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# T lymphocytes in the intestinal epithelium and lamina propria of mice

M. R. McDERMOTT,* P. HORSEWOOD,* D. A. CLARK† & J. BIENENSTOCK* *Host Resistance Program, Departments of †Medicine and *Pathology, McMaster University Health Sciences Centre, Hamilton, Ontario, Canada*

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## SUMMARY

Mesenteric lymphoblasts have a predilection to localize selectively in the murine small intestine within 24 hr after adoptive transfer. In this report, we quantify the localization and intraintestinal distribution of radiolabelled mesenteric lymph node (MLN) T and B blasts in relation to the *in situ* distribution of intestinal T and B cells which were detected immunohistochemically. Our results show that, within 24 hr after transfer, MLN T blasts localized predominantly in the intestinal epithelium and villus lamina propria, whereas B blasts were found mostly in the basal lamina propria of the gut. In the epithelium and villus lamina propria, 100% and 68%, respectively, of labelled were of thymic origin; this cell type comprised 54% of labelled cells in the basal lamina propria. This pattern of localization was the reverse of the distribution of T lymphocytes and B-cell derived plasmacytes residing in the intestinal wall. These results suggest that MLN T lymphocytes may be a component of common mucosal immunological system and may be integrated with peripheral immunity according to the immunological needs of the host.

## INTRODUCTION

The predominance of immunoglobulin A (Ig)-synthesizing plasmacytes beneath secretory epithelia is an important feature of mucosal immunity (reviewed in Befus & Bienenstock, 1982; McDermott, Befus & Bienenstock, 1982). The precursors of these cells originate in the Peyer's patches (PP) (Cebra *et al.*, 1977) and the bronchus-associated lymphoid tissue (Rudzik *et al.*, 1975b). As PP progenitors mature, they become committed to IgA synthesis and migrate to mesenteric lymph nodes (MLN) (Roux *et al.*, 1981; McWilliams, Phillips-Quagliata & Lamm, 1977). Many lymphocytes derived from the MLN undergo blastogenesis and migrate through the thoracic duct (TD) and the circulation, and selectively localize in the small intestine and other mucosal sites (McWilliams, Phillips-Quagliata & Lamm, 1975; Befus & Bienenstock, 1982; McDermott *et al.*, 1982a). These observations have led to the concept of a common mucosal immunological system that links together the gut and distant mucosae through the migration of IgA plasma cell precursors (Befus & Bienenstock, 1982; McDermott *et al.*, 1982a).

Correspondence: Dr Mark R. McDermott, Host Resistance Program, Dept. of Pathology, McMaster University Health Sciences Centre, Hamilton, Ontario L8N 3Z5, Canada.

Abbreviations: EB/FDA, ethidium bromide/fluorescein diacetate; IEL, intraepithelial lymphocytes; Ig, immunoglobulin; LP, lamina propria; MLN, mesenteric lymph nodes; NK, natural killer; PBS, phosphate-buffered saline; PLN, peripheral lymph nodes; TD, thoracic duct.

Whether the mucosal immune system similarly contains various populations of mucosally restricted T lymphocytes is not clear. Cells bearing Thy 1.2 antigen are present in the intestinal lamina propria (LP) (McDermott & Bienenstock, 1979; Rudzik *et al.*, 1975a), and many intraepithelial lymphocytes (IEL) in the intestine and lungs of humans and rodents appear to be T cells (Davies & Parrott, 1981; Ernst, Befus & Bienenstock, 1985a). Indeed, T lymphoblasts derived from MLN and TD selectively localize in the intestine (Guy-Grand, Griscelli & Vassalli, 1978; McDermott, Clark & Bienenstock, 1982b; Heyworth & Owen, 1982), and some IEL in the gut may be derived from PP (Guy-Grand *et al.*, 1978). Many lymphocytes in the intestinal lumen and in the respiratory epithelium and lumen may also be of thymic origin (Heyworth & Owen, 1982; McDermott *et al.*, 1985). However, all of these reports have used mixed populations of lymphocytes in studies of cell localization in the intestine, and none has provided a quantitative assessment of the intestinal T lymphocyte population in regard to site or origin and intravillus distribution. Such an assessment is important, given the apparent variety of T-cell activities present in the intestine (Befus & Bienenstock, 1982; McDermott *et al.*, 1982a) and the abundance of cells synthesizing IgA, an isotype which is highly T-cell dependent (Ebersole, Taubman & Smith, 1979; Elson, Heck & Strober, 1979; Tomasi, 1983).

In this report, we have quantified the distribution of T lymphocytes in the intestinal wall. Further, we have assessed the localization of MLN-derived T and B lymphocytes in this site. Our results show that there is a differential distribution of these cells within different regions of the murine intestinal mucosa,

both in terms of localization and distribution, and that MLN-derived blasts in the epithelium are all of thymic origin.

## MATERIALS AND METHODS

*Purification of lymphocytes*

Suspensions of MLN cells were prepared from 6–12-week-old CBA/J female mice as previously described (McDermott & Bienenstock, 1979). Enrichment of cell suspensions in Thy 1.2-bearing lymphocytes (operationally designated T cells) was accomplished by the petri-dish purification method of Wysocki & Sato (1978). Briefly, the cells were incubated for 30 min in a 1:20 dilution of an anti-mouse Ig reagent consisting of a mixture of affinity chromatography-purified rabbit antisera (Litton Bionetics Inc., Kensington, MD) specific for mouse IgA, IgG, IgM and k-chain. Cells were washed by centrifugation in medium and incubated on plastic bacteriological dishes coated with an anti-rabbit IgG reagent. This reagent was prepared by absorption of a goat anti-rabbit globulin serum (Cedarlane Laboratories Ltd., Hornby, Ontario) to a Sepharose CL-6B column (Pharmacia Canada Ltd, Montreal, Quebec) conjugated with purified rabbit IgG (Bethell *et al.*, 1979) and subsequent elution of goat antibodies at low pH. Non-adherent cells were recovered from dishes by repeated rinsing with medium and recovered by centrifugation.

Routinely, greater than 95% of the recovered cells were specifically lysed by anti-Thy 1.2 serum plus complement (Clark & McDermott, 1978) using ethidium bromide and fluorescein diacetate (EB/FDA) viability stains (Takasugi, 1971). Flow cytometric analysis (Ortho Spectrum III Flow cytometer; Ortho Diagnostic Systems Inc., Westwood, MA) revealed that more than 98% of these cells were stained with fluorescein-conjugated monoclonal anti-Thy 1.2 serum (New England Nuclear, Boston, MA). Furthermore, less than 1% of these cells showed detectable surface membrane staining with fluorescein-conjugated goat anti-rabbit IgG (heavy and light chain-specific) serum (Litton Bionetics Inc.) when assessed by ultraviolet light microscopy or by flow cytometry. Fewer than five cells remaining on each petri-dish were sensitive to lysis by *in situ* treatment with anti-Thy 1.2 serum plus complement when examined by low magnification ultraviolet microscopy after EB/FDA staining. The recovered cells were operationally defined as T cells.

Enrichment of lymphocytes bearing surface Ig (operationally designated B cells) was achieved by treatment of the MLN cell suspension with anti-Thy 1.2 serum and complement (Clark & McDermott, 1978; Takasugi, 1971). Non-viable cells formed clumps during the radiolabelling incubation period at 37° described later, and these clumps were subsequently removed by filtration of the labelled suspension through a 400-mesh stainless steel wire screen, followed by sedimentation over fetal bovine serum (McDermott & Bienenstock, 1979). Routinely, more than 95% of these cells stained with fluorescein-conjugated goat anti-mouse Ig serum (Litton Bionetics Inc.) and less than 2% were stainable with fluoresceinated monoclonal anti-Thy 1.2 serum (New England Nuclear) as assessed by flow cytometry. The viable proportion of cells in the purified T- or B-cell suspensions always exceeded 97%.

*Autoradiographic detection of lymphoblasts*

Unseparated MLN cell preparations, or the purified T- and B-cell fractions of MLN, were radiolabelled for 90 min at 37° with [$^3$H] thymidine and injected intravenously into syngeneic recipient mice (McDermott & Bienenstock, 1979). The recipients' intestines were removed 22–24 hr later and processed for autoradiographic analysis (McDermott & Bienenstock, 1979). Cell smears prepared from the donor cell inocula were used to estimate the numbers of labelled cells transferred. The frequency of labelled cells seen in the basal, villus and epithelial morphological regions of the villus crypt unit was determined (Mirski *et al.*, 1981).

*Immunohistochemical detection of lymphocytes*

Lymphocytes were detected in lyophilized duodenal tissue sections taken from healthy CBA/J mice by using the avidin–biotin complex (ABC) peroxidase staining method. Small intestines were removed, flushed of faeces using phosphate-buffered saline (PBS), and cut into 1-cm lengths. Tissues were rapidly frozen in isopentane cooled in liquid nitrogen and were placed into a lyophilization vessel together with additional liquid nitrogen and paraffin wax pellets (Paraplast®, M.P. 56–57°; Canlab, Toronto, Ontario). Lyophilization was conducted for 12–18 hr at 6·65 Pa. The vessel was removed with maintenance of its internal vacuum and the wax was melted at 56–59° using a shaking waterbath. Tissues were infiltrated with molten wax under vacuum for 30–60 min, embedded in blocks and sectioned at 4–5 μm. Sections were placed directly on albumin-coated slides, briefly heated to 60° and stored overnight at 37° to ensure tissue adherence to the slides in subsequent steps.

Tissues were dewaxed by agitation in ice-cold xylene for 3 min and sequentially rinsed in ice-cold 95% ethanol and PBS. Sections were immunochemically stained using an ABC kit purchased from Vector Laboratories Inc., Burligame, CA. The primary reagents were a 1:200 dilution of rabbit anti-mouse IgA (Litton Bionetics Inc.) or a 1:10 dilution of monoclonal anti-Thy 1.2 (Becton-Dickinson Monoclonal Centre, Mountain View, CA); both of these reagents were diluted with 2% bovine serum albumin in PBS and subsequent steps were conducted according to the kit instructions. Coverslips were applied and the specimens were examined at a magnification of 400 diameters. The frequency of stained cells seen in the basal, villus and epithelial morphological regions of the villus-crypt unit was determined (Mirski *et al.*, 1981). Control experiments (data not shown) indicated that this staining procedure with anti-Thy 1.2 specifically revealed T lymphocytes in the splenic periarteriolar lymphatic sheaths. Similar studies with IgA-specific antisera showed IgA plasmacyte staining in the lamina propria (LP) that was consistent with that seen by immunofluorescence (McDermott & Bienenstock, 1979). Very few cells were revealed by the IgA antiserum on sections of spleen.

## RESULTS

Table 1 shows the frequencies of radiolabelled cells on intestinal tissue taken from mice 22–24 hr after adoptive transfer of [$^3$H] thymidine-labelled MLN lymphoblasts. Preliminary experiments (data not shown) and other published work (Guy-Grand, Griscelli & Vassalli, 1974; Guy-Grand *et al.*, 1978) indicated that 50–65% of MLN lymphocytes were Thy 1.2-bearing lymphocytes, and that the relative proportions of T and B MLN lymphoblasts were almost equal. Therefore, in order to transfer an approximately equal number of either Thy 1.2- or Ig-bearing

**Table 1.** Distribution of [$^3$H]thymidine-labelled mesenteric Thy 1·2- and Ig-bearing lymphoblasts in the small intestine 22–24 hr after adoptive transfer into syngeneic mice*

| | Cells transferred | | Radiolabelled cells per 1000 high-power microscopic fields§ | | | |
|---|---|---|---|---|---|---|
| | Surface phenotype† | Number radiolabelled‡ | Total | Basal lamina propria | Villus lamina propria | Villus epithelium |
| Experiment 1 | | | | | | |
| Unseparated | 57% Thy 1.2$^+$<br>37% Ig | $8{\cdot}2\times10^6$ | $252{\cdot}2\pm18{\cdot}6$ | $177{\cdot}1\pm19{\cdot}7$ | $64{\cdot}2\pm0{\cdot}5$ | $10{\cdot}1\pm1{\cdot}1$ |
| Separated | < 1% Ig$^+$<br>> 95% Thy 1.2$^+$ | $3{\cdot}9\times10^6$ | $144{\cdot}5\pm20{\cdot}3$<br>(42·7) | $81{\cdot}9\pm12{\cdot}1$<br>(53·7) | $54{\cdot}2\pm7{\cdot}2$<br>(15·6) | $9{\cdot}7\pm2{\cdot}1$<br>(0) |
| Experiment 2 | | | | | | |
| Unseparated | 62% Thy 1.2$^+$<br>34% Ig$^+$ | $1{\cdot}61\times10^6$ | $97{\cdot}8\pm8{\cdot}2$ | $62{\cdot}8\pm6{\cdot}7$ | $26{\cdot}3\pm1{\cdot}2$ | $8{\cdot}9\pm2{\cdot}2$ |
| Separated | > 96% Ig$^+$<br>< 1% Thy 1.2$^+$ | $0{\cdot}72\times10^6$ | $37{\cdot}6\pm8{\cdot}9$<br>(61·6) | $28{\cdot}8\pm9{\cdot}6$<br>(54·1) | $8{\cdot}8\pm3{\cdot}7$<br>(67·2) | 0<br>(100) |

* $n=5$ CBA/J female mice per group.

† Thy 1.2-bearing cells were purified by absorption of Ig-bearing cells onto anti-immunoglobulin-coated petri-dishes. Ig-bearing lymphocytes were prepared by treating the unseparated cell preparation with anti-Thy 1.2 serum plus complement. The unseparated lymphocyte inoculum in Experiment 2 was treated with complement alone. Figures represent the approximate percentage of cells displaying a particular surface phenotype.

‡ The number of labelled cells in the inocula was calculated from the percentage of labelled cells detected autoradiographically in smears prepared from each cell suspension. The number of separated Thy 1.2- and Ig-bearing cells transferred was approximately equal to their number present in the unseparated inocula. Differences in the numbers of labelled cells transferred between Experiments 1 and 2 represent routinely observed differences in the number of labelled cells present in the donor cell inocula prepared for different experiments.

§ The methods used to enumerate labelled cells in the morphological regions of the intestinal wall are detailed in reference 23. Figures represent the arithmentic mean ± standard error. Percentages in parentheses represent the mean reduction in the frequency of separated labelled cells compared to that of the unseparated control preparation.

lymphocytes in the separated or unseparated cell inocula, the numbers of purified donor cells of each type were adjusted accordingly. In Experiments 1 and 2, transfer of unseparated cells resulted in the localization of labelled lymphoblasts in the gut wall in frequencies predicted by the dose of transferred cells (Mirski *et al.*, 1981). Removal of Ig-bearing lymphocytes from the inoculum (Experiment 1) resulted in a 43% decrease in the total number of labelled cells localized in the intestine, a finding consistent with the dimunition in the total number of donor lymphoblasts transferred (Mirski *et al.*, 1981). However, there was considerable disparity in the contribution of each morphological region of the intestinal wall to the total decrease in the frequency of labelled cells observed. In the basal LP, the frequency of labelled cells was reduced by 54% but in the villus LP this reduction was only 16%; the frequency of labelled cells observed in the basal and villus epithelium was similar. Thus, the unequivocal ratio of Thy 1.2- to Ig-bearing lymphoblasts in the morphological regions of the intestinal wall MLN suggested that a greater proportion of T than B lymphoblasts localized in the villus lamina propria, and that donor MLN lymphoblasts in the epithelium were exclusively T cells. Surprisingly, no T lymphoblasts were found in the epithelium over the crypts. Lymphoblasts localized in the lower half of the villus epithelium appeared to be randomly distributed.

Support for these findings was acquired in an anti-thetical way. Experiment 2 in Table 1 shows that, in comparison with the transfer of unseparated MLN cells, transfer of purified MLN Ig-bearing cells resulted in a 54% decrease in the frequency of labelled cells found in the basal LP, and in the villus LP this frequency was reduced by 67%. No labelled cells were ever found in the intestinal epithelium after transfer of purified Ig-bearing lymphoblasts. Two independent experiments confirmed the findings presented in Table 1.

Taken together, these results suggest that approximately equal proportions of MLN-derived T and B lymphoblasts localized in the basal parts of the LP, although three- to four-fold more T than B MLN lymphoblasts were found 24 hr after transfer in the higher villus LP. Furthermore, only MLN-derived T and not B lymphoblasts entered the intestinal epithelium above the basement membrane.

In order to determine whether the distribution of T and B cells occurring naturally in the intestinal wall correlated with the localization pattern of MLN-derived T and B lymphoblasts, immunochemical staining methods were used. Figure 1 shows

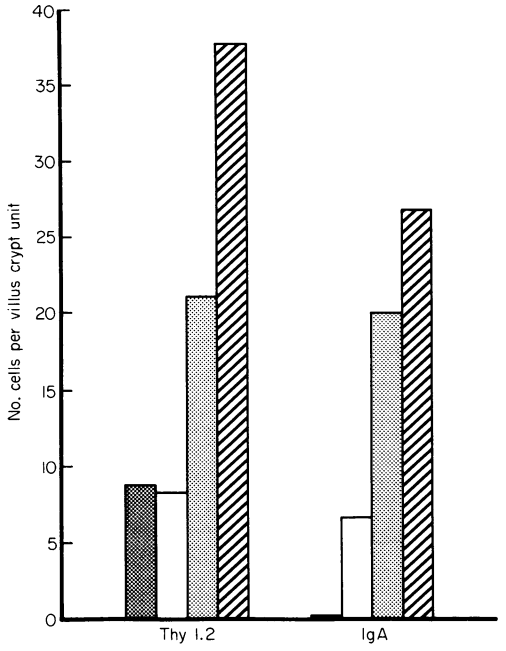


**Figure 1.** Frequency of T lymphocytes and IgA plasma cells in various morphological regions of the mouse small intestine. T lymphocytes bearing Thy 1.2 antigen were detected by an avidin-biotin-horseradish peroxidase immunochemical staining procedure using biotinylated anti-Thy 1.2 monoclonal antiserum. IgA-containing plasma cells were detected in a similar manner using rabbit anti-mouse IgA and biotinylated anti-mouse Ig. (■) Epithelium; (□) basal lamina propria; (□) villus lamina propria; (□) total villus crypt unit. Data shown are based on the examination of 80 villus-crypt units.

the frequency of Thy 1.2-bearing lymphocytes detected in the intestinal wall. The frequency of IgA-containing plasmacytes is also shown, since the majority of these cells are believed to have differentiated from MLN B lymphoblasts and few IgG-containing cells are seen in the mouse intestine (Befus & Bienenstock, 1982; McDermott & Bienenstock, 1979). Large numbers of T cells were found in the intestine, with approximately 55% of the total in the villus LP and 21% in both the basal LP and epithelium. Furthermore, about 74% of IgA plasma cells were found in the villous LP, 14% in the basal LP and none in the epithelium. These results are in striking contrast to the distribution of labelled T and B cells localizing in the intestinal wall (Table 1).

## DISCUSSION

In this paper, we show that, 24 hr after adoptive transfer, the proportion of MLN-derived T lymphoblasts is greater than MLN-derived B lymphoblasts in the intestinal epithelium and villus lamina propria. These results (summarized in Table 2) are in contrast to those found with immunohistochemical techniques.

The mechanisms responsible for the apparent differential localization of MLN T and B lymphoblasts throughout the intestinal wall are not known. Since IgA-containing plasma cells are found in large numbers in the upper villus lamina propria (Fig. 1), it is possible that the apparent compartmentalization of MLN T and B lymphoblasts is artifactual and simply related to the migration time allotted between cell transfer and recipient death. However, the experience of ourselves (Mirski *et al.*, 1981; McDermott *et al.*, 1985) and others (Pierce & Gowans, 1975; Crabbé, Carbonara & Heremans, 1965; Husband, 1982) has clearly shown that transferred IgA plasmablasts always accumulate in the basal LP within 24 hr after transfer. Certainly, IgA-containing cells might be expected to be more frequent around the crypts where glandular secretions originate (Crabbé *et al.*, 1965), Thus, MLN B lymphoblasts may enter the intestinal wall in the basal lamina propria and migrate upward into the villus LP. However, before the migration can occur for the majority of cells, local residence in the basal portions of the villi appears to be necessary.

In contrast, a high frequency of MLN-derived T lymphoblasts were observed in the villus lamina propria and between enterocytes. The observation that these cells were never seen between enterocytes in the crypts suggests that T lymphoblasts cross the basement membrane to intraepithelial locations in the upper villus, and their presence here may be independent of upward epithelial cell migration (Darlington & Rogers, 1966). Furthermore, a portion of these cells may migrate downwards from the villus LP into the basal lamina propria. Certainly, cytotoxic cell activities associated with intestinal cells derived from LP and intraepithelial sites (Davies & Parrott, 1981) may be more frequent in the villus LP. We also recently showed (Ernst *et al.*, 1985b; Tagliabue *et al.*, 1982) that IEL and LP cells displaying T lymphocyte surface antigens possess both natural killer (NK) cell activity and cytotoxic T-cell precursors. Moreover, Ward, Argilan & Reynolds (1983) have found that considerable numbers of IEL in the lung and gut of athymic rats bear surface membrane markers found on NK cells. Thus, a number of MLN-derived cell subpopulations may be distributed in the intestinal wall according to the immunological

**Table 2.** Proportion of cells enumerated in different morphological regions of the small intestine

| | | % total cells detected in defined morphological regions | | |
|---|---|---|---|---|
| Cell type | Detection method | Basal lamina propria | Villus lamina propria | Villus epithelium |
| MLN T blast | Autoradiography | 54 | 68 | 100 |
| MLN B blast | Autoradiography | 54 | 16 | 0 |
| *In situ* T cell | Histochemistry | 28 | 72 | 100 |
| *In situ* IgA plasmacytes | Histochemistry | 16 | 84 | 0 |

Data shown are summarized from the results in Table 1 and Fig. 1.

requirements of the host. For example, a dramatic increase in IEL and mast cell content (Guy-Grand *et al.*, 1984) as well as NK cell activity (Ernst *et al.*, 1985b; Tagliabue *et al.*, 1982) in the gut wall occurs in response to intestinal nematode infection. This observation may represent a developmental and/or functional relationship between T cells, IEL, mast cells and NK cells residing at mucosal sites (Ward *et al.*, 1983; Manning & Parmely, 1980).

We previously showed (McDermott & Bienenstock, 1979) that lymphoblasts from MLN but not those from peripheral lymph nodes (PLN) selectively localized at a variety of anatomical sites included in the common mucosal immunological system, and that the majority of these cells are B lymphoblasts committed to IgA synthesis. Our present work shows that MLN T lymphoblasts move to the intestinal mucosa, and this confirms and extends earlier reports (Guy-Grand *et al.*, 1978; McDermott *et al.*, 1982b). However, our results should not be taken to indicate that mucosally derived T lymphoblasts can at present be incorporated into the concept of a common mucosal immune system. Indeed, Manning & Parmely (1980) have demonstrated that PLN T cells effectively localized in rat mammary tissue. We have recently shown (M.R. McDermott, A.E. Lukacher, V.L. Braciale, T.J. Braciale and J. Bienenstock, submitted) that influenza-specific cloned T cells derived from spleen localize in the respiratory tract parenchyma, mucosal LP and epithelium and, indeed, were capable of entering the respiratory lumen although they were never found in the intestine. Nevertheless, it is conceivable that T cells as well as other Thy 1.2-bearing cells from several anatomical sources move to and differentiate in mucosal sites. This idea is consistent with our earlier suggestion (McDermott & Bienenstock, 1979) that mucosal and peripheral immunity may be integrated according to the immunological needs of the host.

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## REFERENCES

Befus A.D. & Bienenstock J. (1982) Mucosal immunology. *Immunology*, **41,** 249.

Bethell G.S., Ayers J.S., Hancock W.S. & Heam O.T.W. (1979) A novel method of activation of cross-linked agaroses with 1,1′-carbonyldiimidazole which gives a matrix for affinity chromatography devoid of additional charged groups. *J. biol. Chem.* **254,** 2572.

Cebra J.J., Gearhart P.J., Kamat R., Robertson S.M. & Tseng J. (1977) Origin and differentiation of lymphocytes involved in the secretory IgA response. *Cold Spring Harbor Symp. Quant. Biol.* **41,** 201.

Clark D.A. & McDermott M.R. (1978) Impairment of host *vs* graft reaction in pregnant mice. *J. Immunol.* **121,** 1389.

Crabbé P.A., Carbonara A.O. & Heremans J.F. (1965) The normal human intestinal mucosa as a major source of plasma cells containing A-immunoglobulin. *Lab. Invest.* **14,** 235.

Darlington D. & Rogers A.W. (1966) Epithelial lymphocytes in the small intestine of the mouse. *J. Anat.* **100,** 813.

Davies M.D.J. & Parrott D.M.V. (1981) Cytotoxic T cells in small intestine epithelial, lamina propria and lung lymphocytes. *Immunology*, **44,** 367.

Ebersole J.L., Taubman M.A. & Smith D.J. (1979) Thymic control of secretory antibody responses in the rat. *J. Immunol.* **123,** 19.

Elson C.O., Heck J.A. & Strober W. (1979) T-cell regulation of murine IgA synthesis. *J. exp. Med.* **149,** 632.

Ernst P.B., Befus A.D. & Bienenstock J. (1985a) Leukocytes in the intestinal epithelium: an unusual immunological compartment. *Immunol. Today*, **6,** 50.

Ernst P.B., Petit A., Befus A.D., Clark D.A., Rosenthal K.L., Ishizaka T. & Bienenstock J. (1985b) Murine intestinal intraepithelial lymphocytes. II. Comparison of freshly isolated and cultured intraepithelial lymphocytes. *Eur. J. Immunol.* **15,** 216.

Guy-Grand D., Dy M., Luffau G. & Vassalli P. (1984) Gut mucosal mast cells. *J. exp. Med.* **160,** 12.

Guy-Grand D., Griscelli C. & Vassalli P. (1974) The gut-associated lymphoid system: nature and properties of the large dividing cells. *Eur. J. Immunol.* **4,** 435.

Guy-Grand D., Griscelli C. & Vassalli P. (1978) The mouse gut T lymphocyte, a novel type of T cell. Nature, origin and traffic in mice in normal and graft-versus-host conditions. *J. exp. Med.* **148,** 1161.

Heyworth M.F. & Owen R.L. (1982) Harvesting and characterization of lymphocytes from the mouse small intestinal lumen after experimental infection. *Immunobiology*, **163,** 243.

Husband A.J. (1982) Kinetics of extravasation and redistribution of IgA-specific antibody containing cells in the intestine. *J. Immunol.* **128,** 1355.

Manning L.S. & Parmely M.J. (1980) Cellular determinants of mammary cell-mediated immunity in the rat. *J. Immunol.* **125,** 2508.

McDermott M.R., Befus A.D. & Bienenstock J. (1982a) The structural basis for immunity in the respiratory tract. *Int. Rev. exp. Pathol.* **23,** 48.

McDermott M.R. & Bienenstock J. (1979) Evidence for a common mucosal immunologic system. I. Migration of B immunoblasts into intestinal, respiratory and genital tissues. *J. Immunol.* **122,** 1892.

McDermott M.R., Clark D.A. & Bienenstock J. (1982b) Selective localization of mesenteric T lymphoblasts in intestinal respiratory and reproductive tissues. *Fed. Proc.* **41,** 607.

McDermott M.R., Horsely B.A., Warner A.A. & Bienenstock J. (1985) Mesenteric lymphoblast localization throughout the murine small intestine: temporal analysis relating intestinal length and lymphoblast division. *Cell Tiss. Kin.* **18,** 505.

McWilliams M., Phillips-Quagliata J.M. & Lamm M.E. (1975) Mesenteric lymph node cells homing to gut-associated lymphoid tissue in syngeneic mice. *J. Immunol.* **115,** 54.

McWilliams M., Phillips-Quagliata J.M. & Lamm M.E. (1977) Mesenteric lymph node B lymphoblasts which home to the small intestine are precommitted to IgA synthesis. *J. exp. Med.* **145,** 866.

Mirski S.E., McDermott M.R., Befus A.D. & Bienenstock J. (1981) Selective localization of mesenteric lymphoblasts in mucosal tissues: effect of altering the numbers of donor lymphoblasts. *Immunology*, **43,** 69.

Pierce N.F. & Gowans J.L. (1975) Cellular kinetics of the intestinal immune response to cholera toxoid in rats. *J. exp. Med.* **142,** 1550.

ReMine S.G., Bartnik W., Bahn R.C. & Shorter R.G. (1981) Further characterization of lymphocytes from human colonic lamina propria: identification of T cells. *Clin. exp. Immunol.* **46,** 294.

Roux M.E., McWilliams M., Phillips-Quagliata J.M. & Lamm M.E. (1981) Differentiation pathway of Peyer's patch precursors of IgA plasma cells in the secretory immune system. *Cell Immunol.* **61,** 141.

Rudzik O., Clancy R.L., Perey D.Y.E., Bienenstock J. & Singal P. (1975a) The distribution of a rabbit thymic antigen and membrane immunoglobulins in lymphoid tissue with special reference to mucosal lymphocytes. *J. Immunol.* **114,** 1.

Rudzik O., Clancy R.L., Perey D.Y.E., Day R.P. & Bienenstock J. (1975b) Repopulation with IgA-containing cells of bronchial and

intestinal lamina propria after transfer of homologous Peyer's patch and bronchial lymphocytes. *J. Immunol.* **114,** 1599.

TAGLIABUE A., BEFUS A.D., CLARK D.A. & BIENENSTOCK J. (1982) Characteristics of natural killer cells in the murine intestinal epithelium and lamina propria. *J. exp. Med.* **155,** 1785.

TAKASUGI M. (1971) An improved fluorochromatic cytotoxic test. *Transplantation*, **12,** 148.

TOMASI T.B. (1983) Mechanisms of immune regulation of mucosal surfaces. *Rev. Infect. Dis.* **5,** S784.

WARD J.M., ARGILAN F. & REYNOLDS C.W. (1983) Immunoperoxidase localization of large granula lymphocytes in normal tissues and lesions of athymic nude rats. *J. Immunol.* **131,** 132.

WYSOCKI L.J. & SATO V.L. (1978) Panning for lymphocytes: a method for cell selection. *Proc. Natl. Acad. Sci. U.S.A.* **75,** 2844.