

Lysis of colonic epithelial cells by allogeneic mononuclear and lymphokine activated killer cells derived from peripheral blood and intestinal mucosa: evidence against a pathogenic role in inflammatory bowel disease

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SUMMARY A sensitive 4 h ^{51}Cr -release cytotoxicity assay has been developed using as targets colonic epithelial cells obtained by Dispase-collagenase digestion of resected mucosa or colonoscopic biopsies. Peripheral blood mononuclear cells (MNC) from most healthy donors showed low, but significant levels of cytotoxicity for normal epithelial cell target cells of 8.7 (4.4) % (mean (SD)) and similar levels were found in 14 ulcerative colitis (6.5 (4.4) %) and 16 Crohn's disease (6.2 (5.2) %) patients. Neither drug therapy nor disease activity influenced the results. The sensitivity of colonic epithelial cells isolated from inflamed and histologically normal mucosa to lysis by peripheral blood MNC from a single donor was not affected by the underlying disease. Anti-epithelial cell activity did not correlate with anti-K562 activity and the cytotoxic cell was plastic non-adherent and Leu-11b⁻. None of 15 MNC populations isolated from mucosa of normal, tumour bearing, or chronically inflamed intestine exhibited significant lysis of colonic epithelial cells despite killing of K562 target cells in 10. Lymphokine activated killer (LAK) cells, generated by interleukin-2 stimulation *in vitro* of nine intestinal and seven peripheral blood MNC populations, exhibited high levels of lysis of K562 cells but, on every occasion, failed to lyse colonic epithelial cells. These data indicate that spontaneously cytotoxic or LAK cells are unlikely to play a role in the generation of colonic epithelial cell injury by direct cytotoxicity in inflammatory bowel disease.

In ulcerative colitis, colonic epithelial cells exhibit abnormality of cell membrane integrity¹ and increased turnover² both of which occur in remission and relapse and appear to be disease specific.¹⁻³ These findings suggest chronic injury to colonic epithelial cells is occurring in ulcerative colitis but the underlying mediators of such damage are poorly understood. Postulated mechanisms include spontaneous and lymphokine activated cell mediated cytotoxicity. Peripheral blood mononuclear cells (MNC) from ulcerative colitis and Crohn's disease patients, but

seldom from healthy subjects, are reported to lyse allogeneic colonic epithelial cells *in vitro*,^{4,7} the phenotype of the effector cell being consistent with that of a natural killer or killer cell. Moreover, intestinal MNC from mucosa affected by inflammatory bowel disease were found to lyse autologous colonic epithelial cells with markedly increased activity compared with controls.⁸ Interpretation of these studies, however, is difficult because of the uncertain viability of the target cells used and questionable validity of the microcytotoxicity assays used.⁹

Mononuclear cells from intestinal mucosa can be activated by interleukin-2 (IL-2) to exhibit lymphokine activated killer (LAK) cytotoxicity of

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not only NK sensitive and NK resistant cell lines^{10,11} but also fresh colon cancer cells and lamina propria fibroblasts.^{12,13} The ability of such cells to lyse normal colonic epithelial cells has, however, not been examined. Local IL-2 release in the chronically inflamed mucosa of ulcerative colitis or Crohn's disease appears likely and this may lead to the generation *in vitro* of LAK cells. Indeed, evidence of local LAK cell induction has been reported in the intestinal mucosa of immunised mice after oral challenge of large doses of antigen¹⁴ and in synovial fluid of patients with active rheumatoid arthritis.¹⁵ Thus, the potential for LAK mediated injury to colonic epithelial cells is present and requires examination.

In the present study, a sensitive and quantitative cytotoxicity assay has been developed using as targets normal colonic epithelial cells which are known to maintain their structure and metabolic activity over the entire assay period.¹⁶ This has allowed a reexamination of the cytotoxic activity of circulating MNC against allogeneic colonic epithelial cells in inflammatory bowel disease and healthy subjects and an assessment of the ability of MNC and LAK cells derived from intestinal mucosa to lyse fresh colonic epithelial cells. The quantitative nature of this assay system has also allowed comparison of the anti-colonic epithelial cell with that of the classical NK cell. In addition, the effect of the underlying mucosal disease on the sensitivity to lysis of colonic epithelial cells has been examined.

Methods

PATIENTS AND SPECIMENS

Peripheral blood was taken from 18 healthy subjects and from 30 patients with inflammatory bowel disease of whom 14 had ulcerative colitis and 16 Crohn's disease. Their details are outlined in Table 1.

Tissue was obtained from surgically resected colon from patients with colorectal carcinoma, diverticular disease, redundant sigmoid loop, ulcerative colitis, and Crohn's ileocolitis. Colonoscopic biopsies were also studied from patients with normal colons and colons affected by colorectal carcinoma, ulcerative colitis, and Crohn's colitis. Diagnoses were made on standard clinical, radiological, and histopathological criteria. Adjacent mucosa was histopathologically examined to determine normality or, if from inflammatory bowel disease patients, to determine whether inflammation was present or not. This was judged by assessment of MNC and polymorphonuclear leucocyte infiltration and by changes of epithelial and crypt morphology.

Only surgically resected colon was used for the isolation of intestinal MNC and details of these

Table 1 Details of healthy subjects and patients from whom peripheral blood MNC were studied

	Corticosteroid therapy	n	Male/female	Age (mean)	Active disease*
Healthy subjects	No	18	9/9	22-56 (33)	-
Crohn's disease†	Yes	9	0/9	17-31 (25)	8
	No	7	4/3	16-71 (31)	4
Ulcerative colitis‡	Yes	7	5/2	22-43 (31)	7
	No	7	5/2	22-76 (51)	3

*Disease activity assessed on histopathological criteria (see text) and graded as active inflammation either present or absent; †Eight patients with ileitis, six ileocolitis, two colitis; ‡Nine patients with extensive colitis, five with distal colitis.

donors are outlined in Table 2. All patients with inflammatory bowel disease studied had active disease at the time of surgery. Three were receiving immunosuppressive therapy of prednisone >10 mg/day and azathioprine and two patients were receiving prednisone ≤10 mg/day. Three patients were on metronidazole and three sulphasalazine therapy. The protocol for the study was approved by the Ethics Committee of the ACT Health Authority in September, 1986.

ISOLATION OF MONONUCLEAR CELL POPULATIONS

Blood was heparinised immediately after venesection, diluted one in two with Hank's Balanced Salt Solution free of magnesium and calcium (HBSS), and centrifuged over a discontinuous Ficoll-Paque (Pharmacia) gradient as previously described.¹⁷ The interface cells were harvested, washed three times in HBSS, counted in a haemocytometer (Neubauer chamber), and resuspended in supplemented RPMI (RPMI 1640 with 10% fetal calf serum, 2 mmol/l L-glutamine, 100 U/ml penicillin, 50 µg/ml gentamicin). 0.5 to 2.5 million cells were isolated per millilitre of blood and viability (assessed by trypan blue exclusion) was always greater than 98%. For

Table 2 Details of patients and healthy subjects from whom intestinal MNC and/or LAK cells were studied

	n	Male/female	Age range (mean) (years)
Healthy subjects*	7	5/2	26-56 (38)
Carcinoma	8	2/6	36-75 (59)
Miscellaneous†	4	1/3	61-70 (67)
Inflammatory bowel disease‡	6	1/5	23-69 (40)

*Only cells from peripheral blood studied; †Adenoma-1, angiodysplasia-1, redundant sigmoid loop-1, chronic radiation damage-1; ‡Crohn's colitis-3, Crohn's ileo-colitis-2, ulcerative colitis-1.

some studies, a further enrichment for large granular lymphocytes was performed by centrifugation over multilayer discontinuous Percoll (Pharmacia) gradients for 30 min at 500 *g*. Percoll densities used were 1.053, 1.063, and 1.073 g/ml and the 1.063 g/ml interface was harvested, washed three times and resuspended in supplemented RPMI ready for further use.

Intestinal MNC were isolated using a method modified from that of Bookman and Bull.¹⁸ Briefly, intestinal mucosa was dissected from the muscularis of fresh surgically resected intestine and the epithelial cells removed by repeated incubation with EDTA. The remaining lamina propria was digested overnight in supplemented RPMI containing 2 U/ml collagenase (CLSPA, Worthington, New Jersey) and 5 U/ml DNase (Calbiochem Type II Behring Diagnostics, La Jolla, Ca). The resulting cell population was centrifuged over a Ficoll-Paque gradient followed by, in some studies, a multistep Percoll gradient (as for peripheral blood) to obtain the 1.063 interface fraction. The cells were resuspended in supplemented RPMI ready for further use.

ISOLATION OF COLONIC EPITHELIAL CELLS

Tissue was dissected from freshly resected colon and transferred to cold HBSS. In cancer bearing specimens, macroscopically normal mucosa was taken more than 5 cm from the edge of the tumour. Epithelial cells were isolated as previously described.¹⁶ Briefly, the colonic wall was washed with HBSS, the mucosa scraped from the submucosa with a glass slide and then minced using a crossed scalpel technique. The minced mucosa was digested for 1.5 h at 37°C in supplemented RPMI containing 1.2 U/ml

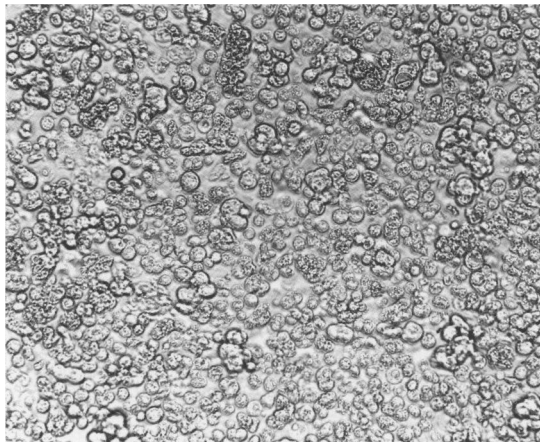


Fig. 1 Phase contrast micrograph of a colonic epithelial cell population following ⁵¹Cr labelling just before cytotoxicity assay.

Dispase I (Boehringer) and 50 units/ml collagenase (Worthington Type IV) followed by trituration through a 21 gauge needle. The resulting cell population was washed, passed through a sieve to remove undigested fragments and mucus, and then the epithelial cells were purified by differential centrifugation twice in 50 ml HBSS at 75 *g* for two to three minutes. The cells were then transferred to supplemented DMEM (DMEM containing 20% Nu serum, 2% luria broth, 4 mmol/l L-glutamine, 25 mmol/l HEPES, 100 U/ml penicillin, 50 µg/ml gentamicin). Epithelial cell purity was always greater than 92% and viability greater than 97% by trypan blue exclusion.

Colonoscopic biopsies were minced with a scalpel blade and digested, triturated, and purified as for surgically resected mucosa. Epithelial cell purity and viability were similar to those from surgical specimens.

GENERATION OF LAK CELLS

Recombinant interleukin-2 (Janssen, Belgium) or tonsillar lymphocyte conditioned medium (TLCM) containing IL-2 activity (kindly produced and supplied by Dr Hilary Warren)¹⁹ were used to generate LAK cells from MNC or Percoll interface cells. Tonsillar lymphocyte conditioned medium was a mitogen free concentrated supernatant of phytohaemagglutinin-stimulated tonsillar lymphocytes and was used in optimal concentrations as previously described.^{11,19} Recombinant IL-2 was used at a concentration of 125 U/ml.¹¹ The cells were cultured in supplemented RPMI in plastic tissue culture flasks at a concentration of 1 to 1.5 million cells/ml at 37°C 5% CO₂ 95% air. Medium was changed at three days. After four to six days' culture, the cells were washed and resuspended in supplemented DMEM ready for assay.

MONOCYTE DEPLETION

Monocytes were depleted from peripheral blood MNC populations by plastic adherence. The MNC were incubated for two hours at 37°C on bacteriological grade plastic Petri dishes in supplemented RPMI medium. The non-adherent population was then harvested by gentle washing of the plate three times. Wright's and non-specific esterase staining showed less than 1% monocytes remaining.

COMPLEMENT MEDIATED LYSIS

Peripheral blood MNC, depleted of monocytes by plastic adherence (as above), were incubated at a concentration of 1 × 10⁷ cells/ml in presence or absence (control cells) of antibody in supplemented RPMI for seven minutes at 37°C. Freshly reconstituted rabbit complement (Cedarline Laboratories)

was then added to a final concentration of 1 in 5 to each cell population and incubated for one hour at 37°C. The cells were then washed twice, counted, and viability (trypan blue exclusion) calculated. The control cells were resuspended in supplemented RPMI at a concentration of 1×10^7 cells/ml and the antibody treated cell populations then resuspended in the same volume.

The cells were then tested for their cytotoxicity of colonic epithelial and K562 cell targets.

Leu-11b (Becton Dickinson), an IgM monoclonal antibody which is a marker for a subpopulation of lymphocytes highly enriched for natural killer cells, was used at a concentration of one in 20. Following complement-lysis of Leu-11b coated cells, 79 (16) % of the control cells were viable by trypan blue exclusion and Leu-11b⁺ cells could not be detected by indirect immunofluorescence.

⁵¹CR-LABELLING OF TARGET CELLS

An aliquot of one to two million freshly isolated epithelial cells from surgically resected colon or all of the cells from colonoscopic biopsies were labelled in 500 μ l of supplemented DMEM containing 100 μ Ci of sodium ⁵¹chromate for one hour. They were washed three times and resuspended in supplemented DMEM, and counted. The cells were then diluted to a concentration of 5×10^4 cells/ml. Resuspension of cells was always done by swirling and repeated tipping of the test tube and not by vigorous pipetting in order to minimise cell damage. A typical colonic epithelial cell population after ⁵¹Cr labelling comprising single cells and partial crypts is shown in Figure 1.

The erythroleukaemic cell line, K562, which is the standard target cell of classical natural killer activity, was also used as target for some studies and was labelled with ⁵¹Cr as previously described.²⁰ The cells were resuspended in supplemented RPMI at a concentration of 5×10^4 cells/ml.

CYTOTOXICITY ASSAY

Assays were undertaken in U-bottomed 96 well microculture plates. 100 μ l of labelled target cells were added to varying numbers of effector cells according to the effector:target (E:T) ratio to be used. These were chosen on the basis of findings of previous studies.^{11 17 20} All MNC populations (stimulated or unstimulated) were tested against K562 at 50:1 and colonic epithelial cells at 200:1 whereas 1-063 Percoll interface cells were used at 25:1 and 100:1 respectively. Other E:T ratios were variably tested (see Results). Each well was made up to a total volume of 200 μ l. Spontaneous release was determined by incubating target cells in culture medium alone and maximal release by adding 100 μ l

5% Triton-X-100 to the target cells. The plate was incubated at 37°C 5% CO₂ 95% air for four hours after which 100 μ l cell free supernatant was aspirated from each well and counted in a gamma counter. The average of six to eight wells for experimental (E) and eight to twelve wells for spontaneous (S) and maximal (M) release were calculated. Cytotoxic activity was expressed as percent specific ⁵¹Cr release and was calculated by the following formula:

$$\% \text{ lysis} = (E - S) / (M - S) \times 100$$

The spontaneous release expressed as a percentage of maximal release was less than 12% for K562 cells. Spontaneous release during 4 h incubation from colonic epithelial cells from histologically normal mucosa was always less than 8% but was 13 (4) % from ulcerative colitis and 8 (3) % from Crohn's colitis. A specific release value was considered to represent significant cytotoxicity when it exceeded the mean plus three SD of the spontaneous release levels. These values were 3% and 4.5% for K562 and colonic epithelial cells respectively. The SD of variation between replicate experimental wells was <10%. Likewise, preliminary experiments showed <10% variability of lysis by a single MNC population when tested against different colonic epithelial cell populations prepared from the same colon.

STATISTICAL ANALYSIS

Data have been expressed as mean (SD) and, where appropriate, statistically compared using a pairing or unpaired *t* test.

Results

ACTIVITY OF PERIPHERAL BLOOD MNC AGAINST EPITHELIAL CELL TARGETS FROM HISTOLOGICALLY NORMAL MUCOSA

Cytotoxicity exerted by peripheral blood MNC against colonic epithelial cells increased with increasing E:T ratios and, in 20 consecutive studies, was 4 (3) % at 50:1, 6 (4) % at 100:1, and 11 (4) % at 200:1. All subsequent results for anti-epithelial cell activity of MNC populations have been expressed for an E:T ratio of 200:1 unless otherwise stated.

Mononuclear cells from most healthy subjects significantly lysed colonic epithelial cells (Fig. 2). Mononuclear cells from ulcerative colitis or Crohn's disease patients showed similar cytotoxicity to those from healthy subjects. Corticosteroid therapy (Fig. 2) or disease activity (data not shown) appeared unrelated to the level of cytotoxicity. Mononuclear cells from one Crohn's disease and two ulcerative colitis patients were tested against autologous and allogeneic colonic epithelial cells and similar cytotoxicity was seen for each target (data not shown).

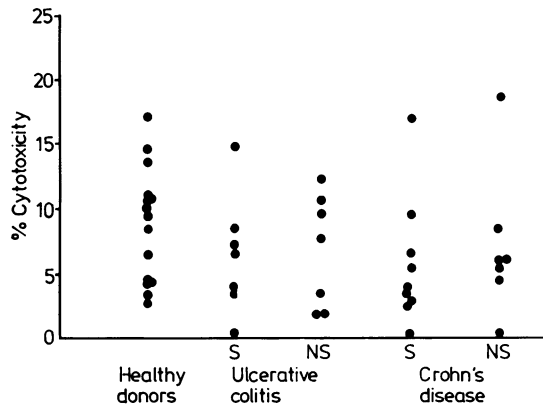


Fig. 2 Cytotoxicity of colonic epithelial cells by peripheral blood MNC from healthy donors or from patients with ulcerative colitis or Crohn's disease receiving (S) or not receiving (NS) steroids.

The results shown in Figure 2 represent the interaction of populations of effector and target cells that come from differing donors. When the effector cell population obtained from a single donor was assayed with epithelial cell targets from different sources, considerable variation in sensitivity of epithelial cells to lysis was seen and similar variability of activity was seen when MNC populations from multiple donors against the same epithelial target cell population were tested (data not shown). In an attempt to determine whether a variability in sensitivity to lysis of epithelial cells may have masked disease related differences of cytotoxic activity, one effector donor (donor 1) was tested on every occasion and the activity of that donor's cells assumed to be relatively constant as previously suggested.²¹ A 'relative cytotoxicity index' was calculated as the ratio of experimental cytotoxicity to that of donor 1 against the same target cell. As shown in Figure 3, donor 1 (represented by a relative cytotoxicity index of one) lies in an approximately average position amongst healthy donors. Patients with ulcerative colitis exhibited an index that did not differ from normal and that was independent of corticosteroid therapy. For patients with Crohn's disease, the index was significantly less than that of healthy subjects whether comparing with that of all patients ($p < 0.02$) or only of those not on corticosteroids ($p < 0.05$). The difference between healthy subjects and patients with Crohn's disease receiving corticosteroids was not significant.

RELATIONSHIP OF THE ANTI-COLONIC EPITHELIAL TO THE ANTI-K562 CELL
Depletion of plastic adherent cells from peripheral blood MNC populations was associated with a slight

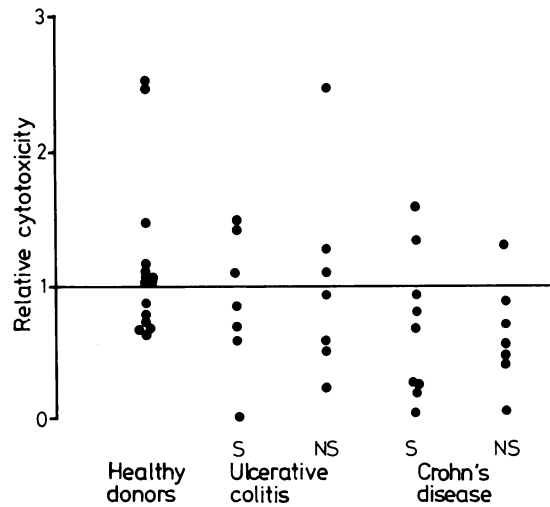


Fig. 3 Cytotoxicity of colonic epithelial cells by peripheral blood MNC from healthy donors and patients with ulcerative colitis or Crohn's disease when compared with cytotoxicity exerted by peripheral blood MNC from Donor 1 tested against the same epithelial cell target at the same time. The relative cytotoxicity index is the ratio of experimental cytotoxicity to that of peripheral blood MNC of donor 1. Patient donors were (S) or were not (NS) receiving steroids.

increase in cytotoxicity against epithelial (14 (8) % to 18 (8) %, $p < 0.001$) and K562 cell targets (49 (24) % to 54 (24) %, $p < 0.001$) in five studies suggesting that lymphocytes rather than monocytes are the effector cells. There was no correlation between the level of anti-K562 and anti-epithelial cell activity of peripheral blood MNC populations when simul-

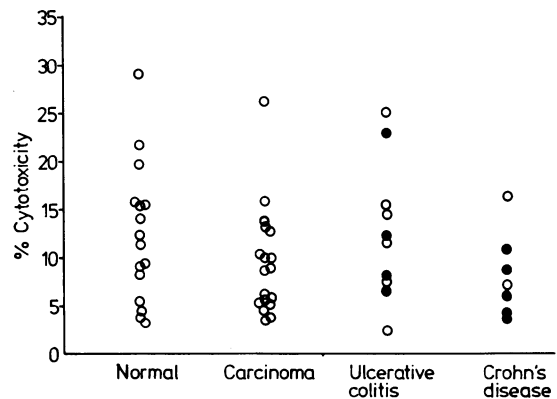


Fig. 4 Cytotoxicity exerted by peripheral blood MNC from a single donor of colonic epithelial cells isolated from normal or cancer-bearing colons or colons affected by ulcerative colitis or Crohn's disease. Shaded symbols represent cells isolated from colons demonstrating active inflammation on histopathological criteria.

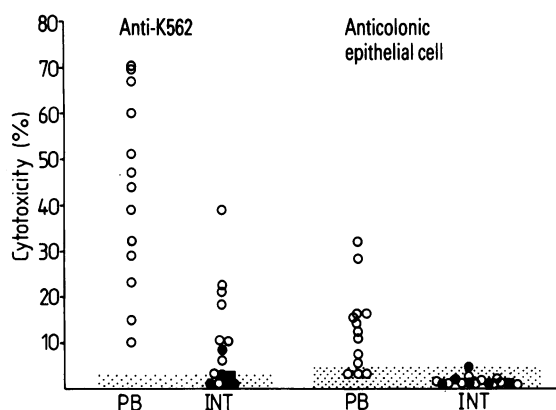


Fig. 5 Cytotoxicity exhibited by peripheral blood (PB) or intestinal (INT) MNC of K562 or fresh colonic epithelial target cells. The peripheral blood was obtained from healthy subjects while the intestinal MNC were isolated from colon involved with Crohn's disease (●), ulcerative colitis (■), or from colon resected because of cancer or miscellaneous diseases (○). Significant lysis of each target cell (see text) is that above the shaded areas.

taneously assayed (data not shown). Complement lysis studies confirmed the majority of anti-K562 activity was confined to the Leu-11b⁺ subpopulation as depletion of Leu-11b⁺ cells reduced activity from 49 (26) % to 12 (11) % in five studies ($p < 0.001$). In contrast, depletion of Leu-11b⁺ cells resulted in no significant change of anti-colonic epithelial cell activity in five experiments (11 (4) % to 12 (5) %, $0.1 < p < 0.05$).

SENSITIVITY OF EPITHELIAL CELLS TO LYSIS BY MNC

Mononuclear cells from a single peripheral blood donor were tested against ⁵¹Cr-labelled epithelial cells from histologically normal mucosa from patients with no colonic pathology or colorectal carcinoma and from mucosa affected by ulcerative or Crohn's colitis. As shown in Figure 3, a wide range of cytotoxicity was seen for all groups but there were no disease related differences.

CYTOTOXIC ACTIVITY OF UNSTIMULATED INTESTINAL MNC (Fig. 4)

Mononuclear cells from normal, tumour bearing or inflamed intestine did not exhibit significant lysis of epithelial cells despite 10 of 15 populations significantly lysing K562 cells. Culture of MNC for 24 or 48 h before assay or the use of high E:T ratios (up to 500:1) had no effect. Control experiments using peripheral blood MNC from healthy subjects demonstrated that the lack of intestinal MNC mediated cytotoxicity was not due to resistance of the target cell to lysis.

CYTOTOXIC ACTIVITY OF LAK CELLS (Fig. 5)

Intestinal or peripheral blood LAK populations whether stimulated with TLMC or recombinant IL-2 (data not shown) or whether prepared from MNC or enriched Percoll interface cells did not exhibit significant lysis of colonic epithelial cells despite high levels of activity against K562 cells (Fig. 6). As for unstimulated MNC, simultaneous control studies using peripheral blood MNC confirmed that the epithelial targets were usually sensitive to MNC mediated lysis.

Discussion

An assay has been developed which can reliably and quantitatively assess the ability of effector cells to lyse normal colonic epithelial cells *in vitro*. Critical to this assay is the use of freshly isolated target cells which are structurally unaltered from that *in vivo* and which maintain viability over the entire assay period. Colonic epithelial cells isolated by the collagenase-Dispase technique do, on multiple criteria, maintain their structural and functional integrity for periods much longer than the four hours used in the ⁵¹Cr release cytotoxicity assay.¹⁶ As many of these cells are in the form of partial crypts rather than single cells, it is possible that some cells may be 'hidden' from effector cells. The colonic epithelial cells exist as single layered sheets, however, rather than clumps, and there should be access by cytotoxic effectors to the cell membrane of all target cells.

The presence of circulating MNC with anticolic epithelial cell activity is a normal phenomenon and no increase in activity was found in patients with ulcerative colitis or Crohn's disease irrespective of their drug therapy. In contrast, antiepithelial cell activity was not detected in any MNC population derived from the intestinal mucosa of patients with malignant, inflammatory and other intestinal diseases. Attempts to correct for possible disaggregation related suppression of activity (by culture before assay)^{22,23} or for possible low proportions of effector cells (by high E:T ratios)¹⁷ were not successful in unmasking activity. In addition, possible inadvertent selection of epithelial target populations that are relatively resistant to MNC mediated lysis was excluded by showing their sensitivity to the action of fresh peripheral blood MNC. For MNC derived from mucosa affected by inflammatory bowel disease, anti-K562 activity was also low or undetectable and it is possible that drug treatment may have played a role. Only three of six inflammatory bowel disease patients studied, however, were receiving moderate to marked immunosuppression.

These results show a marked disparity with those from earlier studies⁴⁻⁸ and reasons for this are not clear. Differences in the nature of the target cell and

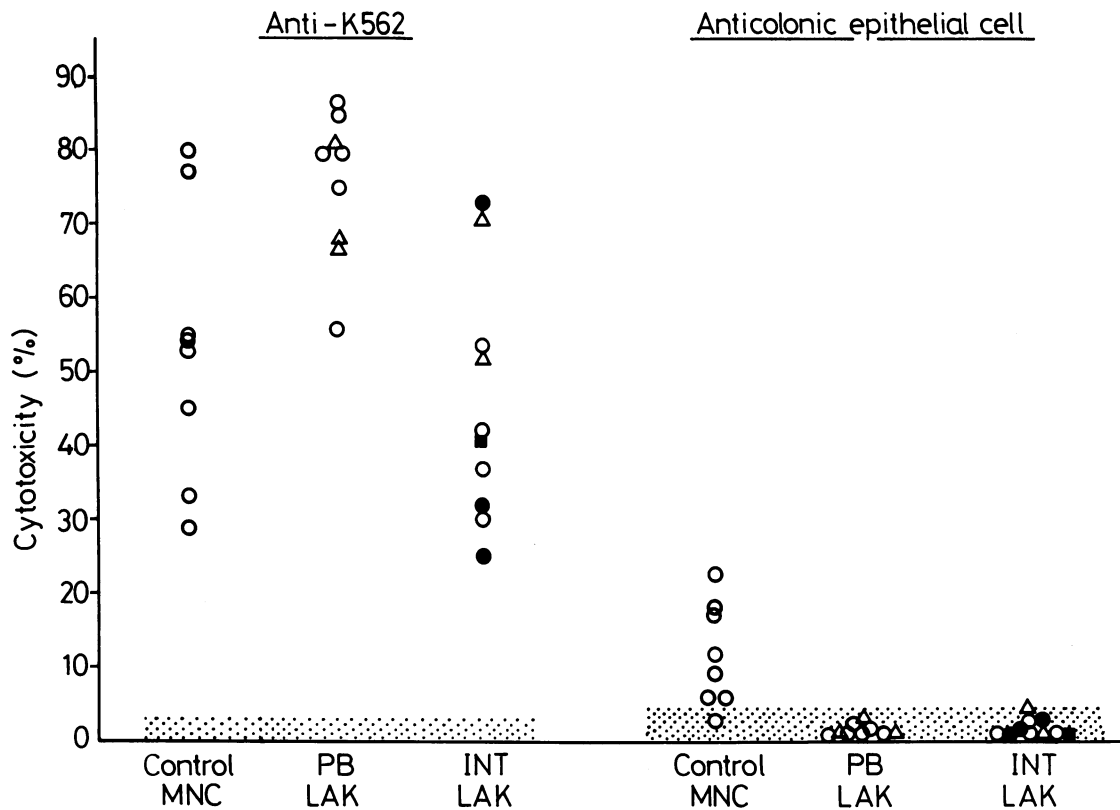


Fig. 6 Cytotoxicity of K562 or fresh colonic epithelial target cells exhibited by fresh peripheral blood (CONTROL) MNC, peripheral blood (PB) LAK cells, or intestinal (INT) LAK cells. Peripheral blood cells were obtained from healthy subjects while intestinal cells were derived from cancer bearing or miscellaneous colons (unshaded) or from colons affected by Crohn's disease (●) or ulcerative colitis (■). Cell populations stimulated with IL-2 were mostly unseparated MNC but for some studies, enriched 1.063 g/ml Percoll fraction cells (△) were used. Significant lysis of each target cell (see text) is that above the shaded areas.

the assay used, however, are likely candidates. Trypsin^{4,6} would be more likely to alter cell membrane structure and receptor expression, the mechanical/collagenase method^{7,8} may favour the isolation of the more mature component of the colonic epithelium, while the collagenase-Dispase method¹⁶ favours the isolation of crypt cells. The major target cell difference, however, lies in cell viability exemplified by the most recent report⁸ in which the investigators were unable to successfully label the colonic epithelial cells with ⁵¹Cr. Such difficulties reflect dead or dying cells rather than a feature peculiar to a particular cell type. Perhaps of even more importance is the validity of the trypan blue microcytotoxicity assay. Until the ability to accurately count all epithelial cells in a mixed epithelial cell-MNC population can be validated, the results of the studies utilising this method should be interpreted with extreme caution.

An additional difference in the study of intestinal MNC is that the present report utilises an allogeneic system in contrast with the autologous system of Shorter *et al.*⁸ Non-MHC restricted cytotoxicity should be detected as well in allogeneic as in autologous systems. Indeed, circulating MNC showed similar activity whether tested against autologous or allogeneic epithelial cells. MHC restricted cytotoxic cells may have contributed to the activity seen in the earlier study⁸ although the results of other reported experiments rendered this less likely.

The identity of the circulating effector cell which lyses colonic epithelial cells has not been elucidated. It is probably a lymphocyte, however, and does not express the Fc receptor for IgG as detected by anti-Leu-11b. This contrasts with the classical natural killer (anti-K562) cell population which is largely Leu-11b⁺. Moreover, there is no correlation between cytotoxicity exerted by any MNC population against

K562 cells compared with that against colonic epithelial cells. Thus, different or at least minimally overlapping cell populations lyse these different cell targets. Such findings suggest that the many reports of the classic NK cell in intestinal mucosa^{17, 20, 24-28} have little relevance to their possible role in damaging colonic epithelial cells in health and disease.

As colonic epithelial cells from patients with ulcerative colitis show abnormal patterns of differentiation and proliferation,^{1, 29-32} their sensitivity to lysis by cytotoxic cells may be likewise altered. The heightened cytotoxicity by intestinal MNC of autologous epithelial cells reported in patients with inflammatory bowel disease⁸ may reflect alterations in the susceptibility of epithelial cells to lysis. This possibility was assessed by testing a single donor's peripheral blood MNC against epithelial cell targets from various disease states. No disease related differences were found indicating that a change in sensitivity to lysis does not follow the alteration in differentiation exhibited by these cells in ulcerative colitis.

The target specificity of LAK cells is an important issue in view of the increasing interest in LAK cells as antitumour therapy^{33, 34} in addition to the possible role of LAK cells generated *in vivo* in injuring colonic epithelial cells in inflammatory bowel disease. Damage to normal cells *in vivo* after intravenous infusion of sufficient numbers of LAK cells to cause tumour regression has not been reported.^{33, 35-37} Fresh lymphocytes are resistant to LAK activity *in vitro*³⁸ though normal fibroblasts from skin³⁸ or intestinal lamina propria¹³ in primary culture are sensitive. The present study, for the first time, has examined the sensitivity of fresh, normal epithelial cells to LAK activity *in vitro* and has demonstrated that allogeneic cells are resistant to lysis by LAK cells derived from peripheral blood or intestinal mucosa. As the LAK phenomenon is non-MHC restricted and as allogeneic and autologous colon cancer cells exhibit similar sensitivity to intestinal LAK cells,¹² it seems likely that colonic epithelial cells would also be resistant to lysis by autologous LAK cells. Thus, LAK cells whether generated *in vivo* in inflammatory bowel disease or whether delivered to the mucosal compartment after exogenous administration are unlikely to injure normal colonic epithelial cells.

In conclusion, the findings of this study clarify the role of spontaneous and lymphokine activated cell mediated cytotoxicity in the pathogenesis of inflammatory bowel disease. The unstimulated effector cells do not exhibit increased activity in peripheral blood and no evidence of their presence in the mucosal compartment can be found. Further studies of cells isolated from mucosa affected by ulcerative colitis are necessary, however, to confirm such data.

The target cells from patients with inflammatory bowel disease have similar sensitivity to lysis by MNC and, thus, abnormal injury produced by an apparently normal effector system is unlikely. In addition, colonic epithelial cells appear resistant to lysis by LAK cells generated from circulating or mucosal compartments. Thus, non-MHC restricted cytotoxic cells whether spontaneously active or lymphokine activated are unlikely to play a pathogenic role in producing colonic epithelial cell injury via direct cytotoxicity in patients with inflammatory bowel disease.

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