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Immunocytochemical Reactions of Serum from Ulcerative Colitis Patients.* (27202)

DAVID KOFFLER, JOHN GARLOCK, AND WALTER ROTHMAN
(Introduced by Hans Popper)
Departments of Pathology and Surgery, The Mount Sinai Hospital, New York City

The association of chronic ulcerative colitis with arthritis, urticaria, erythema nodosum, conjunctivitis, hepatic and renal damage suggests the presence of an altered immunologic apparatus(1). The colon participates in nonspecific allergic reactions of the Arthus(2), and Auer types(3). The serum contains precipitating(4), agglutinating(5) and complement fixing antibodies(6) which react primarily with extracts of colonic mucosa, but also of liver and kidney. However, this has been disputed(10). Antigenic specificity resides in the microsomal fraction of colonic mucosa(7,8) although lesions have been produced with colotoxic serum only rarely(9), and an attempt at eliciting autoimmune disease of the gastro-intestinal tract was not successful(11). Therefore sera of patients with ulcerative colitis and regional enteritis were studied for gamma globulin factors bound to human colonic mucosa and hepatic ductules by the indirect fluorescent antibody technic of Coons.

Material and methods. A few drops of the sera from 25 patients with ulcerative colitis, 8 with regional ileitis, 27 with other diseases, and 26 of normal controls (Table I) were applied to cryostat sections of normal human adult colon at room temperature for 30 minutes(12). Following 3 washes with buffered saline, they were treated for 30 minutes with fluorescinated rabbit anti-human gamma globulin absorbed with liver powder. Control tissues were first blocked by unlabeled rabbit anti-human gamma globulin before use of fluorescinated rabbit anti-human gamma globulin. All sections were then washed, mounted in buffered glycerin and examined under a fluorescence microscope.

Patient's sera which showed positive binding with adult colon were then applied to the following sections using the indirect technics: sterile fetal colon, patient's own resected colon, Brunner's glands from normal duodenum and liver with malignant hepatitis showing proliferation of bile ductules (Table II). Additional sections of adult colon were incubated 30 to 45 minutes with 0.5% periodic acid to inactivate polysaccharide substance and then treated by the indirect method. Isopentane and dry ice fixed surgical specimens of acute ulcerative colitis, regional

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TABLE I. Normal Adult Colon Stained with Patients' Sera.

Type of case	No. of cases	Binding with mucosal glands
Ulcerative colitis	25	3
Regional ileitis	8	0
Other diseases (hospital controls)	27	0
Normal controls	26	0

ileitis, and normal colon were sectioned and stained directly with fluorescinated rabbit anti-human gamma globulin.

Results. The sera of 3 patients with ulcerative colitis exhibited binding to normal colonic glands (Table I). Similar binding was not encountered in sera of patients without ulcerative colitis. Fetal colonic mucosa showed the strongest reaction with patients' sera. Diffuse fluorescent staining was limited to epithelial glands Fig. 1A. In normal adult colon small foci of gamma globulin containing plasma cells were found scattered in the mucosa and submucosa in addition to fluorescent staining of epithelial glands and contiguous mucus. Surgically removed colon when reacted with autologous serum showed positive binding similar to normal colon. Gamma globulin containing cells were increased in active ulcerative colitis and regional ileitis cases. When sections of normal colon were incubated with Periodate solution and treated by the indirect Coon's technic, fluorescent staining was markedly decreased but not eliminated. Brunner's glands of normal duodenum showed no reaction with any serum tested (Table II).

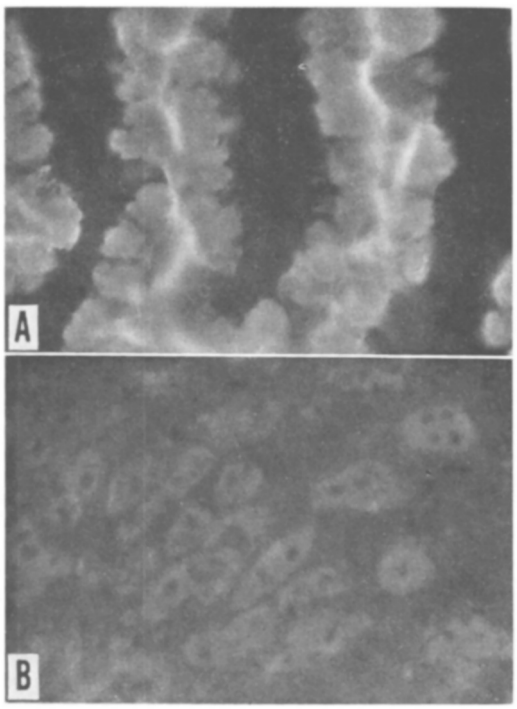

FIG. 1. Fluorescence photomicrographs of cryostat sections stained with sera of ulcerative colitis patients. A. Fetal colon (×143). B. Liver with proliferated bile ductules (×143).

TABLE II. Staining Reactions of Sera Binding with Colonic Mucosal Glands.

Type of tissue	No. of cases	Binding (± - 3+)*
Sterile fetal colon	3	3+
Patient's surgically resected colon	2	1+
Brunner's glands of normal duodenum	3	0
Liver (malignant hepatitis)	3	1+
Normal adult colon before periodic acid treatment	3	2+
Normal adult colon after periodic acid treatment	3	±

* Grading of fluorescence, ± = trace to 3+ bright.

The 3 positive sera were applied to sections of the liver with proliferated ductules Fig. 1B. Binding of these sera to the cytoplasm of ductular cells was found(13).

Discussion. The sera of some patients with ulcerative colitis contain a gamma globulin factor bound specifically to colonic epithelium. The colonic antigen present in sterile fetal colon is not dependent on the presence of bacteria or inflammation. Decreased binding of serum gamma globulin by intestinal mucosa following Periodate treatment suggests that the antigenic substrate is at least in part a polysaccharide. The increased gamma globulin in the intestine with active regional ileitis and ulcerative colitis reflects either non-specific insudation of protein, local formation of antibody or specific localization of gamma globulin, to be determined in the future.

Steroid therapy and disease inactivity may have decreased the titer of circulating antibodies. With young children as subjects, other quantitative technics have revealed a

higher proportion of patients with circulating antibodies to extracts of colonic mucosa(4). The indirect Coon's technic, however, facilitates the study of non-precipitating antibodies and the localization and histochemical properties of antigenic material in the colon.

The serum globulin binding to hepatic ductules may indicate that common or similar cross-reacting antigens reside in colon and liver. A common embryological association of these organs and the known propensity for hepatic damage in ulcerative colitis(14) support this hypothesis.

The demonstration of antibodies to colon mucosa and the definition of their respective antigens form the basis for further study of immunologic processes occurring in ulcerative colitis. Future investigation may determine the pathogenetic significance of these substances.

Conclusion. Increased gamma globulin was demonstrated in the intestinal tract of patients with active regional ileitis and ulcerative colitis. The indirect fluorescent antibody technic revealed a gamma globulin in the serum of 3 of 25 patients with ulcerative colitis which was bound to epithelial glands of colonic mucosa and of hepatic bile ductules. Sera of normals and hospital controls did not bind to colonic mucosa.

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Quantitative Chromatographic Analysis of the Phospholipids of Abnormal Human Red Blood Cells.* (27203)

GERALD B. PHILLIPS AND NORMAN S. ROOME
Depts. of Biochemistry and Medicine, College of Physicians and Surgeons, Columbia University, and Presbyterian Hospital, N. Y. City

The abnormal shape of the red blood cells in patients with hereditary spherocytosis, pernicious anemia, sprue, and thalassemia raises the possibility of a defect in the red blood cell membrane as a basis for the red blood abnormality. Since almost all of the lipid of the red blood cell resides in the membrane (1), a study of the red blood cell lipid offers one approach to this problem. Indeed, Williams *et al.*(2,3), in a study of the red blood cells of patients with pernicious anemia, observed a decreased concentration of lipid phosphorus and free cholesterol, an increased concentration of cholesterol ester, and development of an abnormal distribution of the individual phospholipids with treatment. Moreover, Allison *et al.*(4) and Kates *et al.* (5), studying the red blood cells of 2 patients with hereditary spherocytosis, found an in-

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