IMMUNOLOGICAL STUDIES IN ULCERATIVE COLITIS

IV. Origin of Autoantibodies*

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While the occurrence of autoimmunity in ulcerative colitis is now well established (see 1 of Bibiography), its cause is not yet understood. In most patients the serum contains anti-colon antibodies which react with a heat-stable gastrointestinal antigen of human and animal origin, chemically related to but immunologically distinct from the blood group (ABH) substances (2–4). The antibodies are autoantibodies (5, 6). There is evidence that the elevated anti-colon antibody titer in human ulcerative colitis is not merely the result of chronic colon damage. Thus, antibody titers are low or nonexistent in the sera of patients with unrelated gastrointestinal diseases, even when accompanied by severe colon damage (7). Furthermore, antibody titers are not related to clinical status of the patients, duration of the disease and other clinical variables (5–7).

Autoantibodies can be produced in experimental animals by breakage of natural tolerance by the injection of cross-reacting antigens (8, 9). This has also been achieved in the case of the colon by injecting rabbits with rat colon (10) or rats with newborn rabbit colon (11). These studies have provided conclusive evidence of an immunological relationship between antigen from germ-free gastrointestinal mucins and bacterial antigens. These latter findings are important since they may explain the induction of autoantibody formation in ulcerative colitis as well. Such a mechanism has previously been suggested for the production of autoimmunity in other human diseases, where cross-reactions between bacteria and the organs involved have been seen (12, 13).

In the search for bacterial antigens cross-reacting with colon antigen, we have concentrated our efforts on *Escherichia coli* O14 which contains an active form of a heterogenetic antigen common for most Enterobacteriaceae (14–16). Earlier results with sera from patients, as well as with rats made autoimmune by injecting them with rabbit colon (3, 11) suggested that immunologically active structures in common for *E. coli* O14 and colon do exist. In this paper, further evidence for this cross reactivity and for the possible significance of the heterogenetic antigen for the induction of anti-colon antibodies in ulcerative colitis will be presented.

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Material and Methods

Antigen was extracted from sterile rat colon and from bacteria by phenol-water at 65°C as earlier described (17). Colon antigen was obtained either from pooled homogenates of germ-free rat colon and cecum, or from dried feces. It contains human blood group A and H activity but no B activity. (4, 18). The rats were of a Swedish inbred strain, reared under germ-free conditions for 18 generations and raised on the semisynthetic diet D7 (19, 20). *E. coli* strains were obtained from the International *Escherichia coli* Center in Copenhagen through the courtesy of Dr. F. Ørskov and from the University of Gothenburg through the courtesy of Dr. K. Lincoln. The bacteria were grown for 18 hr at 37°C on a synthetic substrate containing sodium lactate as the carbon source (11). *Clostridium difficile* isolated from normal rat feces was grown anaerobically for 72 hr at 37°C on Difco Bacto Fluid thioglycolate medium (Difco Laboratories, Detroit, Mich.), and *Staphylococcus aureus* S 209 (from the Karolinska Hospital, Stockholm) for 18 hr at 37°C on a dialysate of Trypticase soy broth (Difco Laboratories, Detroit, Mich.). All bacteria were grown in liquid culture, collected by centrifugation, washed in physiological saline, and heat inactivated. *Clostridium difficile* was killed with 0.5% formaldehyde before extraction. None of the bacterial strains used contained measurable blood group A or H activity, as assessed by hemagglutination inhibition experiments with human or eel serum and human erythrocytes.

Sera were collected from randomly selected patients with clinically, rectoscopically, and roentgenologically typical ulcerative colitis. The sera were obtained from the Karolinska Hospital and St. Erik's Hospital, Stockholm (7). Control subjects were sex- and age-matched healthy blood donors and laboratory personnel from Sweden. A second control group consisted of South African patients with amebic dysentery or with liver abscess, and healthy individuals from the same territory. The South African sera were kindly supplied by Dr. S. E. Maddison, Amoebiasis Research Unit, Durban, South Africa. Sera were collected aseptically, heat inactivated at 56°C, divided into small aliquots, and kept at −20°C until titrated. Prior to testing, all sera were absorbed with sheep erythrocytes and human A_1 erythrocytes (7).

The technique of *passive hemagglutination* was the same as that used earlier (4, 7). In each titration series, sera from the same five patients with ulcerative colitis and from five blood-donors were incorporated to assess the reproducibility of the test. The titrations were reproducible within ±1 dilution steps (7). For sensitization of 0.025 ml packed red cells the following antigen concentrations were used: colon 2.0 mg/ml; *E. coli* O14, 0.03 mg/ml; *E. coli* O7, 0.064 mg/ml; *E. coli* O6, 0.25 mg/ml; *E. coli* O8, 0.05 mg/ml; *E. coli* O75, 0.25 mg/ml; *Clostridium difficile*, 0.5 mg/ml; *Staphylococcus aureus* S 209, 2.0 mg/ml. These concentrations gave optimal sensitization of red cells when tested with a few standard ulcerative colitis sera. Sensitization with colon antigen was slightly suboptimal (3). *Bacterial agglutination* of heat-killed *E. coli*, grown in Petri dishes on brain veal agar for 18 hr, was performed by Dr. K. Lincoln.

For *hemagglutination inhibition* tests, sera with a titer of $\geq 1/32$ were chosen. Tests were performed in two ways. (*a*) For screening of a large number of sera; to each twofold dilution of serum inhibitor was added at a constant concentration (usually 2.0 mg/ml). After incubation for ½ hr at room temperature the cells were added and hemagglutination was recorded as usual (4). The titers were compared with those obtained in titrations of aliquots of the same sera, properly diluted with saline. A titer reduction by at least three dilution steps was regarded as indicative of cross-reactivity (3). (*b*) To 0.1 ml aliquots of the sera, diluted to contain four to eight hemagglutinating units (HU) of antibody, varying concentrations (2–2000 μg/ml) of inhibiting antigen were added, as earlier described (4, 11).

E. coli O14 for *immunization of rabbits* were grown as already described, collected by centrifugation, washed in physiological saline, and killed by treatment with 0.5% formalin for 2 days at 37°C. After dialysis against distilled water, the suspension was concentrated.

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1 ml containing 1 mg bacteria (dry weight) was mixed with 1 ml of Freund's incomplete adjuvant and injected intramuscularly. Two injections were given 14 days apart and antiserum was collected 7 days after the last injection.

RESULTS

Antibodies to Rat Colon in Sera from Patients with Ulcerative Colitis and Controls.—Titers in patients with ulcerative colitis and the various control groups are given in Table I. Mean titer height and incidence of elevated titers in the ulcerative colitis group are significantly increased over the controls. The difference between patients with ulcerative colitis and controls either from Sweden or from South Africa is highly significant ($\chi^2 = 27$, $P < 0.01$). Patients with ameba dysentery or ameba liver abscess do not differ from their controls (colored or Asian blood donors from the same territory) who also have elevated titers more often than South African whites. The difference between on one hand, healthy colored and Asian and, on the other, white South Africans is also significant ($\chi^2 = 9$, $P < 0.01$).

TABLE I

Distribution of Hemagglutinating Titers to Rat Colon Antigen in Ulcerative Colitis Patients and Different Categories of Controls

Origin of sera	Clinical group	No. of sera	No. of sera reacting at highest dilution of										Mean titer	Sera with titer ⩾1/32
			1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024		
														%
Sweden	Ulcerative colitis (white)	24	—	—	1	5	8	4	5	—	—	1	$2^{5.8*}$	75
	Healthy (white)	33	1	10	18	3	1	—	—	—	—	—	$2^{2.8}$	3
South Africa	Amoebic dysentery (colored)	29	1	5	13	5	2	3	—	—	—	—	$2^{3.4}$	17
	Amoebic liver abscess (colored)	16	1	2	4	4	5	—	—	—	—	—	$2^{3.6}$	31
	Healthy (colored)	108	3	22	29	20	19	8	4	3	—	—	$2^{3.7}$	31
	" (Asian)	106	2	20	32	21	11	15	5	—	—	—	$2^{3.8}$	29
	" (white)	111	5	41	29	26	5	5	—	—	—	—	$2^{3.1}$	9

* The superscripts represent the mean values of the number of dilution steps at which end point was reached.

Antibiodies to E. Coli O14 in Sera from Patients with Ulcerative Colitis and Controls.—The optimal sensitizing dose of *E. coli* O14 antigen was the same for four high-titered ulcerative colitis sera (0.03 mg/ml). Lower as well as higher doses gave lower titers.

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With this dose, sera of ulcerative colitis patients and controls were investigated. In three independent experiments, mean titers and incidence of elevated titers ($\geqslant 1/128$) against *E. coli* O14 were significantly increased in ulcerative colitis as compared to the controls. Typical results from one experiment are shown in Table II. The difference between patients and controls is highly significant ($\chi^2 = 16.5$, $P < 0.01$).

Independent confirmation of these data was obtained by Dr. K. Lincoln who tested a randomly selected group of our sera from ulcerative colitis patients and Swedish controls both in passive hemagglutination and in bacterial agglutination. In the hemagglutination experiment, sheep cells were sensitized with a

TABLE II

Antibodies to E. coli O:14 in Ulcerative Colitis Patients and Different Categories of Controls

Origin of sera	Clinical group	No. of sera	No. of sera reacting at highest dilution of										Mean titer	Sera with titer $<1/128$
			1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024		
														%
Sweden	Ulcerative colitis (white)	32	—	—	1	2	6	8	6	1	4	4	$2^{6.7}$*	47
	Healthy (white)	32	—	—	4	11	8	7	2	—	—	—	$2^{4.7}$	6
South Africa	Amoebic dysentery (colored)	28	—	4	6	7	6	3	—	2	—	—	$2^{4.2}$	7
	Amoebic liver abscess (colored)	16	—	1	3	5	1	4	2	—	—	—	$2^{4.6}$	13
	Healthy (colored)	19	—	2	4	6	2	3	—	2	—	—	$2^{4.2}$	11

* See Table I.

saline extract of heat-killed *E. coli* O14. 7 of 21 sera from patients with ulcerative colitis had titers of 1/16 or more, but only 1 of 22 controls (difference statistically significant, $\chi^2 = 4.1$, $P < 0.01$). In bacterial agglutination of heat-killed organisms, 9 of 22 patients and 2 of 22 controls had a titer of $\geqslant 1/32$ ($\chi^2 = 4.3$, $P < 0.01$).

Antibodies to E. coli O14 and Clinical Variables.—To investigate the hypothesis that elevated titers against *E. coli* O14 are caused by intensified bacterial stimulation through the ulcerated colon mucosa, antibody titers and clinical variables were compared. The clinical variables were the same as those recorded earlier (7). In sera from 27 unselected ulcerative colitis patients there was no relation between antibody titer to *E. coli* O14 and *duration or severity* of the disease, *extent of colon involvement*, or presence of *extra colonic manifestations.*

Antibodies to Other E. coli, Staphylococcus aureus S 209, Clostridium Difficile and Sheep Red Cells (Forsman Antigen) in Ulcerative Colitis and Swedish Con-

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trols.—Aliquots of the same ulcerative colitis sera and Swedish controls (32 unselected sera in each group) were also titrated against sheep cells sensitized with *E. coli* O7, *E. coli* O75, *E. coli* O6, and *Staphylococcus aureus* S 209. Optimal sensitization doses were determined with the same four ulcerative colitis sera as used in the experiments described above. All four sera gave optimal sensitization at the same concentration when tested with the same antigen. However, the optimal concentrations were different for the three antigens (see Materials and Methods). Fig. 1 shows the incidence of antibody titers $\geqslant 1/64$ to the four bacterial antigens. For comparison, the incidence of anti *E. coli* O14 titers in the same group of sera, as obtained in the experiment of Table II, is also included. No difference between ulcerative colitis and controls was seen when these sera were tested with *E. coli* O75, *E. coli* O7, or with *St. aureus* S

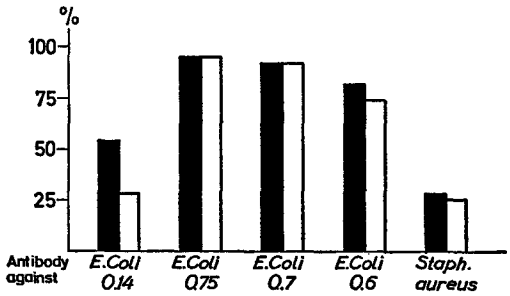

FIG. 1. Percentages of elevated titers ($\geqslant 1/64$) to different strains of E. coli and to Staphylococcus aureus in patients with ulcerative colitis (black bars) and matched controls (white bars).

209. With *E. coli* O6 the difference between the groups was not significant. Comparison of mean titers gave similar results. (Thus, for the patients, mean titers to *E. coli* O14, O75, O6, O7, and *Staph. aureus* were $2^{6.7}$, $2^{8.8}$ $2^{6.2}$, 2^{8}, and $2^{4.8}$ respectively. For the controls these means were $2^{4.6}$, 2^{9}, 2^{7}, $2^{8.6}$, and $2^{4.9}$ respectively.)

Further studies on the antibody pattern in ulcerative colitis and controls revealed that they were not significantly different when tested with extracts of a member of the anaerobic gut flora, *Clostridium difficile*. 23 of 30 of the ulcerative colitis group and 18 of 29 controls had titers $\geqslant 1/128$. The mean titers for the two groups were $2^{7.3}$ and $2^{6.8}$, respectively. When titrated with unsensitized sheep erythrocytes (sera not absorbed with sheep erythrocytes), 2 of 32 ulcerative colitis sera and 2 of 29 controls had anti-Forsman antibody titers of $\geqslant 1/128$ (Mean titers $2^{3.7}$ and $2^{3.6}$, respectively).

Cross-reaction between E. coli O14 and Rat Colon Antigen.—Absorption with *E. coli* O14 antigen inhibited the reaction between colon sensitized sheep cells, and sera from ulcerative colitis patients in approximately one-third of the large

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number of sera tested (Table III). Using this rather insensitive method, no inhibition was found with *E. coli* O7 or *E. coli* O75. In the reverse experiment (*E. coli* O14 antigen as sensitizer and colon antigen as inhibitor), significant inhibition was demonstrated in only 2 of 16 ulcerative colitis sera.

TABLE III

Hemagglutination Inhibition Experiment with Ulcerative Colitis Sera and Antigen from Germ-Free Rat Colon or E. coli

Sensitizing antigen	Inhibiting antigen			
	Rat colon	*E. coli* O:14	*E. coli* O:7	*E. coli* O:75
Rat colon	44/44*	13/44	0/40	0/40
E. coli O:14	2/16	16/16	—‡	—

* No. of patients' sera showing titer reduction of ⩾3 dilution steps following absorption with 2.0 mg/ml of inhibiting antigen (see Material and Methods). Data based on 44 ulcerative colitis sera with hemagglutination titers against colon ⩾1/16. 16 of these have anti-*E. coli* O14-titers ⩾1/16.

‡ Not done.

TABLE IV

Hemagglutination-Inhibition Assay for Cross-Reactivity of Anticolon Antibodies in Ulcerative Colitis Sera with Bacterial Antigens. Erythrocytes Sensitized with Germ-Free Rat Colon Antigen

Inhibitor	Antigen needed for complete inhibition of 4 hemagglutinating units of ulcerative colitis serum*
	μg/ml
Germ-free rat colon	8
E. coli O:14	64
" O:1	1000
" O:2	>1000
" O:3	1000
" O:7 (O:39)	1000
" O:8	1000
" O:9	>1000
" O:18	1000
" O:75	>1000

* The anti-colon titer of this serum was 1/128. Inhibition experiments performed in duplicates. >: No inhibition at highest doses tested.

Since the number of sufficiently high-titered (anticolon) control sera (both Swedish and South African) was limited, no conclusive results were obtained in absorption experiments of this type.

Table IV summarizes a typical hemagglutination inhibition experiment, in which varying concentrations of different antigens were added to four HU of a

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high-titered ulcerative colitis serum, run against colon sensitized sheep erythrocytes. Of the bacterial extracts tested *E. coli* O14 was by far the strongest inhibitor. Of the others, only *E. coli* O3, O7 (039), and 08 also gave weak inhibition when added at approximately 100 times the concentration of the homologous inhibitor. In all, 30 different sera from ulcerative colitis patients have been tested in this way, all with similar results. No hemagglutination inhibition was demonstrated with phenol-water extract of pneumococci or hemolytic streptococci.

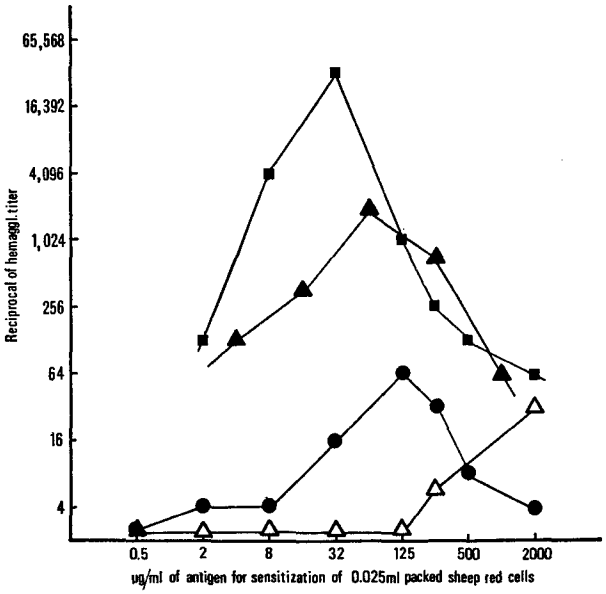

FIG. 2. Hemagglutination with rabbit anti-E. coli O14 serum of sheep red blood cells sensitized with different concentrations of antigen from four different strains of E. coli. ■, E. coli O14; ●, E. coli O1; ▲, E. coli O8; △, E. coli O75. (For further explanations see Material and Methods.)

Relationship of the Rat Colon Antigen to the Heterogenetic Antigen of Enterobacteriaceae.—The relationship between rat colon antigen and the common antigen of Enterobacteriaceae was also tested with rabbit anti-*E. coli* O14 sera. Such sera usually contain antibodies to the type-specific somatic antigen (O14) in high concentration. Therefore the assay for the common antigen requires that the red blood cells be sensitized with antigen from another strain, whose somatic antigen is unrelated to that of *E. coli* O14 (14–16). It is also necessary to choose a strain with a relatively high activity of the common antigen, since, otherwise, the titer may be determined by the animal's natural antibodies to the specific somatic antigen of the sensitizing strain. The activity of the common antigen in other strains than *E. coli* O14 can be determined by measuring

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the optimal sensitizing dose for hemagglutination with *anti-O14* serum. Of the *E. coli* antigens available for this investigation, *E. coli* O8 gave the highest titer at relatively low doses when tested with rabbit anti *E. coli* O14 serum (Fig. 2). It was therefore chosen for the assay of the heterogenetic antigen when it was established that its somatic antigen was unrelated to the type specific O14 antigen.

TABLE V

Hemagglutination Inhibition Assay for Cross-Reactivity between the Common Antigen of Enterobacteriaceae and Antigen from Germ Free Rat Colon. Rabbit Anti-E. coli O:14 Serum Reacted with Sheep Cells Sensitized with E. coli O:8

Inhibitor	Antigen needed for complete inhibition of hemagglutination		
	Serum 1*		Serum 2‡
	Exp. 1	Exp. 2	
	8 HU	8 HU	4 HU
	μg/ml	*μg/ml*	*μg/ml*
Germ-free rat colon	1000	1000	1000
" " " " (purified)§	125	250	250
E. coli O:1	125	125	—¶
" O:4	500	250	—¶
" O:7 (O:39)	125	125	—¶
" O:8	—¶	—¶	64
" O:14	2	4	<2
" O:75	1000	1000	—¶

* Sensitizing dose: 0.064 μg/ml; antibody titer: 1/512.

‡ " " : 0.032 μg/ml; " " : 1/2048.

§ Phenol-water extract further purified by precipitation with ethanol between 51 to 55% concentration.

¶ Not done.

The reaction between two rabbit antisera to *E. coli* O14 and antigen from *E. coli* O8 was inhibited by the colon antigen as shown in Table V. In these experiments, both the phenol-water extracted colon antigen and a purified fraction thereof were added as inhibitors. As can be seen, while the inhibitory power of the original colon extract was weak, the purified preparation was four to eight times more active. When compared with the original extract this preparation was also approximately eight times more active in regard to colon antigen concentration, as revealed by hemagglutination inhibition experiments with patients' sera and colon sensitized red cells (unpublished experiments). However, even the purified rat colon antigen was about 50 times less effective as inhibitor than the homologous *E. coli* O14 antigen (Table V). The Table also

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demonstrates that different strains of *E. coli* contain different concentrations of the inhibitory antigen. It will be noted that *E. coli* O75 is considerably less active than *E. coli* O7.

In all, six rabbit anti-*E. coli* O14 sera were available for these experiments. Most of these reacted as shown in Table V. Such sera also had elevated titers when tested with colon sensitized erythrocytes. When tested with *E. coli* O14 sensitized erythrocytes, the reactions were usually not inhibited with colon or *E. coli* O8 antigen. In a few sera, however, the reaction with *E. coli* O14 sensitized cells was also inhibited with colon and *E. coli* O8. This was the case in those sera in which the antibodies to the common antigen were of a similar or higher concentration than those to the type specific O14 antigen. One serum was also encountered with a titer to *E. coli* O8 which could be inhibited neither with colon nor with *E. coli* O14 but only with *E. coli* O8. These reactions were probably due to antibodies to the type specific O8 antigen present already before immunization. Taken together, these data strongly support the supposition that the cross-reacting factor of the colon extract is very similar to the common antigen of the Enterobacteriaceae.

DISCUSSION

The sera in this investigation was obtained from unselected ulcerative colitis patients and controls, most of whom have not been reported previously. The general level of hemagglutinating antibody titers seen here was higher than that reported earlier because of minor technical modifications. However, the difference in incidence of elevated titers and mean titers between patients and healthy Swedish controls was significant and very similar to that noted previously (7). These results differ from those recently reported by McGiven et al. (21) who found elevated hemagglutination titers equally distributed in 20% of the sera from Australian patients as well as controls. The reason for this discrepancy is not understood, particularly since 24% of their ulcerative colitis sera but only 6% of their controls gave a positive fluorescent antibody staining of rat colon mucosa. The present data provide no clue as to the specificity of the antibodies in the various controls. However, since the rat colon antigen is immunologically heterogenous (11), they may well be directed to other determinants than the antibodies in the patients' sera. This is also supported by previous findings, that no such background activity is seen in normal sera when the assays are performed with sterile human colon extract (2).

Elevated anti–colon antibodies do not appear in the sera of patients with unrelated diseases of the gastrointestinal tract (5–7). Nevertheless, it seemed important to include some patients with severe and long-lasting colon lesions among the controls. We therefore obtained sera from patients with amebic dysentery, a disease which has many clinical and pathological similarities with ulcerative colitis. Neither Wright and Truelove (5) nor Harrison (6) could

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detect anti-colon antibodies in these patients, when using the fluorescent antibody techniques. In this study, these South African sera had similar mean titers and incidence of elevated titers as sera from healthy individuals from the same territory and of the same racial origin (Negroes and Asian). These values were all slightly higher than those typical for the Swedish controls. However, the group of healthy South African whites which was also included did not differ from healthy Swedish controls.

The possibility of autoimmunization in ulcerative colitis, arising from breakage of tolerance by cross-reacting bacterial antigen, was supported by earlier preliminary findings of an increased incidence of elevated titers to phenol-water extracted antigen from *E. coli* O14 in the patients' sera. In contrast, the spectrum of antibodies to some common serotypes of *E. coli* and *Salmonella* was found to be the same in patients and controls (3). This is important because *E. coli* O14 is known to carry a particularly immunogenic form of a heterogenetic antigen, common for most Enterobacteriaceae (14–16, 22–24). Significant amounts of this antigen must therefore always be present in the human intestine, even in the absence of *E. coli* O14. The data presented here confirm and extend previous findings. Evidence for increased incidence of anti-*E. coli* O14 antibodies in ulcerative colitis has now been presented in a second group of patients. No deviations from the normal antibody pattern were observed in regard to antibodies to Forsman antigen, *Staphylococcus aureus*, *Clostridium difficile*, and *E. coli* O75. As noted for anti-colon titers (7), anti-*E. coli* O14 titers in ulcerative colitis patients were not related to the clinical state of the disease in individual patients. Furthermore, the antibody pattern to *E. coli* O14 in patients with amebic dysentery, whose colonic lesions are comparable in severity to those of ulcerative colitis, was indistinguishable from that of the healthy controls.

Taken together, these data do not favor the notion that elevated antibody titers to colon and to *E. coli* O14 are sequelae of colon damage in general. Rather they suggest that these phenomena are in some way causally related. This is strongly supported by the results of hemagglutination inhibition experiments which prove that the phenol-water extracted antigen from germ free rat colon is immunologically related to the antigen in a corresponding extract of *E. coli* O14. Thus, in about 30% of a large group of patients' sera, the anti-colon reaction could be significantly inhibited by means of absorption with *E. coli* O14, and the same was true in a few cases in the reverse system. The results do not imply that cross-reacting antibodies were absent in the sera in which hemagglutination was not inhibited. The absorption procedure used in the screening tests of Table III was rather crude, and hemagglutination would probably have been inhibited in a larger proportion of the sera by using more sensitive tests and more highly purified antigen preparations. Furthermore, the spectrum of antibodies to different determinants in the two antigen preparations may show con-

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siderable variations in individual sera (11). Hemagglutination titers are determined by the antibodies present in highest concentration. Moreover, differences in concentration of active determinants or of their availability in different extracts will affect the outcome of both the hemagglutination and the hemagglutination inhibition tests. Further analysis requires greater knowledge of the immunochemistry of the antigens and of the specificity of the antibodies in individual sera.

Examination of a few selected sera for cross-reactivity with several bacterial antigens by means of a more sensitive hemagglutination inhibition technique indicated that *E. coli* O14 was the single potent inhibitor, only about 10 times less active on a weight basis than the colon extract. A few of the other *E. coli* strains inhibited weakly probably because of a somewhat elevated concentration of the heterogenetic antigen (see below). Since the strains tested were devoid of blood group activity (A and H), no such cross-reactions were involved. It is of considerable interest that anti–colon antibodies induced in rats by immunization with newborn rabbit colon have an identical pattern of reactivity in hemagglutination inhibition tests (11).

Although the data described above show beyond reasonable doubt that colon extract and *E. coli* O14 contain common structures reactive with antibodies in ulcerative colitis sera, they do not prove that the antigen involved is the common antigen of the Enterobacteriaceae. Evidence for this was obtained in hemagglutination inhibition experiments, in which the reaction between rabbit antisera to *E. coli* O14 and *E. coli* O8 sensitized sheep erythrocytes was inhibited with colon-extract, while no inhibition was usually encountered when erythrocytes were sensitized with *E. coli* O14. These experiments also suggested that the concentration (or activity) of the common antigen varied in extracts of different *E. coli* strains.

Asherson and Holborow (25) have previously shown that the serum of rabbits injected with some *E. coli* or related bacteria develop colon-reactive antibodies, demonstrable by the fluorescent antibody test. This staining may, in fact, also have been caused by antibodies to the heterogenetic antigen (26).

The data presented here suggested that anticolon antibody production in ulcerative colitis may be initiated by stimulation with a cross-reacting bacterial antigen. However, if this ubiquitous antigen of Enterobacteriaceae is the responsible antigen, it is obvious that additional factors must account for the occurrence of elevated autoantibodies to colon in this disease. It is possible that a genetic predisposition may play a role, since preliminary data indicate that relatives of ulcerative colitis patients have a higher incidence of anticolon antibodies than is found in the normal population (unpublished observations). The data do not reveal the nature of the genetic lesions which may be involved. These may be entirely nonimmunological (1). Whether or not autoimmunity cause tissue damage in ulcerative colitis or contributes to its maintenance is

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largely unknown, although there are some data in support of potentially harmful immunological mechanisms in this disease (27–29).

SUMMARY

The incidence and height of antibody titers to colon, assayed by indirect hemagglutination with a heat stable colon extract from germ free rats, is significantly higher in sera from patients with ulcerative colitis than in those from healthy controls or from patients with amebic liver abscess or dysentery. While sera from ulcerative colitis patients and controls are indistinguishable in regard to incidence and height of antibody titers to Forsman antigen, *Staphylococcus aureus S* 209, *Clostridium difficile*, and several common strains of *E. coli*, they have elevated titers and increased incidence of antibodies to a heat stable antigen of *E. coli* O14. Patients with amebic dysentery have normal titers of such antibodies.

Absorption of patients' sera with *E. coli* O14 antigen inhibits the colon directed hemagglutination reaction in approximately 30% of the cases tested. Likewise, the anti-*E. coli* O14 reaction can sometimes be inhibited with the colon extract. Other *E. coli* strains and other bacteria are inactive or have only weak inhibitory activity. Hemagglutination inhibition experiments show that germ free rat colon and *E. coli* O14 contain common structures, depicted by antibodies in the patients' sera. This pattern of reactivity closely resembles that seen in rats made autoimmune to colon by injection of newborn rabbit colon.

E. coli O14 is known to carry a heterogenetic antigen present in lower concentration (or activity) in most Enterobacteriaceae. Hemagglutination inhibition experiments with rabbit antisera to *E. coli* O14 suggest that the antigen common for *E. coli* O14 and colon is related to this heterogenetic antigen. The findings imply that this antigen, which is constantly present in low concentrations in the human colon, may give rise to anticolon antibody formation in ulcerative colitis through breakage of tolerance. Since this antigen is present in healthy individuals as well, additional factors are required to explain the induction of anti–colon autoimmunity in ulcerative colitis.

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