Regulation of human gut B lymphocytes by T lymphocytes

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SUMMARY The aims of this study were first, to assess whether or not immunoglobulin secretion from human gut mucosal B lymphocytes can be modified by T lymphocytes, and second whether human gut mucosal T lymphocytes are capable of regulating mucosal B lymphocyte function. T and B lymphocyte enriched cell populations were isolated from gut mucosa and co-cultured in varying proportions. Addition of T lymphocytes to B enriched mucosal cell populations (ratio B:T = 2:1) showed that mucosal B lymphocytes were responsive to T cell 'help'. Addition of more T cells (ratio B:T = 2:10) suppressed immunoglobulin synthesis.

IgA is the predominant class of immunoglobulin synthesised in, and secreted from, human mucosa.¹ Antibody secreted at mucosal sites regulates bacterial proliferation² and antigen uptake,³ and thus itself must be finely tuned to local needs. Disturbed control of the local immune response may lead to a wide range of clinically relevant events such as excessive bacterial proliferation and the 'blind loop syndrome',⁴ excessive antigen absorption and immune complex formation, and autosensitisation.⁵ The level of control of the mucosal immune response has been little studied. Thus, while it is clear that B lymphocytes primed within Peyer's patches by absorbed antigen proliferate,⁶ differentiate,⁷ and migrate to various mucosal surfaces,⁸ the role of polyclonal mitogens and modulating T lymphocytes within the mucosa in determining the rate of antibody secretion is unknown.

In this study, human gut mucosal B cells stimulated by the polyclonal mitogen, pokeweed mitogen (PWM), were co-cultured with T lymphocytes to examine first, whether B lymphocytes located within the gut mucosa can respond to T lymphocyte mediated control, and second, whether mucosal T lymphocytes can mediate help and suppression.

Methods

MATERIALS GUT MUCOSA

Gut was obtained from patients having stomach (eight), ileum (six), or colon (four) resection. Partial gastrectomy was for duodenal or gastric ulceration, resection of ileum was for Crohn's disease (two), Meckel's diverticulum (one) or carcinoma of the right side of the colon (three), and colectomy was for trauma (one) or colon carcinoma (three). Histological examination of the pyloric mucosa showed moderate chronic gastritis in each case. Ileum resected in association with the Meckel's diverticulum and the colonic carcinoma was histologically normal, while mild chronic inflammatory changes were detected in mucosa taken from macroscopically normal bowel resected in association with Crohn's disease, and histologically normal colon resected in association with colon carcinoma was used in these experiments.

CELL ISOLATION, CHARACTERISATION AND SEPARATION

A modification of several techniques has been developed,^{9 10} which provides 7×10^6 lymphocytes per gram of mucosa, with cell viabilities of greater than 95%. Gut was washed in phosphate buffered saline within 30 minutes of resection, the mucosa dissected free from underlying tissue and cut into 1 cm² pieces and incubated in 1 mM DL-dithiothreitol

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(Sigma, USA) for 20 minutes at room temperature. The tissue pieces were then finely minced and incubated in clostridial collagenase (25 U/ml; CSL III, Worthington Biochem, USA) in RPMI 1640 for two hours at 37°C on an Adams Rotator in a humidified atmosphere with 5% CO₂ in air, using 10 ml/g of tissue. The partly digested mucosa was then pressed through a stainless steel mesh (300 gauge) and the resulting cell suspension washed in 1 mM DL-dithiothreitol (Sigma, USA) in RPMI 1640. Cell concentration in RPMI 1640 containing 100 IU/ml penicillin and 100 µg/ml streptomycin (Flow Lab, USA), 50 µg/ml gentamicin (Schering Corp, USA), 0.125 µg/ml Amphotericin B (Squibb, USA), and 50% heat inactivated fetal calf serum (GIBCO, USA), was adjusted to 1×10⁷/ml, added to an equal volume of carbonyl iron (Lymphocyte Separator Reagent, Technicon, USA), and incubated for 20 minutes at 37°C. The cells were washed twice in RPMI 1640, and resuspended at a concentration of 1×10⁶/ml for microculture or 3×10⁶/ml for surface marker studies.

T cells were quantitated according to their capacity to bind to papain treated sheep erythrocytes¹¹ and B cells quantitated according to the presence of surface immunoglobulin (sIg), using fluorescein conjugated F(ab')₂ rabbit anti-human IgG, IgA, and IgM (DAKO Immunoglobulin Ltd, Denmark).¹²

T cell and B cell enriched cell populations were prepared by double centrifugation of E rosetted lymphocytes over a Ficoll-Paque (Pharmacia Fine Chemicals, Sweden) gradient as previously described.¹³ Cells were washed three times and resuspended in supplemented RPMI 1640 at a concentration of 1×10⁶/ml. Mucosal T enriched cell populations contained less than 8% B cells, and mucosal B enriched cell populations contained less than 2% T cells.

CELL CULTURE AND IMMUNOGLOBULIN ESTIMATION

Cell cultures were established in RPMI 1640 supplemented as above, with 10% heat inactivated fetal calf serum. Lymphocyte concentrations were 1×10⁶/ml, with a final volume of 0.2 ml except for the T:B cell collaborative studies, when B cells were standardised at 100 000 cells/culture, and T cells added at T:B ratios of 1:2 or 10:2, as previously described to detect 'help' and 'suppression' respectively.¹³ Cultures were conducted in sterile microtitre plates with U-bottomed wells ('Nunc', Nunc, Denmark), contained PWM (1/200 concentration, determined as 'optimal' from dose response curves: GIBCO, USA), and were incubated for seven days at 37°C in a humidified

atmosphere with 5% CO₂ in air.

The concentrations of IgA, IgG, and IgM in culture supernatants was measured by solid phase radioimmunoassay.¹⁴ Sensitivity of assay is for IgG 10 ng/ml, IgA 20 ng/ml, and IgM 60 ng/ml.

Results

CHARACTERISATION OF MUCOSAL CELL POPULATIONS

Percentage of T cells obtained from pyloric mucosa (72.8±2%) were greater than those obtained from colonic (49.4±6%) and ileal (54.6±12%) mucosa. B cells constituted 29±9% of intestinal mucosal cell preparations.

The spontaneous production of immunoglobulin from non-separated mucosal cell populations is seen in Table I. Polyclonal stimulation with optimal concentrations of PWM was found for IgA (4.0 fold increase), IgG (2.0 fold increase), and IgM (2.0 fold increase) with ileal mucosal cells. No increase in secretion rate for any isotype was seen with gastric mucosal cells.

RESPONSE OF MUCOSAL B LYMPHOCYTES TO T CELL 'HELP' (Table 2)

Little or no immunoglobulin was detected in cultures of 'enriched T cells'. Addition of mucosa T cells to autologous gastric mucosal 'B enriched' populations enhanced immunoglobulin secretion in: IgG, one of four studies; IgA, two of four studies; and IgM, nil of four studies. Depleting T lymphocytes from gastric mucosal cell preparations had no significant effect on immunoglobulin secretion. In 10 similar studies of intestinal B cell preparations, addition of T cells (B:T = 2:1) provided 'help' in three studies with IgG, eight studies with IgA, and eight studies with IgM. Under these specified circumstances, T cell 'help' for IgM was greater than it was for IgG and IgA.

It should be noted that in all studies the three immunoglobulin isotypes were measured in the same supernatant and in each instance at least one immunoglobulin class was detected. Viability studies were routinely performed on terminated cultures using trypan blue exclusion. Viability fell in

Table 1 Spontaneous secretion of immunoglobulin from gut mucosal lymphocytes

	IgG (ng/ml)	IgA (ng/ml)	IgM (ng/ml)
Stomach (5)	53 (0-265)	380 (0-950)	88 (0-440)
Ileum (7)	73 (0-250)	32 (0-235)	247 (0-630)
Colon (2)	80 (0-160)	90 (70-110)	330 (0-660)

Table 2 Mucosal B lymphocytes – response to T lymphocyte ‘help’

Organ	Diagnosis	IgG (ng/ml)		IgA (ng/ml)		IgM (ng/ml)	
		B cells	B:T (2:1)	B cells	B:T (2:1)	B cells	B:T (2:1)
Colon	Normal	0	0	210	260	0	0
	Carcinoma of colon	0	0	0	265	975	925
	Crohn's disease	0	0	230	400	490	1190
	Carcinoma of colon	0	77	410	1350	200	6750
Ileum	Carcinoma of colon	75	980	0	250	0	1900
	Crohn's disease	0	0	0	0	0	375
	Carcinoma of colon	0	440	0	775	0	9500
	Crohn's disease	0	0	0	0	350	1780
	Carcinoma of colon	0	0	0	235	190	4000
	Meckel's diverticulum	0	0	0	500	0	1200
	Duodenal ulcer	135	105	1800	3150	370	0
Stomach	Duodenal ulcer	0	0	375	430	230	0
	Duodenal ulcer	0	85	230	162	205	205
	Gastric ulcer	265	240	0	0	400	0

both blood and mucosal lymphocyte cultures over the seven day period, with viabilities at day seven of 50–60% for blood lymphocytes and 25–30% for mucosal lymphocytes. Treating blood lymphocytes in an identical fashion to that used for mucosal lymphocyte separation had little effect on immunoglobulin secretion, but it did reduce their viability in terminated cultures by about 10%.

Statistical analysis (Student's *t* test) of data converted to percentage with maximum synthesis assigned as 100, for individual isotypes, showed significant ‘help’ provided by T:B lymphocyte combinations, for IgA from colon B cells ($p<0.05$), and for IgA ($p<0.05$) and IgM ($p<0.05$) from ileal B cells. Group analysis for different isotypes secreted from stomach B lymphocytes failed to demonstrate significant ‘help’. If the colon and ileum data are pooled (‘intestinal lymphocytes’), ‘help’ for both

IgA ($p<0.01$) and IgM ($p<0.01$) is significant.

RESPONSE OF MUCOSAL B CELLS TO T CELL SUPPRESSION (Table 3)

Controls for a ‘crowding’ effect, to account for suppression, were (1) addition of killed T lymphocytes to a final cell concentration of $4 \times 10^6/\text{ml}$, and (2) quantitation of immunoglobulin in blood lymphocyte cultures, with concentrations up to $4 \times 10^6/\text{ml}$. In each case, immunoglobulin concentration in supernatants correlated in a linear fashion with the B cell number.

Inhibition of immunoglobulin secretion from mucosal B cells obtained from resected ileum by the addition of T lymphocytes (B:T = 2:10) was shown in nine out of the 10 studies where immunoglobulin secretion was detected at B:T ratios of 2:1. Conversion of data converted to percentage with

Table 3 Mucosal B lymphocytes – response to T lymphocyte ‘suppression’

Organ	Diagnosis	IgG (ng/ml)		IgA (ng/ml)		IgM (ng/ml)	
		B:T = 2:1	B:T = 2:10	B:T = 2:1	B:T = 2:10	B:T = 2:1	B:T = 2:10
Ileum	Carcinoma of colon*	980	55	250	0	1900	0
	Crohn's disease*	0	0	0	0	375	410
	Carcinoma of colon*	440	0	775	0	9500	270
	Crohn's disease*	0	0	500	235	1200	0
Stomach	Duodenal ulcer	1170	270	1270	520	2150	150
	Duodenal ulcer	540	550	7500	5800	340	185
	Gastric ulcer	320	280	258	210	270	130
	Gastric ulcer	240	280	0	0	0	410
	Duodenal ulcer*	0	155	430	530	0	200
	Duodenal ulcer*	85	175	162	177	205	0

* Lymphocytes from sources used for ‘help’ experiments (Table 1).

maximum synthesis assigned as 100, showed significance only for IgM ($p < 0.025$), though pooling of data for all classes of immunoglobulin was significant ($p < 0.01$). No such clear suppressive effect was seen with co-cultures of gastric mucosal T and B cells, as while suppression was noted in nine of the 18 studies, eight showed an increase of immunoglobulin secretion with the greater numbers of added T cells – that is, B:T = 2:10.

Discussion

IgA is the predominant gut immunoglobulin isotype determined by quantitation of class specific immunoglobulin containing cells,¹⁵ secretion from perfused gut,¹⁶ and assay of organ culture supernatants,¹⁷ and the trend seen with our limited data from analysis of supernatants of cultured mucosal lymphocytes separated from stomach and ileum, is consistent with these observations. Restricted studies suggest, however, that IgA may not always be the predominant immunoglobulin secreted from isolated mucosal lymphocytes¹⁰ and in the present study using intestinal B cells in the reconstituted co-culture T cell 'help' experiments, we show that with low T:B cell ratios, IgM may predominate. Although this apparent paradox may be explained by differential cell survival in culture possibly influenced by the extraction procedure, the recent studies by Strober *et al* on T cell regulation of the gastrointestinal immune response suggest that these results may also reflect a culture induced change of control and 'switch' mechanisms normally mediated by a unique mucosal T cell population.¹⁸

Two observations are made in this study. First, the rate of immunoglobulin synthesis from human gut mucosal B lymphocytes is not fixed, and secretion is responsive to local control. Second, mucosal T lymphocytes provide a control mechanism through their capacity to both facilitate and suppress immunoglobulin secretion.

T cell 'help' was clearly seen in B cell preparations taken from intestine where enhanced immunoglobulin secretion was detected in two thirds of the experiments. 'Help' was most clearly observed with IgM. This is of interest in that IgA is the immunoglobulin which traditionally characterises the mucosal immune response, and IgM antibody synthesis is generally considered less 'T cell dependent' than is antibody of other isotypes.¹⁹ It can be stated, however, that human mucosa does contain B lymphocytes capable of responding to local stimuli, and which retain a responsiveness to T cell 'help'. The current study complements *in vivo* studies which indicate that local antigen stimulates relocated gut primed B cells.²⁰ These observations

have important implications with respect to the development of mucosal immunisation strategies in man.

Inhibition of immunoglobulin synthesis at higher T:B cell ratios was regularly detected in cultures containing B cells from intestinal mucosa. High levels of suppression (>50%) were found in all but one of nine studies of intestinal B cells. An imposed limitation on the expansion of B lymphocytes within the mucosa owing to a high level of sensitivity to T cell suppression, would allow for a stimulation of mucosal B cells in response to local needs, but only in a limited fashion, short of that degree of 'inflammation' that would cause pathology.

Gastric mucosal B:T cell co-cultures failed to show significant T cell modulation of B cell function, in the limited number of experiments available for analysis. The considerable variation in results may reflect shifts in the dose response curve not detected with the choice of ratios used, the variable infiltrate of lymphocytes found in all resected gastric mucosa, and the contamination of 'enriched' T and B cell populations which was greater with stomach mucosa than was found for intestinal cell populations. Real differences between mucosal B cell responsiveness to T cell control at different levels of the gut may exist, however, and these remain to be analysed. Differences in luminal content and antigen handling may condition regional variations in T-B cell relationships.

T cells are the dominant lymphocyte type within human gut mucosa²¹ and this is confirmed in our study. Effector functions have been described for human mucosal T cells.^{22, 23} Less is known regarding a regulatory role for mucosal T cells. Regulatory T cells have been identified in the mouse in relation to the afferent limb of the mucosal immune response²⁴ and oral feeding is followed by the appearance of suppressor cells in Peyer's patches.²⁵ Regulatory T cells in the lamina propria limited the effector arm of mucosal immunity by inhibiting lymphocyte division after stimulation,²⁶ and have been shown to enhance secretion of IgM from blood B cells.²⁷ The current study extends these observations by showing that intestinal T cells inhibit immunoglobulin production from polyclonally activated mucosal B cells, as has been described for co-cultured blood lymphocytes.¹³

One previous study showed differences in spontaneous and PWM stimulated immunoglobulin secretion from cells isolated from normal and inflamed gut,²⁸ using culture conditions differing significantly from those used here. In our study no attempt has been made to correlate findings with disease states. Future study of diseased mucosa may show defective T-B cell interaction within the

lamina propria as contributing pathogenic mechanisms.

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