

# Absence of K cells in human gut mucosa

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**SUMMARY** Lymphocytes have been isolated from human rectal biopsies in numbers sufficient for microassay procedures. No antibody-dependent cell-mediated cytotoxic (ADCC) activity could be detected in normal or inflamed human gut mucosa, using two assay systems which detect predominantly K cell activity. The tissue localisation and circulation characteristics of K cells require definition before establishing their role as an efferent immunological mechanism.

Target cell 'kill' by antibody-dependent cell-mediated cytotoxicity (ADCC) is an appealing mechanism for mediating both local resistance in health and mucosal damage in disease. *In vitro* studies using cells obtained from peripheral blood suggest that ADCC may contribute to tissue damage in inflammatory disease of both the small and large bowel (Fakhri and Hobbs, 1972; Shorter, 1974). The immunological potential of the gut mucosa, however, is restricted to those mechanisms which the local apparatus can support, or which can be recruited into the gut mucosa. We describe an absence of ADCC with cells obtained from both normal and inflamed colonic mucosa.

## Methods

Specimens of human gut were obtained following resection, or by rectal biopsy forceps. Rectal biopsies weighed between 22 to 40 mg. Histological diagnosis was made in each subject. A single cell suspension was made from either the entire rectal biopsy or a piece of mucosa-submucosa dissected from resected gut, measuring about 2.5 cm<sup>2</sup> (1 square inch). The gut mucosa was washed twice in phosphate buffered saline (PBS) containing gentamicin (50 µg/ml) and finely minced with scissors. The fragments were ground on a sieve (300 mesh) and cells washed through with RPMI 1640 medium containing 5% foetal calf serum, from a Pasteur pipette. The cell suspension was passed through a siliconised glass bead column. For rectal biopsies, a 10 ml syringe with a 19 gauge needle was packed from the bottom with 1 cm of 3 mm beads, 1 cm of 1 mm beads, and 4 cm of 3 mm beads. For resected

gut, a 50 ml syringe was packed in similar fashion.

The final cell suspension consisted of more than 90% lymphocytes. Cell recovery from the rectal biopsies was 400 000 to 800 000, while about 5-10 × 10<sup>6</sup> cells per 2.5 cm<sup>2</sup> of resected mucosa were obtained. Cell viability was confirmed by mitotic response to pokeweed mitogen and trypan blue exclusion by cultured cells.

Two assays for ADCC were used. In the first the Chang hepatoma cell assay (Hersey *et al.*, 1976) was used with rabbit anti-Chang cell antibody. Rabbit antibody was used at optimal dilution. ADCC was detected as specific release of Cr<sup>51</sup> from labelled target cells according to the formula:

$$\frac{a-b}{c-b} \times 100,$$

where a = loss of Cr<sup>51</sup> from Chang cells in presence of anti-Chang cell antibody (1/100); b = spontaneous loss of Cr<sup>51</sup> from Chang cells; c = maximum releasable Cr<sup>51</sup> from Chang cells (in distilled water).

The second assay used Chang cells labelled with Cr<sup>51</sup> as above and coated with *E. coli* lipopolysaccharide prepared by boiling cultures of six *E. coli* strains (the most common Australian serotypes obtained from Institute of Medical and Veterinary Science, Adelaide). Chang cells were coated by incubating 1 × 10<sup>6</sup> Chang cells in 4 ml supernatant (Neter, 1956). Pooled sera from six normal subjects shown to have agglutinating activity against the *E. coli* lipopolysaccharide by haemagglutination were used in the ADCC assay at an optimal dilution, determined from a dose response curve using peripheral blood lymphocytes. Results were obtained according to the above formula. Incubation in each assay was for 18 hours at 37°C. Consideration of the variation in these systems indicated specific cytotoxicity when more than 5%

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Cr<sup>51</sup> release was detected.

Circulating lymphocytes were obtained from blood taken into heparin (10 µ/ml) at the time of biopsy or resection. Lymphocytes were isolated by centrifugation over Ficoll-Hypaque (Boyum, 1968). Lymphocytes treated with proteolytic enzyme were incubated with trypsin (2 mg/ml) at 37°C for 30 minutes, and washed with RPMI 1640 medium containing 15% AB serum. Trypsin was obtained from bovine pancreas and twice crystallised (Sigma).

## Results

ADCC activity was regularly demonstrated in blood lymphocytes with both assays (Tables 1 and 2). Processing of blood lymphocytes by the technique used to isolate gut lymphocytes caused no loss of ADCC activity.

The spontaneous rate of Cr<sup>51</sup> loss from Chang cells was 28-32% over 18 hours and addition of blood or gut lymphocytes in the ratios expressed in Tables 1 and 2, in the absence of antibody, increased Cr<sup>51</sup> loss by less than 5%. Addition of *E. coli* antigen to the Chang cell surface, however, increased the rate of 'spontaneous' loss of Cr<sup>51</sup> in the absence of lymphocytes to 35-40% over 18 hours. With mucosal lymphocytes ADCC activity was not detected regardless of whether human or

rabbit antibody was used, or whether lymphocytes were obtained from normal or inflamed gut.

The possibility that K cell activity was masked by adsorbed blocking factors was examined by preincubation of gut lymphocytes, and treatment with proteolytic enzyme trypsin (Table 3). In neither case could significant K cell activity be detected. The loss of K cell activity in blood after 24 hours suggests that K cells may have a short lifespan in culture.

Table 3 Failure of incubation and trypsin digestion to enhance ADCC

Cell source	Specific cytotoxicity (%)		Post trypsin digestion
	Preincubation	24 h incubation at 37° C	
Colon	1.5	0	0
Blood	12	0	8
Colon	—	3	—
Blood	—	7	—

Lymphocytes obtained from normal descending colon and blood of two patients with carcinoma of sigmoid colon. Lymphocyte/target cell ratio 25/1.

## Discussion

The 'homing' of cells mediating ADCC is determined

Table 1 Antibody-dependent cell-mediated cytotoxicity in human gut mucosa\*

Diagnosis (treatment)	Source of gut lymphocytes	Lymphocyte/target cell	Specific cytotoxicity (%)	
			Blood lymphocytes	Gut lymphocytes
Normal	Rectum (biopsy)	20/1	67	0
Normal	Rectum (biopsy)	17/1	30	0
Ulcerative colitis	Rectum (biopsy)	10/1	12	0
Ulcerative colitis (steroids)	Rectum (biopsy)	15/1	33	0
Ulcerative colitis (salazopyrin)	Rectum (biopsy)	10/1	10	0
Crohn's disease (steroids)	Asc. colon (colectomy)	10/1	23	0
		20/1	34	0
		50/1	45	0
Ulcerative colitis (steroids)	Asc. colon (colectomy)	10/1	1	1
		20/1	3	0
		50/1	3	1

\*Target cell is Chang cell; antibody is rabbit anti-Chang cell serum at dilution of 1:100.

Table 2 Antibody-dependent cell-mediated cytotoxicity in human gut mucosa\*

Diagnosis (treatment)	Source of gut lymphocytes	Lymphocyte/target cell	Specific cytotoxicity (%)	
			Blood lymphocytes	Gut lymphocytes
Crohn's disease (steroids)	Asc. colon (colectomy)	50/1	10	0
		100/1	—	0
Carcinoma colon	Terminal ileum (R hemicolectomy)	100/1	18	0
Carcinoma colon	Desc. colon (L hemicolectomy)	100/1	23	0

\*Target cell is Chang cell coated with *E. coli* antigen; antibody is pooled normal serum at 1:100 dilution. Gut mucosa was histologically normal.

by their capacity to bind to Fc fragments of antibody bound to target cells (MacLennan, 1976). This provides a sensitive and economical mechanism for target cell destruction. *In vitro* evidence suggests that ADCC may contribute to host resistance to infection (Simone *et al.*, 1972) and to tissue destruction in certain autoimmune diseases (Calder *et al.*, 1973), but clear demonstration of an *in vivo* effect is lacking.

It is important, therefore, that cells capable of mediating ADCC could not be isolated from histologically normal or inflamed human colon mucosa. Parallel studies of peripheral blood lymphocytes regularly demonstrated ADCC. *E. coli* antigen was adsorbed to Chang cells and tested as antigen in ADCC with human antibody specifically to examine the hypothesis that the pathogenesis of ulcerative colitis may include ADCC, involving mucosal K cells and antibody to colon epithelial cells, which crossreacts with antigens common to a number of enterobacterial species (Shorter, 1974). Again, no K cell activity could be detected. The apparent intravascular restriction of K cells emphasises the importance of determining the distribution and circulation characteristics of these cells in man before an effector role can be attributed to them. Absence of such cells from thoracic duct lymph (Holm *et al.*, 1974), mouse Peyer's patches (Nelson *et al.*, 1976), and guinea-pig lung (Hunninghake and Fauci, 1976) indicates a general depletion of this cell population in mucosal lymphoid tissue. The possibility that K cells were present, but that their activity was masked by adsorbed factors, was considered unlikely when incubation at 37°C for 24 hours and treatment with proteolytic enzyme was not followed by the appearance of ADCC. Indeed, ADCC may be undesirable in the gut mucosa as the local immune response to gut contents includes antibody which crossreacts with host antigen, which in the presence of ADCC may induce mucosal damage.

Effector cells vary in different antibody sensitised target cell systems. Chang cell lysis reflects predominantly K cell activity, while monocytes, B cells, and neutrophils mediate lysis of other antibody-sensitised target cells (Holm, 1972; Chess *et al.*, 1974; Kovithavongs *et al.*, 1975). K cells have the appearance of lymphocytes, but lack the unique surface markers of T or B cells (MacLennan, 1976). It can be stated, therefore, that few K cells are present within normal colon mucosa, nor can they be recruited through inflammation. While K cells probably do not contribute to the pathogenesis of inflammatory bowel disease, a contribution by other ADCC mechanisms can not be excluded.

These studies of gut lymphocyte function followed

the development of a simple procedure for isolating lymphocytes from rectal biopsies in numbers sufficient for microassay procedures. This method overcame the problem of cell trapping in mucus and provided a lymphocyte population free from significant contamination with epithelial cells and debris. It may be expected that the direct study of the cellular basis of mucosal defence mechanisms and the pathogenesis of mucosal disease in man may be facilitated by the use of this technique.

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