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Functional properties of lymphocytes isolated from murine small intestinal epithelium

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Summary. Intraepithelial lymphocytes (IEL) were isolated from murine small intestine using a modification of previously published procedures. Analysis of IEL by immunofluorescence using monoclonal antibodies showed they were predominantly Lyt-2$^+$ cells, with relatively few B cells or macrophages present. IEL cultured at sufficiently high cell densities proliferated in response to concanavalin A (Con A), phytohaemagglutin (PHA) and lipopolysaccharide (LPS). IEL were also capable of recognizing alloantigens in an *in vivo* graft-versus-host assay and in an *in vitro* mixed lymphocyte reaction. These studies therefore confirm that murine IEL contain cells with immunologic properties characteristic of typical T lymphocytes.

INTRODUCTION

Lymphocytes in the small intestine are found in Peyer's patches (PP), in the lamina propria (LP), and between the epithelial cells lining the villi. In contrast to lymphocytes from the LP and PP, the majority of murine IEL are Lyt-2$^+$ cells (Parrott *et al.*, 1983). In both rat and man, IEL are also predominately of the cytotoxic/suppressor phenotype (Selby *et al.*, 1981; Lyscom & Brueton, 1982). IEL are also unusual in that they contain a large proportion of granulated lymphocytes (Ferguson, 1977).

It is well known that B cells in the gut secrete IgA with specificity for enteric antigens. However, the role of T lymphocytes in the lamina propria, and especially in the epithelium, remains obscure. It is known, however, that the major stimulus for the appearance of lymphocytes in the intestine is the normal gut flora, because germ-free mice have significantly fewer LP cells (Crabbe *et al.*, 1970) and IEL (Ferguson & Parrott, 1972; Ropke & Everett, 1976) than conventionally reared animals.

Recently procedures have been developed for isolating mucosal lymphocytes from murine small intestine (Davies & Parrott, 1981; Tagliabue *et al.*, 1982). Lamina propria T cells can respond to mitogens and can also regulate B cell responses (Tseng, 1982). Specific cytotoxic T cells can also be detected in LP lymphocyte suspensions isolated from mice 5 days after priming with syngeneic tumour cells (Parrott *et al.*, 1983). Studies on the functional characteristics of IEL have focused on their cytotoxic and natural killer potential (Arnaud-Battandier *et al.*, 1978; Tagliabue *et al.*, 1982; Parrott *et al.*, 1983). Although these activities are present in IEL, their specific role in the mucosal immune response remains undefined.

Since the lymphoid populations found in the lamina propria and in the epithelium are phenotypically distinct, it is important that functional studies do not use mixtures of gut mucosal lymphocytes. In order to

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determine the functional characteristics of murine IEL we have used a protocol to isolate the most complete sampling of this population without contamination by either LP or PP cells. Using this procedure, we now show that murine small intestinal IEL respond to mitogens and can recognize alloantigens *in vitro* and *in vivo*.

MATERIALS AND METHODS

Animals

BDF_1 (C57Bl/6 × DBA/2) F_1 male mice, 5–10 weeks old, fed sterile Lablox Chow (Purina) and acidified water were used for all experiments. C57Bl/6 male mice, 5–10 weeks old, were used as donors in the graft-versus-host (GVH) experiments. C3H/HeJ male mice, 18 months old, were used as a source of splenic stimulator cells in the mixed lymphocyte reaction (MLR). All mice were from Jackson Laboratories, Bar Harbor, ME. Sprague-Dawley male or female rats, 12–16 weeks old, were used to make T cell-growth factor (TCGF).

Isolation of IEL

Small intestines were removed and prepared for isolation of IEL as previously described (Davies & Parrott, 1981). After washing each gut with 60 ml cold Ca^{++}- and Mg^{++}-free balanced salt solution (CMF), the PP, fat and mesentery were removed. The intestines were cut open longitudinally and then cut into 1–2 cm pieces. For isolating IEL, we modified previously published procedures (Davies & Parrott, 1981; Tagliabue *et al.*, 1982) as follows. Gut pieces were gently stirred at 37° in CMF with 10^{-4} M ethylenediamine tetraacetic acid (EDTA) for two 15 min intervals, followed by a series of 15 min incubations in sterile Hanks's balanced salt solution with 5% newborn calf serum, 25 mM HEPES, penicillin and streptomycin (HBSS/NCS). Supernatants were collected each 15 min until they no longer appeared cloudy (a total of six incubations were usually sufficient). The first two supernatants collected were spun at 500 r.p.m. for 5 min to remove large clumps of epithelial cells and debris. Although some lymphocytes were also lost, this preliminary step resulted in a greater recovery of live lymphocytes from subsequent steps in the purification procedure. The cells in all six supernatants were then washed once and cell viability determined by erythrosin B dye exclusion. Pooled supernatants were then washed through 300 mg glass wool columns with HBSS/NCS. To enrich for live IEL, we used a slight modification of a two step Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient previously described by Davies & Parrott (1981). Optimal separation of live lymphocytes was obtained by resuspending the cells ($1–5 \times 10^7$ live lymphocytes and a maximum of 4×10^7 dead cells) in 2 ml 1·057 g/ml Percoll and layering this over 1 ml 1·089 g/ml Percoll. Gradients were spun at 600 ***g*** for 20 min at 4° and cells were collected from the band forming at the 1·057/1·089 g/ml interface. Cells were washed three times in RPMI before culture.

Immunofluorescence

To test for surface immunoglobulin (sIg), cells were directly labelled with fluorescein conjugated $F(ab')_2$ goat anti-mouse IgG (Cappel Laboratories, Cochranville, PA). Monoclonal anti-Lyt-1 (53.7.313, rat IgG2a), anti-Lyt-2 (53.6.72, rat IgG2a), anti-Thy-1.2 (HO.13.4, mouse IgM), or anti-Mac-1 (rat IgG) were used as first step reagents to test for the corresponding surface antigens. Fluorescein-labelled $F(ab')_2$ goat anti-rat IgG or $F(ab')_2$ goat anti-mouse IgG (Cappel) were used as second step reagents. Incubations were at 4° for 15 min and all washes and dilutions were done in phosphate-buffered saline (PBS) with 0·1% sodium azide and 1% bovine serum albumin. In the two step procedure, cells were also tested with the second reagent alone to determine background binding levels. In each experiment, freshly isolated thymocytes, peritoneal exudate cells, or spleen cells were used as positive controls. The Mac-1 hybridoma was obtained from the Salk Institute, all other hybridomas used were obtained from the American Type Culture Collection Hybridoma Bank.

Cytotoxicity

One million lymphocytes were resuspended in 1 ml monoclonal anti-Thy-1.2 for 30 min on ice. Cells were quickly washed once in cold RPMI and resuspended in 3 ml of a 1/10 dilution of freshly thawed guinea-pig serum as a source of complement. After incubation for 30 min in a 37° water bath, cells were washed twice in RPMI and the number of viable cells remaining was determined by dye exclusion.

TCGF production

Rat spleen cells were teased into single cell suspensions, and erythrocytes were lysed with buffered ammonium chloride containing EDTA. The cells were then cultured at 5×10^6/ml in RPMI-1640 supple-

mented with 2·8 mM L-glutamine, 1·0 mM L-asparagine, 10 mM HEPES, 5×10^{-5} M 2-mercaptoethanol, 10% heat-inactivated foetal calf serum (Reheis Chemical Co., Phoenix, AZ), 50 μg/ml gentamycin (Schering Corp., Union, NJ), penicillin and streptomycin. Concanavalin A (Con A, Sigma C-2010) was added to a final concentration of 5 μg/ml. After 48 hr, supernatants were harvested, filter sterilized, and stored at −70°. TCGF activity was measured using an interleukin-2 (IL-2)-dependent cell line, CTLL-16 (kindly provided by Dr Dolores Byrne, Dept. of Biochemistry, Thomas Jefferson University). To prepare lectin-free TCGF, the Con A supernatants were first concentrated 10 times using a Diaflo PM10 membrane (Amicon Corporation, Lexington, MA) and were then absorbed with Sephadex G-50 (Pharmacia Fine Chemicals) as described by Tadakuma *et al.* (1976). To remove residual Con A, the supernatants were next treated as described by Schrader, Scollay & Battye (1983). Saturated ammonium sulphate (SAS) was added to the supernatant to make a 50% solution. After centrifuging to remove the precipitate, SAS was added to the supernatant to make an 80% solution. The precipitate was collected, redissolved in 40% SAS, and dialysed extensively against PBS. After this treatment the supernatants remained active when tested on CTLL-16 cells, and only stimulated minimal thymocyte proliferation in the absence of exogenously added Con A (not shown).

In vitro *proliferation of IEL's to mitogens and alloantigens*

For mitogen and MLR assays, cells were cultured in supplemented RPMI in a final volume of 200 μl in flat-bottomed 96-well microtitre plates (Linbro). Con A, phytohaemagglutinin-P (PHA-P; Difco) or *E. coli* 0111:B4 lipopolysaccharide (LPS; Difco) were diluted in RPMI and used at the concentrations indicated in the text. To prepare stimulator cells for the MLR, spleen cells at 2×10^7/ml were treated with 25 μg/ml mitomycin-C (Sigma) for 30 min at 37° in the dark. After two washes, $2{\cdot}5 \times 10^5$ mitomycin-C treated spleen cells were added per well. Allogeneic or syngeneic responder IEL were added at the cell doses indicated in the text. After 3 days incubation at 37° in 5% CO_2, 1 μCi [^{3}H-methyl] thymidine (New England Nuclear, Boston, MA) was added per well. Cells were collected on a Skatron automatic harvester (Flow Laboratories Inc., McLean, VA) 18–22 hr later. Filters were air-dried and counted in 4 ml Scintiverse II cocktail (Fisher Scientific, King of Prussia, PA) on a Packard Model 3330 scintillation counter. Each test group was assayed in triplicate, and each experiment shown was repeated at least three times with comparable results.

Graft-versus-host assay

IEL isolated from parental (C57BL/6) or F_1 (BDF_1) mice were resuspended in RPMI and 50 μl was injected into the rear footpads of adult BDF_1 mice. Seven days later the popliteal lymph nodes (PLN) were dissected out and weighed. The PLN index was calculated as the average weight of the nodes draining the footpads injected with allogeneic cells divided by the average weight of the nodes draining the footpads injected with syngeneic cells.

Staining methods

Smears were prepared using a Shandon cytocentrifuge and fixed in either absolute methanol for giemsa (Harleco) staining, or in Carnoy's fixative before staining with 0·5% toluidine blue (Sigma) in McIlvaines citrate buffer. Gut sections prepared from tissue preserved in 10% formol saline were stained with haematoxylin and eosin.

Statistics

All results are expressed as mean ± one standard error of the mean (SEM). Differences between means were determined by the unpaired Student's *t*-test.

Table 1. Isolation of murine IEL

Populations	Cell recoveries × 10^7 (±SEM)*		
	Pool of six supernatants	Glass wool columns	Percoll gradient†
Live lymphocytes	7·3±0·73	4·4±0·61	2·4±0·2
Live epithelial cells	4·9±2·0	1·5±0·45	0·3±0·06
Dead cells‡	21·4±5·6	10·4±3·5	0·8±0·12

* Mean recoveries (±one standard error) of IEL isolated from three BDF_1 mice in each of 11 experiments.

† Cells collected from band formed at the interface of 1·057 g/ml and 1·089 g/ml layers.

‡ All cells (lymphocytes and epithelial) that failed to exclude erythrosin B are included. Most dead cells at the 1·057/1·089 g/ml interface appeared to be lymphoid; the majority of epithelial cells (live and dead) were found in the band forming at the top of the 1·057 g/ml layer.

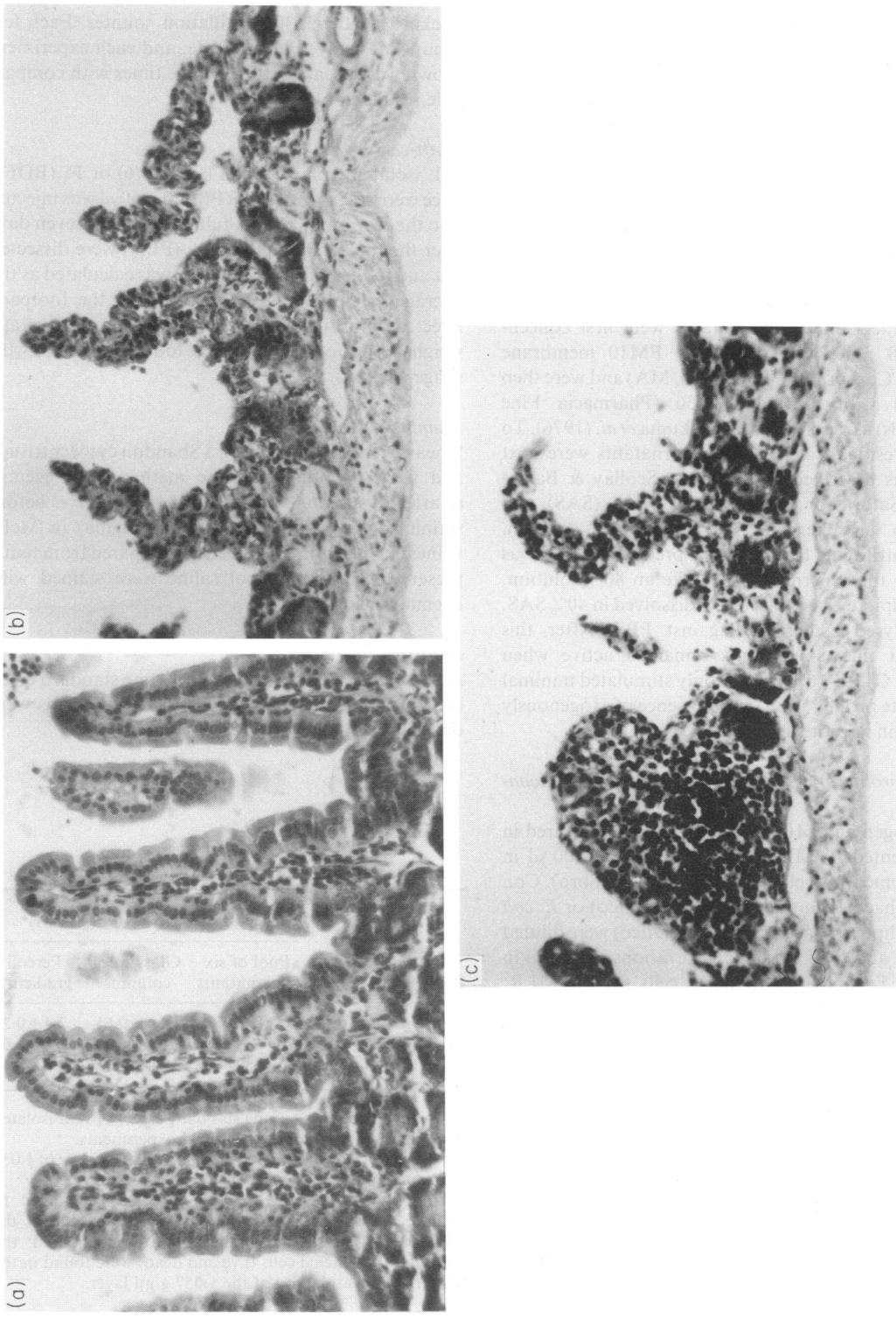

Figure 1. Sections of normal mouse small intestine (a) before and (b, c) after treatment to remove the epithelium. Note that the lymphoid nodule in (c) remained intact. (Haematoxylin and eosin × 280.)

RESULTS

Isolation procedure

Cell recoveries at the various stages of the isolation procedure are shown in Table 1. In the pool of six unpurified supernatants, live lymphocytes only represented about 22% of the total cells recovered. After purification on glass wool columns and on the Percoll gradient, 33% of the live lymphocytes present in the original suspension were recovered, while the dead cells were reduced in number by 96%. Histologic studies of gut pieces that had undergone only two incubations in CMF + 10^{-4} M EDTA showed that a large proportion of the epithelial layer was not removed by this treatment (not shown). However, with additional incubations (usually four) in HBSS/NCS, virtually the entire villus epithelium was removed while the lamina propria and microscopic lymphoid nodules remained intact (Fig. 1). Giemsa stained cytocentrifuge smears of freshly isolated IEL showed that 98% of the cells had a lymphoid morphology, and 37% (27·3–51·8) of these contained cytoplasmic granules. Eosinophils and cells resembling macrophages were occasionally seen.

Surface phenotype of IEL

The distribution of cell surface antigens on murine IEL is shown in Table 2. About 17% of IEL were Lyt-1$^+$ cells, and 57% were Lyt-2$^+$. Only 30% of IEL were Thy-1.2-positive by immunofluorescence, however about 70% of IEL were killed by monoclonal anti-Thy-1.2 plus complement (data not shown). Davies & Parrott (1981) also reported that about 66% of murine IEL were killed by a rabbit anti-mouse T cell serum and complement, while only 18–40% were positive by immunofluorescence using a monoclonal anti-Thy-1.2 antibody (Parrott *et al.*, 1983). The reasons for these differences are unclear.

Table 2. Surface phenotype of IEL determined by immunofluorescence

Surface phenotype	Mean percent positive (Range)*
sIg$^+$	5·9 (1·1–11·0)
Mac-1$^+$	3·3 (1·6– 3·7)
Thy-1.2$^+$	30·6 (27·0–34·0)
Lyt-1$^+$	17·2 (12·5–20·0)
Lyt-2$^+$	57·4 (47·5–72·2)

* A minimum of 200 cells were counted per group in each experiment. Percentages of Mac-1$^+$, Thy-1.2$^+$, Lyt-1$^+$, and Lyt-2$^+$ cells have been corrected for background as described in 'Methods'. Epithelial cells contaminating the IEL never displayed membrane fluorescence, and were disregarded from counts.

IEL contained a small percentage of cells positive for the Mac-1 antigen which is expressed to different degrees on macrophages from various sites (Springer *et al.*, 1979). IEL were also tested for the presence of adherent phagocytic cells (MacDonald & Carter, 1982), but none were detected. When peritoneal exudate cells were placed on the Percoll gradient, neither Mac-l-positive nor adherent phagocytic cells were depleted (data not shown).

Murine IEL respond to mitogens *in vitro*

IEL cultured at $2{\cdot}5 \times 10^5$/well or at higher densities proliferated when cultured with 5 μg/ml Con A, but the response was not as strong at lower cell concentrations (Fig. 2). When lectin-free TCGF was added to Con A stimulated cultures, proliferation could be detected at lower cell concentrations, and was also enhanced at higher cell concentrations when com-

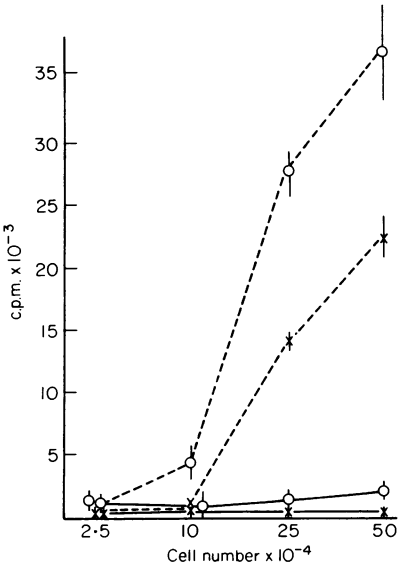

Figure 2. Proliferative response of IEL to Con A on day 4 of culture. Graded doses of IEL were cultured as described in 'Methods', with medium alone (×—×); 50% lectin-free TCGF (○—○), 5 μg/ml Con A (×- - -×) or 5 μg/ml Con A plus 50% lectin-free TCGF (○- - -○). Results represent the mean ± standard error of the mean (SEM) c.p.m. of triplicate wells.

Table 3. Proliferative response of IEL to Con A, PHA, and LPS

$2{\cdot}5 \times 10^5$ IEL cultured in	Exp. 1* c.p.m. (±SEM)	SI	Exp. 2 c.p.m. (±SEM)	SI
Medium	135±6	—	303±43	—
5 μg/ml Con A	4768±2276	35	42,983±506	142
10 μg/ml PHA	1632±182	12	4651±244	15
10 μg/ml LPS	24,106±3773	179	72,275±6489	239

* Results are shown as mean c.p.m. of triplicate wells ± the standard error on day 4 of culture. Stimulation indices (SI) were calculated by dividing the mean c.p.m. in cultures with mitogens by the mean c.p.m. in cultures with medium alone.

pared to IEL stimulated with Con A alone ($P < 0{\cdot}02$–$0{\cdot}01$). However, in the absence of Con A, the proliferative response to lectin-free TCGF was reduced by about 90%. IEL also responded to PHA and LPS (Table 3). The addition of lectin-free TCGF enhanced the proliferative response to PHA, but not to LPS (data not shown).

Phenotype of IEL cultured in TCGF

IEL could be maintained in culture with the addition of crude TCGF. After 1 week, the distribution of cell types was similar to that seen in freshly isolated IEL (Table 4). However, after several weeks, the Lyt-2^+ cells were no longer detectable. The cells remaining were rounded in suspension, non-adherent, and contained granules that stained metachromatically with toluidine blue. These are probably P cells which have been isolated from many lymphoid tissues including the gut mucosa (Schrader *et al.*, 1983).

Table 4. Surface phenotypes of IEL cultured in crude TCGF

Days in culture*	Percent positive† Thy-1.2	Lyt-1	Lyt-2	sIg
Exp. 1 8	ND‡	ND	47	3
Exp. 2 7	53	7	49	1

* In Exp. 1, IEL were cultured at 2×10^4 cells/0·2 ml in microtitre wells (15 wells pooled). In Exp. 2, IEL were cultured at $2{\cdot}5 \times 10^5$ or 5×10^5/ml in 24-well cluster dishes. Total input on day $0 = 7{\cdot}5 \times 10^5$ IEL; on day 7 viable IEL $= 1{\cdot}4 \times 10^6$.

† Determined by immunofluorescence.

‡ Not done.

Response to alloantigens

IEL were tested for their ability to recognize and respond to alloantigens in an *in vitro* one-way mixed lymphocyte reaction and in an *in vivo* graft-versus-host assay. For the MLR, freshly isolated BDF_1 ($H\text{-}2^{b/d}$) IEL from non-immune mice were cocultured with mitomycin-C-treated C3H/HEJ ($H\text{-}2^k$) spleen cells or with syngeneic stimulator cells, at several responder to stimulator ratios. After 4 days in culture, IEL cocultured with allogeneic stimulator cells showed a strong proliferative response in comparison to IEL cocultured with syngeneic stimulator cells ($P < 0{\cdot}01$–$0{\cdot}001$) (Fig. 3).

In the *in vivo* graft-versus-host assay, popliteal lymph nodes removed from BDF_1 mice that received C57BL/6 parental IEL by foot pad injection 7 days earlier were approximately two times the weight of nodes removed from BDF_1 mice injected with syngeneic cells (Fig. 4).

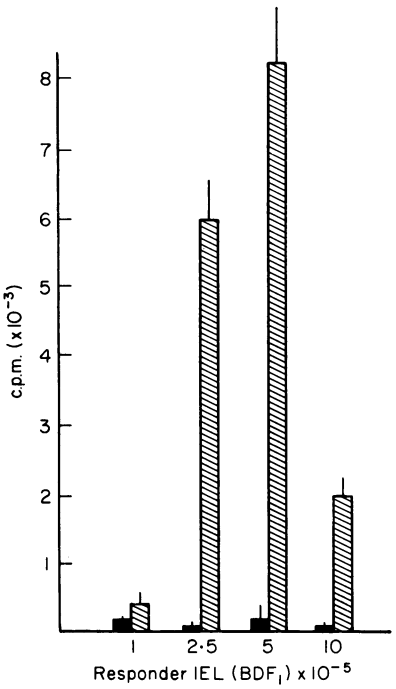

Figure 3. IEL recognize alloantigens in a MLR. Graded doses of BDF_1 IEL were cocultured with syngeneic or allogeneic mitomycin-C-treated splenic stimulator cells: (▨) $2{\cdot}5 \times 10^5$ mitomycin-C-treated C3H spleen cells; (■) $2{\cdot}5 \times 10^5$ mitomycin-C-treated BDF_1 spleen cells. Results shown represent the mean c.p.m. ± SEM of triplicate wells on day 4 of culture.

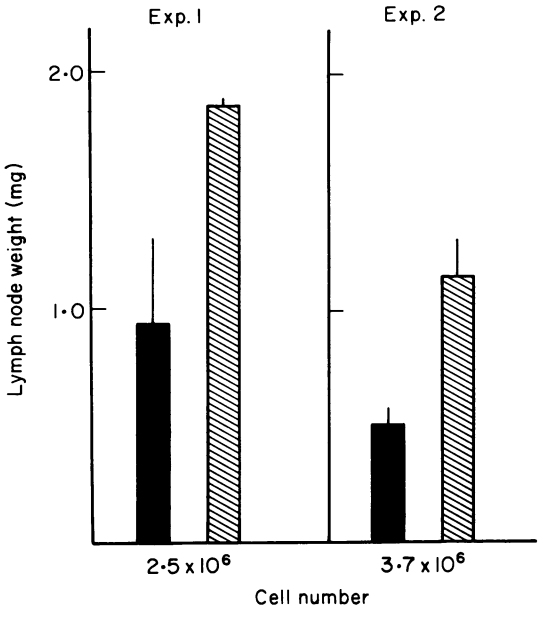

Figure 4. IEL mediate the GVH reaction. Mean weights (±SEM) of PLN seven days after injection of BDF_1 (■) or C57BL/6 (▩) IEL into BDF_1 recipient footpads. PLN index in Exp. 1 = 1·96 and Exp. 2 = 2·30. The difference between PLN weights in control versus experimental groups was significant only in Exp. 2 ($P < 0.01$).

DISCUSSION

In recent years it has become clear that IEL represent a heterogenous population of cells. IEL from normal, non-immunized mice contain mast cell precursors (Petit *et al.*, 1983; Schrader *et al.*, 1983) and also cells with natural killer cell activity (Tagliabue *et al.*, 1982). Earlier studies in mice showed that thymectomy resulted in decreased numbers of small intestinal IEL, although the effects were not dramatic (Ferguson & Parrott, 1972; Ropke & Everett, 1976). More recently, Mayrhofer & Whately (1983) have shown that there are bone marrow-derived cells in rat IEL, and suggest that although maturation of IEL may be T cell- or thymus-dependent, IEL themselves do not contain T cells. Despite this, the present study now provides strong evidence that murine IEL contain a substantial proportion of cells that have functional properties characteristic of T lymphocytes; that is, they have T cell surface markers and they proliferate in response to T cell mitogens and to alloantigens.

The question of whether IEL represent typical lymphocytes is further complicated by the range of Thy-1^+ or T cell marker-positive IEL cited by different investigators. Studies with human intestine have consistently shown very high percentages (80%) of T cell antigens on IEL (Janossy *et al.*, 1980; Selby *et al.*, 1981; Cerf-Bensussan, Schneeberger & Bhan, 1983; Greenwood, Austin & Dobbins, 1983). However, in studies in rats and mice, the proportion of IEL with T cell markers were reported as being high (80–90%) (Guy-Grand, Griscelli & Vassalli, 1978) intermediate (Lyscom & Brueton, 1982; Tagliabue *et al.*, 1982; Parrott *et al.*, 1983) or even absent (Meyrhofer & Whately, 1983). There is general agreement, however, that the majority of IEL bear surface antigens that characterize the suppressor/cytotoxic subset of T cells. It has been previously reported that some murine IEL bear the Lyt-2 antigen, but are Thy-1^- (Lyscom & Brueton, 1982; Parrott *et al.*, 1983). In the present study it was shown that 57% of IEL were Lyt-2^+, but only 30% were Thy-1.2^+ by immunofluorescence, thus confirming the presence of cells with this unique surface phenotype in the gut.

Much of the relevance of the present findings are dependent upon whether or not the cells isolated for study are in fact lymphocytes from the gut epithelium. Based on the histology, cell surface phenotype distribution, and the high percentage of granulated cells we feel that the cells we have isolated represent IEL with little or no contamination by other gut mucosal lymphocytes (e.g. Peyer's patches or lamina propria cells).

In previous work, Schrader, *et al.* (1983) separated Thy-1^+ cells from murine gut mucosa with a fluorescent activated cell sorter and found that these cells proliferated and gave rise to Thy-1^+ blasts when cultured with Con A and TCGF. However, the isolation procedure used in that particular study did not allow the authors to rule out significant contamination of the cell preparation by lamina propria cells. In the present study, IEL cultured at a relatively high cell density ($2{\cdot}5 \times 10^5/0{\cdot}2$ ml) responded to Con A, PHA, and LPS in the absence of exogenously added growth factors (Fig. 2, Table 3). The LPS response was quite high, considering the relatively low numbers of B cells in IEL. It is possible that non-B cells from IEL also contribute to the response as a result of prior *in vivo* exposure to LPS, though no conclusions on this point can be drawn from the present study.

Since it was necessary to add an exogenous source of IL-2 (lectin-free Con A supernatants) to induce pro-

liferation at lower cell densities, it is probable that either macrophages or IL-2 producing T cells are limiting under these conditions. This may explain why other investigators failed to detect mitogen responses by human IEL at low cell numbers of 5×10^4 per culture (Greenwood *et al.*, 1983). It is, however, surprising that the addition of TCGF did not result in responsiveness in that particular study. In an earlier study, murine IEL were also reported to be unresponsive to Con A or to allogeneic stimulator cells (Guy-Grand *et al.*, 1978). However, the procedures used for isolating IEL and for assaying their *in vitro* responses to mitogens and alloantigens differed considerably from those used in the present study. In addition, unpublished studies from this laboratory suggest that contamination of the final IEL preparation by epithelial cells can result in non-responsiveness as a result of non-specific toxicity. In addition to cellular contamination, non-cellular materials carried over from the gut could also interfere with the *in vitro* responsiveness of IEL.

In order to determine whether IEL also responded immunologically in an antigen-specific fashion, the ability of IEL from C57BL/6 mice to mediate a graft-versus-host reaction against the semi-syngeneic BDF_1 strain was tested. With the cell doses of IEL injected, the results were not dramatic, and only one of two experiments showed a significant difference between test and control groups ($P < 0{\cdot}01$) (Fig. 4). However, in a one-way MLR performed in microculture, BDF_1 IEL proliferated vigorously in response to allogeneic (C3H/HeJ) but not syngeneic mitomycin-C-treated stimulator cells (Fig. 3), demonstrating that IEL are capable of recognizing and responding to alloantigens.

These studies thus show for the first time that murine IEL contain immunologically active T lymphocytes. Since IEL decrease dramatically in number when the gut is rendered antigen- and/or germ-free (Ferguson & Parrott, 1972; Ropke & Everett, 1976; Reynolds & Morris, 1983) it is possible that intraepithelial T lymphocytes have specificities for bacterial and other gut antigens as do LP and PP lymphocytes. In view of the fact that IEL are predominantly Lyt-2^+ cells, their role *in vivo* could involve down-regulation of mucosal immune responses, or specific cytotoxicity reactions toward virally infected cells, tumour cells, gut bacteria or parasites. Alternatively, IEL may represent a unique population of cells with functional potentials that differ from those classically associated with peripheral lymphocytes.

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