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Chemotherapy and Radiotherapy of Gastrointestinal Tumors

Edited by H. O. Klein

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Contents

The Importance of Lectin Binding Sites and Carcinoembryonic Antigen with Regard to Normal, Hyperplastic, Adenomatous, and Carcinomatous Colonic Mucosa

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Summary

Using fluorescence microscopy a higher content of lectin binding sites was observed in the mucosubstances of transitional mucosa adjacent to colonic carcinomas than in normal mucosa. The lectin binding pattern of adenomas was similar to that of the transitional mucosa. However, in proliferating parts and areas with cell atypia the amount of lectin receptors was generally reduced. In colonic carcinomas the occurrence of lectin receptors was correlated with the morphological degree of differentiation: well differentiated carcinomas showed more lectin binding sites than undifferentiated ones. Often a concomitant occurrence of FITC-*Ricinus communis* agglutinin with carcinoembryonic antigen was found; the latter is known to be a glycoprotein with a high carbohydrate content. Of special interest was the peanut agglutinin receptor that was only demonstrable in transitional mucosa and carcinomas but was absent in normal mucosa of the colon. The importance of this receptor is discussed with respect to the occurrence of blood group antigens and immunotherapeutical considerations in colonic carcinomas.

The histopathology and classification of tumours of the colon is well established (survey by Morson and Sobin [17]; also [6]). However, in future the morphological differentiation alone may not be adequate for the characterisation of these tumours. With respect to new therapeutical considerations, therefore, further investigations are needed that give more detailed information about the metabolism of tumour cells. This development in oncology requires a more sensitive microscopical diagnosis with histochemical methods that allow the demonstration of distinct cell components. In this context the analysis of the mucins in the epithelial cells of the colon seems to be of great importance [3, 4, 7, 10, 12, 13, 16, 18]. There is still much, to be learned about the role of the different monosaccharides to determine the significance of mucins in the physiology and pathology of the bowel.

In this study different carbohydrate structures have been investigated by fluorescence microscopy in normal, hyperplastic, adenomatous, and carcinomatous colonic mucosa. Lectins that are well known for their carbohydrate specificity (survey by Sharon and Lis [21]), were used for visualisation of these sugar components; the lectins were labelled by conjugation with fluorescein isothiocyanate (FITC). Additionally, in our histochemical investigations the occurrence of lectin receptors was compared with

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the localisation of carcinoembryonic antigen (CEA), a tumour marker that possesses a high content of carbohydrates [15].

Material and Methods

The investigations were performed on formalin-fixed and paraffin-embedded tissue specimens of the colon obtained by surgery. Adult normal colonic mucosa ($n = 15$) was taken from macroscopically normal resection margins from cases of colonic carcinomas. The transitional mucosa ($n = 26$) adjacent to colonic mucosa was separately investigated. Tissues were also obtained from three hyperplastic polyps and 16 adenomas (tubular and villous, with and without cell atypia) of the colon. Finally, cancerous samples were investigated from 33 colonic carcinomas with various degrees of differentiation.

Representative sections were taken from each specimen for HE and PAS staining. Conjugates of FITC with *wheat germ agglutinin* (WGA), *Ricinus communis* agglutinin (RCA, MW 120,000), and *peanut agglutinin* (PNA) were used in this study (labelled lectins were commercially obtained from Medac, Hamburg). After deparaffinisation, the tissue sections were exposed (30 min, 20° C) in a moist chamber to these FITC-lectin conjugates at a concentration of 100 µg/ml [see also 11]. Thereafter they were washed with PBS (0.01 *M* sodium phosphate buffer pH 7.4 containing 0.15 *M* sodium chloride) and examined under the fluorescence microscope (Zeiss, epifluorescence, 435–490 nm spectrum filter, HBO 50-W mercury lamp). Regularly, one tissue section in each sample was pretreated with neuraminidase (*Vibrio cholerae*, 500 units/ml, Behringwerke, Marburg) for demonstration of sialic substituted lectin binding sites. In control experiments the binding specificity for distinct sugars was tested: FITC-RCA was inhibited by D-galactose (100 m*M*), whereas fluorescein-labelled PNA was best inhibited by desialylated glycophorin, which is known to possess a high amount of the disaccharide D-galactose-(1-3)N-acetyl-galactosamine. The specificity of RCA and PNA for the above-mentioned sugars was also confirmed by simultaneous incubation of tissue sections with rhodamine-labelled PNA and FITC-labelled RCA. The two lectins often showed a different distribution pattern of receptors according to their affinity for the appropriate sugar. FITC-WGA was used for the demonstration of neuraminic acid (electrostatic binding). This lectin, however, was only conclusive for this sugar when the reaction was blocked by pretreatment of the tissue sections with neuraminidase in a control slide.

The demonstration of CEA was performed by immunoperoxidase and immunofluorescence technique (according to Huitric et al. [8] and Burtin et al. [1]). CEA and rabbit antibodies against CEA were prepared as described in detail by Uhlenbruck et al. [24]; the antibodies were diluted 1:10 in PBS. Controls of CEA staining were performed with anti-CEA globulin absorbed with the antigen.

Results

Wheat Germ Agglutinin Binding

The mucus of goblet cells in normal colonic epithelium (resection margins from cases of carcinoma of the colon) showed a slight increasing fluorescence towards the top of

the crypt after incubation of tissue sections with FITC-labelled WGA. Additionally, the labelled lectin reacted with the apical portion of epithelial cells at the mucosal surface and with cells lining the upper portion of crypt lumen. A similar distribution of WGA binding was observed in the transitional mucosa. In comparison with the normal mucosa the fluorescence here was more pronounced. WGA receptors were also detected in mucinous substances of carcinomas, especially in mucinous carcinomas. These fluorescences were usually blocked by preincubation of the tissue sections with neuraminidase, whereas WGA receptors on the surface of the epithelial cells in transitional mucosa and carcinomas were often resistant to neuraminidase.

Ricinus Communis Agglutinin Binding

Free receptors for RCA were predominantly found in the lower parts of the crypts in normal mucosa. In contrast, RCA reacted with the goblet cells of the upper parts of the crypts (Fig. 1) only after preincubation of the tissue sections with neuraminidase, indicating the occurrence of sialic acid substituted binding sites. Generally the transitional mucosa possessed an increased amount of RCA receptors that partly

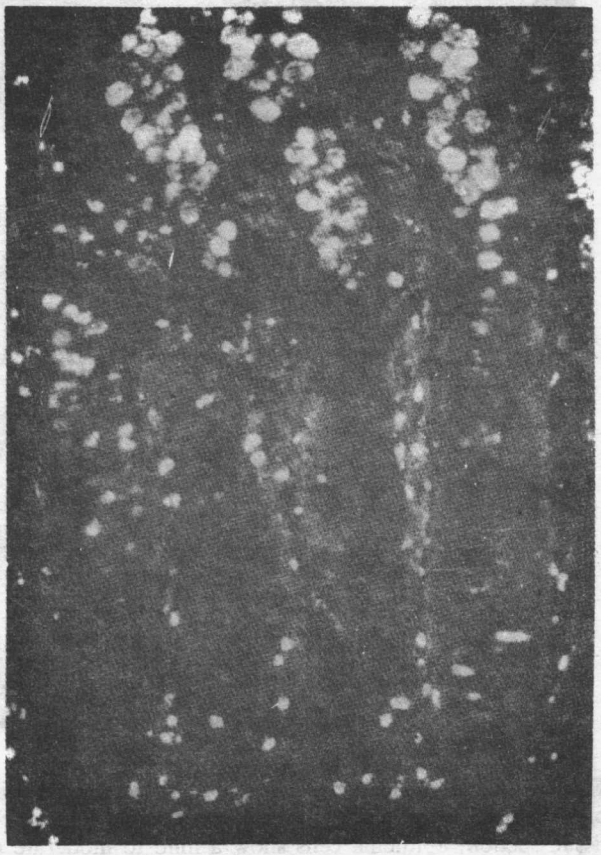

Fig. 1. Normal colonic mucosa with RCA receptors in the goblet cells of the upper part of the crypts; pretreatment of the tissue section with neuraminidase (*Vibrio cholerae*)

showed a moderate disturbance of distribution, which was more pronounced in carcinomas. In well and moderately differentiated colonic carcinomas, RCA mainly reacted with mucus substances, whereas in undifferentiated tumours a membranous fluorescence was sometimes observed.

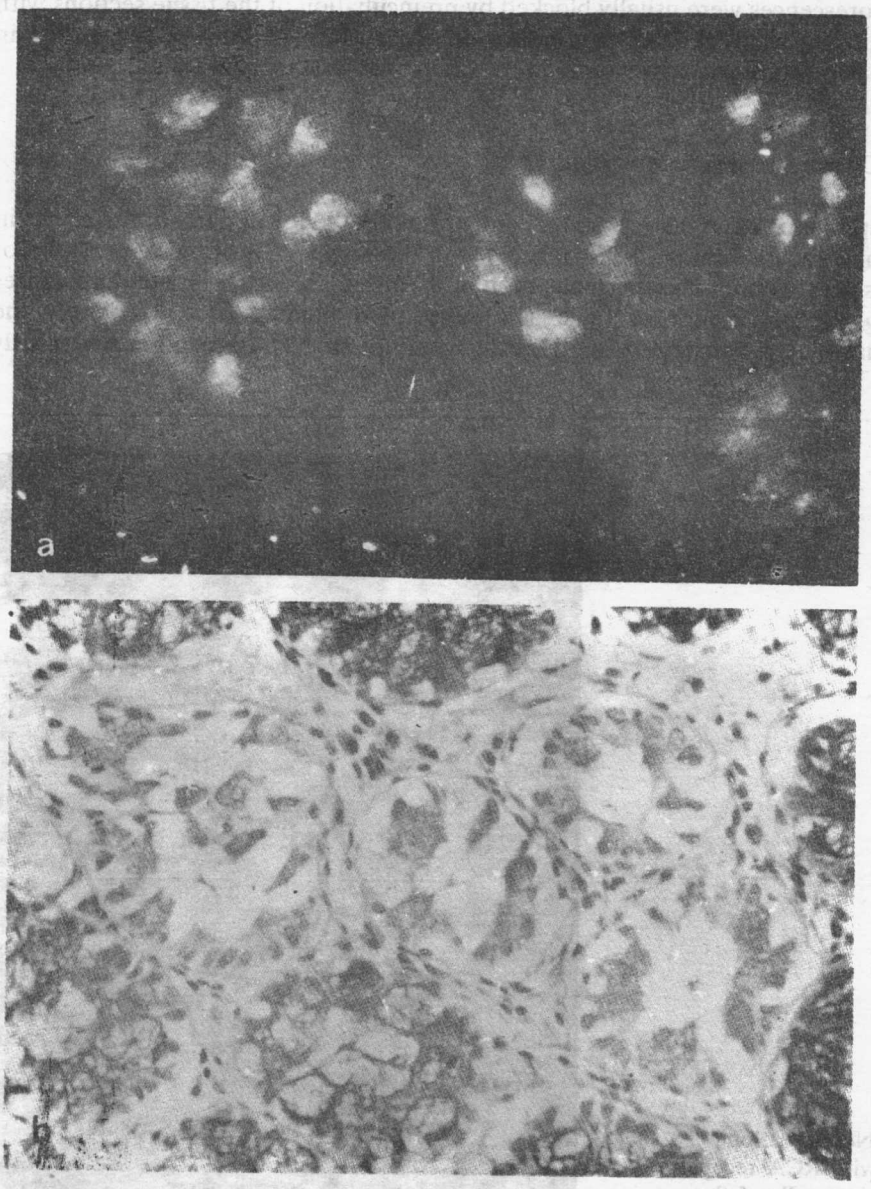

Fig. 2. a Transitional mucosa adjacent to a carcinoma showing fine granular cytoplasmatic PNA binding sites in some crypts of colonic mucosa. b Area corresponding to a in a PAS-stained tissue section; epithelial cells show a mild to moderate degree of atypia

Peanut Agglutinin Binding

The normal mucosa of the colon exhibited no PNA binding sites, even after preincubation of the tissue sections with neuraminidase. In the transitional mucosa, only a few cells reacted with PNA, showing a fine granular fluorescence in the goblet cells (Fig. 2a and b), that increased after desialylisation of tissue sections. In well

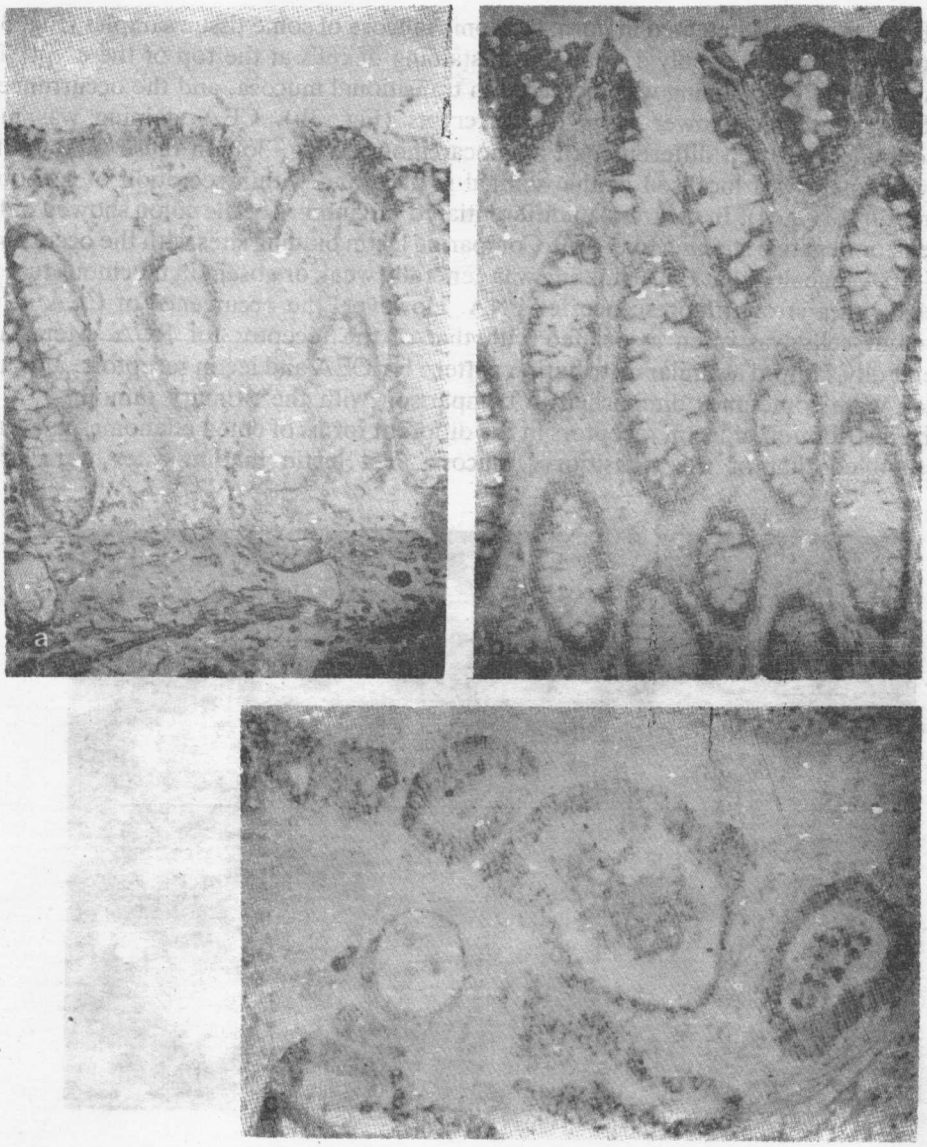

Fig. 3a–c. Occurrence of CEA (peroxidase staining) in **(a)** normal mucosa of the colon; **b** transitional mucosa adjacent to a carcinoma; **c** adenocarcinoma

differentiated carcinomas a heterogenous pattern of fluorescence was observed. The incubation of tissue sections revealed areas with mucinous cells that contained either PNA or RCA receptors alone. Furthermore groups of tumour cells occurred that exhibited binding sites for both lectins. In addition, the mucus within glandular structures also showed a positive reaction with PNA.

Carcinoembryonic Antigen

CEA was already detected in normal colonic mucosa of some tissue samples (Fig. 3a). However, there was only a weak apical staining of cells at the top of the crypt. The amount of CEA staining was increased in transitional mucosa, and the occurrence of CEA extended to lower parts of the crypts (Fig. 3b). CEA staining was most pronounced in well differentiated adenocarcinomas (Fig. 3c). In these tumours the glycoprotein was localised at the luminal surface and within secretion of glandular structures (Fig. 4). In contrast, undifferentiated carcinomas of the colon showed only a weak or negative staining for CEA. Comparing lectin binding sites with the occurrence of CEA revealed that CEA staining was generally weak or absent in mucinous tumour cells with a strong fluorescence for PNA. However, the occurrence of CEA in the tumour cells was often associated with that of the receptor for RCA. Metastases generally showed a similar distribution pattern for CEA and lectin receptors, although they were sometimes diminished in comparison with the primary tumour.
The distribution of lectin receptors in the different forms of colon adenomas generally resembled that of the transitional mucosa. Yet lectin binding sites were often

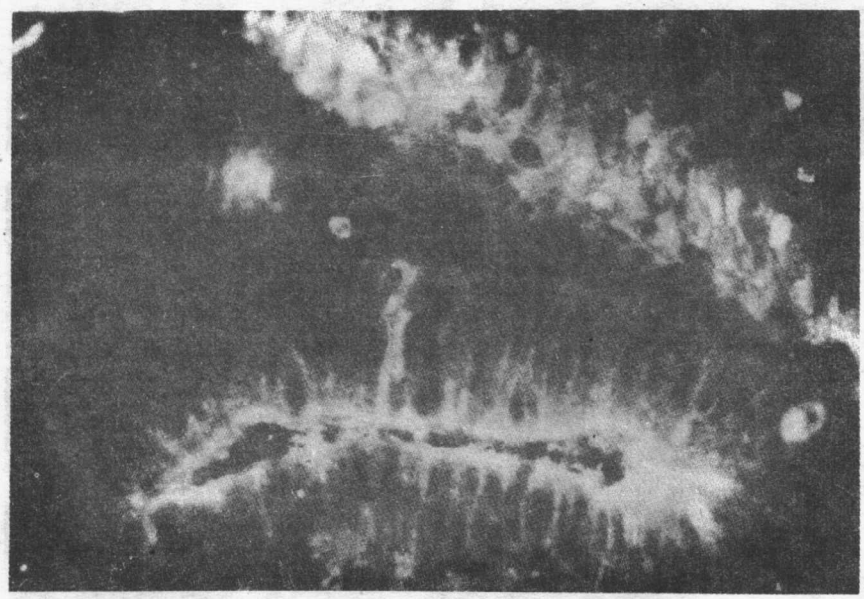

Fig. 4. Demonstration of CEA with a FITC-labelled antibody in an adenocarcinoma of the colon. Fluorescence is observed in apical parts of the cells and in the secretion lying within the lumen of the glandular structures

diminished in those parts of the adenomas where the goblet cells were reduced and the epithelium showed an increased proliferation. The same observations were made in areas of polyps with cell atypia. These findings contrasted to a certain extent with the occurrence of CEA, which was also found in regions of the polyp with an increased proliferation and moderate to severe cell atypia. In hyperplastic (metaplastic) polyps of the colon, both the lectin binding sites and the CEA were concentrated on the apical parts of the crypts.

Discussion

Our histochemical findings with labelled lectins and anti-CEA antibodies revealed differences between normal colonic mucosa and colonic carcinoma that mainly consisted in the amount of binding sites for different lectins [see also 3] and anti-CEA [2, 8, 9, 19, 25]. Therefore it should be emphasised that there exists no tumour-specific staining. The specificity of the lectin binding sites was proven by the appropriate inhibitory sugar.

The difference between normal and transitional mucosa consisted in an increased number of lectin and CEA binding sites, accompanied by an increased production of mucinous substances. This phenomenon was also observed in adenomas, with the exception that the amount of lectin receptors was reduced in proliferating areas. Carcinomas of the colon showed a wide range of lectin binding patterns, partly correlated with the morphological degree of differentiation of the tumours. Well differentiated and especially mucinous carcinomas showed the highest content of lectin receptors, whereas in undifferentiated tumours the number of lectin binding sites and CEA was reduced. The comparison of lectin binding sites with the occurrence of CEA often showed a similar distribution of RCA receptors and CEA. This may be explained by the high carbohydrate content of CEA [15], which also possesses galactose, the main binding site for RCA.

Of special interest are the findings with the peanut lectin, which was only observed in the transitional mucosa and in carcinomas, but was absent in normal colonic mucosa. This lectin exhibits a high affinity for the disaccharide galactose-galactosamine [14, 23], which is thought to be an important part of the immunodominant group of the Thomsen-Friedenreich antigen that is localized on the erythrocyte membrane. Furthermore it is proposed that there is a relationship to the blood group M and N [22]. Some authors suggested the importance of the Thomsen-Friedenreich antigen for immunodiagnostic and immunotherapeutic concepts in cancer (survery by Sedlacek and Seiler [20]).

Recently Fox [5] presented an interesting hypothesis concerning the glycoproteins in mucosubstances of the gastrointestinal tract. He emphasises the protective function of the mucosubstances and proposes that the shed membrane glycoproteins within the mucus close to the cell surface block the binding of lectins to membrane receptors. Maybe a disturbance in the production or composition of glycoprotein in the colonic mucosa represents an initial state in bowel cancer. In this connection it should be considered that lectins, a regular component of food, can directly bind to cell membranes and possibly induce cell proliferation – an essential factor of tumour genesis. It should be emphasised that lectins can possess mitogenic factors.

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