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# Anti-colon antibody and lymphocytophilic antibody in ulcerative colitis

T. HIBI*, S. AISO*, T. YOSHIDA*, M. WATANABE*, H. ASAKURA*, S. TSURU† & M. TSUCHIYA* *Department of Medicine, School of Medicine, Keio University, Tokyo, Japan and † Department of Bacteriology, National Defence Medical College, Saitama, Japan*

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## SUMMARY

The presence of anti-colon antibody in the sera from patients with ulcerative colitis was demonstrated by antibody-dependent cell-mediated cytotoxicity (ADCC) assay. In addition, the high prevalence of lymphocytophilic antibody in the sera from patients with ulcerative colitis was obtained by fluorescence activated cell sorter (FACS) analysis. This lymphocytophilic antibody was absorbed by rat colon epithelial cells. Moreover the lymphocytes from ulcerative colitis showed lower binding capacity to this antibody, but acquired higher binding capacity after 20 hr incubation at 37°C *in vitro*. These data suggest that ADCC may have some role in the pathogenesis of ulcerative colitis.

## INTRODUCTION

Although several investigators have described abnormalities of cellular and humoral immunity in patients with ulcerative colitis, the pathogenesis of ulcerative colitis has remained unresolved. In the past two decades, circulating anti-colon antibodies have been detected in the sera of patients with ulcerative colitis (Broberger & Perlmann, 1959; Marcussen, 1978). However, these anti-colon antibodies have not been cytotoxic for the colonic epithelial cells (Broberger & Perlmann, 1963). Shorter and his colleagues have accumulated impressive evidence that a class of circulating lymphocytes in patients with ulcerative colitis exerts specific cytotoxicity against the colonic epithelial cells *in vitro* (Shorter, Spencer & Huizenga, 1968), and that the effector cells may be Fc-receptor bearing cells (Stobo *et al.*, 1976). On the other hand, the presence of serum factors with apparently combining capacity and cytotoxic activity for lymphocytes (lymphocytophilic and lymphocytotoxic antibodies) has been reported in patients with ulcerative colitis (Korsmeyer *et al.*, 1974) as well as in patients with some autoimmune diseases (Mittal *et al.*, 1970). Therefore these antibodies are thought to play some role in the pathogenesis of ulcerative colitis.

In an attempt to elucidate the role of anti-colon antibody and lymphocytophilic antibody in the pathogenesis, we investigated the nature of these antibodies in the sera of patients with ulcerative colitis utilizing antibody-dependent cell-mediated cytotoxicity (ADCC) assay and fluorescence activated cell sorter (FACS) analysis. Moreover the characteristics of the circulating lymphocytes from patients with ulcerative colitis were also examined.

## MATERIALS AND METHODS

*Sera.* Sera from 21 patients with ulcerative colitis, five patients with systemic lupus erythematosus (SLE) and five patients with rheumatoid arthritis (RA) were examined. Sera from 13

Correspondence: Dr Masaharu Tsuchiya, Department of Medicine, School of Medicine, Keio University, 35 Shinano-machi, Shinjuku-ku, Tokyo 160, Japan.

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apparently healthy individuals were used as controls. All of the patients had active disease. Fifteen of 21 patients with ulcerative colitis were treated with salicylazosulphapyridine, and the other six patients were treated with salicylazosulphapyridine and corticosteroid. There was no history of previous blood transfusion in any of the patients studied. All the sera used for the ADCC assay and the FACS analysis, were heat-inactivated for 30 min at 56°C and centrifuged (100,000 ***g***; 90 min) before use.

*Lymphocyte preparation.* Heparinized blood was obtained from six apparently healthy individuals and five patients with ulcerative colitis. Peripheral blood lymphocytes were purified on Ficoll-Hypaque density gradient ($\rho = 1{\cdot}076$) by centrifugation. After being washed in phosphate-buffered saline (PBS) at pH 7·4, the lymphocytes from healthy controls were used for the ADCC assay. For the staining, the cells were then purified further by passage through a nylon wool column to delete B cells (Julius, Simpson & Herzenberg, 1973). Contamination of surface immunoglobulin (Ig) bearing cells (B cells) as detected by staining with fluorescein conjugated rabbit anti-human Ig was less than 5%.

*Epithelial cell preparation.* Epithelial cells from the rat colon and small intestine were prepared as described previously (Aiso *et al.*, 1981). Fresh specimens of the colon and small intestine were obtained from rats of Wistar strain weighing 200–250 gm. The mucosa was dissected off, washed several times with RPMI 1640 medium (GIBCO, New York, USA) and finely minced with iris scissors. The tissue was subjected to trypsinization (0·25% trypsin, GIBCO,) for approximately 1 hr at 37°C. The suspension of epithelial cells was then washed three times with RPMI 1640 medium and resuspended at a concentration of $1 \times 10^6$ cells/ml. Viability estimated by trypan blue exclusion, exceeded 85%. A cell line intestine 407 of human fetal small intestine epithelial cell origin (Henle & Deilnhardt, 1965) was subjected to trypsinization (0·25% trypsin) for 5 min at 37°C. The suspension of cells was then washed three times with RPMI 1640 medium and resuspended at a concentration of $1 \times 10^6$ cells/ml. All of the epithelial cells were used 8 hr after trypsinization for tests.

*ADCC assay.* Target epithelial cells ($1 \times 10^5$) were suspended in 1 ml of medium containing 100 $\mu$Ci $Na_2{}^{51}CrO_4$ (Daiichi Radioisotope, Tokyo) and incubated for 45 min at 37°C. These labelled cells were washed three times with medium and resuspended at a concentration of $1 \times 10^5$ cells/ml. One tenth of a millilitre of them was dispensed into microtest plate wells, and incubated with 0·05 ml of the sera for 30 min at 37°C. After 0·05 ml of normal lymphocyte suspension ($2 \times 10^6$ cells/ml) was added to the wells, the plates were incubated for 8 hr at 37°C in a humidified atmosphere of 5% $CO_2$ and 95% air. The final ratio of effector to target cells was 10:1. All cultures were performed in triplicate. $^{51}Cr$ released from the labelled target cells was collected with Titertek supernatant collection system (Flow Laboratories, McLean, Virginia, USA) and was measured with a gamma-counter (Aloka, ARC 251). Maximum possible $^{51}Cr$ release was determined by three freeze–thaw cycles. The results were expressed in % cytotoxicity as follows,

$$\% \text{ cytotoxicity} = \frac{\text{c.p.m. of experimental release} - \text{c.p.m. of spontaneous release}}{\text{c.p.m. of maximum release} - \text{c.p.m. of spontaneous release}}$$

*Fluorescene staining of cells.* For the routine FACS analysis of peripheral blood lymphocytes from healthy controls and patients with ulcerative colitis, 0·1 ml of T cell enriched preparations ($1 \times 10^6$ cells/ml) was reacted with 0·1 ml of the sera from normal controls, patients with ulcerative colitis, SLE and RA for 30 min at room temperature. After being washed in PBS, the lymphocytes were incubated with 0·1 ml of fluorescein isothiocyanate (FITC)-conjugated anti-human Ig (G + A + M), anti-human IgG, anti-human IgA or anti-human IgM $(Fab')_2$ fragments from rabbit (Cappel Laboratories, Downington, Pennsylvania, USA) for 30 min at room temperature. The F/P ratio was between 2 and 3. FITC-conjugated serum was centrifuged at 100,000 ***g*** for 90 min immediately before use. Cells were resuspended at $1 \times 10^6$ cells/ml and held in an ice bath for a cellular analysis. The T cell enriched preparations from patients with ulcerative colitis were stained just after passage through a nylon wool column or after 20 hr culture at 37°C in a humidified atmosphere of 5% $CO_2$ and 95% air. In an experiment for absorption of antibody, the epithelial cells from the rat colon, rat small intestine or human small intestine were incubated in the sera from normal controls, patients with ulcerative colitis, SLE and RA for 1 hr at 37°C. After that procedure these sera were also examined as described above.

*Analysis of cell preparations.* Analysis of fluorescence-binding cells was carried out with a fluorescence activated cell sorter (FACS-II, Becton Dickinson Electronics Laboratory, Mountain View, California, USA) (Bonner *et al.*, 1972). Cells were processed at 2,000–3,000 cells per second and the intensity of fluorescence was recorded for each individual cell on the pulse height analyser. The level of background fluorescence was determined by analysing T cell enriched preparations treated with FITC-conjugated anti-human Ig but without the intermediate sera from normal controls, patients with ulcerative colitis, SLE and RA as negative control. Thus the percentage of labelled cells was determined by counting the number of cells giving fluorescence signals above the background and dividing by the total number of viable cells examined. Light scattering signals distinguished live from dead lymphocytes. The calculation by the FACS analyser was based on analysis of $5–10 \times 10^4$ individual viable cells.

## RESULTS

*ADCC assay. (Fig. 1)*

Significantly higher levels of cytotoxicity against rat colon epithelial cells were observed using the sera from nine patients with ulcerative colitis (% cytotoxicity = 32·5 ± 6·4), in comparison to the sera from 13 healthy controls (% cytotoxicity = 23·4 ± 3·6). But the cytotoxic activity obtained with the patients' sera did not differ significantly from that obtained with the sera of healthy controls when rat or human small intestinal cells were used as target cells in the cytotoxicity assay.

*Reactivity of the sera from healthy controls, patients with ulcerative colitis, SLE and RA against the lymphocytes from healthy controls. (Fig. 2)*

The fluorescence histograms of negative control cells, which were stained with FITC-conjugated anti-human Ig serum but without intermediate sera, appeared as a narrow peak with fluorescence intensity lower than 40. Therefore, cells with fluorescence intensity more than 40 were defined as

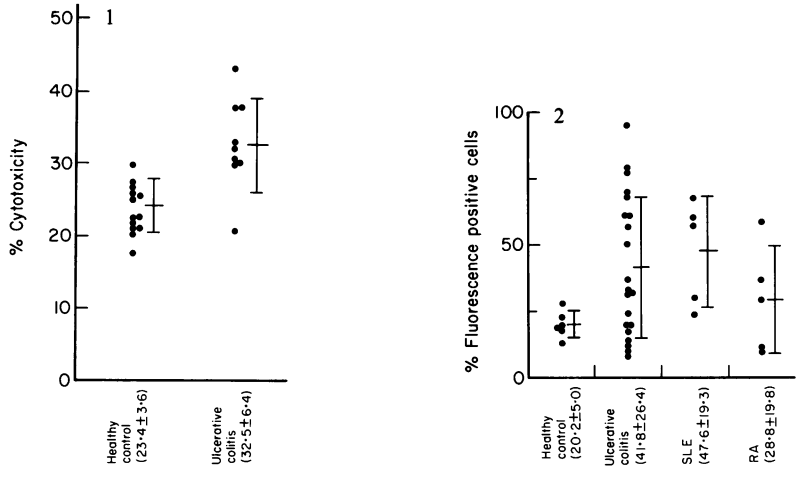


**Fig. 1.** Antibody-dependent cell-mediated cytotoxicity against rat colon epithelial cells, using the sera from healthy controls and patients with ulcerative colitis. Rat colon epithelial cells were used as target cells and lymphocytes from healthy controls were used as effector cells. The effector/target ratio was 10:1. The results were expressed as mean ± s.d. ($P < 0·01$). Statistical analysis was performed according to Student's *t*-test.

**Fig. 2.** Lymphocytophilic antibody in the sera from healthy controls, patients with ulcerative colitis, SLE and RA. The percentage of fluorescence positive cells in total population was measured by FACS analysis. The results were expressed as mean ± s.d. Controls vs ulcerative colitis patients: $p < 0·05$; controls vs SLE patients: $p < 0·01$. Statistical analysis was performed according to Student's *t*-test.

positive cells. The percentage of fluorescence-positive cells was 20·2 ± 5·0 in six sera of healthy controls, 41·8 ± 26·4 in 21 sera of ulcerative colitis, 47·6 ± 19·3 in five of SLE and 28·8 ± 19·8 in five sera of RA. The immunoglobulin class of the antibody binding to the lymphocytes was IgG. Staining of more than 30% of the normal lymphocytes was obtained with sera of 13 of 21 patients with ulcerative colitis, four of five patients with SLE and two of five patients with RA.

*Reactivity of the sera from patients with ulcerative colitis against the lymphocytes from patients with ulcerative colitis*

Lowered fluorescence histograms were obtained, when the sera of five patients with this disease, all showing clear fluorescence against normal lymphocytes, were treated with lymphocytes from patients with ulcerative colitis. But when these lymphocytes were incubated for 20 hr at 37°C, these were found to be well stained with the same sera, as shown in Fig. 3. There was no difference in the T cell populations before and after 20 hr incubation.

*Reactivity of the sera from patients with ulcerative colitis, SLE and RA before and after incubation with epithelial cells*

The sera from patients with ulcerative colitis showing clear fluorescence against normal lymphocytes, lost the staining activity after incubation with rat colon epithelial cells (Fig. 4). After

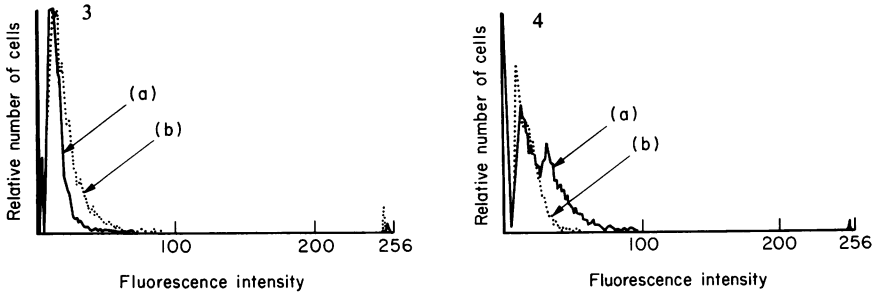


**Fig. 3.** The fluorescence histograms of antibody binding lymphocytes from ulcerative colitis. T cell enriched preprations without *in vitro* incubation (a) and after 20 hr *in vitro* incubation at 37°C (b), were incubated with the sera of ulcerative colitis patients for 30 min at room temperature. After being washed, the cells were incubated with a fluorescein-conjugated anti-human Ig serum from rabbit. The cells were then washed and analysed with the FACS. The brightly stained cells appeared after the *in vitro* incubation.

**Fig. 4.** The fluorescence histograms of antibody binding lymphocytes from healthy controls. T cell enriched preparations were incubated for 30 min at room temperature with the sera from patients with ulcerative colitis before (a) and after (b) the sera were incubated with colonic epithelial cells. After washed and incubated for additional 30 min at room temperature with fluorescein-conjugated anti-human Ig serum from rabbit, the cells were then washed and analysed with the FACS. The populations of brightly stained cells decreased, using the serum incubated with colonic epithelial cells, in comparison to the non-treated serum.

incubation with epithelial cells obtained from the small intestine of rat or human, the loss of staining activity was not observed. The staining activity of the sera from patients with SLE and RA was not removed by incubation with epithelial cells from the rat colon or small intestine.

## DISCUSSION

The presence of the anti-colon antibody in the sera of patients with ulcerative colitis has been revealed by various methods (Broberger & Perlmann, 1959: Marcussen, 1978). The anti-colon antibody has been reported to react with antigen in the sterile human colon as well as rat colon or faeces (Langercranz *et al.*, 1968). But this anti-colon antibody has not been shown to be cytotoxic

for colonic epithelial cells in ulcerative colitis (Broberger & Perlmann, 1963) or in the animal model of colitis (Rabin & Herrington, 1980). In the present study, we demonstrated a cytotoxic effect of the patients' sera on rat colon cells in collaboration with peripheral lymphocytes from healthy controls. Das, Dubin & Nagai (1978) have isolated anti-colon antibodies bound to the colonic tissue of patients with ulcerative colitis. The findings cited above suggest that antibody-dependent cell-mediated cytotoxicity has a role in the sustained inflammation in the colon of such patients.

Lymphocytophilic or lymphocytotoxic antibody has been demonstrated in the sera of patients with ulcerative colitis by Korsmeyer *et al.* (1974) and Strickland *et al.* (1977). They have reported that this type of antibody is detected in the sera of almost 50% of the patients studied according to Terasaki's microcytotoxicity assay. By using FACS analysis, we observed a higher prevalence of this antibody in patients with ulcerative colitis than that previously reported. This high prevalence may support the participation of immunological mechanisms in the pathogenesis of the disease. The precise clinical significance of this antibody still remains unknown. One of the conceivable explanations is that there might exist some common exposure to a potentially aetiological agent such as a virus in these patients. Thus, new antigens expressed on the surface of lymphocytes as a result of viral infection might lead to a loss of tolerance to neighbouring normal cell antigens (Obota *et al.*, 1975). This is supported by the possible involvement of transmissible agents in the pathogenesis of inflammatory bowel disease (Cave, Mitchell & Brooke, 1975). However, other investigators have reported negative results concerning transmissible agents (Bolton *et al.*, 1973). Another possible explanation is that suppressor T cells might be killed selectively by the antibody as in the case of SLE (Sakane *et al.*, 1979). Decreased suppressor T cell activity in inflammatory bowel disease has been demonstrated by Hodgson, Wands & Isserbacher (1978). Such depression of suppressor activity may result in the production of anti-colon antibody as autoantibody. However, there is contrary evidence that few of the other autoantibodies as seen in SLE sera has been detected in the sera from patients with ulcerative colitis.

One of the most striking findings in this study is that the sera from patients with ulcerative colitis lost their binding capacity to lymphocytes after incubation with colonic epithelial cells. Furthermore this phenomenon was not observed after incubation with the epithelial cells of the small intestine. The loss of binding capacity was not observed with the sera from patients with SLE after incubation with the epithelial cells of the colon or small intestine. Thus, it is conceivable that the anti-colon antibody with a combining capacity for lymphocytes was absorbed by the colon-specific antigens. The antibody shown to be lymphoctyophilic by FACS analysis of the sera from patients with ulcerative colitis is not specific for lymphocytes and may be different from the 'anti-lymphocyte antibody' as detected in SLE sera.

Another interesting finding is that the sera from patients with ulcerative colitis showed a much lower combining capacity for the lymphocytes from the patients as compared with the high combining capacity for the lymphocytes from healthy controls. The lymphocytes of patients with ulcerative colitis may be modified *in vivo* to disturb the attachment of cytophilic or cytotoxic antibody. For the resolution of this mechanism, we performed the following experiment. Twenty hours after incubation of the lymphocytes from patients with ulcerative colitis *in vitro*, the lymphocytes became capable of binding to the antibody. This phenomenon may be due to the removal of either bound immune complexes reported by Jewell & MacLennan (1973), or some factors which protect against binding of the antibody.

The presence of a close reaction between lymphocytophilic antibody and anti-colon antibody in the pathogenesis of the colonic inflammation in patients with ulcerative colitis, is established in this series of experiments. The findings in the present study lead us to advance a tentative hypothesis that circulating anti-colon antibodies in the sera of these patients may have the binding capacity for a class of peripheral lymphocytes, especially Fc receptor bearing cells, which in turn may cause antibody-dependent cell-mediated cytotoxicity in the colon. Further studies will be performed to establish definitely the validity of this hypothesis.

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## REFERENCES


AISO, S., ASAKURA, H., TANAKA, T. & TSUCHIYA, M. (1980) Lymphocyte response to colonic antigen. Its role in the pathogenesis of ulcerative colitis. *Biochem. exp. Biol.* **16,** 287.

BOLTON, P.M., OWEN, E., HEATLEY, R.V., JONES WILLIAMS, W. & HUGHES, L.E. (1973) Negative findings in laboratory animals for a transmissible agent in Crohn's disease. *Lancet*, **ii,** 1122.

BONNER, W.A., HULLET, H.R., SWEET, R.G. & HERZENBERG, L.A. (1972) Fluorescence activated cell sorting. *Rev. Sci. Instrum.* **43,** 404.

BROBERGER, O. & PERLMANN, P. (1959) Autoantibodies in human ulcerative colitis. *J. exp. Med.* **110,** 657.

BROBERGER, O. & PERLMANN, P. (1963) *In vitro* studies of ulcerative colitis. I. Reactions of patients' serum with human fetal colon cells in tissue culture. *J. exp. Med.* **117,** 705.

CAVE, D.R., MITCHELL, D.N. & BROOKE, B.N. (1975) Observations on the transmissibility of Crohn's disease and ulcerative colitis. *Gastroenterol.* **68,** 871.

DAS, K.M., DUBIN, R. & NAGAI, T. (1978) Isolation and characterization of colonic tissue-bound antibodies from patients with idiopathic ulcerative colitis. *Proc. Natl. Acad. Sci.* **75,** 4528.

HENLE, G. & DEILNHARDT, F. (1965) The establishment of strains of human cells in tissue culture. *J. Immunol.* **79,** 54.

HODGSON, H.J.F., WANDS, J.R. & ISSERBACHER, K.J. (1978) Decreased suppressor cell activity in inflammatory bowel disease. *Clin. exp. Immunol.* **32,** 451.

JEWELL, D.P. & MACLENNAN, I.C.M. (1973) Circulating immune complexes in inflammatory bowel disease. *Clin. exp. Immunol.* **14,** 219.

JULIUS, M.H., SIMPSON, E. & HERZENBERG, L.A. (1973) A rapid method for the isolation of functional thymus derived murine lymphocytes. *Eur. J. Immunol.* **3,** 645.

KORSMEYER, S., STRICKLAND, R.G., WILSON, I.D. & WILLIAMS, R.C. (1974) Serum lymphocytotoxic and lymphocytophilic antibody activity in inflammatory bowel disease. *Gastroenterol.* **67,** 578.

LANGERCRANZ, R., HAMMARSTROM, S., PERLMANN, P. & GUSTAFFSON, B.E. (1968) Immunological studies in ulcerative colitis. IV. Origin of autoantibodies. *J. exp. Med.* **128,** 1339.

MARCUSSEN, H. (1978) Fluorescent anti-colon and *E. coli* antibodies in ulcerative colitis. *Scand. J. Gastroent.* **14,** 277.

MITTAL, K.K., ROSSEN, R.D., SHARP, J.T., LIDSKY, M.D. & BUTLER, W.T. (1970) Lymphocyte cytotoxic antibodies in systemic lupus erythematosus. *Nature*, **225,** 1255.

OBOTA, Y., HISHOMI, I., STOCKERT, E. & BOYSE, E.A. (1975) Relation of $G1_X$ antigen of thymocytes to envelope glycoprotein of murine leukemia virus. *J. exp. Med.* **141,** 188.

RABIN, B.S. & HERRINGTON, E. (1980) Antibody-mediated complement-dependent cytotoxicity in immunologically induced experimental colon disease. *Int. Arch. Allerg. appl. Immunol.* **63,** 205.

SAKANE, T., STEINBERG, A.D., REEVES, J.P. & GREEN, I. (1979) Studies of immune functions of patients with systemic lupus erythematosus. Complement-dependent immunoglobulin M anti-thymus-derived cell antibodies preferentially inactivate suppressor cells. *J. clin. Invest.* **63,** 954.

SHORTER, R.G., SPENCER, R.J. & HUIZENGA, K.A. (1968) Inhibition of *in vitro* cytotoxicity of lymphocytes from patients with ulcerative colitis and granulomatous colitis for allogeneic colonic epithelial cells using horse anti-human thymus serum. *Gastroenterol.* **54,** 227.

STOBO, J.D., TOMASI, T.B., HUIZENGA, K.A., SPENCER, R.J. & SHORTER, R.G. (1976) *In vitro* studies of inflammatory bowel disease. Surface receptors of the mononuclear cell required to lyse allogeneic colonic epithelial cells. *Gastroenterol.* **70,** 171.

STRICKLAND, R.G., MILLER, W.C., VOLPICELLI, N.A., GAEKE, R.F., WILSON, I.D. & KIRSNER, J.B. (1977) Lymphocytotoxic antibodies in patients with inflammatory bowel disease and their spouses-evidence for a transmissible agent. *Clin. exp. Immunol.* **30,** 188.