Effect of *E. coli* antigens, tuberculin, and phytohaemagglutinin upon ulcerative colitis lymphocytes

S. STEFANI AND S. FINK

From the Veterans Administration Hospital and Loyola Stritch School of Medicine, Hines, Illinois, U.S.A.

EDITORIAL COMMENT Some very interesting observations showing that lymphocytes from the peripheral blood in patients with active ulcerative colitis differed from other lymphocytes tested in their response to *E. coli* extracts.

Human peripheral blood lymphocytes respond *in vitro* to a wide range of agents by undergoing blastoid transformation and mitosis (Pearmain, Lycette, and Fitzgerald, 1963; Elves, Roath, and Israels, 1963). It is believed that specific antigen-antibody reactions within the growing cells are responsible for these changes (Hashem, 1965). As a result of these observations, Hirschhorn, Back, Kolodny, Firschein, and Hashem (1963), among others, have stated that the human peripheral lymphocyte provides a readily available system representative *in vitro* of the donor's immune status.

It has been suggested that the destruction of colonic mucosa in ulcerative colitis is the result of an immunological reaction in which *E. coli* may play a role (Perlmann, Hammarström, Lagercrantz, and Gustafsson, 1965). It was, therefore, decided to study *in vitro* the morphological reaction of blood lymphocytes obtained from patients with ulcerative colitis and from normal donors to extracts of a pathogenic strain of *E. coli* (0 119: B 14) as well as to old tuberculin and phytohaemagglutinin.

METHOD

Lymphocytes were obtained from three groups of ambulatory adult male donors. Two groups of patients had known ulcerative colitis established by barium enema and sigmoidoscopy and confirmed by compatible rectal biopsy. The first of these was judged to have active ulcerative colitis by the presence of diarrhoea, rectal bleeding, and gross proctoscopic abnormalities, graded three and four (Dragstedt, Dack, and Kirsner, 1941) at the time of the study *in vitro*. The patients in the second group were free of symptoms, had normal mucosal appearance on proctoscopy and were, therefore, classified as being in remission. Patients with mild or no

symptoms but with mucosal abnormalities were not included in this investigation. The third group consisted of normal donors. None of the patients studied had received corticosteroid therapy during the six months preceding the investigation. As indicated in Table I, 10 patients were taking salicylazosulphapyridine (Azulfidine) and had been receiving the drug for periods of six to 24 months.

Twenty millilitres of blood were obtained from each donor and sedimentation was carried out for 90 minutes at 37°C. The leucocytes in the supernatant plasma were centrifuged, washed, and resuspended in the final medium which consisted of equal parts of TC 199 (Difco) and a compatible human serum. The suspension was then introduced into a flat-bottomed T flask and incubated for 30 minutes at 37°C. During this procedure, the granulocytes and monocytes adhere to the glass. In the supernatant, 90 to 99% of the nucleated cells are seen to be lymphocytes. The supernatant was divided into 0.9 ml. aliquots and placed in small test tubes. From each patient, one group of lymphocyte suspensions was treated with 0.1 ml. of saline and served as a control, another group with phytohaemagglutinin (PHA-P Difco) at a final concentration of 4 µl. per ml., another with old tuberculin (Parke-Davis & Co.) at a final concentration of 1 : 100. The last three groups were treated with each of three extracts derived from cultures of *E. coli* 0 119: B 14. Two of the three *E. coli* extracts, a protein and a polysaccharide, were prepared by the phenol extraction method of Westphal *et al.*, (1952); the third, a K antigen, was kindly supplied by the Sylvania Company, Millburn, New Jersey. Rabbit antisera to each of the *E. coli* extracts were prepared and the antigenic potency of the three preparations was verified by precipitin-ring tests. The suspensions were incubated for six days at 37°C. On the sixth day, several slide chambers were prepared from each suspension and the viable cells were examined, photographed, and counted by use of an inverted phase contrast microscope (Schrek and Stefani, 1964). Blastoid cells were arbitrarily defined as those having 10 µ as

their smallest diameter. At least 1,000 lymphocytic cells were counted in each group of suspensions derived from any given donor and the number of blastoid cells and mitotic figures present was expressed as a percentage of this total lymphocytic cell count. The suspensions were classified according to the total percentage yield of blastoid cells and mitotic figures as follows:

++++ = > 20% blastoid cells or mitotic figures
 +++ = 10% to 20% blastoid cells or mitotic figures
 ++ = 5% to 10% blastoid cells or mitotic figures
 + = < 5% blastoid cells or mitotic figures
 0 = no blastoid cells and mitotic figures, or only a few, but not in all slides examined

The cells were classified without foreknowledge on the part of the examiner either of the donor's diagnosis or of the agent used.

RESULTS

The results are presented in Table I. The saline-treated suspensions produced no blastoid transformation while suspensions treated with phytohaemagglutinin showed a uniform marked response regardless of the donor's diagnosis and stage of the

disease. Old tuberculin produced constant blastoid transformation and formation of mitosis in all lymphocyte suspensions tested from purified protein derivative (P.P.D.) positive patients but not in suspensions from patients who were P.P.D. negative. The morphological response was irrespective of the phase of the patient's disease. This response to old tuberculin of lymphocytes from patients with ulcerative colitis was comparable to that observed many times in this laboratory in lymphocytes from normal donors (Stefani, 1966). Lymphocyte suspensions exposed to *E. coli* extracts showed a different pattern of response. Lymphocytes obtained from the control group and from patients with ulcerative colitis in remission showed consistent blastoid transformation with all the *E. coli* extracts although this reaction was usually most marked in the presence of the protein extract. In contrast, all three *E. coli* extracts induced little or no blastoid transformation in the lymphocyte suspensions obtained from patients with ulcerative colitis in an active phase.

TABLE I

REACTIVITY *in vitro* OF NORMAL AND ULCERATIVE COLITIS LYMPHOCYTES¹

Patient's Initials	Diagnosis	Current Therapy ²	P.H.A.	Old Tuberculin ³	<i>E. Coli</i> 0119: B14 Extracts
E.E.	Ulcerative colitis (active)	None	++++	0 (-)	+
W.J.	Ulcerative colitis (active)	Azulfidine	++++	0 (-)	+
*V.K.	Ulcerative colitis (active)	None	++++	++++ (+)	0
R.L.	Ulcerative colitis (active)	None	++++	+++ (+)	0
F.V.	Ulcerative colitis (active)	Azulfidine	++++	++ (+)	0
R.C.	Ulcerative colitis (active)	None	++++	++ (+)	0
C.C.	Ulcerative colitis (active)	Azulfidine	++++	++ (+)	+
*K.G.	Ulcerative colitis (active)	Azulfidine	++++	+++ (+)	0
W.E.	Ulcerative colitis (active)	Azulfidine	++++	+++ (+)	0
W.D.	Ulcerative colitis (active)	Azulfidine	++++	+++ (+)	0
A.B.	Ulcerative colitis (in remission)	None	++++	0 (-)	+++
*K.G.	Ulcerative colitis (in remission)	Azulfidine	++++	0 (-)	+++
J.W.	Ulcerative colitis (in remission)	Azulfidine	++++	+++ (+)	++
*V.K.	Ulcerative colitis (in remission)	None	++++	++++ (+)	++
R.R.	Ulcerative colitis (in remission)	None	++++	+++ (+)	+
G.K.	Ulcerative colitis (in remission)	None	++++	+++ (+)	+
C.P.	Ulcerative colitis (in remission)	None	++++	+++ (+)	++
B.D.	Ulcerative colitis (in remission)	None	++++	+++ (+)	++
E.M.	Ulcerative colitis (in remission)	Azulfidine	++++	0 (-)	++
A.S.	Ulcerative colitis (in remission)	None	++++	0 (-)	++
A.H.	Ulcerative colitis (in remission)	Azulfidine	++++	0 (-)	++
R.D.	Ulcerative colitis (in remission)	None	++++	++ (+)	++
F.R.	Normal	None	++++	*	++
G.G.	Normal	None	++++	*	+++
R.M.	Normal	None	++++	*	+++
W.W.	Normal	None	++++	*	+++
J.S.	Normal	None	++++	*	++
F.W.	Normal	None	++++	*	++
W.N.	Normal	None	++++	*	++
C.P.	Normal	None	++++	*	++
R.B.	Normal	None	++++	*	++
S.R.	Normal	None	++++	*	++

¹See classification of reactivity in text.

²During the six months preceding the investigation.

³The sign in parenthesis in this column represents the status of the tuberculin test with intermediate P.P.D. strength.

*These two patients changed to a different phase of their disease during the investigation.

*All lymphocyte suspensions from normal donors studied in this laboratory by this technique gave a ++ to ++++ response if P.P.D. positive and 0 if P.P.D. negative (Stefani, 1966).

Some patients both in remission and in the active phase of the disease were receiving Azulfidine treatment at the time of the investigation. No correlation was observed between this treatment and the degree of *E. coli*-induced blastoid transformation.

Two patients, V. K. and K.G., were examined twice at three-and five-month intervals respectively. During the first investigation, V.K. was classified as having active ulcerative colitis and no morphological response to *E. coli* extracts was detected. At the time of the second study, he was in remission and blastoid transformation (++) was observed. On the other hand, K.G. was first classified as being in remission and full blastoid transformation (+++) was observed. No reaction was detected when this patient was examined five months later during an exacerbation of the disease.

The possibility that a serum factor might interfere with the blastoid transformation in patients with active ulcerative colitis was investigated. Sera from two patients with active ulcerative colitis were added to lymphocyte suspensions taken from two patients in the control group, and no reduction in the extent of blastoid transformation was observed. Similarly, the addition of normal sera to suspensions of lymphocytes from patients with active ulcerative colitis failed to modify the reaction, *i.e.*, no blastoid transformation occurred.

DISCUSSION

The phenomenon of blastoid transformation of lymphocytes *in vitro* in the presence of a specific antigen such as old tuberculin or of phytohaemagglutinin is well known (Pearmain *et al.*, 1963; Elves *et al.*, 1963). Human blood lymphocytes from all of our patients with ulcerative colitis exhibited a normal reaction to old tuberculin and phytohaemagglutinin. The present experiment revealed, however, that peripheral blood lymphocytes from patients with active ulcerative colitis differed from the other lymphocytes tested in their response to *E. coli* extracts, *i.e.*, they did not show the expected blastoid transformation. This phenomenon varied in the same patient according to the stage of his disease and seemed to be independent of Azulfidine therapy. Moreover, this inhibitory effect was not due to a factor present in the serum of ulcerative colitis patients as shown by the experiments with media prepared with ulcerative colitis serum. Hence, the most probable explanation for this finding is that ulcerative colitis lymphocytes have at least one dysfunction demonstrable only during the acute stage of the disease when the cell is challenged with *E. coli* extracts.

On the basis of experimental and clinical observations, it has been suggested that ulcerative colitis is an autoimmune disease in which the lymphocyte plays a central role (Kirsner, 1965). The finding of some cross reactivity between certain *E. coli* extracts and colonic mucosal antigens (Perlmann *et al.*, 1965) suggests that *E. coli* may sensitize the lymphocytes of patients with ulcerative colitis with subsequent damage to the colon by these cells (Kirsner, 1965). Our finding that ulcerative colitis lymphocytes present an impaired reaction to *E. coli* antigens would seem to weaken the hypothesis of a high lymphocyte reactivity to *E. coli* as an essential factor in the pathogenesis of the condition. Two alternative possibilities should be considered: one, that this impaired reaction may indicate a diminished defence, and thereby permit damage of the colonic mucosa by pathogenic strains of *E. coli* such as 0 119: B 14. The second alternative is that the diminished lymphocyte response is caused by lymphocyte 'exhaustion' due to prolonged stimulation from *E. coli* which freely enters the body through the ulcerating mucosa. This impaired reaction would follow the destruction of the mucosa and would be a secondary phenomenon in the course of acute ulcerative colitis. However, it should be emphasized that only one strain of *E. coli* was used in this experiment; it is possible that the ulcerative colitis lymphocytes will respond differently to extracts obtained from other strains.

SUMMARY

Peripheral blood lymphocytes of 20 patients with ulcerative colitis and 10 normal patients were cultured in the presence of old tuberculin, phytohaemagglutinin, and extracts of *E. coli* 0 119: B 14. After six days of incubation, the degree of blastoid transformation and mitosis was determined by phase contrast microscopy. Lymphocytes from all ulcerative colitis patients studied exhibited a normal reaction to phytohaemagglutinin and old tuberculin. However, lymphocytes from patients with active ulcerative colitis differed from lymphocytes of patients with the disease in remission and from normal donors in their reaction to *E. coli* extracts. The observed absent or diminished reaction of lymphocytes from active ulcerative colitis patients was found to be independent of Azulfidine therapy and of serum factors. The degree of reactivity varied according to the change in stage of the disease for two patients in whom a second study was made.

We thank Mr. M. Carino, Mr. C. Kirsh, and Mrs. I. Zielonka for technical assistance. This work was partially supported by research grant AM 097930-3 from the National Institutes of Health, Bethesda, Maryland.

REFERENCES

- Dragstedt, L. R., Dack, G. M., and Kirsner, J. B. (1941). Chronic ulcerative colitis. A summary of evidence implicating bacterium *necrophorum* as an etiologic agent. *Ann. Surg.*, **114**, 653-662.
- Elves, M. W., Roath, S., and Israels, M. C. G. (1963). The response of lymphocytes to antigen challenge in vitro. *Lancet*, **1**, 806-807.
- Hashem, H. (1965). Mitosis: Induction by cultures of human peripheral lymphocytes. *Science*, **150**, 1460-1462.
- Hirschhorn, K., Back, F., Kolodny, R. L., Firschein, I. L., and Hashem, H. (1963). Immune response and mitosis of human peripheral blood lymphocytes in vitro. *Ibid.*, **142**, 1185-1187.
- Kirsner, J. B. (1965). The immunologic response of the colon. *J. Amer. med. Ass.*, **191**, 809-814.
- Pearmain, G., Lycette, R. R., and Fitzgerald, P. H. (1963). Tuberculin-induced mitosis in peripheral blood lymphocytes. *Lancet*, **1**, 637-638.
- Perlmann, P., Hammarström, S., Lagercrantz, R., and Gustafsson, B. E. (1965). Antigen from colon of germ-free rats and antibodies in human ulcerative colitis. *Ann. N.Y. Acad. Sci.*, **124**, 377-394.
- Schrek, R., and Stefani, S. (1964). Radioresistance of phytohaemagglutinin-treated normal and leukemic lymphocytes. *J. nat. Cancer Inst.*, **32**, 507-521.
- Stefani, S. (1966). Old tuberculin-induced radioresistance on human lymphocytes in vitro. *Brit. J. Haemat.*, **12**, 345-350.
- Westphal, O., Luderitz, O., and Bester, F. (1952). Über die Extraction von Bakterien mit Phenol/Wasser. *Z. Naturforsch.*, **70**, 148-155.