Secretory Immunoglobulins in Colonic Neoplasms

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Secretory immunoglobulins are found in nongoblet columnar cells of normal intestinal epithelium. These molecules consist of a secretory component portion, which is synthesized in the columnar cells, and an immunoglobulin portion which enters the columnar cells from plasma cells in the adjacent lamina propria. In the present work, the synthesis and transport of these various subunits have been studied by immunofluorescence in benign polyps and cancers of the colon. In both the epithelium and plasma cells of benign and malignant tumors, as well as in normal tissue, IgA is the principal immunoglobulin, followed by IgM. However, when compared to normal tissue, neoplastic epithelium contains less immunoglobulin and also less secretory component; the decrement usually inversely parallels the degree of differentiation. Thus, benign polyps closely resemble normal colonic mucosa in so far as the secretory immunoglobulin system is concerned. In contrast, atypical areas of benign polyps and carcinomas exhibit greatly decreased or absent synthesis and transport of secretory IgA. Plasma cells tend to be markedly decreased in the stroma of carcinomas, suggestive of an alteration in the normal mechanism for attracting the circulating precursors of local IgA plasma cells. Whenever neoplastic epithelium contained IgA, plasma cells with IgA could be observed in the vicinity; this is in keeping with the concept of local synthesis of secretory IgA. In some instances in which local plasma cells were plentiful, neoplastic cells were deficient in secretory component and IgA, which suggested impairment of the mechanisms for transporting IgA across epithelium. The possible role of secretory component in such transport and in attracting lymphoblasts to mucous membranes is discussed. (Am J Pathol 85:303–316, 1976)

SECRETORY IMMUNOGLOBULIN A (IgA), the body's first line of immunologic defense in mucous membranes, is composed of an immunoglobulin portion (heavy, light, and J chains), synthesized and secreted by local plasma cells, and a nonimmunoglobulin portion, secretory component (SC), a 70,000 molecular weight glycoprotein synthesized by the lining epithelial cells. Assembly of secretory IgA is thought to occur in or on the surface of the cells that make SC; the complete secretory IgA molecule then passes through the epithelial cell to be elaborated into the lumen.[1] Not all the SC fulfills this role, however; under normal conditions SC is apparently produced in excess of what is needed to complex with IgA, and this excess is elaborated as free SC.

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Supported by Grants AI-09738 and CA-08627 from the National Institutes of Health and by a grant from The Council for Tobacco Research—USA, Inc.

Accepted for publication June 30, 1976.

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In a previous study the production of SC by benign and malignant epithelial neoplasms of the colon was examined.[2] In general, synthesis of SC by neoplastic epithelial cells was proportional to the degree of histologic differentiation. Well-differentiated areas in benign polyps uniformly produced secretory component, whereas atypical zones in polyps and poorly differentiated carcinomas tended to be deficient. It was also observed that production of SC tended to correlate with the production of mucin even though in both normal and neoplastic epithelium SC and mucin are produced in different types of cells.

In the present work we have extended the earlier findings by investigating the occurrence of different classes of immunoglobulins in neoplastic colonic epithelium and in plasma cells in the stroma of tumors. The distribution of immunoglobulins in relation to SC production is presented. In benign polyps the expression of immunoglobulins and SC remains close to normal. In atypical areas of polyps and in carcinomas, the expression becomes deranged; the functional implications of this derangement are discussed.

Materials and Methods

Tissue

Specimens were obtained fresh from segments of colon resected for polyps and carcinomas.

Preparation of Fluorescent Conjugates

Free SC was isolated from colostrum and used to immunize rabbits. The specificity and reactivity of the resulting antisera have been described.[3,4] The γ-globulin fraction was isolated [5] and conjugated to fluorescein and rhodamine according to the methods of The and Feltkamp [6] and Amante *et al.*,[7] respectively. The fractions used had ratios of fluorochrome to protein of about 2. Fluoresceinated rabbit antihuman IgA and IgE, and fluoresceinated goat anti-IgM, IgD, and IgG were obtained from Behring Diagnostics and Kallestad Laboratories, respectively. Rhodaminated rabbit antihuman IgA was obtained from Cappel Laboratories.

Ulex europaeus anti-H^c (chitobiose-cellobiose inhibited) lectin [8] and a modified mucin stain were used to detect goblet cells. The lectin detects blood group H-activity, which in intestinal epithelium is selectively produced by goblet cells.[9] Purified lectin, kindly supplied by Dr. Joel Oppenheim, at 2 mg ml in 0.1 M cellobiose (to protect the binding sites) was conjugated with fluorescein isothiocyanate (FITC).[6] The conjugate was dialyzed extensively against 0.15 M NaCl–0.01 M sodium phosphate, pH 7.5, in phosphate-buffered saline (PBS) to eliminate unbound fluorescein and cellobiose.

Preparation of Immunohistologic Sections

Pieces of neoplasm with adjacent normal tissue were embedded in Tissue-Tek II OCT Compound (Lab-Tek Products), frozen, sectioned at 4 to 6 μ, fixed in acetone for 10 minutes, and stored at −30 C. Sections to be stained for IgG were washed in PBS for 15 minutes before fixation to decrease background fluorescence. Staining of the sections was

performed as follows. After soaking in PBS for a few seconds, the slides were stained with one drop of fluorescent antibody or lectin solution at 1 mg ml and incubated for 30 minutes in a moist chamber at room temperature. Double staining was performed by adding one drop of fluoresceinated reagent and one drop of rhodaminated reagent simultaneously and incubating for 30 minutes. Controls were: staining with antisera specifically absorbed for the antibody under study, staining with fluorescent normal rabbit and goat γ-globulins, and blocking by prior treatment with unconjugated antibody. The normal tissue on each section served as an additional control.

The sections were washed twice in PBS, 5 minutes each time, mounted in 5% PBS in glycerol, and kept at 4 C until examined. All slides stained in the routine fashion with fluoresceinated antibody showed nonspecific fluorescence of eosinophils; therefore, a level of each block was stained with Lendrum's stain for 30 minutes before staining with fluoresceinated antibody.[10,11] Lendrum's stain could not, however, be used when double fluorescence was employed because it interfered with rhodamine fluorescence. To permit the observation of mucin-producing cells by bright-field microscopy and fluorescent cells by ultraviolet microscopy in the same section, a modified mucin stain [12] was used. A stock solution of alcian blue was diluted five times with PBS, brought to pH 5 by the addition of 1 N sodium hydroxide, and filtered. Sections were stained for 10 minutes, washed in PBS, counterstained with hematoxylin in PBS, and washed. They were then treated with fluoresceinated antibody in the usual way.

Preparations were examined with a Leitz Orthoplan microscope illuminated by a 200-W ultra-high pressure mercury lamp. For fluorescein, KP490 and K480 excitation filters plus a K510 suppression filter and, for rhodamine, BG36 and S546 excitation filters plus a K610 suppression filter were used. Photographs were taken with Kodak Tri-X panchromatic (ASA 400) film or GAF (ASA 200) color film. Double stained sections were photographed by double exposure.

Each section was evaluated for the proportion of the lesion which fluoresced and for the intensity of fluorescence. All specimens were also examined after conventional hematoxylin and eosin staining. Polyps were diagnosed as adenomatous, villous, or mixed, and carcinomas were graded as well differentiated, moderately well differentiated, or poorly differentiated according to the criteria of Evans [13] as applied to the predominant morphologic pattern.

Results

Normal Colonic Mucosa

Secretory component was identified in columnar cells, predominantly in the Golgi zone and the apical portion of columnar cells, but was absent from goblet cells. This differential location was confirmed by staining the goblet cells with fluoresceinated *Ulex europaeus* lectin (or alcian blue) and the columnar cells with rhodaminated (or fluoresceinated) anti-SC and or anti-IgA. There was no evidence of mucin in columnar cells or of SC in goblet cells.

The immunoglobulins IgA and IgM were also prominent in nongoblet columnar cells, most conspicuously in the apical portion, but were not present in goblet cells. A predominance of IgA staining over that of IgM was noted. By double immunofluorescence IgA and IgM could be demonstrated in the same cell. A comparison between SC and immunoglobulin

(IgA and IgM) staining showed that SC occupied both the Golgi zone and the apical portion of the epithelial cell, whereas the immunoglobulins occurred mostly in the apical portion. These staining characteristics of SC and the immunoglobulins agree with previous findings.[4,14]

In normal colonic mucosa the proportions of plasma cells containing the different classes of immunoglobulins were about 80% IgA, 15% IgM, 3% IgE, and 2% IgD and IgG, in general agreement with the results of other authors.[15-18] The plasma cells were distributed throughout the lamina propria and were most abundant surrounding the upper portions of the crypts.

Benign Polyps

Twenty-five adenomatous polyps were studied (Table 1). For the most part they were composed of well-differentiated glands in which the intracellular localization of the components of the secretory immunoglobulin system was similar to the normal colonic mucosa. The majority of the polyps showed normal or somewhat decreased epithelial fluorescence for SC and immunoglobulins as compared to normal epithelium, and in some glands no immunoglobulin was noted (Figures 1 and 2). Some atypical glands in these polyps lacked SC and immunoglobulins, or lacked only immunoglobulins, or exhibited a marked decrease in these proteins. Occasional atypical glands with decreased SC showed a normal intensity of IgA; however, IgA was never observed in glands devoid of SC. In atypical glands IgM and the other immunoglobulins were always absent. Mucin-producing cells were plentiful in well-differentiated areas, though usually in lesser quantities than in normal colonic epithelium. In atypical areas, mucin-producing cells were greatly decreased.

The lamina propria within polyps showed equal or slightly increased numbers of plasma cells compared to normal mucosa (Figure 1), with a similar percentage distribution among classes of immunoglobulin. Whenever epithelial staining for SC, IgA, and IgM was observed in the glands, subjacent plasma cells were always identified. Usually the intensity of the epithelial staining correlated with the number of underlying plasma cells. In certain instances where secretory component was positive and IgA was negative in the epithelium, IgA-positive plasma cells were seen in the adjacent stroma (Figure 2). Some atypical areas, in which the epithelium was negative for SC, IgA, and IgM, showed a decreased population of IgA and IgM plasma cells in the stroma.

Nine villous polyps were studied. For the most part they exhibited similar staining characteristics to the adenomatous polyps. However, many glands exhibited atypical patterns of immunofluorescence, espe-

cially where the epithelium was pseudostratified and composed of cells with poor polarity. Such areas were often negative for SC and intraepithelial immunoglobulins, and showed normal or decreased numbers of plasma cells as seen in the atypical areas of adenomatous polyps.

Nine mixed polyps showed characteristics similar to those described above, with a decrease in epithelial SC, IgA, and IgM, as well as decreased numbers of plasma cells in the atypical areas.

Carcinomas

Thirty-eight carcinomas were classified into well-differentiated, moderately well-differentiated (the predominant category), and poorly differentiated carcinomas. Some features of these lesions are listed in Table 1. In the case of carcinomas the characteristics given apply to the areas showing the major morphologic component, upon which the diagnosis was based. In general, the components of the secretory immunoglobulin system were much less prominent than in normal or benign neoplastic colonic tissue, especially in the least well differentiated tumors. The presence of intraepithelial SC was variable but tended to correlate with the degree of histologic differentiation and with the production of mucus as observed previously.[2] The normal segregation of the production of SC and mucus to different cells was maintained; none of the tumors in this study exhibited cells which contained mucin, secretory component, and immunoglobulin together within the same cell.

In the four well-differentiated carcinomas there was a decrease in SC production. Decreased amounts of IgA and absence of the other immunoglobulins were also noted in the neoplastic epithelium. Slightly decreased numbers of plasma cells were present within these tumors, but the junctions with normal mucosa showed increased numbers of plasma cells.

Thirty moderately well-differentiated carcinomas showed decreased numbers of IgA plasma cells, especially in relation to glands lacking SC and immunoglobulins. Occasionally, however, IgA plasma cells were readily visualized close to such glands (Figure 3). The percentage distribution among the different classes of immunoglobulin in plasma cells was normal. In general IgA was greatly diminished in the epithelium. Most cells containing SC lacked IgA, and SC-negative cells never showed prominent amounts of IgA. The remaining immunoglobulin classes were not observed in the epithelium. Whenever intraepithelial IgA was present, there were nearby plasma cells synthesizing IgA. Two of the moderately well-differentiated tumors contained large mucinous areas, which lacked plasma cells and immunoglobulins (Figure 4).

In four poorly differentiated carcinomas the bulk of the lesions tended

Table 1—Some Characteristics of Colonic Neoplasms

Tissue	Epithelial Markers*			Plasma cells†					Number of specimens
	Columnar cells		Goblet cells						
	SC	IgA	Mucin	IgA	IgM	IgD	IgE	IgG	
Adenomatous polyps	2+	2+	2+	5+	2+ to 3+	1+	1+	1+	25
Villous polyps	1+ to 2+	1+ to 2+	2+	3+ to 4+	1+ to 2+	–	–	–	9
Mixed polyps	1+ to 2+	1+ to 2+	2+	5+	2+ to 3+	1+	1+	1+	9
Well-differentiated carcinomas	2+	2+	2+	3+ to 4+	1+ to 2+	–	–	–	4
Moderately well-differentiated carcinomas	1+	1+	– to 2+	1+ to 3+	– to 1+	–	–	–	30
Poorly differentiated carcinomas	–	–	–	–	–	–	–	–	4
Normal	3+	3+	3+	4+	2+	1+	1+	1+	81

* Intraepithelial secretory component (SC), IgA, and mucin were graded from – (absent) to 3+ (normal) according to the proportion of positive cells and the intensity of fluorescence. These two criteria were parallel.

† Population densities of plasma cells containing the various classes of immunoglobulins graded as follows per microscopic field: – (none), 1+ (1 to 5 cells), 2+ (5 to 15 cells), 3+ (15 to 30 cells), 4+ (30 to 60 cells), and 5+ (greater than 60 cells). Wide-field 10 × oculars and a 25 × oil immersion objective were used.

to be devoid of SC, intraepithelial immunoglobulins, and mucin. Occasional plasma cells containing IgA were observed at the deepest margins of invasion, and near the better differentiated glands (Figure 5). For the most part, plasma cells were markedly decreased or absent.

Discussion

The synthesis, packaging, and secretion of several macromolecules by normal and neoplastic intestinal epithelial cells have been investigated.[2,19-23] Some of these can be used as markers to distinquish between goblet-type and columnar cells. For example, mucin and the ABO blood group substances occur only in the former,[9] whereas SC and immunoglobulins are limited to columnar cells,[4,14,24] which are integral to the transport of immunoglobulins from the lamina propria to the lumenal contents. In the present study the production and transport of the various components of the secretory immune system and of mucin have been assessed by immunofluorescence in polyps and cancers of the colon.

Mucin-producing cells in normal colonic mucosa never contained any immunoglobulins. The latter together with SC were always found in the columnar cells, most conspicuously in the apical portion of the cytoplasm. IgA was the most noticeable immunoglobulin in columnar cells, in keeping with the predominance of underlying plasma cells containing this immunoglobulin. The presence of IgM in columnar cells was less conspicuous, and the other immunoglobulins were present in only minimal quantities, again consistent with the distribution of local plasma cells. Within epithelium, IgA and IgM could be demonstrated in the same cell.

The secretory immunoglobulin system in benign polyps generally closely resembled that of normal colonic mucosa. However, in atypical areas of polyps and in most cancers there was usually a disruption of one or more of the steps involved in the assembly and transport of secretory immunoglobulins. In the case of neoplastic epithelium there tended to be a decrease in both the intensity and geographic extent of secretory component production which paralleled the degree of histologic and cytologic differentiation, as reported earlier.[2] The number of local plasma cells could also be correlated with the histologic appearance of the tumor. In relation to benign tumors and well-differentiated carcinomas there were approximately normal numbers of plasma cells. However, plasma cells were much reduced in the stroma of moderately well and poorly differentiated cancers. In all cases, for the plasma cells that were present, the ratios of cells containing the different classes of immunoglobulin followed the normal pattern, i.e., mostly IgA, followed next by IgM. As far as intraepithelial immunoglobulins in neoplasms are concerned, again IgA,

and to a lesser extent IgM, predominated. These intraepithelial immunoglobulins were less visible as the degree of differentiation of the neoplasm diminished.

The variations in numbers of plasma cells associated with benign versus malignant lesions, and with the degree of differentiation of the latter, are intriguing. They could reflect differences in the production of substances which normally influence the homing of lymphocytes to mucous membranes or that affect the movement of leukocytes more generally.[25,26] Variations in nonspecific or tumor-specific antigen content could also play a role. Several investigators have correlated degree of differentiation and prognosis with the number of lymphocytes and plasma cells in the vicinity of malignant tumors.[27-29] Although not conclusive, most authors believe that higher populations of lymphocytes and plasma cells, as found in the well-differentiated lesions in the present study, are associated with a better prognosis. Certainly in the present group of tumors, the poorly differentiated invasive carcinomas exhibited relatively few plasma cells.

Under normal conditions the IgA that occurs in mucous membrane secretions is produced by local plasma cells which are thought to be the terminal step in an IgA cell cycle which begins with lymphocytes already committed to IgA in the organized lymphoid tissue of the gut, such as occurs in Peyer's patches and the appendix.[1] Via the mesenteric lymph nodes, thoracic duct, and blood, these cells reach the lamina propria of the intestine where they quickly differentiate into plasma cells. Presumably a local receptor attracts the homing cells to the lamina propria. Appreciable evidence also exists that transport of the locally secreted IgA through the intestinal epithelium is selective. Study of the secretory immune system in disease can potentially shed light on some of these points.

It has been suggested that secretory component, an exocrine mucosal epithelial cell product, could mediate the homing of IgA-committed lymphocytes to mucosal sites. We have previously shown that in large bowel neoplasia there tends to be a decrease in the production of SC which correlates with the degree of differentiation.[2] In the present work a correlation was found between the number of local plasma cells and the morphology of the tumor. The observations suggest a relationship between the loss of epithelial SC and the diminished numbers of plasma cells in the lamina propria. In a recent case report, evidence was provided for defective production of SC and local IgA.[30] The former lack was considered to be the fundamental problem, and it was hypothesized that a deficiency of local IgA production stemmed from a deficiency in local receptor, namely SC, necessary to attract the precursors of the IgA plasma cells. It should be noted, though, that in an experimental system some

evidence inconsistent with this view has been obtained.[31] Also, under normal conditions a consistent relationship between production of SC by epithelial cells in mucous membranes and the local presence of IgA plasma cells is lacking. For example, the epithelium of the gallbladder produces SC, but in the absence of inflammation IgA plasma cells are not conspicuous.[4] Conversely, in the gastric mucosa, IgA plasma cells are evident but the glands contain negligible amounts of SC as evaluated by immunofluorescence.[32] Consistent observations were made in the present study that IgA plasma cells could be plentiful in the stroma of atypical areas of colonic neoplasms which synthesized little or no SC and which revealed no epithelial IgA. Also, areas in carcinomas which were positive for SC sometimes lacked plasma cells.

A basic observation concerning the transport of IgA into exocrine secretions has been the associated mucosal IgA-secreting plasma cells. In all the normal and neoplastic tissues examined in the present work, wherever IgA was present in glandular epithelial cells, plasma cells containing IgA were found in the adjacent stroma, in keeping with the concept of local synthesis of secretory IgA. However, since IgA plasma cells could be observed in relation to glands lacking intraepithelial IgA in some instances, it seems clear that the mere presence of IgA-secreting plasma cells, although necessary, is not sufficient for the passage of IgA across epithelial surfaces. This transport is thought to be selective. Another postulated function for secretory component is to mediate this transport process since SC is synthesized by the columnar cells through which IgA passes and since the secretory IgA molecule contains one SC polypeptide chain. The frequent observation in the present studies of approximately normal numbers of IgA (and IgM) plasma cells underlying a neoplastic epithelium devoid of SC, and also immunoglobulin, is certainly consistent with such a view. Nevertheless, if SC does play a role in transport, its mere presence in epithelial cells is not sufficient. Thus, in both villi and crypts of the normal small intestine, SC is produced in the epithelium and abundant IgA plasma cells are present in the adjoining stroma, yet IgA only enters the epithelial cells of the crypts.[4] In the present investigation the numbers of plasma cells observed in association with carcinomas were in general fewer than in normal mucosa. This decrement in plasma cells thus paralleled the reduced content of intraepithelial immunoglobulin and suggested a cause-and-effect relationship. Yet in certain instances the stroma did contain IgA plasma cells but the overlying neoplastic epithelium lacked IgA even though it was synthesizing SC. Conversely, rare neoplastic glands showed dramatic reductions in SC but only a moderate reduction in IgA. There was no instance,

however, in which epithelial cells that were devoid of SC contained prominent amounts of IgA. Thus, a role for SC in the transepithelial passage of IgA remains open. Clearly much needs to be learned concerning the mechanisms by which the precursors of IgA plasma cells reach mucous membranes and how IgA is transported across epithelial surfaces.

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Legends for Figures

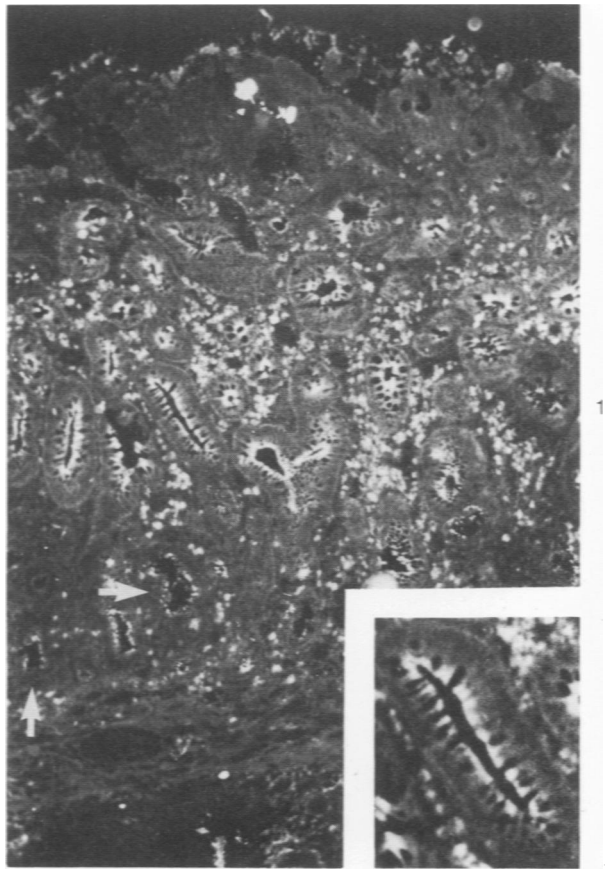
Figure 1—Adenomatous polyp treated with fluoresceinated anti-IgA. Plasma cells producing IgA are present in the lamina propria. IgA is also seen within the epithelial columnar cells. This distribution corresponds to that in normal mucosa. Although IgA is present in most glands, there are scattered glands with markedly decreased or no immunoglobulin (*arrows*). (× 50) **Insert**—A higher magnification of one gland illustrating that IgA is localized to the apical portions of columnar cells and is absent from goblet cells as in the normal (× 100).

Figure 2—Adenomatous polyp. **A**—Treated with fluoresceinated anti-SC; SC is synthesized throughout the adenomatous epithelium in nongoblet columnar cells (× 50). **B**—Neighboring section treated with fluoresceinated anti-IgA. (*Arrows* mark the same gland in the two sections). Plasma cells producing IgA are abundant in the lamina propria. In this region, which is histologically indistinguishable from other areas, the glands show a marked decrease or absence of epithelial IgA, an unusual occurrence in well-differentiated glands. (× 90).

Figure 3—Moderately well differentiated carcinoma doubly stained with rhodaminated anti-IgA and fluoresceinated anti-SC and photographed under rhodamine fluorescence. The glands are negative or but faintly positive for IgA and are negative for SC (not shown). Many IgA plasma cells, an unusual situation in cancers, lie in the stroma. (× 120)

Figure 4—Mucinous adenocarcinoma. The section was treated with fluoresceinated *Ulex europaeus* lectin to stain mucin-producing cells (**A**) and with rhodaminated anti-IgA (**B**). **A**—Normal glands are seen at the lower right; these show mucin-positive goblet cells (*arrows*). The left side shows nests of mucin-producing tumor cells. **B**—The same field showing the normal glands on the right (*arrows*) with apical IgA in columnar cells and negative goblet cells. At the left, nests of tumor cells are negative for IgA, and there are no IgA plasma cells. (× 240)

Figure 5—Moderately to poorly differentiated carcinoma treated with rhodaminated anti-IgA. The poorly differentiated area (*above*) does not appear because it is completely negative for IgA. The moderately well differentiated gland (*below*) shows apical staining. Several IgA plasma cells lie between these two zones. (× 240)



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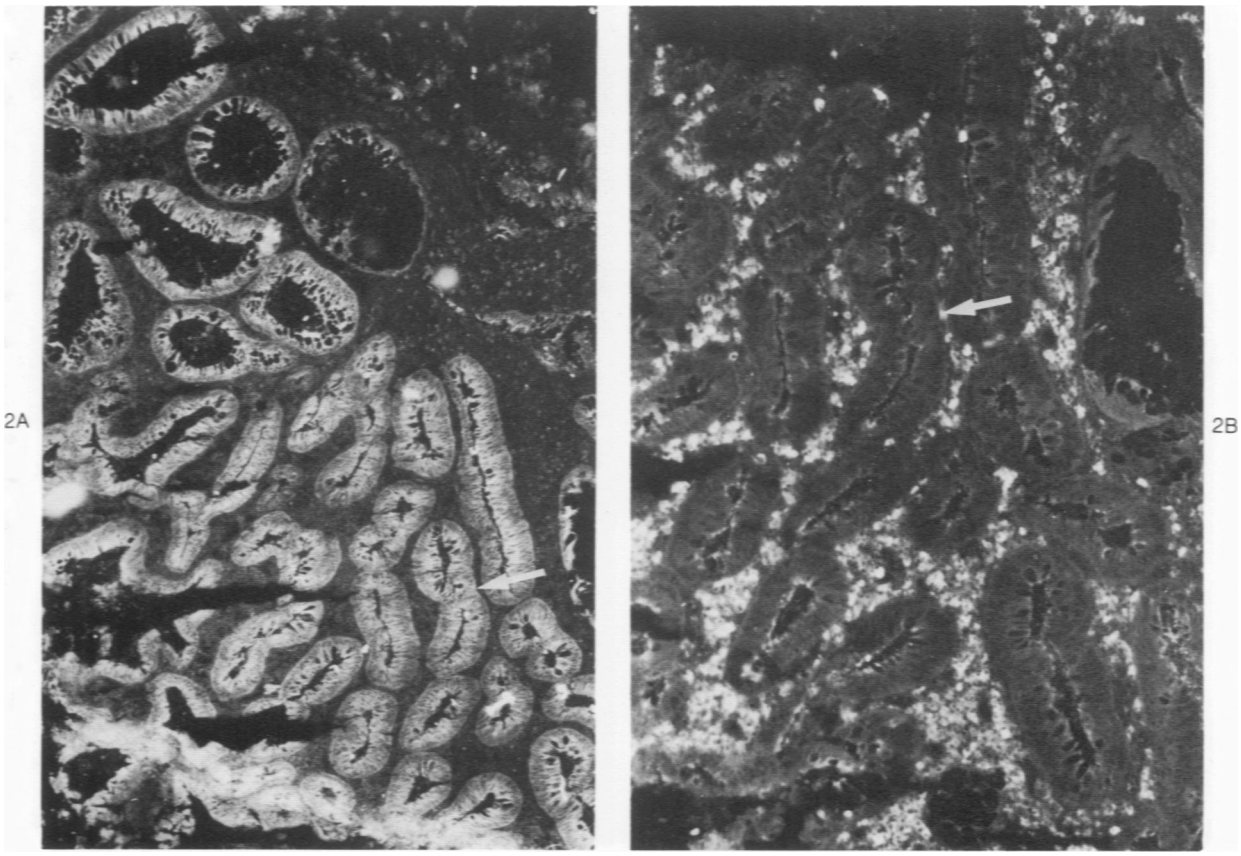

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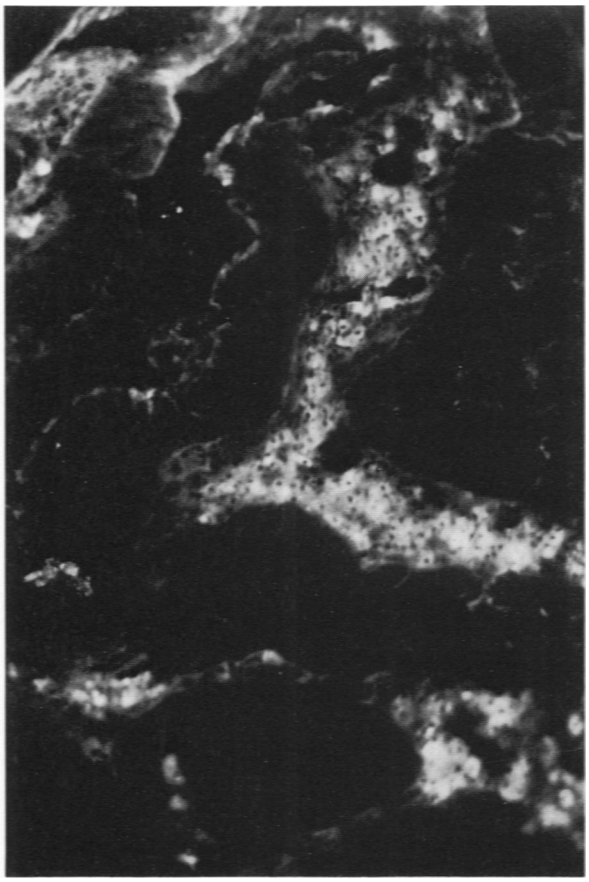

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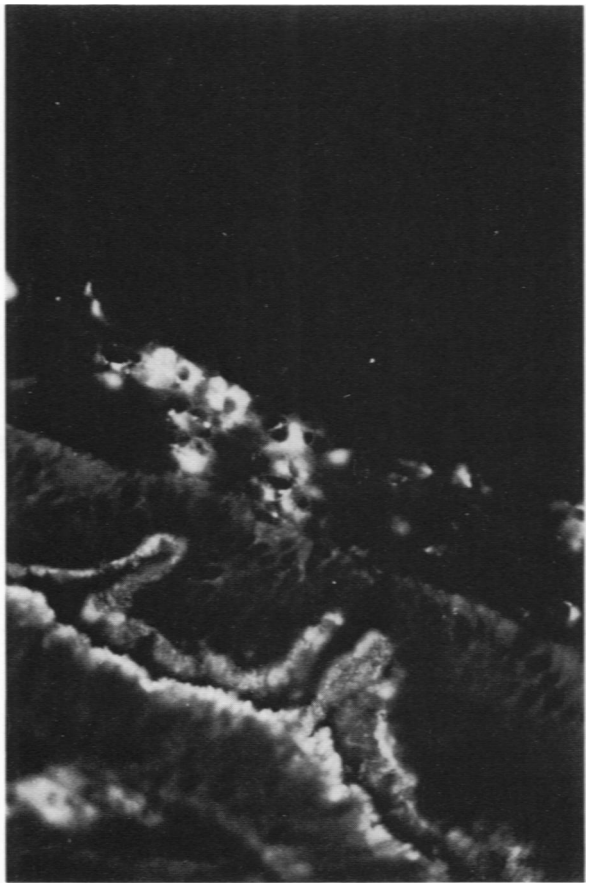

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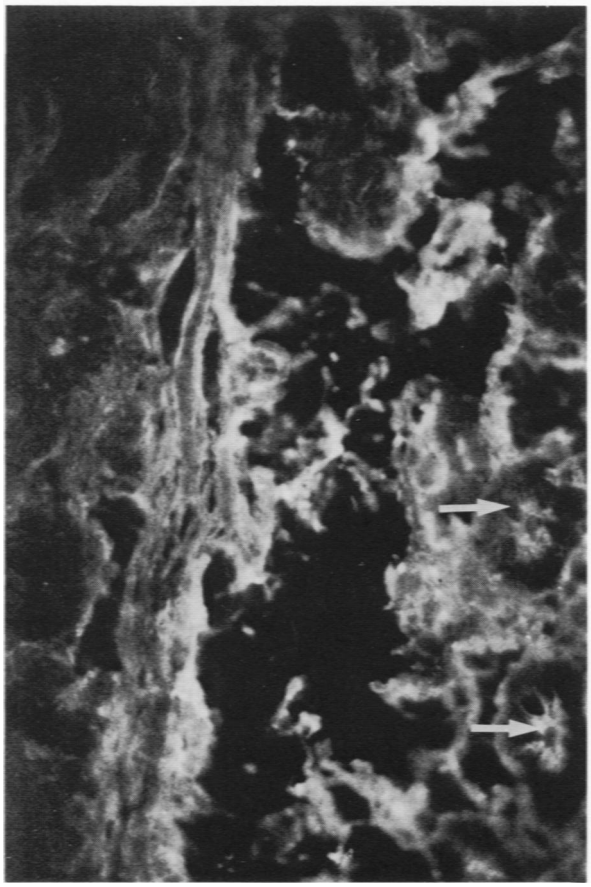

4B