 in PBS) was used for strains Va 068/1 and Ty 7717. This antiserum bound to the *E. coli* strains as shown by indirect immunofluorescence microscopy. For strain Va 084, a homologous rabbit immune serum (1:1500 in PBS) was applied. The adherent bacteria were incubated with the antisera for 1–2 h at 37°C. After washing, wells were incubated with a goat anti-rabbit IgG washing, wells were incubated with a goat anti-rabbit IgG antiserum conjugated to horseradish peroxidase at a dilution of 1:250 in PBS (Bio-Rad, München, FRG) for 1 h at room temperature (100 μl/well). The color reaction was developed as described [17]. In control experiments, (i) immobilized glycoproteins, and (ii) bacteria were omitted. Background OD was <0.1 with the homologous antiserum to strain Va 084, and in the range of 0.2–0.3 with the crossreacting pool antiserum in both controls. Background OD values were subtracted from the values obtained in the presence of attached bacteria. As additional detection method, bacteria attached to microtiter plates were stained with 50 μl per well of an Acridine orange solution (Api Bio Merieux, Nürtingen, FRG) for 30 min, washed and examined for bacteria in an UV inverted microscope.

3. RESULTS

3.1. *Binding of* E. coli *to CEA and NCA*

Three different strains of *E. coli* were tested with respect to the adhesion to immobilized CEA and NCA. Each strain adhered to CEA and NCA. An example for the binding of strain Va 084 to CEA as revealed by the Acridine orange stain is given in fig.1a. As shown in fig.2, the binding of the different strains increases with increasing amounts of immobilized CEA. Binding plateaus are reached at CEA amounts of approximately 100 ng added per well. With NCA binding of strain Va 084 reached a plateau at about 300 ng antigen added per well (fig.3). Binding of the remaining strains was tested at constant amounts of NCA (250 ng added per well). For both strains, OD values of >0.5 were obtained after subtraction of background staining. In order to exclude that mannosyl residues on the bacteria may bind to CEA or NCA, or that trace amounts of concanavalin A (ConA) in the CEA preparation are responsible for the binding activity, control experiments were performed with immobilized ConA (500 ng per well). Since ConA bound antibodies, binding of bacteria was determined microscopically using the Acridine orange stain (cf. fig.1). No binding could be detected.

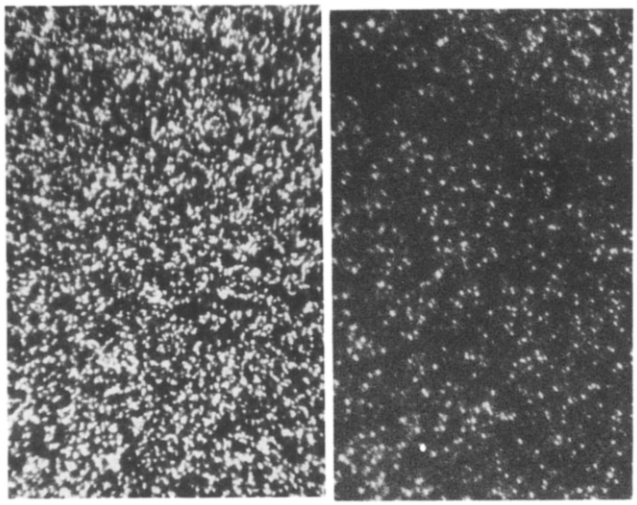

Fig.1. Binding of *E. coli* strain Va 084 to CEA absorbed to microtiter plates in the presence of different amounts of α-methyl D-mannopyranoside. Acridine orange stain (250 ng CEA per well). Concentrations of α-methyl D-mannopyranoside: (a) 0.2 mmol/l; (b) 1.6 mmol/l; at concentrations of >10 mmol/l, binding was completely inhibited. × 947.

3.2. *Inhibition studies*

The different strains of *E. coli* were added to immobilized CEA or NCA in the presence of increasing amounts of α-methyl D-mannopyranoside. The effect was monitored by enzyme immunoassay and by direct staining of the attached bacteria. For each strain, the binding to CEA was completely abolished at 10 mM α-methyl D-mannopyranoside (fig.4). For strain Va 084, the corresponding Acridine orange stain is shown in fig.1b. The binding of strain Va 084 to NCA was

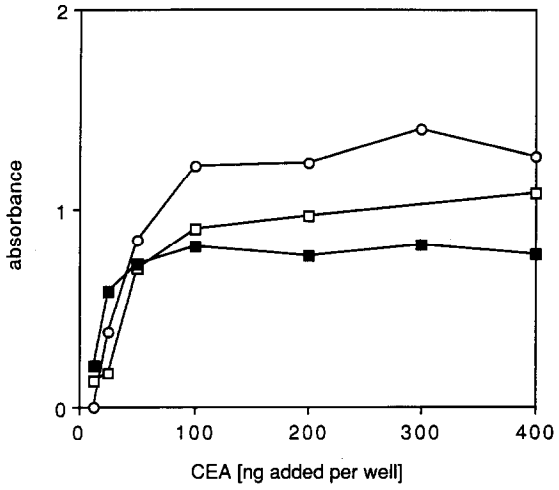

Fig.2. Binding of different strains of *E. coli* to CEA absorbed to microtiter plates. Wells were coated with CEA amounts indicated on the abscissa. The absorbance was read at 492 nm. The values refer to the difference of the absorbance in the presence and absence of bacteria. (○) Strain Va 068/1; (□) Va 084; (■)Ty 7717.

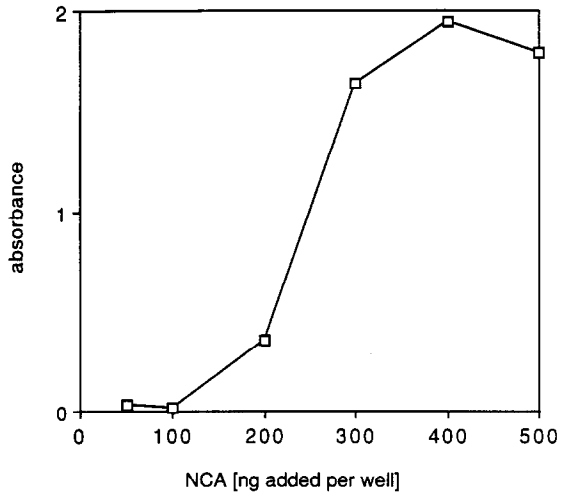

Fig.3. Binding of *E. coli* strain Va 084 to NCA absorbed to a microtiter plate. Plates were coated with NCA amounts indicated on the abscissa. Further experimental details as in fig.2.

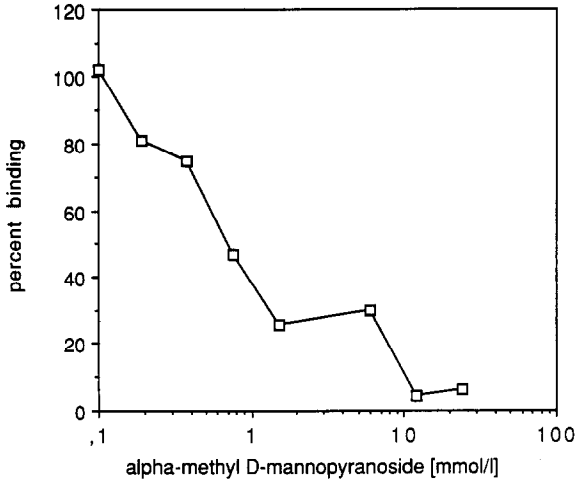

Fig.5. Binding of *E. coli* strain Va 084 to immobilized NCA in the presence of increasing concentrations of α-methyl D-mannopyranoside (250 ng NCA added per well).

similarly inhibited by α-methyl D-mannopyranoside (fig.5). For reasons of lack of material, the remaining strains were not tested. As shown in fig.6, sugars other than α-methyl D-mannopyranoside did not inhibit binding of strain Va 084 to CEA.

3.3. *Hemagglutination assay*

The *E. coli* strains were further investigated in a hemagglutination assay in which type 1 fimbriated bacteria show a mannose-sensitive agglutination. Following culture on DST agar for 18 h at 37°C, only strain Ty 7717 showed a mannose-sensitive agglutination. The remaining two strains did not agglutinate. When these strains were further cultured under conditions optimal for the expression of type 1 fimbriae (Luria broth, 48 h, 37°C), strain Va 68/1 showed a faint and strain Va 084 showed a strong mannose-sensitive agglutination.

3.4. *Binding to deglycosylated CEA*

Microtiter plates were coated with deglycosylated CEA at amounts which resulted in MAb T84.1 binding comparable with native CEA. About 10-fold the amount of deglycosylated CEA was used for coating (2.5 μg per well). Compared with native CEA, a significantly lower binding is observed (table 1). The increase in non-specific binding was much more pronounced when the low titer crossreacting antibodies were used for the remaining strains. Due to the high background activity, enzyme immunoassays could not

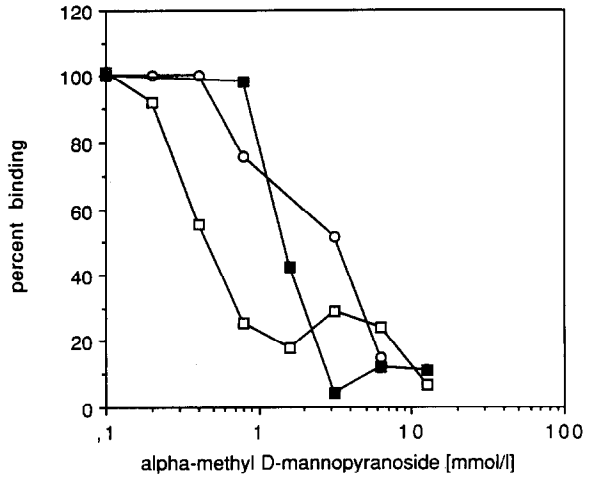

Fig.4. Binding of different strains of *E. coli* to immobilized CEA in the presence of increasing concentrations of α-methyl D-mannopyranoside (250 ng CEA added per well). (○) Strain Va 068/1; (□) Va 084; (■) Ty 7717.

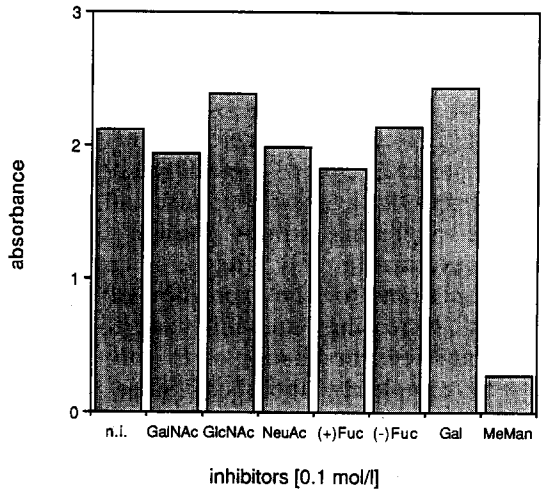

Fig.6. Binding of *E. coli* strain Va 084 to immobilized CEA in the presence of different inhibitors. n.i., no inhibitor; MeMan, α-methyl D-mannopyranoside.

Table 1

Binding of *E. coli* strain Va 084 to native and deglycosylated CEA[a]

	Absorbance	
	Native CEA	Deglycosylated CEA
Bacteria added	>2.5	0.68
No bacteria added	0.09	0.22

[a] Amounts of antigens added per well were chosen to give comparable binding of monoclonal anti-CEA antibody T84.1. Native CEA = 250 ng per well; deglycosylated CEA = 2500 ng per well

be evaluated. In the Acridine orange stain, strain Va 068/1 did not bind, whereas some residual binding was observed with strain Ty 7717.

4. DISCUSSION

CEA is a highly glycosylated protein which is actively synthesized by normal colonic mucosa. In the colonic epithelium of infants and adults, CEA shows a polar membrane localization at the brush border cells lining the lumen of the large intestine [2,3]. Furthermore, the degree of expression of CEA-specific mRNA in normal colonic mucosa is comparable with colonic carcinoma cells [6], and normal colonic mucosa actively produces CEA in vivo [5] as well as in organ culture [6]. NCA, a glycoprotein with extensive structural homology to CEA [7], is produced by normal granulocytes in a non-polar fashion [11]. Since both the colonic epithelium and granulocytes are involved in the binding of bacteria, we considered bacterial recognition as a putative unifying concept for the biological function of both CEA and NCA.

Here we provide evidence that different strains of *E. coli* from human origin bind to immobilized CEA and NCA. The binding was dose dependent and saturable. Furthermore, the binding seems to be of relatively high avidity since binding plateaus were reached with amounts of CEA or NCA of less than 1 μg used to coat the wells of microtiter plates. In a hemagglutination test routinely used for the detection of type 1 fimbriated strains of *E. coli* [13], one of the strains showed a mannose-sensitive agglutination after culture on DST agar. The remaining strains showed a mannose-sensitive agglutination only after being cultured under optimal conditions for the expression of type 1 fimbriae. At increasing amounts of α-methyl D-mannopyranoside, the binding of *E. coli* to CEA and NCA was completely abolished. Since the *E. coli* strains did not bind to ConA and, in addition, their binding to deglycosylated CEA was abolished or significantly reduced, it is concluded that the bacteria bind to D-mannosyl residues in CEA and NCA [18].

It is well established that colonic epithelial cells as well as granulocytes and macrophages bind type 1 fimbriated bacteria via D-mannosyl residues [19–23]. Our results suggest that CEA may be involved in the recognition of *E. coli* by human colonic epithelial cells, and that NCA may be involved in bacterial recognition by human granulocytes. Recently, Benchimol et al. [24] provided evidence that CEA may function as a homophilic cell adhesion molecule. However, the localisation of CEA at the colonic brush border cells in the colon of infants and adults is difficult to reconcile with this hypothesis. Similarly, a possible involvement of NCA in homophilic cell adhesion would be difficult to explain since NCA is produced by non-stimulated, non-adherent human granulocytes. Furthermore, the low conservation of the CEA family members during evolution [25,26] has to be taken into account when biological functions of these glycoproteins are considered. Bacterial recognition would be compatible with the tissue distribution and the low conservation of CEA and NCA. Additional studies are being performed in order to prove that CEA and NCA function as bacterial recognition molecules in colonic epithelia and granulocytes in vitro and in vivo.

Acknowledgement: The technical assistance of Irmgard Kalf is gratefully acknowledged.

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