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Natural cytotoxicity, responsiveness to interferon and morphology of intra-epithelial lymphocytes from the small intestine of the rat

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Summary. The expression of natural cytotoxicity in the gut-associated lymphoid tissue of the rat was investigated. Intra-epithelial lymphocytes (IEL) isolated from the small intestine had a similar frequency of cytotoxic and tumour target binding cells as the spleen. However, cytotoxic activity was low in Peyer's patches and mesenteric lymph node. Cytotoxic IEL exhibited similar target-cell specificity to splenic natural killer (NK) cells, and substantial antibody-dependent cell-mediated cytotoxicity. However, their cytotoxicity and lytic efficiency was not rapidly increased following exposure to interferon and IEL probably contain only a relatively small pool of pre-NK cells. IEL contained approximately 50% of cells with basophilic cytoplasmic granules, compared to approximately 2% of granule-containing mononuclear cells in the spleen. Direct observation using the uptake of acridine orange to detect cytoplasmic granules established that some cytotoxic IEL were granular. However, substantial cytotoxicity was also mediated by non-granular IEL and not all granular IEL which bound to target cells were cytotoxic. Counts of granulated cells in Giemsa-stained preparations of spleen cells and results from single-cell assays using unfractionated splenocytes suggest that the majority of target-binding cells and at least half of the naturally cytotoxic cells must also be non-granular.

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INTRODUCTION

The epithelium of the villi in the small intestine contains large numbers of lymphocytes (intra-epithelial lymphocytes, IEL), some with prominent cytoplasmic granules and others that are non-granular (Collan, 1972). Granular IEL are present in nude mice and thymectomized, irradiated, bone-marrow reconstituted (ATX.BM) rats (Mayrhofer, 1980a) and also in nude rats (Mayrhofer & Bazin, 1981). These findings do not support the suggestion that granular cells represent differentiated T lymphocytes (Guy-Grand, Griscelli & Vassalli, 1978). However, non-granular IEL may be thymus-dependent (Mayrhofer, 1980a). Although it has been suggested that granular IEL may be precursors of mucosal mast cells (Guy-Grand *et al.*, 1978; Mayrhofer, 1980b), the function of IEL is still unknown. Their close proximity to the gut suggests that they may be active in host defence and their numbers have been shown to increase during intestinal parasitic infestations (Ferguson, 1977).

Isolated granular IEL resemble the large granular lymphocytes of peripheral blood which exhibit natural

cytotoxicity in man (Saksela, Timonen, Ranki & Häyry, 1979) and in the rat (Reynolds, Timonen & Herberman, 1981) but a comprehensive histochemical analysis of the granules of large granular lymphocytes has not been reported. Natural killer (NK) cells show spontaneous cytotoxicity to a variety of virus-infected or tumour cells *in vitro* (Santoli & Koprowski, 1979) and their activity is rapidly augmented by interferon (Trinchieri & Santoli, 1978; Djeu, Huang & Herberman, 1980; Shellam, Winterbourn & Dawkins, 1980; Flexman & Shellam, 1981). These properties suggest that they may be important *in vivo* in early defence against virus infections and tumour growth. Whilst most studies have been concerned with NK cells from the blood and lymphoid organs, naturally cytotoxic cells have recently been detected in the IEL population of guinea-pigs (Arnaud-Battandier, Bundy, O'Neill, Bienenstock & Nelson, 1978) and in the lungs of mice (Puccetti, Santoni, Riccardi & Herberman, 1980) suggesting that NK cells may also constitute a defence mechanism at various mucosal surfaces.

Accordingly, the IEL population of the rat gut has been examined for NK-cell activity. The relationship between granular IEL and cytotoxic cells has been directly examined at the single-cell level on unfractionated cells, to avoid selective cell losses that might occur during enrichment procedures. It was found that the IEL exhibited much greater natural cytotoxicity than the other gut-associated lymphoid tissues, that NK cells from this site were relatively refractory to stimulation by interferon and that not all of the lytic activity was associated with granular lymphocytes.

MATERIALS AND METHODS

Animals

Inbred female WAG rats bred at the University of Western Australia were conventionally housed and used at 24–33 weeks of age. Unless otherwise stated, all rats received 50 mg of metronidazole daily by intragastric instillation for 3 days, 1 week prior to use, to remove endemic infections with *Hexamita muris* and *Giardia muris* (Underdown, Roberts-Thomson, Anders & Mitchell, 1981).

Tumours

The *in vitro* culture of RBL-5, P815, K562 and the Gross virus-induced rat lymphoma W/FuG-1 in RPMI 1640 (GIBCO, New York, U.S.A.) with 10% foetal calf serum (FCS), which is hereafter referred to as medium, is described elsewhere (Dawkins & Shellam, 1979). Unless otherwise stated, W/FuG-1 cells were routinely used as target cells in all assay systems.

Preparation of cell suspensions

Spleen cells and IEL or other lymphoid cells were always obtained from the same rat, and unless otherwise stated procedures were carried out at room temperature. Spleen-cell suspensions were prepared as described by Flexman & Shellam (1981, 1982) and mesenteric lymph node (MLN) cells and Peyer's patch (PP) cells as described by Allan & Mayrhofer (1981). The method for preparing IEL will be described in detail elsewhere (Mayrhofer & Whately, 1983). The small intestine was removed, divided into three equal parts and IEL were isolated from the two proximal portions. After perfusion with cold (4°) phosphate-buffered saline (PBS; Dulbecco's phosphate-buffered saline, solution A; Oxoid, U.K.), they were then everted, distended until tense and ligated. Each portion was divided between ligatures into three smaller segments and gently vortexed in 20 ml of PBS containing 2 mM dithiothreitol (DTT, Sigma) to remove mucus. The segments were then vigorously vortexed with about 20 ml of Dulbecco's solutions A and B, containing 5% newborn calf serum (DAB/NCS), until most of the epithelium had been removed. The supernatants were extracted three times by addition of teased absorbent cotton wool to remove dead epithelial cells. Clumps of epithelial cells were allowed to settle at unit gravity for 20 min before the supernatants were passed through loosely packed cotton wool. The cells were collected, washed twice with DAB/NCS by centrifugation, and resuspended in 10 ml of 30% Percoll (Pharmacia, Sweden) in DAB. They were then passed through cotton wool, which retained the remaining dead epithelial cells in the absence of serum. After addition of 0·5 ml of NCS and 3 mg of DTT, the cells were diluted to 15 ml with 30% Percoll and distributed on three step-gradients each consisting of 9 ml of 35% Percoll and 6 ml of 80% Percoll. After centrifugation at 1000 ***g*** (maximum) in an angle head rotor for 30 min, the cells overlying the 80% Percoll were collected and washed by centrifugation. The mean cell yield from the proximal two-thirds of the small intestines from ten rats was $4{\cdot}04 \pm 0{\cdot}49$ (SE) $\times 10^7$ IEL per rat, with 98–100% viability and contamination with 5–10% viable epithelial cells.

Newcastle disease virus (NDV)

NDV was prepared and titrated by an egg infectious

dose titration, 50% end point (EID_{50}) method (Fazekas de St. Groth and White, 1958). The EID_{50} of the NDV was $10^{8.2}$ per ml.

Preparation and titration of interferon (IFN)
Adult W/Fu rats were injected intraperitoneally (i.p.) with 5 ml of 2·95% w/v thioglycollate broth (BBL, Becton, Dickinson and Co., U.S.A.), and 72 hr later the peritoneal exudate cells were harvested in RPMI 1640 containing 5 units of heparin/ml. Five millilitres of cells at 10^7/ml and 1 ml of NDV ($10^{8.2}$ EID_{50}) were added to each 75 cm^2 flask (Lux, Calif.) which was incubated for 24 hr at 37°. The supernatant was harvested by centrifugation at 250 ***g***, dialysed against 0·1 M glycine-HCl buffer, pH 2·0 at 4° for 2 days, further dialysed against PBS at pH 7·4 at 4° for 2 days, and, finally, the supernatant was sterilized by filtration. The resulting supernatant had an antiviral titre of 160,000 units/ml. The assay for anti-viral activity is described elsewhere (Shellam, Winterbourn & Dawkins, 1980). For stimulation with IFN, lymphoid cells were incubated in medium at 10^7/ml at 37° for 4 hr in the presence of 4000 u/ml of IFN (Flexman & Shellam, 1983) unless otherwise stated. The cells were then washed once before assay for cytotoxicity.

^{51}Chromium release assay (CRA)
The 4-hr CRA using microtitre trays (Falcon, Oxnard, Calif.) and 10^4 ^{51}Cr-labelled target cells/well, and the calculation of percentage ^{51}Cr release, has been described in detail elsewhere (Dawkins & Shellam, 1979). Data are expressed in cytotoxic units (CU), which are defined as the slope of the linear regression curve which is the best fit to the points obtained by plotting percentage ^{51}Cr release against the number of effector cells, and is expressed as percentage ^{51}Cr release per 10^6 lymphoid effector cells. The correlation coefficient of the slope invariably exceeded 0·93. The spontaneous release was routinely 5–10% of the total counts.

Assay for antibody-dependent cell-mediated cytotoxicity (ADCC)
This assay was performed exactly as previously described (Flexman & Shellam, 1980) using P815 cells coated with hyperimmune rat anti-P815 antibody.

Assay for target-binding cells (TBC)
The TBC assay was performed as previously described (Flexman & Shellam, 1982).

Enumeration of cytotoxic effectors (CE) in agar
The agar assay is described in detail elsewhere (Flexman & Shellam, 1982). The percentage of cytotoxic effector (CE) cells was calculated by counting the number of conjugates containing dead tumour cells in a total number of 100 conjugates. Tumour cells alone in agar served as a control for spontaneous tumour cell death. The percentage of bound effector cells that were cytotoxic was calculated as follows: (percentage of dead targets in conjugates) − ([fraction of spontaneous dead targets] × [the percentage of dead targets in conjugates]). In addition, the frequency of cytotoxic leucocytes in the original population was calculated by: (percentage of bound cells that were cytotoxic at 4 hr) × (percentage of total leucocytes bound to the targets) × 10^{-2}.

Detection of intra-cellular granules by acridine orange (AO)
Acridine orange (M-N87, Gurr, Searle, High Wycombe, U.K.) was used at a final concentration of 1:400,000 w/v in PBS. Cells were examined as wet preparations by incident light using standard illumination for fluorescein fluorescence microscopy. Cytoplasmic granules of lymphocytes exhibited metachomatic red fluorescence and granular cells were scored as any cells with red-staining granules, irrespective of the number, size or staining intensity of the granules.

This stain was employed in three ways. (i) The percentage of granular cells in leucocyte preparations was determined after incubation with AO for 15 min at 37° followed by a single wash in medium at 250 ***g*** and enumeration of leucocytes with red granules by fluorescence microscopy. (ii) Effector-target cell conjugates were formed as described above, resuspended and granular TBC were counted after incubation in AO for 15 min at 37°. (iii) To detect granules in leucocytes bound to lysed target cells, conjugates were first incubated at 37° for 4 hr, resuspended, and AO and trypan blue were added, the latter at a final concentration of 0·2% w/v. After a further incubation for 15 min, cells were examined by fluorescence microscopy. AO staining did not affect cell viability and was used at the end of the assay period to avoid any influences on cytotoxic activity.

RESULTS

Comparison of cytotoxicity of IEL and spleen cells

As shown in Table 1, a similar percentage of target binding cells and frequency of cytotoxic cells towards

Table 1. Natural cytotoxic activity in the gut-associated lymphoid tissue

Group		^{51}Cr release assay		Single cell assay		
		Cytotoxic units (±SE)*	Total cytotoxic units/organ†	TBC (%)	Frequency of C.E.	Total no. of C.E./organ
(a)	Spleen cells	48·1±2·3	7311	15·0	3·24	$4·92 \times 10^6$
	IEL	24·9±2·6	996	16·8	3·32	$1·33 \times 10^6$
(b)	Spleen‡	36·0±1·1	5220			
		17·2±0·3	2508			
		19·2±0·9	3669			
	MLN	3·6±0·3	162			
		3·5±0·1	150			
		5·1±0·3	204			
	PP	2·4±0·2	29			
		1·1±0·4	14			
		0·6±0·4	7			

* Cytotoxic units = % ^{51}Cr release/10^6 effector cells.

† Data was derived thus: Total cytotoxic units = cytotoxic units × mean cell count ($\times 10^{-6}$) per organ. Intraepithelial lymphocytes (IEL) were obtained from only the proximal 2/3 of the small intestine.

‡ Spleen cells, mesenteric lymph node cells (MLN) and Peyer's patch cells (PP) were assessed together for cytotoxicity in 3 separate experiments. The data derived from each experiment is listed in descending order.

W/FuG-1 tumour cells was repeatedly observed in IEL and spleen cell preparations using the single-cell assay (Tables 1, 4). The total number of cytotoxic effector cells in the IEL obtained from the proximal two-thirds of the small intestine was about one-third that of the spleen. Considerable cytotoxicity towards target cells was also observed in the CRA, but the cytotoxicity of IEL in the CRA showed greater inter-experiment variability in ten experiments than did that of spleen cells. These variations are unexplained, but were not due to metronidazole, which did not alter the frequency of granular IEL (Table 5) or the cytotoxicity of IEL or spleen cells (not shown). Furthermore exposure of spleen cells to gut washings, DTT or Percoll did not affect their cytotoxicity (not shown). In contrast to the IEL, very low levels of cytotoxicity were detected in the CRA using cells from either the mesenteric lymph node or Peyer's patches (Table 1).

Specificity of natural cytotoxicity and measurement of ADCC

Cytotoxic cells in the IEL and spleen-cell preparations showed similar patterns of target cell selectivity (Table 2), with lysis of W/FuG-1 > K562 > RBL-5, P815 target cells. In addition, both cell populations exhibited ADCC against antibody-coated P815 cells. These characteristics have previously been shown for rat NK cells (Shellam & Hogg, 1977; Flexman & Shellam, 1980, 1981).

Table 2. Specificity of cytotoxicity. ^{51}Chromium release from target cells (cytotoxic units ±SE)

Group	W/FuG-1	P815	āP815*	RBL-5	K562
Spleen cells	56·0±5·2	6·8±0·5	55·3±1·6	7·6±1·0	26·2±1·1
IEL	35·8±3·2	1·4±0·3	43·5±2·6	5·8±0·6	15·0±1·1

* Antibody-coated P815 cells were used for measurement of ADCC. The lysis of all target cells was measured in a 4-hr CRA.

Table 3. Kinetics of lysis in the ^{51}CRA

Assay time	Cytotoxic units ±SE		
	Spleen cells	IEL	Ratio Spleen:IEL
4 hr	42·5±0·3	36·1±2·5	1·18
8 hr	59·7±1·6	49·5±1·9	1·21
18 hr	91·5±8·0	62·1±7·8	1·47

* Cytotoxicity was measured using W/FuG-1 target cells.

Kinetics of lysis

Cytotoxic cells were present in similar frequency in the IEL population and spleen, but IEL were less cytotoxic in the CRA (Table 1), suggesting that NK cells in the IEL population were either less lytically efficient or less able to take part in multiple lytic interactions than were NK cells in the spleen. Further evidence of a relative deficiency in cytotoxicity of IEL in the CRA is shown in Table 3, where spleen cells exhibited a greater rate of lysis of W/FuG-1 cells than did IEL.

The responsiveness of NK cells in the IEL population to IFN was examined by pretreatment of spleen cells and IEL with IFN before cytotoxicity was measured either by ^{51}Cr release (Table 4a) or in the single cell assay (Table 4b). IFN produced only a small increase in the cytotoxicity of IEL in either the 3-hr or 9-hr assay (Table 4a), while the cytotoxicity of spleen cells was enhanced at both times. The greater response of spleen cells to IFN was due to an increase both in lytic efficiency and in the frequency of cytotoxic effector cells compared with the IEL (Table 4b). Therefore the spleen has a greater frequency, and therefore a larger pool, of pre-NK cells than the IEL population.

Morphological studies on spleen cells and IEL

Fig. 1a illustrates granular cells in an IEL preparation which range from small to large lymphocytes with either a clear or a slightly basophilic cytoplasm. Typically, the majority of granular cells have a cluster of large basophilic granules in the cytoplasm. Some cells (e.g. Fig. 1a, arrow) contain fewer granules which are smaller and more scattered and others were non-granular. Differential counts are presented in Table 5. Treatment with metronidazole did not affect the ratio of granular to non-granular cells (Table 5).

Approximately 2% of spleen mononuclear cells contained fine basophilic cytoplasmic granules (Table 5). These are mainly medium-sized lymphoid cells with a pale cytoplasm, frequently with a slightly indented nucleus (Fig. 1b), closely resembling some of the finely granulated cells in IEL preparations (e.g. Fig. 1a,

Table 4. Effect of interferon on cytotoxicity*

(a) *^{51}Cr release assay*

	Group	Assay time (cytotoxic units ±SE) 3 hr	9 hr
Untreated:	Spleen cells	5·6±0·4	23·3±0·8
	IEL	2·4±0·2	23·1±0·8
IFN-treated:	Spleen cells	11·6±0·2	38·1±1·0
	IEL	3·7±0·3	25·2±1·3

(b) *Single cell assay*

	Group	TBC (%)	Assay time (frequency of C.E.) 2 hr	4 hr	8 hr	12 hr
Untreated:	Spleen cells	22·0	1·25	4·00	4·53	5·28
	IEL	20·7	0·99	3·95	4·64	4·80
IFN-treated:	Spleen cells	22·0	3·76	6·60	6·99	7·74
	IEL	20·7	1·57	4·91	5·69	5·63

* Cells were pretreated for 4 hr before assay with 4000 u/ml of IFN at 10^7 cells/ml. Experiments (a) and (b) were performed on separate occasions.

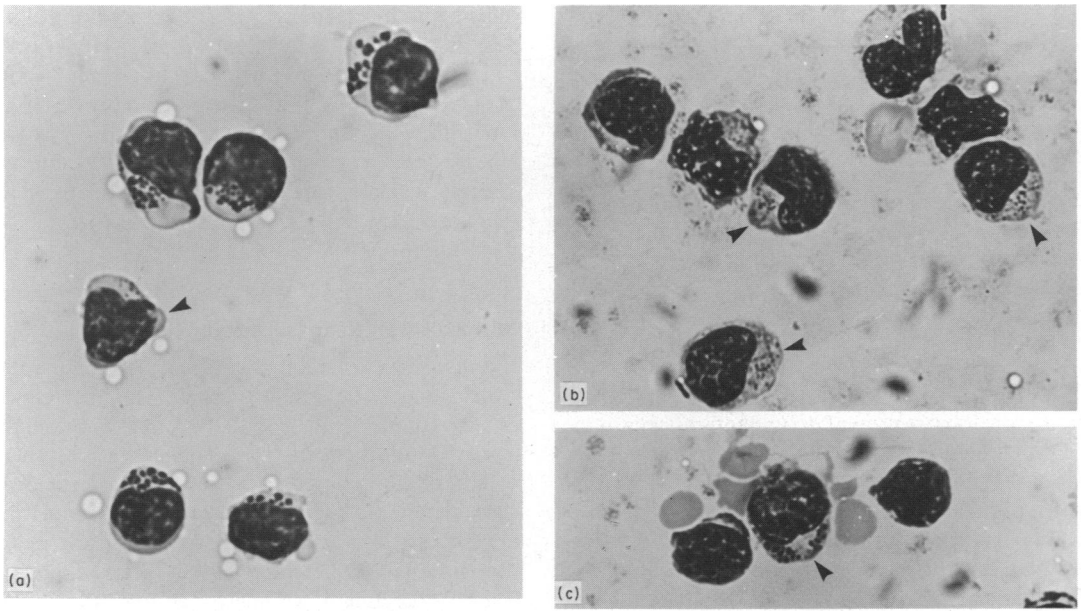

Figure 1. Giemsa-stained smears showing granular lymphoid cells.

(a) Rat IEL. Note the characteristic clusters of large basophilic cytoplasmic granules in most cells, which range in size from small to large-sized lymphocytes. One cell (arrowed) contains only a few fine granules. Only cells with large granules have been scored as typical granular IEL in differential counts.

(b) Rat spleen cells, showing three granular lymphoid cells (arrows). They are medium-sized lymphocytes, often with indented nuclei and contain a cluster of basophilic cytoplasmic granules. Most granular spleen lymphoid cells have smaller granules than those seen in typical granular IEL.

(c) A granular lymphoid cell from rat spleen (arrowed). The granules in this cell are large and closely resemble in size and number those seen in typical granular IEL. Such cells are rare in spleen compared to the more finely granular cells arrowed in B. All $\times 1100$.

Table 5. The percentage of granular cells in preparations of IEL and spleen lymphoid cells

		IEL differential count, % (300–400 cells)				
	Cell recovery $\times 10^{-7}$	Typical granular*	Few fine granules*	Total granular	Non-granular	Spleen cells differential count, % granular (800 cells)
	3·8	36·9	12·6	49·5	50·5	3·3
	5·9†	31·8	13·1	44·9	55·1	1·9
	3·6†	25·2	14·7	39·9	60·1	2·0
	4·7†	28·9	18·1	47·0	53·0	1·5
	4·4§	30·5	15·2	45·7	54·3	ND
	ND§	35·9	17·9	53·8	46·2	ND
	3·4†	36·6	11·5	48·1	52·0	ND
	1·7	40·3	14·6	54·9	45·1	ND
	5·1‡§	40·8	18·9	59·7	40·3	ND
	3·8‡	42·8	16·3	59·1	40·9	ND
mean	4·04 ± 0·4 (SE)	35·0 ± 1·8 (SE)	15·3 ± 0·8 (SE)	50·3 ± 2·0 (SE)	49·8 ± 2·0 (SE)	2·2 ± 0·4 (SE)

* Typical granular IEL had a cluster of characteristic large granules (Fig. 1a). Cells with only one to six small granules (arrowed Fig. 1a) or with fine granules scattered throughout the cytoplasm were counted separately.

† Mean yield per rat, calculated for pooled cells from two rats. Differential counts were performed on the pooled cells.

‡ Littermates, one treated with metronidazole and IEL prepared on the same day.

§ No metronidazole treatment.

arrowed). Rare spleen cells contain large basophilic granules and are similar in appearance to typical granular IEL (Fig. 1c).

Morphology of cytotoxic IEL

Cytotoxic cells in the IEL population were examined for evidence of cytoplasmic granules (Table 6). To avoid selective losses that can occur during cell-fractionation procedures, the morphology of TBC and lytic cells in conjugates was assessed directly on unfractionated cells, using AO to stain cytoplasmic granules. A considerable proportion of rat thoracic duct lymphocytes and spleen cells also contain one to two fine granules that stain by this method but not by the Giemsa method (unpublished observations) and macrophages are also stained by AO (Allison, Harrington & Birbeck, 1966). Therefore, more importance is attached to the absence of AO-stained granules than to their presence. Nevertheless, following AO staining, typical granular IEL are readily recognized by their characteristic cluster of large granules.

In unconjugated preparations, approximately 50% of the IEL contained fluorescent granules, in close agreement with estimations of total granular IEL in Giemsa-stained smears (Table 5), suggesting that the two methods detect the same cell populations. Approximately equal proportions of granular and non-granular IEL bound to W/FuG-1 target cells and following conjugate formation, approximately one-third of the leucocytes bound to dead tumour cells were granular and two-thirds were non-granular cells. Therefore, by direct observation some granular cells have clearly been shown to be cytotoxic, but not all cytotoxic cells were granular. Conversely, approximately 60% of granular IEL were bound to viable target cells. Therefore, among the IEL not all granular cells are cytotoxic under these assay conditions and some cells with natural cytotoxicity are non-granular.

Table 6. Morphology of unconjugated, target-binding and cytotoxic IEL*

1. By acridine orange staining, 47 and 52% of the unconjugated IEL were granular compared with 40–60% in Giemsa smears (Table 5).
2. Of all the TBC, 48 and 45% were granular on immediate testing using acridine orange, and 52% and 55% were non-granular respectively.
3. Of the IEL bound to dead target cells after 4 hr, 31% and 36% were granular, 69% and 64% were non-granular cells respectively.
4. Of the granular IEL bound to target cells after 4 hr, 40% were bound to dead targets, 60% to live targets†
5. In a representative experiment:†
   Frequency of C.E.‡ in the IEL = 4·00%
   Frequency of granular C.E. in the IEL = 1·25%
   Frequency of non-granular C.E. in the IEL = 2·75%

* Results of two experiments, except as indicated.
† Results from a single experiment.
‡ C.E., cytotoxic effector cells.

DISCUSSION

In this study, natural cytotoxicity was detected at higher levels in the IEL population than in any other gut-associated lymphoid tissue examined. These results confirm an earlier report of substantial NK activity in the IEL population in the guinea-pig, with low activities in the mesenteric lymph node and Peyer's patch cells (Arnaud-Battandier *et al.*, 1978). Lamina propria lymphocytes were not examined in the present study, but in the guinea-pig very little NK activity was found in this population (Arnaud-Battandier *et al.*, 1978). Thus, IEL exhibit the highest level of natural cytotoxicity of all the gut-associated lymphoid tissues. Gross contamination of IEL by cells from Peyer's patches or lamina propria is unlikely, as also suggested by the similar proportions of granular cells in preparations of IEL (approximately 35%) and in tissue sections of jejunum (approximately 30%; Mayrhofer, 1980a).

The natural cytotoxicity of the IEL closely resembled that of splenic NK cells in their selective pattern of tumour target cell lysis. Analysis of the frequency of cytotoxic cells using a single-cell assay showed IEL to contain naturally cytotoxic cells with a frequency similar to spleen cells, although IEL usually exhibited less cytotoxicity than spleen cells in the ^{51}Cr-release assay. IEL also exhibited substantial ADCC, a property of these cells also reported in the guinea-pig (Arnaud-Battandier *et al.*, 1978). Indirect evidence suggests that splenic NK cells in rodents also exhibit ADCC (Ojo & Wigzell, 1978; Flexman and Shellam, 1980, 1981). However, NK cells in the IEL population differ from splenic NK cells in two respects. Firstly, only weak stimulation of natural cytotoxicity was obtained in either the single-cell assay or in the ^{51}Cr-release assay by pretreatment of the IEL with IFN, suggesting that IEL are deficient as compared to spleen cells in interferon-inducible pre-NK cells.

Secondly, the IEL exhibited slower kinetics of target-cell lysis than spleen cells although the IEL and the spleen contained equal frequencies of cells that would form conjugates with target cells. This suggests that IEL were less efficient than spleen cells either in their lytic interactions with target cells or in their ability to recycle to new target cells after lytic interactions. The relative inability of IEL to respond to IFN, despite their relative richness in NK activity, appears to be a property of these cells not shared by NK cells in other lymphoid organs (Flexman & Shellam, 1983). It is possible that stimulation of local IFN production in the gut-associated lymphoid tissue by antigens or by polyclonal mitogens is responsible for the relatively high levels of endogenous NK activity in freshly isolated IEL, leaving few cells capable of responding to further exogenous IFN.

Approximately 35% of isolated IEL contain typical basophilic granules and a further 15% contain fine granules. In other studies, most NK activity in man (Timonen & Saksela, 1980; Timonen, Ortaldo & Herberman, 1981), rats (Reynolds *et al.*, 1981) and mice (Luini, Boraschi, Alberti, Aleotti & Tagliabue, 1981) has been associated with a cell fraction enriched in large granular lymphocytes of the type illustrated in Figure 1b. However, in these studies the cytotoxicity of NK cells was measured by ^{51}Cr-release assays on fractionated cells, where any contribution by the small number of non-granular cells could not be assessed. Direct examination of the morphology of IEL involved in lytic interactions with target cells using acridine orange staining showed that IEL with AO-positive granules could kill target cells, the morphology in many cases suggesting that the effector cells were typical granular IEL. More importantly, granular and non-granular cells were equally represented in the target binding population and 60% of the lytic interactions were mediated by non-granular IEL. The possibility that the latter cells could have become degranulated during the lytic interaction has not been excluded. The present study suggests that isolation and purification methods used by other workers may have led to selective losses of a non-granular subpopulation of cells with NK activity. This possibility can also be inferred by inspection of the differential counts of granular cells in spleen cell preparations only, where approximately 2% of mononuclear cells contained cytoplasmic granules. The majority of TBC and perhaps half of the cytotoxic effector cells in the spleen enumerated in the single cell assay are probably non-granular lymphoid cells.

It has been suggested that most IEL in the mouse (Guy-Grand, Griscelli & Vassalli, 1974) and man (Janossy, Tidman, Selby, Thomas, Granger, Kung & Goldstein, 1980) are T lymphocytes. However, recent studies have shown that granular IEL are rapidly regenerated in ATX.BM rats (Mayrhofer, 1980a), and that they are present in normal numbers in nude mice (Mayrhofer, 1980a) and in nude rats (Mayrhofer & Bazin, 1981). It has also been shown that IEL (granular and non-granular) are derived from donor cells in ATX.BM rats reconstituted with genetically marked bone marrow (Mayrhofer & Whately, 1983). It is now important to explore possible relationships between granular and non-granular IEL and also between granular IEL and granular cells in the spleen, the bone marrow and the blood. A relationship between granular IEL and mast cells remains possible.

The present studies strongly suggest a cytotoxic function for IEL. IEL are common in the gastrointestinal mucosa (Collan, 1972), and have been described in other epithelia (Jeffery & Reid, 1975). Infections with protozoa such as *Giardia* have been associated with increased numbers of IEL in the intestinal epithelium (Ferguson, 1977) and infestation of rats with *Nippostrongylus brasiliensis* leads to accumulation of IEL in the gut and an increase in the proportion of granular IEL (Pitts & Mayrhofer, unpublished observation). NK cells have been shown to destroy virus-infected cells (Santoli & Koprowski, 1979) and to inhibit the growth of *Cryptococcus neoformans* (Murphy & McDaniel, 1982), in addition to their well-known ability to lyse certain tumour cells. It is therefore speculated that IEL may function in the defence of epithelial surfaces against infective agents and neoplasia. As only a small proportion of the granular cells exhibited natural cytotoxicity to tumour cells, it seems possible that some may have natural effector functions against other forms of target and studies to investigate this possibility are in progress.

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