Auto-immune reactions in ulcerative colitis¹

RALPH WRIGHT AND S. C. TRUELOVE

From the Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford

EDITORIAL SYNOPSIS This is a useful review of studies on auto-immune reactions in relation to ulcerative colitis. In this series 15.8% of patients were found to have auto-antibodies but there was no correlation with the length or severity of the illness.

The aetiology of ulcerative colitis is unknown but an underlying immunological disturbance has frequently been implicated. This might be due to an auto-immune reaction (Broberger and Perlmann, 1959, 1962, 1963; Wright, 1964) or to reactions to an exogenous antigen (Andresen, 1925, 1942; Truelove, 1961; Wright, 1964). We have published our studies on the possible role of dietary allergy elsewhere (Wright and Truelove, 1965a and b) and the present report deals with the investigations which we have carried out to assess the role of auto-immune mechanisms in this disease.

It has been suggested that auto-immune disorders form part of a broad spectrum of diseases which are often associated clinically; there is an even wider association of auto-antibodies (Hijmans, Doniach, Roitt, and Holborow, 1961).

This hypothesis is of value when considering the possibility that a disease might be due to an auto-immune disturbance. Suggestive evidence implicating the operation of auto-immune processes in a disease might be obtained, first by demonstrating antibodies to the organ or organs primarily involved in the disease and by determining their specificity, and secondly, by studying its clinical and serological overlap with other auto-immune disorders.

Antibodies to a phenol-water extract of human foetal colon were first demonstrated by Broberger and Perlmann (1959) in the sera of children with ulcerative colitis but not in healthy controls. These haemagglutinating antibodies are found less frequently in adults with ulcerative colitis and in patients with systemic lupus erythematosus and liver disease (Asherson and Broberger, 1962). Using haemagglutination, collodion particle, and complement-fixation techniques, with various methods of extracting the antigen, the finding of antibodies to colon in

ulcerative colitis has been confirmed by some workers (Polcak and Vokurka, 1960; Bregman and Kirsner, 1960; Bernier, Lambling, and Cornelius, 1960; Maratka and Wagner, 1961) but not by others (Edgar, 1961; Gray, Walker, and Thompson, 1961; Henriksen, Gundersen, and Opsahl, 1962). Bacterial contamination of some of the colonic extracts used as antigens may explain the conflicting reports of the incidence of antibodies to colon in ulcerative colitis when such techniques are used.

By means of the more specific fluorescent antibody technique Broberger and Perlmann (1962) showed that sera from some patients with ulcerative colitis react with an antigen present in the cytoplasm of colonic epithelial cells. This has been confirmed by Klavins (1962, 1963) and Koffler, Minkowitz, Rothman, and Garlock (1962) but the relationship of the antibodies to the clinical features of the disease and their incidence in other disorders has been poorly studied.

There have been several reports of the occurrence, in the same patient, of ulcerative colitis and disorders which may be the result of an auto-immune disturbance. These include systemic lupus erythematosus (Brown, Shirey, and Haserick, 1956; Zetterström and Berglund, 1956; Kurlander and Kirsner, 1964; Alarcón-Segovia, Herskovic, Dearing, Bartholomew, Cain, and Shorter, 1965), lupoid hepatitis (Gray, Mackay, Taft, Weiden, and Wood, 1958; Mackay and Wood, 1962), auto-immune haemolytic anaemia (Lorber, Schwartz, and Wasserman, 1955; Balint, Hammack, and Patton, 1962; Fong, Fudenberg, and Perlmann, 1963), Hashimoto's thyroiditis (Brearley and Spiers, 1962), myasthenia gravis (Galbraith, Summerskill, and Murray, 1964), and pernicious anaemia (Perillie and Nagler, 1959; Edwards and Truelove, 1964). However, it is difficult to know whether such associations are not due to chance alone (Masi, Hartmann, Hahn, Abbey, and Shulman, 1965). On the other hand it is possible that ulcerative colitis is not a single disease but a syndrome with

¹This paper is based on a thesis by one of us (R.W.) accepted for the degree of Doctor of Philosophy in the University of Oxford, 1964, and was presented in part at the combined meeting of the British Societies of Allergy and Immunology, October 1964.

several different causes, auto-immune processes only being involved in a minority, in which case such associations may take on a greater significance.

There are few studies of the incidence, in ulcerative colitis, of auto-antibodies frequently found in some of the diseases mentioned above. Calabresi, Thayer, and Spiro (1961) reported an increased incidence of antinuclear globulins in the sera of patients with ulcerative colitis. On the other hand, rheumatoid factor and antibodies to thyroglobulin occur no more frequently in patients with ulcerative colitis than in healthy controls (Thayer and Spiro, 1963).

In the present study the sera of a large number of patients with ulcerative colitis have been examined for organ-specific and non-organ-specific auto-antibodies.

The incidence of antibodies to colon has been related to certain clinical features of the ulcerative colitis and the sera of patients with a variety of other disorders have been tested for such antibodies.

In addition the organ specificity of the antigen found in colonic epithelial cell cytoplasm has been investigated by testing the reactions of human sera and sera from immunized rabbits known to have a strongly positive colonic antibody against various human and rabbit tissues. The purpose of this investigation has been to determine whether the distribution of the colonic antigen, if it occurs in organs other than the colon, corresponds to that of the extracolonic lesions seen clinically.

The possibility that there is an association between ulcerative colitis and other auto-immune diseases has been examined by testing the sera of patients with ulcerative colitis for antinuclear factor and antibodies to thyroid and gastric-parietal cells. For convenience the immunological techniques used will be described together and the results presented and discussed separately later.

MATERIAL AND METHODS

HUMAN SERA Specimens of serum were obtained from a large number of patients with ulcerative colitis at different clinical stages of the disease. Visitors to a general medical ward and medical and laboratory staff were used as a healthy control group. Sera from patients with Crohn's disease (regional enteritis), idiopathic steatorrhoea, amoebic dysentery, rheumatoid arthritis, uveitis, and systemic lupus erythematosus have also been used in some of the tests.

RABBIT ANTISERA Antisera were produced in rabbits to extracts of rat colon as described by Holborow, Asherson, and Wigley (1963). Using a similar method, rabbits were immunized with saline extracts of rat uvea dissected free from other eye structures under a dissecting microscope. Antisera were also produced to human lens and cow uvea

by repeated intramuscular injection with Freund's complete adjuvant.

Specimens of serum were stored at $-20^{\circ}\text{C}.$, usually in several small aliquots, until subsequent immunological testing.

FLUORESCENT ANTIBODY TESTS The indirect 'sandwich' technique described by Coons and associates (Coons and Kaplan, 1950; Weller and Coons, 1954) was used throughout.

TISSUE SECTIONS All human tissues were obtained from biopsy or operation specimens and were snap-frozen on copper plates or in iso-Pentane (British Drug Houses, Ltd.) at approximately $-70^{\circ}\text{C}.$, using a dry-ice-acetone freezing mixture.

Specimens of rectal mucosa were obtained by biopsy at sigmoidoscopy from patients with ulcerative colitis or the irritable colon syndrome. Specimens of colonic mucosa were obtained at operation. Tissues from patients, irrespective of blood group, were used initially, but all sera showing positive staining of epithelial glands were later retested against tissue from a patient of blood group O.

Specimens of small intestine were obtained by biopsy from the jejunum or upper ileum and gastric, thyroid, uveal tissue, and skeletal muscle at operation.

Specimens of rabbit colon, stomach, small intestine, gall bladder, liver, spleen, kidney, and uvea were frozen in the same way as human tissue.

Sections $6-10\ \mu$ thick were cut at a temperature of -16° to $-20^{\circ}\text{C}.$ in a Pearse-Slee cryostat.

Calf thymus smears and smears of rectal mucus were used in some of the tests.

STAINING PROCEDURE The stain employed was a commercially available preparation (Sylvana or Wellcome) of fluorescein isothiocyanate conjugated with anti-human globulin. When rabbit antisera were tested anti-rabbit globulin conjugate was used.

Unfixed tissue sections were treated with undiluted serum for 30 minutes in a moist chamber, washed in two changes of Coon's buffer, pH 7.2, treated with the appropriate anti-human or anti-rabbit fluorescein anti-globulin conjugate, then washed for a further 10 minutes in buffer. Sections were then mounted in equal parts of glycerol and Coon's buffer and examined under a Zeiss photomicroscope with a dark-ground condenser, the source of illumination being an Osram Hb 200 ultraviolet lamp.

This method closely follows that used by Holborow, Brown, Roitt, and Doniach (1959) for the detection of thyroid cytoplasmic antibodies and by Taylor, Roitt, Doniach, Couchman, and Shapland (1962) for the detection of gastric-parietal cell antibodies. Calf thymus smears were used in addition when testing for antinuclear factor as described by Widelock, Gilbert, Siegel, and Lee (1961). Where possible known positive controls were included in each batch of tests and direct staining with antiglobulin conjugate alone was sometimes used, especially with sections of large and small intestine.

Initially the method used by Broberger and Perlmann (1962) was adopted when comparing the incidence of

antibodies to colon in the different groups of patients so that sections were treated with serum and conjugate at 37°C. It was subsequently found that similar results could be obtained at room temperature. All other immunofluorescent tests were carried out at room temperature.

RESULTS

ANTIBODIES TO COLON Specimens of serum from 273 patients with ulcerative colitis were examined for antibodies to colon using the fluorescent antibody technique. Specific fluorescence of the epithelial cell cytoplasm of sections of rectal biopsy specimens or colon was found with 43 sera (15.8%). Some sera showed a stronger reaction than others and with two the fluorescence was very powerful (Fig. 1). Specific staining of epithelial cell cytoplasm could not be demonstrated with any of the sera from patients with a variety of other diseases (Table I).

The type of staining observed appears to be the same as that reported by Broberger and Perlmann (1962), Klavins (1962, 1963), and Koffler *et al.* (1962). Staining is confined to the cytoplasm of the epithelial

cells and is quite unlike that seen when the nuclei are stained with a serum containing a strongly positive antinuclear factor. When serum from a rabbit immunized with rat colon according to the method described by Holborow and associates (1963) was tested against human rectal biopsy sections an identical appearance was observed.

In order to determine whether the weaker fluorescence seen with most of the sera represented a similar but weaker reaction to that observed with the two sera showing very powerful fluorescence, one of the strongly positive sera was diluted serially with saline before testing. In this way it could be shown that, at a dilution of 1 : 32, staining with the strongly positive serum was of the same intensity as that given by an undiluted serum regarded as being weakly positive.

ENHANCEMENT OF THE REACTION USING A PARTICULAR RECTAL BIOPSY SPECIMEN Sera have been tested against autologous rectal biopsy tissue. This did not appear to make any difference in four of the five patients studied. However, one of the strongly positive sera (W.L.) when tested against autologous rectal biopsy tissue appeared to enhance what was already a very powerful reaction. An unexpected finding was that there was considerable enhancement of the reaction obtained with sera from other patients with ulcerative colitis when tested against biopsy sections from patient W.L. In addition many ulcerative colitis sera which had previously shown no reaction now showed fluorescence of epithelial cells and positive reactions were also obtained in some sera from healthy subjects. The incidence of positive reactions using a biopsy from this patient is compared in patients with ulcerative colitis and control subjects in Table II.

TABLE I

INCIDENCE OF COLONIC ANTIBODIES DETECTED BY THE FLUORESCENT ANTIBODY TECHNIQUE IN ULCERATIVE COLITIS, HEALTHY CONTROLS, AND VARIOUS DISORDERS

	Negative	Weak Positive	Strong Positive	Total
Ulcerative colitis	230	25 (9.2%)	18 (6.6%)	273
Healthy controls	50	0	0	50
Amoebic dysentery	22	0	0	22
Crohn's disease	38	0	0	38
Rheumatoid arthritis	39	0	0	39
Systemic lupus erythematosus	20	0	0	20
Uveitis	19	0	0	19

¹ Kindly provided by Dr. S. J. Powell, Amoebiasis Research Unit, Durban.

FIG. 1. Immunofluorescence of a cryostat section of human rectal mucosa showing a strongly positive reaction.

TABLE II

INCIDENCE IN PATIENTS WITH ULCERATIVE COLITIS AND IN CONTROLS OF BINDING OF γ -GLOBULIN TO EPITHELIAL CELL CYTOPLASM OF A RECTAL BIOPSY SPECIMEN FROM A PATIENT (W.L.) WITH A STRONGLY POSITIVE ANTIBODY IN HIS SERUM

	Negative	Doubtful	Weak Positive	Strong Positive	Total
Ulcerative colitis	38	21	26	8	93
			36.6%		
Healthy controls	47	41	9	2	99
			11.1%		

REACTIONS TO COLON IN RELATION TO THE CLINICAL FINDINGS IN PATIENTS WITH ULCERATIVE COLITIS The relationship between fluorescent antibodies to colon

and the clinical findings has been studied in a group of patients in whom a survey had been carried out to determine the incidence of two extra-colonic manifestations of ulcerative colitis-uveitis and sacro-iliac abnormality (Wright, Lumsden, Luntz, Sevel, and Truelove, 1965). In this study it was shown that 17 (11.8%) of 144 patients with ulcerative colitis had evidence of past or present anterior uveitis and 25 (17.4%) had abnormalities of the sacro-iliac joints. The two conditions were strongly associated among the patients with ulcerative colitis. The incidence of both conditions among control subjects was negligible.

The incidence of antibodies to colon was similar in patients with sacro-iliac or uveal involvement to that in the remainder of the cases of colitis who showed no such abnormality. Similarly no relationship could be found between the incidence of colon antibodies and the age or sex of the patient or the length of history or radiological extent of the disease (Table III).

TABLE III

ANTIBODIES TO COLONIC EPITHELIAL CELL
CYTOPLASM IN PATIENTS WITH ULCERATIVE
COLITIS IN RELATION TO AGE, SEX, LENGTH OF HISTORY
OF THE DISEASE, EXTENT OF COLONIC INVOLVEMENT,
AND THE PRESENCE OF SACRO-ILIAC AND
UVEAL ABNORMALITIES

	Negative	Weak Positive	Strong Positive	Total
<i>Age in years</i>				
30	21	1	1	23
30-39	31	2	0	33
40-49	24	1	2	27
50+	31	2	7	40
Total	107	6	10	123
<i>Sex</i>				
Male	41	6	5	52
Female	66	0	5	71
Total	107	6	10	123
<i>Length of history of ulcerative colitis</i>				
First attack	9	0	0	9
3 years	12	0	3	15
3-9 years	52	3	6	61
10+ years	34	3	1	38
Total	107	6	10	123
<i>Extent of colonic involvement</i>				
Distal	42	2	2	46
Substantial	34	3	5	42
Universal	31	1	3	35
Total	107	6	10	123
<i>Sacro-iliac or uveal abnormalities</i>				
Present	24	1	3	28
Absent	83	5	7	95
Total	107	6	10	123

THE ORGAN-SPECIFICITY OF THE COLONIC ANTIGEN
Serum from a patient which showed a strongly positive reaction with colonic epithelial cell cyto-

plasm and serum from rabbits immunized to rat colon, rat uvea, and calf uvea were used to study the distribution of the colonic antigen in various organs, and its relationship to uveal antigen. By means of the indirect fluorescent antibody technique the sera were tested against several different human and rabbit organs as well as against smears of rectal mucus from a patient with the irritable colon syndrome. Normal human and rabbit sera were used as controls and sections were also tested with conjugate alone.

Table IV shows that serum from patient W.L. and serum from the rabbit immunized with rat colon showed specific staining of colonic epithelial cell cytoplasm, of goblet cells in the small intestine, and of rectal mucus. Specific fluorescence of other organs, including the gastric mucosa, could not be demonstrated. Furthermore, sera from rabbits immunized to rat or calf uvea did not show specific staining of any of the organs tested.

TABLE IV

REACTIONS OF SERA FROM A PATIENT WITH A
STRONGLY POSITIVE ANTIBODY TO COLON,
IMMUNIZED RABBITS, AND CONTROLS TO VARIOUS
HUMAN AND RABBIT TISSUES¹

Tissue	Serum			
	Human Positive to Colon	Rabbit Immunized to Rat Colon	Rabbit Immunized to Rat Uvea	Rabbit Immunized to Calf Uvea
<i>Human</i>				
Rectal mucosa	+++	++	—	—
Colonic mucosa	+++	++	—	—
Small-intestinal mucosa	++	+	—	—
Gastric mucosa	—	—	—	—
Rectal mucus	+++	NT	NT	NT
Thyroid	—	NT	NT	NT
Skeletal muscle	—	NT	NT	NT
Uvea	—	—	—	—
<i>Rabbit</i>				
Rectal mucosa	+++	+++	—	—
Colonic mucosa	+++	+++	—	—
Small-intestinal mucosa	++	++	—	—
Gastric mucosa	—	—	—	—
Uvea	—	—	—	—
Kidney	—	—	—	—
Spleen	—	—	—	—
Liver	—	—	—	—
Gall bladder	—	—	—	—
NT = not tested				

¹Sera from normal subjects showed uniformly negative reactions.

ANTINUCLEAR FACTORS Table V shows that there is no significant increase in the incidence of antinuclear factor using calf thymus smears and human thyroid sections in patients with ulcerative colitis when compared with healthy controls. The slightly higher incidence in the patients with ulcerative colitis is compatible with the rare association found

TABLE V

INCIDENCE OF ANTINUCLEAR FACTOR USING
CALF THYMUS AND HUMAN THYROID NUCLEI IN
PATIENTS WITH ULCERATIVE COLITIS AND CONTROL
SUBJECTS

	Negative	Positive	Total
Ulcerative colitis	83	6 (7%)	89
Healthy controls	49	1 (2%)	50

clinically between ulcerative colitis and systemic lupus erythematosus.

THYROID AND GASTRIC ANTIBODIES The incidence of thyroid cytoplasmic and gastric-parietal cell antibodies in patients with ulcerative colitis is compared with that in healthy control subjects of similar age and sex in Table VI. This shows that the incidence of thyroid cytoplasmic antibodies is similar in the two groups whereas gastric-parietal cell antibodies are nearly twice as common in the patients with ulcerative colitis as in the controls, although the difference is not statistically significant ($\chi^2 = 1.367$; $P < 0.05$).

DISCUSSION

In the present study auto-antibodies to colon have been detected in 15.8% of patients with ulcerative colitis but not in healthy control subjects or in patients suffering from Crohn's disease, amoebic dysentery, rheumatoid arthritis, systemic lupus erythematosus, or uveitis. When the biopsy from a patient whose serum had shown a strongly positive reaction was used as the source of antigen the incidence of staining of the epithelial cell cytoplasm was increased in the patients with ulcerative colitis but positive reactions were also obtained in control subjects.

There does not appear to be a correlation between the presence of the antibody and the age or sex of the patient, the length of history of the disease, the extent of colonic involvement, or the incidence of uveal and sacro-iliac involvement.

The staining of colonic epithelial cells described here closely resembles that reported by other workers. Nuclear structures and the basement membrane remain unstained whereas the epithelial cell cytoplasm shows specific fluorescence. The

TABLE VI

INCIDENCE OF GASTRIC-PARIETAL CELL AND THYROID CYTOPLASMIC ANTIBODIES IN PATIENTS
WITH ULCERATIVE COLITIS AND HEALTHY CONTROL SUBJECTS

Antibody	Disease	40 Years		40-59 Years		60 Years and Over		Total		Grand Total		
		Females	Males	Females	Males	Females	Males	Females	Males			
Gastric-parietal cell	Ulcerative colitis	Neg.	48	21	33	22	7	6	88	49	137	
		Pos.	5	1	7	1	6	Nil	18	2	20	
	Healthy controls	Neg.	24	13	24	5	3	1	51	19	70	
		Pos.	Nil	Nil	2	1	1	1	3	2	5	
Thyroid cytoplasmic	Ulcerative colitis	Neg.	26	11	31	20	9	3	66	34	100	
		Pos.	5	3	4	2	5	Nil	13	5	18	
	Healthy controls	Neg.	14	9	13	5	2	1	29	15	44	
		Pos.	1	Nil	3	Nil	2	Nil	6	Nil	6	
										(17.0%)	(3.9%)	(12.7%)
										(5.6%)	(9.5%)	(6.7%)
								(16.5%)	(12.8%)	(15.3%)		
								(17.1%)		(12%)		

The incidence of antibodies to thyroglobulin using Wellcome preserved tanned sheep cells according to the method of Fulthorpe, Roitt, Doniach, and Couchman (1961) was similar in the two groups (Table VII).

TABLE VII

INCIDENCE OF ANTIBODIES TO THYROGLOBULIN
USING THE TANNED-RED-CELL TEST IN PATIENTS
WITH ULCERATIVE COLITIS AND HEALTHY CONTROL
SUBJECTS

	Negative	1/5	1/25	1/250	1/2,500	Total
Healthy controls	28	10	8	3	1	50
Ulcerative colitis	31	6	7	3	3	50

reactions of sera showing strongly positive fluorescence were reproduced on numerous occasions. However, the weaker reactions were occasionally negative or doubtful when repeated and this may have been due to the antigen being lost during the staining procedure. It would seem that the stronger reactions, which presumably involve a greater quantity of antibody, bind the antigen in the colonic epithelial cells and this might prevent their loss by mechanical means during the staining procedure. Broberger and Perlmann (1962) suggested that a higher incidence of positive reactions might be obtained by using fixatives but this has not been examined in the present study.

Broberger and Perlmann (1962) showed, by means of inhibition tests, that the reaction is specific. They were able to prevent, or greatly diminish, the fluorescence by previously absorbing positive sera with phenol-water extracts of foetal colon.

Although the fluorescent staining with ulcerative colitis sera is similar to that produced by antisera to blood group substance, Broberger and Perlmann (1962) have shown that there is no immunological relationship between the two antigens. This is confirmed by the absence of staining of the gastric mucosa by positive sera observed in the present study since blood group antigen is present in gastric as well as in colonic and ileal mucosa (Holborow, Brown, Glynn, Hawes, Gresham, O'Brien, and Coombs, 1960). Koffler and associates (1962) obtained reactions with sterile foetal colon, thus excluding the possibility that the antibody was reacting with bacterial contaminants.

The explanation for the higher incidence of cytoplasmic fluorescence which has been found when the biopsy from a patient with a strongly positive serum is used as the source of antigen is unknown. One possibility is that in this patient the antigen is more securely bound in the epithelial cells than in most other patients. The more likely explanation is that the increased frequency of staining is due to an unusually high concentration of antigen in the cells. It has been shown that thyrotoxic thyroids give a higher incidence of positive reactions than normal thyroids because they contain ten times the quantity of antigen (Doniach and Roitt, 1964).

The distribution in the body of the antigen or antigens found in colonic epithelial cell cytoplasm has not been fully studied. This would be of interest as it is possible that some of the extra-colonic manifestations of ulcerative colitis result from involvement of organs which share a common antigen with the colon. Broberger (1961) has shown that sera from children with ulcerative colitis contain haemagglutinating antibodies which react with multiple antigens in colon, liver, and kidney but that some of the antigens are confined to the colon. Using immunofluorescence, Koffler and associates (1962) found that sera from patients with ulcerative colitis which reacted with colonic epithelial cell cytoplasm also reacted with mucosal cells in the ileum and proliferating hepatic bile ductules. This finding is of interest because both the liver and the small intestine may be abnormal or affected in ulcerative colitis. On the other hand, in the present study, human and rabbit sera containing antibodies to colon failed to react with human or rabbit uvea. Furthermore, specific staining of colonic epithelial cell cytoplasm could not be demonstrated with sera

from patients with uveitis or from rabbits immunized with calf or rat uvea.

The finding of circulating auto-antibodies to colon in ulcerative colitis does not necessarily mean that they are of importance in the aetiology or pathogenesis of the disease. When considering the significance of circulating auto-antibodies, the crucial question which needs to be answered is whether they are the cause or the consequence of the tissue damage.

In general it has been found that circulating auto-antibodies can only produce lesions in tissues where the antigen is easily accessible (Waksman, 1962). The cytotoxic effect of circulating auto-antibody on the cellular elements of the blood is a good example (Dacie, 1962). It is possible that in solid tissues factors in addition to the auto-antibody may be necessary. For example, thyroid antibodies are cytotoxic for trypsinized cells in tissue culture (Forbes, Roitt, Doniach, and Solomon, 1962; Irvine, 1962) but have no effect on well-established tissue cultures of thyroid cells (Pulvertaft, Doniach, Roitt, and Hudson, 1959).

Studies using sera from children with ulcerative colitis have failed to show a cytotoxic effect of anticolon antibodies on foetal colon cells in tissue culture (Perlmann and Broberger, 1962).

Koffler and associates (1962) stained sections of colon from patients with severe acute ulcerative colitis directly with fluoresceinated antisera to human γ -globulin, fibrinogen, and complement but failed to demonstrate antibodies in colonic epithelial cells in any of 12 colons examined. Similarly, in the present study no binding of γ -globulin to epithelial cells was observed with direct staining of rectal biopsy specimens. In other words, it has not been possible to demonstrate the attachment of antibodies to the colonic epithelial cell antigen.

An alternative explanation is that the colonic antibodies are a consequence of the disease, the antibody response being provoked by damaged tissue which has been altered antigenically. This is thought to be the source of the auto-antibodies to heart sometimes found in patients with myocardial infarction (Ehrenfeld, Gery, and Davies, 1961).

The present study does not help to resolve the problem. If antibodies to colon are the cause of the disease a higher titre might be expected just before or during a relapse of the colitis. Little fluctuation in the intensity of fluorescence has been observed on the few occasions when serial specimens of serum from the same patient have been examined during remission and during relapse of the disease, but this is an unsatisfactory way of judging antibody titre and haemagglutination techniques might be more appropriate. It has been shown that serum from some

patients with ulcerative colitis and serum from a rabbit immunized with rat colon will produce identical reactions with both human and rabbit colonic and small-intestinal epithelial cell cytoplasm, as well as with rectal mucus; but the production of the antibody in rabbits is not associated with the development of lesions in the colon or small intestine.

If antibodies to colon are a consequence of the disease a correlation with the severity, or the chronicity, of the illness might be expected, but was not found. Strong evidence in favour of tissue damage alone being responsible would have been forthcoming if antibodies to colon had been demonstrated in the sera of patients with amoebic dysentery. On the other hand, the negative results in this group do not warrant the conclusion that colonic damage *per se* does not produce circulating antibodies to colon, as relatively few sera from patients with dysentery were tested.

Recent studies suggest that cellular antibody rather than circulating antibody is responsible for the tissue damage in some experimental auto-immune disease, thus implicating a delayed type of hypersensitivity response. It has been shown that allergic encephalomyelitis (Paterson, 1960), nephrosis (Hess, Ashworth, and Ziff, 1962), and thyroiditis (Felix-Davies and Waksman, 1961) may be produced by the passive transfer of lymphocytes or spleen cells from animals with these experimental auto-immune diseases to normal recipients.

The most powerful evidence so far available implicating auto-immune mechanisms in ulcerative colitis is the finding of Perlmann and Broberger (1962) that leucocytes from patients with ulcerative colitis are cytotoxic for foetal colon cells in tissue culture. The cytotoxic effect appeared to be specific for colon cells, and pre-treatment of the lymphocytes with colon antigen inhibited the reaction. As yet the transfer of experimentally induced ulcerative colitis from an affected animal to a normal recipient by means of lymphocytes has not been reported.

An alternative approach, adopted in the present study, has been to investigate the incidence, in ulcerative colitis, of auto-antibodies commonly found in other diseases suspected of having an auto-immune aetiology. For example, in Sjögren's syndrome there is an increased incidence of thyroid antibodies and of antinuclear factor (Bunim, 1961; Anderson, Gray, Beck, and Kinnear, 1961). This has been taken as additional evidence favouring an auto-immune disturbance in this disorder.

The present study has shown that there is no significant increase in the incidence of thyroid or gastric antibodies or of antinuclear factor in patients with ulcerative colitis compared with control subjects. Although not reaching a level which is

statistically significant, gastric-parietal cell antibodies were twice as common in patients with ulcerative colitis as in controls. This may, however, reflect an acquired lesion of the stomach, possibly resulting from long-continued medication or chronic iron-deficiency anaemia. Iron-deficiency anaemia is common in ulcerative colitis (Beal, Skyring, McRae, and Firkin, 1963; Edwards and Truelove, 1964) and may be a factor in the development of gastritis (Badenoch, Evans, and Richards, 1957).

Antinuclear factor was a little more common in patients with ulcerative colitis than in the controls, the incidence being compatible with the rare association of systemic lupus erythematosus with this disease. The discrepancy between the incidence of antinuclear factor found in the present study and that found by Calabresi and associates (1961) might be accounted for by the different nuclear sources used. The fact that they too failed to detect an increased incidence of antinuclear factors using calf thymus smears raises the possibility that they were measuring some other substance when using white cell nuclei. For example, Alexander and Potter (1961) have suggested that the 'antinuclear factor' found in some patients with rheumatoid arthritis might be due to a lysozyme-like factor in the serum and not an antibody.

The low incidence of gastric and thyroid antibodies and of antinuclear factor does not support the view that ulcerative colitis belongs to the group of disorders in which there is an association of auto-antibodies. This does not mean that a condition indistinguishable from idiopathic ulcerative colitis is not sometimes a component of a widespread auto-immune disturbance such as systemic lupus erythematosus, but whether the aetiology is the same, in such cases, as in the more usual form of the disease, is unknown.

The possibility that there is a relationship between hypersensitivity to autoantigens and hypersensitivity to exogenous antigens requires consideration. For example, it is possible that hypersensitivity to dietary or bacterial antigens may initiate auto-immune responses. A feature of some of the relapses provoked by cow's milk which we have observed (Truelove, 1961; Wright and Truelove, 1965a) has been that once the relapse has occurred the elimination of milk by itself may not achieve a remission. This suggests that other factors, such as auto-immune reactions, may have been perpetuating the tissue damage.

It has often been observed clinically that an attack of ulcerative colitis may be provoked by gastrointestinal infection. The possibility, first suggested by Taylor and Truelove (1962), that an immunological response against bacterial antigens might trigger off an auto-immune response against

related antigens in the colon has recently received some support from the work of Perlmann, Hammerström, Lagercrantz, and Gustafsson (1964). Their preliminary studies suggest that there is a close relationship between the colonic antigen and an antigen present in *Esch. coli* 014. This may be the heterogenetic lipopolysaccharide antigen present in Enterobacteriaceae described by Kunin (1962).

Finally, it is possible that reactions to exogenous antigens, either dietary or bacterial, as well as reactions to auto-antigen, may not be the primary factor in the production of tissue damage in ulcerative colitis, but may be responsible for perpetuating an abnormality initiated in some other way.

Despite extensive investigation using several different methods of approach it is clear that the role of immunological disturbances in the aetiology and pathogenesis of ulcerative colitis and its extracolonic manifestations remains uncertain. Further study, particularly of delayed hypersensitivity response to bacterial, dietary, and auto-antigens is necessary in this disease but such studies are at present hampered by inadequate methods of detecting these disturbances *in vitro*.

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