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Circulating antibodies to the surface antigens on colon epithelial cells in ulcerative colitis

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SUMMARY

The fluorescence activated cell sorter (FACS) was used for detecting circulating antibodies to the surface antigens on isolated colon epithelial cells (anti-colon antibodies) by indirect immunofluorescence. Anti-colon antibodies were found in the serum of 30 of 41 (73%) patients with ulcerative colitis. This incidence is much higher than one established in earlier reports by application of indirect immunofluorescence to colon tissue using the fluorescence microscope. The results suggest that FACS analysis is very useful for detecting antibodies to colon specific antigen.

Keywords ulcerative colitis anti-colon antibodies FACS indirect immunofluorescence

INTRODUCTION

The impairment of immune regulation may play an important role in the pathogenesis of ulcerative colitis (UC) since autoimmune phenomena have been described in some patients with this disease. Circulating anti-colon antibodies have been detected in patients' sera using sterile human fetus colon (Broberger & Permann 1963), adult normal or diseased colon (Lagercratz *et al.*, 1966), germ free rat colon (Perlmann *et al.*, 1965), and *Escherichia coli* (*E. coli*) 014 (Perlmann *et al.*, 1967) as antigens. Although these heterogeneous antibodies do not participate in antibody-mediated complement-dependent cytotoxicity (Perlmann *et al.*, 1967), it has been suggested that antibody-dependent cell-mediated cytotoxicity (ADCC) might cause destruction of colon epithelial cells and thus contribute to the pathogenesis of UC (Stobo *et al*, 1976; Nagai & Das, 1981). Therefore, to clarify the role of the ADCC mechanism in the pathogenesis of this disease, it is important to detect circulating anti-colon antibodies in the sera of patients with UC. In the present study, circulating antibodies to surface antigens on isolated colon epithelial cells from germ free rats were examined with a fluorescence activated cell sorter (FACS) and the results were compared with those of the indirect tissue immunofluorescence technique.

MATERIALS AND METHODS

Patients' sera. Sera were obtained from 41 adult patients (19 of them males) with clinical, radiological, endoscopic and biopsy findings typical of UC. Nineteen patients had been treated with

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salicylazosulphapyridine, eight with corticosteroids, 12 with both and two with neither of them. Eleven of the 41 patients were in the active stage and the others in the inactive stage. Control sera were obtained from nine healthy laboratory workers and hospital staff. The sex and age distribution of the healthy control group was comparable to that of the UC group. Sera were stored at −80°C without preservatives and were centrifuged at 105,000*g* for 90 min before use.

Indirect tissue immunofluorescence. Tissue specimens (1 cm^3) from the colon or ileum of germ free Wistar rats weighing 200–250 g were frozen in a mixture of dry ice and acetone at −70°C, and then cut into 4 μm thick sections in the cryostat. The sections were dried at room temperature by an electric fan, and then treated with undiluted human serum for 30 min at room temperature, followed by incubation with fluorescein isothiocyanate (FITC) conjugated $(Fab')_2$ fragments of rabbit anti-human immunoglobulin (Ig) antibody (Cappel Laboratories, Cochranville, Pennsylvania, USA).

After each step, the slides were washed three times in phosphate-buffered saline, pH 7·2 (PBS). The sections were finally mounted in a drop of phosphate-buffered glycerine, pH 7·2, and examined by a Zeiss fluorescence microscope with an Osram 200 W mercury lamp, two BG-12 exciter filters and one OG-5 barrier filter. Two controls were prepared in each experiment: (i) by omitting the human serum or (ii) by replacing the patient's serum with normal human serum. The identity of the sera was unknown until the sections had been examined and the results recorded.

Fresh specimens of colon or ileum were obtained from Wistar rats weighing 200–250 g. The mucosa was separated, washed several times with Hanks' balanced salt solution (HBSS), and finely minced with iris scissors. The tissue was subjected to trypsinization (0·25% trypsin, GIBCO, Grand Island, New York, USA) for approximately 1 h. The suspension was filtered through a 400 μm nylon mesh to remove large pieces of tissue, washed three times in HBSS, and then resuspended in RPMI 1640 medium (Flow Laboratories, Rockville, Maryland, USA) at a final concentration of 1×10^6 cells/ml. The percentage of viability estimated by trypan blue exclusion was more than 85% in each instance.

Indirect epithelial cell immunofluorescence. One million cells were incubated with 0·1 ml of undiluted human serum at room temperature for 30 min, and then washed three times with HBSS. Washed cells were incubated with 0·1 ml of FITC conjugated $(Fab')_2$ fragments of rabbit anti-human Ig(G+A+M), anti-human IgG, anti-human IgM or anti-human IgA antibody (Cappel Laboratories) at room temperature for 30 min. After three washes with HBSS, the cells were resuspended at a concentration of 1×10^7 cells/ml in RPMI medium, and then filtered through a 400 μm nylon mesh. The cell suspensions were then analysed with the FACS-II (Becton-Dickinson, Mountain View, California, USA).

RESULTS

Indirect tissue immunofluorescence

Specific fluorescence of the colon epithelial cells was found with six of 27 sera (22·2%) from patients with UC. A distinct staining was detected in the apical as well as lateral membrane of individual colon epithelial cells (Fig. 1). Specific staining of colon epithelial cells could not be demonstrated with any of the sera from healthy controls. Some of the sera from healthy controls weakly stained goblet cells and glandular luminal contents, but these staining patterns were regarded as negative.

Immunofluorescence staining of ileum did not occur with any of the sera from 27 patients with UC. The six sera which reacted with colon epithelial cells were then subjected to testing with each of FITC conjugated anti-human IgG, anti-human IgM and anti-human IgA. All of the six sera contained antibodies of IgG class, and did not show specific staining with anti-human IgM or anti-human IgA.

Indirect epithelial cell immunofluorescence

The average relative cell number of colon epithelial cells with fluorescence intensity higher than the background was 9·1 ± 5·0% (mean ± s.d.) in experiments with healthy control sera and 44·1 ± 26·1% in experiments with sera from patients with UC (Fig. 2). When the patients' sera were used as

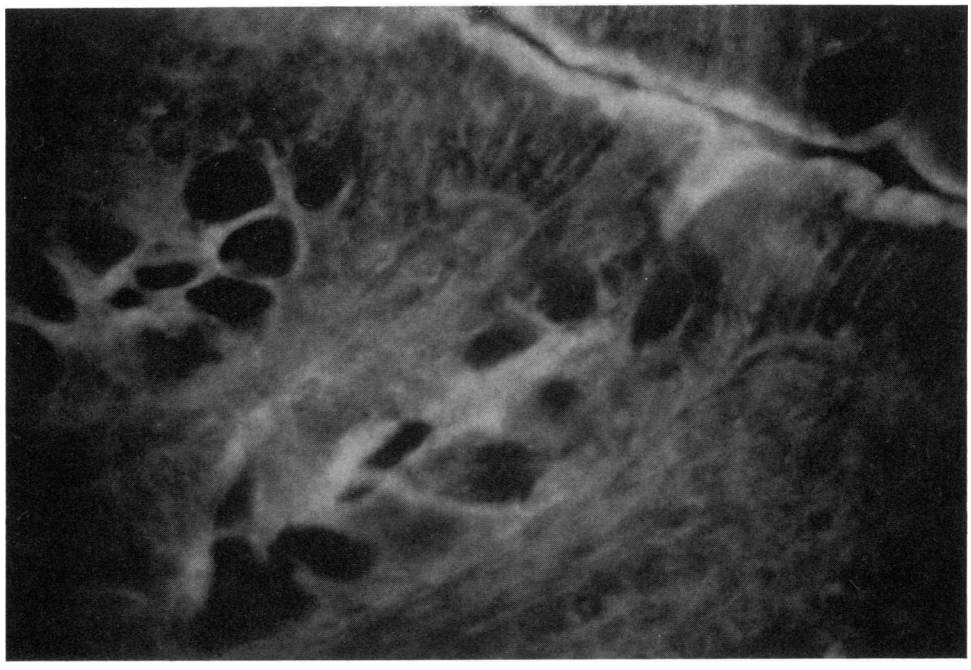

Fig. 1. Indirect tissue immunofluorescence. Colon tissue from Wistar rat was stained with the serum from patient with UC and FITC conjugated F(ab′)$_2$ fragment of rabbit anti-human Ig antibody. The apical and lateral membrane of colon epithelial cells was fluorescence positive.

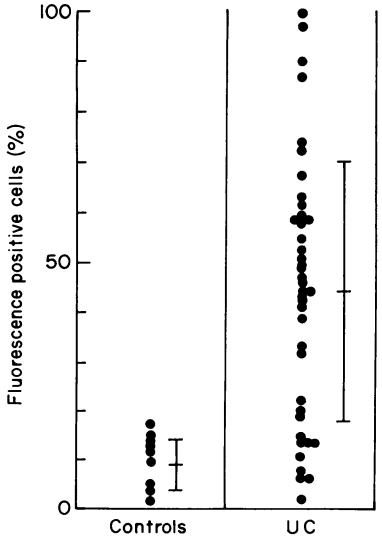

Fig. 2. Indirect epithelial cell immunofluorescence. Colon epithelial cells isolated from Wistar rat were stained with the serum from a healthy control or patient with UC, and then with FITC conjugated F(ab′)$_2$ fragments of rabbit anti-human Ig antibody. The percentage of fluorescence positive cells was significantly higher using the sera from patients with UC (44·1 ± 26·1%) than those from healthy controls (9·1 ± 5·0%).

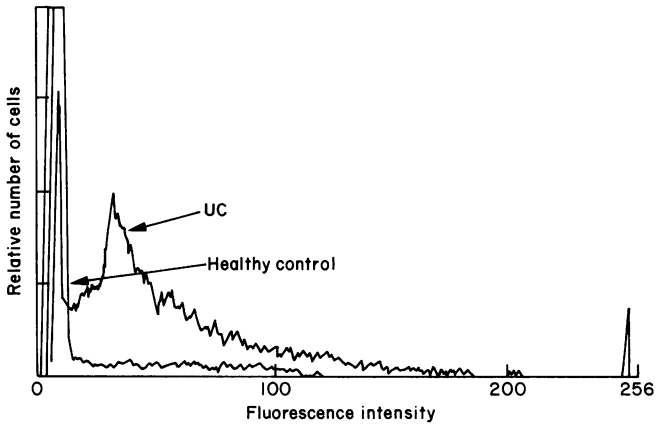

Fig. 3. Fluorescence histogram of colon epithelial cells stained by the serum from a patient with UC and a healthy control. The horizontal axis represents the fluorescence intensity and the vertical axis means numbers of cells with given degree of fluorescence. A large peak of cells with a low-to-intermediate degree of fluorescence intensity was seen.

probes, the fluorescence profile of the stained cells was characterized by a large peak of cells which had a low to intermediate degree of fluorescence intensity (Fig. 3). Circulating anti-colon antibodies were found in 30 of 41 (73%) patients with UC when the sera staining more than 19·1% of colon epithelial cells (above mean ± 2 s.d. for healthy controls) were regarded as positive. All of the six sera which stained tissue in the indirect immunofluorescence tests showed positive staining by FACS analysis. All of the positive sera contained anti-colon antibodies of the IgG class. With regard to disease activity, seven of 11 patients (64%) in the active stage and 23 of 30 (77%) in the inactive stage had positive sera. With regard to the influence of drug administration, 15 of 19 (79%) patients administered with salicylazosulphapyridine, four of eight (50%) with corticosteroids, nine of 12 (75%) with a combination of salicylazosulphapyridine and corticosteroids and one of two without medication had positive sera. No significant difference was found with regard to percentage of positive cells between sera from patients with UC and those from healthy control, when ileal epithelial cells were used instead of colon cells.

DISCUSSION

Stobo *et al.* (1976) have reported that peripheral Fc receptor positive mononuclear cells from patients with UC are cytotoxic *in vitro* for isologous or allogeneic colon epithelial cells. These results suggest that *in vitro* lysis of colon epithelial cells by mononuclear cells from patients with UC represents ADCC. It has also been shown that prior coating Fc receptor positive cells with antigen–antibody complexes specifically arms these cells for lysis of target cells bearing the antigens which are present in the complexes (Perlmann, Perlmann & Biberfeld, 1972; Greenberg & Shen, 1973). Circulating immune complexes have been detected in patients with UC in some reports (Jewell & MacLennan, 1973); Kemler & Alper, 1980), although specific antigens in the immune complexes have not yet been identified. As well, lymphocytophilic antibodies have been demonstrated in serum from patients with UC by Korsmeyer, Strickland & Williams (1974). In an earlier communication, we have shown that lymphocytophilic antibodies can be absorbed by isolated colon epithelial cells and that anti-colon antibodies which participate in the ADCC mechanism *in vitro* have some relationship to lymphocytophilic antibodies (Hibi *et al.*, 1982).

In order to clarify the role of the ADCC mechanism in the pathogenesis of ulcerative colitis, it has become important to detect circulating antibodies which are specific for colon epithelial cells. In the earlier reports circulating anti-colon antibodies were found in 15–40% of patients with UC (Broberger & Perlmann, 1963; Lagercrantz *et al.*, 1966; Harrison, 1965; Wright & Truelove, 1966;

McGiven, Ghose & Nairn, 1967; Marcussen, 1978). The incidence of circulating anti-colon antibodies obtained by the indirect tissue immunofluorescence method in our study was similar to that in previous reports, although some differences were observed in the staining patterns of the tissue. In some previous studies, anti-colon antibodies were reported to stain goblet cells and cytoplasm of colon epithelial cells (Broberger & Perlmann, 1963; Harrison, 1965; Wright & Truelove, 1966; Marcussen, 1978). In contrast, in our present study anti-colon antibodies were observed to stain apical and lateral membrane of colon epithelial cells.

The results of the present study obtained by FACS analysis indicate that the incidence of circulating anti-colon antibodies in patients with UC is much higher than suggested by the earlier reports. The specificity of this technique was demonstrated by the fact that there was no significant staining of the isolated ileal epithelial cells. It seems possible that FACS analysis detects more anti-colon antibodies than the other techniques. Various methods have previously been used to identify circulating anti-colon antibodies, including indirect haemoagglutination (Lagercrantz *et al.*, 1966) and indirect immunofluorescence (Harrison, 1965; Wright & Truelove, 1966; McGiven *et al.*, 1967; Marcussen, 1978). These methods, however, have certain disadvantage as compared to the FACS analysis employed in the present study. When tissue homogenates or slices of colon tissue are analysed, antibodies in UC serum might react with intracellular or cell surface antigen, and non-specific reactions might also occur. Also, staining patterns obtained in indirect tissue immunofluorescence are sometimes difficult to interpret. One advantage of the FACS analysis is that it can detect antibodies to antigen determinants exposed on the surface of viable colon epithelial cells. Moreover, this technique is more sensitive than the other techniques using the fluorescence microscope. Thus, the FACS technique, which we have utilized, appears to be a very useful one for detecting antibodies to colon specific antigens on single viable epithelial cells.

Our finding that colon specific antibodies in sera of UC patients were exclusively of the IgG class thus confirms previous reports which have suggested the IgG class antibodies might play a special role in the pathogenesis of UC (Das, Dubin & Nagai, 1978; Nagai & Das, 1981; Baklien & Brantzaeg, 1970). Further studies should be carried out with peripheral and/or intestinal effector cells (lymphocytes) from patients with UC in order to clarify role of the immune system in this disease.

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