Human colonic intraepithelial and lamina propria lymphocytes: cytotoxicity *in vitro* and the potential effects of the isolation method on their functional properties

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SUMMARY Colonic mucosal lymphoid cells, selectively enriched for intraepithelial (IEL) or lamina propria lymphocytes (LPL), were isolated by sequential EDTA-collagenase treatment of resected human colons. Cytotoxic activities of colonic and peripheral blood lymphoid cells (PBL) were tested in three different assays, using chicken erythrocytes (CRBC) and Chang cells as targets. Antibody-dependent cell-mediated cytotoxicity (ADCC) and PHA-induced cytotoxicity (MICC) for both targets were shown by all the isolates of PBL, as was spontaneous cell-mediated cytotoxicity (SCMC) for Chang cells. However, no SCMC or ADCC for Chang cells was found with LPL, and IEL showed minimal or no activity in either assay. PBL, LPL, and IEL demonstrated MICC for Chang cells but, contrasting with PBL and LPL, IEL showed no MICC for CRBC. No significant differences were found between the cytotoxic capabilities of colonic lymphoid cells from patients with inflammatory bowel disease and those from patients with other colonic diseases. Importantly, control studies with PBL showed that SCMC for Chang cells and ADCC for CRBC and Chang cells were reduced by the collagenase treatment used in the isolation of LPL. Also, SCMC for Chang cells was reduced by the treatment of PBL with EDTA. In contrast, neither EDTA nor collagenase reduced MICC for CRBC or Chang cells. Both forms of treatment induced variable degrees of cell losses in the PBL. By analogy, it can be implied that the isolation of intestinal mononuclear cells using EDTA and collagenase may influence some of their cytotoxic activities *in vitro*. This raises an important caveat in the interpretation of such studies.

Immunological mechanisms have been implicated in the pathogenesis of a number of gastrointestinal diseases, including gluten-sensitive enteropathy and non-specific inflammatory bowel disease (IBD).^{1,2} In this regard, we developed a hypothesis for the pathogenesis of IBD which included a role for cell-mediated immune responses in the bowel wall.³ However, this proposal was based on observations using mononuclear cells from peripheral blood and a

question can be raised as to whether such cells reflect the functions of intestinal lymphoid tissues.^{4,5} Accordingly, firstly we isolated lamina propria lymphoid cells from the human colon and concluded that these were capable of mediating two different antibody-dependent cell-mediated cytotoxicity assays *in vitro*.⁶

Arnaud-Battandier *et al.*⁷ reported that intraepithelial lymphocytes from the small bowel of guinea-pigs showed cytotoxic properties different from those of lamina propria lymphocytes. As no similar studies of the human have been described, we recently developed an EDTA-collagenase method for the selective isolation of enriched populations of colonic intraepithelial and lamina propria lymphocytes from patients with various colonic diseases.⁸ The study was then extended to involve three assays of cytotoxicity for two different target cells. These

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were necessary to screen for the activity of various subsets of effector cells in the heterogeneous populations of colonic lymphoid cells. Our purpose was to seek evidence to support a role for local cellular immune responses in the pathogenesis of IBD.

Methods

STUDY GROUPS

Lymphoid cells were isolated from samples of surgically resected colons and from the peripheral blood of patients with IBD or other diseases of the large bowel. The IBD group comprised 10 patients (six women; four men), aged 16–53 years, with chronic ulcerative colitis (CUC), and 10 patients (nine women; one man), aged 16–67 years, with Crohn's disease. Eight patients (chronic ulcerative colitis, four; Crohn's disease, four) were receiving daily systemic steroids at the time of study (20 mg prednisone or less). Of 35 patients (16 women; 19 men), aged 44–85 years, with other colorectal diseases there were 21 with colorectal carcinoma, six with single, large colonic polyps, five with chronic diverticulitis, two with carcinoids, and one with chronic rectal prolapse. The colonic samples from the patients with IBD were taken from sites showing no ulceration and, from the patients with colonic tumours, from areas which were grossly and histologically normal. Isolates of lymphocytes were prepared from peripheral blood samples from a total of 32 healthy volunteers (12 women; 20 men), aged 21–54 years. The project was approved by the institutional Human Studies Committee and informed consent for blood sampling was obtained.

EFFECTOR CELLS

The methods for the isolation of colonic lymphoid cells, enriched for intraepithelial lymphocytes (IEL) or lamina propria lymphocytes (LPL), and for the isolation of lymphoid cells from the peripheral blood (PBL) have been detailed recently.⁸

Isolation of IEL

Colonic mucosa, 20–40 cm² in area, was dissected from the underlying muscularis, cut into small strips, and incubated for three minutes in calcium and magnesium-free Hank's balanced salt solution (CMF-HBSS) containing 1 mM dithiothreitol (DTT; Grand Island Biological Co., Grand Island, NY). The mucosal pieces then were incubated with stirring at 37°C in air for 90 minutes in 100 ml CMF-HBSS containing 0.7 mM EDTA (BDH Chemicals Ltd., Poole, England), pH 7.4, to strip off epithelial cells together with the intraepithelial lymphocytes. The resulting supernatant was collected, passed through nylon mesh (100 µ), and spun in centrifuge tubes. The

cell pellets then were washed twice in CMF-HBSS, pooled, and resuspended in TC 199 (Grand Island Biological Co., Grand Island, NY) containing 10% v/v fetal calf serum (FCS; Grand Island Biological Co., Grand Island, NY) and antibiotics (penicillin 100 units/ml; streptomycin 100 µg/ml; gentamicin 40 µg/ml; amphotericin B 50 µg/ml). The cell suspension then was incubated with iron filings (Lymphocyte Separator Reagent, Technicon Instruments Corp., Tarrytown, NY) for 45 minutes at 37°C, followed by removal of the iron-containing macrophages using bar magnets.⁸ The cells then were washed twice, counted, and resuspended in various concentrations in TC 199 containing 10% v/v FCS and antibiotics (TC-FC-A), as described above. This technique gave mononuclear cell yields of $2.4\text{--}7.5 \times 10^6$ (mean $\pm$ SEM = $5.6 \pm 0.5 \times 10^6$) per 20 cm² mucosa, with 77–94% viability (mean = $88 \pm 9\%$) as estimated by trypan blue exclusion (0.1%). For differential counting, smears of the isolates were prepared by cytocentrifugation and stained with Wright's stain and for non-specific esterase.⁹ The final IEL-enriched isolates contained $94 \pm 2.7\%$ lymphocytes, $4 \pm 2.7\%$ eosinophils, and less than 1% of neutrophils, mast cells, and macrophages.

Isolation of LPL

The colonic mucosal strips, used initially to obtain IEL, were further incubated at 37°C in room air for 90 minutes in 100 ml of fresh CMF-HBSS containing 0.7 mM EDTA, pH 7.4. This step was repeated three to four times, each for a 90 minute period. The pieces of mucosa then were washed in CMF-HBSS and incubated with stirring for 12 hours at 37°C in room air in 100 ml TC-FC-A and 3000 units of purified collagenase, type VI (Sigma Chemical Co., St. Louis, MO), with the pH adjusted to 7.4. After this, the supernatant was passed through nylon mesh (100 µ) and spun in centrifuge tubes. The resulting cell pellets were washed twice, pooled, and resuspended in TC-FC-A. The cell suspension was then depleted of macrophages using the method described above. This technique gave mononuclear cell yields of $2\text{--}10 \times 10^7$ (mean = $3.8 \pm 1.6 \times 10^7$) per 20 cm² mucosa with 88–96% viability (mean = $92 \pm 1\%$) as estimated using light microscopy and trypan blue exclusion (0.1%). Differential counts of smears prepared by cytocentrifugation and stained with Wright's stain or for non-specific esterase⁹ showed that the final isolates of LPL contained $91 \pm 0.5\%$ lymphocytes, $7 \pm 2.1\%$ plasma cells, and less than 1% macrophages, neutrophils, eosinophils, basophils, and mast cells.

The duration of the two-staged procedure for the isolation of colonic lymphoid cells was approximately 24 hours.

Isolation of PBL

PBL were isolated from heparinised venous blood samples using a Ficoll-Hypaque gradient (Lymphoprep, Nyegaard & Co., Oslo, Norway), as described by Bøyum.¹⁰ Macrophage depletion was achieved and evaluated as described above. The yields of lymphoid cells were $4\text{--}10 \times 10^6/10$ ml of blood and the viabilities exceeded 95% (trypan blue 0.1%). Differential counts of cytocentrifuge smears stained with Wright's stain or for non-specific esterase⁹ consistently showed more than 99% lymphocytes and less than 1% macrophages in the final isolates.

To evaluate the potential effects of the chemical and enzymatic treatments on the gut-derived lymphoid cells, aliquots of macrophage-depleted PBL from 10 healthy subjects were incubated *in vitro* with EDTA for 90 minutes or with collagenase for 12 hours, as described above for the isolation of IEL and LPL. After washing, each aliquot of PBL was counted and resuspended in various concentrations in TC-FC-A. The viabilities were estimated using trypan blue exclusion (0.1%).

CHARACTERISATION OF T AND B LYMPHOCYTES

The macrophage-depleted isolates of PBL, LPL, and IEL were characterised for T and B cell markers as described previously.⁸ Immunofluorescent staining for membrane immunoglobulin was performed after the cell suspensions were incubated for 45 minutes and subsequently washed in TC 199 at 37°C to remove cytophilic antibody.¹¹

CYTOTOXICITY ASSAYS

Macrophage-depleted PBL, either freshly isolated or treated with EDTA or collagenase, and the macrophage-depleted colonic isolates enriched for IEL or LPL were tested in different cytotoxicity assays using two different target cells—namely, chicken erythrocytes (CRBC) and Chang cells.

Cytotoxicity assays with CRBC

(1) Antibody-dependent cell-mediated cytotoxicity (ADCC)

The method used essentially was that described by Perlmann and Perlmann.¹² Briefly, 0.25 ml of each of the following was added to tissue culture tubes (no. 2058; Falcon, Oxnard, CA): (1) effector cells (5×10^6 viable cells/ml), (2) CRBC, labelled with Na_2CrO_4 (Sp. Act. 50–400 mCi/mg Cr; Amersham Corp., Arlington Heights, IL) by a method described previously,⁶ in a concentration of 2×10^6 cells/ml, (3) heat-inactivated rabbit anti-CRBC serum (Cappel Laboratories, Cochranville, PA) diluted 10^{-6} , an optimal dilution obtained from preliminary experiments with PBL from healthy volunteers. The viable

effector to target ratio used was 25 to 1, a ratio which had been shown by preliminary experiments with PBL to be appropriate for screening for ADCC in this assay; this ratio also permitted conservation of cells for the other testing. Controls were: (1) tubes containing ^{51}Cr -labelled CRBC and non-labelled CRBC, (2) ^{51}Cr -labelled CRBC with CRBC-anti-serum and non-labelled CRBC, (3) ^{51}Cr -labelled CRBC with normal rabbit serum and non-labelled CRBC, (4) tubes containing ^{51}Cr -CRBC and effector cells with heat-inactivated normal rabbit serum (titre 10^{-6}).

All testing was performed in duplicate. After 18 hours' incubation at 37°C in a humidified atmosphere containing 5% CO_2 , the tubes were centrifuged, 0.5 ml of the supernatant was removed from each tube, and the radioactivities in the supernatant (0.5 ml) and in the pellet (0.25 ml) were counted in a well-type gamma scintillation counter. The cytotoxicity was derived from the percentage of ^{51}Cr released and was calculated by the following formula: % cytotoxicity = $(a \times 1.5/a + b) \times 100$, where a = cpm in supernatant and b = cpm in pellet. Corrections were made for the spontaneous release of ^{51}Cr by the CRBC, and cytotoxicity was expressed as the mean of duplicate tests.

(2) Mitogen-induced cell-mediated cytotoxicity (MICC)

0.25 ml of each of the following was added to tissue culture tubes: (1) effector cells (5×10^6 viable cells/ml), (2) ^{51}Cr -labelled CRBC (2×10^6 /ml), (3) purified PHA (Wellcome Reagents Ltd., Beckenham, England) in a final concentration of $1 \mu\text{g}/\text{ml}$, which, using tritium-labelled thymidine incorporation as the measure of response, was shown in six preliminary experiments to induce blastoid transformation (approximately 6,000–10,000 cpm after four days *in vitro*) in PBL from healthy volunteers and in colonic lymphoid isolates from IBD or cancer patients. The effector to target ratio was kept constant at 25 to 1, and was appropriate for screening for MICC.¹³ Controls were: (1) tubes containing ^{51}Cr -labelled CRBC, PHA, and non-labelled CRBC, (2) ^{51}Cr -labelled CRBC and non-labelled CRBC, (3) tubes containing ^{51}Cr -CRBC and effector cells, but no PHA. All testing was done in duplicate. The methods of incubation, final processing, calculation and expression of cytotoxicity were those described above for ADCC.

Cytotoxicity assays using Chang cells as targets

(1) ADCC

The technique was modified from that described by MacLennan *et al.*¹⁴ Briefly, Chang cells (American

Type Culture Collection, Rockville, MD)* were harvested after six minutes' incubation of monolayers in a solution containing 0.25% trypsin (Nutritional Biochemical Corp., Cleveland, OH) and 0.1% EDTA in CMF-HBSS (pH 7.4). The resulting suspension was washed twice and then kept at 37°C for two hours in TC 199 containing 10% v/v FCS. Then the Chang cells were labelled with $\text{Na}_2^{51}\text{CrO}_4$ for one hour at 37°C, washed three times, and resuspended in a concentration of 1×10^4 cells/ml TC-FC-A. 0.1 ml of each of the following was added to the wells of flat bottom type microtitre plates (Titertek; Flow Laboratories Inc., Hamden, CT): (1) ^{51}Cr -labelled Chang cells (1×10^4 /ml), (2) effector cells in different concentrations appropriate to achieve final viable effector to target ratios of 1:1, 5:1, 10:1, 25:1, 50:1, 100:1, and (3) heat-inactivated rabbit anti-Chang serum* in a dilution of 10^{-4} , an optimal dilution which had been determined by preliminary experiments with PBL from healthy human volunteers. Controls included: (1) ^{51}Cr -labelled Chang cells incubated with antiserum, but without effector cells, (2) ^{51}Cr -Chang cells, effector cells, and heat-inactivated normal rabbit serum (titre 10^{-4}), (3) ^{51}Cr -Chang cells in TC-FC-A for evaluation of spontaneous release of ^{51}Cr .

All testing was performed in triplicate. After 18 hours' incubation at 37°C in a humidified atmosphere containing 5% CO_2 , the microtitre plates were centrifuged (800 g) and 0.1 ml aliquots of the supernatants were placed in separate counting tubes. Cytotoxicity was calculated using the following formula:

$$\% \text{ cytotoxicity} = \frac{\text{experimental cpm} - \text{spontaneous release cpm}}{\text{total cpm} - \text{spontaneous release cpm}} \times 100,$$

where total cpm = mean cpm obtained from counts of six 0.1 ml aliquots of the radiolabelled target cell suspension. The cytotoxicity was expressed as the mean of triplicate tests.

(2) MICC

0.1 ml of each of the following was added to wells of microtitre plates: (1) ^{51}Cr -labelled Chang cells (1×10^4 /ml), (2) effector cells (2.5×10^5 /ml), (3) PHA in a concentration of 1 $\mu\text{g}/\text{ml}$. The viable effector to target ratio was 25 to 1, which, in preliminary experiments with PBL, was found to be adequate for screening for such activity, in agreement with others.¹³ Controls included: (1) ^{51}Cr -Chang cells with PHA, but without effector cells, (2) ^{51}Cr -Chang cells with effector cells, but without PHA, (3) ^{51}Cr -Chang

cells in TC-FC-A for evaluation of spontaneous release of ^{51}Cr . All testing was performed in triplicate. The methods of incubation, final processing, and calculation of cytotoxicity were those described above for ADCC for Chang cells.

(3) *Spontaneous cell-mediated cytotoxicity (SCMC)*
0.1 ml of ^{51}Cr -Chang cells (1×10^4 /ml) was incubated in microtitre wells with 0.1 ml of effector cells in different concentrations appropriate to achieve final viable effector to target ratios of 1:1, 5:1, 10:1, 25:1, 50:1, and 100:1. For spontaneous release of ^{51}Cr , labelled Chang cells were incubated alone in TC-FC-A. All testing was done in triplicate. The methods of incubation, processing, calculation, and expression of cytotoxicity were those described for ADCC for Chang cells.

STATISTICAL ANALYSIS

The results of all experiments were expressed as the mean $\pm$ one standard error of the mean (SEM). Analysis for the significance of difference between means was done using Student's *t* testing. A *P* value of 0.02 or less was demanded for significance. As no apparent differences were found between those with CUC or CD, the IBD results were considered together.

Results

MEMBRANE CHARACTERISTICS OF LYMPHOID CELL POPULATIONS

The numbers of T cells, B cells, and cells lacking these membrane characteristics (null cells) are shown in Table 1. In comparison with the peripheral blood, IEL and LPL populations each contained less T cells and more B cells and these differences were significant ($P < 0.001$). Also, differences were found when the two colonic lymphoid cell fractions were compared: the percentage of T cells in the IEL was

Table 1 *Membrane characteristics of macrophage-depleted colonic mucosal and peripheral blood lymphoid cells*

Source of cells	(no.)	Mean $\pm$ SEM %		
		T	B	Null
PBL				
Healthy controls	10	66 $\pm$ 2.7	13 $\pm$ 1.2	21 $\pm$ 2.3
Patients	26*	62 $\pm$ 1.7	14 $\pm$ 1	24 $\pm$ 1.8
IEL	30†	32 $\pm$ 2	34 $\pm$ 2.4	34 $\pm$ 2
LPL	44‡	52 $\pm$ 1.3	22 $\pm$ 1.1	26 $\pm$ 1.1

No.: number of individuals studied. *inflammatory bowel disease, 12; other colonic diseases, 14. †Inflammatory bowel disease, 10; other colonic diseases, 20. ‡Inflammatory bowel disease, 12; other colonic diseases, 32.

*Kindly supplied by Drs D L Nelson and W Strober, Metabolism Branch, National Cancer Institute, Bethesda, MD, USA.

lower ($P < 0.001$) than in the LPL. The content of null cells, calculated as $[100 - (T + B)]\%$, was similar in the IEL, LPL, and PBL. None of the various values apparently was influenced by disease. Other qualities of these isolates have been detailed recently elsewhere⁸ which support that we were testing isolates enriched for IEL and LPL respectively.

EFFECTS OF TREATMENT WITH EDTA OR COLLAGENASE ON CYTOTOXICITIES OF PBL

Ninety minutes' treatment with EDTA of aliquots of macrophage-depleted PBL from 10 healthy subjects resulted in variable cell losses of 5–20% but did not modify their MICC for CRBC or Chang cells, all relative changes being less than or equal to $\pm 2\%$ of the cytotoxicity values shown by freshly isolated, untreated aliquots. The viabilities of the mononuclear cells exceeded 90% (trypan blue) after the EDTA treatment.

However, similar EDTA treatment of other aliquots increased their ADCC for CRBC and for Chang cells, the relative changes being $+16 \pm 3\%$ (SEM) and $+18 \pm 4\%$, from the respective values with untreated aliquots. Lastly, EDTA-treated PBL showed decreased SCMC for Chang cells, the mean relative change being $-28 \pm 2\%$ of the % cytotoxicities obtained with the untreated aliquots.

Twelve hours' collagenase treatment of aliquots of the PBL resulted in viabilities in excess of 90% with variable cell losses of 6–18%, and decreased ADCC for CRBC and for Chang cells. The relative changes were $-52 \pm 6\%$ and $-30 \pm 5\%$ of the respective cytotoxicity values with untreated aliquots. Collagenase treatment also led to a mean relative decrease of $-59 \pm 8\%$ in the SCMC of PBL for Chang cells in respect to the values with fresh aliquots. In contrast, the same treatment of PBL led to a mean relative increase of $+20 \pm 4\%$ in their MICC for Chang cells. However, collagenase treatment did not modify the MICC of PBL for CRBC, the relative change being only $\pm 3\%$.

ADCC FOR CRBC

The mean value for the spontaneous release of ^{51}Cr from CRBC was $3.7 \pm 0.7\%$, which was not exceeded when target cells were incubated either with anti-

Table 2 Antibody dependent cell-mediated cytotoxicity for chicken erythrocytes at an effector to target ratio of 25:1.

Source of effector cells	No.	% cytotoxicity (mean $\pm$ SEM)
PBL		
Healthy controls	25	59.2 ± 3.7
IBD	8	51.9 ± 5.5
Other diseases	6	71.9 ± 8.1
Total	39	59.7 ± 3.0
LPL		
IBD	18	39.0 ± 4.1
Other diseases	17	32.4 ± 3.7
Total	35	35.8 ± 2.8
IEL		
IBD	6	4.9 ± 1.7
Other diseases	8	2.1 ± 1.6
Total	14	3.3 ± 1.2

No.: number of individuals tested. PBL: peripheral blood lymphocytes; LPL: enriched isolates of colonic lamina propria lymphocytes; IEL: isolates enriched for colonic intraepithelial lymphocytes. All isolates were macrophage-depleted.

CRBC serum alone or with effector cells but without the specific antiserum.

The results are summarised in Table 2. All the PBL tested showed ADCC for CRBC at the screening E:T of 25:1 and there was no significant difference between the mean value for cytotoxicity shown by the PBL from 25 healthy subjects and that with PBL from a total of 14 patients. Also, all the LPL isolates, either from patients with IBD or from those with other colonic diseases, demonstrated ADCC for CRBC, an activity which, as emphasised above, may have been diminished by the isolation procedure. In contrast, the activity of IEL was unimpressive, even though the method of isolation potentially might have increased their activity. Six of the total of 14 samples of IEL showed zero activity, and in the remaining eight the cytotoxicities were modest, only one achieving approximately 10%. In addition, the finding of no apparent relationship between the ADCC for CRBC shown by IEL and LPL isolated from the same colonic mucosa suggested that these were functionally distinct populations of cells (Table 3).

ADCC AND SCMC FOR CHANG CELLS

The mean spontaneous release of ^{51}Cr from Chang cells was $18.1 \pm 1.7\%$ which was not exceeded with

Table 3 Comparison between ADCC activities in CRBC system shown by representative IEL and LPL populations isolated from same colons (effector to target ratio, 25:1)

Source of cells	% specific cytotoxicity										
	Colorectal cancer (5 cases)					IBD (6 cases)					Total cases Mean $\pm$ SEM
IEL	12.7	2.7	0	0	0	10.8	7.6	6.6	3.4	1.2	4.1 ± 1.4
LPL	58.8	33.6	48.2	39.9	20.7	24.8	50.6	61.6	32.3	21.8	38 ± 4.5

Fig 1 ADCC for Chang cells shown by PBL, LPL, and IEL at different effector to target ratios. All values represent the mean $\pm$ 1 SEM. The values for LPL are uncorrected for SCMC (see text).

the addition of anti-Chang serum or normal rabbit serum.

Figs. 1 and 2 show the results in the Chang cell ADCC and SCMC assays. In Fig. 1 the ADCC values are not corrected for SCMC. The two tests were performed simultaneously using: (1) PBL isolates from five healthy subjects and nine patients, (2) LPL isolates from four patients with IBD and 10 with other colonic diseases, and (3) IEL isolates from four patients with IBD and three others. No ADCC or SCMC was observed using PBL, LPL, or IEL at effector: target ratios (E:T) of 1:1 and 5:1, so these are excluded from the graphs. At higher ratios, all the PBL isolates showed ADCC for Chang cells, with a mean cytotoxicity value of $65.1 \pm 6\%$ at E:T of 100:1 (Fig. 1). On testing the colonic lymphoid cell isolates, LPL appeared to be modestly active in mediating ADCC with a mean value of $10.4 \pm 2.8\%$, but the IEL were inactive.

In the SCMC assay, PBL from healthy controls

Fig. 2 SCMC for Chang cells shown by PBL, LPL, and IEL at different effector to target ratios. All values represent the mean $\pm$ 1 SEM.

and from patients, regardless of the nature of the disease, were cytotoxic for Chang cells. The mean SCMC for the 14 PBL so tested was $31.2 \pm 5.6\%$ at E:T of 100:1 (Fig. 2). With LPL, two patients out of the total of 14 showed no activity but in the remaining 12 the SCMC ranged from 4 to 35% at E:T of 100:1. The overall mean value for the SCMC shown by the 14 isolates of LPL tested was $8.7 \pm 2.6\%$, which was considered to be low but definite cytotoxicity. In contrast, all the IEL were inactive with a mean cytotoxicity value of $1.2 \pm 0.5\%$ at the same E:T.

However, because SCMC may have contributed to the cytotoxicity found in the Chang cell assay for ADCC, a subtraction of the % SCMC from the ADCC obtained with the same lymphocytes permitted a simple correction. When this was done, all the PBL tested showed 'corrected' ADCC for Chang cells, with a mean value of $39.9 \pm 6.1\%$ at E:T of 100:1. In contrast, of the 14 samples of LPL, seven showed no 'corrected' ADCC for Chang cells at the same E:T, and for the remaining seven the mean value was only $3.4 \pm 0.9\%$.

MICC FOR CRBC AND CHANG CELLS

The spontaneous release of ^{51}Cr from CRBC or Chang cells, given above, was not exceeded when target cells were incubated only in the presence of PHA.

MICC for CRBC and Chang cells shown by LPL were similar to that demonstrated by the freshly isolated PBL (Fig. 3) at the screening E:T of 25:1. While the IEL showed no MICC activity in the CRBC assay, MICC for Chang cells was demonstrable. The differences between the mean values for MICC for Chang cells shown respectively by PBL, LPL, and IEL ($30.7 \pm 6.3\%$, $23.5 \pm 5.1\%$, and $11.6 \pm 4.4\%$) were not significant. In contrast, MICC for CRBC shown by IEL was essentially zero (mean

Fig. 3 PHA-induced MICC for CRBC and Chang cells shown by PBL, LPL, and IEL at an effector:target ratio of 25:1. All values represent the mean $\pm$ 1 SEM.

$3 \pm 1.7\%$) in contrast with that found with PBL ($17.5 \pm 3.9\%$) or with LPL ($26.1 \pm 4.6\%$) at the same E:T. These comparisons of MICC demonstrated by the different lymphoid isolates were valid, as control studies with PBL showed that neither EDTA nor collagenase treatment reduced their MICC activity.

Lastly, the mean values for ADCC, SCMC, or MICC for Chang cells did not differ depending on the nature of the colonic disease and the same was true when CRBC were used as targets for ADCC and MICC. In addition, neither ADCC, SCMC, nor MICC shown by PBL, LPL, or IEL was demonstrably influenced in the patients with IBD who were receiving steroid treatment.

Discussion

Sequential treatment of human colonic mucosa with EDTA and collagenase (EDTA-C) was used to isolate lymphoid cells enriched for intraepithelial lymphocytes (IEL) or lamina propria lymphocytes (LPL). The IEL contained approximately equal proportions of T, B, and null cells, whereas the LPL showed a higher percentage of T cells and somewhat lower proportions of B and null cells, and these values did not differ with disease. Treatment with EDTA-C has been shown to have only modest effects (relative change: -7 to $+9\%$) on the proportion of T cells in similar characterisations of control PBL, with negligible relative change in the percentage of B cells.⁸ Thus, as emphasised in our previous report,⁸ we concluded that we were obtaining preparations selectively enriched for IEL and LPL. Our findings in LPL contrasted somewhat with those of Bull and Bookman⁵ who reported a lower percentage of B cells and that null cells were not present, and with those of MacDermott *et al.*¹⁵ in their patients with IBD. Goodacre *et al.*,¹⁶ who used mechanical isolation, found a lower percentage of T cells in human colonic LPL compared with those observed either by Bull and Bookman⁵ or by Bartnik *et al.*⁸ using EDTA-C isolation. However, neither Bull and Bookman⁵ nor Goodacre *et al.*¹⁶ studied colonic IEL. Lastly, MacDermott *et al.*¹⁷ showed that 'K' lymphocytes were present in their EDTA-C isolates of human colonic LPL and Strickland *et al.*¹⁸ found null cells in tissue sections of human colonic lamina propria. Thus, the major classes of lymphocytes—namely, T, B, and null cells—are represented in EDTA-C isolates of human colonic IEL and LPL, but relatively little is known of the nature and properties of their subsets.

Several groups have found that ADCC and SCMC for various targets, including CRBC and Chang cells, can be mediated by subsets of Fc receptor-

bearing null cells,^{13 19–22} and also by subsets of T lymphocytes,^{23–25} particularly those possessing receptors for IgG.²⁶ The present results imply that such subsets were well represented in the peripheral blood, as evidenced by the high cytotoxicity values obtained with the macrophage-depleted PBL isolates in the ADCC and SCMC assays. Our finding that ADCC for CRBC and Chang cells shown by PBL did not vary with the disease agrees with the observation of Britton *et al.*²⁷ using mouse lymphoma cells as targets, but contrasts with results showing that CRBC-ADCC shown by PBL from patients with active IBD was decreased²⁸ or increased²⁹ compared with healthy controls. The reasons for these discrepancies are not apparent. Importantly, we demonstrated that human colonic LPL, but not IEL, mediated strong ADCC for chicken erythrocytes. Because our isolates were depleted of polymorphonuclear leucocytes and macrophages, it can be inferred that 'K' lymphocyte activity was present in the LPL.¹³ This finding of ADCC for CRBC with LPL is in agreement with our previous study⁶ and those of others.^{17 30} In contrast, also using an enzymatic isolation technique, Fiocchi *et al.*³¹ found essentially no ADCC for CRBC with human colonic LPL (Table 4).

Although colonic LPL mediated SCMC for Chang cells, IEL were inactive. However, in the Chang-ADCC system, while IEL were inactive, the apparent ADCC of LPL was also reduced effectively to zero when corrected for SCMC. This finding contrasts with a previous study in our laboratory in which ADCC for Chang cells was shown by some isolates of colonic LPL.⁶ We conclude that the discrepancies between the Chang-ADCC results in the two studies were due to technical factors, including particularly the use of different antisera, and the lower ratio of effector to target cells in the previous SCMC assays. We consider now that ADCC for Chang cells was not demonstrated by colonic LPL isolated by the EDTA-C technique. However, in agreement with our previous findings,⁶ MacDermott *et al.*³⁰ also found ADCC for Chang cells with some suspensions of LPL isolated using EDTA-C, but could detect no such activity in a subsequent study.¹⁷ Furthermore, in an earlier report³⁰ they found SCMC for Chang cells with some isolates of LPL, as in the experiments reported herein, but failed to confirm this subsequently.¹⁷ Nevertheless, we and MacDermott *et al.*¹⁷ now generally agree on the findings with EDTA-C isolated human colonic LPL in the CRBC and Chang cytotoxicity assays, with the exception of the conclusions from the Chang-SCMC experiments (Table 4).

Others also have tested human colonic LPL for ADCC for Chang cells,^{32 33} and the somewhat con-

troversial results are summarised in Table 4. In addition, human LPL also have shown variable SCMC and ADCC using other cell lines as targets.^{17 30 34 35}

In our experiments, MICC for CRBC was shown by colonic LPL but not by IEL. However, MICC for Chang cells was demonstrated by LPL and by IEL and, to our knowledge, no previous reports exist of the testing of human colonic IEL for such activity. Of importance to the interpretation of these findings, others concluded that MICC for CRBC, using PBL depleted of polymorphonuclear leucocytes and macrophages, can be mediated by subsets of T, B, or null cells, whereas MICC for Chang cells is a property of a T-cell subset.¹³ Lastly, human LPL also were found to mediate MICC for human erythrocytes, using colonic mononuclear cells isolated by the EDTA-C method.^{17 30}

In this study the cytotoxic activities of IEL, and those of colonic LPL, differed from those found by Arnaud-Battandier *et al.*⁷ in guinea-pigs; they reported that small intestinal IEL, isolated mechanically, mediated ADCC, SCMC, and MICC for Chang cells, whereas LPL, isolated using collagenase, showed only MICC. The discordance between the findings in the two studies cannot be explained by limited viability of our cells but may reflect species differences and the different loci from which the cells were derived. However, it is possible that influences of the isolation procedures also may be involved, a point which will be discussed later.

It is clear that agreement and disagreement exist on the cytotoxic properties *in vitro* of isolates of human colonic LPL, especially in their SCMC and ADCC for cell-lines (Table 4). While these discrepancies might be explicable in part by technical variations in the cytotoxicity assays or by differences

in the patients studied, the methods of isolation of colonic mononuclear cells probably influence their functional behaviour *in vitro*. To illustrate this, Bland *et al.*³³ found synthesis of PGE₂ during the mechanical isolation of human colonic LPL. In discussion they emphasised that many studies³⁶⁻⁴³ have shown that E series prostaglandins may inhibit some functions of lymphocytes *in vitro*. Thus, the production of PGE₂ must be recognised in the interpretation of any future experiments using their mechanical method for the isolation of LPL. An inhibitory effect of PGE₂ might explain the delayed and reduced mitogenic response to PHA of human LPL *in vitro*, compared with PBL, which was found by Goodacre *et al.*¹⁶

Another variable is the possible loss of cells during isolation. To reduce such losses, Bland *et al.*³³ stressed the merit of isokinetic gradient purification compared with discontinuous gradients. Importantly, their mechanical isolation of LPL was similar to that used by Goodacre *et al.*¹⁶ but differed from the 'rubbing' technique used by Arnaud-Battandier *et al.*⁷ In our study, EDTA or collagenase treatment of control PBL caused variable cell losses and reduced certain cytotoxic activities. Accordingly, similar effects might be exerted on colonic mononuclear cells by this isolation method, although it must be stressed that our control findings differed from those in other reports,^{6 17 35} for unexplained reasons. Unfortunately, in our experiments the sizes of the colonic mucosal samples available were insufficient to permit the use of a mechanical¹⁶ and the EDTA-C techniques in parallel. Nevertheless, our observations, and those of Bland *et al.*,³³ raise the important question of whether technical artefacts, resulting from current isolation techniques, may be introduced into the subsequent testing of the

Table 4 Summary of data from various studies of *in vitro* cytotoxicities of human colonic lamina propria lymphocytes (LPL) or intraepithelial lymphocytes (IEL) for Chang cells or chicken red blood cells (CRBC)

Reference	Effector cells	Isolation method	TC	ADCC	SCMC	MICC
This study	LPL	EDTA-C	Chang	0	+	+
	IEL	EDTA	Chang	0	0	+
	LPL	EDTA-C	CRBC	+	nt	+
	IEL	EDTA	CRBC	0	nt	0
Fiocchi <i>et al.</i> ³¹	LPL	C-R	CRBC	±	nt	nt
Chiba <i>et al.</i> ⁸	LPL	EDTA-C	Chang	+	±	nt
	LPL	EDTA-C	CRBC	+	nt	nt
MacDermott <i>et al.</i> ³⁰	LPL	EDTA-C	Chang	+	+	nt
	LPL	EDTA-C	CRBC	+	nt	nt
MacDermott <i>et al.</i> ¹⁷	LPL	EDTA-C	Chang	0	±	0
		EDTA-C	CRBC	+	0	nt
Clancy and Pucci ²⁸	LPL	M	Chang	0	nt	nt
Bland <i>et al.</i> ³³	LPL	EDTA-C	Chang	+	?	nt
	LPL	M	Chang	0	?	nt

TC: target cell; EDTA-C: EDTA-collagenase; M: mechanical; C-R: collagenase-ribonuclease; ADCC: antibody-dependent cell-mediated cytotoxicity; SCMC: spontaneous cell-mediated cytotoxicity; MICC: mitogen-induced cytotoxicity; nt: not tested.

cytotoxic properties of human colonic mononuclear cells *in vitro*.

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