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MIGRATION *and* HOMING *of* LYMPHOID CELLS

Volume I

Alan J. Husband

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Migration and Homing of Lymphoid Cells

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Editor

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PREFACE

Lymphoid cell migration is crucial to successful immune defense. The continued recirculation of small lymphocytes maximizes the opportunity for antigen-presenting cells, effector-cell precursors, and regulatory cells of appropriate specificity to cooperate in response to antigen encounter, and the subsequent migration of effector cells to target sites ensures an appropriate dissemination of the response. Considerable evidence has accumulated in recent years proving that, while this process may be random within specified compartments, there are pools of lymphocytes, and perhaps even of antigen-presenting cells, defined by nonrandom patterns of migration with respect to tissue specificities and antigen-influenced events. An understanding of the restrictions on cell migration is essential to the development of effective immunization strategies.

This book addresses the issues of lymphocyte recirculation leading to inductive interactions in the immune response to antigen, the sites of these interactions, and the subsequent migration and homing of effector cells generated from these responses. In view of the lack of success in establishing effective vaccines against diseases at mucosal sites, particular attention is given to the apparent contrasts between systemic and mucosal lymphoid cell pools, and explanations are sought for mechanisms mediating selectivity of migration and homing.

The contributors to this book represent a wide range of expertise from many research centers ensuring coverage of a diversity of interests on cell-traffic research and providing a broad perspective on this key function of the immune system. Regrettably, a contribution by Professor W. L. Ford was prevented by his untimely death, but his additions to our understanding of cell migration remain an enduring bequest to immunology.

Alan J. Husband, Ph.D.

THE EDITOR

Alan J. Husband, Ph.D., is Associate Professor of Immunology in the Faculty of Medicine of the University of Newcastle in Australia. Dr. Husband received his B.Sc.Agr. degree from the University of Sydney in 1972 and subsequently was awarded a Ph.D. degree from the same university for studies in ruminant immunity. He then spent a period of overseas postdoctoral study in the Sir William Dunn School of Pathology at the University of Oxford and returned to Australia in 1977 to the position of Research Scientist with the New South Wales Department of Agriculture. In 1980 he accepted his appointment at the University of Newcastle.

Dr. Husband's research interests in immunology have focused primarily on problems of immune function at mucosal surfaces, particularly the role of cell migration in mucosal effector responses, and he has published extensively in this area. He is a Member of the Australian Society for Immunology, the Australian Society for Microbiology, and the American Association of Immunologists.

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Chapter 1

LYMPHOCYTE TRAFFIC — HISTORICAL PERSPECTIVES AND FUTURE DIRECTIONS

Delphine M. V. Parrott

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I. INTRODUCTION

It is now almost 30 years since the first publications by James Gowans[1,2] revolutionized our thinking about the physiology of lymphocyte traffic and established it as one of the cornerstones of modern immunology. Until that time, it had been considered that all lymphocytes were short-lived cells which were destroyed within a few hours of entering the blood, and the notion that lymphocytes could survive for many months, even years, and could circulate continuously from blood to lymph was indeed revolutionary. But Gowans would be the first to admit that his observations did not come as "a bolt from the blue" but were dependent upon others, so it is appropriate that his discoveries should be placed in historical prospective. Prospects past and future is the theme for this short introductory chapter and it is a personal viewpoint which many readers may dismiss in whole or in part, but if I succeed in provoking some reaction then my intention will have been achieved.

II. LIFE SPAN OF LYMPHOCYTES

A major incentive to research in the 1950s was the prevailing argument over whether lymphocytes were short-lived and rapidly destroyed or long-lived. This argument had its origins in the early 1885 studies of Fleming,[3] whose name is usually associated with the discovery of germinal centers, in spleen, lymph nodes, and tonsils, but who also made far-sighted proposals to explain the observed difference in the numbers of lymphocytes in efferent lymph which was always much richer in cells than lymph arriving at a node in afferent lymphatics. He proposed that *either* there was continuous cell division within the node (and, remember, he had observed considerable cell division in germinal centers) *or* that lymphocytes could enter the lymph node by crossing the walls of blood vessels so that there was continuous circulation of cells from the blood into the lymph node and back to the blood from the lymph. At that time, he insisted, there was no means of deciding between either proposal. During the first decade of this century, Davis and Carlson[4] carried out extensive studies on leukocytes in the blood and the major lymphatics of the dog. They measured the number of lymphocytes entering the blood per hour and the number of lymphocytes in the blood and calculated that lymphocytes must be replaced at least once and possibly 3 to 4 times every 24 hr. Since the level of lymphocytes remained constant, Davis and Carlson concluded that lymphocytes may be destroyed rapidly, developed further, used for repair purposes, or "they may circulate from lymph to blood and from blood, through the capillary endothelium, into the tissue lymph, and thence back into the lymph, of the larger lymphatic trunks".[4] The idea that lymphocytes might recirculate from blood to lymph was restated by Sjovall in 1936[5] but dismissed by Yoffey and Drinker in 1939.[6] After World War II, technical developments occurred which led to the resolution of at least the main argument over lymphocyte life span. Bollman and his colleagues at the Mayo Clinic[7] developed the techniques of thoracic duct cannulation in the rat and designed a restraining cage in which rats could be maintained with an indwelling cannula for several days. This permitted Mann and Higgins[8] to show that drainage from the thoracic duct resulted in the progressive depletion of lymphocytes from the lymph. Another very important technical development followed the use of radioactively labeled compounds for biological research. In 1954, Ottesen[9] used radioactive phosphorus to estimate the life span of lymphocytes in human blood and calculated that the majority of lymphocytes had an average life span of 100 to 200 days — at that time a reasonable conclusion from the data, but, nevertheless, an astonishing finding. In 1957, Gowans[1] repeated the experiments of Mann and Higgins,[8] but extended them to show that, by reinfusing lymphocytes into the blood, the level of circulating lymphocytes could be maintained. It is interesting that he tested an idea which had been put forward by Davis and Carlson and then reiterated by Mann and Higgins that lymphocytes and lymph have a function

in providing essential nutrients by infusing cell-free lymph or lymph containing killed lymphocytes. Both were ineffective when compared with live lymphocytes. Gowans suggested "that the continuous entry of living lymphocytes into the blood may be essential for maintaining the output of lymphocytes from the thoracic duct".[1] He proceeded to address that suggestion by infusing ^{32}P-labeled lymphocytes into rats which were rapidly recovered from the thoracic duct."[1] Furthermore, he infused tritium-labeled thymidine which would be incorporated into the DNA of newly divided cells and showed that "the number of new lymphocytes found each day in the rat amounts to only a small fraction of the normal output of lymphocytes from the thoracic duct".[2]

Gowans was a pupil of Lord Howard Florey, an Australian who shared a Nobel prize for the discovery of penicillin and had a life-long interest in the physiology of lymphocytes. Florey headed the Sir William Dunn School of Pathology and inspired many young collaborators to work on lymphocytes.. Another former pupil of Howard Florey in Oxford was Bede Morris who, with a series of colleagues, developed the necessary techniques of lymphatic cannulation in the sheep which have permitted many problems to be addressed which would be impossible to resolve in small laboratory rodents.[10,11] In the John Curtin School of Medical Research, Canberra, Australia, Bede Morris, with his Ph.D. student, Joe Hall, carried out experiments[12,13] which beautifully complemented those of Gowans. Hall and Morris infused ^{3}H-thymidine into a popliteal lymph node and showed by the labeling pattern of lymphocytes in the efferent lymph that under normal conditions without antigenic stimulation not more than 4% of those lymphocytes were actually produced in the node, and since so few cells entered the node from efferent lymph then at least 85% of cells in efferent lymph must have entered from the blood.[12] In other experiments Hall and Morris[13] showed that the destruction of lymphocytes within an isolated lymph node by irradiation was followed by prompt restoration at far too rapid a rate to be explained by production of new lymphocytes.

At the beginning of the 1960s, as far as lymphocyte "traffickers" were concerned, lymphocytes were divided into long-lived and short-lived, small lymphocytes and large lymphocytes or lymphoblasts.[14] There were also mobilizable and nonmobilizable pools of lymphocytes, that is, those lymphocytes which could be mobilized by thoracic duct drainage and those which could not.[15] There were, however, concurrent lines of investigation which were directed towards the source rather than the life span of lymphocytes and these culminated in the major discoveries concerning the functions of the thymus and the bursa of Fabricius and, ultimately, into the division of lymphocytes into T and B. Such discoveries were, of course, directly relevant to lymphocyte traffic studies, but the immunological hot-bed of enthusiasts which indulged in such nonphysiological pursuits as excision of whole organ, whole body irradiation, and the in vitro "torture" of lymphocytes prepared from lymph nodes provoked an exasperated Bede Morris to exclaim that B and T were most appropriate as the first and last letters of "bullshit".[78] Nevertheless, the division of lymphocytes in T and B prompted much careful and systematic work on lymphocyte traffic.[16-18] At first it was proposed that T cells were long-lived and could recirculate, but B cells could not. We now know that lymphocytes do vary in their life span and in their ability to recirculate from blood to lymph, but these properties cut across the B-T division. Space does not permit a detailed account, and I trust that those who also spent many years on those problems will forgive my scant acknowledgment of their labors. At the present time, life span and lymphocyte recirculation are still very much live issues which are provoking controversy and experiment.[79]

III. LYMPHOCYTE TRAFFIC — THE IMMUNE RESPONSE AND MEMORY

The stimulus to study lymphocyte traffic may have been a need to resolve the question of life span, but within a short space of time it was demonstrated that the immune respon-

siveness of the whole animal is dependent upon lymphocyte circulation. In 1963, McGregor and Gowans[19] demonstrated that removal of the recirculating pool by thoracic duct drainage seriously impaired the ability of rats to initiate a primary immune response to sheep red blood cells or to skin grafts and that the deficit could be restored by reinfusion of lymphocytes. Three years later, Gowans and Uhr[20] extended the studies to show that memory in the absence of an ongoing immune response is also a property of circulating lymphocytes. Sam Strober in 1968[21] pointed out that both circulating and noncirculating lymphocytes were important in the initiation of an immune response. Bill Ford devised the ingenious technique of isolating a rat spleen whole and adjusted the perfusate to contain a variable number of lymphocytes.[22] He was thus able to demonstrate that the response to sheep red blood cells was directly related to the number of lymphocytes in the perfusate, not the number of lymphocytes in the isolated spleen.

In many ways, however, the most telling demonstrations of the importance of lymphocyte traffic in immune responses were those which utilized the technique of the isolated perfused lymph node. Hall and his colleagues in 1967[23] were the first to demonstrate the function of the cells leaving a lymph node from the efferent lymphatic. They immunized sheep with *Salmonella typhi* 'O' antigen and demonstrated that removal of efferent lymph and lymphocytes inhibited the production of a systemic immune response. Furthermore, they took efferent lymph cells from an immunized sheep and transferred into an unimmunized recipient twin sheep. The recipient responded with the prompt production of antibody in the absence of added antigen. The authors also made careful and detailed electron microscopy studies on the ultrastructure of the blast cells in efferent lymph and were somewhat "put off" by that failure to find classical plasma cells in the lymph. With the benefit of hindsight we would nowadays reinterpret all of the early experiments on initiation of immune responsiveness and memory in the light of separate T and B cells, but that in no way diminishes the impact which these early experiments made.

A different, though equally significant, manifestation of the importance of lymphocyte migration in immune responses is illustrated by local mucosal immunity. Gowans and Julie Knight[24] first noted that large lymphocytes for the thoracic duct migrated into the small intestinal mucosa and transformed into plasma cells. It was several years, though, before these observations were followed up, beginning with Griscelli, Vassalli, and McCluskey in 1969[25] who showed that although mesenteric lymphoblasts migrated to the gut, lymphoblasts from peripheral lymph nodes did not, and Guy-Grand et al.[26] identified two types of migrating lymphoblast, IgA precursors and T blasts, in thoracic duct lymph. But the crucial experiments in respect to lymphocyte traffic and immune responsiveness must be credited to Nat Pierce and Gowans[27] in 1975. They compared the number of specific antibody-containing cells in the lamina propria of the intestine of primed rats with or without a thoracic duct fistula after intraduodenal challenge with cholera toxin. Drainage of lymph from the thoracic duct drastically reduced the numbers of specific anticholera toxin antibody-containing cells in the lamina propria, but infusion of lymph that was rich in antitoxin-containing cells resulted in the prompt appearance of such cells in the lamina propria, thus demonstrating unequivocally the function of lymphocyte traffic in local mucosal immunity.

IV. THE ROUTE OF ENTRY OF LYMPHOCYTES INTO LYMPH NODE

The first experiments of Gowans[1,2] and Hall and Morris[11-13] showed that lymphocytes move from blood to lymph, but the route which each lymphocyte took could not be followed in detail without the technique of labeling small thoracic duct lymphocytes with tritiated adenosine and using autoradiography to clarify their whereabouts in the lymphoid tissues. Earlier workers had noticed that lymphocytes were often to be seen in the walls of the postcapillary venules often characterized by plump endothelial walls, but it was Gowans

and Knight in 1964[24] who identified these vessels as the route by which lymphocytes enter lymph nodes. They rapidly infused labeled lymphocytes intravenously over 12 min and immediately placed lymph nodes in fixative. At that time, large numbers of lymphocytes could be seen in walls of the postcapillary vessels. Others followed to show that lymphocytes from other sources, namely thymus and spleen, also use this same route to enter the paracortex of the lymph node, an area termed thymus dependent.[28]

The distinct relationship of HEV (as the characteristic postcapillary, high endothelial venules of lymph nodes and gut-associated tissues are usually termed) with lymphocytes was emphasized by Gowans and Knight[24] since not only was it the preferred route of migration of lymphocytes, but under normal conditions it excluded other leukocytes. Vince Marchesi and Gowans[29] attributed this difference to the capacity of lymphocytes to cross the HEV by emperipolesis, i.e., they moved intracellularly rather than intercellularly. We now know that the majority of lymphocytes do not take the intracellular route (Schoefl[30] and Anderson and Anderson[31]) and there is less emphasis on the capacity for HEV to exclude other leukocytes, since during inflammation they probably do not. Importance of the unidirection of lymphocyte traffic (i.e., from blood to node but not vice versa) is now also less emphasized because bidirectional traffic is normal in the pig. Gowans and Knight postulated that "the rapid 'homing' of labeled cells into the lymph nodes presumably has its basis in the special affinity of small lymphocytes for the endothelium of the post-capillary".[24] The search for the molecular basis of that receptor remains an obsession to this day. Gesner and Ginsburg in 1966[32] first started the search in terms of the carbohydrates of the lymphocyte membrane, but most experiments required that lymphocytes should be preincubated in various enzymes or lectins prior to intravenous injection. Such treatments which are known to alter the migration of lymphocytes through most tissues, including lungs, liver, and spleen, could not be narrowed down to focus only on the interaction of lymphocytes with HEV,[33,34] although experiments along these lines nevertheless persist. At present, the techniques devised by Woodruff and her colleagues[34,35] to examine the adherence of lymphocytes to HEV in vitro are proving to be very profitable in the HEV-receptor search. Chin et al.[35] have identified a factor in thoracic duct lymph which can block the binding of lymphocytes to rat HEV in vitro. Gallatin and his colleagues,[36] using the Woodruff technique, have developed an antibody which identifies a surface component on lymphocytes, and both groups are working assiduously to demonstrate how these factors and antibodies identified by in vitro methods are, nevertheless, applicable to lymphocyte-traffic studies in vivo.

V. THE RECOGNITION OF PLACE: THE DEFINITION OF MICROENVIRONMENT AND COMPARTMENTS RECOGNIZED BY LOCOMOTING CELLS

The use of autoradiography as a means of following the progress of labeled lymphocytes into lymphoid organs offered a way in which another then-current pressing problem could be approached. Many groups were investigating the differing effectiveness of whole thymus grafts vs. separated preparations of thymocytes, lymph node lymphocytes, or splenic lymphocytes in restoring the impaired immunological responsiveness of neonatally thymectomized mice. The particular deficiency of thymocytes was especially puzzling in this respect. In 1964, the question was posed to Maria de Sousa, a young Portuguese pathologist recently arrived at the Imperial Cancer Research Fund, Mill Hill, London, did thymocytes and spleen cells "differ in efficiency because they reached different destinations within the lymphoid organs of neonatally thymectomized mice".[28] The question prompted the careful examination of many sections of lymph nodes and spleens of normal and thymectomized mice and the infusion of radiolabeled suspensions of thymocytes or spleen cells into mice using the same isotope, ^{3}H-adenosine as had been used by Gowans and Knight[24] in their experiments in

rats. There followed a description of the areas of depletion of the lymphocytes from thymectomized mice in both lymph nodes and spleen and these were termed thymus-dependent areas.[28] It was shown that ^{3}H-adenosine-labeled thymus cells localized preferentially in the thymus-dependent areas, but that spleen cells contained an additional population of lymphocytes which migrated to the outer cortex of lymph nodes and the periphery of the splenic follicle. It is ironic that Gowans and Knight[24] failed to detect a comparable separate population of lymphocytes in the inoculum of thoracic duct lymphocytes which they injected because of the metabolic differences between T and B lymphocytes in thoracic duct lymph. The B cells took up far less ^{3}H-adenosine than the T cells,[18] differences which do not occur in mouse lymphocytes.[37] In a subsequent publication from Gowan's laboratory by Jonathan Howard utilizing rat thoracic duct lymphocytes the length of exposure of autoradiographs was prolonged and lightly labeled B lymphocytes were detected in lymph node nodules.[38] Careful examination of autoradiographs and sections of neonatally thymectomized mice by de Sousa had permitted the postulate that the segregation of two separate migrating populations of cells, thymus-dependent and thymus-independent, occurred and, subsequently, that the ability to segregate is a characteristic of long-lived rather than short-lived cells.[16,28] It required, however, the preparation of reasonably pure separated populations of T and B lymphocytes which could be labeled in vitro and injected before the concept that T and B cells have separate migration pathways within lymphoid organs became accepted.[38,39] Later discernment of segregation was possible without preparing thymus-less animals, e.g., the binding of sheep erythrocytes to human T cells and the definition of B cells by the presence of surface immunoglobulin and of Fc-receptors and C3 receptors.[40] At the present time, monoclonal antibodies identify not only T and B cells, but various subsets of lymphocytes in many species, man, mouse, rat, and sheep.

The nature of the phenomenon of segregation of lymphocytes was described clearly in 1971[16] thus:

> ". . . having entered the peripheral lymphoid tissues it would seem reasonable to expect all lymphocytes by virtue of their intrinsic mobility to spread themselves evenly over all territories yet in neonatally thymectonised animals, or in adult thymus-deprived mice, they do not; vast areas normally occupied by the thymus derived cells are left void, with the nodules and medulla in the lymph nodes, and the other layer of the Malpighian body and the red pulp in the spleen fully populated."

It was realized that understanding segregation would be a major step in identifying the sites of cell-cooperation in vivo, but progress has been slow and has come mainly through perception of interaction of lymphocytes with nonlymphoid cells rather than interactions between T and B lymphocytes.

Veldman in 1970[41] first identified the interdigitating cell in the T areas of the lymph node, and it now appears that the different compartments of the spleen and the lymph node each have their own individual type of reticulum cell and their own mononuclear/macrophage type of cell, the reticulum cell being part of the fixed stroma of the tissue while the mononuclear/macrophage cell is the mobile element, and it seems likely that by interacting with these types of cells that lymphocytes position themselves.[42] There is plenty of morphological evidence of such interactions and, of course, a wealth of in vitro experiments demonstrating the importance of lymphocyte/macrophage/dendritic cell interactions in the initiation of immune responses, but hard evidence that such interactions are relevant to lymphocyte traffic studies is more difficult to find. Fossum et al.[43] attributed the slow progress of lymphocytes in the nude rat to the increased number of interdigitating cells in these animals. Hendriks[44] and his colleagues observed that severing of afferent lymphatics to a lymph node resulted in the disappearance of the nodular aggregates of B lymphocytes and terminal centers and the reduction in height of HEV. Drayton and Ford[45] extended these observations to show that despite careful maintenance of blood supply the delivery of

lymphocytes and blood flow was dramatically reduced in deafferentized lymph nodes. Such studies cannot distinguish between the relative importance of the different components of lymph, whether cellular or not, to these changes, but the evidence points to the importance of both antigen in maintaining the HEV and the nonlymphoid cells in the maintenance of structure.

VI. THE RELEVANCE OF LYMPHOCYTE LOCOMOTION TO LYMPHOCYTE TRAFFIC

The studies on the segregation and interaction of lymphocytes with other cells assumed that lymphocytes can and do locomote in a purposeful way[12,16,24] (see above), though this was not proven at the time. In an early review on lymphocyte traffic Ford and Gowans made a simple statement on the subject of lymphocyte locomotion, "the motive force for pushing lymphocytes through the labyrinthine reticulum of the white pulp of the spleen and the lymph nodes is not known".[46] Later, after referring to Bill Ford's experiments on the perfusion of isolated spleens they concluded that the "intrinsic motility of lymphocytes would seem to be far more important than any vis a tergo in propelling the cells through the organ",[46] an assumption which was repeated by Parrott and de Sousa[16] but not given any serious consideration. Presumably, at that time we had in mind the experiment carried out many years before by Lewis[47] and McCutcheon[48] who had given good descriptions of the locomotor morphology of lymphocytes though we did not refer to them! Later, Schoefl[30] and Anderson and Anderson[31] remarked that lymphocytes assume locomotor morphology when squeezing between the endothelial walls of HEV, though the morphology is constrained under these circumstances and the "hand mirror"-type morphology may not be obvious. Anderson and Anderson[31] describe the lymphocyte as making contact with the endothelial cells by means of its pseudopod and microvilli, then adhering by numerous points of contact, and flattening by moving across the endothelial wall.

The indifference of most lymphocyte traffickers to the way in which lymphocytes "get around" inside lymphoid organs and other tissues was and is quite extraordinary. Enthusiasm to study lymphocyte locomotion has come mostly, however, from cell biologists who are primarily interested in the machinery which a cell employs to move itself and in the stimuli which provoke cell movement in vitro, chemokinesis, and chemotaxis.[49] When investigations into this topic began in Glasgow, we reasoned that since activated lymphoblasts,[49] isolated from lymph or lymph nodes, had been observed to migrate in a nonspecific way in vivo into sites of inflammation and were apparently responding to the same stimulus as monocytes then lymphoblasts would be the most likely population of lymphocytes to respond to chemoattractants — and so they did.[50] Cultured lymphoblasts and mitogen-stimulated lymphoblasts also responded to chemoattractants such as casein, endotoxin-activated serum, and denatured protein.[49,50]

The study of normal lymphocyte as distinct from lymphoblast locomotion in vitro using the filter methods commonly used for other leukocytes was unsatisfactory, however, because lymphocytes, unlike neutrophils and monocytes, attach poorly to glass or plastic.[49,51] Recently, however, three-dimensional matrices, including collagen gels which mimic the reticular framework of a lymph node, have been developed.[51] Lymphocytes can move well on collagen gels because they do not require to make adhesions, but can insert blebs into gaps in the gel matrix and use the gel rather like a climbing frame.[51] The techniques open up an entirely new prospective for lymphocyte traffic because there is now convincing visual evidence of chemotaxis in normal lymphocytes.[52] Using lipopolysaccharide (LPS)-activated serum or the supernatant fluids from the culture of monocytes as a chemotactic source, lymphocytes have been demonstrated by their morphology to orient themselves and locomote directionally.[52]

VII. THE EFFECT OF ANTIGEN ON LYMPHOCYTE CIRCULATION

In complete contrast to the lack of enthusiasm for lymphocyte locomotion studies is the avid pursuit for a specific effect of antigen on lymphocyte traffic.

The idea of specificity is embedded in immunology despite ample evidence to the contrary. Many traffic studies have been (and still are) designed with the expectation that some degree of direction and specificity in relation to antigen would be revealed. Most of the expectations were doomed to disappointment!

A. "The Lymphocyte Trap"

Hall and Morris had observed that following administration of antigen there was a rapid increase in the number of lymphocytes leaving via the efferent lymph, but they also observed that the very first response (in the first few hours) was a transient *drop* in the output of lymphocytes in the efferent lymph and concluded that "this immediate fall in cell output was a specific response of the lymph node to the presence of antigenic material and could best be explained in terms of a temporary reduction in the rate at which lymphocytes recirculate from blood to lymph".[53] Carefully chosen words! They speculated but did not explore the role of the vascular system in this response. Dresser et al.,[54] using a different system in the mouse, studied the effect of injecting sheep red blood cells and adjuvant materials on the migration of ^{51}Cr-labeled lymph node cells at 24 hr to local lymph nodes draining the site of antigen injection. They observed that there were much larger amounts of radioactivity present in the draining than contralateral nodes, but did not find any change in blood volume to the draining node. There was no evidence of antigenic-specific "homing" to any draining lymph node. In a thoughtful discussion they proposed that the changes could be due to an "increase in the efficiency of the mechanical trapping of circulating lymphocytes" or to "secretion of a chemotactic agent which activates stimulated cells to migrate to a draining node".[54] The subject of chemotaxis of lymphocytes did not arouse immediate interest, but the first of those phrases fired the imagination of many groups of workers and there followed a spate of papers about "lymphocyte trapping",[55] all based on the assumption that lymphocytes were inhibited from leaving the spleen or lymph nodes rather than inhibited from entering as Hall and Morris[53] proposed. Later, Hall[56] modified his earlier position, but it was left to Cahill and his colleagues[57] to readdress the subject of "lymphocyte trapping". They carefully measured the actions of several different antigens in the cell input and output from the popliteal and prefemoral lymph nodes of sheep and came to the following conclusions: "There are at least two distinct mechanisms controlling the migration of circulating lymphocytes through an antigen stimulated lymph node. The first controls the increased input of recirculating lymphocytes which occurs only through high endothelial PCV. The second controls the immediate decrease in cell output which . . . does not occur at the PCV . . . they can occur independently and are possibly controlled by different mechanisms." Cahill et al.[57] make the point that the term "lymphocyte trapping" is inappropriate in the context of these phenomena and should be reserved for the trapping of a small minority of specific antigen-reactive cells.

At this time (1976), Peter Herman,[58] who had been interested in the microvasculative of lymph nodes, was measuring the changes in blood flow which occurred in the antigenically stimulated rabbit popliteal lymph node. Subsequently, Hay and Hobbs,[59] who noted that the increased output in the efferent lymph of sheep occurred at the same time as the regional blood flow was increased, proposed that vascular changes and increased lymphocyte traffic may be related phenomena. Ottaway and Parrott[60] set the seal on this topic by devising a technique for measuring blood flow and lymphocyte traffic simultaneously and showed that the migration of small lymphocytes to lymph nodes is directly related to blood flow. Thus, the notion that antigen-induced chemotaxis or increased trapping could account for the large

increase in lymphocytes in a draining lymph was set aside in favor of increase in blood flow.

The brief period of shut down that Cahill et al.[57] identified as a separate phenomenon was a concept followed up by other researchers, and the agent causing it was identified as prostaglandin E_2, though the mode of action was yet to be elucidated.[61]

B. Antigen-"Directed" Migration

A different "yearning" to reveal antigen-directed migration is also to be found in attempts to unravel the mechanism of cell-mediated immune responses, especially in response to allografts or tumor, and also to understand the assembly of effector cells in the mucosa of the intestine. There were claims and counter claims in respect to antigen-specific accumulations of lymphocytes and lymphoblasts into the site of rejecting skin allografts, but, on the whole, the results were unsatisfactory. More consistent findings came under category of migration into sites of inflammation, though they provided scant support for antigen-specific migration. Asherson and Allwood[62] were concerned with the study of the passive transfer of contact sensitivity and were puzzled by the effectiveness of peritoneal exudates presumed to contain mostly monocytes/macrophages in this respect, because the most obvious response to a contact-sensitizing agent was in the draining lymph nodes. They showed that ^{51}Cr-labeled lymphocytes from draining lymph nodes could move not only into sites of the application of a contact sensitizer, but also into skin sites nonspecifically inflamed with croton oil. A series of experiments summarized in a review by Parrott and Wilkinson[49] culminated in the identification of T lymphoblasts in S-phase, but not of small lymphocytes which could move nonspecifically into sites of inflammation whether caused by the contact-sensitizing agent which initiated the production of the T lymphoblasts, a noncross-reacting contact sensitizer, or a simple inflammatory agent. A parallel line of thought and experimentation was followed by MacGregor and Logie.[63] They were studying the collaborative role of macrophages and lymphocytes, especially those cells present in peritoneal exudates in rats in transferring resistance to *Listeria monocytogenes*. They also identified T lymphoblasts in S-phase and not small lymphocytes as being effective in transferring resistance and showed that these cells could be encouraged to extravasate into the peritoneal cavity by a nonspecific inflammatory stimuli, e.g., glycogen or thioglycollate, as well as by killed bacteria. Subsequent experiments along the lines set by Asherson and Allwood[62] and MacGregor and Logie[63] did detect significant elements of increased localization of lymphoblasts which were accredited to the presence of specific antigen, but major elements of nonspecific accumulation were always present.[60] Nevertheless, these experiments do indicate circumstances in which one type of lymphocyte is prompted to leave the bloodstream and was the starting point for one series of experiments on lymphocyte locomotion[50] (see above).

Another line of thought is to be found in the observations of Griscelli and his colleagues[25] who identified two different migration patterns of large lymphoblasts. They found that "the distribution of labeled cells was found to depend upon the source of the donor cells. Cells from mesenteric lymph nodes or thoracic duct lymph shared a marked preferential accumulation in lymphoid tissue within or adjacent to the intestine, whereas cells from peripheral nodes accumulated preferentially in peripheral lymph nodes".[25] Small lymphocytes did not display a similar preferential localization nor could Griscelli et al. determine whether the selective accumulation of large dividing cells "was due to an antigen recognition mechanism or was the result of two different populations of cells with different 'homing' mechanisms".[25] But this was the seminal paper which introduced the concept of nonrandom migration which could be influenced by mechanisms other than antigen. Griscelli et al.[25] also implanted the thought in the minds of others that antigen in the gut lumen may not be the only determining factor in inducing mesenteric or thoracic duct lymphoblasts to migrate into the intestinal mucosa. This was reinforced by the demonstration that segments of "antigen-free" gut grafts