FACTORS AFFECTING THE SELECTIVE LOCALIZATION OF MUCOSAL LYMPHOBLASTS IN MUCOSAE

By

SHELAGH ELIZABETH LINK MIRSKI, B.Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

SELECTIVE LOCALIZATION OF MUCOSAL LYMPHOBLASTS

DOCTOR OF PHILOSOPHY (1983)
(Medical Sciences)

McMASTER UNIVERSITY
Hamilton, Ontario

TITLE: Factors Affecting the Selective Localization of Mucoal Lymphoblasts in Mucosae

AUTHOR: Shelagh Elizabeth Link Mirski,
B.Sc. (University of Toronto)

Supervisor: Professor J. Bienenstock

NUMBER OF PAGES: 202

Abstract

The selective localization in mucosal tissues of lymphoblasts derived from the mesenteric lymph node (MLN) compared to those from peripheral lymph nodes (PLN: axial, brachial and inguinal) was studied using an adoptive transfer model in syngeneic CBA/J mice. The localization of lymphoblasts which had been labelled *in vitro* with ^{3}H-thymidine or ^{125}I-deoxyuridine was assessed using autoradiography or radiocounting.

I found that the number of MLN lymphoblasts which localized in the small intestine, lung, Peyer's patches, MLN, PLN, spleen, and liver was directly proportional to the number transferred. This relationship was also present in intestinal epithelium and basal and villus lamina propria and in pulmonary parenchyma, BALT and bronchial epithelium. Even at doses of MLN lymphoblasts which approximated four times the daily output of blasts in thoracic duct lymph, I could not saturate the capacity of these tissues to accommodate MLN blasts, nor was their intra-intestinal distribution altered. Because of this dose relationship it is necessary to control the number of blasts transfered when comparing the localization of lymphoblasts from different organ sources using autoradiography. Thus my dose studies show that, contrary to earlier studies which were not controlled in this manner, MLN blasts do not selectively localize compared to PLN blasts in pulmonary parenchyma. In addition the results suggest that any method of increasing the number of lymphoblasts released from MALT (e.g. by

mucosal immunization) or increasing the delivery of these cells to a particular organ (by altering the proportion of the cardiac output which it receives) will increase the number of immunoblasts in a tissue.

Although the enumeration of radiolabelled or fluorescent cells in tissue sections has been used extensively in the literature to assess localization, the variation in these results has rarely been stated. I found that this variation can be quite high and is largely due to the probability of detecting a low number of cells in a small volume of tissue.

I found that the sex of the recipient had no effect on the number of MLN lymphoblasts which localized in the small intestine 24 h after transfer. In contrast, initial experiments showed that lymphoblasts from male donors localized two to three times more frequently than those from female donors in the small intestines of recipients of either sex. However this phenomenon disappeared between September 1980 and August 1981 and neither its initial existence nor its subsequent loss have been explained. I found that the gonadal hormone environment in which the MLN lymphoblasts developed did not influence their capability to localize in the small intestine 24 h after transfer. However, I do not know whether this observation relates to the phenomenon of greater localization of male lymphoblasts because this phenomenon had likely already disappeared when the experiments involving hormonally altered donors were performed.

Using autoradiography in a series of experiments analyzing the kinetics of lymphoblast localization, I demonstrated that MLN

lymphoblasts selectively localize compared to PLN blasts in the small intestine by 0.5 h after transfer. This suggests that one factor in selective localization is the selective entry of MLN blasts from the vasculature, perhaps mediated by specific receptors on blasts and tissue of localization.

The concentration of MLN lymphoblasts was the same in the lamina propria adjacent to the Peyer's patch and distant from the Patch at both 0.5 and 24 h after transfer. This suggests that lymphoblasts extravasate in the lamina propria rather than extravasating in the Peyer's patch and subsequently migrating into the lamina propria. In addition, the distribution of blasts in the basal and villus lamina propria was the same at 0.5 and 24 h after transfer but labelled cells appeared in the intestinal epithelium after 0.5 h.

The evidence presented in this thesis suggests that the selective localization of MLN lymphoblasts is mediated at the vascular endothelium in the lamina propria, perhaps by specific receptors.

Acknowledgements

I would like to express my appreciation to my Supervisor, Dr. John Bienenstock and the other members of my supervisory committee, Drs. Dean Befus, Myron Szewezuk and Ken Rosenthal for their support and guidance throughout this project. I would like to add special thanks to Dr. Dean Befus who acted as my Supervisor during Dr. Bienenstock's absence on sabbatical and who was, along with Dr. Bienenstock, particularly helpful in resolving some of the difficulties encountered in the project.

I would like to thank Dr. Mark McDermott for introducing me to the laboratory and the adoptive transfer and autoradiographic techniques. Without the technical assistance of many people this project would not have been possible. I would like to thank Mrs. Daljeet Banwatt, Mrs. Angie Chaput, Mrs. Nancy Dyck, Dr. Peter Ernst, Dr. Tom Egwang, Mr. Ralph Haspeiller, Mrs. Barney McLaughlin, Ms. Laurie Nielsen, Ms. Agnes Petite, Mrs. Sharon Putns, Mr. Carl Richards, Ms. Ann Robson, Ms. Renata Slapsys, Ms. Jane-Ann Smith, Mr. Andy Stadnyk, Ms. Allison Warner, and Ms. Gillian Woodall.

The friendship of my fellow students and the technicians and faculty who worked in the lab were truely appreciated and have left me with many fond memories of fun times and intense discussions.

The financial support which I recieved from Ontario Graduate Scholarships, McMaster University Graduate Scholarships and the Medical Research Council of Canada was appreciated.

Most of all I would like to thank my husband John for his emotional support and for his patience with a wife who was for much of this endeavour living in a different city.

Table of Contents

Table of Contents (cont'd)

Table of Contents (cont'd)

Table of Contents (cont'd)

Table of Contents (cont'd)

List of Tables

List of Tables (cont'd)

List of Tables (cont'd)

List of Figures

List of Plates

List of Abbreviations

No.	Title
BALT	bronchus-associated lymphoid tissue
blast	lymphoblast
BLN	bronchial (mediastinal) lymph node
dpm	disintegrations per minute
FCS	fetal bovine serum
GALT	gut associated lymphoid tissue
GGfHS	Gamma globulin free horse serum
HBSS	Hanks' balanced salts solution
HEV	high endothelial post-capillary venules
HPF	high power microscopic fields (area 0.1mm^2)
^{3}H-Tdr	(^{3}H)-thymidine
Ig	immunoglobulin
^{125}I-Udr	(^{125}I)-deoxyuridine
MALT	mucosa - associated lymphoid tissue
MLN	mesenteric lymph node(s)
PBS	phosphate buffered saline
PLN	peripheral lymph node(s)
PP	Peyer's patch(es)
SC	secretory component
TDL	thoracic duct lymph (lymphocyte)

Foreword

The intention of this foreword is to provide the reader with an overview of the thesis. In the Introduction I note that the immune system which is active at mucosal surfaces is important in host defense, that responses in the mucosal immune system are somewhat separate from those in the systemic immune system and that mucosal responses are disseminated from one mucosal tissue to others. Possible mechanisms for both the dissemination and the limitation of mucosal responses are discussed, including the mechanism which is the subject of this thesis, that is, the selective mucosal migration of antigen-reactive, mucosally-derived lymphoblasts. I note that in contrast to lymphoblasts small lymphocytes do not display this selective localization in mucosal tissues but continually recirculate between blood and lymph. To emphasize the distinction between lymphocytes and lymphoblasts I will use the term blast to mean lymphoblast.

The ultimate goal of understanding the mechanism which results in selective blast localization has been approached by studying the factors which affect this phenomenon. Although the traffic of lymphocytes and blasts clearly differs in a number of ways, analogies have been drawn between them in the search for the factors which affect selective localization. In the Introduction I therefore describe some of the factors affecting lymphocyte traffic through lymph nodes including: delivery, kinetics, antigen, cell surface structures and an *in vitro* model for studying the factors affecting lymphocyte adherence

to the vascular endothelium which they migrate across in lymph nodes.

Sex hormones have generalized effects on the immune system and turn on the mucosal immune system in target tissues, in part by affecting blast localization. I suggest that they might also influence blast localization in those mucosae which are not sex hormone target tissues. I propose to investigate some of the problems described in the Introduction by using an adoptive transfer model in syngeneic mice.

The Materials and Methods describes the adoptive transfer procedure, the autoradiographic and gamma-counting detection of transferred cells, and the castration procedures.

The Results are divided into five chapters. The first (chapter 3) describes the accuracy and reproducibility of the system. Chapter 4 describes localization using the adoptive transfer model at base line conditions, approximating those which had been used previously in our laboratory and in other laboratories. To investigate factors affecting blast localization, I then perturbed this system by altering the number of lymphoblasts transferred (chapter 5), the time of study of blast localization (chapter 6), the gender of donor and recipient mice, and gonadal hormone levels in blast donors (chapter 7).

In the Discussion (chapter 8) I consider these results in the context of the literature and make suggestions for future research on selective mucosal blast localization. Topics discussed include: the site of entry of blasts into the small intestine, the delivery of blasts to this vascular site, and potential mediators of selective localization of blasts such as antigen, immunoglobulin isotype and

other cell surface molecules, diet, parasitic infection, gender, and gonadal hormones.

Chapter 1

Introduction

1.1 The Mucosal Immune System

1.1.1 Importance in host defense and isolation from systemic responses

Mucosal surfaces are the major portal of entry for most infectious agents and foreign antigens. Therefore, the mucosae constitute the first line of defense, initially by nonspecific mechanisms of resistance such as ciliary action, peristalsis, mucus, acid, enzymes and macrophages and subsequently by specific immunological responses. Indeed, it has been estimated that more than half of the antibody - producing cells in the lymphoid system are located in the mucosa of the intestinal tract (Hanson *et al.*, 1980). More than 90% of the plasma cells in this site produce immunoglobulin A (IgA). Because plasma cells which produce IgA (the major antibody isotype in secretions (Chodirker & Tomasi, 1963) are present in the mucosae, it seems likely that antibodies active in mucosal defense could be produced locally.

Besredka (1919) postulated that local humoral immunity existed in the gut which was independent of systemic antibody responses, following observations that resistance to bacillary dysentery in orally immunized rabbits was unrelated to serum antibody titre. Davies (1922) observed anti- *B. dysenteriae* agglutinins in the feces (coproantibodies) of humans with bacillary dysentery. Similarly,

resistance to Vibrio cholera was associated with the presence of coproantibodies and the titre of these was independent of the serum titre (Burrows et al., 1947). In the lungs, antibodies to influenza virus were stimulated by local application of antigen and protection correlated better with the presence of antibodies in bronchial secretions than with the serum antibody titre (Fazekas de St. Groth & Donnelley, 1950). Similarly, vaginal and uterine antibody titers to Brucella abortus were found in genital tract secretions following intrauterine immunizations, but not following parenteral immunization, in spite of high serum titres (Kerr, 1953). Subcutaneous immunization in the vicinity of the parotid and submandibular glands of experimental animals with glucosyltransferase (an enzyme involved in the pathogenesis of dental lesions) resulted in IgA and IgG anti-glucosyltransferase antibodies in saliva (and IgG antibody in serum) and a lower incidence of caries (Taubman & Smith, 1977). Salivary IgA anti-glucosyltransferase activity has been detected in humans (Klein et al., 1977). These are only a few of the many studies which have demonstrated that resistance to viral and bacterial pathogens is primarily mediated by antibodies produced locally, i.e. in the mucosa where these agents are encountered rather than systemically by antibodies in the blood (reviewed by Ganguly & Waldman, 1980). Although less extensively studied, cell mediated immunity also functions locally, at least partially independent of systemic cell-mediated immunity. For example, in BCG-immunized animals, protection against infections with M. tuberculosis correlated with the presence of

cell mediated immunity in cells from the lung but not with its presence in cells from the peritoneal cavity (Yamamoto et al., 1970). In addition macrophage migration inhibition factor (MIF) was produced in either the lung or the spleen depending on whether the immunization was intranasal or parenteral (Henney & Waldman, 1970). This evidence and similar experiments (reviewed by Ganguly and Waldman, 1980) clearly suggest that there are both humoral and cellular immune functions which are generated locally in mucosal tissues and are at least partially independent of the systemic immune system.

Differences in the predominant immunoglobulin isotypes represent another distinction between mucosal and systemic immune responses. Whereas IgG has the highest concentration in serum, in secretions dimeric IgA predominates (Chodirker & Tomasi, 1963). The synthesis of secretory IgA and its components has been demonstrated in vitro in the salivary glands (Hochwald et al., 1964; Hurlimann & Darling, 1971), mammary glands (Asofsky & Small, 1967; Lawton et al., 1970), gut (Bull et al., 1971; Kagnoff et al., 1973), and upper respiratory tract (Morgan, 1980). IgA was also synthesized by cells in the lower respiratory tract but IgG synthesis predominated (Morgan, 1980). Since most of the plasma cells in the mucosae contain IgA it is likely that these represent the local source of much of the IgA in secretions (Tomasi & Bienenstock, 1968). Dimeric IgA with its associated J-chain is transported across the secretory epithelium in association with secretory component (SC), an epithelial cell product (Brandtzaeg, 1982). The serum represents an important source of IgA in some secretions eg. in the liver dimeric IgA is selectively transported

into bile (Vaerman et al., 1982; Brown, 1982), and in the salivary gland into saliva (Montgomery et al.,1977), and this is mediated by SC.

In addition to the lymphocytes which are distributed in the mucosal epithelium and lamina propria, there are organized lymphoid tissues in the mucosae (mucosa-associated lymphoid tissue, MALT). These are the Peyer's patches (PP), solitary lymphoid nodules and appendix in the small intestine (gut-associated lymphoid tissue, GALT) (Faulk,et al., 1971; Waksman et al., 1973; Abe & Ito, 1977) and the morphologically and functionally similar bronchus-associated lymphoid tissue (BALT) in the lungs (Bienenstock et al., 1973a,b). These tissues are well suited for sampling the lumenal environment, being covered by a lymphoepithelium which is characterized by a paucity of goblet cells and the presence of epithelial cells which have surface microfolds (M-cells) and are both actively pinocytotic and allow lymphocytes to closely approach the lumen (Owen et al., 1981). These lymphoid structures would seem to be a likely source of precursors for the extensive lymphoid population of the mucosae. If this were the case they would require a high proportion of B cells which are surface α positive and / or μ positive cells, the precursors of IgA producing cells (Jones et al., 1974a,b). Indeed, Peyer's patches contain a higher proportion of B cells (74%) relative to T cells (13%) than do lymph nodes (B:T of 47:48). 14% of the lymphocytes and 38% of the blasts in the Peyer's patch had α-chains on their surface and 21% had μ chains (Guy-Grand et al., 1974). Thus the Peyer's patches contain a large number of precursors for IgA plasma cells. These cells will

repopulate the gut and lung with IgA producing cells after transfer into irradiated recipients, but lymphocytes from peripheral lymph nodes or peripheral blood do not (Craig & Cebra, 1971,1975; Rudzik et al., 1975b; Tseng, 1981). Although B cells from Peyer's patch can be induced to synthesize specific antibody and T cells make an allograft response in vitro (Kagnoff & Campbell, 1974), plasma cells were not observed in the Peyer's patch after direct stimulation with antigen (Bienenstock & Dolezel, 1971). Thus the cells which are stimulated by antigen in the mucosae leave the GALT and BALT to mature elsewhere and, as in the experimental model, populate the mucosae with antigen specific cells.

Another mechanism which seems to operate to separate the mucosal and systemic immune systems is selective suppression. After oral administration of antigen, systemic responses are often suppressed. Although the mechanism regulating this oral tolerance is not fully understood, suppressor cells often seem to be involved. After oral administration of antigen, specific suppressor cells for the IgG response were generated in the PP and migrated to the spleen, while specific helper cells for the IgA response were found in PP and mesenteric lymph node (MLN) but not spleen (Mattingly & Waksman, 1978; Richman et al., 1981). Conversely, after parenteral administration of antigen, specific suppressor cells which interfered with a primary mucosal response, have been detected initially in the spleen and subsequently in Peyer's patches (Koster & Pierce, 1983). The relative contributions of selective localization of responder cells, antigen and/or isotype specific suppressor cells, and antigen presenting

cells, to the separation of the systemic and mucosal immune systems is unknown.

The underlying basis for the predominance of IgA plasma cell precursors in MALT is unclear.. Kawanishi et al., (1982) found T cell clones from Peyer's patch, but not from spleen, which dramatically decreased the number of Peyer's patch B cells which bore surface IgG and contained cytoplasmic IgM or IgG. These T cell clones dramatically increased the number of cells which bore surface IgA but only slightly increased the number which contained IgA. However both the spleen and Peyer's patch - derived T cell clones had similar suppressive effects on LPS stimulated IgG synthesis and small helper effects on LPS stimulated IgA responses by PP B cells. Kawanishi et al. (1982) suggested that T cells derived from PP could induce class switching to sIgA bearing cells but did not provide help for terminal differentiation. Tseng (1982) found that when T-cells from spleen or Peyer's patch were titrated against B cells from either tissue there was no significant difference induced by the T cells in the proportion of cells with cytoplasmic IgM, IgA or IgG . Therefore Tseng (1982) suggested that B cells, but not T cells specific for IgA production are preferentially localized in Peyer's patch.

2

1.1.2 Dissemination of the mucosal response to distant mucosae.

Although largely isolated from the systemic immune system, the immune response generated at one mucosal surface does not remain localized but disseminates to other mucosae. For example, after oral immunization of pregnant rabbits with DNP-pneumococcal vaccine, anti-

IgA antibody was detected in colostrum but not in serum (Montgomery et al., 1974). Ingestion of a distinctive serotype of E. coli led to the appearance of specific IgA antibody in human milk (Goldblum et al., 1975). Similarly, ingestion of Streptococcus mutans by rats led to the appearance of specific IgA antibodies in saliva and milk but not in serum, and a decline in the incidence of dental caries (Michalek et al., 1976). Although there are other possible explanations, lack of a serum response was consistent with the suggestion that dissemination of the mucosal response was not due to antigen gaining access to the circulation. Antigen might have been transported selectively to distant mucosae by migrating macrophages. However Montgomery et al., (1981) found identical spectrotypes of IgA in distant mucosal secretions after mucosal immunization at one site, suggesting that unless the resident cells in all mucosae were identical, it was likely that clones of cells triggered in one mucosal tissue migrated to other mucosae. This dissemination of the mucosal immune response has obvious protective value. Analogies with the systemic immune system suggest that this information might be disseminated by lymphocytes stimulated in one mucosal tissue migrating to other mucosae.

1.2 Lymphocyte Traffic and Selective Lymphoblast Localization

1.2.1 Importance of lymphocyte recirculation

Few of the lymphocytes in a lymph node react with a given antigenic determinant. The continuous recirculation through lymphoid

organs of immunocompetent cells (migrating between blood and lymph, Gowans, 1959; Sorent, 1973) enhances the probability of a specific lymphocyte contacting the relevant antigenic determinant. Indeed it has been estimated that of the cells in the efferent lymph, 80% are cells which had entered the lymph node from the blood by migrating across the morphologically distinct high endothelial post-capillary venules (HEV), 10 to 15% are from afferent lymph and only 2 to 4% are due to division within the node (Issekutz et al., 1981). Upon stimulation by antigen the lymphocytes begin to differentiate and, in the form of antigen specific lymphoblasts, migrate into the efferent lymph (Frost et al., 1976) and to distant nodes and tissues where they mature into antibody producing plasma cells and other effector cells. As an aid to the reader in distinguishing comments about lymphoblasts from those about lymphocytes, I will henceforth use the term blast to mean lymphoblast.

1.2.2 Localization differences between small lymphocytes and large lymphocytes (blasts)

There is an analogous circulation among the mucosae whereby lymphocytes stimulated in MALT beneath one mucosal surface undergo blastogenesis, enter the lymph and then migrate via the blood to mucosal tissues where they extravasate and complete differentiation, primarily into IgA-secreting plasma cells. Gowans and Knight (1964) found that approximately 98% of transferred small lymphocytes from thoracic duct lymph (TDL) were recovered in the lymph over the 4 days following transfusion. In contrast only 5% of the labelled large

lymphocytes from TDL were recovered in TDL after transfer (Gowans & Knight, 1964; Smith et al., 1980). Of these labelled cells, 4.3% were large lymphocytes and 0.7% small lymphocytes (Gowans & Knight, 1964). Similarly, Howard (1972) found that B blasts from TDL appeared in much smaller numbers and peaked earlier (at 9 to 12 h) than small B or mixed B and T lymphocytes from TDL (15 to 18 h) in the lymph of cannulated recipients. In marked contrast with the small lymphocytes, many of the large lymphocytes were observed in the small intestine as early as 1 h (Hall & Smith,1970) and at 1 and 4 days after transfer (Gowans & Knight, 1964). The observation that blasts from the MLN displayed the same tendency to localize in the small intestine as TDL blasts (Griscelli et al., 1969) was consistent with the estimate that TDL is derived almost entirely from the intestine via the MLN (Yoffey & Courtice, 1970).

1.2.3 Selective localization of mucosally derived blasts

Most interesting was the observation of Griscelli et al., (1969) that although MLN and TDL blasts displayed a strong propensity to accumulate in the gut mucosa at 20 h after transfer, blasts derived from peripheral (non-mucosal) lymph nodes (PLN) did not. The observation that, in contrast to mucosally derived blasts which accumulate in the small intestine, PLN blasts accumulate in recipient PLN and spleen and are only infrequently observed in the mucosae has been confirmed repeatedly (Griscelli et al., 1969; Guy Grand et al., 1974; Parrott & Ferguson, 1974; McWilliams et al., 1975; Hopkins & Hall, 1976; Hall et al., 1977; McDermott & Bienenstock, 1979; Ottaway &

Parrott, 1980; Smith et al, 1980). This phenomenon was even more apparent when autoradiography, rather than radiocounting, was used to assess the location of the transferred cells (Griscelli et al., 1969; Befus et al, 1980). The marked accumulation in mucosal tissue after adoptive transfer of mucosally derived blasts compared to peripherally derived blasts has been termed selective localization. This selective localization of cells which had been stimulated by antigens crossing mucosal epithelia is an underlying basis for the observations of local immune responses being separate from systemic immune responses and for the dissemination of an immune response from one mucosal surface to another. This applies to both T blasts and immunoglobulin containing blasts.

In contrast to their differential localization in mucosae, blasts from MLN, TDL and PLN had an equal tendency to accumulate in the spleen, and small lymphocytes from all three sources had similar distribution patterns in recipient MLN, PLN and Peyer's patches at 20 h after transfer (Griscelli et al., 1969). Many of the blasts in the MLN (and TDL) were likely derived from lymphocytes which had been stimulated by enteric antigens in the Peyer's patch. Indeed many more anti-toxin containing cells were observed in the TDL when cholera toxoid was instilled into an intestinal loop with a Peyer's patch than when the loop did not include a patch, but removal of the MLN before challenge had no effect on the response (Husband & Gowans, 1978).

Many of the mucosally-derived blasts which localized beneath mucosal epithelia bore surface IgA (Guy-Grand et al., 1974; McWilliams et al., 1977) and differentiated into IgA-containing plasma cells

beneath mucosal surfaces including: small intestine (Guy Grand et al., 1974; McWilliams et al., 1977; McDermott & Bienenstock, 1979), lung (McDermott & Bienenstock, 1979; Weisz-Carrington et al., 1979), lactating mammary gland (Roux et al., 1977; McDermott & Bienenstock, 1979; Weisz-Carrington et al., 1979), genital tract (McDermott & Bienenstock, 1979) salivary glands (Weisz-Carrington et al.,1979; Jackson et al., 1981) and lacrimal glands (Montgomery et al., 1983).

Double transfer studies have further elucidated the migration pathway. Using an IgA alloantigenic marker in congenic mice, Tseng (1981) found that Peyer's patch lymphocytes migrated to the small intestinal lamina propria following a 5 to 7 day interval during which they resided elsewhere, primarily in the spleen. Transfers using splenectomized recipients demonstrated that the spleen was not an obligatory intermediate site. Although quantitatively much less than the spleen, PP cells also were found in the MLN as an intermediate site (Tseng, 1981). Roux et al., (1981) found that radiolabelled Peyer's patch blasts were equally distributed between MLN and PLN but upon secondary transfer a higher proportion of those from the intermediate site of MLN than those from PLN localized in the small intestine and contained IgA. At least some of the cells which initially accumulated in MLN seemed to have arrived via lymph, suggesting that they had extravasated in the gut,(Roux et al., 1981), perhaps at HEV in Peyer's patches.

Presumably T blasts and B blasts of isotypes other than IgA are generated beneath mucosal epithelia. Indeed, Peyer's patch lymphocytes

had a small but significant potential to differentiate into IgM and IgG plasma cells in irradiated recipient rabbits (Craig & Cebra, 1975). Indeed this group (Cebra et al., 1983) has shown, using the splenic-focus assay that PP or BALT cells gave rise to clones producing IgM, IgG, IgE and/or IgA. Thus selective localization would presumably not be limited to IgA-plasma cell precursors. After transfer of MLN blasts, more IgG containing radiolabelled cells were observed in the small intestine than after transfer of ^{3}H-Tdr labelled PLN blasts (McDermott et al., 1979). T-blasts appeared in the gut mucosa after transfer of labelled MLN or TDL blasts (Guy Grand et al, 1974; McWilliams et al.,1975; Rose et al., 1976a; Sprent, 1976; Freitas et al., 1980). As predicted, T-blasts from MLN or TDL selectively localized in the small intestine compared to those from PLN or spleen (Guy Grand et al., 1978; Ottaway & Parrott, 1980; Smith et al., 1980).

Similarly blasts from the bronchial lymph node (BLN) which drains the lung selectively localized (compared to PLN blasts) and differentiated into IgA and IgG containing cells in the lung (McDermott & Bienenstock, 1979). There seemed to be a relative preference for blasts derived from one mucosal tissue to return to that tissue. Thus although blasts from the BLN accumulated and contributed IgA positive cells to the small intestine, the quantity was much less than after MLN transfer and not different from PLN transfers. MLN blasts accumulated in the lungs more than PLN blasts but less than BLN blasts (McDermott & Bienenstock,1979). However, as McDermott and Bienenstock noted (1979) there were large differences in the numbers of blasts transferred from the various donor cell sources and their data could not be corrected

for these differences because of lack of information on the effect of altering the number of cells transferred. One of the purposes of this thesis will be to describe the effect of altering the number of blasts transferred so that the localization of blasts from different sources and in different experiments can be compared.

In contrast with blasts, rodent small lymphocytes from TDL or PLN have similar patterns of recirculation, (Hall et al., 1978; Freitas et al., 1980) and demonstrate no tendency to localize in intestinal mucosa (Guy Grand et al., 1974; McWilliams et al., 1975; Freitas et al., 1980). Smith et al., (1980) found no difference in the concentration of rat small TDL lymphocytes in either MLN and caecal lymph node or PLN. Although small lymphocytes and blasts from both MLN and alloantigen stimulated PLN (95% T cells) accumulated in large numbers in the coeliac lymph node, this seemed to be due to arrival in afferent lymph from the liver, rather than to a different tendency of lymphocytes to leave the blood and enter these nodes compared to other nodes (Smith et al, 1980). However selective migration of small lymphocytes has been demonstrated in the sheep. Small lymphocytes from intestinal lymph in sheep traffic selectively through the small intestine and back into intestinal efferent lymph but those from peripheral lymph do not (Scollay et al., 1976; Cahill et al., 1977; Hall, 1977; Chin & Hay ,1980). Also, Issekutz et al., (1982) showed that small T lymphocytes (but not blasts, B cells or macrophages) from afferent lymph recirculated preferentially from blood through skin or a granuloma and back into afferent lymph, but small T lymphocytes from efferent lymph recirculated from blood through the lymph node and back into efferent lymph.

This fundamental difference between traffic of small lymphocytes in the rodents and sheep is unexplained but might reflect species differences. Unlike rodents, no paricular class of blood vessel can be identified in sheep lymph node as being responsible for the transmission of lymphocytes from blood to lymph (Morris & Courtice, 1977). Perhaps there is a different distribution of various organ directed memory cells in lymph of sheep compared to rodents. In addition, the differences might result from the cell sources used for comparison, intestinal and peripheral lymph in sheep versus TDL and lymph nodes in rodents. Indeed Reynolds et al., (1982) found that the differential circulation was not as great when lymphocytes were obtained from, and comparisons made between, mesenteric and prescapular lymph node rather than intestinal and prescapular efferent lymph of sheep. However, as in rodents by 24h after transfer, sheep blasts from intestinal lymph had virtually all disappeared from the lymph and blood, and displayed selective localization in the small intestine compared to blasts from peripheral lymph (Hall et al., 1977).

Thus lymphocyte migration experiments in rodents and sheep show that immune responses in the mucosae are linked into a common mucosal immune system (Bienenstock, 1974) and are at least partially separated from those in the systemic immune system, by the selective localization of blasts which have been stimulated in MALT.

1.3 Factors Affecting Lymphocyte Traffic

The mechanism which underlies the selective mucosal localization of MALT-derived blasts is unknown. In an attempt to understand this process there has been a search for factors which affect the accumulation of blasts in mucosae. This was the purpose of my thesis project and both my results and the relevant literature will be discussed in chapter 8. Much of the direction of research into selective blast localization has been drawn from analogies with lymphocyte traffic through lymph nodes. However it should be stressed that these are not identical processes since, in rodents, lymphocytes enter lymph nodes via morphologically distinct vascular sites (HEV) and, in rodents and sheep, most do not remain in the node but enter the lymph, return to the blood and continue to recirculate. In contrast, MALT-derived blasts enter non-lymphoid mucosal tissue which does not contain HEV and remain and differentiate within that tissue, tending not to recirculate. Nevertheless there may be similarities in the factors influencing these two processes and in the mechanism of entry of lymphocytes into lymph nodes and blasts into mucosae. I will therefore review the factors which affect lymphocyte traffic through lymph nodes including their delivery to the node, the effect of specific antigen, evidence for lymphocyte and HEV cell surface structures involved in localization, and the kinetics of localization.

1.3.1 Delivery of lymphocytes to lymph nodes - effect on lymphocyte traffic through a node

The early studies of Gowans (1959) showed that the normal high

output of lymphocytes from the thoracic duct could be more than doubled by a large intravenous transfusion of lymphocytes. This suggested that the mechanism for the circulation of lymphocytes from blood to lymph allowed a certain proportion of the lymphocytes in the blood to cross into lymph, and that the mechanism was not readily saturable. Indeed, it has been shown using isolated spleen (Ford, 1969) or MLN (Sedgley & Ford, 1976) that the number of cells entering the tissue was directly proportional to the concentration of cells in the perfusate. Approximately 11% of the lymphocytes in the arterial blood entered the isolated MLN (Sedgley & Ford, 1976). Similarly, Hall (1967) estimated that 12% of the lymphocytes presented by the blood entered sheep lymph nodes. Hay and Hobbs (1977) also found a direct relationship between delivery and entry but estimated a somewhat more efficient uptake, 25% of the lymphocytes delivered to the node. Presumably the number of lymphocytes entering a node could be increased either by increasing the concentration of lymphocytes in the blood or by increasing the amount of blood flowing to that node. Ottaway and Parrott (1979) found that the probability of finding ^{51}Cr-labelled lymphocytes in a particular lymph node was related to the proportion of the cardiac output which that node received. In addition, the increased localization of lymphocytes in the node draining a site of inflammation correlated with increased blood flow to that node (Cahill et al., 1976). Similarly, Drayson et al., (1981) found the following sequence of events after stimulating rat popliteal lymph node with SRBC: blood flow increased, then lymph node weight increased, then lymphocyte accumulation during

the first hour after transfer (primarily a reflection of influx) increased. However these authors found that after irradiation, lymphocyte influx into antigen stimulated nodes increased without a significant increase in blood flow. They suggested that there might have been a redistribution of blood flow within the node to HEV since the opening of arteriovenous shunts, perhaps leading to greater flow to HEV, was prominent after antigenic stimulation (Herman et al., 1978). The evidence regarding uptake of a proportion of the lymphocytes presented to the node combined with the correlation with blood flow, strongly suggests that the delivery of lymphocytes to the HEV of lymph nodes directly influences the number which will enter the node under physiological conditions. Perhaps a similar relationship exists in nonlymphoid tissues. This has not been controlled in previous investigations of blast localization. Experimental manipulations have been made (such as intestinal parasitic infection or sex hormone treatments) which might have effected localization by altering blood flow. The quantitative localization of different blast populations have been compared without controlling the number of blasts transferred. One of the purposes of this thesis was to address this problem and to discuss the earlier literature in light of my results.

1.3.2 The effect of antigen on lymphocyte localization

Antigen is another factor which influences lymphocyte localization. There has been some evidence of selective localization of antigen specific cells in skin allografts undergoing rejection but there was no specific localization in the lymph nodes draining the

grafts (Lance & Cooper, 1972; Tilney & Ford 1974). However much of the antigen - associated influences on lymphocyte traffic seem likely to have been initially stimulated by specific immunologic interactions and subsequently to act on the larger population of lymphocytes with irrelevant specificities. Thus increased lymphocyte trapping was observed in the spleen after i.v. antigen and in the draining lymph node after footpad injection of antigen (Zatz & Lance 1971; Zatz, 1975). This trapping or "cell shutdown" was not immunologically specific (Zatz & Lance, 1971), although the trapping of specifically sensitized lymphocytes can be demonstrated in spleen but not lymph nodes (Emeson & Thrush 1974). Cell shutdown was induced with complement activation in sheep lymph nodes (McConnell & Hopkins, 1981) or by infusion of PGE_2 (Hopkins et al., 1981). PGE_2 levels were greater in efferent lymph after cell shutdown and inhibition of PGE_2 synthesis in the node abolished cell shutdown induced by antigen (Hopkins et al., 1981). These investigators suggested that complement activation initiated shutdown by stimulating the production of PGE_2 which then mediated the response, perhaps in part by enlarging PCV and opening direct arterio - venous shunts into HEV. Because of the large nonspecific component of lymphocyte trapping in antigen - stimulated nodes and regions of inflammation, careful checkerboard designs are essential in order to detect any antigen - specific localization.

1.3.3 Kinetics of lymphocyte localization

Experiments in which labelled small lymphocytes were transferred and their location at various times assessed by

autoradiography, led to the conclusion that both T and B lymphocytes enter lymph nodes from the blood via HEV and that this process requires between 15 and 30 min (Gowans & Knight, 1964). Most studies of selective blast localization have assessed the accumulation of cells at one time, between 18 to 24 h after transfer. If blasts can enter mucosae as rapidly as lymphocytes enter lymph nodes then many influences on, and changes in, their position could have taken place during 24 h. The few studies of blast localization kinetices which had been performed when I began the work described in this thesis demonstrated that blasts entered the mucosa much earlier than 24 h. However there had been no direct comparison of the kinetics of localization in the mucosae of mucosally, as compared to peripherally, derived blasts. Such a study might suggest the mechanism of selective blast localization. Therefore I have compared the localization of MLN and PLN blasts in the small intestine, lung and Peyer's patch at various times after transfer.

1.3.4 In vivo evidence for involvement of cell surface structures in lymphocyte traffic

The first suggestions that cell surface molecules were involved in lymphocyte traffic came from observations of the effects of enzyme treatments. Trypsin treatment temporarily interfered with the ability of lymphocytes to enter lymph nodes (Woodruff & Gesner, 1968; Jacobson & Blomgren, 1972) but not tissues where HEV were not involved such as liver, gut, spleen and bone marrow (Woodruff & Gesner, 1968; Rannie et al.,1977). Glycosidases from Clostridium perfringens (Gesner & Ginsburg, 1964) and neuraminidase caused the transient sequestration of

treated lymphocytes in the liver (Woodruff & Gesner, 1969; Freitas & de Sousa, 1976). When lymphocytes were allowed to reach the node (in an isolated, perfused node), those treated with neuraminidase migrated across HEV as well as controls but the migration of trypsin treated cells was inhibited (Ford et al., 1976). The putative receptor seemed to undergo rapid turnover since both puromycin (Woodruff, 1974) and mitomycin C (Romano et al.,1976) treatment of lymphocytes interfered with their migration into lymph nodes. Schlesinger (1976) suggested that both the effects of trypsin and neuraminidase might have been due to changes in electrophysical properties of the membrane which altered adhesiveness, rather than the cleaving of a specific molecule involved in a lock-and-key type receptor. Similarly, changes in surface charge and adhesion have been suggested as explanations for the lymphocytosis induced by treatment with sulphated polysaccharides such as heparin and dextran sulphate (Ford et al., 1978; de Sousa, 1981), although in this case the major effect seemed to be on the HEV rather than the lymphocyte (Ford et al., 1978). The effect of nonspecific mitogens on lymphocyte localization has been studied quite extensively but no meaningful patterns have yet emerged. Some mitogens inhibit migration into lymph nodes, perhaps by increasing trapping in the spleen, whereas others have no effect (reviewed by Schlesinger et al., 1976; Ford et al., 1978; deSousa, 1981). Sodium azide treatment of lymphocytes decreases their localization in lymph nodes at 30 min after transfer (Ford et al., 1978) suggesting that an active response of the cell may be required for binding to HEV.

The different mechanisms involved in localization in lymph nodes via HEV and spleen via vessels lined with low endothelia, is emphasized by the fact that of the three agents which most significantly inhibit lymphocyte localization in lymph nodes (trypsin, sodium azide and dextran sulphate), only dextran sulphate administered to the recipient had any effect on localization in the spleen and this was much less than its effect on lymph node localization (Ford et al., 1978). Mitomycin C interfered with localization in the lymph nodes but not spleen (Romano et al., 1976). Thus one must be aware that differences exist in the mechanisms for localization of lymphocytes in different tissues and care must be taken when drawing analogies between potentially different systems such as lymphocyte migration into lymphoid tissue or non lymphoid tissue or blast localization in mucosae.

1.3.5 An in vitro model of lymphocyte adherence to HEV

An in vitro model has been developed to study the factors which affect the adherence of lymphocytes to HEV. Lymphocytes layered over glutaraldehyde fixed or frozen sections of lymph node adhere preferentially to HEV. This adherence was maximal at 7 °C. The adherence of TDL lymphocytes in vitro correlated with the % of injected radioactivity recovered in lymph nodes (Stamper & Woodruff, 1977). Similarly, the adherence of MLN lymphocytes in vitro correlated with their localization during a single-pass perfusion through a lymph node at 37 °C and in vivo at 15 min after intravenous injection as assessed by fluorescence and autoradiography (Butcher et al., 1979). Adherence was energy dependent, being inhibited in vitro by iodoacetate and azide

and was sensitive to trypsin treatment of the TDL lymphocytes but not to neuraminidase treatment (Woodruff et al., 1977). Thus the observed effects of trypsin and azide on lymphocyte localization in vivo are likely due to interference with their binding to HEV. In contrast and as expected, the in vivo effects of neuraminidase were not due to interference with lymphocyte binding to HEV. The in vitro system also revealed that lymphocyte binding was calcium but not magnesium requiring, and involved the microfilament but not the microtubule system (Woodruff et al., 1977). These authors argued that surface receptor movement was likely necessary since ionophore A-23187, which inhibits cap formation in mouse B cells, inhibited adherence. However this drug promotes the transport of divalent cations across cell membranes and therefore its effect might have been mediated by altering the availability of calcium ions (Woodruff et al., 1977). There was no difference in vitro in the adherence of lymphocytes from different individual mice, different sexes, different ages, syngeneic or allogeneic donors or after treatment of lymphcyte donors with hydrocortisone acetate (Butcher et al., 1979). In vivo localization in irradiated recipients or isolated, perfused nodes was also not affected by histocompatibility differences (Zatz et al., 1972). Differences which have been reported seem to have been due to removal of allogeneic cells by lungs and liver, rather than an inability to enter lymph nodes from blood or afferent lymph (Frost et al., 1975).

The specificity of localization might be determined at the level of the vascular endothelium. T cells and B cells enter lymph

nodes by the same HEV route (Nieuwenhuis & Ford, 1976) and do not differ perceptibly in their locations until at least an hour after they have left the blood (Ford et al., 1978). This does not necessarily imply that they are binding to the same putative HEV receptor. In contrast to blasts, the organ source (PLN, PP, spleen) of T or B lymphocytes did not influence their localization pattern in lymph nodes at 2 h after transfer (Stevens et al., 1982). However, T and B cells do show different localization patterns from one another (Stevens et al., 1982). T cells localized five fold more frequently than B cells in PLN but T and B were equally numerous in the Peyer's patch. B cells localized somewhat more frequently in MLN than in PLN and B cells were 1.5 fold more frequent than T cells in the spleen (Stevens et al., 1982). The distribution of in vitro adherence to HEV was similar to in vivo localization except that B cells (relative to T cells) bound more efficiently in vitro than they localized in vivo. Stevens et al. (1982) stressed that the in vivo situation involved many more factors than just adherence to the HEV. These authors noted that the proportion of T and B cells in a tissue paralleled the migratory and endothelial interaction tendencies of T and B cells for that tissue. They suggested that the availability of these lymphocyte classes, and thus the immune response in a lymphoid organ, could be influenced by lymphocyte migration, determined largely by HEV adherence (Stevens et al., 1982).

The in vitro model of lymphocyte localization in lymph nodes has provided evidence which suggests that this process may be mediated by specific receptors. Both at 30 min after transfer and in vitro, PP

cells adhered more to PP and less to PLN and mediastinal LN than did MLN or PLN cells (Butcher et al., 1980). PP cells adhered to MLN less than to PP but more than to PLN. In contrast, PLN cells adhered equally to PP, MLN and PLN. Some thymic lymphoma clones were selectively adherent in either PP or lymph node, whereas others adhered equally well to both. Butcher et al. (1980, 1982) suggested that these different patterns of localization reflected the differential expression of node or Peyer's patch - specific receptors on T and B cells, and determinants on HEV. They also suggested that MLN HEV which were unique in binding both PLN-specific and PP-specific cells, probably express both PLN and PP HEV determinants.

Chin et al. (1980a,b, 1982) detected a factor in rat TDL which can bind to HEV and inhibit lymphocyte attachment. The factor appeared to be a 160,000 MW glycoprotein antigenically related to surface components of TDL lymphocytes (Chin et al., 1980a,b). Adherence of TDL to lymph node sections was inhibited by treating the lymphocytes (but not sections) with anti-inhibitory factor Ig or the $F(ab')_2$ or Fab fragments of anti-inhibitory factor Ig. Adherence was not inhibited by the $F(ab')_2$ fragments of anti-thymocyte or anti-Ig antibodies (Chin et al., 1980b, 1982). Anti-inhibitory factor Ab treatment of TDL lymphocytes decreased the 2 h in vivo localization in cervical and axillary lymph node by 70%, and in MLN by 20 to 40% but did not decrease localization in PP nor change localization in spleen, liver, lung, or blood (Chin et al., 1982). Both T and B cells from TDL gave similar results. Anti-thymocyte or anti Ig $F(ab')_2$ had no effect on in

vivo migration (Chin et al., 1982). The authors concluded that specific adherence molecules are responsible for the entry of recirculating lymphocytes into lymph nodes, and they suggest that different molecules may mediate their entry via HEV into Peyer's patch and lymph nodes (Chin et al., 1982). Thus there is evidence from two laboratories which suggests that specific determinant - receptor interactions are involved in small lymphocyte localization in lymph nodes. Perhaps a similar mechanism directs selective blast localization in the mucosae.

1.4 Sex Hormone Influences on the Immune System

The observation that selective localization of gut-derived blasts in the mammary gland was associated with its development during pregnancy and lactation or after stimulation with estrogen and prolactin (Roux et al., 1977; Weisz-Carrington et al., 1978) stimulated the interest of mucosal immunologists in sex hormonal influences on the mucosal immune system. In general, males have a lower resistance to infection and a weaker immune response than females, while females have a higher incidence of some autoimmune diseases. The mechanisms underlying these differences are not understood but seem likely to be related to sex hormone differences, since variations in immune indices have been noted during the estrus cycle in both experimental animals and humans (Krzych et al., 1978; Mathur et al., 1979). Cohn (1979) critically reviewed the literature and found that, at that time, no unifying conclusion could be drawn concerning the effects of sex

hormones on immune responsiveness. In both sexes goradectomy has been reported to have either no effect, a detrimental effect or to enhance resistance to disease. Even when the same doses of gonadal hormone were used there have been conflicting reports regarding their effect on immune responses. Cohn (1979) stressed that inconsistencies in conclusions from different investigators are not surprising since the literature is complicated by inconsistencies in techniques, inappropriately controlled experiments and the different effects of different hormone doses, suspending vehicles, routes of administration, age, physiologic state of the animal and strain, including strain associated differences in hormone sensitivity. Another complication is added by the fact that androgens can serve as estrogen precursors and can function through estrogen receptors.

In spite of these problems, some information regarding gonadal hormone interactions with components of the immune system seems clear. Receptors for estradiol, dihydrotestosterone, progesterone and dexamethasone are present on chicken bursal epithelial cells (Sullivan & Wira, 1979). However, although glucocorticoid receptors are present on bursal lymphocytes, sex hormone receptors are not (Sullivan & Wira, 1979). Similarly, estrogen (Gillette & Gillette, 1979; Kalland & Forsberg, 1980) and dihydrotestosterone (Sasson & Mayer, 1981) receptors are present on thymic epithelial cells but not thymic lymphocytes. Thus gonadal hormones could influence the immune system through effects on the epithelial cells of primary lymphoid tissues.

Although specific receptors have not been detected on mixed lymphocyte populations gonadal hormones may effect subpopulations

directly. Kenny et al. (1976) found that in vitro doses of estradiol which approximated physiological serum levels in females, enhanced the plaque-forming cell response of male splenic lymphocytes from animals which had been immunized with E. coli 3 days previously. This effect was also observed after in vivo administration of physiologic doses of estrogen (Kenny et al., 1976). Progesterone also increased the plaque-forming cell response (Kenny & Diamond, 1977). Similarly, Paavonen et al. (1981) observed that physiological concentrations of estradiol (but not testosterone) in vitro increased the formation of plaque-forming cells, primarily those containing and secreting IgM, rather than IgG or IgA in pokeweed mitogen stimulated cultures of human peripheral blood lymphocytes without affecting the proliferative response. Estradiol seemed to act by inhibiting the suppressive activity of radiosensitive, Fc(IgG) receptor positive T lymphocytes. Paavonen and coworkers suggested that this might be the cellular basis for the differences in immune responses between males and females.

1.4.1. Sex hormone influences on the mucosal immune system

Hormonal variations influence the accumulation of lymphoctyes in target tissues. Estrogen and prolactin treatment of recipients enhanced the selective localization of MLN compared to PLN blasts in the mammary gland (Weisz-Carrington et al., 1978). The movement of small lymphocytes into the uterus was increased by a 3 day estradiol treatment of ovariectomized rats (Wira et al., 1980). The localization of MLN compared to PLN blasts in the cervix and vagina was greater at proestrus and estrus than at metaestrus and diestrus (McDermott et al.,

1980a). Whether these observations were due to hyperemia and the resultant increased delivery of lymphocytes to these tissues and/or to direct influences on the vasculature is not known. There are sex differences in the height of the endothelial cells in the HEV of lymph nodes where lymphocytes extravasate (Henry & Beverley, 1975). These cells are higher in female than male mice, and the height in males but not females could be increased by gonadectomy (Kittas & Henry, 1979). Pharmacologic doses of hexoestrol for 7 days increased the height of these cells in both male and female mice (Kittas & Henry, 1979). This seemed to be independent of the decreased number of lymphocytes crossing HEV as a result of the estrogen treatment since this would have been expected to result in a decrease in cell height (Kittas & Henry, 1979). The significance of endothelial cell height to lymphocyte localization is unclear since lymph nodes and PP are able to concentrate circulating lymphocytes better than other organs at a time in their developement when small lymphocytes first appeared within them and before the cuboidal appearance of the PCV was evident (Goldschneider & McGregor, 1968; Miller et al., 1969). Thus there are sex and gonadal-hormone related differences in both the accumulation of blasts in mucosae which are sex hormone target tissues and in the morphology of the vasculature where lymphocytes leave the blood and enter lymph nodes. Perhaps sex differences and hormones also influence the accumulation of blasts in mucosae which are not target tissues.

1.5 Research Proposal

Blasts stimulated by antigen at one mucosal surface leave that area and, after maturing, return selectively to that tissue and to the other mucosae. This seems to be a major mechanism which results in the dissemination of immune information to all the regions where it is likely to be required but limits the response in the systemic immune system where it might be detrimental rather than protective, perhaps by enhancing the probability of autoimmune reactions. An understanding of the mechanism which results in this selective localization would be invaluable when attempting to alter immune responses in the mucosae. The purpose of this thesis was therefore to examine factors which might potentially influence this phenomenon. I chose to use the adoptive transfer of MLN or PLN blasts in syngeneic CBA/J mice as the model for selective mucosal localization since this was a system which had been used extensively by our laboratory and by others, so comparisons were facilitated. Although some aspects of the system were already well documented (see Disscusion), some important basic issues had not been addressed. The dose of labelled blasts transferred could not be controlled and therefore, in the past, comparisons had been made between different populations without controlling for, or knowing the effect of, altering the dose of cells transferred. Experiments involving the transfer of different doses of cells would also give an indication of the capacity of the mucosal tissues and their various morphologic regions to accumulate blasts in the absence of any specific antigenic or inflammatory stimulus. This could be important if one

wished to enhance the lymphoid population in the mucosae without altering the recipient tissue by direct stimulation. For example, if for the purposes of prophylaxis, large numbers of mucosally derived blasts were obtained from a specifically immunized donor and transferred into a naive recipient, what limits would the recipient tissue place on the number of those blasts which might enter it? Another issue was what contribution selective entry, compared to selective retention, played in the selective localization of blasts in mucosal tissues. The site in the mucosa at which the blasts extravasated was also unknown. Was the selective localization of MLN blasts apparent at all times after transfer or was it partially a reflection of different rates of localization compared to PLN blasts? I attempted to address these questions by examining the kinetics of selective localization. Sex hormones enhance some aspects of the mucosal immune system, including blast localization, in the genital tract and mammary glands. I investigated whether sex differences and gonadal hormones influenced the localization of MLN blasts in a tissue, the small intestine, not known to be a sex hormone target.

CHAPTER 2

Materials and Methods

2.1 Animals

CBA/J male and female mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Age matched mice were used in all experiments. Normally, mice were 6 to 12 weeks old when used in adoptive transfer experiments. Mice which had been altered surgically were up to 17 weeks when used. Mice were housed in a 12h light/12h darkness regime and fed Purina lab chow and watered ad libitum

2.2 Oophorectomy and Orchidectomy

Anaesthetic for surgery was 0.3 ml intraperitoneal of the following: 0.25g Tribromoethanol (ICN Pharmaceuticals,Inc. Life Sciences Group Plainview, N.Y.) dissolved in 0.15 ml 2-Methylbutan-2-ol (BDH Chemicals Ltd. Poole England) and then mixed with 10 ml phosphate buffered saline (PBS). Prior to surgery, the fur was wetted with 70% ethanol. To minimize hemorrhage, blood vessels were tied with synthetic suture silk (Davis and Geck, Cyamid Canada Inc., Montreal, Que.) or clamped with small hemoclips (Weck Surgical Co., Weston, Ontario). Wound clips (Becton, Dickinson and Co. Parsippany N.J. 07054) were used to close the incision. Following surgery, mice were housed under standard conditions except that tetracycline was added to drinking water for 5 days.

For oophorectomy, each ovary was removed through a small

dorsal incision lateral to the erector spinae muscle. For sham oophorectomy the ovary and uterus were pulled out through the incision and then reinserted. For orchidectomy, the testes were removed through a midline incision in the scrotum. For sham orchidectomy the incision was made, the testes manipulated and the wound closed.

The success of the oophorectomy or orchidectomy in decreasing sex steroid hormone levels was assessed by visual examination of the degree of atrophy of the uterus (Greiger, 1973) or seminal vesicles respectively (Chai,1956). The occasional animal in which these organs were not atrophied was excluded from the experiment.

2.3 Preparation of Lymphocyte Suspensions

Mice were killed by cervical dislocation and their mesenteric (MLN) or peripheral (PLN: axial, brachial and inguinal) lymph nodes removed. Lymph nodes were placed in Hanks' balanced salts solution (HBSS; Grand Island Biological Co., Grand Island, N.Y.) supplemented with 10% v/v fetal bovine serum (FCS, Grand Island Biological Co.) or gammaglobulin free horse serum (GGfHS, Grand Island Biological Co.) and 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES, Sigma Chemical Co., St Louis, Mo.) and then adjusted to pH 7.2 and 310 mOsM/kg. Lymph nodes were rolled on bibulous paper to remove fat and then disrupted by pressing through 60-mesh stainless steel sieves into supplemented HBSS. To remove cell aggregates and fine debris, cell suspensions were layered over FCS (or GGfHS) and sedimented at 1g and room temperature for 1 h. The suspension was subsequently centrifuged through FCS (or GGfHS) at 200xg and 4°C for 12 min. (Shortman, 1972).

To wash, the cell pellet was resuspended in supplemented HBSS and centrifuged again. Viable nucleated cells were enumerated in a hemocytometer using 0.4% w/v trypan blue dye in saline (Pappenheimer,1917).

2.4 Radiolabelling of Dividing Cells

Dividing cells were radiolabelled by the addition of 5uCi/ml [^{3}H]-thymidine (^{3}H-Tdr, 6.7 Ci/mM; New England Nuclear Corp.,Boston, Mass,) or [^{125}I]-deoxyuridine (^{125}I-Udr,>2000Ci/mM; New England Nuclear Corp.) to cell suspensions which had been adjusted to 1×10^{7} viable nucleated cells per ml. Cell suspensions were incubated at 37^{o}C for 90 min and then centrifuged at 200xg and 4^{o}C for 12 min. The cells were then washed three times in supplemented HBSS to remove unincorporated radiolabel.

2.5 Adoptive Transfer of Radiolabelled Cells

Viable nucleated cells were enumerated in 0.4% (w/v) trypan blue dye. Cell suspensions were adjusted to a concentration such that the total volume of inoculum per recipient would not exceed 0.4 ml. Mice under physical restraint with no anaesthetic or under light ether anaesthesia were injected with a pre-determined number of cells via a lateral tail vein. If the radioactivity in the recipient was to be detected by gamma counting, the radioactivity in the syringe was counted before and after injection of the inoculum in order to

determine the disintegrations per minute (dpm) of radioactivity transferred. If the radioactivity in the recipient was to be detected by autoradiography, an aliquot of the cell suspension was used to prepare cell smears so that, after autoradiography, the proportion of cells in the inoculum which had incorporated radioactivity could be determined.

2.6 Autoradiographic Detection of ^{125}I-Udr or ^{3}H-Tdr Associated Radioactivity

2.6.1. Tissue processing

The time allowed for blast localization after adoptive transfer was 22 to 24 h unless the purpose of the experiment was to study the kinetics of localization. Mice were killed by cervical dislocation.

The small intestine was removed and flushed of fecal material with 0.85% NaCl. It was then coiled, with the duodenal end in the centre, in a plastic tissue cassette (Lab-Tek Products, Miles Laboratories Inc., Naperville, Ill.) so that the entire gut could be sectioned longitudinally. In some experiments the Peyer's patches were removed from the small intestine before it was coiled so that they could be processed separately. I attempted to position Peyer's patches so that sections included both the lumenal and serosal surfaces. The lungs were oriented with the dorsal surface on the bottom of the block, so as to result in longitudinal sections of the primary bronchi. All tissues were fixed in 10% buffered (pH7.2) formalin (BDH Chemicals, Toronto), embedded in paraffin and sectioned at 5um. A solution of 50%

albumin:50% glycerin was used to adhere tissue sections to the glass microscope slides. Paraffin was removed by washing in xylene, and then the tissues were dehydrated in alcohol and the slides were air dried.

2.6.2 Autoradiography

Autoradiography was performed in complete darkness. The slides were dipped in a 50% dilution of NTB/2 photographic emulsion (Kodak Canada Ltd., Toronto, Ont.) and distilled water at 40 C. They were air dried with a blow drier, placed in a light-proof slide box containing a packet of Drierite (Fisher Scientific Co. Ltd., Toronto, Ont,) and stored at -20 C until developed.

Autoradiography is a powerful tool for examining the localization of radiolabelled cells but it suffers from the disadvantage that the exposure time is measured in weeks. To eliminate this problem, the exposure can be enhanced by secondary photo-emission from fluors in a liquid scintillator in contact with the photographic emulsion (Panayi & Neill, 1972: Durie & Salmon, 1975: McDermott & Bienenstock, 1979). Therefore many of the tissues were processed for high speed autoradiography by immersing the emulsion coated slides, after drying, in a dark adapted toluene-based scintillation fluid (6g 2,5-diphenyloxazole (PPO) and 75 mg 1,4-bis(2-(5-phenyloxazolyl))-benzene (POPOP) per litre of toluene) for a period of 24h at -20 C. Dr. M. McDermott determined in our laboratory, using cell smears, that this was the optimal exposure period to observe all the labelled cells (Ph.D. thesis). Therefore, although similar within an experiment, no attempt was made to be consistent from one experiment to the next, in

the exposure period in air at -20°C before their exposure in scintillation fluid. Recently, I observed that a 24h exposure of tissue sections (rather than cell smears) in scintillation fluid did not result in exposure of as many silver grains (and therefore not as many cells labelled heavily enough to be scored as positive) as did a 3 week exposure in air (Table 1). Therefore for any experiments in which the exposure method might have been inadequate, new tissue sections were processed for autoradiography with a 3 week exposure. In all experiments but one, although the absolute number of radiolabelled cells varied from the high speed exposure, the conclusions of the experiments were unaltered (Table 2).

Tissues were lightly stained with Harris' hematoxylin and eosin.

2.6.3 Microscopy

The concentration of radiolabelled cells in a tissue was determined by recording the area, in high power microscopic fields (HPF) at 400 diameters, which was scanned and the number of radiolabelled cells which were observed. The actual area of each microscopic field was 0.159 mm^2 and usually 1000 of these were scanned for each tissue. Thus the quantity of tissue sampled from each animal was approximately an area of 159 mm^2. The volume of tissue examined was 0.795 mm^3 (159mm^2 area x 0.005mm section thickness) but the volume of intestine surveyed for radiolabelled cells might have been as much as twice this amount because the nuclei of radiolabelled cells not entirely contained within the section would also have been scored. For ease of comparison with data generated by other individuals in our

Table 1

The Effect on Grain Density of Exposing Autoradiographs For 24 h in Scintillation Fluid or 3 weeks in Air

Experiment	Exposure[a]	Method	Grain Density[b] Light	Normal	Heavy
K1	0.5h	H.S.	85.3+/-0.4[c]	14.7+/-0.4	0
		3 WK	6.6+/-1.8	64.5+/-5.2	25.6+/-3.7
	24h	H.S.	94.3+/-3.0	5.7+/-3.0	0
		3 WK	18.0+/-3.5	57.0+/-5.5	25.0+/-2.9
K8	0.5h	H.S.	19.6+/-0.7	45.3+/-15.6	35.1+/-16.3
		3 WK	4.4+/-0.5	41.7+/-13.2	54.0+/-12.7
	24h	H.S.	35.0+/-12.3	46.1+/-8.4	18.9+/-5.2
		3 WK	7.2+/-2.8	42.7+/-6.6	50.5+/-9.0

a) Exposure method was either high speed (H.S.) i.e. 24 h in scintillation fluid, or a 3 week exposure in air (3 WK).

b) The density of grains over labelled cells was classified as: heavy, cell totally obscured by silver grains; normal, intermediate number of grains, cell not totally obscured; light, 5 to 10 grains per cell when background was 2.

c) Figures represent mean +/- SE of the percent of cells with light, normal or heavy silver grain density from three individuals per experimental group.

Table 2

Effect on Experimental Conclusions of Exposing Autoradiographs for 24h in Scintillation Fluid or 3 weeks in Air

Experiment	Method of Exposure				Diff c)
	High Speed		3 weeks		
	data a)	conclude b)	data a)	conclude b)	
K1 d) 0.5h	8.2+/-1.4		46.8+/-3.6		
24h	12.3+/-5.1	1/2h=24h	129.1+/-3.5	1/2h<24h	yes
K8 0.5h	25.2+/-12.4	1/2h=24h	49.1+/-20.9	1/2h=24h	
24h	13.1+/-0.7	trend 1/2h>24h	25.2+/-3.0	trend 1/2h>24h	no
S4 e) ♂=♂	185.3+/-16.0		184.4+/-25.0		
♀→♀	80.2+/-4.1	♂=♂>♀→♀	98.7+/-4.4	♂=♂>♀→♀	no
S8 ♂=♂	32.5+/-6.0		41.6+/-2.2		
♀→♀	28.1+/-1.4	♂=♂=♀→♀	42.1+/-2.1	♂=♂=♀→♀	no
S9 ♂=♂	27.9+/-5.6		30.2+/-2.1		
♀→♀	28.8+/-3.4	♂=♂=♀→♀	37.6+/-6.2	♂=♂=♀→♀	no

Table 2 (footnotes)

a) Number of radiolabelled MLN cells per 10^3 HPF of small intestine, mean +/- S.E., n=3 per group

b) Conclusion based on Student's t test; α =0.05

c) Did the different exposure methods produce a difference in the conclusion of the experiment?

d) Experiments with a code beginning with K compared MLN lymphoblast localization in the small intestine at different times after transfer, 0.5 h and 24 h.

e) Experiments with a code beginning with S compared the localization of MLN lymphoblasts from male (♂) or female (♀) donors in ♂ or ♀ recipients.

laboratory using microscopes with different field areas, all data has been expressed as the number of radiolabelled cells per 1000 HPF with a field area of $0.1mm^2$.

The intestinal wall was divided, for descriptive purposes, into three morphological regions; the epithelium, the villus lamina propria and the basal lamina propria (Fig 1). Only longitudinally sectioned, intact villus-crypt units (Jarrett et al, 1968) were examined to ensure that each of these regions was as consistently represented as possible. Peyer's patches and solitary lymphoid nodules were not included when enumerating the cells which had localized in the intestinal wall.

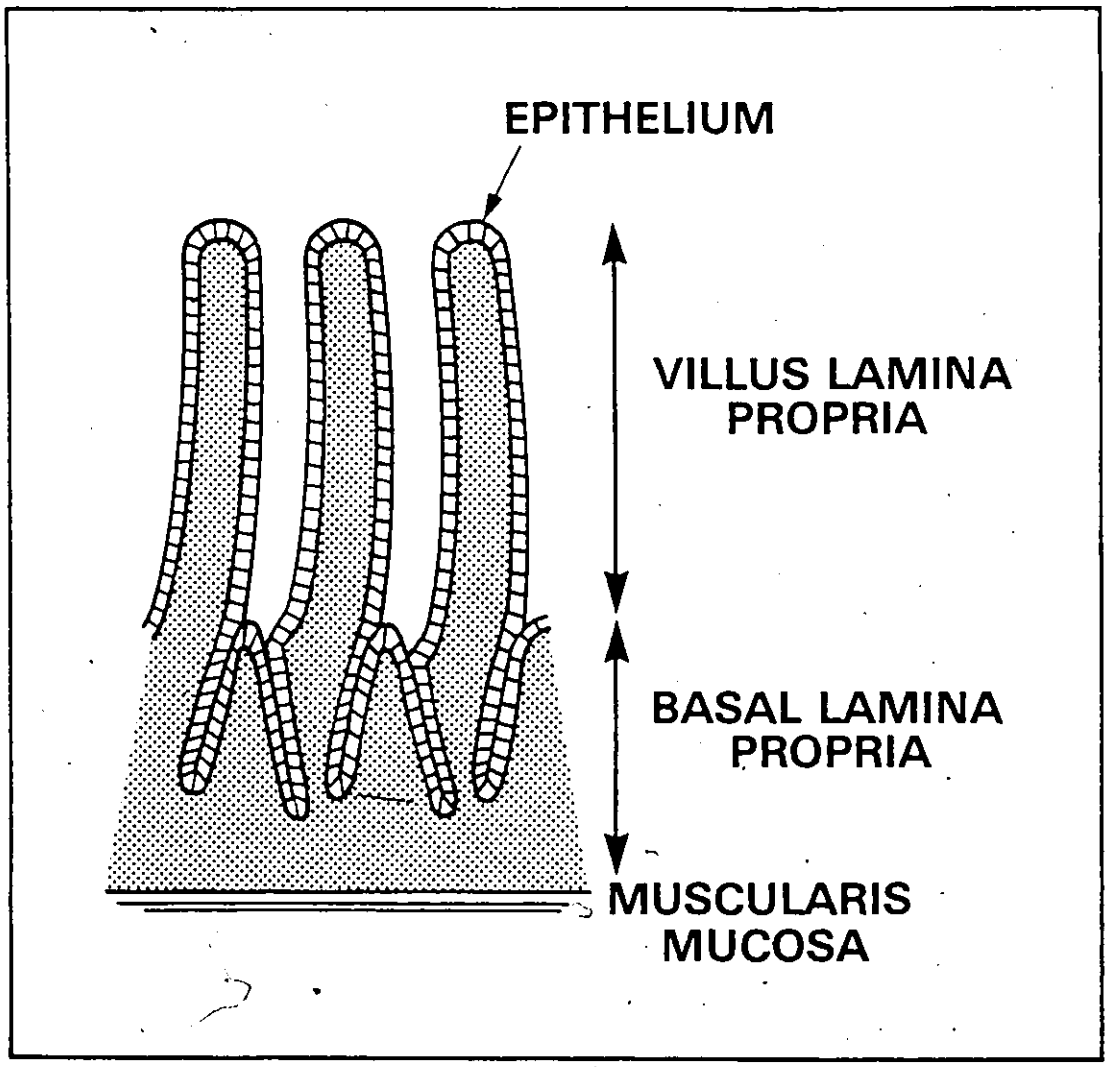
The entire area of each lung section was examined and the location of radiolabelled cells was recorded as the epithelium, the bronchus-associated lymphoid tissue (BALT, Bienenstock et al, 1973) or the parenchyma. The parenchyma comprised more than 90% of the area examined and was defined as non-epithelial, non BALT lung tissue including alveolar spaces, blood vessels and subepithelial mucosa. The location of cells in Peyer's patches was designated as within the follicle, thymus dependent area (TDA), dome or the epithelium (Faulk et al , 1971; Waksman et al, 1973).

2.7 Gamma-Counting Detection of ^{125}I-Udr Associated Radioactivity

All tissues were freed of fat and blood clots, rinsed with saline and fixed in formalin-filled scintillation vials before assaying for radioactivity in a Beckman Gamma 8000 and Gamma 9000 Counting System. The small intestine was flushed of fecal contents and, if to

Figure 1

Morphologic regions of the small intestinal mucosa. The regions studied were the epithelium, the villus lamina propria (defined as the region between the tip of the villus and the top of the crypts of Lieberkuhn), and the basal lamina propria (defined as the region between the top of the crypts of Lieberkuhn and the muscularis mucosae).


EPITHELIUM
VILLUS LAMINA PROPRIA
BASAL LAMINA PROPRIA
MUSCULARIS MUCOSA

be embedded with paraffin and processed for autoradiography, it was coiled and fixed in a tissue cassette before being placed in a scintillation vial for counting. After fixation and counting, tissues were blotted dry and weighed. Since the entire carcass of each mouse was counted, it was meaningful to express the data both as a percentage of the total dpm recovered and as a percentage of the total dpm transfered. These percentages were then divided by the weight of the tissue to obtain figures which represented the relative concentration of radioactivity in each tissue.

2.8 Statistical Analyses

To fit regression lines to some of the data, computer-assisted regression analyses (Draper & Smith, 1966) were performed by Dr. Gerry Chan, Dept. Epidemiology and Biostatistics, McMaster University, Hamilton, Ont.. Student's unpaired t test was used to test for significant differences between groups and the paired difference test was used for differences between separate scorings of the same tissues.

RESULTS

CHAPTERS 3,4,5,6,7

Chapter 3 Accuracy and Reproducibility of the Estimation of the Numbers of Labelled Cells in Inocula and in Tissue Sections

3.1 Confidence in Estimating the Percentage of Radiolabelled Cells in the Inoculum

During adoptive transfer experiments the concentration of cells in each inoculum was known, but the proportion of these cells which were radiolabelled (and therefore detectable after transfer) was unknown. This proportion was determined by preparing autoradiographs of inoculum cell smears and counting the proportion of radiolabelled cells. It was necessary to know the reliability of this estimate to compare results from different experiments. The level of confidence that this estimate of the proportion of radiolabelled cells represented the true proportion was determined using the t statistic and the formula $D=t_{\alpha/2}^{0.5}\,100(p(1-p)/n)$ where p=proportion, D=deviation and n=number of cells counted (Brown & Hollander, 1977). This statistic allows one to determine with 95% certainty (α=0.05) that the true proportion lay within the range 100p+/-D. For example, in experiment S8 autoradiographs of cell smears of the inoculum (^{3}H-Tdr labelled MLN from male mice) were prepared. When these were examined, 9143 cells were counted of which 148 were labelled i.e. 1.62% radiolabelled cells

(blasts). The confidence in this proportion was calculated as follows:

$$D = t_{\alpha/2}\,100(p(1-p)/n)^{0.5}$$

$$= (1.96)(100)(0.0162(1-0.0162)/9143)^{0.5}$$

$$= 0.26$$

Therefore we were 95% certain that the true proportion of radiolabelled cells in the inoculum was within the range 1.36% to 1.88%. The deviation in estimates of this proportion was similar in all experiments.

3.2 Reproducibility of Scoring the Number of Cells per 10^3 HPF

When examining autoradiographs of tissue sections it was necessary to find the appropriate balance between maximizing the validity of the estimate by increasing the number of HPF counted and minimizing the time required to obtain the data by reducing the number of HPF counted. It had been the convention in our laboratory to examine 1000 HPF of a particular tissue for each animal. When examining this number of fields the difference in the number of radiolabelled cells per 10^3 HPF detected by replicate scoring of the same tissue sections (Table 3) was less than or equal to 25% for 31 of 37 individuals (i.e. in 83.8% of the individuals the variation between scorings was less than or equal to 25% of the number of labelled cells observed). The tissues from four experiments were scored twice and in each experiment both scorings indicated the same conclusion (Table 3). One experiment was scored four times and three out of four times the conclusion was the same (Table 3). In addition, three other experiments (S4, S8, S11) tissues were resectioned, processed for

Table 3

Reproducibility of Results When Scoring 10^3 HPF of Tissue per Individual

Experiment group (n)	Scoring a) 1st	2nd	Difference b) between scorings
S7			10.2%+/-3.0% c)
normal (3)	64.8+/-10.3	68.9+/-8.3 d)	No change in conclusion of experiment
sham (3)	91.7+/-5.1	83.7+/-9.7	
cast (3)	65.6+/-10.7	66.5+/-5.8	
K1			15.8%+/-7.1%
1/2 h (3)	64.9+/-5.8	83.2+/-4.8	No change in conclusion of experiment
24 h (3)	205.4+/-5.5	202.6+/-4.0	
K9			20.1%+/-11.0%
1/2 h (3)	32.3+/-6.0	33.0+/-4.9	No change in conclusion of experiment
24 h (3)	12.3+/-2.4	15.0+/-4.1	

Table 3 (continued)

Experiment group (n)	Scoring a) 1st	2nd	Difference b) between scorings
First processing e)			
K8			30.0%+/-11.3%
1/2 h (2)	73.3+/-21.1	96.9+/-19.0	
24 h (3)	62.7+/-5.3	49.8+/-14.5	
Second processing			
K8			14.3%+/-4.4%
1/2 h (2)	108.4+/-33.3	119.0+/-32.7	Same conclusion in 3 of the 4 scorings of the same tissues from experiment K8
24 h (3)	40.1+/-4.7	47.3+/-6.0	
S4 (6)			13.2%+/-4.9% No apparent change in conclusion, not enough individuals per group in second scoring to test statistically

a) The number of radiolabelled cells per 10^3 HPF of small intestine were determined on two separate occasions, 1st and 2nd scorings.

b) The conclusions of each scoring of each experiment were based on a Student's T test at $\alpha=0.05$.

Table 3 (continued)

c) The mean +/- S.E. of the % difference between scorings for each individual in the experiment.

d) Mean +/- S.E. of the number of radiolabelled cells per 10^3 HPF in the small intestine.

e) The same tissues were sectioned and processed twice for autoradiography, first and second processing, and each set of slides was scored twice.

autoradiography and scored and the conclusions of the experiments were the same in both the first and second processings. Thus, using a number of different approaches I determined that the variation in results after counting 1000 HPF was not greater than 25%.

Much of this variation could be accounted for by the probability of detecting radiolabelled cells given their low frequency. Since observing more than one cell per field was a very rare event I have chosen to ignore this possibility for simplicity and have defined as positive, a field in which I observed a labelled cell. From the dose curve for the small intestine (Fig. 4) I would predict that for a dose of 1.48×10^6 labelled MLN blasts transferred, their frequency in the small intestine 24 h later would be 35 per 1000 HPF i.e. 3.5% positive HPF. Using the formula for estimating confidence in proportions (Section 4.1) I calculated the 95% confidence limits to be 3.5%+/-1.14% that is, $(35+/-11.4) \times 10^6$ labelled cells per 1000 HPF. This range is remarkably close to the 95% confidence limits for the line of best fit through the dose experiments, $(35+/-8.6) \times 10^6$. Thus the majority of the variation between individual animals and between experiments was due not to biological variation but to errors in estimating a proportion from a relatively small sample. Increasing either the number of radiolabelled cells in the small intestine (by transferring more cells) or the number of HPF scored, would reduce the variability. However, this would have resulted in dramatically increased cost of animals and technical assistance and was therefore not feasable. I think that the nature of the information obtainable by autoradiography favours its use in spite of the relatively high

variation in autoradiographic results.

Chapter 4

The Prototype Adoptive Transfer Model of Selective Localization

Before attempting to manipulate the adoptive transfer model of blast localization we established a prototype set of conditions with which other situations could be compared. This set of conditions was similar to that used previously in our laboratory and in other laboratories. It included the use of female CBA/J mice, the transfer of 1.0×10^8 cells and the removal of tissues 24 h after the adoptive transfer of radiolabelled blasts.

4.1 Gamma Counting Examination of the Prototype

Gamma counting of tissues after transfer of ^{125}I-Udr labelled MLN blasts gave a picture of the relative distribution of these cells throughout the body. This type of data has been commonly expressed as the percentage of injected radioactivity which was recovered in an organ. Data expressed in this manner (Table 4) revealed a distribution similar to that described previously in our laboratory (McDermott, Ph.D. thesis 1979). The small intestine contained the greatest amount of recovered radioactivity. Approximately half as much radioactivity was recovered in the spleen and a quarter as much in the liver. Trace amounts were detected in the lungs, Peyer's patches, MLN and PLN. However, because this distribution may have merely reflected the relative size of the organs, we also expressed the data as the percentage of injected radioactivity in an organ divided by the weight

of that organ. This resulted in a description of the relative concentration of radioactivity in an organ (Table 4). Unfortunately this useful method of expressing data has rarely been used in the literature. I found that the concentration of radioactivity in the liver and lungs was low compared to that in the small intestine and Peyer's patches. The concentration in the Peyer's patches was about twice that in the small intestine. This pattern and the concentrations were similar to those observed by Smith and coworkers (1980) after the transfer of ^{125}I-Udr labelled MLN blasts in the rat. Unlike Smith et.al. (1980), I found that the concentration of radioactivity in the MLN and PLN was slightly higher than in the Peyer's patches and there was no selective accumulation of MLN blasts in MLN compared to PLN. I found that after MLN blast transfer, the radioactivity was highest in the spleen. Smith et al. (1980) found roughly comparable levels of MLN blast associated radioactivity in MLN, PP and spleen. Both McWilliams et al. (1977) in the mouse and Smith et al. (1980) in the rat observed a greater % of injected radioactivity in recipient MLN than PLN after MLN blast transfer. The differences between my results and those of Smith et al. (1980) may be due to species differences or to the low number (2) of recipients used by Smith et.al. (1980). However, in addition to the radiocounting results of McWilliams et al. (1977), autoradiography has suggested that MLN blasts localize more in MLN than in PLN in the mouse (Guy-Grand et al., 1974; McDermott & Bienenstock, 1979) and rat (Griscelli et al., 1969). My results, when expressed on a per organ basis suggest greater localization of MLN blasts in recipient MLN than PLN (Table 4). This again stresses the importance

Table 4

Distribution and Concentration of ^{125}I-Udr Labelled MLN Lymphoblasts 24h after Adoptive Transfer in Female C8A/J Mice

Tissue	% of injected radioactivity a) per organ	b) per gram
Small Intestine	4.30+/-0.36 c) (6; 24) d)	3.34+/-0.33 c) (2; 8) d)
Small Intest. less Peyer's patches	3.20+/-0.34 (2; 8)	3.13+/-0.30 (2;8)
Peyer's patches	0.42+/-0.06 (2; 8)	7.20+/-1.21 (2; 8)
Lung	0.27+/-0.39 (4; 15)	1.51+/-0.28 (2; 8)
MLN	0.34+/-0.03 (4; 15)	14.62+/-2.88 (2; 8)
PLN	0.20+/-0.04 (2; 8)	17.05+/-3.60 (2; 8)
Spleen	1.94+/-0.33 (1; 5)	32.62+/-4.41 (1; 5)
Liver	0.96+/-0.09 (1; 5)	1.11+/-0.11 (1; 5)
Total Animal	23.9+/-1.9 (6; 23)	1.24+/-0.12 (2; 8)

Table 4 (continued)

a) % of injected radioactivity/organ = (dpm recovered in the organ/ dpm injected)x100

b) % of injected radioactivity/gram = 100(dpm recovered in the organ/ (dpm injected)(organ weight))

c) Figures represent mean +/- S.E.

d) (number of experiments; total number of individuals)

of expressing the same data in different ways and suggests that the small size of the lymph nodes in the mouse might have resulted in errors in determining tissue weight.

4.2 Autoradiographic Examination of the Prototype

Autoradiographic studies of adoptive transfer experiments have the advantage that in addition to providing information about the concentration of cells in a tissue, they also show the histologic location of the cells.

Table 5 shows the distribution, on a per unit volume basis, of ^{3}H-Tdr labelled MLN or PLN blasts in the small intestine. The tendency of MLN blasts, rather than PLN blasts, to selectively localize in the small intestine is well known and is clearly demonstrated by the data in this Table. Of the labelled MLN cells which localized in the small intestine, 64.71+/-1.86% (mean +/- SE of 23 recipients) were in the basal lamina propria (Plate 1), 32.07+/-1.80% in the villus lamina propria (Plate 2) and 3.53+/-0.55% in the epithelium (Plate 3). Did this distribution reflect a difference in the concentration of the cells in the various regions, or a difference in the proportional representation of these regions in a HPF? We estimated that of the tissue area in a HPF, 32.4%+/-0.57% (mean+/-S.E. of 30 estimates) was villus lamina propria, 43.9%+/-0.87% was basal lamina propria and 23.6%+/-0.56% was epithelium. Clearly the proportion of labelled cells in the epithelium was much less than the proportional representation of this region in a HPF. Therefore we concluded that the MLN blasts accumulated to a lesser extent in the epithelium than in the lamina

Table 5

Frequency and Distribution of Radiolabelled MLN or PLN Cells in the Small Intestine 24h after Transfer of 100×10^6 cells per Recipient Female Mouse.

Expt[a] (n)	Dose[b]	Epithelium	Lamina Propria Villus	Lamina Propria Basal	Total Gut
MLN transfers					
D1 (3)	2.5[c]	2.5+/-0.65	9.6+/-1.1	28.3+/-3.0	40.3+/-3.5
D2 (3)	1.35	3.13+/-0.9	17.1+/-5.0	33.4+/-5.3	53.6+/-11.0
D5 (3)	1.35	0.4+/-0.2	7.0+/-1.7	10.8+/-2.6	18.3+/-3.5
S4 (5)	1.36	2.1+/-0.2	24.8+/-2.2	53.3+/-2.8	80.2+/-4.1
S8a (3)	1.10	1.4+/-0.7	7.6+/-1.1	19.1+/-1.9	28.1+/-1.4
S8b (3)	1.30	0.2+/-0.2	11.3+/-0.5	31.3+/-5.5	42.7+/-5.5
S5 (3)	1.24	1.4+/-0.5	22.3+/-1.2	27.1+/-6.2	50.3+/-6.5
MLN mean+/-SE (23)		1.6+/-0.3	15.2+/-[illegible].6	31.2+/-3.1	47.9+/-4.6

Table 5 (continued)

Expt (n)	Dose a)	Epithelium b)	Lamina Propria Villus	Lamina Propria Basal	Total Gut
PLN transfers					
D4 (3)	1.12	0.2+/-0.2	0.4+/-0.4	0.2+/-0.2	0.8+/-0.6
D5 (3)	0.30	0	0	0	0
K2 (2)	0.15	0	0	0	0
PLN mean+/-SE (8)		0.08+/-0.07	0.16+/-0.15	0.08+/-0.07	0.31+/-0.22

a) experiment code, (n)

b) The percentage of cells in the inoculum which were radiolabelled.

c) Figures represent the mean +/- S.E. of the number of radiolabelled cells per 10^3 HPF of total small intestine.


20μ

Plate 1

A radiolabelled MLN lymphoblast in the basal lamina propria of the small intestine.


20μ

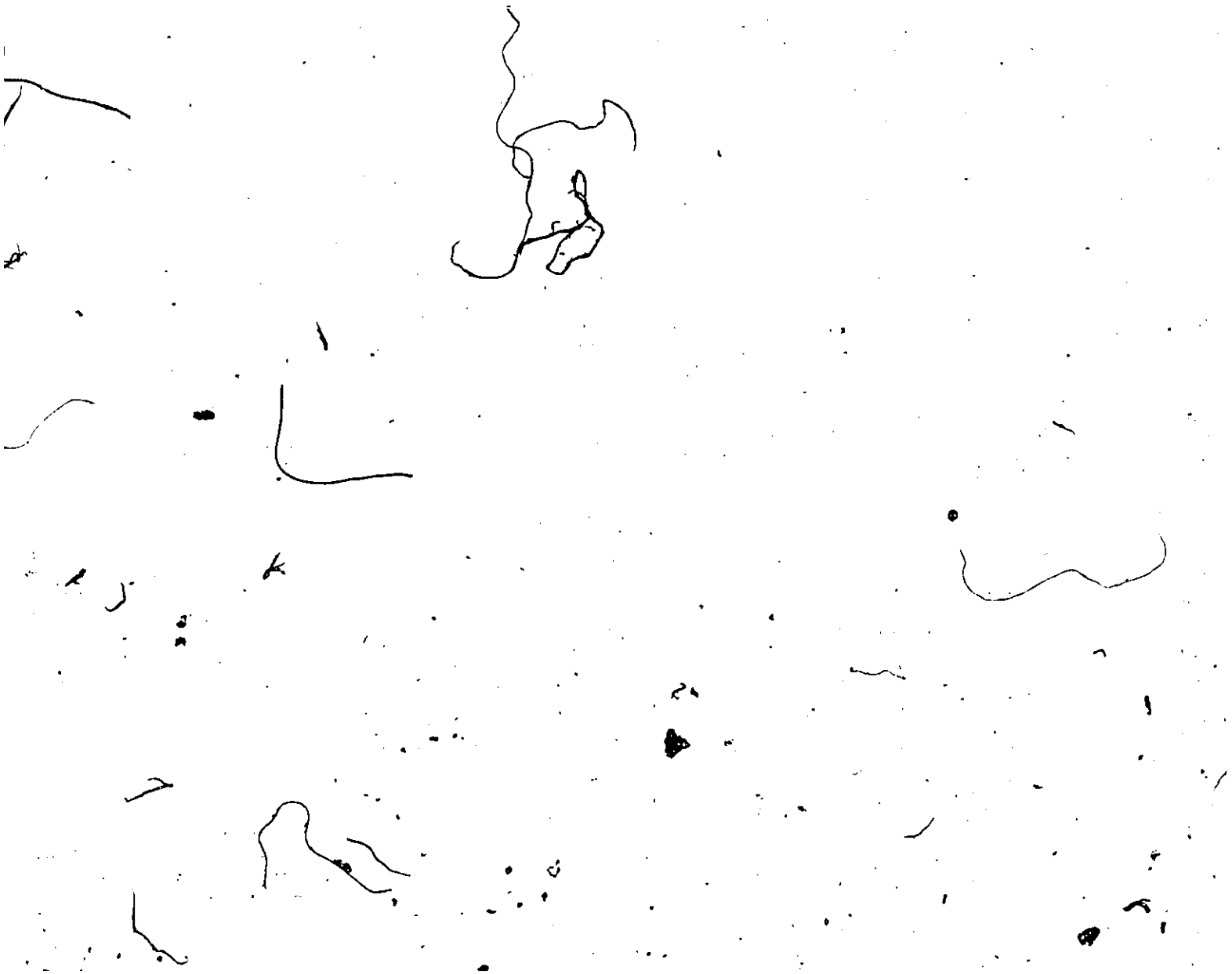
Plate 2

A radiolabelled MLN lymphoblast in the villus lamina propria of the small intestine.


20 μ

Plate 3

A radiolabelled lymphoblast in the intestinal epithelium. Note the position of the radiolabelled cell relative to other intraepithelial lymphocytes (◁).



propria. When considered separately, the area of lamina propria could be divided into 42.4%+/-0.75% villus and 57.5%+/-1.14% basal lamina propria. Of the labelled cells observed in the lamina propria, 34.20%+/-1.92% were in the villus and 69.0%+/-1.98% were in the basal lamina propria. Thus, although the concentration of radiolabelled cells in both these regions was similar, blasts did accumulate more in the basal lamina propria than in the villus lamina propria.

There was no selective localization of MLN compared to PLN blasts in the lungs (Table 6). The frequent appearance of labelled cells in the pulmonary parenchyma (Plate 4) but only occasional appearance in the BALT (Plate 5) and bronchial epithelium (Plate 6) likely reflected the relative abundance of these morphologic regions (see Section 6.1.2). Since over 90% of the cells in the lung were in the parenchyma, it seemed likely that these cells were trapped in the capillary network. Alternatively, this might have represented an integration, in the lung, of the systemic and mucosal immune systems.

In the Peyer's patch at 24h after transfer, the largest number of radiolabelled MLN blasts were observed in the TDA (Plate 7, Table 11). Although the area examined was small, the concentration of radiolabelled cells in the dome at 24h (Plate 8) appeared to be similar to that in the TDA. Very few radiolabelled cells were observed in the follicles (Plate 9) and only one intraepithelial blast was observed in the epithelium of the Peyer's patches from five animals.

Table 6

Frequency and Distribution of Radiolabelled MLN or PLN Cells in the Lung 24h after Transfer of 100×10^6 Cells per Recipient Female Mouse

Expt	Dose [a]	Bronchial Epithelium [b]	BALT	Pulmonary Parenchyma	Total Lung
MLN transfers					
D2 (3)	1.35	0.97+/-0.8 [c]	1.0+/-0.3	10.1+/-1.4	12.0+/-2.3
D5 (2)	1.35	0	0	8.1+/-4.0	8.1+/-4.0
K1 (4)	1.58	0	0	12.7+/-7.3	12.7+/-7.3
K2 (2)	0.36	0.3+/-0.3	0.6+/-0.6	7.5+/-3.6	8.4+/-3.4
S8 (3)	1.10	0	0	38.2+/-4.2	38.2+/-4.2
PLN transfers					
D4 (2)	1.12	0.7	0.5	5.5+/-1.1	6.6+/-1.31
D5 (3)	0.30	0	0	3.2+/-2.3	3.2+/-2.3
K2 (2)	0.15	0	0	12.4+/-3.4	12.4+/-3.4

Table 6 (continued)

a) experiment code, (n)

b) The percentage of cells in the inoculum which were radiolabelled.

c) Figures represent mean +/- S.E. number of radiolabelled cells per 10^3 HPF of total lung.

Plate 4

A radiolabelled MLN lymphoblast in the pulmonary parenchyma.

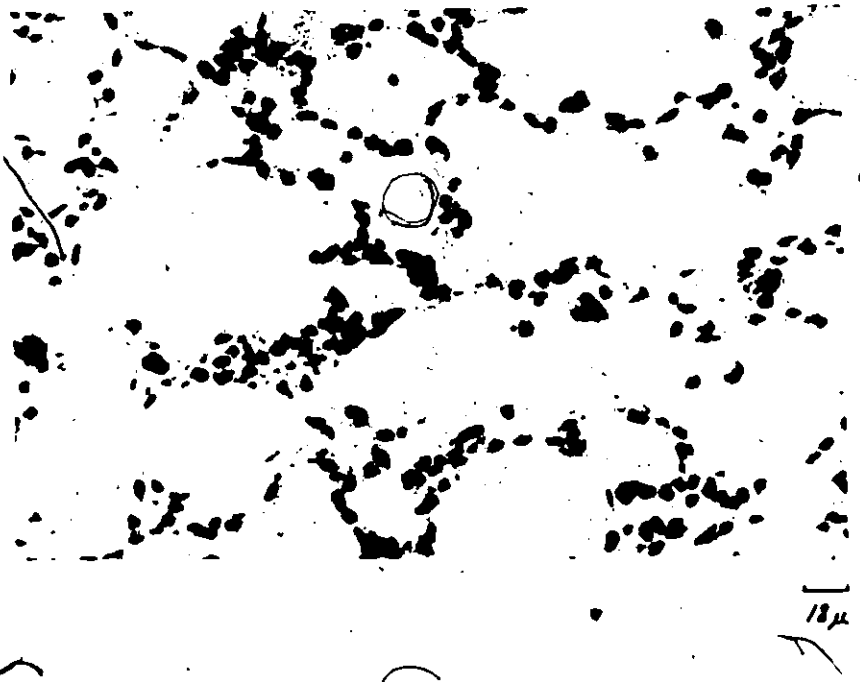
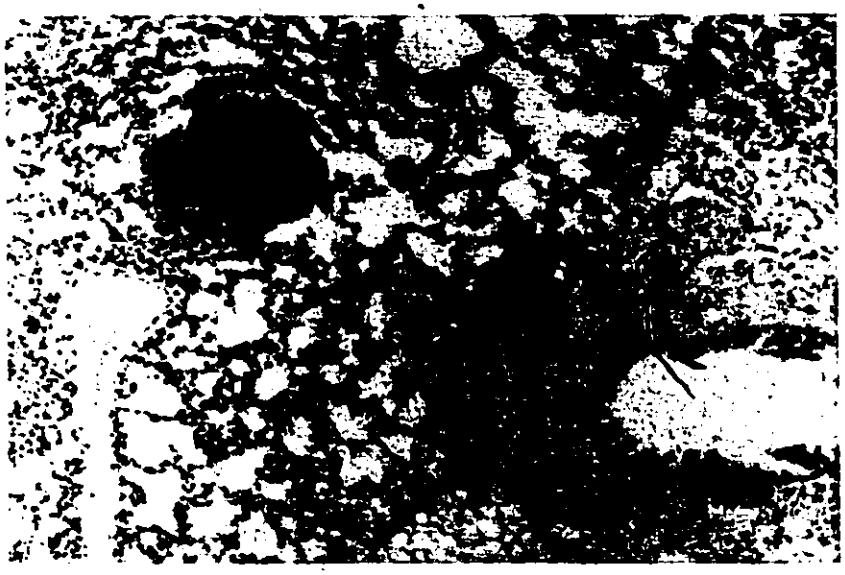

18μ

Plate 5

A radiolabelled lymphoblast in a lymphoid aggregate in the lung. Note the proximity of a bronchus. This cell would have been enumerated as a radiolabelled blast in BALT. The same cell is indicated by an arrow in a) and b).

a



20μ

b

20μ

Plate 6

A radiolabelled MLN lymphoblast near or within bronchial epithelium.

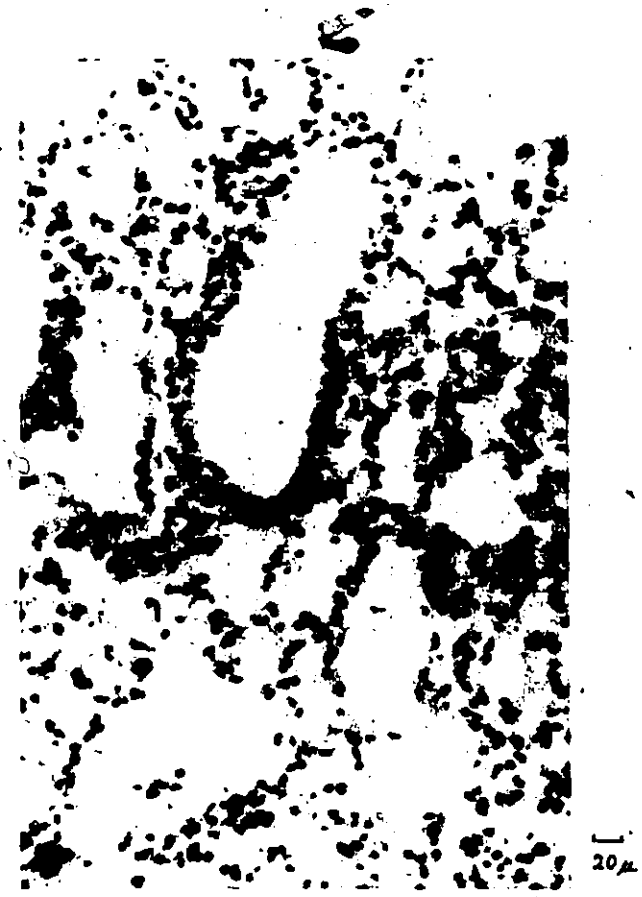

20μ

Plate 7

A radiolabelled MLN lymphoblast in the thymus dependent area of the Peyer's patch. The same cell is indicated by an arrow in a low power (a) and high power (b) magnification.

a

b

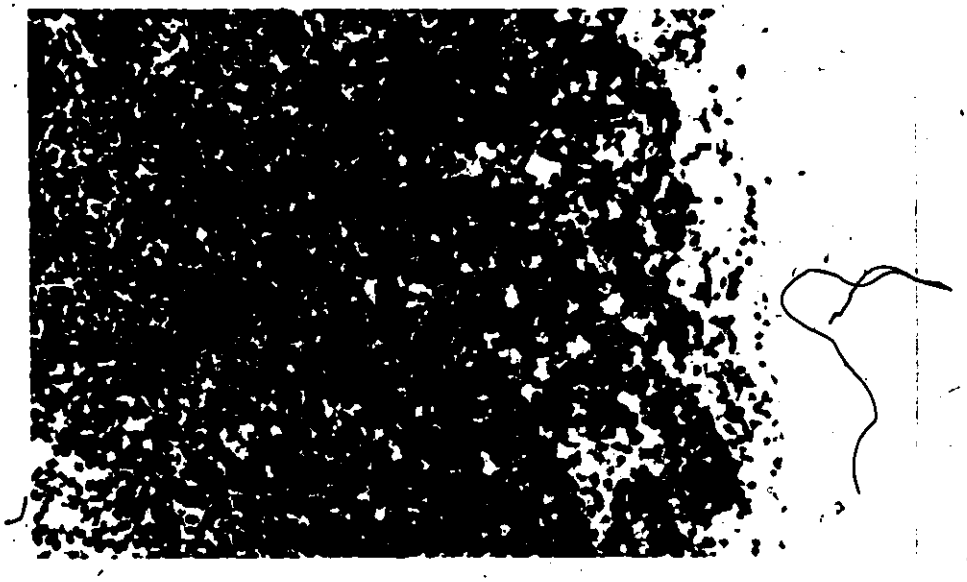
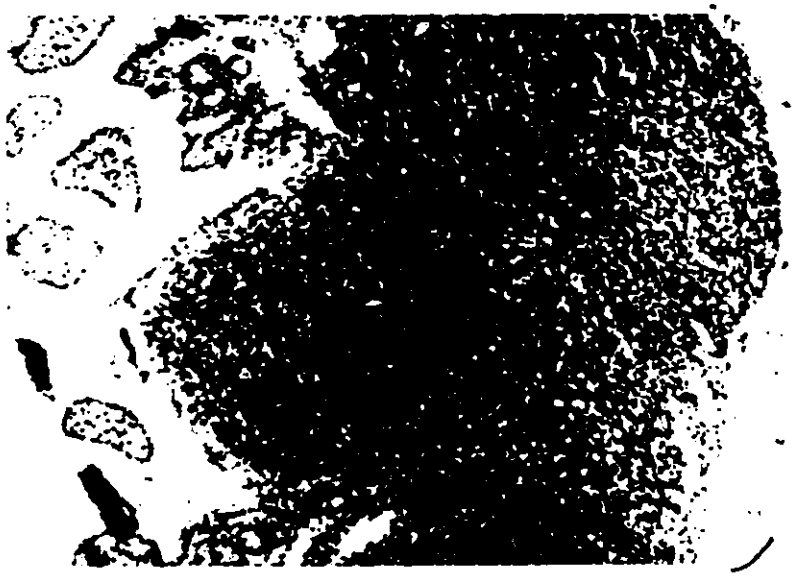


Plate 8

A radiolabelled MLN lymphoblasts in the dome of a Peyer's patch. The same cell is indicated by an arrow in a low power (a) and higher power (b) magnification.

a



b

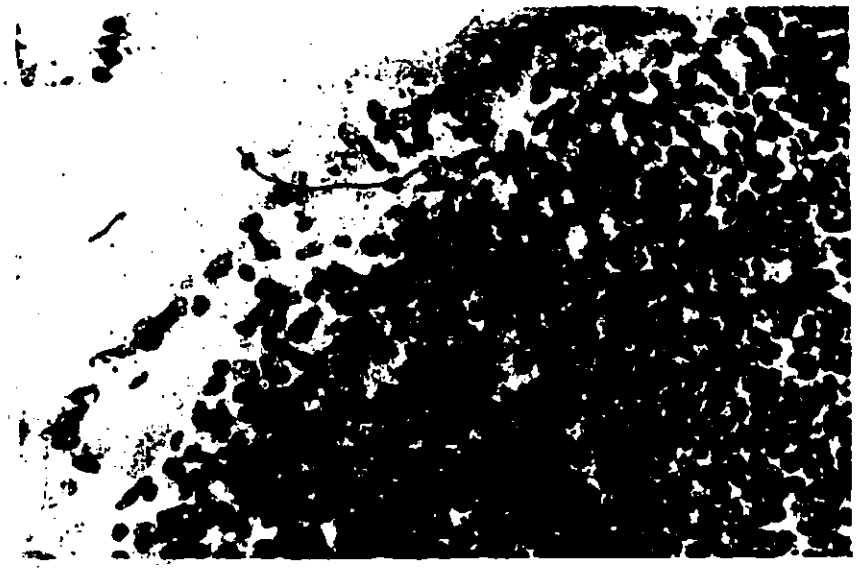


Plate 9

A radiolabelled MLN lymphoblast in the follicle of a Peyer's patch.


20μ

Thus, I established a clear picture of the prototype adoptive transfer model of blast localization in mucosal and other tissues. From this base I proceeded to manipulate the model in an attempt to ascertain what factors influenced the quantity of blasts which appeared in mucosal tissues.

Chapter 5

Influence of the Number of Blasts Transferred on Localization

There were two reasons for studying the influence of the number of blasts transferred on their accumulation in mucosal tissues 24h later. First, in previous investigations which showed selective mucosal accumulation of blasts from MLN (McWilliams et.al., 1975; McDermott & Bienenstock, 1979), approximately twice as many radiolabelled MLN as PLN blasts were transferred and this might, in part, have explained the differences in the localization of these populations. This problem often resulted because the number of cells but not the number of radiolabelled cells transferred could be set during an experiment. Thus determining the influence of the number of blasts transferred would allow comparison of results from different experiments and different cell sources. Secondly, the shape of the dose curve, particularly with large numbers of blasts transferred, would indicate the capacity of the localization mechanism to accumulate cells in the mucosae.

5.1 Radioactivity in Tissues After Transfer of Increasing Numbers of ^{125}I-Udr Labelled MLN Blasts

Table 7 shows that transfer of 100, 300, or 500x10^6 MLN cells resulted a in similar percent of injected radioactivity per gram in each organ. The somewhat greater percentages after transfer of 100x10^6 cells may have been largely due to greater viability since these

Table 7

Effect of Transferring Increasing Numbers of ^{125}I-Udr Labelled MLN Blasts on Lymphoblast Localization (Radioactivity Recovered) in Female Mice[a] at 24 h

Tissue	Localization after Blasts Doses:[b] 0.78×10^6	2.34×10^6	3.90×10^6
Small Intestine	3.26+/-0.45[c]	2.42+/-0.13	2.63+/-0.20
Peyer's patches	9.03+/-1.33	5.91+/-0.34	5.63+/-0.74
Lung	2.01+/-0.22	1.29+/-0.08	1.93+-0.37
MLN	18.89+/-3.14	10.93+/-1.21	10.74+/-1.62
PLN	23.50+/-2.79	13.25+/-0.96	15.12+/-0.38
Spleen	32.62+/-4.41	21.06+/-1.08	20.61+/-0.98
Liver	1.11+/-0.11	0.98+/-0.06	1.09+/-0.03
Carcass	1.01+/-0.13	0.93+/-0.15	0.88+/-0.10

a) A second experiment using male donors and recipients yeilded similar results.

b) Inoculum was 0.78% lymphoblasts; transferred 100, 300 or 500×10^6 cells per recipient.

c) Figures represent mean+/-S.E. radioactivity expressed as % of injected per gram = dpm recovered per organ/(dpm injected)(organ weight). There were no significant differences in the % of injected radioactivity in each organ after the transfer of different doses of radiolabelled MLN blasts except in recipient PLN (Student's t test, $\alpha=0.05$

differences were greatly diminished when the data were expressed as percentages of the total recovered radioactivity in the animal. When viewed in a different manner (Fig.2), the same data showed a linear increase in the dpm recovered in each organ with an increasing dose of cells transferred. Therefore the transfer of a large number of cells, comparable to the total daily output of the thoracic duct (Sprent, 1973) did not saturate the mechanism which allowed the accumulation of MLN blasts in these tissues over a 24 h period.

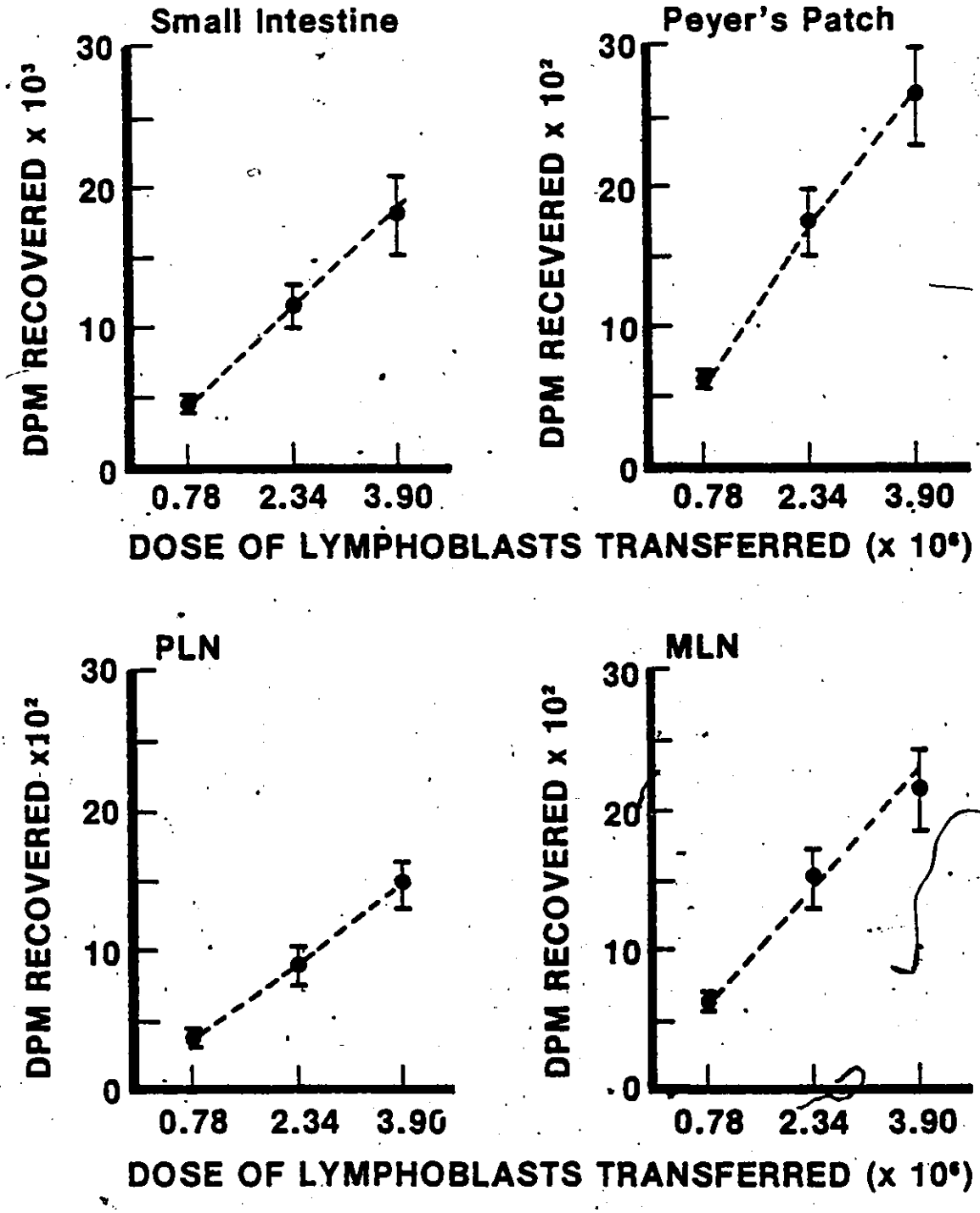
5.2 Autoradiographic Studies of the Effect of Number Transferred on Frequency and Distribution of MLN and PLN Blasts

5.2.1 Dose effects in small intestine

The relationship between the number of labelled MLN blasts transferred and the number which localized in the small intestine 22-24 h later (Fig.3) was linear ($P_{\alpha}=0.05$) over a dose range of 2.5×10^{5} to 5.6×10^{6} blasts transferred, a 22.4-fold increase. There were no significant differences ($P_{\alpha}=0.05$) among three experiments when individually evaluated and, thus, for analyses and presentation, the data from these experiments were pooled. This linear relationship also held when the basal lamina propria, or villus lamina propria, or the epithelium was considered independently ($P_{\alpha}=0.05$ in each region; Fig.3b,c,d). Data obtained from animals that received greater than 5.6×10^{6} blasts were not included in the statistical analyses because

Figure 2

Effect of transferring increasing numbers of ^{125}I-Udr labelled MLN blasts on radioactivity recovered 24h later. Data is from the same experiment as that in Table 7. Data with error bars represent mean +/- S.E. of results for 3 recipients at doses 2.34 and 3.90×10^6 and 5 recipients at dose 0.78×10^6. Straight lines were fitted by eye.


Small Intestine
DPM RECOVERED x 10³
30
20
10
0
0.78
2.34
3.90
Peyer's Patch
DPM RECEVERED x 10²
30
20
10
0
0.78
2.34
3.90
DOSE OF LYMPHOBLASTS TRANSFERRED (x 10⁶)
PLN
DPM RECOVERED x10²
30
20
10
0
0.78
2.34
3.90
MLN
DPM RECOVERED x 10²
30
20
10
0
0.78
2.34
3.90
DOSE OF LYMPHOBLASTS TRANSFERRED (x 10⁶)

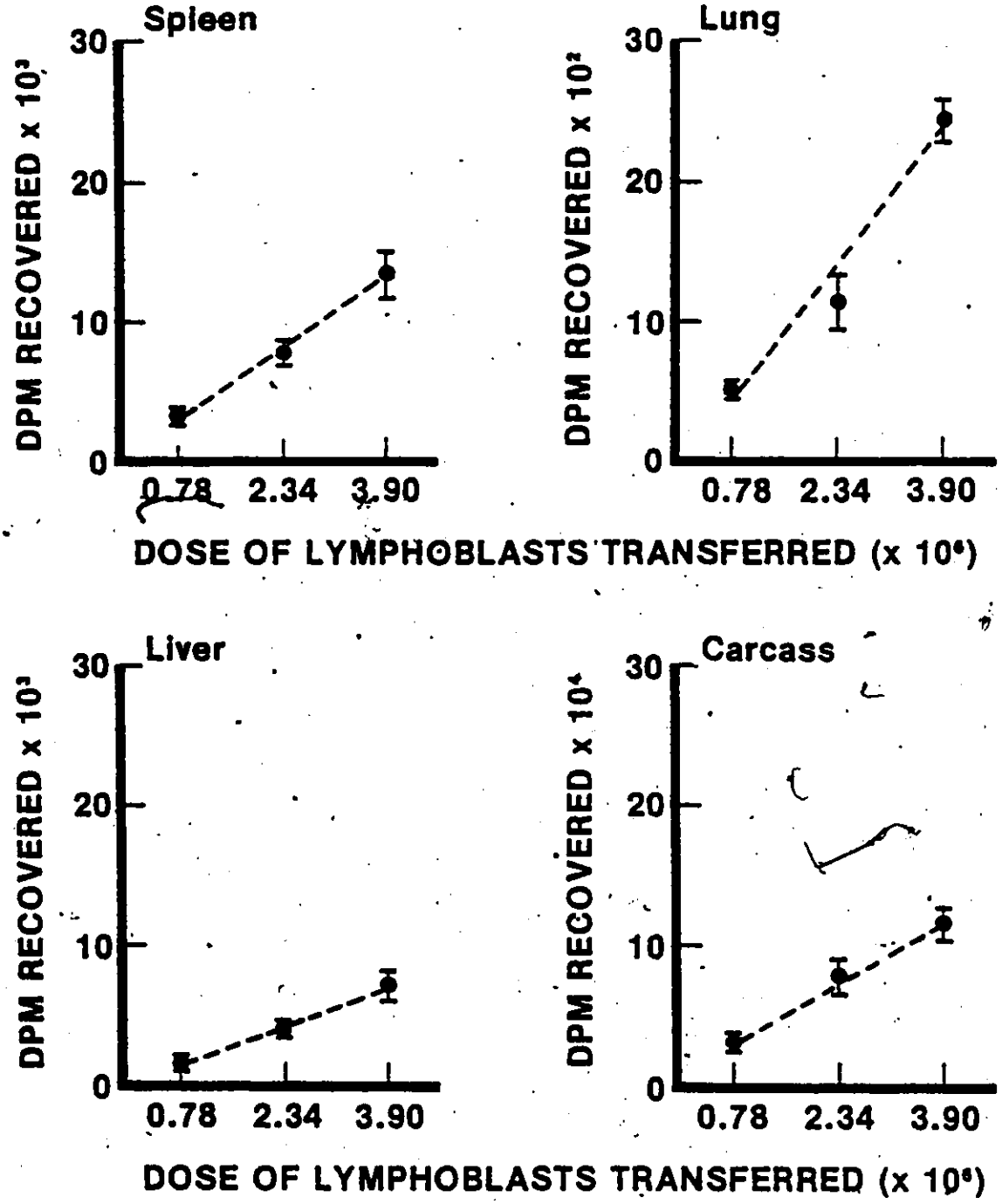
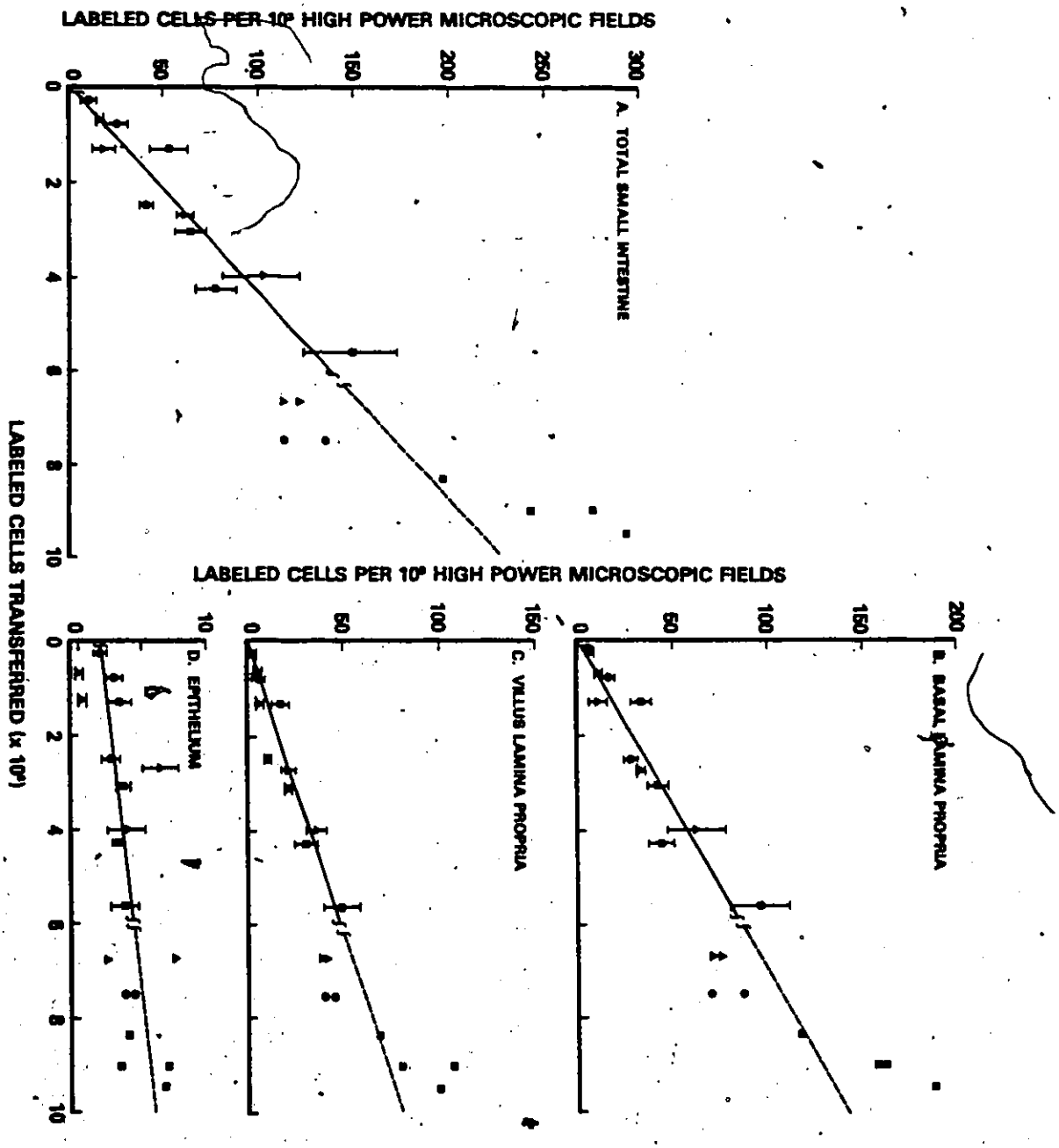

Spleen
DPM RECOVERED x 10³
30
20
10
0
0.78
2.34
3.90
Lung
DPM RECOVERED x 10³
30
20
10
0
0.78
2.34
3.90
DOSE OF LYMPHOBLASTS TRANSFERRED (x 10⁶)
Liver
DPM RECOVERED x 10³
30
20
10
0
0.78
2.34
3.90
Carcass
DPM RECOVERED x 10⁴
30
20
10
0
0.78
2.34
3.90
DOSE OF LYMPHOBLASTS TRANSFERRED (x 10⁶)

Figure 3

The relationship between the number of (^{3}H)-thymidine-labelled mesenteric blasts adoptively transferred and the frequency of labelled cells in the intestinal mucosa 22-24h later. The regions of the intestine are described in Fig. 1. Experiments 1, ● ; 2, ■ ; and 3, ▲ . Data with error bars represent the mean +/- S.E. of results from 3 recipient animals. Single points represent results from one recipient. Statistical analysis of these data are described in the text.


LABELED CELLS PER 10⁶ HIGH POWER MICROSCOPIC FIELDS
A. TOTAL SMALL INTESTINE
LABELED CELLS PER 10⁶ HIGH POWER MICROSCOPIC FIELDS
B. BASAL LAMINA PROPRIA
C. VILLUS LAMINA PROPRIA
D. EPITHELIUM
LABELED CELLS TRANSFERRED (x 10⁶)

the entire high dose range was not well represented in more than one experiment and no high dose of blasts was administered to more than two recipients. However, except in one case, the frequencies at these high doses were within or greater than the values of the 95% prediction interval surrounding the extrapolation of the linear regression curve.

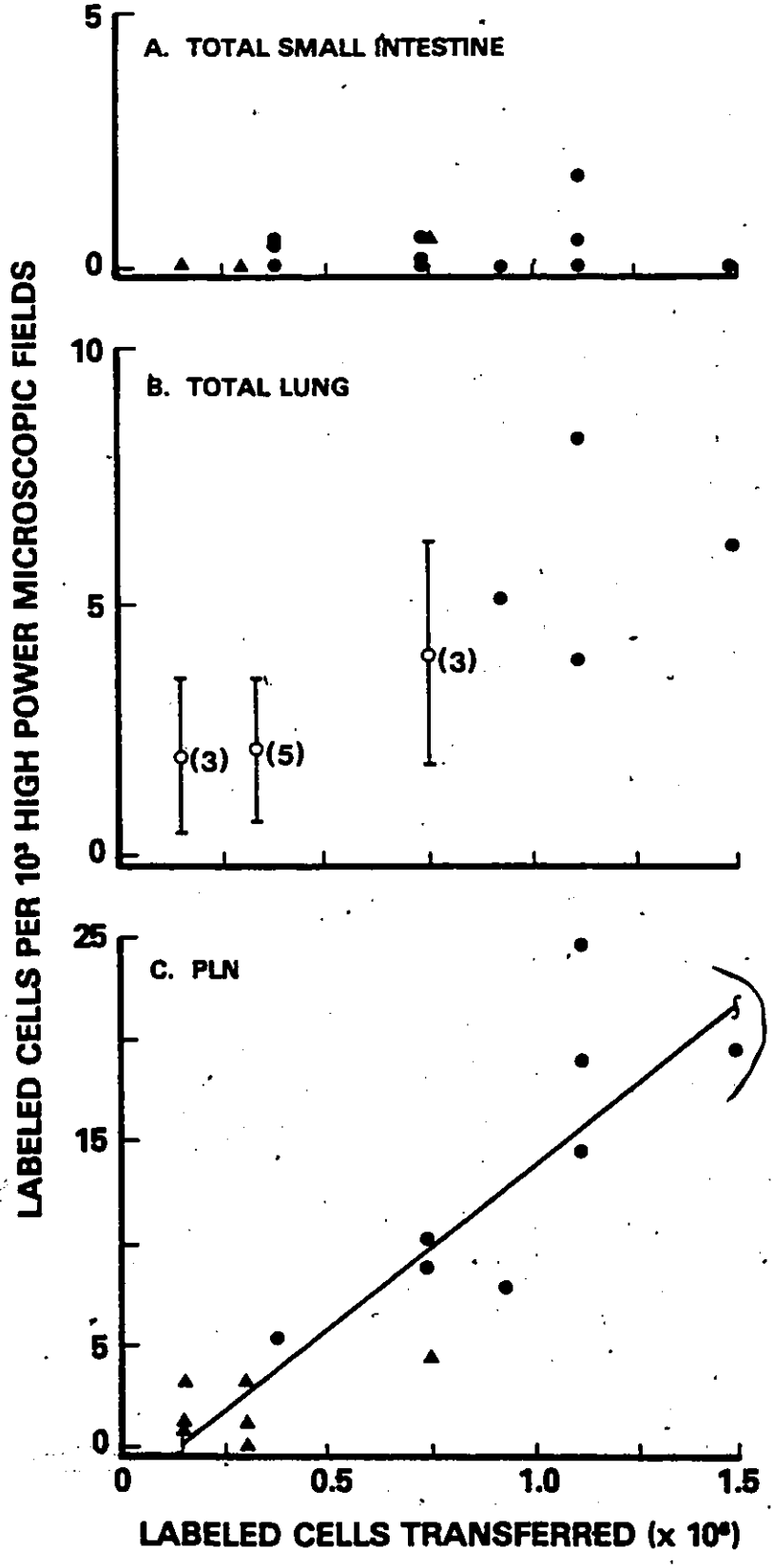
The relative distribution of radiolabelled cells among the morphological regions of the small intestine did not change as the dose transferred was increased (Fig. 3). Of the labelled cells in the small intestine, 62.5%+/-0.9% (mean+/-SE of forty-one recipients) appeared in the basal lamina propria, 32.5%+/-0.9% in the villus lamina propria and 5.0%+/-0.6% in the epithelium. Many of the radiolabelled cells in the basal lamina propria were located lateral to the crypts while the majority of blasts in the villus lamina propria were in the basal portion of the villi.

Thus, as with radiocounting, autoradiographic studies suggested that the mechanism of blast localization in the intestine had a large capacity which was not saturated even at doses estimated to be four times the normal daily output of thoracic duct blasts (Sprent, 1973).

In marked contrast to MLN blasts, very few PLN blasts localized in the small intestine (Fig. 4A). For example, (compare Figs. 3A & 4A) at a dose of 1.5×10^6 blasts transferred, approximately 35.4 labelled MLN blasts but only one or two, if any, labelled PLN blasts were observed per 1000 HPF of small intestine. Thus, at any dose of cells transferred, MLN blasts but not PLN blasts selectively localize in the small intestine 24h after adoptive transfer.

Figure 4

The relationship between the number of (^{3}H)-thymidine-labelled peripheral blasts adoptively transferred and the frequency of labelled cells in various tissues 22-24h later. Experiments 1, ●○ and 2, ▲ . Data points with error bars represent the mean +/- S.E. of results from the number of animal indicated in brackets. Single points represent results from one recipient.


A. TOTAL SMALL INTESTINE
B. TOTAL LUNG
C. PLN
(3)
(5)
(3)
5
0
10
5
0
25
15
5
0
0
0.5
1.0
1.5
LABELED CELLS PER 10³ HIGH POWER MICROSCOPIC FIELDS
LABELED CELLS TRANSFERRED (x 10⁶)

5.2.2 Dose effects in lung

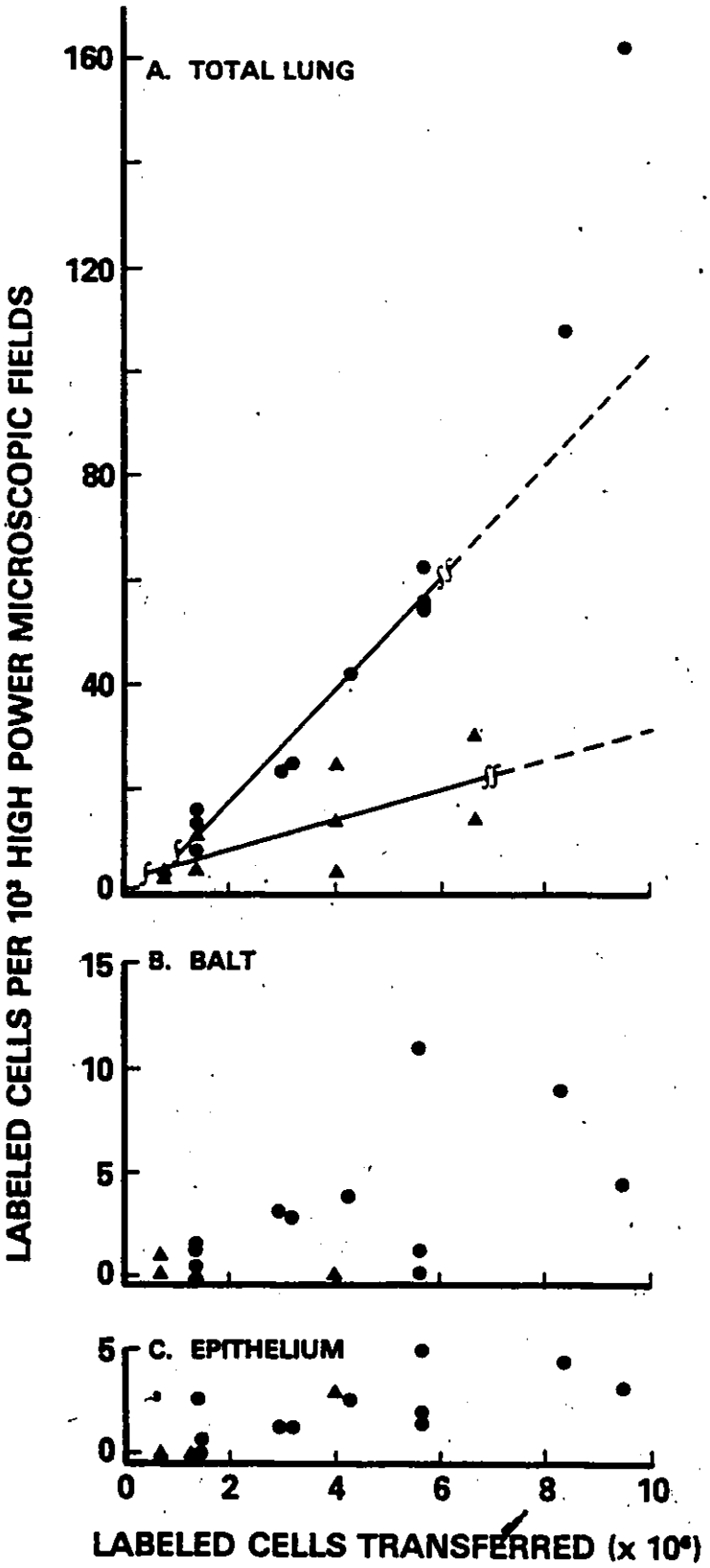
Unlike the results obtained from the replicate experiments in the intestine, two experiments examining MLN blast localization in the lungs yielded regression lines with significantly different slopes (P_{α} =0.05) and, thus, the results of each experiment are presented individually (Fig. 5A). This relationship prevailed in the parenchyma. The data obtained from recipients which received greater than 6.7×10^6 blasts were not included in the statistical analyses for reasons similar to those described for MLN blasts in the small intestine. The data obtained concerning the localization of MLN blasts in BALT and bronchial epithelium was not statistically analysed because too many data points had a value of zero. However, as the dose of donor blasts increased, there tended to be an increase in the accumulation of blasts in the BALT (Fig. 5B) and in the bronchial epithelium (Fig. 5C).

The number of PLN blasts that accumulated in the lung parenchyma (Fig. 4B) was variable but in two experiments there seemed to be a trend towards an increase with higher doses of donor blasts. No labelled PLN cells were seen in the BALT or bronchial epithelium. The frequencies of labelled PLN compared with MLN blasts in the lungs did not seem to differ over a range of 1.5×10^5 to 1.5×10^6 blasts transferred (see Figs. 4B and 5A).

Since the number of PLN blasts which localized in the lungs and small intestine was so small, we used localization of these cells in PLN as a positive control. A linearly increasing number of labelled PLN blasts (P_{α} =0.05) were oberved in recipient PLN when increasing numbers of cells were transferred (Fig.4C).

Figure 5

The relationship between the number of (^{3}H)-thymidine-labelled mesenteric blasts adoptively transferred and the frequency of labelled cells in the respiratory tract 22-24h later. Experiments 1, ● and 2, ▲ . Each point represents the results obtained from one animal.


A. TOTAL LUNG
B. BALT
C. EPITHELIUM
160
120
80
40
0
15
10
5
0
5
0
0
2
4
6
8
10
LABELED CELLS PER 10³ HIGH POWER MICROSCOPIC FIELDS
LABELED CELLS TRANSFERRED (x 10⁶)

I concluded that over the narrow and low dose range which I was able to study, the localization of MLN and PLN blasts in the lung parenchyma did not differ. It is possible that MLN blasts might have selectively localized in the mucosal tissues in the lungs (BALT and bronchial epithelium) but the area of these regions examined was too small for conclusions to be drawn.

CHAPTER 6

Influence of the Time Available for Localization on the Frequency and Distribution of MLN and PLN Blasts

Studies of the kinetics of chemical reactions yield information about their mechanism. Therefore, to investigate the mechanism of blast localization, I designed experiments to determine the kinetics of blast localization at mucosal surfaces. In addition, I reasoned that if cells were extravasating in one location and then migrating through the tissue to other morphological regions I should be able to observe a change, over time, in the distribution of labelled cells within a tissue.

6.1 Localization of MLN and PLN blasts in small intestine at various times after transfer

Examination of the small intestine by radiocounting after transfer of ^{125}I-Udr labelled MLN blasts (Table 8), revealed that the accumulation of blasts was as great by 6 h as at any subsequent time and that by 24 h there was significantly less radioactivity in the gut in two of three experiments ($P = 0.05$). In all three experiments there was a significant increase after 6 h in the percent of the total recovered radioactivity (% recovered) which was present in the small intestine. Thus, the net accumulation of MLN blasts in the small

Table 8

Radiocounting Kinetics of MLN Blast Localization [a] in the Small Intestine

Experiment (n)	Time [b] (h)	Radioactivity in Small Intestine [c] % of Injected	% of Recovered
K5 (4)	6	7.7+/-0.81	8.56+/-1.38
	12	5.91+/-0.47	13.38+/-3.13
	18	4.72+/-0.38	18.82+/-2.28
	24	4.64+/-0.27	29.66+/-2.22
K6 (4)	6	4.14+/-1.32	12.56+/-0.44
	12	4.73+/-0.41	16.16+/-0.88
	18	4.75+/-0.20	24.03+/-0.18
	24	7.06+/-1.19	23.16+/-1.29
	30	3.21+/-0.55	20.11+/-0.69
K7 (3)	6	6.4+/-0.3	13.2+/-0.8
	12	5.4+/-0.2	15.0+/-1.4
	18	3.7+/-0.2	14.2+/-0.8
	24	3.8+/-0.2	17.1+/-1.0

Table 8 (continued)

a) MLN lymphoblasts were labelled with ^{125}I-Udr and adpotively transferred into syngeneic recipients.

b) Time after transfer when recipient was killed.

c) Figures represent mean+/-S.E. of the radioactivity recovered in the small intestine, expressed as a % of the injected radioactivity or as a % of the total radioactivity recovered in the recipient.

intestine was maximal by 6 h after transfer, but MLN blast associated radioactivity was lost more slowly from the small intestine than from the rest of the body. This might reflect either all the MLN blasts localizing in the small intestine by 6 h or a greater exit from, than entry into the small intestine after 6 h.

Table 9 shows the autoradiographic data from kinetics experiments to study MLN and PLN blast localization in the small intestine. The ability of blasts to localize rapidly in the mucosae was remarkable. In each MLN experiment the kinetics pattern differed (numbers increased, decreased or remained the same from 0.5 h to 24 h) but the important feature was that many, if not all, of the MLN blasts had already localized in the small intestine by 0.5 h after transfer. The cells which were in the intestine at 24 h might have been the same ones which were observed at 0.5 h or the similar numbers at these times might have reflected an equilibrium between cell entry, retention and loss from the intestine established by 0.5 h. In contrast, few PLN blasts were observed in the small intestine at 0.5 h or at later times after transfer. Thus the selective localization in the small intestine of MLN compared to PLN blasts which was observed at 24 h (Section 4.2) was also demonstrated as early as 0.5 h after transfer.

If blasts extravasated in a particular location in the mucosae, analogous to the extravasation of lymphocytes at high endothelial post-capillary venules in the TDA of lymph nodes, then their distribution

Table 9

Autoradiographic Kinetics of MLN or PLN Blast [a] Localization in the Small Intestine

Experiment	(n)	Cell Source	Dose [b]	Time (hours) [c]	Cells in Small Intestine [d]
K1	(3)	MLN	1.58	0.5	52.3+/-3.0
	(3)	MLN		24	127.4+/-2.5
K2	(3)	MLN	0.36	12	80.6+/-34.0
	(3)	MLN		18	39.5+/-3.1
	(3)	MLN		24	68.3+/-18.2
	(3)	MLN		30	47.8+/-18.9
	(3)	PLN	0.15	12	0.63+/-0.36
	(2)	PLN		18	0.31+/-0.18
	(2)	PLN		24	0
	(2)	PLN		30	0
K8	(2)	MLN	2.5	0.5	46.1+/-9.4
	(3)	MLN		1	47.7+/-3.3
	(3)	MLN		3	52.3+/-4.8
	(3)	MLN		24	39.4+/-3.4
	(2)	PLN	2.3	0.5	5.7+/-0.5

Table 9 (continued)

Experiment (n)	Cell Source	Dose b)	Time (hours) c)	Cells in Small Intestine
K9 (3)	MLN	0.86	0.5	20.7+/-3.1
(3)	MLN		24	9.4+/-2.5
(3)	PLN	0.76	0.5	3.4+/-1.9

a) Lymphoblasts from either MLN or PLN were labelled with ^{3}H-Tdr and adoptively transferred into syngeneic recipients.

b) Number of lymphoblasts transferred per recipient.

c) Time after transfer when recipient killed.

d) Figures represent mean +/- S.E. of the number of radiolabelled cells per 10^{3} HPF.

would change over time. One would expect to find, soon after transfer, a higher concentration close to the point of extravasation and a lower concentration in more distant locations. This gradient would then diminish after all the cells had extravasated and migrated through the tissue. However, as the data in Table 10 demonstrate, the distribution of MLN and PLN blasts in the epithelium, villus and basal lamina propria of the small intestine (Fig. 1) was the same at all times after transfer. However, there was a tendency for more cells to appear in the epithelium later than 0.5 h. Therefore, either both extravasation and migration through the tissues occurred very rapidly (within 0.5 h) or blasts left the vasculature in both the basal and villus lamina propria.

It has been shown that lymphocytes extravasate via the HEV in lymphoid tissue (Gowans & Knight, 1964). Because these structures are present in Peyer's patches but not in intestinal lamina propria there was a possibility that blasts extravasated in the Peyer's patches and then migrated through the lamina propria. Our observations of the frequency of blasts in the villus lamina propria at 0.5 h made this potential route of entry seem unlikely (Section 6.1.1). Nevertheless 65% of MLN blasts which localize in the small intestine become IgA containing cells (McWilliams et al., 1977; McDermott & Bienenstock, 1979) and there have been observations of a higher concentration of IgA positive cells in the lamina propria adjacent to the Peyer's patches than in the lamina propria distant from the patches (Crabbe et al., 1970; Rudzik et al., 1975b; Befus et al., 1978). There have also been observations of TDL (Hall et al., 1972) and T-TDL (Sprent, 1976) blasts

Table 10

Distribution of Adoptively Transferred MLN or PLN Blasts in the Morphologic Regions of the Small Intestine at Various Times After Transfer

Expt (n)	Cell Source	Time (hrs) a)	% of Cells in Intestinal Regions: Epithelium	Lamina Propria Villus	Lamina Propria Basal b)
K1 (3)	MLN	0.5	0.57+/-0.57	39.1+/-2.7	60.3+/-2.1
(3)	MLN	24	4.4+/-0.7	26.2+/-2.8	70.8+/-2.4
K2 (3)	MLN	12	5.2+/-2.6	29.3+/-0.94	65.5+/-3.4
(3)	MLN	18	4.5+/-2.5	36.2+/-4.2	59.2+/-2.6
(3)	MLN	24	4.7+/-0.8	32.6+/-6.2	62.7+/-5.4
(3)	MLN	30	2.7+/-2.7	35.7+/-4.1	61.6+/-1.7
(3)	PLN	12	0	16.7+/-16.7	50+/-28.9
(2)	PLN	18	0	0	50+/-50
(2)	PLN	24	0	0	0
(2)	PLN	30	0	0	0

Table 10 (continued)

Expt (n)	Cell Source	Time (hrs) [a]	% of Cells in Intestinal Regions: [b] Epithelium	Lamina Propria Villus	Lamina Propria Basal
K8 (2)	MLN	0.5	0	29.7+/-3.2	70.3+/-3.3
(3)	MLN	1	1.3+/-0.8	32.4+/-3.0	66.2+/-3.7
(3)	MLN	3	1.0+/-1.0	23.5+/-0.9	75.8+/-1.6
(3)	MLN	24	4.1+/-2.7	36.1+/-4.1	59.7+/-4.7
(2)	PLN	0.5	0	41.9+/-8.2	58.2+/-8.2
K9 (3)	MLN	0.5	0	22.6+/-4.6	77.4+/-4.6
(3)	MLN	24	6.4+/-3.2	41.7+/-10.7	51.9+/-9.9
(3)	PLN	0.5	2.7+/-2.7	25.2+/-14.4	72.1+/-16.8

a)Time after transfer when recipient killed.

b)The proportion (%) of cells in each morphologic region of the small intestine (see Figure 1); figures represent mean +/- S.E.

concentrated near PP. However, we found that the frequency of MLN blasts in the HPF immediately adjacent to Peyer's patches and in the mushroom of lamina propria between the follicles was the same as in the remainder of the gut at both 0.5 h and 24 h after transfer (Table 11). This was further evidence that the Peyer's patches were not likely the portal of entry of most blasts into the lamina propria.

6.2 Localization of MLN and PLN blasts in lungs at various times after transfer

Following transfer of ^{125}I-Udr labelled MLN blasts, both the % injected and % recovered radioactivity in the lungs decreased progressively from 6 to 24 h (Table 12). Similarly, the kinetics of blast accumulation in the lungs, studied with autoradiography, showed a rapid decrease from a high at 0.5 h after transfer of MLN blasts (Fig. 6). The accumulation of PLN blasts in the lungs (Fig. 6) likely also had a similar kinetics since their numbers at 0.5 h were comparable to those after MLN transfer and there were few PLN blasts in the lungs by 12 h with a trend to a decrease to 24 h. Thus at any time after transfer there was no preferential accumulation in the lungs of cells from either lymphoid source. Cells seemed to be retained initially in the lungs, perhaps by trapping in the capillary bed, and to leave subsequently for an unknown destination.

In one experiment the area of BALT and epithelium scanned was recorded separately from the area of the parenchyma. BALT was observed in only 3 of 15 recipients and the largest area was 15.5 HPF. In spite of the small sample, we found that, upon converting the data to number

Table 11

Kinetics of Localization of MLN and PLN Blasts in Lamina Propria Adjacent to Peyer's Patches

Expt	a)	Time	b) (n)	Number of Lymphoblasts in L.P.		
				Mushroom	Adjacent to P.P.	Distant from P.P.
K8	PLN	0.5h	(2)	0	30.9+/-7.6 c)	5.7+/-0.5
	MLN	0.5h	(3)	75.5+/-37.8	72.5+/-38.7	46.1+/-9.4
	MLN	24h	(3)	65.5+/-37.5	39.8+/-9.6	39.4+/-3.4
K9	PLN	0.5h	(3)	14.8+/-10.4	0	3.4+-1.9
	MLN	0.5h	(3)	42.8+/-25.3	14.2+/-7.9	20.7+/-3.1
	MLN	24h	(3)	15.0+/-7.5	5.1+/-5.1	9.4+/-2.5

a) Source of ^{3}H-Tdr labelled lymphoblasts transferred

b) Time after transfer at which animals were killed

c) Mean +/- S.E. number of radiolabelled cells per 10^{3} HPF of each region. Actual scoring used a square with area 0.079mm^{2} divided into a grid of 100 smaller squares; data was then converted to number of cells per 10^{3} HPF (HPF area 0.1mm^{2})

Actual area scored was only: Mushroom 11 to 67 HPF

Adjacent PP 33 to 121 HPF

Distant from PP approx 1000 HPF

Table 12

Radiocounting Kinetics of MLN Blast[a] Localization in the Lungs

Experiment (n)	Time[b] (h)	Radioactivity in Lungs[c] % of Injected	% of Recovered
K7 (3)	6	2.2+/-0.3	4.6+/-0.6
	12	1.0+/-0.08	2.6+/-0.2
	18	1.4+/-0.9	1.8+/-0.1
	24	0.4+/-0.08	1.7+/-0.4

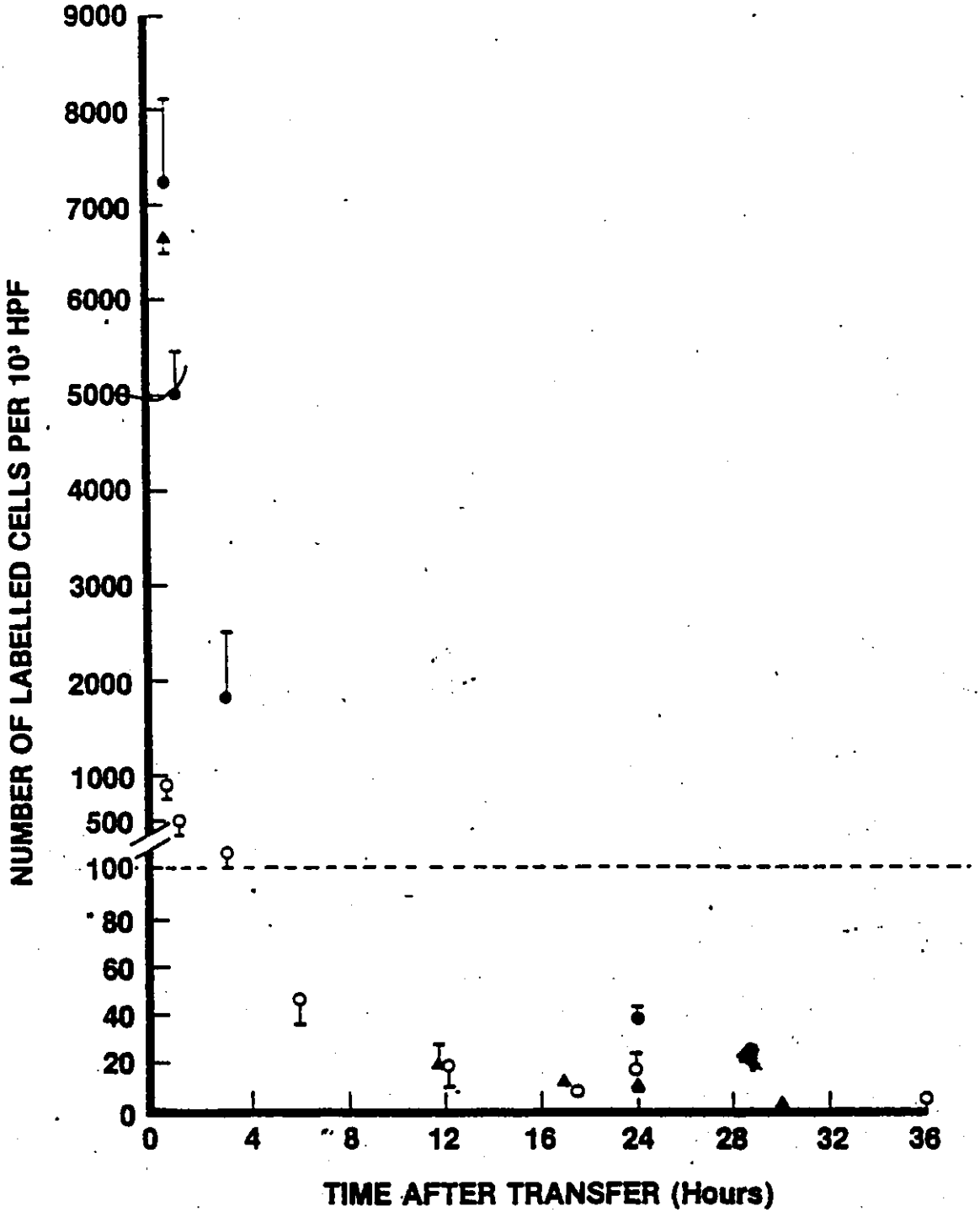
a) MLN lymphoblasts were labelled with ^{125}I-Udr and adoptively transferred into syngeneic recipients.

b) Time after transfer when recipient was killed.

c) Figures represent mean+/-S.E. of the radioactivity recovered in the lungs, expressed as a % of the injected radioactivity or as a % of the total radioactivity recovered in the recipient.

Figure 6

The relationship between the time after transfer and the number of ^{3}H-Tdr labelled MLN or PLN blasts in the lung parenchyma. Symbols with error bars represent mean +/- S.E. Where no error bars appear, the S.E. was not larger than the symbol. Transfer of 1.5×10^{6} MLN blasts in experiment K1 (○) (n=3 for time points 3, 6, 12, 36 h; n=4 for 0.5, 1, 24 h; n=5 for 19 h) or 2.5×10^{6} MLN blasts experiment K8 (●) (n=3). Two other MLN experiments (not shown) covering the periods 6 to 36 h and 12 to 24 h showed a similar tendency for the blasts to accumulate in the lungs at early times after transfer and to subsequently leave the lungs. Transfer of PLN lymphoblasts (×), experiment K2 0.15×10^{6} blasts transferred (n=3 for time points 18, 24 h, n=2 for 12 h, n=1 for 30 h) and experiment K8 2.5×10^{6} blasts transferred (n=3 for time point 0.5 h).


NUMBER OF LABELLED CELLS PER 10³ HPF
9000
8000
7000
6000
5000
4000
3000
2000
1000
500
100
80
60
40
20
0
0
4
8
12
16
24
28
32
36
TIME AFTER TRANSFER (Hours)

of cells per 10^3 HPF, the concentration of labelled MLN cells observed in the BALT was the same order of magnitude as in the parenchyma at the same time after transfer (3 hr). The area of BALT observed in recipients of PLN was not large enough to determine whether PLN blasts localized in this region. The area of epithelium observed ranged from 5 to 43 HPF per animal (scored as cells per field diameter and converted by using 12 field diameters of epithelium per HPF). In recipients of either PLN or MLN blasts, cells were only occasionally observed in the bronchial epithelium.

6.3 Localization of MLN and PLN blasts in Peyer's patches at various times after transfer

As was observed in the lungs and small intestine, the radiolabelled cells which localized in the Peyer's patches had already done so by 0.5 h after transfer (Table 13). At 0.5 h after transfer, MLN and PLN blasts accumulated in the Peyer's patch with a similar distribution and frequency. The number of MLN blasts in the various morphologic regions was the same at 0.5 h and 24 h with the exception of the dome. MLN blasts were observed in the dome at 24 h but not at 0.5 h. By 24 h their concentration in the dome was similar to that in the TDA. Since the blasts appeared first in the TDA and subsequently in the dome they might have extravasated in the TDA and then migrated through the patch to the dome.

Table 13

Kinetics of Localization of MLN and PLN Blasts in Peyer's Patches

Expt [a]	Time [b]	(n)	Number of Blasts in Peyer's Patch: Follicle	Dome	TDA [c]
K8 PLN	0.5h	(2)	31.2+/-31.2 [d]	0	182.0+/-120.8
MLN	0.5h	(3)	4.5+/-4.5	0	341.3+/-136.6
MLN	24h	(3)	10.8+/-10.8	243.8+/-191.2	393.9+/-32.6
K9 PLN	0.5h	(3)	0	0	22.1+/-11.1
MLN	0.5h	(3)	6.8+/-3.5	0	121.2+/-63.9
MLN	24h	(3)	23.5+/-12.9	117.9+/-61.6	204.7+/-38.7

a) Source of ^{3}H-Tdr labelled lymphoblasts transferred

b) Time after transfer at which animals were killed

c) TDA = thymus dependent area

d) Mean +/- S.E. number of radiolabelled cells per 10^{3} HPF of each region. Actual scoring used a square with area of 0.079mm^{2} divided into a grid of 100 smaller squares; data was then converted to number of cells per 10^{3} HPF (HPF area 0.1mm^{2})

Actual area scored was only: Follicle 29 to 96 HPF

Dome 1.9 to 8.1 HPF

TDA 8.3 to 72.4 HPF

CHAPTER 7

Gender and Gonadal Hormone Influences on MLN Blast Localization in the Small Intestine

Gonadal hormones influence both the immune system in general and, of particular interest, the localization of MLN blasts in mucosal sex hormone target tissues (Section 1.4). I therefore designed experiments to determine whether there were any sex differences (potentially hormonally mediated) in the localization of MLN blasts in a mucosal tissue which is not a sex hormone target tissue, the small intestine.

7.1 MLN Blast Localization with Male or Female Donors and Recipients.

7.1.1 Autoradiographic studies of gender influences on localization

The data from all experiments which compared the localization of MLN blasts from male or female donors in the small intestines of male or female recipients is presented in Table 14a & b. Table 14a shows the first five experiments. The first two experiments, with a total of 32 recipients, showed conclusively that at least twice as many blasts from male donors as from female donors localized in the small intestine. In addition, in three experiments (total of 16 recipients) using male donors but without female donor controls, localization was greater than would have been expected if a similar dose of female

Table 14

Effect of the Sex of the Donor and the Recipient on the Number of Radiolabelled Cells in the Small Intestine 24 h After Adoptive Transfer of MLN Blasts

Expt	Dnr [a]	Recip (n) [b]	Dose ($\times 10^6$) [c]	Blasts in Small Gut [d]	Conclusions [e]
Table 14a					
S4	♂	♂ (5)	1.01 - 1.59	185.3+/-16.0	
	♂	♀ (5)		154.5+/-17.1	((♂→♂)=(♂→♀))
	♀	♀ (5)	1.09 - 1.63	80.2+/-4.1	>((♀→♀)=(♀→♂))
	♀	♂ (5)		79.7+/-8.3	
S5	♂	♂ (3)	0.88 - 1.72	97.2+/-7.8	
	♂	♀ (3)		120.3+/-10.4	((♂→♂)=(♂→♀) >((♀→♀)=(♀→♂))
	♀	♀ (3)	1.37 - 2.23	50.3+/-6.5	
	♀	♂ (3)		55.0+/-3.5	
P1	♂	♂ (2)	6.87	466.9+/-21.7	high comp. to expect (157.8)
S1	♂	♂ (4)	2.3	148.9+/-6.2	high comp. to
	♂	♀ (4)		120.0+/-11.7	expect (53.6)
S7	♂	♂ (3)	0.55 - 0.89	43.3+/-5.2	high comp. to expected
	sc♂ [a]	♂ (3)	0.72 - 1.22	52.6+/-10.6	(17.6 and 22.1)

Table 14 continued

Expt	Dnr a)	Recip b) (n)	Dose c) ($\times 10^6$)	Blasts in Small Gut	Conclusions e)
Table 14b					
S8b	♂	♂ (3)	1.12 - 1.58	31.9+/-3.3	no difference
	♂	♀ (2)		36.7+/-4.7	among the groups
	♀	♀ (3)	1.07 - 1.53	42.7+/-5.4	
	♀	♂ (2)		40.7+/-7.8	
S8a	♂	♂ (3)	1.36 - 1.88	32.5+/-6.0	
	♂	♀ (3)		20.1+/-1.8	no diff. between ♂→♂ and ♀→♀ even with most
	♀	♀ (3)	0.95 - 1.35	28.1+/-1.4	extreme dose correction
	♀	♂ (1)		34.3	♂→♀ < ♀→♀
S11	♂	♂ (4)	0.66 - 1.02	27.9+/-5.6	no difference between the
	♀	♀ (3)	0.75 - 1.15	28.8+/-3.4	groups
S13	♂	♂ (3)	0.61 - 0.96	4.6+/-1.9	no difference
	♀	♀ (3)	0.58 - 0.98	5.2+/-0.4	between ♂ and ♀
	♂	♂ (3)	1.83 - 2.90	11.6+/-0.4	at any dose
	♀	♀ (3)	1.74 - 2.94	20.7+/-2.7	of cells
	♂	♂ (3)	3.0 - 4.8	28.5+/-2.0	transferred
	♀	♀ (3)	2.9 - 4.9	31.6+/-1.9	

Table 14 (continued)

Expt	Dnr a)	Recip b) (n)	Dose c) ($\times 10^6$)	Blasts in Small Gut	Conclusions e)
S9	♂	♂ (3)	1.02 - 1.44	38.9+/-2.1	not different
	sc♂	♂ (3)	1.19 - 1.95	29.1+/-2.8	from expected (29 and 36)

a) Sex of blast donor: (♂) male, or (♀) female, or (sc♂) sham castrate male

b) sex of blast recipient: (♂) male, or (♀) female.

c) Number of labelled blasts transferred; single numbers represent mean; range represents 95% confidence limits about the mean

d) mean +/- S.E. number of radiolabelled cells per 10^3 HPF small intestine

e) conclusions based on Student's t test, $\alpha = 0.05$ and comparison to dose curve established using female mice (Fig. 3); ♂→♂ means the number of radiolabelled cells in the small intestine 24 h after transfer of labelled MLN blasts from male donors into male recipients.

blasts had been transferred (Fig. 3). The sex of the recipient did not influence the number of blasts which localized in the small intestine. This data convinced us that there was a difference in MLN blasts from male donors which enabled them to localize in the small intestine (at 24 h after transfer) in greater numbers than female MLN blasts.

Gonadal hormones seemed a likely mediator of the difference in localization of male compared to female blast populations. We therefore investigated the effect on blast localization of altering sex hormone levels of the blast donors. Unfortunately, because the size of the experiments would have been prohibitive, all the possible opposite sex controls were not included in these experiments. Therefore all but one of the experiments using castrated donors were complete before I noticed that the phenomenon of increased localization of male blasts seemed to have disappeared. Because they are of interest, I have decided to present the results of these investigations even though they are negative and I cannot comment on their relevance to the initial phenomenon. Table 14b shows four experiments with opposite sex controls (total 45 recipients) in which the localization of male MLN blasts in the intestine was not greater than the female controls or the expected value from the female dose curve (Fig. 3). To check for possible technical sources of the change, tissues from one positive experiment (S4) and two negative experiments (S8, S11) were re-sectioned, processed simmultaneously for autoradiography and scored. The conclusions from this second processing were the same as the first eliminating changes in autoradiographic or scoring technique as an explanation for the appearance and /or loss of the phenomenon.

7.1.2 Radiocounting studies of gender influences on localization

In two experiments MLN blasts were labelled with ^{125}I-Udr and localization assessed by counting radioactivity in the tissues after adoptive transfer (Table 15). Neither of these experiments showed any significant difference in the recovered radioactiviy in the small intestine after transfer of male or female blasts. However the relevance of this information is not clear because when the same tissues were assessed by autoradiography a male/female difference was detected in the first (Table 14a, S5) but not the second experiment (Table 14b, S8).

7.2 Effect of Oophorectomy of Female Donors on MLN Blast Localization in the Small Intestine.

If the presence or absence of estrogen during development was a factor in determining the ability of blasts to accumulate in the small intestine then we would expect a difference in the localization of MLN cells derived from oophorectomized or sham oophorectomized donors. The ovaries were removed from donor mice a minimum of 3 weeks before the adoptive transfer experiment. Therefore blasts were stimulated and began to differentiate in an environment with very low levels of estrogen compared to that in a normal female mouse. The data in Table 16 show that there was no effect of oophorectomy of female donors on MLN blast localization in the small intestine.

Table 15

Effect of the Sex of the Donor and the Recipient on the Radioactivity and the Number of Radiolabelled Cells in the Small Intestine 24h After Adoptive Transfer of ^{125}I-Udr labelled MLN Blasts

Expt	Donor	Recip	(n)	Radioactivity in Small Gut [a]	Labelled Cells in Small Gut [b]
S5 [d]	♂	♂	(3)	22.0+/-2.1 [c]	97.2+/-7.8 [e]
	♂	♀	(3)	21.4+/-1.6	120.3+/-10.4
	♀	♀	(3)	19.9+/-0.7	50.3+/-6.5
	♀	♂	(3)	17.9+/-1.6	55.0+/-3.5
S8b	♂	♂	(3)	13.9+/-1.11	31.9+/-3.3 [f]
	♂	♀	(2)	14.95+/-0.38	31.7+/-5.7
	♀	♀	(3)	12.10+/-1.28	42.7+/-5.4
	♀	♂	(2)	13.1+/-1.16	40.7+/-7.8

a) Percent recovered radioactivity = dpm in small intestine / dpm in total mouse

b) Number of radiolabelled cells per 10^3 HPF

c) Figures represent mean +/- S.E.

d) Autoradiographic data for these experiments was also presented in Table 14.

e) $((♂ \rightarrow ♂)=(♂ \rightarrow ♀))>((♀ \rightarrow ♀)=(♀ \rightarrow ♂))$ based on Student's t test, $\alpha = 0.05$

f) No difference among the groups based on Student's t test, $\alpha = 0.05$

Table 16

Effect of Oophorectomy of Female Donors on MLN Blast Localization in the Small Intestine [a]

Expt	Donor [d]	Dose [b]	Recip ♂ (n)	Labelled Cells in Small Gut [e]	Conclusion [c]
S6	sham ♀	1.3	♂ (3)	20.9+/-9.5	no difference
	ooph ♀	1.2	♂ (3)	18.2+/-5.7	between groups
S10	normal ♀	0.77	♂ (4)	42.5+/-3.8	no difference
	sham ♀	0.87	♂ (4)	29.3+/-4.9	among the groups
	ooph ♀	0.87	♂ (4)	33.1+/-2.0	

a) Localization of ^{3}H-Tdr labelled MLN blasts in small intestine 24h after adoptive transfer.

b) Number of labelled blasts transferred ($\times 10^{6}$)

c) Based on Student's t test; $\alpha = 0.05$

d) Donors were female CBA/J mice, either normal, or oophorectomized (ooph) or sham oophorectomized (sham) at least 3 weeks prior to experiment.

e) Figures represent mean+/-S.E. of the number of radiolabelled cells per 10^{3} HPF.

7.3 Effect of Castration of Male Donors on MLN Blast Localization in the Small Intestine.

Similarly, if the simple presence or absence of testosterone during developement was a factor in determining blast localizing ability then castration of male donors would result in a change in the quantity of MLN blasts which accumulated in the small intestine. The testes were removed a minimum of 3 weeks prior to the adoptive transfer experiment. The data in Table 17 show that there was no effect of castration of male donors on MLN blast localization in the small intestine.

Table 17

Effect of Castration of Male Donors on MLN Blast Localization[a] in the Small Intestine

Expt	Donor[d]	Dose[b]	Recip (n)	Labelled Cells in Small Gut[e]	Conclusion[c]
S7	normal ♂	0.72	♂ (3)	40.7+/-6.5	correction for dose not necessary
	sham ♂	0.97	♂ (3)	57.6+/-3.0	no difference between sham & castrate or normal & sham
	cast ♂	1.11	♂ (3)	41.3+/-6.8	
S9	normal ♂	1.23	♂ (3)	38.9+/-2.1	after correct for dose: castrate=normal castrate>sham normal>sham
	sham ♂	1.57	♂ (3)	29.1+/-2.8	
	cast ♂	2.13	♂ (3)	67.5+/-9.2	
S11	normal ♂	0.84	♂ (4)	27.9+/-5.6	correction for dose not necessary no differences among groups
	sham ♂	1.09	♂ (4)	27.0+/-3.8	
	cast ♂	0.65	♂ (4)	21.3+/-3.5	
	normal ♀	0.95	♂ (4)	28.8+/-5.9	

a) Localization of ^{3}H-Tdr labelled MLN blasts in small intestine 24h after adoptive transfer

b) Number of labelled blasts transferred ($\times 10^{6}$)

c) Based on Student's t test; $\alpha = 0.05$

d) Donors were male CBA/J mice, either normal, castrated (cast) or sham castrated (sham) or normal females.

e) Mean +/- S.E. number of radiolabelled cells per 10^{3} HPF

CHAPTER 8

Discussion

8.1 Preamble

The existence of a common mucosal immune system which integrates local resistance at mucosal surfaces and is, at least in part, separate from the systemic immune system, is maintained by the selective traffic of immunocompetent cells from their site of stimulation beneath mucosal epithelia to other mucosae. It has been repeatedly demonstrated that lymphoblasts (blasts) from the lymph nodes which drain a mucosal tissue selectively localize in that tissue and other mucosal tissues when compared to blasts from peripheral lymph nodes. The mechanism underlying this localization is unknown. It must involve one, or a combination of, the following processes: selective entry into the tissue from the vasculature, selective retention in the tissue after entry, or selective proliferation in the tissue.

In an attempt to understand better the phenomenon of selective mucosal localization, the discussion to follow will review my work and the current literature in the context of these processes. Components of these processes include the site of entry of blasts into the tissue, the delivery of blasts to this vascular site, potential specific mediators such as antigen, immunoglobulin isotype, secretory component, and other cell surface molecules. Other factors which influence the selective localization of blasts include diet, parasitic infection, sex differences and gonadal hormones. Table 18 summarizes the conclusions

Table 18

Factors Affecting the Selective Localization of Mucosal Lymphoblasts in Mucosae [a]

Factor	Net Effect [b]	Component of Blast Localization Influenced by Factor			
		delivery	emigrat	retent	prolif [c]
mucosal vs non-mucosal blast source	+		+	+?	
antigen	↑/-	+?	-	+?	+
SC	-				
histocompatibility & other surface Ag	?				
immunoglobulin isotype	?				
elemental diet recipient gut	↓	+			
protein-calorie malnourished blast donor	↓				
chemical inflammation or gut parasite in recipient	↑T blast	+	+	+ -	
localization in mucosal sex hormone target tissues: hormone effects	↑	+			

Table 18 (continued)

Factor	Net Effect b)	Component of Blast Localization Influenced by Factor c)			
		delivery	emigrat	retent	prolif
gender & hormones in recipient small intestine	-				
gender & hormones in blast donor: assay localization in small intestine	+/-				

a) This table summarizes tha conclusions from the Discussion regarding the mechanism of action of factors which influence lymphoblast localization in mucosae.

b) The net effect of the factor on localization: ↑ increase; ↓ decrease; + produces difference; - no effect; ? effect unknown or uncertain

c) Possible mechanisms involved in the alteration of lymphoblast localization.

delivery = delivery to mucosae

emigrat = emigration from vasculature

reten = retention in mucosae after emigration

prolif = proliferation in the mucosae (or after transfer)

+ = produces effect (or contributes to effect)

- = not responsible for effect

? = conclusion uncertain

reached in the Discussion about the mechansisms of action of these factors. A better understanding of mucosal traffic would certainly be valuable in attempts to manipulate the immune system to prevent and treat disease.

8.2 Technical Considerations

Several important technical issues have been highlighted during the work described in this thesis. These will be described in some detail, so the reader can appreciate better the methods employed and so that other investigators might consider them when designing investigations of blast localization.

8.2.1 Scoring the entire length of small intestine

It is well recognized that the distribution along the gastrointestinal tract of IgA containing cells and the blasts from which they arise, is not uniform. Gowans and Knight (1964) observed a higher concentration of ^{3}H-Tdr labelled TDL in recipient upper and middle small intestine than in the lower portion. Crabbe and Heremans (1966) found the density of IgA containing cells in the human duodenal and jejunal mucosae was about twice that in the rectal mucosae. In the calf, IgA and IgM containing cells were more numerous in the duodenum and jejunum than ileum (Porter,1972). Similarly, Husband and Gowans (1978) observed approximately twice the density of IgA containing cells in the rat duodenum than in the ileum, with an intermediate frequency in the jejunum. After adoptive transfer of ^{125}I-Udr labelled MLN blasts, half of the radioactivity in the small intestine was in the proximal third of the gut and successively less was recovered in the intermediate and distal thirds (McWilliams et al., 1975). Thus, to obtain a good estimate of the concentration of cells which localized in the small intestine, it was necessary to consistently sample all regions. I therefore prepared sections which each contained the whole length of the small intestine and scanned approximately 7 sections to

count 1000 HPF.

8.2.2 Autoradiography vs Radiocounting

Differences between two of the techniques commonly used in lymphocyte traffic experiments, autoradiography and radiocounting have been noted previously (Griscelli et al., 1969; Befus et al., 1980) but the existence and significance of these differences has generally not been stressed. Examination of data from other investigators revealed these differences. For example, the difference in the number of ^{3}H-Tdr labelled MLN compared to PLN blasts which localized in the small intestine 24 h after transfer (homing index, the relative tendency for labelled MLN compared to PLN blasts to appear in a tissue) was greater when assessed by autoradiography (homing index of 10.9) than by radiocounting (homing index of 2.3) (McDermott, Ph.D. thesis 1979). Similar discrepancies were also noted in recipient MLN, PLN, and lung (McDermott, Ph.D. thesis 1979). The data of Roux et al (1981), re-expressed in terms of a homing index, shows a greater tendency for MLN than PLN blasts to localize in MLN at 24 h when assessed by autoradiography (6.2) than by radiocounting (1.6). Some of these discrepancies might have been due to differences in the concentration of blasts in the inoculum since this is internally controlled in radiocounting but not in autoradiography. However my work suggests that this is not a complete explanation. I performed two experiments in which the same dose of ^{125}I-Udr labelled blasts from male or female donors were adoptively transferred and their localization assessed by radiocounting and then by autoradiography of the same tissues (section

7.1.2). In both experiments radiocounting showed no differences between the experimental groups however autoradiographic analysis revealed a two fold difference between the groups in the first experiment and no difference in the second experiment. The reason for the inconsistencies between these two techniques is not known. One possibility is that a portion of the cells which localize in the small intestine divide (Befus et al, 1980; Roux et al.,1981), thereby producing more radiolabelled cells but no more radioactivity. Recently Dr. M. McDermott (personal communication) in our laboratory observed two populations of cells with different mean silver grain density in autoradiographs of ^{3}H-Tdr labelled MLN blasts in the small intestine at 6 h after adoptive transfer but only one population in the innoculum. Thus the amount of radioactivity per cell is neither uniform nor constant and this would produce differences in the results of experiments when assessed by autoradiography compared to radiocounting. In addition, radiocounting assesses the amount of radioactivity (assumed to be cell associated) per organ or per unit weight of tissue, whereas autoradiographic information is in terms of numbers of cells which contain radioactivity per unit volume of tissue. Thus although a laborious procedure, autoradiography is a valuable technique because it provides a different perspective on the processes which lead to the selective enrichment of the lymphoid population of mucosal tissues with cells derived from MALT.

The counting of cells in tissue sections, whether radiolabelled or labelled with fluorescent antisera, is labour-intensive and apparently meticulous. However, care must be taken not to generalize

too early from a particular case and to consider the sources of variation both between individual animals and experiments and those due to the technique. I have estimated a 75% confidence in scoring autoradiographs by rescoring sets of slides and determining the variation between scorings (section 2.2). Much of this variation can be accounted for by simple probability. I have calculated that most of the variation within and between experiments can be accounted for by the sample size (number of HPF or, in smears the number of cells counted) and the relatively low concentration of radiolabelled blasts. It was not economically feasible to increase either the number of HPF or cells counted or the number of radiolabelled cells transferred to reduce the variation. I concluded that the advantages of autoradiography, especially the ability to describe the morphological location of transferred cells, outweighed the difficulties of man-hours and variability. It should also be noted that because of the low frequency of radiolabelled cells, even seemingly qualitative statements such as their location must be made on a quantitative basis including a careful estimate of the area of a particular morphological region.

8.3 Blast Source and Localization Kinetics

The time available for localization is an important factor when determining whether cells contribute to the lymphoid population of a tissue or not. Perhaps one of the clearest examples of this was that Peyer's patch lymphocytes, but not PLN lymphocytes repopulated the gut of an irradiated recipient with IgA containing cells but not until approximately 6 days after their adoptive transfer into rabbits (Craig & Cebra, 1971; Rudzik et al., 1975) or mice (Roux et al., 1981; Tseng, 1981). Therefore if analysis had been only at day 1 after transfer no selective accumulation would have been observed. Similarly, Roux et al. (1981) demonstrated that Peyer's patch blasts had equal affinity for MLN and PLN at 24 h after transfer but by 90 h the cells accumulated more in MLN. Clearly the time of assessment is crucial when determining whether a population of cells will accumulate in a given site.

However the factor of time has not often been considered in studies of selective blast localization, perhaps in part because of the magnitude of such experiments. Gowans and Knight (1964) observed an accumulation of large TDL blasts but not small TDL lymphocytes in the lamina propria of the small intestines of recipient rats by 1 day after transfer. The first observation of selective mucosal localization compared the accumulation in the small intestine at 20 h after transfer of ^{3}H-Tdr labelled rat TDL or MLN to that after transfer of PLN blasts (Griscelli et al., 1969). Since then, most studies have assessed the selective blast localization of MLN compared to PLN blasts at 18 to 24

h after adoptive transfer. These included studies of the selective localization of MLN compared to PLN blasts or precursors of antibody producing cells in sites other than the gut, such as lung (McDermott & Bienenstock, 1979), cervix and vagina (McDermott & Bienenstock, 1979), lactating mammary glands (Roux et al., 1977), and the salivary glands (Weisz-Carrington et al., 1979; Jackson et al., 1981). It seems unlikely that these studies detected merely a momentary difference in cell localization rather than an absolute tendency for mucosally derived cells, in contrast to those from a peripheral source, to accumulate in mucosal tissues because when comparing the repopulation of gut with PP or PLN derived cells, because these differences were apparent over 1 week later (Craig & Cebra, 1971; Rudzik, 1975; Roux et al., 1981; Tseng, 1981). However, it was not clear how rapidly the differential accumulation of MLN compared to PLN blasts occurred. Using autoradiography, Guy-Grand et al. (1974) observed a preferential migration of MLN blasts to rat gut associated lymphoid system and gut mucosa rather than spleen as early as 90 min after transfer. Although they did not make direct comparisons of MLN and PLN blast localization at times earlier than 20 h after transfer, their earlier studies (Griscelli et al., 1969) showed large accumulations of Bacillus pertussis stimulated PLN blasts in the spleen and PLN but not GALT or gut mucosa from 30 min to 36 h after transfer. Thus selective localization may be apparent as early as 90 min after transfer. Whether selective accumulation represents selective entry and/or selective retention of MLN rather than PLN blasts in mucosal tissues, can only be assessed by looking at various times after transfer.

8.3.1 Kinetics of MLN accumulation

When I began the work described in this thesis there were only a few studies of the localization kinetics of mucosally derived blasts. Most of the kinetics investigations used radiocounting to assess localization. These will be described first, followed by autoradiographic studies of localization kinetics (section 8.3.2). Hall and co-workers studied the localization of ^{125}I-Udr or ^{3}H-Tdr labelled TDL blasts from unstimulated rats (Halstead & Hall, 1972) or rats stimulated in the caudal lymph nodes by injection in the hindquarter with BCG, washed sheep red blood cells, human influenza vaccine and suspensions of sarcoma or kidney tumour cells (Hall & Smith, 1970; Hall et al., 1972). They found most of the recovered radioactivity at 15 min after transfer in the lungs and liver, although 10% of injected radioactivity was already in the small intestine at this early time. Over time there was an increase in the % of recovered radioactivity in the gut (from 10% at 15 min to 25% at 4 h and 30% at 21 h) and a decrease in the lungs (from 38% at 15 min to 3% at 4 h and 1% at 21 h) with a less rapid decrease in the liver and a slight increase in the spleen to 4 h before a decrease. Thus by 4 h after transfer of TDL blasts most of the recovered radioactivity was in the small intestine . At 4 h after TDL blast transfer there was approximately 20% as much radioactivity in the Peyer's patches as in the remainder of the small gut, a large amount, considering the relative weights of these tissues.

Sprent (1976) observed very similar patterns of localization of

radioactivity after the transfer of TDL from irradiated F_1 mice which had been injected with parental strain T cells so that nearly all of the TDL were donor-cell derived, host-reactive T blasts (T-TDL). In addition he found that the kinetics of localization, as assessed by recovered radioactivity, was essentially the same for T-TDL blasts as for B blasts obtained by treating normal TDL with anti-theta serum. Sprent also observed that the radioactivity recovered in the small intestine decreased to about 7% of injected by 48 h from about 20% at 4 and 24 h.

Rose and coworkers (1976) were the first to study the kinetics of MLN blasts localization, however these were derived from T. spiralis infected donor mice and transferred to infected recipients. These investigators observed localization from 1 to 24 h and found a similar pattern of recovered radioactivity as that just described for TDL.

I have extended these studies by observing the kinetics of localization of MLN blasts from normal CBA/J mice in normal syngeneic recipients (Tables 8, 12). The results of these experiments showed that the kinetics of localization of MLN blasts was similar to that observed by others with TDL or with MLN from T. spiralis infected mice. My work revealed maximal accumulation of radioactivity in the small intestine (as a percentage of injected) by 6 h (first time studied) and a trend to decrease thereafter (30 h last time studied). However the radioactivity recovered in the small intestine as a percentage of that recovered in the total animal increased over this period indicating that it was being lost more slowly from the small intestine than from other tissues. In contrast, loss from the lung was more rapid than for

the whole body.

The kinetic pattern I observed for MLN blasts in the small intestine has been confirmed and extended by Ottaway et al. (1980) who described a slight increase in the mouse small intestine from 8% of injected radioactivity at 1 h to 12% at 6 h after transfer and then a decrease to about 6% at 24 h which was the last time point studied. Rose et al. (1978) found that the percentage of injected radioactivity in the small intestine decreased from 24 to 72 h and, during this period, was greater in recipients of ^{125}I-Udr labelled MLN than PLN blasts. Smith et al. (1980) found that for both TDL and MLN blasts in the rat, at least 1/3 of the maximum % of injected radioactivity per gram was in the small intestine by 30 min after transfer and localization was maximal for TDL by the next time point of 2.5 h and for MLN by 9 h. There was no decrease to 24 h. For both cell sources the expected high early localization in the lungs and liver with subsequent decrease was observed. In addition, Smith & Ford (1983) observed that after the first 5 min following injection of ^{51}Cr labelled TDL, there was a very close correspondence between the % of injected radioactivity in the blood and in the lungs, suggesting that the initial high levels were due to nonspecific trapping. Indeed, a recent unpublished study by Rattray and Ford (described in Smith & Ford, 1983) showed that almost all of the ^{3}H-uridine labelled TDL lymphocytes in the lung 10 min after injection were tightly surrounded by capillary endothelium. Thus I conclude that after adoptive transfer many MLN and TDL blasts became nonspecifically trapped in the lung

capillaries but then rapidly left the lung. In marked contrast to the lung, the radioactivity in the small intestine at 30 min after blast transfer was nearly as great as it would be over the next 24 h at which time the amount of radioactivity would begin to decrease.

Although substantial, MLN blast accumulation was likely not complete by 30 min after transfer. Even if the number of blasts in the intestine did not change after this time, cells might still be entering the intestine, balanced by the number leaving. In three experiments I observed an increase (sometimes small) in the number of labelled cells per 1000 HPF in the small intestine after 30 min (Table 9). My radiocounting observations (table 8) and those of other investigators (see above) showed that the percentage of injected radioactivity in the small intestine did not increase after 6 h, suggesting that localization was complete, or had reached an equilibrium of cell entry and loss by that time, if not earlier. Some of the large population of blasts which rapidly left the lungs after 30 min might have entered the small intestine, thus contributing to the increase in recovered radioactivity which some investigators (Hall et al., 1972; Ottaway & Parrot, 1980; Smith et al., 1980) have observed between 30 min and 4 to 6 h after transfer. This increase was not always present (Rose et al., 1976; Sprent et al., 1976).

The differences in kinetic patterns observed by different investigators is unexplained but may reflect the use of different cells sources (MLN or TDL) and different methods of stimulating blasts which would expand different subpopulations of cells. If these different subpopulations have different localization properties, their expansion

would alter the apparent localization of the whole population. In addition, division of labelled blasts after transfer might have augmented the number of labelled cells in the small intestine. Counting of silver grains in autoradiographs of small intestine from a kinetics experiment has suggested that 80 % of these cells divide within 6 h after transfer (Dr. M. McDermott, personal communication). The examination of the same tissues by both grain-counting autoradiography and radiocounting might give some indication as to whether division of blasts after transfer contributes significantly to the lymphoid population of the small intestine and to the different results obtained using these two techniques.

8.3.2 Comparison of kinetics of localization of MLN and PLN blasts

When I began this work no comparison of the kinetics of MLN and PLN blast localization had been performed. I found (using autoradiography) that at all times examined after transfer, from 30 min to 30 h, MLN blasts localized in the small intestine in greater numbers than PLN blasts (Table 9). PLN blasts were only rarely seen at any time after transfer but were perhaps slightly more frequent at 30 min than at other times. Perhaps this indicates a small amount of non-specific trapping in the vasculature of the small intestine at this time. Nevertheless, the seven fold higher frequency of MLN than PLN blasts at 30 min indicated that there was already significant selective mucosal localization by this early time. No other comparison of MLN and PLN blast localization kinetics has been performed using

autoradiography, but Smith et al. (1980) have studied this question using radiocounting. In direct agreement with my results, they found 18-fold more TDL and 10-fold more MLN than alloantigen-stimulated PLN blasts in the small intestine at 30 min after transfer. They also found no increase in the concentration of alloantigen-stimulated PLN blasts and a decrease in the concentration of complete Freund's adjuvant-stimulated PLN blasts to 24 h. Recently, Phillips-Quagliata et al. (1983), using radiocounting, have also observed selective intestinal localization of MLN compared to PLN blasts by 30 min after transfer. Thus I conclude that the selective localization of MLN blasts in the small intestine was a rapid process, already evident at 30 min after adoptive transfer.

8.3.3 Suggestions from kinetics studies regarding a mechanism for selective accumulation

Kinetics studies might also provide information regarding the mechanism(s) of selective localization. Ottaway and Parrott (1981) developed a mathematical model of localization which, after certain assumptions, used kinetic data to estimate the rates of blast entry and length of retention in (or rate of loss from) tissues. Their assumption that the rate of lymphoid cell entry into a tissue was proportional to the number of lymphoid cells presented by the blood has been supported by evidence from myself and others which will be discussed later (section 8.5). Their assumption that the rate of cell loss from a tissue was proportional to the number of these cells which were present in the tissue seems reasonable, although the experimental evidence to support it did not include mucosal tissues and

blasts but was limited to small lymphocytes in lymphoid tissue (Ottaway & Parrott, 1981).

Ottaway and Parrott's analysis indicated that small lymphocytes, MLN blasts and PLN blasts had the same probability (efficiency) of entry into the mouse small intestinal tissue. However, MLN blasts remained in the gut longer ($t_{1/2}$ =3.01 to 6.3 h) than PLN blasts ($t_{1/2}$ =0.59 h), or MLN or PLN small lymphocytes ($t_{1/2}$ =0.85 and 0.71 h respectively). The authors did not present the data used in their calculations (based on radiocounting experiments) which is unfortunate because seeing the data might have suggested why they concluded that selective retention was most important, whereas my data and those of Smith et al. (1980) and Phillips-Quagliata et al. (1983) suggest a different hypothesis. I do not have the information, such as the rate and pattern (linear or exponential) of disappearance of cells from the blood, which would be required to perform these calculations using my data. Therefore, no direct comparison is possible. However my data and those of Smith et al. (1980) and Phillips-Quagliata et al. (1983) seem more consistent with a difference in the probability of entry of MLN and PLN blasts rather than, or in addition to a difference in the probability of loss from the small intestine. Both these sets of data, from radiocounting studies in the rat and mouse and autoradiographic studies in the mouse, showed an accumulation of radioactivity or labelled cells at 30 min which was much greater for MLN than PLN blasts, even after correcting for differences in the number of labelled cells transferred. Therefore

unless substantial numbers of PLN blasts entered the gut lamina propria and left it before 30 min after transfer, the major difference in localization of MLN and PLN blasts in this tissue would seem to be due to their ability to enter it from the vasculature. Investigations of localization before 30 min after transfer would test this hypothesis.

Although we do not know the rate at which blasts cross the vasculature in mucosae, it might be similar to the rate at which lymphocytes cross HEV in lymph nodes. Although not directly addressing this question, the work of Hall et al. (1977) is suggestive that the kinetics of movement of lymphoid cells through the intestine is similar to that through lymph nodes. Hall et al. (1977) collected lymph for 2 h intervals up to 24 h after blast transfers in sheep and found that the recirculation of blasts from intestinal lymph through the intestine and back into intestinal lymph occurred to the same extent and had the same kinetics as the recirculation of blasts from peripheral lymph, through a lymph node and back into peripheral lymph. Gowans and Knight (1964) observed that 15 min after the start of an intravenous transfusion of labelled small TDL, the cells were localized almost exclusively in the walls of post-capillary venules and the few which had penetrated were still in the immediate vicinity of HEV. None were observed in either the marginal or medullary sinuses. Thus if the rate of entry into and migration through lamina propria is similar to that in lymph nodes, I would expect the majority of the cells which extravasated to still be close to that site by 15 min and likely still at 30 min. However, Roux et al. (1981) observed Peyer's patch derived blasts in the subcapsular sinus of MLN by 30 min after transfer and the

concentration of these cells in recipient Peyer's patches compared to adjacent lamina propria (Phillips-Quagliata et al., 1983) suggested that these cells had extravasated in the Peyer's patch and migrated via lymph to the MLN within 30 min. Therefore it is possible that large numbers of PLN blasts extravasated in the small intestine or Peyer's patches and left it before 30 min. However, there was no large accumulation of PLN blasts in MLN at 30 min after transfer (Griscelli et al., 1969; Smith et al., 1980; Phillips-Quagliata et al., 1983), nor were many of these cells recovered in the TD lymph collected during the first 24 h after transfer (Griscelli et al., 1969). Therefore if PLN blasts did extravasate early in the lamina propria in large numbers (comparable to MLN blasts) they must have left the gut via the blood stream, not the lymphatic circulation. Thus it seems unlikely that PLN enter the lamina propria immediately after transfer. My results suggest that selective entry, rather than selective retention, is the mechanism which results in the differential localization of MLN compared to PLN blasts.

8.3.4 Localization properties of mucosal blast population and subpopulations

Although blasts generated in one mucosal tissue circulate and localize in other mucosal tissues they seem to localize most in the tissue from which they originated. McDermott and Bienenstock (1979) found few bronchial lymph node (BLN) blasts localized in the small intestine compared to the number of MLN blasts which localized in the small intestine, or the number of BLN blasts which localized in the

lungs. Although MLN blasts accumulated more in the small intestine than lung (McDermott & Bienenstock, 1979), the differences were not as great as when BLN localization in these two tissues was compared. This is consistent with the observation that dissemination of immunologic memory (due to migrating lymphocytes) to the duodenum or colon was only slight following intra tracheal priming compared to either colonic or duodenal priming (Pierce & Cray, 1981). Similarly Pierce and Cray (1981) observed greater secondary responses in the colon or duodenum when the challenge site was the same as the priming site, although some of this effect might have been due to resident (as opposed to migrating) memory cells.

Just as blasts from MLN or PLN differ in their localization properties, different subpopulations of blasts from a particular node localize differently. Thus, any change in their proportional representation would appear as a change in the localization of the whole population. For example, ^{51}Cr labelled TDL from normal mice localized in the small intestine less than ^{51}Cr labelled TDL from irradiated F$_1$ mice which had been reconsituted with parental (H-2 incompatible) T cells (T-TDL; Sprent, 1976). Most of the apparent localization of this population was due to the localization of only a subpopulation of the TDL cells, i.e. the blasts. Since blasts represented a larger proportion of T-TDL than TDL, a greater proportion of the former population localized in the small intestine. T-TDL blasts but not small lymphocytes traffic selectively through rodent small intestine (Freitas et al.,1980). Thus even though cells from the blast subpopulation of T-TDL and TDL had the same potential to localize

in the small intestine, cells from T-TDL appeared to localize selectively compared to those from TDL because of the different proportional representation of a specific subpopulation. This potential source of confusion should be kept in mind when studying the selective localization of mucosally derived blasts beneath mucosae. Certainly this population is heterogeneous in that it contains T and B blasts, that these cells would be directed against different antigens and that they will likely progress along different pathways of differentiation, to effector cells or short or long term memory cells. The proportional representation of these subpopulations would doubtless be influenced by many factors and cannot be expected to remain constant. However, in the past, investigators have compared the localization of MLN, TDL or PLN blast populations, each stimulated by different antigens which would expand different subpopulation of cells. This problem may have been somewhat minimized in my system by the use of the endogenously stimulated blast populations of MLN and PLN. Recently some investigators have attempted to circumvent this problem by limiting their studies to T or B blasts, or IgA containing cells or to cells containing antibody against a specific antigen. This approach has revealed differences in the distribution of T and B cells within the small intestine (section 8.4.2) and influences of antigen on the kinetics of accumulation of antigen specific cells (section 8.7.1).

In conclusion, the source of a population of blasts is the major factor determining their ultimate destination after release from lymph nodes. At any time after transfer, MLN or TDL derived blasts but not PLN blasts localized in the small intestine. When studying this phenomenon by adoptive transfer experiments it is important to be cognisant of the fact that one is observing a "still photograph" of an ongoing process and that kinetic studies are crucial to gain a clear impression of this process. My kinetic studies suggested that selective entry of MLN compared to PLN blasts was likely a major mechanism resulting in the selective accumulation of MLN blasts in intestinal mucosae, unless large numbers of PLN blasts both enter and leave the lamina propria via the vasculature within 30 min after transfer. From a teleological point of view this seems unlikely. Factors which influence the proportional representation of subpopulations of blasts released from the node will obviously influence the lymphoid population in the tissue in which they localize, but may also influence the apparent selective localization of the blast population if these subpopulations all have the same label but have different localization characteristics.

8.4 Sites of Blast Localization

The location in the mucosal lamina propria at which blasts leave the vasculature is unknown. In lymph nodes, small lymphocytes (Gowans & Knight, 1964) and blasts (Griscelli et al., 1969) leave the vasculature by migrating between the cuboidal epithelial cells of post capillary venules in the cortex (Schoefl & Miles, 1972; van Ewijk et al., 1975; Anderson et al., 1976). These morphologically distinctive high endothelial venules (HEV) are not present in the lamina propria of the small intestine but occur in Peyer's patches (Parrott & Ferguson, 1974; Blau, 1977). Therefore, blasts which selectively localize in the small intestine must either leave the vasculature at sites which are morphologically indistinct or at HEV in Peyer's patches followed by migration through the tissue into the lamina propria.

8.4.1 Peyer's patches - the site for blast entry into lamina propria?

There is evidence which pertains to the potential role of PP as the site of blast extravasation in the small intestine. Blasts localized normally in the small intestines of rats from which all the PP had been removed (McDermott et al., 1980). In addition, specific antibody containing cells migrated into surgically isolated loops of intestine in which no PP had been included (Husband & Gowans, 1978). However solitary lymphoid nodules in the gut (Keren et al., 1978) could not be excluded or removed might have provided a portal of entry for blasts. If blasts extravasated in the PP prior to migrating into the lamina propria I would have expected to observe a high

concentration of these cells in the PP early after transfer with a subsequent decrease. However, I observed no difference in the concentration of MLN blasts in the PP at 30 min and 24 h (Table 13). My observation that the concentration of blasts in the lamina propria immediately adjacent to a PP was not different to that in the remainder of the gut at both 30 min and 24 h after transfer (Table 11) also sugested that the PP and the adjacent lamina propria were not important sites for extravasation of mucosal blasts enroute to the lamina propria. It is possible that the blasts had already extravasated in the PP and moved to their final destination in the lamina propria by 30 min after transfer but this seems unlikely because lymphocytes take about 5 to 10 min just to cross the HEV (Smith & Ford, 1983). However it its not known how rapidly lymphocytes can migrate through the lamina propria. Collectively these data suggest that the PP is not a major extravasation site for blasts destined for the lamina propria.

It has been repeatedly observed that IgA containing cells are present in higher concentration immediately adjacent to the PP than in distant lamina propria in normal mice (Crabbe et al., 1970) and after repopulation of irradiated recipients with cells from PP (Rudzik et al., 1975b; Befus et al., 1978), BALT (Rudzik et al., 1975b) or intestinal lamina propria (Befus et al., 1978). In addition, Hall et al. (1972) observed a halo of labelled cells in the lamina propria around the PP from 4 to 24 h after transfer of TDL blasts stimulated by caudal injection of various antigens. Sprent (1976) noted that most of the labelled cells in the intestinal lamina propria were near PP 2 to 3

days after transfer of T-TDL which had been labelled in vivo with ^{3}H-Tdr for 48 h before transfer. Although it is not known how these observations can be reconciled with mine, there are several possibilities. The MLN blast population contained both B and T cells which might localize differently along the intestine. Alternatively, the concentration of IgA containing cells adjacent to the PP might not be due to selective localization of precursor blasts but to some other mechanism such as the local expansion of memory cells (Pierce & Cray, 1982) due to the concentration of antigens absorbed by the PP.

I conclude that the PP is not a significant portal of entry for blasts from the vasculature into the lamina propria and thus blasts must selectively localize in the small intestine by extravasating in the lamina propria.

8.4.2 The extravasation site in the lamina propria

The site of extravasation in the lamina propria might be investigated by studying the distribution soon after transfer compared to the distribution at later times. In lymph nodes, post capillary venules with high cuboidal endothelium (HEV) are the exit site for lymphocytes and receive their blood flow both from the capillary plexus of the marginal sinus and from direct arteriovenous anastomoses (Anderson & Anderson, 1975; Herman et al., 1979; de Sousa, 1981). Although HEV do not exist in the lamina propria, perhaps blasts leave the vasculature in post capillary venules with continuous thin endothelium. Even in lymph nodes, lymphocytes have occasionally been observed passing through the larger smooth walled low endothelial

venules (Cho & de Bruyn, 1979).

The blood flow to the intestinal mucosa is as follows: a central arteriole originates from submucosal vessels and proceeds, without branching, to near the tip where it gives off two branches, one which empties directly into the central villus vein, and another which connects to the marginal arterioles (one or two) from which arise a subepithelial capillary net. The subepithelial capillaries join the central vein below the tip. In the crypt region two or more branches of the submucosal arteries, called marginal arterioles (smaller than central villus arteriole) form a capillary net which joins with the subepithelial capillary net of the villus (Mohiuddin, 1966; Miller et al., 1969; Levitt et al., 1979). Thus if blasts leave the vasculature in post capillary venules in the small intestinal lamina propria, I would expect them to be distributed all along the villus and in the basal lamina propria. To test this hypothesis I examined the distribution of MLN blasts in the morphological regions of the small intestine (Fig. 1) at 30 min and 24 h after transfer (Table 10). I found that there was a small tendency for blasts to accumulate more in the basal than villus lamina propria (even after allowing for the differences in the proportional representation of each morphological region in a HPF, sections 4.2 & 6.1.1). This was the pattern expected if blasts extravasated in the post capillary venules in the lamina propria.

The observed distribution was the same at 30 min and 24 h after transfer (Table 10). Therefore I conclude that the MLN blast population most likely has a similar propensity to cross the vascular

endothelium in both the villus and basal lamina propria. This also suggested that the majority likely remained close to the point of extravasation. The slight difference in distribution might have represented a small difference in the ability of blasts to adhere to and cross endothelium in these two regions but other explanations are possible.

It has been shown that the number of blasts presented to a tissue, either by changing the number of blasts transferred (analogous to changing the output from the MLN) or by changing blood flow, is an important factor in determining the concentration of blasts in that organ (discussed in section 8.5). Perhaps the delivery of blasts to the villus and basal lamina propria differs. Indeed, blood flow is not evenly partitioned between the villi and the crypts. Lifson et al. (1979) injected 9 μm polystyrene microspheres into rabbits and assessed their distribution immediately thereafter by counting in cleared sections under a dissecting microscope or after digestion of tissue. These investigators calculated that about 60% of the beads were in the crypt region and two fifths in the villi. In a second group of rabbits the distribution differed, perhaps due to a change in oral antibiotic, and was approximately 40% in the crypts and 60% in the villi (Lifson et al., 1979). The same group constructed a theoretical model of the vascular network by measuring lengths and radii microscopically and calculating segmental resistances (Levitt et al., 1979). Their theoretical model was in close agreement with microsphere measurements. They suggested that 44% of the mucosal flow was carried by marginal

arterioles and that most of this went to the crypts. Fifty-six percent of the mucosal flow entered the mucosa via the central arteriole to go to the villus. Two thirds of this (38% of total) bypassed the villus capillaries to reach the central vein at the tip of the villus while one third (18% of the total) flowed to the subepithelial capillary network (Levitt et al., 1979). After injection of 7 to 10 μm spheres into dogs, virtually all of those in the villi were at the tips (Bond & Levitt, 1979). If a similar partition of flow exists in the mouse, and if blasts lodged in a manner similar to the spheres and primarily in capillaries, we would have expected approximately twice as many in the crypts as in the villi (44% vs 18%) and most of those in the villi to be near the tips. I did observe about twice as many labelled blasts in the basal lamina propria (69.0+/-1.98%) as in the villi (34.2%+/-1.92%). Although some of this distribution was an artifact of the relative areas of villus and basal lamina propria, localization was greater in the basal lamina propria (section 4.2). This distribution could have been explained by the differential delivery of blasts to these two regions. However most of the blasts in the villi were not near the tips indicating either a slight difference in the vascular network of the mouse or that the site for blast extravasation was not the same as that for microsphere trapping (presumably pre-capillary sphincters and capillaries). Perhaps the blasts lodged in the postcapillary venules, or perhaps their somewhat larger size might have resulted in more shunting through the arteriovenous anastomosis in the villi, rather than trapping in capillaries. The relative absence of this shunt in the basal lamina propria might explain the different

concentration there. It would be interesting to compare the distribution of blasts with that of microspheres of the same size in the small intestinal vasulature of the mouse.

Alternatively, the small difference in the propensity of the MLN blast population as a whole to localize in the basal or villus lamina propria might be comprised of large differences for the various subpopulations of the MLN blast population. Dr. M. Mc Dermott (personal communication) has recently performed adoptive transfers using separated B and T blasts from MLN., He found that at 24 h after transfer there were equal proportions of B and T blasts in the basal lamina propria but in the villus there were 70% T and only 30% B blasts. The distribution of B blasts was consistent with the many observations of concentrations of IgA containing cells around the crypts of Lieberkuhn and bases of villi (Crabbe et al., 1965; Pierce & Gowans, 1975; Rudzik et al., 1975a). This distribution of IgA producing cells and B blasts was also consistent with the observation that IgA secretion occurred primarily through crypt epithelial cells. Perhaps T blasts have a greater ability to extravasate in the villus and B blasts in the basal lamina propria. Alternatively these cells may have left the vasculature with the same distribution and relocated later. Husband (1982) found that after autologous transfer, specific antibody containing cells from TDL were equally distributed between villus and basal lamina propria at 3 and 6 h but then began to accumulate in the basal lamina propria. This redistribution was antigen associated since it was only observed with cells containing

antibody for the antigen which was introduced into the lumen of the gut. The number of cells in the loop producing antibody to irrelevant antigen decreased after 6 h to zero by 18 to 24 h (Husband, 1982). (The influence of antigen on localization is discussed further in section 8.7.1.) This work supported my conclusion that blasts extavasate throughout the vasculature in the lamina propria, but also suggested that after extravasation, different subpopulations may become distributed differently within the lamina propria.

8.4.3 Blast localization in intestinal epithelium

The kinetics of localization of MLN blasts in the intestinal epithelium differed markedly from that in the lamina propria (Table 10). While a significant proportion of the localizing blasts had accumulated in the lamina propria by 30 min after transfer, this was not the case for the epithelium. In eight recipients, only one labelled blast was observed in the epithelium at 30 min after transfer. The conclusion that localization in the epithelium requires more time than in the lamina propria is not surprising since these cells would first have to migrate across the lamina propria and basal lamina.

The number of blasts which localized in the epithelium per unit area also differed from that in the lamina pripria. Although the epithelium was 23.6%+/-0.56% of the tissue area of a HPF, MLN blasts in the epithelium accounted for only 3.53%+/-0.55% of the MLN blasts in the small intestine (for explanation of the estimate of area see section 4.2). This paucity of cells localizing in the epithelium is unexplained. Perhaps only a small subpopulation of MLN blasts have the ability to localize in this site.

Intraepithelial lymphocytes are granulated lymphocytes and have been shown by some investigators to have cell membranes positive for T cell antigens in the mouse (Guy-Grand, et al., 1978), human (Selby et al., 1981), and rat (Lyscom & Brueton, 1982). However they do contain subpopulations which are granulated and thymus-independent (Mayrhofer, 1980) or nongranulated and thymus-dependent (Ferguson & Parrott, 1972; Mayrhofer, 1980) as assessed in neonatally thymectomized or nude mice and B rats. Mayrhofer and Whately (1983) found normal numbers of granular IEL in jejunal sections from nude rats or adult thymectomized, irradiated, bonemarrow reconstituted rats. The granulated IEL have been suggested as precursors of the mucosal mast cell (Guy-Grand et al., 1978; Mayrhofer, 1980). However recent evidence in the mouse (Schrader et al., 1983) using selective growth factors and surface antigenic markers suggests that intramucosal granulated lymphocytes were not likely mast cell progenitors. Only 10 to 20% of the granulated cells expressed Thy-1 at levels comparable to cortical thymocytes and, although T cells could be grown from intraepithelial lymphocytes, whether these were derived from the $Lyt\text{-}2^{+}$, $Thy\text{-}1^{-}$ granulated cells remains to be established (Schrader et al., 1983). In the rat there were higher concentrations of OX8 positive (T-suppressor/cytotoxic) cells in the IEL (73%) than in the lamina propria (26%) and the IEL were unusual in containing a subpopulation which was negative for W3/13 (T cell marker) but positive for OX8 (Lyscom & Brueton, 1982). In the human, the suppressor-cytotoxic phenotype also predominates (84%) in IEL and only 1/3 of these reacted with an antigen

found on human peripheral blood T cells (Selby et al., 1983). The remaining IEL had the helper phenotype. In contrast the helper phenotype predominated in the lamina propria (Selby et al., 1983). Thus the IEL population differs from the lamina propria lymphocyte population in the type of cells it contains and therefore also presumably differs in the type of blasts which would be the precursors of these cells. This concept helps explain why only a small number (a limited subpopulation) of MLN blasts localize in the epithelium.

Another explanation for the minimal localization of MLN blasts in epithelium is that blast localization might represent only a minor mechanism for the maintenance of this intraepithelial lymphocyte population. Darlington and Rogers (1966) studied the rate of replacement of intraepithelial lymphocytes by injecting ^{3}H-Tdr into mice and noting the number and location on the villus of labelled cells at various times thereafter. These authors suggested that either epithelial lymphocytes formed a self-perpetuating cell line with a low rate of cell division, and a correspondingly low rate of loss, or that they were supplemented by cells from other tissues. If the latter hypothesis is the major mechanism then the pattern of labelling was predominantly that of the parent population. Much evidence suggests that IEL are derived from PP lymphocytes. Sprent (1976) observed the localization of T-TDL blasts in intestinal epithelium. Guy-Grand et al (1978) detected radiolabelled cells in the epithelium after direct application of ^{3}H-Tdr to PP. In addition, thoracic duct drainage on days 5 and 6 of mice undergoing a graft-versus-host reaction (irradiated and reconstituted with allogeneic thymocytes) almost

entirely depleted gut T lymphocytes. Also, Guy-Grand et al. (1978) found that local irradiation with ^{32}P-disks of PP or MLN resulted in a progressive decrease in the number of IEL T cells from 23+/-3 per microscopic field in normals to 2.3 per field at day 17 of irradiation. ^{32}P-irradiation of spleens caused only a moderate decrease, to 17 IEL per field at day 17. Thus, as well as being a major source of lamina propria lymphocytes, the PP are the major source of IEL, and blasts stimulated in the PP traffic via MLN and TDL to enter the intestinal epithelium.

8.4.4 Blast localization in the lungs

The evidence that the lungs are part of the common mucosal immune system has been reviewed in the Introduction (sections 1.1.1 and 1.2.3). However the lung is composed of both mucosal (BALT and bronchus) and nonmucosal (parenchymal) tissue. Unfortunately most studies have not attempted to distinguish between localization in these two sites. In radiocounting studies the lung was, of necessity, considered as a whole organ. Using autoradiography, McDermott and Bienenstock (1979) made the qualitative statement that MLN blasts seemed to lodge adjacent to the BALT and that PLN blasts showed a similar intraorgan distribution. For most of the work performed for this thesis there was no attempt made to assess quantitatively the concentration of blasts in these regions of the lungs. However, blasts were not observed in either BALT or bronchial epithelium in 84.4% of the 101 individuals in which the localization of MLN blasts was assessed even though other individuals at the same times and donor cell

doses had cells localized in these regions. The explanation for this observation may simply be the paucity of BALT and bronchial epithelium compared to lung parenchyma. Indeed, in one experiment where the areas of these regions was recorded, BALT was observed in only 3 of 15 recipients and the largest area was only 15.5 HPF (1.5% of the area normally sampled). In spite of the small area, labelled cells were observed in both of the MLN recipients in which BALT was observed (3 h post-transfer). Similarly, the area of epithelium was small, ranging from 5 to 43 HPF per animal, so it was not surprising that labelled MLN blasts were observed in the epithelium of only 2 of the 12 recipients. I conclude that MLN blasts do localize in BALT and bronchial epithelium. However, because of the small area of tissue available for examination it was not possible to conclude whether MLN blasts selectively localized in the bronchial mucosa compared to PLN blasts.

As discussed in section 8.5, transfer experiments to assess the effects of blast dose and the kinetics of localization suggested that neither MLN nor PLN blasts accumulated <u>selectively</u> in the lung parenchyma. Blasts from bronchial lymph node localized in the lung, perhaps slightly more than MLN or PLN blasts (McDermott & Bienenstock, 1979; my discussion 8.5). These patterns of blast migration are consistent with the observation that immunologic memory is disseminated to the trachea by migrating cells following intracolonic or intraduodenal priming but that these responses are not as great as those following tracheal priming (Pierce & Cray, 1981).

8.4.5 Blast localization in Peyer's Patch

Since the information is not available elsewhere in the literature I will describe the localization kinetics of MLN blasts in PP using autoradiography (Table 13). However it must be stressed that the tissue area examined was small so variation was high. At both 30 min and 24 h, the majority of blasts were observed in the TDA with very few in the follicles or dome. This was similar to the distribution of N. brasiliensis stimulated MLN blasts in the PP at 24 h observed by Parrott & Ferguson (1974). Guy-Grand et al (1974) also noted that the majority of labelled cells in the rat PP 20 h after MLN blast transfer were in the interfollicular areas and that most were T lymphocytes and none contained IgA. Labelled cells also predominated in the interfollicular areas 24 h after rabbit TDL blast transfer (Perey & Milne, 1975) and 2 to 3 days after T-TDL transfer (Sprent, 1978). No cells were observed in the dome at 30 min after transfer but they were present in this area at 24 h. Although the area of dome scored was small, the concentration of labelled cells appeared to be similar to that in the TDA. Perhaps some of the blasts which entered the PP via HEV in TDA subsequently migrated into the dome.

MLN and PLN blasts accumulated in the PP with the same distribution and frequency at 0.5 h after transfer. This observation was in contrast with the radiocounting data of Smith et al. (1980) who found that MLN and TDL blasts localized approximately eight times more in PP than alloantigen or complete Freund's adjuvant stimulated PLN. The differences between these two sets of results might have been due

to the antigenic stimulation of PLN in the studies of Smith *et al* (1980).

8.5 The Relationship Between Blast Delivery in Nonlymphoid Tissue and Their Localization

The number of lymphocytes entering lymphoid tissue is directly proportional to the number presented to that tissue in the vasculature (Introduction). The rate at which blasts are delivered to the mucosae would presumably also affect the number which extravasated during a particular time interval. The magnitude of the effect would depend upon the nature of the relationship between the number of blasts passing the extravasation site and the number which will cross it.

I performed experiments in which different numbers of ^{125}I-Udr labelled blasts were transferred and found that the radioactivity recovered in the small intestine, PP, lung, MLN, PLN, spleen and liver was directly proportional to the number of MLN blasts transferred (Fig. 2). This linear relationship was also demonstrated for MLN blast localization in the small intestine and lung using autoradiography (Fig.3). In confirmation, inspection of results presented by Husband and Gowans (1978) revealed a linear increase in the number of antigen specific TDL localized in the small intestine after transfer of increasing numbers of TDL. Similarly, Freitas et al (1980) found that a greater percentage of blasts in the ^{51}Cr-labelled T-TDL population was associated with a greater recovery of radioactivity in the small intestine. Both the small intestine and lung had a large capacity to accumulate blasts since transfer of 9.5×10^8 MLN blasts (estimated to be four times the normal daily output of thoracic duct blasts (Sprent,1973)) did not result in saturation. Where increased accumulation was evident in the villus and basal lamina propria, the

intestinal epithelium, lung parenchyma, BALT and bronchial epithelium and none of these morphological regions were saturated after transfer of large numbers of MLN blasts.

The number of PLN blasts in the recipient lung and PLN also increased after transfer of more cells. This relationship might also have existed in the small intestine, however too few labelled PLN cells were observed to be certain (Fig. 4). Thus it seems that for each blast population (MLN or PLN) a certain proportion of the blasts transferred (i.e. present in the blood) will localize in a tissue. Ottaway and Parrott (1980) used a different approach and obtained evidence which suggested the same conclusion. They found that the localization along the small intestine of unseparated or T-enriched MLN or PLN blasts all showed a similar correlation with the fraction of cardiac output delivered to that portion of the gut, in spite of marked differences in the tendency of these populations to accumulate in the gut. Subsequent work by the same group (Ottaway and Parrott, 1981) emphasized the importance of this mechanism for altering blast localization. They observed that MLN blasts localized more in the small intestines of mice fed a standard rather than an elemental diet. This was not a direct effect of dietary antigen but rather was mediated through increased blood flow to the intestines of mice on a conventional diet.

It should be stressed that, as expected, when equal numbers of MLN or PLN blasts were transferred, at least ten fold more of the former than the latter localized in the small intestine (Figs. 3a and

4a). In contrast no differences occurred in the number of cells which localized in the lungs following transfer of equal numbers of MLN or PLN blasts (Figs.4b and 5a). These results suggested that previous reports by McDermott & Bienenstock (1979) of autoradiographic results suggesting selective pulmonary localization of MLN compared to PLN-derived blasts (localization ratio 3:1) were due to differences in cell dose (blast ratio 2:1). Similarly, since the proportion of blasts in the innoculum from bronchial lymph nodes (BLN) was five times that of PLN (McDermott & Bienenstock, 1979), most if not all of the apparent selective pulmonary localization of BLN blasts compared to PLN blasts (blast ratio 10:1) was likely also an artifact of differences in cell dose. There may have been a slightly greater localization (by a factor of two) of BLN blasts than MLN or PLN blasts in the lungs. However this is much less than the homing indices of three and ten respectively which are suggested when cell dose differences are not considered. My observation that both MLN and PLN blasts accumulated in the pulmonary parenchyma is consistent with the integration of mucosal (IgA) and peripheral (IgG) immune responses in the lungs. However I cannot exclude the possibility that this comparable accumulation was due to nonspecific trapping of cells in pulmonary capillaries. Although MLN blasts were observed in BALJ and bronchial epithelium, the paucity of these tissues did not allow me to conclude whether MLN blasts selectively localized in these regions compared to PLN blasts (section 8.4.4).

In conclusion, the supply of blasts to a tissue in which a certain proportion will localize can be altered by changing the

concentration of blasts in the blood and/or the delivery of these cells by changing the regional blood flow. Alteration of the number of blasts passing through the vasculature of a particular tissue will alter the number of blasts which accumulate in that site. This has implications in the design of experiments and perhaps more importantly in creating immunization protocols. Immunization protocols designed to increase the output of MLN blasts or to provide more blood-borne MLN cells to a site by expansion of the regional circulation would likely enhance MLN blast localization in nonlymphoid mucosal tissues even in the absence of specfic antigen or inflammation. For example, oral immunization would increase the output of MLN blasts. These antigen - specific blasts would localize in the gut but also in distant mucosal tissues in greater numbers than blasts generated by parenteral immunization. Thus immunization of mucosal surfaces would be favoured over parenteral immunization for protection against genital tract infections or to enhance the quantity of specific antibodies in colostrum in order to passively protect the suckling infant. Oral immunization combined with hormone treatment to increase blood flow and therefore increase delivery of blasts would be expected to enhance immunity in the genital tract.

8.6 Gender Differences and Sex Hormone Influences on Localization

When I began the work described in this thesis it was recognized that blasts stimulated in the small intestine would localize in sex hormone target tissues. This localization was augmented after treatment with the appropriate hormone. I investigated whether sex or sex hormonally induced differences were also present in the accumulation of MLN blasts in the small intestine.

8.6.1 Localization in sex hormone target tissues

IgA antibodies specific for gastrointestinal flora and antigens presented orally have been found in the colostrum and milk of lactating animals (see Introduction). Oligomeric, J-chain containing IgA (and IgM) were transported selectively from serum into colostrum when compared to IgG (Halsey et al., 1980). Thus selective transport from serum may represent an important source of IgA in colostrum and milk. However local synthesis is likely also important since plasma cells containing IgA lie adjacent to the breast epithelium. During late pregnancy and lactation there was a dramatic increase in the number of plasma cells in the mammary gland (150 fold per unit area) in excess of the organ's increase in weight (6 fold) and over 90% of these cells contained IgA (Weisz-Carrington, 1977). The plasma cells in the breast might have been derived from blasts which originated in MALT. Indeed, TDL blasts accumulated 15 fold more in lactating than in non-lactating mammary gland, although there was only a five fold increase in weight (Love & Ogilvie, 1977). When compared to PLN blasts, MLN blasts from virgin donors selectively localized in the mammary gland of mice 1 day antepartum and throughout lactation but not before or after this period

(^{125}I-Udr; % of injected/gram) (Roux et al., 1977; Jackson et al., 1981). The majority of the MLN blasts which selectively localized in the mammary gland had surface IgA and contained IgA 16 to 24 h after transfer (Roux et al., 1977). This enhanced localization in the mammary gland could be obtained by mimicking lactation using hormone treatments with combined progesterone and estrogen for 10 days followed by prolactin for 30 days. Although the percent of injected radioactivity recovered was much lower than in naturally lactating recipients, an MLN:PLN ratio of 2.4+/-0.5 was observed (Weisz-Carrington et al., 1978). However Rose et al., (1978) found that MLN from untreated mice and oxazolone stimulated PLN blasts accumulated to a similar extent in the mouse mammary gland at mid lactation. The apparent disparity between the work of Rose et al. and that of Weisz-Carrington, Roux and coworkers might have been due to the differences in the peripheral blast population. Indeed, Manning and Parmely (1980) found that although with the onset of lactation, there was a 3 to 4 fold increase in T blast migration into rat mammary tissue on a per unit weight basis (7 to 10 fold per organ), there was no difference in the accumulation of unseparated or nylon wool purified T blasts from MLN compared to those from superficial cervical lymph node. Perhaps some of the increased localization was due to increased blood flow per unit weight of tissue. Perhaps only B blast display selective mucosal localization to the mammary gland. Interpretation of these results is complicated by the use of the superficial cervical lymph node as a source of blasts to compare to MLN blasts. Tilney (1981) noted that

the superficial cervical node of the rat drains the nasolabial lymphatic plexus and the tongue. Therefore this node might be a source of mucosal blasts which would be expected to localize in the mammary gland. Thus no explanation for the discrepancies between the results of Rose and coworkers and those of other research groups can be offered.

IgA is the principal immunoglobulin in genital tract secretions (Waldman et al., 1971). Perhaps, like in the breast, the immune system of the genital tract is influenced by sex hormones. Wira and Sandoe (1977) observed that the IgA concentrations in rat uterine washings were highest at pro-estrus but remained partially elevated for estrus. IgG concentrations in uterine secretions were 10 to 15 fold higher at pro-estrus than throughout the rest of the cycle. No such fluctuations were observed in serum immunoglobulin levels. Similar elevations could be induced by administration of estradiol to castrated rats for 2 to 3 days, but not 6 to 14 days (Wira & Sandoe, 1980). In the human, IgA and IgG levels in cervical secretions have been reported to vary during the menstrual cycle (Hulka & Omran, 1969) or with the use of oral contraception with the combined pill (Chipperfield & Evans, 1975). At least some of these changes were due to alterations in the accumulation of plasma cells in the genital tract. When estradiol treated, ovariectomized rats were compared to untreated castrates, there was a greater accumulation of IgA in uterine tissues preceding an increase of IgA in the lumen, an accumulation of IgA-positive cells in the uteri and an increase in the localization of ^{51}Cr - labelled pooled MLN, para-aortic and peripheral lymph-node lymphocytes in uteri

(Wira et al., 1980). Similarly, the selective accumulation of MLN compared to PLN blasts in the cervix, vagina and uterus was maximal at proestrus and estrus, and 60% of these MLN-derived cells contained IgA (McDermott & Bienenstock, 1979; McDermott et al., 1980a). Most of the increased accumulation of lymphoid cells in the sex hormone target tissues was likely due to alterations in the tissue since in transfer experiments cells were derived from virgin animals unselected for the stage of estrus. However substantial numbers of IgA positive cells were present in the uteri at all stages of estrus and uterine tissue IgA levels remained elevated throughout estrus (Wira et al., 1980). Estradiol also induced transudation of IgA and IgG from blood into the uterus (Sullivan & Wira, 1983b). In addition to cell localization, the effect of estrus on IgA accumulation in the uterine lumen might also be mediated at the level of secretion. Indeed, the levels of free and IgA-bound secretory component in uterine lumen parallelled those of IgA during the estrous cycle and in estradiol-treated, ovariectomized rats. Estradiol stimulated SC synthesis by uterine tissue in vitro (Sullivan et al., 1983). No such variations in SC levels during the estrous cycle occurred in saliva or small intestinal secretions (Sullivan & Wira, 1983a). Dexamethasone diminished the estrogen effect of the accumulation of IgA in the uterine lumen, but did not alter its effect on SC levels suggesting that estrogen regulation of uterine SC may be at least partially independent of IgA (Sullivan et al., 1983). These authors suggested that in spite of the increased levels of SC, dexamethasone likely interfered with the accumulation of IgA and/or IgA

containing cells in uterine tissue, thereby decreasing the amount of IgA available for transport into the lumen.

Thus estrogen may act by a number of mechanisms which all result in increased levels of IgA in uterine lumen at proestrous and estrus and in the mammary gland during lactation. In addition to the effects of hormones on serum transudation of immunoglobulins and the availability of SC for transport of IgA into secretions, there seem to be effects on the local lymphocyte population in sex hormone target tissues which are due, at least in part, to alterations in the accumulation of IgA plasma cell precursors. Although the increased localization was greater than the increase in weight in both the breast and genital tract, the possibility that increased localization was due to increased blood flow and therefore increased delivery of blasts to the tissue, has not been ruled out. If SC is important in augmenting IgA blast accumulation (section 8.7.5) then the increased synthesis of SC stimulated by estrogen might be a factor resulting in increased accumulation of MLN blasts in these tissues at proestrus and estrus. Franklin et al., (1978) found that after hormonal stimulation, IgA positive cells predominated in mammary autografts located in the anterior chamber of the rabbit eye and that there were twice as many IgA positive cells in the uveal tract adjacent to the mammary gland compared to inflammatory sites (due to intravitreal ovalbumin), distant from the gland and in nonimplanted eyes. These authors suggested that perhaps a diffusable factor was responsible for IgA plasma cell precursor localization in and around the mammary gland. Thus hormones might in some way alter the tendency of MLN blasts (or perhaps

primarily IgA positive MLN blasts) which are delivered to the tissue to extravasate. In lymph nodes, differences in the number of lymphocytes crossing HEV and the height of endothelial cells in HEV have been observed in males and females and after hormonal alteration (Kittas & Henry, 1979a,b; see section 1.4.1).

8.6.2 Gender and gonadal hormone influences on intestinal tissue as a site for blast localization

Do sex differences or hormonal changes result in alterations in the attractiveness of the small intestine for MLN blasts? Love and Ogilvie (1977) observed a 2.5 fold increase in weight of the small intestines of lactating rats but no increase in the percent of injected radioactivity recovered after transfer of TDL blasts. Similarly, there was no difference in localization of MLN compared to PLN blasts in the small intestines of virgin, pregnant or lactating recipients (radiocounting; Roux et al., 1977). Also, using autoradiography, McDermott et al., (1980a) found no difference in the accumulation of radiolabelled MLN blasts or IgA positive radiolabelled cells in the small intestine during the estrous cycle although there was a small increase in the number of IgG-containing radiolabelled cells at estrus and diestrus. Therefore, in contrast to sex hormone target tissues, different levels of female hormones did not alter the small intestine as a site for blast localization. My observation that MLN blasts have an equal propensity to accumulate in either the male or female small intestine 24 h after adoptive transfer lends further support to this suggestion (Tables 14 & 15) and suggests that male hormones do not

alter the small intestine as a site for localization.

8.6.3 Influences of gonadal hormones and gender on localization properties of the blast

Although considerable evidence suggests that sex hormones influenced blast localization in hormone target tissues by alterations of the tissue, the hormones might have also altered the blast population destined to localize in these tissues. Similarly, although these factors did not influence the small intestine as a localization site, they might have influenced the blast population which had the ability to localize in the small intestine. Although not clearly understood there certainly are sex differences in, and gonadal steroid influences on immune phenomena and lymphoid cells (section 1.4). I therefore compared the intestinal localization of MLN blasts from male or female donors. I found that MLN blasts from male donors localized more than those from female donors in the small intestine of either sex recipient (Table 14a).

This phenomenon might have been due to hormonal differences between the male and female blast donors. I therefore performed adoptive transfers of cells from donors which had been depleted of gonadal steroids by castration for a minimum of 3 weeks. Neither oophorectomy (Table 16) or orchidectomy (Table 17) of MLN blast donors had any effect on the tendency of these cells to accumulate in the small intestine compared to sex matched normal or sham castrated donors. Unfortunately, because the size of the experiments would have been prohibitive, opposite sex controls were not included in these experiments. Therefore considerable work was done before it became

apparent that the phenomenon of greater localization of male as compared to female blasts which had been clearly demonstrated (Table 14a), seemed to have disappeared. Thus hormone levels might or might not have been involved in the initial phenomenon.

Further adoptive transfer experiments using male or female donors of MLN blasts confirmed that there was no longer any difference in their accumulation in the small intestine (Table 14b). Thus, sometime between September 1980 (last experiment showing difference) and August 1981 (first experiment showing no difference) the phenomenon disappeared.

There does not seem to be any technical explanation for the loss of this phenomenon. There was no change in the adoptive transfer technique. To eliminate any possibility in variations due to autoradiography or scoring, tissues from one positive and two negative experiments were re-sectioned, processed simultaneously for autoradiography and scored. The results from this second processing were the same as from the first, eliminating changes in autoradiographic or scoring technique as an explanation for the appearance and / or loss of the phenomenon. This re-scoring also removed the remote possibility that the observed differences were due to the sampling error inherent in the autoradiographic technique (Section 2.2).

Over the period when the phenomenon disappeared, there were no known environmental changes in the McMaster University animal quarters. At the Jackson Laboratory (supplier; Bar Harbor, Maine) there were no

specific changes such as in food or bedding nor were there any indications of any infectious diseases in any of the rooms. However between August 1980 and August 1981 the production expansion colonies were relocated from Morrell Park room #4 to room #3 and then room #2. The production colonies remained in Morell Park rooms #6 and#7. It is possible that there was some undetected change in the environment, such as the appearance or disappearance of a sub-clinical infection, perhaps associated with the change in rooms. Viral infections can induce transient lymphocytopenia, and Woodruff and Woodruff (1970, 1974) suggested that this was due to viral enzyme induced alterations in the lymphocyte surface which interfered temporarily with their ability to recirculate. If an infection was more intense in one sex than the other and if it altered blast surface characteristics important in localization in the small intestine, then the appearance or disappearance of such an infection could explain the disappearance of the phenomenon of enhanced localization of male blasts.

Alternatively, a genetic alteration in the CBA/J population might have occurred and resulted in the loss of the phenomenon. Although there is no evidence that genetic alterations occurred in the CBA/J population, there has been a report of commercially supplied BALB/c mice being heterozygous, not consistently expressing either the expected H-2 haplotype or glucose phosphate isomerase type (Kahan et al., 1982).

There is little information in the literature regarding sex and gonadal hormone effects on the lymphocyte in lymphocyte localization. Jackson et al., (1981) reported no difference in the radioactivity

recovered after localization of ^{125}I-Udr labelled MLN or PLN blasts from virgin compared to those from lactating C57Bl/6J mice in the small intestines of virgin recipients. Butcher et al., (1979) found that the sex of the lymphocyte donor had no effect on binding to HEV in vitro. Therefore, there seems to be no information which consistently suggests an effect of gender or gonadal hormones on blasts which selectively localize in the small intestine.

In conclusion, sex hormones certainly affect the local immune system of sex hormone-target tissues and some of this alteration is due to changes in the localization of MALT-derived blasts. The mechanism which results in this alteration is unknown. The effect may be entirely on the tissue and its blood supply but might also be on the localizing blast. Sex differences and gonadal steroids do not alter the small intestine as a site for blast localization. In my data there was at one time an indication that sex differences influence the ability of MLN blasts to localize in the small intestine, however this phenomenon disappeared over a period of 1 year and neither its original existence nor its disappearance have been explained. I do not know whether the phenomenon was associated with gonadal hormone differences. Experiments which altered the gonadal hormone levels in MLN blast donors did not change the localization of these cells in the small intestine but since no positive control (opposite sex donor) was included I do not know whether the phenomenon might have already disappeared at the time of these experiments.

8.7 Other Influences on Mucosal Blast Localization

8.7.1 Influences of antigen on blast localization in mucosae

Since the cells which selectively localize in the small intestine are blasts which have been stimulated by antigen in the gut, antigen would seem to be a likely candidate as a factor directing selective localization. However, although antigen may augment accumulation it does not seem to be necessary for the ontogeny of the secretory immune system or for the localization of antigen specific cells. IgA plasma cells were present in the intestines of germ-free mice (Crabbe et al., 1970) and in spite of lack of intraluminal antigen, isografts of foetal small intestine implanted under the kidney capsule or skin were populated, in the Peyer's patches, lamina propria and epithelium, by thymus dependent and thymus independent lymphocytes (Ferguson & Parrott, 1972; Milne et al., 1975). Similarly, BALT and a few IgA containing cells were observed in transplanted fetal lung (Milne et al., 1975). Furthermore, TDL blasts localized in the small intestine of both neonatal suckled and unsuckled rats (Halstead & Hall, 1972) and in implants of foetal gut (Moore & Hall, 1972). It therefore seemed likely that MALT derived blasts could selectively localize in mucosal tissue even in the absence of the antigens against which they were directed. Indeed, in the absence of enteric antigens, IgA positive MLN blasts selectively localized (compared to PLN blasts) in the lactating mammary gland, uterus, cervix and vagina. Compared to PLN blasts, MLN blasts selectively localized in the intestines of germ free rats (Griscelli et al., 1969). Similarly, MLN blasts selectively

localized, compared to PLN blasts. as well or better in grafts of foetal intestine as in normal gut (Guy-Grand et al., 1974; Parrott & Ferguson, 1974). Also of interest, the presence of ferritin in the gut did not result in the intestinal localization of PLN blasts which had been stimulated by this antigen (Guy-Grand et al., 1974). In addition, Hall et al., (1977) found that immunoblasts from intestinal lymph localized in the sterile gut of fetuses in utero. Specific antibody containing cells appeared in either immunized or in non-immunized recipients of MLN (from animals which had been orally immunized with horse ferritin), in the small intestine, lactating mammary gland, parotid gland, respiratory tract and MLN (Weisz-Carrington et al., 1979). Most of these cells contained IgA except in PLN and spleen where IgM and IgG outnumbered IgA positive cells. In contrast, after stimulation and transfer of PLN, most anti-ferritin containing cells in all locations were IgG positive. The transfer of immune cells circumvented the possibility that antigen was absorbed into the circulation and stimulated the appearance of specific antibody producing cells at sites distant from the gut (Weisz-Carrington et al., 1979). Similarly, Pierce and Gowans (1975) found that after transfer of TDL from cholera toxoid - immunized rats into normal rats there were large numbers of anti-toxin containing cells in the small intestine. The numbers were no greater at 5 to 6 h after cell transfer in recipients which simultaneously received toxoid orally. When a graft versus host reaction was produced by thymocytes from C57Bl$_6$ mice in irradiated (C57Bl$_6$ xDBA)F$_1$ recipients bearing a graft of fetal intestine of either parental genotype. both syngeneic or allogeneic grafts and

the recipient's gut showed a similar degree of T-blast accumulation (Guy-Grand et al., 1978). Thus there is conclusive evidence that selective mucosal localization is not dependent upon the presence in the mucosae of the antigens against which the blasts are directed. In addition to not being obligatory, the presence of antigen is also not sufficient to induce the localization of PLN blasts in mucosal tissues.

However, antigen might act as a second signal to augment the accumulation of MALT - derived blasts in the mucosae. Crabbé et al (1970) noted that germ-free mouse intestines were sparse with respect to IgA plasma cells but that these were augmented by placing the mice in a conventional habitat. Similarly, Milne et al., (1975) found the numbers of IgA containing cells in isografts of small intestine and the intestines of germ-free mice were only 1/3 that in the normal mouse gut. Ferguson and Parrott (1972) also noted decreased numbers of intraepithelial lymphocytes, lamina propria lymphocytes and plasma cells in grafts of small intestine compared to normally sited gut. These authors argued that the close parallels between the growth and development of grafted and normal gut justified their assumptions that differences in the lymphoid population were due to immunological processes, although they did not formally rule out differences in the vasculature or nervous connections. However, alterations in blood flow might have been an important factor in these observations. In mice fed an elemental diet, the decreased intestinal localization of MLN blasts was due not to a direct effect of diminished dietary antigen but rather to decreased blood flow (Ottaway & Parrott, 1981). Thus in all cases

where an antigen-related effect is observed, it must not be assumed to be directly immunologic and antigen specific since it might have been mediated through generalized mechanisms such as alterations in blood flow, which admittedly may have been indirectly changed by antigen specific mechanisms.

There is evidence which does suggest an antigen specific enhancement of the mucosal lymphoid population. Local immunization of a segment of human colon with polio vaccine was followed by the appearance of specific IgA entirely confined to the immunized segment (Ogra & Karzon, 1969). Husband and Lascelles (1974) found that in sheep with two intestinal loops, IgA antibody against an antigen only appeared in the immunized loop. Similarly, although Pierce and Gowans (1975) found that the localization of anti-cholera toxin specific TDL shortly after transfer was not enhanced by the presence of cholera toxoid in the gut, after intraintestinal boosting the greatest density of anti-toxin containing cells occurred at, or distal to, the boosted site and that this occurred at the expense of ACC in distant regions of the gut. In addition, Husband and Gowans (1978) observed more anti-cholera containing cells in an intestinal loop challenged with toxin than in a nonchallenged loop. Since the total number of IgA-containing cells was the same in both loops (and considerably less than in the rest of the intestine) it seems likely that this was a direct effect of antigen on the lymphoid population. In accordance with the work of Pierce and Gowans (1975), this group also noted that localization of TDL (autologous transfer) was the same in both challenged and unchallenged loops until 6 h after transfer. However, from 12 to 36 h

the number of ACC increased in the challenged loop and decreased in the nonchallenged loop. Similarly, Husband (1982) performed cross-over experiments to examine the localization of tetanus toxoid specific cells and albumin specific cells in rats with two loops, each of which contained only one of the antigens. Husband (1982) found that there was an early equal localization which peaked at 6 h and was followed by a decrease of ACC in the loop with irrelevant antigen to zero by 18 to 24 h. In contrast, the number of ACC specific for the immunizing antigen continued to increase, peaking between 24 and 36 h and then decreased. Some of this augmentation of ACC in the loop with the appropriate antigen appeared to be due to cell division in situ (Husband & Gowans, 1978; Husband, 1982). These antigen induced differences may be transient since Pierce and Cray (1982) found an increase in the number of ACC in the cholera toxin stimulated loop from 6 to 24 h but no difference at 2 days compared to mucosa not stimulated with toxin. They did not compare localization in the presence or absence of antigen prior to 2 days after transfer. However they did find an increased number of ACC at 6 days in rats given an intra-duodenal challenge of toxin and a sharp decrease in those not challenged. Even when TDL were drained from the time of challenge there was still an ACC response in the lamina propria, confined almost entirely to the site of challenge. This might have been due to local division of memory cells (Pierce & Cray, 1981, 1982) or to continued migration of primed cells from other mucosal sites such as lung (Mayrhofer & Fisher, 1979). Thus the antigen related differences in

accumulation may be due to augmented division or augmented retention rather than enhanced entry of antigen specific cells.

In conclusion, selective localization of MLN blasts is not an antigen directed phenomenon. Localization early after transfer is not augmented by the presence of relevant antigen. The expansion of the local mucosal immune system observed in the presence of antigen thus seems to be due to enhanced retention and proliferation of blasts and memory cells which localized in the mucosae under the influence of non antigen specific influences.

8.7.2 Influences of parasitic infection and inflammation on blast localization in mucosae

Parasitic infection is another factor which affects blast localization. Enhanced intestinal localization has been observed with MLN blasts in *T. spiralis* infected mice (Rose *et al.*, 1976), TDL blasts in *T. spiralis* and *N. brasiliensis* infected rats (Love & Ogilvie, 1977), TDL and MLN blasts in coccidia infected rats (Rose *et al.*, 1980) and peripheral blood blasts in *coccidia* infected chickens (Rose *et al.*, 1980). This effect was not antigen specific because it occurred whether the blasts were derived from infected or uninfected donors or donors infected with a different organism (Rose *et al.*, 1976; Love & Ogilvie, 1977; Rose *et al.*, 1980). This enhanced localization in parasitized gut was primarily due to T blasts since the localization of ^{125}I-Udr labelled MLN from T-depleted mice was not enhanced in *T. spiralis* infected mice (Rose *et al.*, 1976b). Rose *et al.* (1976b) found that the localization of PLN blasts stimulated with oxazolone or

picrylchloride and the localization of bone marrow blasts but not spleen blasts was enhanced in 4 day T. spiralis infected mice compared to normal recipients. MLN blasts still localized in parasitized gut in greater numbers than PLN blasts. These authors suggested that an explanation for these observations was the nonspecific attraction of these inflammatory cells to the parasite-induced inflammation in the gut. In addition, the enhanced localization of MLN blasts, MLN T-blasts and oxazolone stimulated PLN blasts was most apparent in the region of the small intestine in which the worms were located, and changed as the worms moved down the gut (Manson-Smith et al., 1979). Rose et al., (1976) noted that the increased weight of the gut at 4 days post infection was not sufficient to account for the differences in recovered radioactivity. Furthermore, Ottaway et al., (1980) found that more MLN blasts accumulated in the small intestines of T. spiralis infected mice at stages in the infection when there was not yet any alteration in the proportion of the cardiac output received by the small intestine. These authors performed kinetics experiments which suggested that the enhanced localization in parasitized small intestine was due to both facilitated entry and retention of blasts.

8.7.3 Influences of nutrition on blast localization in mucosae

Both the incidence and the severity of infections are increased in the protein-calorie malnurished and the vitamin A deficient (Scrimshaw et al., 1968; Chandra & Newberne, 1977). This may be due in part to defective localization of mucosal blasts., McDermott et al., (1982) found a decrease to two-thirds of normal localization when MLN

blasts were obtained from protein-calorie malnourished rats. When MLN blasts were obtained from protein-calorie and vitamin-A deficient rats the intestinal localization was even less, being only one-third of normal. As with my observations of sex related differences (section 8.6), this phenomenon was associated with differences in the blast, not the small intestine since MLN blasts from normal animals localized equally in protein-calorie malnourished or combined protein and vitamin-A deficient recipients.

8.7.4 Importance of immunoglobulin isotype in blast localization in mucosae

Since IgA producing cells predominate in the mucosae, perhaps this immunoglobulin is involved in selective localization. Guy-Grand et al., (1974) argued that IgA was not directly responsible since IgA positive cells constitute a larger proportion of Peyer's patch blasts than MLN blasts, but the former only localized one eigth as much as the latter in the gut at 20 h post-transfer. In addition, since MLN T blasts selectively localized in the gut, selective localization is not likely due to the different classes of immunoglobulin which predominate in MLN compared to PLN (Rose et al., 1978). In addition, blasts producing IgG accumulate in greater numbers in the small intestine after MLN than after PLN transfer (McDermott & Bienenstock, 1979). However, whether this represented selective localization of MLN IgG blasts or simply accumulation cannot be determined since the number of IgG positive blasts in the MLN compared to PLN innocula was not

determined. In spite of the evidence which suggests that IgA is not responsible for selective MLN blast localization in general, different types of blasts might respond to different localization stimuli and surface IgA molecules might be involved in the localization of IgA-producing blasts. There is no evidence to support this hypothesis since free IgA *in vivo* did not interfere with the localization of MLN blasts in the small intestine.

8.7.5 Importance of secretory component in blast localization in mucosae

Since secretory component is an epithelial cell product (Poger & Lamm, 1974) associated with the transport of dimeric IgA and polymeric IgM into secretions (Brandtzaeg, 1982) it might be a factor in the localization of blasts displaying surface IgA or IgM. The lack of IgA plasma cells in the intestinal lamina propria, in spite of circulating IgA, in a patient with absolute SC deficiency seems to support this hypothesis (Strober *et al.*, 1976). However, IgA and IgM are expressed as monomers on the cell surface (Sigal & Klinman, 1978) and the monomers of these immunoglobulins had no affinity for SC (Weicker & Underdown, 1975). Indeed, SC did not bind to the surface of living TDL *in vitro* despite its avid binding to IgA-containing TDL cells in fixed smears (Grosse, 1978). In addition, neither free IgA nor anti-SC serum interfered with the localization of MLN blasts (McWilliams *et al.*, 1975). Thus there is little support for the hypothesis that SC might be a receptor in mucosae for localizing blasts.

8.7.6 Histocompatibility and blast localization in mucosae

Since histocompatibility antigens are involved in lymphocyte interactions and tissue recognition, perhaps they are involved in the blast-endothelial interactions necessary for extravasation in the mucosae. However, Moore and Hall found that ^{125}I-Udr labelled prefemoral node blasts from sheep extravasated in neonatal rat small intestine. Thus whatever the exact nature of the putative complementary receptors on immunoblasts and endothelial cells, they act across species barriers, and therefore there is no evidence that histocompatability antigens are involved in selective mucosal localization. However, Curtis and Renshaw (1982) showed that allogenicity in histocompatibility antigens can lead to increased lymphocyte adhesion to pulmonary endothelia *in vitro* and suggest that histocompatability antigens might influence lymphocyte traffic by mediating their adherance to vascular endothelia.

8.8 Summary

Several technical considerations have been stressed in this thesis and it is hoped that this will be helpful in the design of further studies on selective blast localization.

The different information obtained by using either autoradiography or radiocounting to assess adoptive transfer experiments argues for the use of both of these techniques. For example, radiocounting provides data in terms of the recovered radioactivity, either for an entire organ or per unit weight of organ, as a percentage of that injected (an internal control for the dose of blasts transferred) or as a percentage of the total recovered in the body or in a particular organ or set of organs. The radioactivity is assumed to be cell - associated and uniformly distributed between all cells. These conditions are not always met. In contrast autoradiography provides data in terms of the number of radiolabelled cells (including any produced by division after transfer) per unit volume of tissue and allows the morphologic location of the cells to be described. However, as demonstrated in this thesis, autoradiographic experiments must be carefully controlled for the number of labelled cells transferred and the reproducibility of replicate determinations on the same samples should be stated since this can be quite high, particularly when the concentration of cells in the tissue is low. Some of these differences between autoradiography and radiocounting may produce the unexplained smaller differences observed in selective localization when radiocounting rather than autoradiography is used to evaluate localization.

I have shown, by transferring different doses of blasts, that a certain proportion of those transferred will localize in each tissue. This proportion is a characteristic both of the blast population and the recipient tissue. This is an important consideration in experimental design since I found that contrary to earlier work in which the dose of blasts transferred was not controlled, MLN compared to PLN blasts did not selectively localize in pulmonary parenchyma. Both my dose studies and the studies of other investigators on the effect of blood flow on blast localization, show that delivery of cells to a tissue is a factor which directly influences the number which will enter that tissue. This has implications in prophylaxis or therapy since it shows that increased accumulations of immune cells can be expected if immune MALT - derived blasts are delivered to a mucosal tissue in greater numbers. either by direct stimulation of MALT, by transfer of blasts, or by increasing blood flow to a particular organ.

The lungs have often been considered as one tissue in localization studies (of necessity in radiocounting experiments). However, the greatest volume of tissue is parenchymal and localization in this region may reflect both non - specific trapping in capillaries and its non mucosal nature. In contrast BALT and bronchial epithelium and lamina propria comprise a small proportion of the total organ and in the past no attempt was made to record the area of these regions when documenting the appearance of blasts after adoptive transfer. I found that in the mouse, BALT was only present in 3 of 15 recipients (in one experiment) and that the area of this region available for scoring was only 1.5% of that normally sampled in autoradiographs of

small intestine. Similarly, the area of bronchial epithelium was only 0.5% to 4.3% of the area normally sampled per animal. Therefore I was unable to suggest whether MLN blasts compared to PLN blasts selectively localize in pulmonary mucosae. I suggest that the question of mucosal localization in the lung be addressed in a larger animal than the mouse, such as the rabbit in which BALT and bronchus can be discected and assessed separately from parenchymal tissue, both by radiocounting and autoradiography.

The importance of characterizing the population of blasts under study cannot be overly stressed. The literature is complicated by the use of the same terminology (MLN, PLN, TDL) to describe populations of cells which undoubtedly differ in composition because of the numerous antigenic stimuli and routes of immunization which have been used to enhance the proportion of blasts in these populations. Populations from one organ source which were not specifically stimulated have been compared to specifically stimulated populations from another organ source, without attempts to control for the different proportions of various subpopulations. The trend to more fully characterize the population being studied has begun and must continue.

Many questions can be addressed by following these subpopulations individually. We know that MALT derived blasts which produce antibody isotypes other than IgA localize in mucosae, but do they localize selectively when compared to a population which contains the same number of blasts of the same isotype stimulated in PLN by the same antigen? Similarly, T blasts from MALT selectively localize in

mucosae. But do subpopulations of T blasts have different migratory characteristics? The route of immunization influences the population of specific T helper and T suppressor cells in mucosal or peripheral tissues. Is this mediated by selective localization?

In the female genital tract and mammary gland the mucosal immune system is influenced and the localization of MALT - derived cells is enhanced by female sex hormones. However the small intestine is not altered as a localization site for these cells by pregnancy or at the various stages of estrous. Similarly, I have shown that the small intestines from male or female animals are equally attractive for MLN blasts from donors of either sex. In contrast with the lack of effect on the tissue in which these blasts localize, I detected a two to three fold greater localization of MLN blasts from male compared to female donors in the intestines of either sex recipient. This phenomenon dissappeared between September 1980 and August 1981 and neither its initial existance nor its subsequent loss have been explained. The gonadal hormone environment in which the blasts developed did not influence their localization in the small intestine 24 h after transfer. I cannot comment on the relevance of this observation to the phenomenon of increased localization of male blasts since this phenomenon may have already dissappeared when the experiments involving hormonally altered donors were performed.

My autoradiographic and radiocounting studies and the radiocounting studies of other investigators showed a rapid accumulation in ~~and~~ subsequent loss of blasts from the lungs. One likely explanation for these observations would be the temporary

trapping of transferred cells in the pulmonary capillaries.

Both my autoradiographic studies and the radiocounting studies of others demonstrate selective localization of MLN compared to PLN blasts in the small intestine by 30 min after transfer. This information, combined with data from others which show no large accumulation of PLN blasts in the lymph draining the intestine. suggest that selective localization is initially determined at the time of extravasation. Perhaps complementary receptors are present on vascular endothelial cells in the mucosae and on mucosally derived blasts. Since such receptors seem to be involved in the traffic of lymphocytes into lymph nodes, perhaps experimental approaches similar to those used to investigate lymphocyte traffic would be informative with respect to the mechanism of selective blast localization. What effect would trypsin treatment have on blast localization in mucosae? Perhaps an <u>in vitro</u> assay for blast adherence to mucosal vascular endothelium could be developed.

Investigations by others using surgical procedures suggested that the PP was not likely an important site for blast entry into intestinal lamina propria. In support of this hypothesis, I have found that the concentration of MLN blasts was the same in the lamina propria immediately adjacent to and distant from a PP at both 30 min and 24 h after transfer. In addition I found that the distribution of MLN blasts was the same in the basal and villus lamina propria at both 30 min and 24 h. I suggest that blasts likely extravasate in the lamina propria and may remain close to the site of extravasation. Dr.

McDermott in our laboratory has shown that T and B blasts are present in different proportions in the villus and basal lamina propria at 24 h after transfer. It would be interesting to examine their distribution soon after transfer. This would suggest whether the distribution of T and B blasts was determined at extravasation (presumably by specific receptors) or later (perhaps by selective retention or selective proliferation). Indeed, although specific antigen is neither necessary nor sufficient to cause selective localization, Husband (1982) has found that anti-toxin producing cells were retained longer in and were distributed preferentially to the basal lamina propria in an intestinal loop containing the specific antigen. In contrast cells directed against an irrelevant antigen were distributed more uniformly between the basal lamina propria and the villus and remained in the loop for less time.

To conclude, blasts are endowed by virtue of the site in which they encounter antigen, with the ability to extravasate and accumulate in particular tissues. I predict that we will find this process to be directed primarily by specific receptors in the vasculature. However there will not likely be a simple relationship between the site of generation and the site of localization of a complement of cells stimulated by contact with a particular antigen. Certainly precursors for antibody producing cells, memory cells, helper and suppressor T cells would be expected to be directed to different locations. Investigations of specific subpopulations of blasts should lead to a better understanding of selective mucosal localization and of immune responses generated by exposure to antigens at mucosal surfaces.

References

Abe, K., and T. Ito. 1977. A qualitative and quantitative morphologic study of Peyer's patches of the mouse. Arch. histol. jap. 40: 407-420.

Anderson, N.D., A.O. Anderson, and R.G. Wyllie. 1976. Specialized structure and metabolic activities of high endothelial venules in rat lymphatic tissues. Immunology 31: 455-473.

Asofsky, R., and P.A. Small. 1967. Colostral immunoglobulin-A: Synthesis *in vitro* of T-chain by rabbit mammary gland. Science 158: 932-933.

Befus, A.D., M. McDermott, S. Mirski, and J. Bienenstock. 1980. Amplification mechanisms in mucosal immunity. In: 81st Ross Conference Pediatric Research. Feb. 10-13, pp. 44-58.

Befus, A.D., M. O'Neill, and J. Bienenstock. 1978. Immediate IgA precursor cells in rabbit intestinal lamina propria. Immunol. 35, 901-906.

Besredka, A. 1919. Ann de l'Inst. Pasteur xxxiii May, 301; Aug 557; Dec 882; Brit. Med. Jour. 1920 ii 325, 402, 404. cited in Davies, A. 1922. Lancet 2: 1009-1012.

Bienenstock, J. 1974. The physiology of the local immune response and the gastrointestinal tract. Prog. Immunol. II, 4: 197-207.

Bienenstock, J., D. Befus, M. McDermott, S. Mirski, and K. Rosenthal. 1983. Regulation of lymphoblast traffic and localization in

mucosal tissues, with particular emphasis on IgA. Fed. Proc., in press.

Bienenstock, J., and J. Dolezel. 1971. Peyer's patches: lack of specific antibody - containing cells after oral and parenteral immunization. J. Immunol. 106: 938-945.

Bienenstock, J., N. Johnston, and D.Y.E. Perey. 1973a. Bronchial lymphoid tissue. I. Morphologic characteristics. Lab. Invest. 28: 686-692.

Bienenstock, J., N. Johnston, and D.Y.E. Perey. 1973b. Bronchial lymphoid tissue. II. Functional characteristics. Lab. Invest. 28: 693-698.

Blau, J.N. 1977. A comparative study of the microcirculation in the guinea-pig thymus, lymph nodes and Peyer's patches.

Bond, J.H., and M.D. Levitt. 1979. Use of microspheres to measure small intestinal villus blood flow in the dog. Am. J. Physiol. 236: E577-E583.

Brandtzaeg, P. 1982. Review and discussion of IgA Transport across mucosal membranes. In. Recent Advances in Mucosal Immunity. Eds. Strober, W., L.A. Hanson, and K.W. Sell. Raven Press, N.Y. pp 267-285.

Brown, B.W., and M. Hollander. 1977. Statistics. A Biomedical Introduction. John Wiley & Sons. Toronto. pp150-158.

Brown, W.R. 1982. Ultrastructural studies on the translocation of polymeric immunoglobulins by intestinal epithelium and liver. In Recent Advances in Mucosal Immunity. Eds. W. Strober, L.A. Hanson, and K.W. Sell. Raven Press. N.Y. pp 251-266.

Bull, D.M., J. Bienenstock, and T.B. Tomasi. 1971. Studies on human intestinal immunoglobulin A. Gastroenterology 60: 370-380.

Burrows, W., M.E. Elliot, and I. Havens. 1947. Studies on immunity to asiatic cholera. J. Infect. Dis. 81: 261-281.

Butcher, E.C., R.G. Scollay, and I.L. Weissman. 1979. Lymphocyte adherence to high endothelial venules: characterization of a modified in vitro assay and examination of the binding of syngeneic and allogeneic lymphocyte populations. J. Immunol. 123: 1996-2003.

Butcher, E.C., R.G. Scollay, and I.L. Weissman. 1980. Organ specificity of lymphocyte migration: mediation by highly selective lymphocyte interaction with organ-specific determinents on high endothelial venules. Eur. J. Immunol. 10: 556-561.

Butcher, E.C., S.K. Stevens, R.A. Reichert, R.G. Scollay and I.L. Weisman. 1982. Lymphocyte - Endothelial cell recognition in lymphocyte migration and the segregation of mucosal and non-mucosal immunity. In Recent Advances in Mucosal Immunity. Eds. W. Strober, L.A. Hanson, and K.W. Sell. Raven Press., N.Y. pp 3-24.

Cahill, R.N.P., H. Frost, and Z. Trnka. 1976. The effects of antigen on the migration of recirculating lymphocytes through single lymph nodes. J. Exp. Med. 143: 871-888.

Cahill, R.N.P., D.C. Poskitt, H. Frost, and Z. Trnka. 1977. Two distinct pools of recirculating T lymphocytes: Migratory

characteristics of nodal and intestinal T lymphocytes. J. Exp. Med. 145: 420-428.

Cebra, J.J., E.R. Cebra, E.R. Clough, J.A. Fuhrman, J.L. Komisar, P.A. Schweitzer, and R.D. Shahin. 1983. IgA commitment: models for B-cell differentiation and possible roles for T-cells in regulating B-cell development. Ann. N.Y. Acad. Sci. 409: 25-38.

Chai, C.K. 1956. Comparison of two inbred strains of mice and their F_1 hybrids in response to androgen. Anat. Rec. 126: 269-282.

Chandra, R.K., and P.M. Newberne. 1977. In Nutrition, Immunity and Infection. Mechanisms of Interactions. Plenum Press, N.Y.

Chin, Y.-H., G.D. Carey, and J.J. Woodruff. 1980a. Lymphocyte recognition of lymph node high endothelium. I. Inhibition of in vitro binding by a component of thoracic duct lymph. J. Immunol. 125, 1764-1769.

Chin, Y.-H., G.D. Carey and J.J. Woodruff. 1980b. Lymphocyte recognition of lymph node high endothelium. II Characterization of an in vitro inhibitory factor by antibody affinity chromatography. J. Immunol. 125, 1770-1774.

Chin, Y.-H., G.D. Carey, and J.J. Woodruff. 1982. Lymphocyte recognition of lymph node high endothelium. IV. Cell surface structures mediating entry into lymph nodes. J. Immunol. 129: 1911-1915.

Chin, W., and J.B. Hay. 1980. A comparison of lymphocyte migration through intestinal lymph nodes, subcutaneous lymph nodes, and chronic inflammatory sites of sheep.

Gastroenterology 79: 1231-1242.

Chipperfield, E.J., and B.A. Evans. 1975. Effect of local infection and oral contraception on immunoglobulin levels in cervical mucus. 1975. Infect. Immun. 11: 215-221.

Cho, Y. and P.P.H. De Bruyn. 1979. The endothelial structure of the postcapillary venules of lymph node and the passage of lymphocytes across the venule wall. J. Ultrastructrue Research 69: 13-21.

Chodirker, W.B., and T.B. Tomasi. 1963. Gamma-globulin: Quantitative relationships in human serum and nonvascular fluids. Science 142: 1080-1081.

Cohn, D.A. 1979. High sensitivity to androgens as a contributing factor in sex differences in the immune response. Arthritis Rheumatism 22: 1218-1233.

Crabbé, P.A., A.O. Carbonara, J.F. Heremans. 1965. The normal human intestinal mucosa as a major source of plasma cells containing A-Immunoglobulin. Lab. Invest. 14: 235-248.

Crabbé, P.A., and J.F. Heremans. 1966. The distribution of immunoglobulin - containing cells along the human gastrointestinal tract. Gastroenterology 51: 305-316.

Crabbe, P.A., D.R. Nash, H. Bazin, H. Eyssen, and J.F. Heremans. 1970. Immunohistochemical observations on lymphoid tissues from conventional and germ-free mice. Lab. Invest, 22: 448-457.

Crabbe, P.A., H. Bazin, H. Eyssen, and J.F. Heremans. 1969. The

normal microbial flora as a major stimulus for proliferation of plasma cells synthesizing IgA in the gut. Int. Arch. Allergy 34: 362-375.

Craig, S.W., and J.J. Cebra. 1971. Peyer's patches: and enriched source of precursors for IgA - producing immunocytes in the rabbit. J. Exp. Med. 134: 188-200.

Craig, S.W., and J.J. Cebra. 1975. Rabbit Peyer's patches, appendix, and popliteal lymph node B lymphocytes: a comparative analysis of their membrane immunoglobulin components and plasma cell precursor potential. J. Immunol. 114: 492-502.

Curtis, A.S.G., and R.M. Renshaw. 1982. Lymphocyte - endothelial interactions and histocompatibility restriction. Adv. Exp. Med. Biol. 149: 193-198.

Darlington, D., and A.W. Rogers. 1966. Epithelial lymphocytes in the small intestine of the mouse. J. Anat. 100: 813-830.

Davies, A. 1922. Serological properties of dysentary stools. Lancet 2: 1009-1012.

de Sousa, M. 1981. Lymphocyte Circulation, Experimental and Clinical Aspects. John Wiley & Sons., New York.

Draper, N.R. & Smith, H. 1966. Applied Regression Analysis. John Wiley & Sons., New York.

Drayson, M.T., M.E. Smith, and W.L. Ford. 1981. The sequence of changes in blood flow and lymphocyte influx to stimulated rat lymph nodes. Immunol. 44: 125-133.

Durie, B.G.M., and S.E. Salmon. 1975. High speed scintillation autoradiography. Science 190: 1093-1095.

Durkin, H.G., L. Caporale, and G.J. Thorbecke. 1975. Migratory patterns of B lymphocytes. I. Fate of cells from central and peripheral lymphoid organs in the rabbit and its selective alteration by anti-immunoglobulin. Cell. Immunol. 16: 285-300.

Emeson, E.E., and D.R. Thursh. 1974. Antigen - induced trapping of immunospecific lymphocytes in the spleen. J. Immunol. 113: 1575-1582.

Faulk, W.P., J.N. McCormick, J.R. Goodman, J.M. Yoffey, and H.H. Fundenberg. 1971. Peyer's patches: Morphologic studies. Cell. Immunol. 1: 500-520.

Fazekas de St. Groth, S., and M. Donnelly. 1950. Studies in experimental immunology of influenza. IV. The protective value of active immunization. Aust. J. Exp. Biol. Med. Sci. 28: 61-75.

Ferguson, A., and D.M.V. Parrott. 1972. The effect of antigen deprivation on thymus-dependent and thymus-independent lymphocytes in the small intestine of the mouse. Clin. exp. Immunol. 12: 477-488.

Ford, W.L. 1969. The kinetics of lymphocyte recirculation within the rat spleen. Cell. Tiss. Kinet. 2: 171-191.

Ford, W.L., M.E. Smith, and P. Andrews. 1978. Possible clues to the mechanism underlying the selective migration of lymphocytes from the blood. Symp. Soc. Exp. Biol. 32: 359-392.

Ford, W.L., M. Sedgley, S.M. Sparshott, and M.E. Smith. 1976. The migration of lymphocytes across specialized vascular

endothelium. II. The contrasting consequences of treating lymphocytes with trypsin on neuraminidase. Cell. Tiss. Kinet. 9: 351-361.

Franklin, R.M., R.A. Prendergast, and A.M. Silverstein. 1978. Nonspecific signals for B-cell localization and activation. J. Exp. Med. 146: 1705-1710.

Freitas, A.A., and M. de Sousa. 1976. The role of cell interactions in the control of lymphocyte traffic. Cell. Immunol. 22: 345-350.

Freitas, A.A., M. Rose, and B. Rocha. 1980. Random recirculation of small T lymphocytes from thoracic duct lymph in the mouse. Cell. Immunol. 56: 29-39.

Frost, H., D.G. Braun, D. Poskitt, R.N.P. Cahill, and Z. Trnka. Antipolysaccharide antibodies of restricted heterogeniety secreted by a single lymph node. 1976. J. Exp. Med. 143: 707-711.

Frost, H., R.N.P. Cahill, and Z. Trnka. 1975. The migration of recirculating autologous and allogeneic lymphocytes through single lymph nodes. Eur. J. Immunol. 5: 839-843.

Ganguly, R., and R.H. Waldman. 1980. Local immunity and local immune responses. Prog. Allergy 27: 1-68.

Geiger, S.R. (executive editor). 1973. Handbook of Physiology. Section 7. Endocrinology. Volume II. Female Reproductive System. American Physiological Society, Washington. Part 1, pp13; Part 2 pp 234.

Gesner, B.M., and V. Ginsburg. 1964. Effect of glycosidases on the

fate of transfused lymphocytes. Proc. Natl. Acad. Sci. 52: 750-755.

Gillette, S., and R. W. Gillette. 1979. Changes in thymic estrogen receptor expression following orchidectomy. 1979. Cell. Immunol. 42: 194-196.

Goldblum, R.M., S. Ahlstedt, B. Carlsson, L.A. Hanson, U. Jodal, G. Lidin-Janson, and A. Sohl-Akerlund. 1975. Antibody-forming cells in human colostrum after oral immunization. Nature 257: 797-799.

Goldschneider, I. and D.D. McGregor. 1968. Migration of lymphocytes and thymocytes in the rat. I. The route of migration from blood to spleen and lymph nodes. J. Exp. Med. 127: 155-168.

Gowans, J.L. 1959. The recirculation of lymphocytes from blood to lymph in the rat. J. Physiol. 146: 54-69.

Gowans, J.L., and E.J. Knight. 1964. The route of recirculation of lymphocytes in the rat. Proc. R. Soc. Lond. B. Biol. Sci. 159: 257-282.

Griscelli, C., P. Vassalli, and R.T. McCluskey. 1969. The distribution of large dividing lymph node cells in syngeneic rats after intravenous injection. J. Exp. Med. 130: 1427-1442.

Grosse, H.J. unpublished results reported by Husband, A.J., and J.L. Gowans. 1978. J. Exp. Med. 148: 1146-1160.

Guy-Grand, D., C. Griscelli, and P. Vassalli. 1974. The gut-associated lymphoid system: nature and properties of the

large dividing cells. Eur. J. Immunol. 4: 435-443.

Guy-Grand, D., C. Griscelli, and P. Vassalli. 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: 1661-1677.

Hall, B.M., S. Dorsh and B. Roser. 1978. The cellular basis of allograft rejection in vivo. I. The cellular requirements for first-set rejection of heart grafts. J. Exp. Med. 148, 878-889.

Hall, J.G. 1967. Quantitative aspects of the recirculation of lymphocytes: an analysis of data from experiments on sheep. Quart. J. exp. Physiol. 52: 76-85.

Hall, J.G., and M.E. Smith. 1970. Homing of lymph-borne immunoblasts to the gut. Nature 226: 262-263.

Hall, J.G., D.M. Parry, and M.E. Smith. 1972. The distribution and differentiation of lymph-borne immunoblasts after intravenous injection into syngeneic recipients. Cell. Tissue Kinet. 5: 269-281.

Hall, J.G., J. Hopkins, and E. Orlans. 1977. Studies on the lymphocytes of sheep. III. Destination of lymph-borne immunoblasts in relation to their tissue of origin. Eur. J. Immunol. 7: 30-37.

Halsey, J.F., B.H. Johnson, and J.J. Cebra. 1980. Transport of immunoglobulins from serum into colostrum. J. Exp. Med. 151: 767-772.

Halstead, T.E., and J.G. Hall. 1972. The homing of lymph-borne

immunoblasts to the small gut of neonatal rats. Transplantation 14: 339-346.

Hanson, L.A., S. Ahlstedt, B. Andersson, B. Carlsson, U. Dahlgren, G. Lidin-Janson, I. Mattsby-Baltzer, and C. Svanborg-Eden. 1980. The biologic properties of secretory IgA. J. Reticuloendo. Soc. 28 (Suppl.) 1s-9s.

Hay, J.B., and B.B. Hobbs. 1977. The flow of blood to lymph nodes and its relation to lymphocyte traffic and the immune response. J. Exp. Med. 145: 31-44.

Henney, C.S., and R.H. Waldman. 1970. Cell-mediated immunity shown by lymphocytes from the respiratory tract. Science 169: 696-697.

Henry, L. and J.K.A. Beverley. 1976. Age and sex differences in response of lymph node post-capillary venules in mice infected with Toxoplasma gondii. Br. J. exp. Path. 57: 274-280.

Herman, P.G., R. Utsunomiya, and S.J. Hessel. 1979. Arteriovenous shunting in the lymph node before and after antigenic stimulus. Immunol. 36: 793-797.

Hochwald, G.M., E.B. Jacobson, and G.J. Thorbecke. 1964. ^{14}C-amino acid incorporation into transferrin and β 2A-globulin by ectodermal glands in vitro. Fed. Proc. 23: 557.

Hopkins, J., and J.G. Hall. 1976. Selective entry of immunoblasts into gut from intestinal lymph. Nature 259: 308-309.

Hopkins, J., I. McConnell, and J.D. Pearson. 1981. Lymphocyte

traffic through antigen - stimulated lymph nodes. II. Role of prostaglandin E_2 as a mediator of cell shutdown. Immunol. 42: 225-231.

Howard, J.C. 1972. The life - span and recirculation of marrow - derived small lymphocytes from the rat thoracic duct. J. Exp. Med. 135: 185-199.

Hulka, J.F., and K.F. Omran. 1969. The uterine cervix as a potential local antibody secretor. Am. J. Obstet. Gynecol. 104: 440-442.

Hurliman, J., and H. Darling. 1971. In vitro synthesis of immunoglobulin-A by salivary glands from animals of differing species. Immunol. 21: 101-111.

Husband, A. 1982. Kinetics of extravasation and redistribution of IgA - specific antibody - containing cells in the intestine. J. Immunol. 128: 1355-1359.

Husband, A.J. and A.K. Lascelles. 1974. The origin of antibody in intestinal secretion of sheep. Aust. J. Exp. Bio. Med. Sci. 52: 791-799.

Husband, A.J., and J.L. Gowans. 1978. The origin and antigen - dependent distribution of IgA - containing cells in the intestine. J. Exp. Med. 148: 1146-1160.

Issekuz, T.B., G.W. Chin, and J.B. Hay. 1981. Lymphocyte traffic through chronic inflammatory lesions: differential migration versus differential retention. Clin. exp. Immunol. 45: 604-614.

Issekutz, T.B., W. Chin, and J.B. Hay. 1982. The characterization

of lymphocytes migrating through chronically inflamed tissues. Immunol. 46: 59-66.

Jacobsson, H., and H. Blomgran. 1972. Studies on the recirculating cells in the mouse thymus. Cell. Immunol. 5: 107-121.

Jackson, D.E., E.T. Lally, M.C. Nakamura, and P.C. Montgomery. 1981. Migration of IgA - bearing lymphocytes into salivary glands. Cell. Immunol. 63: 203-209.

Jarrett, W.F.H., E.E.E. Jarrett, H.R.P. Miller, and G.M. Urquhart. 1968. Quantitative studies on the mechanism of self cure in Nippostrongylus brasiliensis infections. In Reaction of the Host to Parasitism. Edited by E.J.L. Soulsby Elwert, Marberg. Lahn. pp. 191-198.

Jones, P.P., and J.J. Cebra. 1974. Restriction of gene expression in B lymphcytes and their progeny. III. Endogenous IgA and IgM on membranes of different plasma cell precursors. J. Exp. Med. 140: 966-976.

Jones, P.P., S.W. Craig, J.J. Cebra, and L.A. Herzenberg. 1974. Restriction of gene expression in B lymphocytes and their progeny. II. Commitment to immunoglobulin heavy chain isotype. J. Exp. Med. 140: 452-469.

Kagnoff, M.F., and S. Campbell. 1974. Functional characteristics of Peyer's patch lymphoid cells. J. Exp. Med. 139: 398-406.

Kagnoff, M.F., D. Serfilippi, and R.M. Donaldson. 1973. In vitro kinetics of intestinal secretory IgA secretion. J. Immunol. 110: 297-300.

Kahan, B., R. Auerbach, B.J. Alter and F.H. Bach. 1982. Histocompatibility and isoenzyme differences in commercially supplied "BALB/c" mice. Science 217: 379-381.

Kalland, T., and J.-G. Forsberg. 1980. Occurrence of estrogen binding components in mouse thymus. A study using fluorescence technique. Immunol. Lett. 1: 293-297.

Kawanishi, H., L.E. Saltzman, and W. Strober. 1982. Characteristics and regulatory function of murine Con A - induced, cloned T cells obtained from Peyer's patches and spleen: mechanisms regulating isotype - specific immunoglobulin production by Peyer's patch B cells. J. Immunol. 129: 475-483.

Kenny, J.F., and M. Diamond. 1977. Immunological responsiveness to Escherichia coli during pregnancy. Infect. Immun. 16: 174-180.

Kenny, J.F., P.C. Pangburn, and G. Trail. 1976. Effect of estradiol on immune competence: in vivo and in vitro studies. Infect. Immun. 13: 448-456.

Keren, D.F., P.S. Holt, H.H. Collins, P. Gemski, and S.M. Formal. 1978. The role of Peyer's patches in the local immune response of rabbit ileum to live bacteria. J. Immunol. 120: 1892-1896.

Kerr, W.R. 1955. Cited by Tomasi, T.B., and J. Bienenstock. 1968. Secretory Immunoglobulins. Adv. Immunol. 9: 1-96.

Kittas, C., and L. Henry. 1979. An electron microscopic study of the changes induced by oestrogens on the lymph-node post-capillary venules. J. Path. 129: 21-29.

Klein, J.P., M. Scholler, and R.M. Frank. 1977. Inhibition of

glucosyl transferase by human salivary immunoglobulin A. Infect. Immun. 15: 329-331.

Koster, F.J., and N.F. Pierce. 1983. Parenteral immunization causes antigen - specific cell - mediated suppression of an intestinal IgA response. J. Immunol. 131: 115-119.

Krzych, U., H.R. Strausser, J.P. Bressler, and A.L. Goldstein. 1978. Quantitative differences in immune responses during the various stages of the estrous cycle in female BALB/c mice. J. Immunol. 121: 1603-1605.

Kuttner, B.J. and J.J. Woodruff. 1979. Adherence of recirculating T and B lymphocytes to high endothelium of lymph nodes in vitro. J. Immunol. 123: 1421-1422.

Lance, E.M., and S. Cooper. 1972. Homing of specifically sensitized lymphocytes to allografts of skin. Cell. Immunol. 5: 66-77.

Lawton, A.R., R. Asofsky, and R.G. Mage. 1970. Synthesis of secretory IgA in the rabbit. II. Production of alpha, light and T chains by in vitro cultures of mammary tissue. J. Immunol. 104: 388-396.

Levitt, D.G., B. Sircar, N. Lifson, and E.J. Lender. 1979. Model for mucosal circulation of rabbit small intestine. Am. J. Physiol. 237: E373-E382.

Lifson, N., B. Sircar, D.G. Levitt, and E.J. Lender. 1979. Heterogeneity of macroscopic and single villus blood flow in the rabbit small intestine. Microvascular Res. 17: 198-180.

Love, R.J., and B.M. Ogilvie. 1977. Nippostrongylus brasiliensis

and Trichinella spiralis: localization of lymphoblasts in the small intestine of parasitized rats. Exp. Parasit. 41: 124-132.

Lyscom, N., and M.J. Brueton. 1982. Intraepithelial, lamina propria and Peyer's patch lymphocytes of the rat small intestine: isolation and characterization in terms of immunoglobulin markers and receptors for monoclonal antibodies. Immunol. 45: 775-783.

Manning, L.S., and M.J. Parmely. 1980. Cellular determinants of mammary cell - mediated immunity in the rat. I. The migration of radioisotopically labelled T lymphocytes. J. Immunol. 125: 2508-2514.

Manson - Smith, D.F., R.G. Bruce, M.L. Rose, and D.M.V. Parrott. 1979. Migration of lymphoblasts to the small intestine. III. Strain differences and relationship to distribution and duration of Trichinella spiralis infection. Clin. exp. Immunol. 38: 475-482.

Mathur, S., R.S. Mathur, J.M. Goust, H.O. Williamson and H.H. Fudenberg. 1979. Cyclic variations in white cell subpopulations in the human menstrual cycle: correlations with progesterone and estradiol. Clin. Immunol. Immunopath. 13: 246-253.

Mattingly, J.A., and B.H. Waksman. 1978. Immunologic suppression after oral administration of antigen. I. Specific suppressor cells formed in rat Peyer's patches after oral administration

of sheep erythrocytes and their systemic migration. J. Immunol. 121: 1878-1883.

Mayrhofer, G. 1980. Thymus - dependent and thymus - independent subpopulations of intestinal intra-epithelial lymphocytes: a granular subpopulation of probable bone marrow origin and relationship to mucosal mast cells. Blood 55: 532-535.

Mayrhofer, G., and R. Fisher. 1979. IgA-containing plasma cells in the lamina propria of the gut: failure of a thoracic duct fistula to deplete the numbers in rat small intestine. Eur. J. Immunol. 9: 85-91.

Mayrhofer, G., and R.J. Whately. 1983. Granular intraepithelial lymphocytes of the rat small intestine. I. Isolation, presence in T lymphocyte-deficient rats and bone marrow origin. Int. Archs. Allergy appl. Immun. 71: 317-327.

Michalek, S.M., J.R. McGhee, J. Mestecky, R.R. Arnold and L. Bozzo. 1976. Ingestion of Streptococcus mutans induces secretory immunoglobulin A and caries immunity. Science 192: 1238-1240.

Miller, J.J. 1969. Studies of the phylogeny and ontogeny of the specialized lymphatic tissue venules. Lab. Invest. 21: 484-490.

Miller, D.S., M.A. Rahmann, R. Tanner, V.I. Mathan and S.J. Baker. 1969. The vascular architecture of the different forms of small intestinal villi in the rat (Rattus norvegicus). Scand. J. Gastroenterol. 4: 477-482.

Milne, R.W., J. Bienenstock, and D.Y.E. Perey. 1975. The influence of antigenic stimulation on the ontogeny of lymphoid

aggregates and immunoglobulin - containing cells in mouse bronchial and intestinal mucosa. J. Reticuloendothelial Soc. 17: 361-369.

Mohiuddin, A. 1966. Blood and lymph vessels in the jejunal villi of the white rat. Anat. Rec. 156: 83-90.

Montgomery, P.C., A. Ayyildiz, I.M. Lemaitre-Coelho, J.-P. Vaerman, and J.H. Rockey. 1983. Induction and expression of antibodies in secretions: the ocular immune system. Ann. N. Y. Acad. Sci. 409: 428-440.

Montgomery, P.C., B.R. Rosner, and J. Cohn. 1974. The secretory antibody response. . Anti-DNP antibodies induced by dinitrophenylated type III pneumococcus. Immunol. Commun. 3: 143-156.

Montgomery, P.C., I.M. Lemaitre - Coelho, and J.-P. Vaerman. 1981. The induction and expression of IgA anti-DNP antibodies in rat bile and secretions. Scand. J. Immunol. 13: 587-595.

Montgomery, P.C., S.A. Khaleel, J. Goudswaard, and G. Virella. 1977. Selective transport of an oligomeric IgA into canine saliva. Immunol. Commun. 6: 633-642.

Moore, A.R., and J.G. Hall. 1972. Evidence for a primary association between immunoblasts and small gut. Nature 239: 161-162.

Morgan, K.L., A.M. Hussein, T.J.Newby, and F.J. Bourne. 1980. Quantification and origin of the immunoglobulins in porcine respiratory tract secretions. Immunol. 41: 729-736.

Morris, B., and F.C. Courtice. 1977. Cells and immunoglobulins in

lymph. Lymphology 10: 62-70.

McConnell, I., and J. Hopkins. 1981. Lymphocyte traffic through antigen - stimulated lymph nodes. I. Complement activation within lymph nodes initiates cell shutdown. Immunol. 42: 217-223.

McDermott, M.R. 1979. Studies of the cellular basis of immunity at mucosal surfaces. Doctoral Thesis McMaster University, Hamilton, Ontario.

McDermott, M.R., and J. Bienenstock. 1979. Evidence for a common mucosal immunologic system. I. Migration of B immunoblasts into intestinal, respiratory, and genital tissues. J. Immunol. 112: 1892-1898.

McDermott, M.R., D.A. Clark, and J. Bienenstock. 1980a. Evidence for a common mucosal immunologic system. II. Influence of the estrous cycle on B immunoblast migration into the genital and intestinal tissues. J. Immunol. 124: 2536-2539.

McDermott, M.R., D.A.Mark, A.D. Befus, B.S. Baliga, R.M. Suskind, and J.Bienenstock. 1982. Impaired intestinal localization of mesenteric lymphoblasts associated with vitamin A deficiency and protein - calorie malnutrition. Immunol. 45: 1-5.

McDermott, M.R., R.V. Heatley, A.D. Befus, and J. Bienenstock. 1980. Lack of influence of Peyer's patches on the intestinal localization of mesenteric lymphoblasts. Cell. Immunol. 55: 240-245.

McWilliams, M., J.M. Phillips - Quagliata, and M.E. Lamm. 1975. Characteristics of mesenteric lymph node cells homing to

gut-associated lymphoid tissue in syngeneic mice. J. Immunol. 115: 54-58.

McWilliams, M., J.M. Phillips - Quagliata, and M.E. Lamm. 1977. Mesenteric lymph node B lymphoblasts which home to the small intestine are precomitted to IgA synthesis. J. Exp. Med. 145: 866-875.

Nieuwenhuis, P., and W.L. Ford. 1976. Comparative migration of B - and T - lymphocytes in the rat spleen and lymph nodes. Cell. Immunol. 23: 254-267.

Ogra, P.L., and D.T. Karzon. 1969. Distribution of poliovirus antibody in serum, nasopharynx and alimentary tract following segmental immunization of lower alimentary tract with poliovaccine. J. Immunol. 102:1423-1430.

Ottaway, C.A., and D.M.V. Parrott. 1979. Regional blood flow and its relationship to lymphocyte and lymphoblast traffic during a primary immune reaction. J. Exp. Med. 150: 218-230.

Ottaway, C.A., and D.M.V. Parrott. 1980. Regional blood flow and the localization of lymphoblasts in the small intestine of the mouse. I. Examination of normal small intestine. Immunol. 41: 955-961.

Ottaway, C.A., D.F.Manson - Smith, R.G. Bruce, and D.V.M. Parrott. 1980. Regional blood flow and the localization of lymphoblasts in the small intestine of the mouse. II. The effect of a primary enteric infection with Trichinella spiralis. Immunol. 41: 963-971.

Owen, R.L., C.L. Allen. and C.P. Stevens. 1981. Phagocytosis of Giardia muris by macrophages in Peyer's patch epithelium in mice. Infect. Immun. 33: 591-601.

Paavonen, T., L.C. Andersson, and H. Adlercreatz. 1981. Sex hormone regulation of in vitro immune response. Estradiol enhances human B cell maturation via inhibition of suppressor T cells in pokeweed mitogen - stimulated cultures. J. Exp. Med. 154: 1935-1945.

Panayi, G.S., and W.A. Neill. 1972. Scintillation autoradiography. A rapid technique. J. Immunol. Meth. 2: 115-117.

Pappenheimer, A.M. 1917. Experimental studies on lymphocytes. I. The reactions of lymphocytes under various experimental conditions. J. Exp. Med. 25: 633-650.

Parrott, D.M.V., and A. Ferguson. 1974. Selective migration of lymphocytes within the mouse small intestine. Immunol. 26: 571-588.

Perey, D.Y.E. and R.W. Milne. 1975. Rabbit gut-associated lymphoid tissue. Major pathway for thoracic duct lymphocyte circulation. Lab. Invest. 33: 678-686.

Phillips-Quagliata, J.M., M.E. Roux, M. Arny, P. Kelly-Hatfield, M. McWilliams, and M.E. Lamm. 1983. Migration and regulation of B-cells in the mucosal immune system. Ann. N. Y. Acad. Sci. 409: 194-203.

Pierce, N.F., and W.C. Cray. 1981. Cellular dissemination of priming for a mucosal immune response to cholera toxin in rats. J. Immunol. 127: 2461-2464.

Pierce, N.F., and W.C. Cray. 1982. Determinants of the localization, magnitude and duration of a specific mucosal IgA plasma cell response in enterically immunized rats. J. Immunol. 128: 1311-1315.

Pierce, N.F., and J.L. Gowans. 1975. Cellular kinetics of the intestinal immune response to cholera toxoid in rats. J. Exp. Med. 142: 1550-1563.

Poger, M.E., and M.E. Lamm. 1974. Localization of free and bound secretory component in human intestinal epithelial cells. A model for the assembly of secretory IgA. J. Exp. Med. 139: 629-642.

Porter, P., N.E. Noakes, and W.D. Allen. 1972. Intestinal secretion of immunoglobulins in the preruminant calf. Immunol. 23: 299-312.

Rannie, G.H., M.E. Smith, and W.L. Ford. 1977. Lymphocyte migration into cell - mediated immune lesions is inhibited by trypsin. Nature 267: 520-522.

Reynolds, J., I. Heron, L. Dudler, and Z. Trnka. 1982. T-cell recirculation in the sheep: migratory properties of cells from lymph nodes. Immunol. 47: 415-421.

Richman, L.K., A.S. Graeff, R. Yarchoan, and W. Strober. 1981. Simultaneous induction of antigen - specific IgA helper T cells in the murine Peyer's patch after protein feeding. J. Immunol. 126: 2079-2083.

Romano, T.J., N.M. Ponzio, and G.J. Thorbecke. 1976. Graft-vs-host

reactions in F_1 mice induced by parental lymphoid cells: nature of recruited F_1 cells. J. Immunol. 116: 1618-1623.

Rose, M.L., D.M.V. Parrott, and R.G. Bruce. 1976a. Migration of lymphoblasts to the small intestine. I. Effect of Trichinella spiralis infection on the migration of mesenteric lymphoblasts and mesenteric T lymphoblasts in syngeneic mice. Immunol. 31: 723-730.

Rose, M.L., D.M.V. Parrott, and R.G. Bruce. 1976b. Migration of lymphoblasts to the small intestine. II. Divergent migration of mesenteric and peripheral immunoblasts to sites of inflammation in the mouse. Cell Immunol. 27: 36-46.

Rose, M.L., D.M.V. Parrott, and R.G.Bruce. 1978. The accumulation of immunoblasts in extravascular tissues including mammary gland, peritoneal cavity, gut and skin. Immunol. 35: 415-423.

Rose, M.E., P. Hesketh, and B.M. Ogilvie. 1980. Coccidiosis: localization of lymphoblasts in the infected small intestine. Parasite Immunol. 2: 189-199.

Roux, M.E., M. McWilliams, J.M. Phillips-Quagliata, and M.E. Lamm. 1981. Differentiation pathway of Peyer's patch precursors of IgA plasma cells in the secretory immune system. Cell. Immunol. 61, 141-153.

Roux, M.E., M. McWilliams, J.M. Phillips-Quagliata, P. Weisz-Carrington, and M.E. Lamm. 1977. Origin of IgA - secreting plasma cells in the mammary gland. J. Exp. Med. 146: 1311-1322.

Rudzik, O., D.Y.E. Perey, and J. Bienenstock. 1975a. Differential

IgA repopulation after transfer of autologous and allogeneic rabbit Peyer's patch cells. J. Immunol. 114: 40-44.

Rudzik, R., R.L. Clancy, D.Y.E. Perey, R.P. Day, and J. Bienenstock. 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: 1599-1604.

Sasson, S., and M. Mayer. 1981. Effect of androgenic steroids on rat thymus and thymocytes in suspension. J. Steroid. Biochem. 14: 509-517.

Schlesinger, M. 1976. Cell surface receptors and lymphocyte migration. Immunol. Commun. 5: 775-793.

Schoefl, G.I., and R.E. Miles. 1972. The migration of lymphocytes across the vascular endothelium in lymphoid tissue. J. Exp. Med. 136: 568-588.

Schrader, J.W., R. Scollay and F. Battye. 1983. Intramucosal lymphocytes of the gut: Lyt-2 and Thy-1 phenotype of the granulated cells and evidence for the presence of both T cells and mast cell precursors. J. Immunol. 130: 558-564.

Scollay, R., J. Hopkins, and J. Hall. 1976. Possible role of surface Ig in non-random recirculation of small lymphocytes. Nature 260: 528-529.

Scrimshaw, N.S., C.E. Taylor, and J.E. Gördon. 1968. In Nutrition and Infection. World Health Organization Monograph Series No. 57, Geneva., cited by McDermott et al., 1982.

Sedgley, M., and W.L. Ford. 1976. The migration of lymphocytes across specialized vascular endothelium. I. The entry of lymphocytes into the isolated mesenteric lymph - node of the rat. Cell. Tissue Kinet. 9: 231-243.

Selby, W.S., G. Janossy, and D.P. Jewell. 1981. Immuno-histological characterisation of intraepithelial lymphocytes of the human gastrointestinal tract. Gut 22: 169-176.

Selby, W.S., G. Janossy, M. Bofill and D.P. Jewell. 1983. Lymphocyte subpopulations in the human small intestine. The findings in normal mucosa and in the mucosa of patients with adult coeliac disease. Clin. exp. Immunol. 52: 219-228.

Shortman, K.N., N. Williams, and R. Adams. 1972. The separation of different classes of cells from lymphoid organs. V. Simple procedures for the removal of cell debris, damaged cells and erythroid cells from lymphoid cell suspensions. J. Immunol. Meth. 1: 273-287.

Sigal, N.H., and N.R. Klinman. 1978. The B-cell clonotype repertoire. In Advances in Immunology. Vol. 26. Eds. F.J. Dixon and H.G. Kunkel. Academic Press, N.Y. pp 255-337.

Smith, M.E., and W.L. Ford. 1983. The recirculating lymphocyte pool of the rat: a systematic description of the migratory behaviour of recirculating lymphocytes. Immunol. 49: 83-94.

Smith, M.E., A.F. Martin and W.L.Ford. 1980. Migration of lymphoblasts in the rat. Preferential localization of DNA-synthesizing lymphocytes in particular lymph nodes and other sites. Monogr. Allergy 16: 203-232.

Sprent, J. 1973. Circulating T and B lymphocytes of the mouse. I. Migratory properties. Cell. Immunol. 7: 10-39.

Sprent, J. 1976. Fate of H2-activated T lymphocytes in syngeneic hosts. I. Fate in lymphoid tissues and intestines traced with ^{3}H-thymidine, ^{125}I-deoxyuridine and ^{51}chromium. Cell. Immunol. 21: 278-302.

Stamper, H.B., and J.J. Woodruff. 1977. An *in vitro* model of lymphocyte homing. I. Characterization of the interaction between thoracic duct lymphocytes and specialized high-endothelial venules of lymph nodes. J. Immunol. 119: 772-780.

Stevens, S.K., I.L. Weissman, and E.C. Butcher. 1982. Differences in the migration of B and T lymphocytes: organ-selective localization *in vivo* and the role of lymphocyte-endothelial cell recognition. J. Immunol. 128: 844-855.

Strober, W., R. Krakauer, H.L. Klaeveman, H.Y. Reynolds, and D.L. Nelson. 1976. Secretory component deficiency. A disorder of the IgA immune system. New Engl. J. Med. 294: 351-356.

Sullivan, D.A., and C.R. Wira. 1979. Sex hormone and glucocorticoid receptors in the bursa of fabricius of immature chicks. J. Immunol. 122: 2617-2623.

Sullivan, D.A., and C.R. Wira. 1981. Estradiol regulation of secretory component in the female reproductive tract. J. Steroid Biochem. 15: 439-444.

Sullivan, D.A., and C.R. Wira. 1983a. Variations in free secretory component levels in mucosal secretions of the rat.

J. Immunol. 130: 1330-1335.

Sullivan, D.A., and C.R. Wira. 1983b. Mechanisms involved in the hormonal regulation of immunoglobilins in the rat uterus. Uterine response to a single estradiol treatment. Endocrinol. 112: 260-268.

Sullivan, D.A., B.J. Underdown, and C.R. Wira. 1983. Steroid hormone regulation of free secretory component in the rat uterus. Immunol. 49: 379-386.

Taubman, M.A., and D.J. Smith. 1977. Effects of local immunization with glucosyl-transferase fractions from Streptococcus mutans on dental caries in rats and hamsters. J. Immunol. 118: 710-720.

Tilney, N.L. 1971. Patterns of lymphatic drainage in the adult laboratory rat. J. Anat. 109: 369-383.

Tilney, N.L., and W.L. Ford. 1974. The migration of rat lymphoid cells into skin grafts. Some sensitised cells localize preferentially in specific allografts. Transplantation 17: 12-21.

Tomasi, T.B.Jr., and J. Bienenstock. 1968. Secretory immunoglobulins. Adv. Immunol. 9: 1-96.

Tseng, J. 1981. Transfer of lymphocytes of Peter's patches between immunoglobulin allotype congenic mice: repopulation of the IgA plasma cells in the gut lamina propria. J. Immunol. 127: 2039-2043.

Vaerman, J.P., I.M. Lemaitre-Coelho, J.N. Limet, and D.L. Delacroix.

1982. Hepatic transfer of polymeric IgA from plasma to bile in rats and other mammals: a survey. In Recent Advances in Mucosal Immunity. Edited by W. Strober, L.A. Hanson, and K.W. Sell. Raven Press. New York. pp. 233-250.

van Ewijk, W., N.H.C. Brons, and J. Rozing. 1975. Scanning electron microscopy of homing and recirculating lymphocyte populations. Cell. Immunol. 19: 245-261.

Waksman, B.H., H. Ozer, H.E. Blythman. 1973. Appendix and M-antibody formation. VI. The functional anatomy of the rabbit appendix. Lab. Invest. 28: 614-626.

Waldman, R.H., J.M. Cruz, and D.S. Rowe. 1971. Immunoglobulin levels and antibody to Candida albicans in human cervicovaginal secretions. Clin. Exp. Immunol. 10: 427-434.

Weiker, J., and B. J. Underdown. 1975. A study of the association of human secretory component with IgA and IgM proteins. J. Immunol. 114: 1337-1344.

Weisz-Carington, P., M.E. Roux, and M.E. Lamm. 1977. Plasma cells and epithelial immunoglobulins in the mouse mammary gland during pregnancy and lactation. J. Immunol. 119: 1306-1309.

Weisz-Carrington, P., M.E. Roux, M. McWilliams, J.M. Phillips-Quagliata, and M.E. Lamm. 1978. Hormonal induction of the secretory immune system in the mammary gland. Proc. Natl. Acad. Sci. U.S.A. 75: 2928-2932.

Weisz-Carrington, P., M.E. Roux, M. McWilliams, J.M. Phillips-Quagliata, and M.E. Lamm. 1979. Organ and isotype distribution of plasma cells producing specific antibody

after oral immunization: evidence for a generalized secretory immune system. J. Immunol. 123: 1705-1708.

Wira, C.R., and C.P. Sandoe. 1977. Sex steroid hormone regulation of IgA and IgG in rat uterine secretions. Nature 268: 534-536.

Wira, C.R., and C.P. Sandpe. 1980. Hormonal regulation of immunoglobulins: influence of estradiol on immunoglobulin A and G in the rat uterus. Endocrinol. 106: 1020-1026.

Wira C.R., E. Hyde, C.P. Sandoe, D. Sullivan, and S. Spencer. 1980. Cellular aspects of the rat uterine IgA response to estradiol and progesterone. J. Steroid Biochem. 12: 451-459.

Woodruff, J. 1974. Role of lymphocyte surface determinants in lymph node homing. Cell. Immunol. 13: 378-384.

Woodruff, J.J., I.M. Katz, L.E. Lucas. and H.B. Stamper. 1977. An in vitro model of lymphocyte homing. II. Membrane and cytoplasmic events involved in lymphocyte adherence to specialized high-endothelial venules of lymph nodes. J. Immunol. 119: 1603-1610.

Woodruff, J., and B.M. Gesner. 1968. Lymphocytes: circulation altered by trypsin. Science 161: 176-178.

Woodruff, J.J., and B.M. Gesner. 1969. The effect of neuraminidase on the fate of transfused lymphocytes. J. Exp. Med. 129: 551-567.

Woodruff, J.F., and J.J. Woodruff. 1970. Virus,- induced alterations of lymphoid tissues. I. Modifications of the recirculationg pool of small lymphocytes by Newcastle Disease Virus.

Cell. Immunol. 1: 333-354.

Woodruff. J.J., and J.F. Woodruff. 1974. Virus - induced alterations of lymphoid tissues. IV. The effect of Newcastle Disease virus on the fate of transfused thoracic duct lymphocytes. Cell. Immunol. 10:78-85.

Yamamoto, K., R.L. Anacker, and E.Ribi. 1970. Macrophage migration inhibition studies with cells from mice vaccinated with cell walls of Mycobacterium bovis BCG: relationship between inhibitory activity of lung cells and resistance to airborne challenge with Mycobacterium tuberculosis. H37RV. Infect. Immun. 1: 595-599.

Yoffey, J.M., and F.C. Courtice. 1970. Lymphatics, lymph and the lymphomyeloid complex. Academic Press. London. p 702.

Zatz, M.M. 1975. Effects of cortisone on lymphocyte homing. Isr. J. Med. Sci. 11: 1368-1375.

Zatz, M.M., and E.M. Lance. 1971. The distribution of Cr-labeled lymphocytes into antigen - stimulated mice. J. Exp. Med. 134: 224-241.

Zatz, M.M., R. Gingrich, and E.M. Lance. 1972. The effect of histocompatibility antigens on lymphocyte migration in the mouse. Immunol. 23: 665-675.