The Distribution of *Escherichia Coli* Antigens in Rectal Mucosa (35378)

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(Introduced by Y. T. Oester)

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Speculation that ulcerative colitis (UC) may be the result of an immunological process has heightened interest in the nature of the antigens in the colon (1). This report describes our findings of antigens which react with antibodies against *Escherichia coli* in normal and diseased rectal mucosa.

Materials and Methods. Subjects. Five ambulatory adult male patients with UC were selected on the basis of mild to moderate proctoscopic changes (2). Three of the patients had been receiving salicylazosulfapyridine (Azulfidine) for several months. None of the patients had received steroids for at least 2 years. Seven healthy adult males served as control subjects. Blood types A, B, and O were represented in both groups. Informed consent was obtained from each subject prior to rectal biopsy.

Proctoscopic mucosal biopsies were obtained from rectal valves approximately 10 cm proximal to the anus. The specimens were rinsed in sterile saline and fixed in cold (5°) 95% ethanol for about 30 min, rinsed in water, frozen and sectioned in a cryostat. Specimens were mounted to one side of a glass slide, permitting adjacent smears of *E. coli* O 14.L 7 and O 119:B 14 for comparison. Hematoxylin–eosin stained slides documented the extent of UC (2).

The *E. coli* antigens were determined by the direct fluorescent antibody technique. Commerical fluorescein conjugated antisera were used with the exception of anti *E. coli* O 14:L 7 which was prepared in the laboratory.

Control and immune serum were obtained from a rabbit injected with formalin killed *E. coli* O 14:L 7. The total globulins were prepared by a cold methanol fractionation procedure (3). The globulins were conjugated with fluorescein as described by Klugerman (4). Passage through Sephadex G 25 with buffered saline removed excess dye and restored the conjugate to pH 7.4. The presence of immunoglobulins in the conjugate was verified by immunoelectrophoresis. Slide agglutination tests (5) were performed on the conjugates and corresponding antisera to verify immunochemical activity.

The general scheme for examination of serial sections for *E. coli* antigenic determinants included the following: (a) untreated native tissue control, (b) conjugated preimmune rabbit serum as a test of innate activity, (c) conjugated rabbit anti-*E. coli* serum globulins, (d) equal mixtures of conjugated and untreated antiserum as a test for specific competitive inhibition, (e) conjugated antiserum containing 1 part in 20 of lissamine-rhodamine conjugated bovine serum to define nonspecific staining (6). Because of the special interest in the O 14 antigen the following were performed in addition to the above tests: (f) conjugated *E. coli* O 14 antiserum absorbed with a suspension of the specific O 14 antigen-bacteria were removed by passage through a Millipore GS filter of 0.22-μ porosity resulting in a 70% reduction of the fluorescent protein, (g) a heterologous antiserum (group G streptococcus) was used as a control of the inhibition test.

The bacterial smears were fixed to the tissue slides by immersion in 95% ethanol. The specimens were exposed to the stains for 20–30 min at 25° in a humid chamber. The conjugates were shaken off the slides which were then washed twice for 5 min in buffered saline (pH 9.3). They were air dried after a final rinse in distilled water. A drop of

buffered glycerine [9 parts glycerine:1 part 0.05 *M* Tris buffer (pH 9.5.)] was added to the slide and a 1.5 mm cover slip cemented in place with Permount.

A Zeiss large fluorescence microscope was used to examine the immunofluorescent specimens. It consisted of a cardioid dark field condenser, an Osram HBO-200 light source, a BG-12 exciter filter with BG-38 heat filter, and a Zeiss 47,-65 barrier filter. Photographs were made with Kodak 135 high speed Ektachrome film using the Zeiss automatic exposure device.

Results. Normal specimens, rabbit globulins. The characteristic pattern of normal, unstained tissue showed intense blue autofluorescence of the glandular structures superimposed upon a dull, light-green background. Staining with labeled nonimmunized rabbit globulins altered this appearance, by giving the glandular structures a general light-green appearance in some cases. Positive staining by labeled immune rabbit globulin was defined as (a) fluorescent staining in excess of that produced by application of the labeled preimmune rabbit serum to the same tissue, and (b) fluorescence which was appreciably diminished or entirely eliminated by the simultaneous application of the same serum in unlabeled form. These criteria were filled in each instance in which *E. coli* O 14:L 7 and the combined OB antisera "Poly A" and "Poly B" were used. When antisera to individual OB strains were used, positive results were obtained in 5 of 7 experiments with O 119:B 14 and in 6 of 7 experiments with O 111:B 4. In the case of *E. coli* O 14:L 7, the conjugated antiserum which had been absorbed with the homologous antigen stained the colon only to about 5% the intensity obtained without absorption. The *E. coli* O 14:L 7 cells were not stained by this preparation. The brilliance of the deposited stain varied in different normal subjects; this variation was greater than the variation when different *E. coli* effects on the same individual's tissue were compared. Despite differences in intensity and extent of staining, a consistent pattern was seen, in which the basement membrane, basal portion of the epithelial cells, and the interstitial leukocytes attracted the labeled globulin. The basement membrane stained heavier, and more consistently, than other areas. In sections of tissue which took up very little stain, only portions of the basement membrane were always seen, and the other areas in varying degrees. In control experiments, when inhibition was incomplete, portions of the basement membrane retained fluorescence when the rest of the tissue showed none. In cases where inhibition was complete the basement membrane also failed to absorb labeled antibodies. In contrast, the apical portion of the acinar goblet cells failed to stain and specific staining of material in the glandular lumina was very rarely observed. These areas remained black or autofluorescent blue, surrounded, in positive specimens, by the shell of bright green fluorescence at the base of the acinar glands.

Lissamine–rhodamine counterstain confirmed and delineated specific reaction by staining the rest of the tissue fluorescent orange-yellow to orange. The counterstain was attracted to the lymphocytes, but did not affect the intense staining of the acini or basement membrane.

There was no correlation between the patient blood group and the intensity of staining. As noted above, very little staining was observed at the apex of the goblet cells or in the glandular lumen, the areas where blood group antigens in colon have been described (7).

Black and white photographs do not accurately reproduce the effect of the blue autofluorescence, green fluorescein, and orange-red lissamine–rhodamine, but an impression may be obtained from Fig. la and b.

UC specimens, rabbit antisera. The autofluorescence of ulcerative colitis tissues resembled that of the normal subjects, although there was mild distortion of the architecture, and increased interstitial leukocyte infiltration. Reaction with labeled rabbit globulins altered this appearance, with positive staining in every instance. The interstitial leukocytes were not only increased in number, but in many areas, masses of leukocytes absorbed the labeled antibodies, causing fluorescence in the interstitial tissues and alongside the

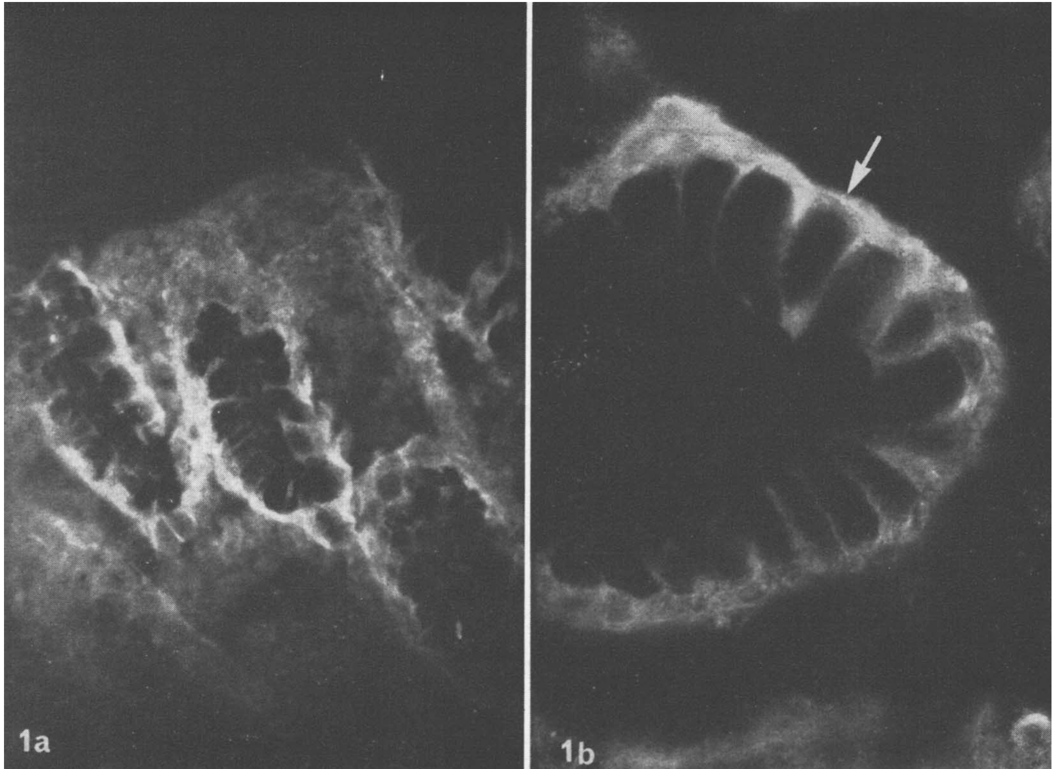

FIG. 1a. Rectal mucosal gland stained with conjugated (FITC) rabbit anti-*E. coli* O 119:B 14 serum; ×256. (b) Rectal mucosal gland stained with conjugated (FITC) rabbit anti- *E. coli* O 14:L 7 serum; ×640; (arrow) fluorescent basement membrane.

basement membrane. This exceeded that seen in the normal subjects. Damage to the basement membrane is a hallmark of UC (8), but it was possible to identify portions of basement membranes on all slides, and these stained as well as the basement membrane in normal tissue. We noted no differences between sections from patients receiving Azulfidine therapy and those who were not, or in comparing sections of patients with different blood types.

All specimens, human gamma globulin. The histochemical pattern of the interaction of conjugated human gamma globulin with colon was identical to that for the *E. coli* immune sera except as to intensity. Four of the 12 specimens (three normal and one UC) were nonreactive.

Discussion. We may assume that some of the antigens we stained, particularly those in leukocytes, were acquired from enteric bacteria. The gram-negative intestinal bacteria are rich sources of potent antigens (9), the human exposure to *E. coli* is universal (10), and in UC this *E. coli* concentration is even greater than normal (11). Recently, an amino acid component of bacterial cell walls has been found in the rectal mucosa of patients with UC (12), suggesting a particularly high concentration of bacterial products in the mucosa, a possibility correlating with the marked staining of the phagocytic cells which we observed in UC tissue sections.

It is also possible that human colon tissue shares some antigenic determinants with *E. coli* supporting this possibility, hemagglutination tests have shown common antigenic determinants in germ free rat colon and an extract of *E. coli* O 14 (13). The persistent glandular staining pattern we observed, with maximal concentration in one area (the basement membrane) supports the view that the colon itself shares antigenic determinants with *E. coli.*

Sera from patients with UC may show low titers against an OB *E. coli* strain (14), but show normal titers against *E. coli* O 14. One would predict then, that attempts to stain rectal tissue with human UC sera would produce staining similar to that produced by our immune rabbit sera and pooled human gamma globulin. This has been attempted but the results differed from ours in that staining of the basement membrane was not observed, and control (normal) sera did not stain tissue at all (15). In view of the ubiquity of *E. coli* antibodies, the failure of those human sera to stain basement membranes must be explained. The labeled globulins applied in that study were not tested for antibodies against *E. coli*, and it is possible that titers, as a result of the methods used in preparation, were low. It is also possible that soluble *E. coli* or *E. coli*-like antigens were displaced and lost during the staining process since those authors used unfixed tissues. Our experience with the cold ethanol fixation of colon biopsies shows the cytologic detail intact while the mucopolysaccharide antigens are insolubilized. It is unlikely that immunochemical reactivity is significantly altered with this procedure (16). It has been shown that human UC and normal sera fail to stain areas of blood group substance concentration in unfixed rectal mucosa. We were able to extend this observation to fixed tissues, and to rabbit anti-*E. coli* O 86:B 5 and O 111:B 4 globulins, which strongly cross-react with blood group substances (17).

Our finding that antibodies against *E. coli* react with antigens in human colon are of particular interest because of the maximal concentration which we found in the area of the acinar gland basement membrane. The basement membrane is particularly susceptible to injury in ulcerative colitis (8) and it is tempting to speculate that an immune reaction between *E. coli* antibodies, and these antigens, plays a role in the pathogenesis of this disease. Immunoglobulins are present in the rectal mucosa (18), and the damaging effects of antigen–antibody reactions in tissue have been described. An immune defect against at least one OB *E. coli* strain is present in active UC (19) and we believe that further study of the immune reaction in UC is indicated.

Summary. Human and rabbit globulins possessing *Escherichia coli* agglutinating activity reacted with antigens present in normal and diseased (ulcerative colitis) rectal mucosa. The direct immunofluorescent staining technique gave positive results when OB *E. coli* strains were tested. Staining was most marked in the basement membrane of the nucosal glands, and in the basal portion of the acinar epithelial cells. Sections of ulcerative colitis rectal tissue showed increased staining of interstitial leukocytes. The observed effects were independent of the donor blood types and did not follow the distribution of blood group substance in rectal mucosa.

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