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Examination of the low proliferative capacity of human jejunal intraepithelial lymphocytes

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SUMMARY

The proliferation of human jejunal intraepithelial lymphocytes (IEL) was examined to determine how it differed from that of peripheral blood (PB) T lymphocytes. The IEL were mainly T lymphocytes of the cytotoxic-suppressor ($T8^+$) phenotype. They demonstrated lower proliferative responses to various stimuli ($2,501 \pm 565$ ct/min with phytohaemagglutinin; PHA) compared to unseparated PB T lymphocytes ($73,678 \pm 2,495$) or the $T8^+$ subset ($68,939 \pm 10,053$ ct/min) ($P < 0{\cdot}001$). This low proliferative response was also a characteristic of the $T8^+$ T lymphocytes in the lamina propria ($4,606 \pm 1,226$ ct/min) but not the $T4^+$ subset ($43,447 \pm 10,188$ ct/min) ($P < 0{\cdot}05$).

These findings were not due to isolation techniques or to differences in kinetics. Mixing experiments revealed that the IEL did not contain cells which suppressed proliferation. In addition, the IEL could be stimulated by mitogens, as they produced the same amount of interleukin 2 (IL-2) and IL-2 receptors as did PB T lymphocytes. Although the lectin-induced proliferative response of IEL was unaltered by the addition of autologous macrophages and minimally increased by IL-2, it was markedly enhanced by the addition of sheep red blood cells (SRBC). The enhancing effect of SRBC was not due to T cell recognition of xenogenic antigens on the erythrocytes since neither allogeneic non-T lymphocytes nor other xenogenic erythrocytes produced the same effect. Both intact SRBC and membrane fragments from osmotically lysed cells augmented lymphocyte proliferation. Thus, jejunal IEL could be activated by mitogen and proliferated as much as PB T lymphocytes if exposed to a membrane component found on SRBC.

Keywords intraepithelial lymphocytes intestinal mucosa T lymphocyte proliferation

INTRODUCTION

The human intestinal mucosa is rich in lymphocytes. These cells are divided into two groups anatomically, those interspersed among epithelial cells and those in the lamina propria. The intraepithelial lymphocytes (IEL) are almost entirely T lymphocytes of the $T8^+$ cytotoxic-suppressor phenotype with rare macrophages and B cells (Cerf-Bensussan, Schneeberger & Bhan, 1983; Selby *et al.*, 1984; Cerf-Bensussan, Guy-Grand & Griscelli, 1985). The lamina propria lymphocytes (LPL) contain 45 to 91% T lymphocytes (largely of the $T4^+$ helper phenotype) as well as variable percentages of B cells, macrophages, and null cells (Bookman & Bull, 1979; Eade *et al.*, 1980; MacDermott *et al.*, 1981; Cerf-Bensussan *et al.*, 1983; Selby, *et al.*, 1984).

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Although the IEL contain a large number of T lymphocytes, they demonstrate only a minimal proliferative response to alloantigens or mitogens (Greenwood, Austin & Dobbins, 1983). Furthermore, they possess no natural killer activity in man even though many contain cytoplasmic granules (resembling natural killer cells) (Cerf-Bensussan *et al.*, 1985). Despite this lack of activity *in vitro*, the IEL are more abundant in such jejunal enteropathies as celiac sprue (Ferguson, 1974; 1977) or giardiasis (Ferguson, McClure & Townley, 1976) than in normal bowel, suggesting that they have a pathophysiologic role in certain disease states.

This study evaluates the ability of normal jejunal IEL to proliferate in response to mitogens or alloantigens. Since over 90% of the jejunal lymphocytes were T cells, proliferation in response to phytohaemagglutinin (PHA) was studied as a simple model system to evaluate T cell function. The IEL had low proliferative responses to this potent mitogen compared to PBL. This finding was further dissected to determine whether it was due to incomplete T cell activation by mitogen, to a scarcity of macrophages, or to lack of soluble factors.

MATERIALS AND METHODS

Patients. Jejunal tissue was obtained from 15 patients undergoing gastric bypass operations. There were nine females and six males in the study with a mean age of 35. Peripheral blood was drawn from the patients so that autologous lymphocytes or macrophages could be studied along the jejunal cells. Peripheral blood was also obtained from healthy volunteers to serve as controls.

Digestion of jejunal mucosa. The jejunal tissue was immediately emersed in RPMI-1640 medium (GIBCO, Grand Island., NY, USA) containing 10% heat-inactivated fetal calf serum (FCS) (Microbiological Associates Bioproducts, Walkersville, MD, USA), 10 mM *N*-2 hydroxyethylpiperazine-*N*'2 ethanesulfonic acid (HEPES) (Sigma Chemical Co., St Louis, MO, USA), 2 mM L-glutamine (GIBCO, Grand Island, NY, USA), and 1% antibiotic-antimycotic solution containing 100 u/ml penicillin, 0·25 μg/ml fungizone, and 100 μg/ml streptomycin (GIBCO, Grand Island, NY, USA) (nutrient medium). The mucosal surface was rinsed vigorously with phosphate buffered saline, bluntly dissected from the submucosa, and then minced. Tissue occupying no more than a 5 ml volume was placed in each 50 ml plastic tube (Corning Glass Works, Corning, NY, USA). The minced tissue was incubated in 10 ml of 1 mM dithiothreitol (DTT) (Sigma Chemical Co., St Louis, MO, USA) in calcium and magnesium free Hanks buffered salt solution (GIBCO, Grand Island, NY, USA) containing 5% FCS, antibiotic-antimycotic solution, and HEPES (serum supplemented CMF-HBSS) at 37°C, 95% air-5% CO_2 for 15 min and then washed twice with unsupplemented CMF-HBSS. The tissue was then incubated in 10 ml of 0·75 mM ethylenediamine tetraacetic acid (EDTA) (Sigma, Chemical Co., St Louis, MO, USA) in supplemented CMF-HBSS for a total of 270 min in a 37°C shaking water bath (160 oscillations per minute) (Precision Instruments, GCA Corporation, Chicago, IL, USA). After each 45 min incubation, the tissue was washed twice with CMF-HBSS and the cells in the supernatant collected. The cells released by the first three treatments were saved to obtain the IEL, while cells from the last three were discarded. The tissue was then digested with 10 ml of a solution of 0·01% deoxyribonuclease (DNAase) and 20 u/ml collagenase (Worthington Scientific, Malver, PA, USA) in nutrient medium for 3 h in a 37°C shaking water bath (Bull & Bookman, 1977). A wire mesh was put over the remaining tissue, pressed, and scraped with forceps. The resulting suspension was passed over a 100-mesh wire grid (Thomas Scientific Co., Philadelphia, PA, USA) to remove cell clumps and debris and served as the source of LPL. Cell count and viability (trypan blue exclusion) were determined for the nonerythroid cells in each preparation.

Isolation of jejunal lymphocytes by Percoll gradient. To separate lymphocytes from epithelial cells, the IEL or LPL (10 to 40 × 10^6 cells) were resuspended in a 16 × 125 mm tube (Corning Glass Works, Corning, NY, USA) in 2 ml of 100% Percoll, containing 9 parts Percoll (Pharmacia Fine Chemicals, Piscataway, NJ, USA) and 1 part 10 × Hanks buffered salt solution (HBSS) (GIBCO, Grand Island, NY, USA) adjusted to pH 7·4 and osmolality 290 mOsm/kg H_2O. Above this were successively layered 2 ml each of 60, 40 and 30% Percoll, all prepared from 100% Percoll diluted with 1 × HBSS and adjusted to pH 7·4. The tube was spun at 400 ***g*** for 20 min at room temperature,

and the cells immediately above the 60% layer were collected and washed (Tagliabue *et al.*, 1981).

Isolation of peripheral blood lymphocytes (PBL), T lymphocytes, and subsets. Peripheral blood lymphocytes were isolated from heparinized blood by Ficoll density gradient centrifugation (Ebert *et al.*, 1984). PB T lymphocytes were separated by nylon wool columns as previously described (Julius, Simpson & Herzenberg, 1973; Ebert *et al.*, 1984). The $T4^+$ and $T8^+$ T lymphocyte subsets from PB or lamina propria were isolated by antibody and complement lysis (Ebert *et al.*, 1985a).

Immunofluorescence. Indirect immunofluorescence (Ebert *et al.*, 1984; 1985a; Ebert, 1985b) was performed using the mouse monoclonal antibodies OKT11, OKT4, OKT8, B1, Mo2, Ia, (Coulter Immunology, Hialeah, FL, USA), Leu-7 (Becton-Dickinson, Mountain View, CA, USA) or Tac (generous gift of Warner Greene), followed by goat anti-mouse IgG conjugated with fluorescein isothiocyanate (Coulter). At least 200 cells were counted with a Zeiss fluorescent microscope to determine the percentage of positive cells. In some experiments, mean fluorescent intensity of positive cells was measured by a flow cytometer (Ortho System 50 Cytofluorograf).

T lymphocyte proliferation in response to various stimuli. Peripheral blood lymphocytes, IEL, or LPL (2×10^5/well) were cultured in triplicate in flat-bottomed Microtest tissue culture plates (No. 3072, Falcon Labware, Oxnard, CA, USA) in 0·2 ml nutrient medium containing 1 μg/ml PHA (Burroughs Wellcome Co., Greenville, NC, USA), 22 μg/ml Concanavalin A (Con A) (ICN Pharmaceuticals, Inc., Cleveland, OH, USA), or 12·5 u/ml recombinant interleukin 2 (IL-2) (Amgen Corp., Thousand Oaks, CA, USA) (units as defined by the manufacturer). Alternatively, mitomycin-C treated allogeneic PBL (2×10^5) were added to produce an allogeneic mixed lymphocyte reaction (Ebert *et al.*, 1985a). T cells were cultured at 37°C in a humidified atmosphere of 95% air-5% CO_2 for 4 days with mitogens, 5 days with allogeneic cells, and 4 or 8 days with IL-2. Twelve hours before termination of the culture, 1 μCi of tritiated thymidine (^{3}H-Tdr) (2 Ci/mmol, Schwarz/Mann, Spring Valley, NY, USA) was added to each well. At the end of the incubation period, the cultures were harvested (PHD Cell Harvester, Cambridge Technology, Cambridge, MA, USA) and the samples counted in a liquid scintillation spectrophotometer (Ebert, *et al.*, 1984).

Various supplements were added to PHA-activated IEL to try to augment their proliferative response: 20% autologous PB macrophages isolated by Petri dish adherent (Ebert, 1985b), IL-2 (125 u/ml), or supernatants from activated macrophages or PBL. The first supernatant was prepared by incubating 5×10^5 macrophages/ml with 20 μg/ml lipopolysaccharide (LPS) (Sigma Chemical Co., St Louis, MO, USA). The culture medium was collected after 24 h of incubation at 37°C and passed through a 0·45 μm filter (Nalgene Labware, Nalge, Co., Rochester, NY) before storage at −70°C. To obtain the second supernatant, PBL (1×10^6/ml) were incubated with PHA (1 μg/ml) for 2 h at 37°C, washed extensively, and then incubated at 1×10^6/ml in nutrient medium for 24 h. The culture medium was collected and filtered. In some experiments, neuraminidase-treated sheep, bovine (GIBCO, Grand Island, NY, USA) or human red blood cells (SRBC, BRBC, or HRBC) (either intact or osmotically lysed) were added to jejunal lymphocytes at a 50:1 ratio as described previously (Ebert, 1985b).

Measurement of suppression by mixing experiments. To test for suppressor cells in the IEL, PB T lymphocytes (5×10^4) were cultured with or without various numbers of IEL in 200 μl of medium containing 1 μg/ml PHA (final concentration). Proliferation was measured by tritiated thymidine incorporation after a 5-day incubation. The control cultures were IEL with PHA and IEL with PB T lymphocytes.

IL-2 production and assay. T lymphocytes or IEL (1×10^6/ml) were incubated with 1 μg/ml PHA at 37°C in 95% air-5% CO_2 for 24 h in 12 × 75 mm culture tubes (Fisher Scientific, Pittsburgh, PA, USA). The supernatant, which was collected, filtered, and stored −70°C, was then tested for IL-2 activity using a CTLL II line (gift of Dr Defreitus, Wistar Institute, Philadelphia, PA and originally from Dr Kendall Smith) (Gillis *et al.*, 1978). The level of IL-2 was determined by probit analysis at 50% of the IL-2 standard, 1 unit/ml of human recombinant IL-2.

Statistical analysis. Control and test values were analysed by calculating arithmetic means and standard error of means for each set of data and by comparing paired data using the two-tailed Student's *t*-test (Winer, 1979).

RESULTS

Characterization of jejunal lymphocytes

Two populations of lymphocytes were isolated from jejunal mucosa: the intraepithelial lymphocytes (IEL) and the lamina propria lymphocytes (LPL). The IEL contained 91·0±1·2% T lymphocytes, almost entirely of the cytotoxic-suppressor phenotype ($T8^+$) (Table 1). None reacted with anti-Tac antibody which detects the IL-2 receptor. There were no B cells ($B1^+$) or natural killer cells (Leu 7^+) and rare macrophages ($Mo2^+$) (0·3±0·2%; three experiments). Similarly, this preparation contained only 0·8±0·3% Ia^+ ($HLA\text{-}Dr^+$) cells (such as B cells, macrophages, or activated T cells; three experiments). The LPL contained 89·0±9·0% T lymphocytes, 45±4% $T4^+$ (helper) and 45±9% $T8^+$, with an average T4/T8 ratio of 1·4±0·5. (In contrast, the T4/T8 ratio of colonic LPL was greater, 2·9±1·1, $P<0·05$.) It is unlikely that the $T8^+$ T lymphocytes in the jejunal LPL were contaminating IEL since the LPL before percoll separation contained 85±3% $T11^+$ cells. This indicates that the EDTA treatment removed most of the epithelial cell layer (including the IEL). In addition, paraffin sections of jejunum after EDTA treatment revealed little residual epithelium, as shown previously (Greenwood, *et al.*, 1983).

Proliferation of jejunal or PB T lymphocytes in response to a variety of stimulants

The IEL, LPL, and PB T lymphocytes were cultured with PHA, Con A, or mitomycin-C treated allogeneic cells, and the resulting proliferation was measured. Kinetics experiments revealed that the jejunal lymphocytes demonstrated their highest mitogen-induced proliferative response when cultured at 2×10^5 cells/0·2 ml for 4 days. Proliferation of either IEL or LPL in response to these stimuli was significantly less than that of PB T lymphocytes ($P<0·001$) (Fig. 1). Recently, IL-2 alone has been shown to trigger PBL or LPL proliferation, with a maximal response around day 8 (Fiocchi & Youngman, 1985; Hauptman *et al.*, 1985). When IL-2 was used as the stimulus, the proliferation of LPL was equivalent to that of PB T lymphocytes whether the duration of culture was 4 or 8 days, while the response of IEL to IL-2 was significantly less ($P<0·001$).

T cell subsets from the LPL were then studied. The $T4^+$ fraction proliferated vigorously in response to PHA (43,477±10,188 ct/min) whereas the $T8^+$ fraction did not (4,606±1,226 ct/min) ($P<0·05$), suggesting that the low proliferative response was common to all intestinal $T8^+$ cells, not just the IEL. Subsequent experiments were performed with IEL only, since the LPL $T8^+$ cells were tedious to isolate.

Table 1. Characterization of jejunal T lymphocytes*

	IEL (per cent positive cells, mean±s.e.)	LPL (per cent positive cells, mean±s.e.)	PBL (per cent positive cells, mean±s.e.)
T11	91±1 (4)†	89±9 (3)	70±3 (6)
T4	5±2 (3)	45±4 (6)	45±4 (3)
T8	80±6 (3)	45±9 (6)	26±4 (3)
Tac	0±0 (2)	0±0 (2)	0±0 (4)

* The following lymphocyte markers on IEL, LPL, or PBL were detected by immunofluorescence using a fluorescent microscope: T11 (on T lymphocytes); T4 (on helper T lymphocytes); T8 (on cytotoxic-suppressor T lymphocytes); Tac (on T and B lymphocytes with IL-2 receptors).

† The number of experiments performed are in parentheses.

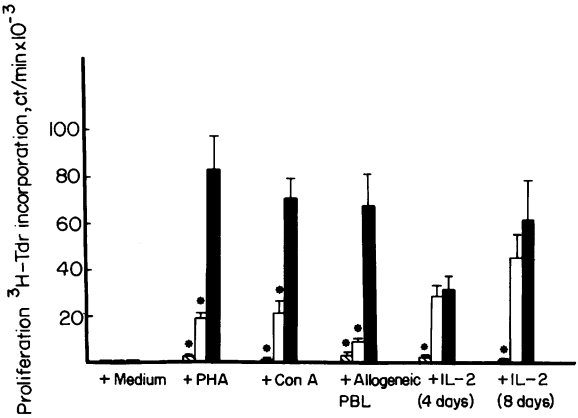

Fig. 1. Jejunal and PB T lymphocytes proliferation in response to a variety of stimulants. IEL, LPL, or PB T lymphocytes (2×10^5/well) were cultured for 4 days with medium, PHA (1 μg/ml), or Con A (22 μg/ml); for 5 days with mitomycin-C treated allogeneic cells (2×10^5/well); or for 4 or 8 days with IL-2 (12·5 u/ml). Proliferation was measured by total ct/min of ^{3}H-Tdr incorporation (mean ± s.e.). Each bar represents the mean of at least five experiments. Values significantly less than control are marked. (▨) IEL; (□) LPL; (■) PBT; (*) $P < 0{\cdot}001$.

The low proliferation of jejunal IEL was not due to cell damage caused by the mucosal digestion. Viability of cultured lymphocytes was similar, whether from the jejunum or the PB. In addition, two experiments revealed that PBL treated with EDTA proliferated as well as untreated PBL (not shown).

Relationship of the low IEL proliferation to their T8$^+$ cytotoxic-suppressor phenotype

Perhaps the low IEL proliferation is related to their T8$^+$ phenotype. However, T8$^+$ T lymphocytes obtained from PB had the same PHA-induced proliferative response as the T4$^+$ T cell subset or unseparated T cells (68,939 ± 10,053 ct/min; 83,909 ± 14,658 ct/min, and 73,678 ± 2,495 ct/min, respectively), as shown previously (Meuer *et al.*, 1982).

Mixing experiments were then performed to determine whether jejunal lymphocytes contained a subpopulation of cells capable of suppressing proliferation. Tritiated thymidine uptake of PB T lymphocytes, cultured with PHA, was the same whether or not autologous IEL were added at a 1:1 ratio (Fig. 2). Similar results were obtained when IEL were added to PB T lymphocytes at ratios of 0·5:1 or 1:0·5. The proliferation of the control cultures, IEL with PHA and IEL with PB T cells, was always less than 200 ct/min. These experiments show that low IEL proliferation was not due to spontaneous suppressor activity.

Generation of IL-2 and IL-2 receptors by PHA-activated IEL

Since T cell proliferation depends upon the generation of IL-2 and its receptor, diminished production of either could account for the low PHA-induced IEL proliferation. T lymphocytes were cultured for 24 h with PHA and the supernatant tested for IL-2 activity using an IL-2 dependent mouse cell line (CTLL assay). Jejunal IEL produced the same amount of IL-2 as PB T lymphocytes (Fig. 3). The T8$^+$ subset of PBL produced less IL-2 (11 ± 5 u/ml) than the unseparated PB T cells or jejunal IEL, but this difference did not reach statistical significance.

The presence of IL-2 receptors was measured by immunofluorescence using anti-Tac antibody. The percentage of Tac$^+$ cells was the same for PHA-activated IEL or PB T lymphocytes (Fig. 3). Moreover, the density of the IL-2 receptors, as judged by the distribution of the intensity of fluorescence obtained in histograms of flow cytometric analysis, was comparable for PHA-activated PB T lymphocytes and IEL in each of three experiments (not shown). The T8$^+$ PBL developed a similar percentage of Tac$^+$ cells after PHA stimulation (48 ± 16%).

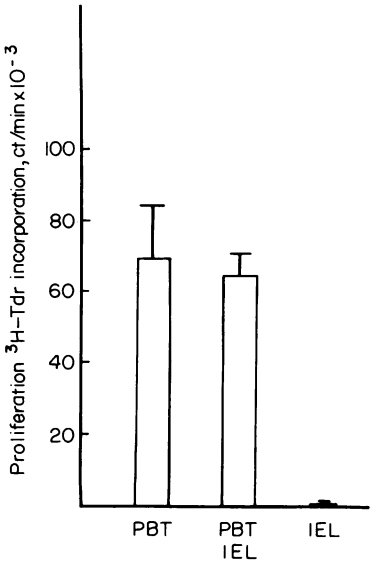

Fig. 2. Mixing experiments to measure suppressor activity in IEL. PB T lymphocytes (5×10^4/well) were cultured with 1 μg/ml PHA with or without autologous IEL (5×10^4/well). In four experiments, proliferation was measured after a 5-day culture by ^{3}H-Tdr incorporation, total ct/min, mean ± s.e. Mitogen-induced PB T cell proliferation was unchanged by the addition of IEL.

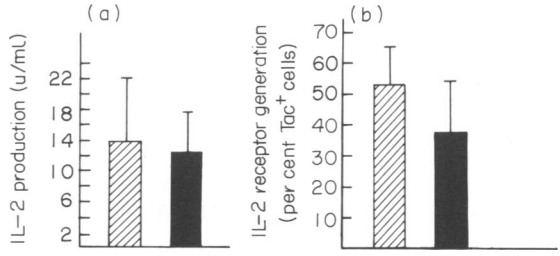

Fig. 3. IL-2 production and receptor generation by jejunal IEL and PB T lymphocytes. (a) IEL or PB T lymphocytes (1×10^6/ml) were cultured with PHA (1 μg/ml) for 24 h. The culture medium was then collected and tested for IL-2 activity using the CTLL assay (four experiments). (b) IEL and PB T lymphocytes, activated with PHA for 24 h, were tested for IL-2 receptor generation by immunofluorescence with anti-Tac antibody. The percentage of Tac$^+$ cells is depicted (mean ± s.e.) (six experiments). (■) IEL; (■) PBT.

Effect of supplements on jejunal IEL proliferation

PHA-activated IEL were supplemented with autologous PB macrophages, IL-2, or supernatants from activated macrophages or PBL. Neither the macrophages nor the supernatants significantly increased the PHA-induced proliferation of IEL (Table 2). The addition of IL-2 increased PHA-induced IEL proliferation from 2,501 ± 565 ct/min to 8,658 ± 1,486 ct/min ($P < 0{\cdot}001$), but not to levels reached by mitogen-stimulated PB T lymphocytes ($P < 0{\cdot}001$). None of these supplements significantly altered the proliferation of PBL to PHA.

The effect of SRBC on IEL proliferation was then measured. Previous experiments revealed that SRBC enhanced the proliferation of PB T lymphocytes when cocultured with those stimulants which result in low levels of proliferation, e.g. autologous cells, soluble antigens, or suboptimal amounts of mitogen (Ebert, 1985b). When the stimulus produced high levels of proliferation (such

Table 2. Effect of supplements on jejunal IEL proliferation

IEL + PHA*	Proliferation: ^{3}H-Tdr incorporation (ct/min)	
+ medium	2,501 ± 565 cpm	(10)†
+ IL-2	8,658 ± 1,486‡§	(5)
+ macrophages	2,730 ± 1,107	(4)
+ macrophage sup't	4,561 ± 1,189	(3)
+ PBL sup't	2,394 ± 848	(3)
+ SRBC	53,158 ± 9,854§	(6)
+ SRBC ghosts	42,928 ± 3,731§	(4)
+ BRBC	13,967 ± 2,440§	(5)
+ BRBC ghosts	4,066 ± 3,955	(3)
+ HRBC	2,193 ± 111	(3)
+ allogeneic PBL	14,903 ± 1,528§	(5)

* IEL (2×10^5 cells per microwell) were cultured with PHA (1 μg/ml final concentration) for 4 days with the supplements listed. Proliferation was the total ct/min, mean ± s.e.
† The number of experiments performed are in parentheses.
‡ Values significantly greater than that of the control (IEL + PHA + medium) are marked (§$P < 0{\cdot}001$). The responses with SRBC or SRBC ghosts were significantly greater than that with any other supplement ($P < 0{\cdot}03$).

as optimal doses of mitogens), there was no further enhancement with SRBC. Here, SRBC markedly increased the PHA-induced proliferation of IEL (53,158 ± 9,854 ct/min) so that it reached the same level as that of PHA-activated PB T lymphocytes supplemented with SRBC (Table 2) (53,158 ± 9,854 ct/min). Such a marked increase in proliferation also occurred when SRBC were added to Con A- or IL-2-activated IEL (not shown).

Xenogenic antigens on the surface of the SRBC may stimulate lymphocytes. However, the IEL proliferated minimally when cultured with SRBC alone (663 ± 222 ct/min). Perhaps, SRBC activate lymphocytes to a small extent, and this effect may be potentiated by a strong mitogenic stimulus. To evaluate this, IEL were cultured with mitogen and allogeneic cells since allogeneic Ia determinants are potent stimuli of T lymphocytes. PHA and mitomycin C-treated allogeneic cells had a synergistic effect on the proliferation of jejunal IEL (2,501 ± 565 ct/min with PHA; 2,651 ± 1,425 ct/min with allogeneic cells; 14,903 ± 1,528 ct/min with both combined) (five experiments) (Table 2), but this stimulation was significantly less than that induced by mitogen and SRBC ($P < 0{\cdot}03$). This suggests that the SRBC effect is due to more than simply T cell recognition of foreign cell surface antigens.

To determine whether the SRBC effect was common to other erythrocytes, BRBC were added to T cell cultures. Jejunal IEL proliferation with PHA and BRBC was similar to that with PHA and allogeneic cells, but much less than that with PHA and SRBC ($P < 0{\cdot}01$) (Table 2). The addition of HRBC did not significantly alter the proliferation of IEL to PHA. These experiments suggest that the SRBC effect could not be duplicated by all types of erythrocytes. To determine whether intact cells or membrane fragments alone were required to augment T lymphocyte proliferation, SRBC were osmotically lysed and then added to the cultures. The SRBC ghosts produced the same

enhancing effect as intact cells. In contrast, BRBC ghosts had no effect on the proliferation of mitogen-stimulated lymphocytes.

DISCUSSION

Intraepithelial lymphocytes are found between the basal part of intestinal epithilial cells throughout the gastrointestinal tract (Ferguson, 1977). Their numbers increase in certain jejunal enteropathies such as celiac sprue, tropical sprue, giardiasis, and cow's milk intolerance, but are normal in inflammatory bowel disease (Ferguson, 1974; Montgomery & Shearer, 1974; Ferguson, *et al.*, 1976; Ferguson, 1977; Phillips *et al.*, 1979). This suggests that the elevated IEL counts are not just the result of nonspecific inflammation but rather may reflect a specific role that IEL may have in certain disease processes.

Analysis of tissue section revealed that the majority of human IEL are T lymphocytes, over 80% of which have the cytotoxic-suppressor phenotype (OKT8$^+$) (Cerf-Bensussan, *et al.*, 1983; Selby, Janossy & Jewell, 1981; Selby, *et al.*, 1984). There are no B cells and rare macrophages. Characterization of the IEL isolated in this study agrees with results from tissue sections. The LPL from the proximal jejunum contained a lower T4/T8 ratio than that from the colon, $1{\cdot}4 \pm 0{\cdot}6$ and $2{\cdot}9 \pm 1{\cdot}1$, respectively. Previous estimates of T4/T8 ratios, mostly derived from colon or distal ilium, range from 1·8 to 3·1 (Greenwood *et al.*, 1983; Fiocchi *et al.*, 1984; Selby *et al*, 1984), agreeing with the data obtained here with colonic mucosa.

This study examines IEL proliferation. The jejunum was chosen since the same area of bowel could be obtained during gastric bypass operations from young individuals with no underlying malignancy or other immunosuppressive disorders. The proliferation of IEL in response to a variety of stimuli was significantly less than that of PB T lymphocytes, as shown previously (Greenwood *et al.*, 1983). LPL demonstrated a lower response to mitogens compared to PB T lymphocytes, a finding not uniformly agreed upon. Other studies found LPL proliferation to be the same (MacDermott *et al.*, 1981), higher (Goodacre & Bienenstock, 1982), or lower (Bull & Bookman 1977; Bland *et al.*, 1981; Greenwood *et al.*, 1983) than that reported here. The differences could be attributed to various culture conditions (dose of mitogen, cell concentration etc) or to the type of tissue studied (jejunum or colon). When T cell subsets from the lamina propria were isolated, the T4$^+$ cells demonstrated brisk proliferation, equivalent to PB T lymphocytes, while the T8$^+$ LPL proliferated poorly. Thus, the low proliferative responses were found with all jejunal T8$^+$ cells, not just with IEL. The only other known compartment of poorly proliferating T lymphocytes is the immature cortical thymocytes (Lopez-Botet & Moretta, 1985), which are T11$^+$ and T6$^+$, but T3$^-$. In contrast, IEL do have T3 antigens (Cerf-Bensussan *et al.*, 1985). The IEL response was evaluated and found not to be due to the enzymatic dissociation of the mucosa or to a shifted kinetic curve. Despite their T8$^+$ phenotype, these cells displayed no spontaneous suppressor cell activity.

In order for T lymphocytes to proliferate, they must first be activated by mitogens or antigens or by IL-2 alone (Hauptman *et al.*, 1985). Cell division depends upon the generation of the soluble polypeptide, IL-2, and the development of IL-2 receptors. Once IL-2 binds to its receptor, proliferation is triggered. The jejunal IEL were activated by PHA and produced the same amount of IL-2 and IL-2 receptors as did PB T lymphocytes, and yet they proliferated minimally. Even the addition of IL-2 or autologous macrophages to the cultures did not reconstitute the response, as shown previously (Greenwood *et al.*, 1983). Perhaps, the IEL generate IL-2 receptors with a low affinity for IL-2 (Robb, Greene & Rusk, 1984), a possibility presently being investigated. Alternatively, these cells may not produce all the necessary growth-promoting factors.

The IEL were able to proliferate briskly when cultured with SRBC and PHA. These red cells also enhanced the proliferation of PB T lymphocytes stimulated by autologous cells, soluble antigens, or suboptimal amounts of mitogen, but not resting T lymphocytes (Ebert, 1985b). The PBL proliferation was augmented by either intact or osmotically lysed SRBC. The effect occurred even if SRBC were added on day 2 of a 5-day culture of PB T lymphocytes and PHA, suggesting that they augment T cell division rather than activation. Finally, the action of SRBC was dependent upon an available SRBC or T11 receptor on the T lymphocyte since it was blocked by anti-T11a

antibody. This was demonstrated by culturing PB T lymphocytes with PHA, washing them, and then reculturing with IL-2 or IL-2 and SRBC, with or without anti-T11a antibody. Only the SRBC-induced enhancement of proliferation was blocked by the antibody, not the IL-2-driven response. Thus, the intact SRBC or its membrane fragments attach to the T11 receptor on activated PB T lymphocytes and promote cell division.

Similarly, IEL, activated by PHA, Con A, or IL-2, proliferated vigorously with the addition of intact or osmotically-lysed SRBC, an effect not reproduced by BRBC. Presumably, the SRBC effect on IEL also required an available T11 receptor. Although the proliferation of PHA-activated IEL could be greatly enhanced by SRBC and blocked by anti-T11a, the specificity of this response could not be demonstrated since PHA-activated IEL responded minimally to IL-2. It is likely, however, that the mechanism of SRBC enhancement would be the same whether PB T lymphocytes or IEL were studied.

When PB T lymphocytes are cultured with two monoclonal antibodies directed at distinct epitopes of the T11 receptor ($T11_2$ and $T11_3$), they become activated and proliferate briskly (Meuer *et al.*, 1984). In the SRBC enhancing effect, the SRBC may attach to the epitope recognized by anti-$T11_3$ which appears only after the T lymphocyte has been activated. SRBC may trigger cell division in a similar manner to these T11 monoclonal antibodies. The effect of these antibodies on IEL proliferation will be tested once they become commercially available.

The SRBC enhancing effect on IEL proliferation may serve as a convenient technical tool. For example, it may be a necessary component in the cloning of antigen-activated IEL. In addition, since the SRBC enhancing effect is much more marked on IEL than on PBL, the IEL would be a good model to use in the study of this interaction. The SRBC may alter some known structure, such as the affinity of IL-2 receptors for IL-2. Alternatively, the membrane component on SRBC may resemble some naturally-occurring growth factor, either produced by cells or found in the intestinal lumen. Since epithelial cells bind SRBC (Ebert, 1985c), these factors may be anchored close to the IEL and result in IEL proliferation *in vivo*.

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