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THE RELEVANCE OF LYMPHOID CELL MIGRATION TO IMMUNODEFICIENCY SYNDROMES

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ABSTRACT

It has been known for some time that antigen stimulation can alter lymphocyte traffic patterns and that viruses are particularly potent in this respect; such alterations may be a consequence of host-derived factors. The retention of lymphocytes in lymph nodes can be sustained for several hours with locally administered interferon (IFN)α. The extravasation of lymphocytes from blood into non-lymphoid tissues can be induced in the skin with IFNγ and particularly tumor necrosis factor (TNF)α. Recent evidence supports the concept that the migratory capacity of CD_4^+ cells differs from the capacity of CD_8^+ cells. Agents (cytokines?) which differentially affect the traffic of these two sub-sets have not yet been described but such a possibility has not been adequately tested. Several new molecules have been defined which alter the interactions between lymphocytes and blood vascular endothelial cells, and these may be important in the critical adhesive event in lymphocyte traffic. In both rat and sheep, it has been possible to cultivate post-capillary endothelial cells from lymphoid tissue, and this may be a helpful approach to studying the mechanisms and molecules involved in adhesion. New cell tracking dyes recently available (Zynaxis Cell Science) permit more significant, long-term studies on the life span of lymphocyte sub-sets and their migratory status. In our experiments, labeled lymphocytes can be followed in vivo *for over 30 days. Traffic alterations may explain some of the abnormalities in immunodeficiency states.*

There are several aspects of lymphocyte migratory behavior which may be significant to the pathogenesis of immunodeficiency states. The headings which follow in this text illustrate some of these relevant aspects.

It has been recognized for over 20 years (1) that antigenic challenge can alter the magnitude and patterns of lymphocyte traffic. Furthermore, viruses are particularly effective at modulating this migration (2). Recent evidence indicates that host-derived factors (cytokines) and not viruses directly, are responsible for these effects (3,4). Since we now know that lymphocyte traffic through all tissues is not the same and that different lymphocyte sub-sets have different migratory properties, more specific examples with relevance to AIDS can be cited (5-7).

The process of lymphocyte recirculation

The physiological traffic of lymphocytes between blood and lymph is more fully described in sheep than in any other species. Most of these traffic studies have been a direct consequence of experiments using chronic lymph drainage techniques described by Morris and colleagues (1,8). For example, this traffic has been measured in a variety of tissues like skin (9-

12), bowel (5,6,13), thymus (14), liver (15), kidney (16), ovary/uterus (17), testis (18), and lung (19,20). Various types of immune and inflammatory lesions have been studied such as chronic granulomas of skin (6,11,21), delayed hypersensitivity lesions (10,22), skin and organ allografts (23,24), hydronephrotic kidneys (21), aerosol challenged lung (20) and bacterial-induced lesions (25-27).

A standard lymphocyte circulation experiment involves the surgical implantation of lymphatic catheters in a variety of efferent or afferent lymphatics, labelling of lymph-derived cells *in vitro* and then their subsequent return to the blood. Such cells are quite rapidly cleared from the blood and they appear to take some time to traverse microcirculatory beds. Some reappear in the lymph within an hour but the greatest number reappear in lymph about 1 day after they are injected into the blood.

Most of this blood to lymph migration occurs within lymph nodes and, because of the large mass, the mesenteric lymph nodes accommodate the greatest traffic. Experiments performed on popliteal lymph nodes have shown that under resting conditions, only 2-4% of the cells in efferent lymph are formed within the node and that only about 5-10% of efferent lymph cells can be accounted for by measuring the input from afferent lymph (28). It seems likely that most of the lymphocytes entering via efferent lymph actually do traverse the node and enter afferent lymph but there is little experimental data on this issue (29). It is certain that the monocytes/macrophages in afferent lymph do not successfully negotiate passage to efferent lymph. They probably become fixed cells and some of them undoubtedly play an important role in antigen presentation. Some of these macrophages have high levels of MHC Class II antigens on their surface and are probably derived from Langerhans cells in the skin or connective tissues (30,31). The relative proportion of lymphocytes to monocytes is about the same in afferent lymph as it is in the blood (approximately one monocyte for every 10 lymphocytes). Perhaps the mechanisms responsible for this low grade emigration of mononuclear leukocytes from the blood are similar for both of these cell types. It is not known whether monocytes leave the blood in lymph nodes in a similar ratio to lymphocytes.

There is a possibility that mesenteric lymph nodes have a different makeup of incoming and exiting cells. The cell concentration in afferent intestinal lymph is higher than that from most other tissues (32,33) and there has been speculation that the blood to lymph traffic within the lymph node is somehow regulated by the magnitude of the input from afferent lymph. A high afferent input may lead to a reduction in the traffic across the postcapillary venules within the node (34). Studies in rodents have shown that lymph nodes deprived of afferent lymph input do not have as much traffic across the postcapillary venules (35).

Experiments by Smith, Cunningham, Lafferty, and Morris (36) showed that an immune response could be confined to a single lymph node if the efferent lymph was diverted from the body. Such a lymph node would undergo a secondary immune response if challenged while the other nodes in the same animals showed no evidence of being primed. Such experiments strongly support the conclusion that recirculating memory cells do not migrate directly back into the blood and must be disseminated via the lymphatic system. Even more convincing data was obtained by Chin and Cahill (37) by directly labelling lymph nodes with fluorescein isothiocyanate, draining the efferent lymph and showing that labeled lymphocytes could not be found outside of the labeled lymph node and its efferent lymph.

During vigorous immune responses large numbers of stimulated lymphocytes were recovered in efferent lymph (25,38). In some examples, 40% of the cells were blasts and the majority of these contained specific antibody (39). The magnitude of the immune response disseminated in the lymph was greater than the residual response in the lymph node.

Selective migration pathways

There is a selective migration of certain lymphocytes through the gut-associated lymphoid tissue as described by Cahill et al (5). This is due to small (6) T cells, both $CD4^+$ and $CD8^+$ cells (7) and the proportion of selectively migrating cells can be enriched by following the same cells on successive journeys from blood to lymph (40). A large difference exists between sub-population migration through the gut and through the skin (6). Although blast cell migration to mucosal surfaces in different tissues may be similar (20,41) the situation with small lymphocytes is less clear (20,42). Is the traffic through the post-lactating mammary gland comparable to migration through the gut? This needs to be directly tested.

Using a quantitative lymphocyte localization assay (43), Teare (unpublished observations) has shown that lymphocyte entry into lesions in the skin and joint synovium are comparable to one another but quite different from the entry into lesions in the bowel or cecum.

Differences in the migratory properties of lymphocyte sub-sets

The distribution of lymphocyte subsets defined by monoclonal antibodies directed against cell surface antigens is different for different regions like blood, lymph nodes and afferent versus efferent lymph (44-46). Surface Ig positive cells (B cells) make up about 25% of the lymphocytes in efferent lymph and only about 6% of the lymphocytes in afferent lymph (47). Experiments have been designed which directly test the redistribution of recirculated lymphocyte sub-sets. These were done by determining the phenotype of a collection of lymph cells (with respect to CD4 and CD8). A separate aliquot of the same cells was directly labeled with fluorochrome and returned to the blood. As they subsequently re-entered lymph over the next hours or days their phenotypic analysis was carried out. Regardless of the lymph compartment from which the cells were originally obtained for *in vitro* labeling and regardless of the lymph compartment into which the labeled cells recirculated, the CD4/CD8 ratio of the recirculated population was consistently and, in some cases, an order of magnitude greater than the CD4/CD8 ratio of this starting population. This suggests that small $CD4^+$ T cells migrate more efficiently or with different kinetics than small $CD8^+$ T cells (7). This redistribution of sub-sets could not explain the tissue-specific patterns already described (5,6) (Abernathy, NJ, JB Hay: The non-random migration of CD4 and CD8 cells is not related to tissue specificity. Submitted for publication).

Experiments by Hopkins et al (48) have determined the proportions of CD4/CD8 cells in lymph during immune responses. $CD4^+$ cells predominate early relative to late in the response. The opposite occurs with $CD8^+$ cells. Cahill and colleagues have proposed that there are at least two types of receptors for lymphocytes on the surface of vascular endothelial cells: one set to convey tissue specificity and a second set to confer sub-set specificity (49).

Molecules which modify lymphocyte migration

Specific molecules which promote the extravasation of neutrophils have been known for many years. In many cases potential mediators (like C5a or IL-1) modified the behavior of neutrophils in chemotactic chambers *in vitro* and also promoted extravasation *in vivo* (50,51). However, the correlation between *in vitro* and *in vivo* data was not always seen. For example, endotoxin is not chemotactic for rabbit neutrophils *in vitro* but it is extremely potent *in vivo* (52). The production of secondary molecules like IL-1 and TNFα almost certainly explains the data obtained following endotoxin challenge *in vivo* (53,54).

Although there are reports of *in vitro* experiments describing lymphocyte chemotaxis, this has been more complicated

and controversial than the situation with neutrophils (55). Our laboratory therefore has standardized an objective, quantitive radiolabeled-lymphocyte extravasation assay *in vivo* in sheep (43). To summarize briefly--1) only lymphocytes are labeled with 111-indium, 2) there is insignificant cell-free radioactivity, 3) acute inflammatory reactions do not accumulate labeled lymphocytes, and 4) entry into delayed hypersensitivity lesions is high and the kinetics of accumulation complement data obtained by microscopy. After screening a variety of molecules such as f-met-lev-phe (fmlp), complement components, interleukin (IL)-1, IL-2, and prostaglandins, we were unable to promote lymphocyte extravasation (Kalaaji, AN, JB Hay, unpublished observations). More recently, however, Kalaaji (3) has obtained interesting results with recombinant bovine interferon (IFN) gamma and particularly with tumor necrosis factor (TNF)α (but not ß). TNFα from either bovine or human sources was potent. Following intradermal injections the greatest extravasation was found near 12-15 hours later. The same sites could be restimulated to accumulate lymphocytes (unlike the situation of tachyphylaxis reported with neutrophils) (56). The local administration of cyclosporin A could inhibit lymphocyte localization in tuberculin reactions (O'Hara, LS, W Phillipson, WJ Hunter, et al: Inhibition of lymphocyte entry into dermal DTH lesions by the local administration of cyclosporin A. manuscript in preparation) but not in TNFα injected sites. We therefore conclude that TNFα is potentially a significant mediator which promotes lymphocyte traffic *in vivo*, possibly by increasing the adhesiveness of postcapillary venule endothelial cells for blood-borne lymphocytes. This has also been observed in other species (57,58). In other experiments we (59) and others using sheep (4) have found that IFNα is potent at preventing lymphocytes from leaving lymph nodes in the efferent lymph but not at preventing their entry into lymph nodes from post capillary venules. These molecules may be useful as immunological adjuvants; for example, it may be feasible to recruit lymphocytes to molecules such as TNFα and to retain them at these sites with the other molecule (IFNγ). Further experiments in the area may be informative.

Other combinations of potential mediators have produced some interesting results. Vascular permeability and neutrophil accumulation can be greatly enhanced by producing a local hyperemia (60,61). Vadas (62) and Borgs (63) showed that PGE_2 alone could significantly enhance the blood flow to skin test sites. When PGE_2 was injected into delayed hypersensitivity lesions the blood flow to the lesion could be enhanced. However, when lymphocyte localization was measured simultaneously the increased blood flow (and therefore labeled lymphocyte delivery) did not enhance lymphocyte localization--this is quite contrary to experiments showing the enhancement of neutrophil accumulation and enhanced vascular permeability when PGE_2 is mixed with other mediators (61,62).

Lymphocyte-endothelial cell interactions

Some investigations have been led to the conclusion that neutrophil-endothelial cell interactions are more important than chemotactic gradients in promoting neutrophil extravasation (53,54). Since the electron-microscopic observations of Marchesi and Gowans (64) and others including Morris (24), a crucial role for the endothelial cell has been implicated to explain lymphocyte extravasation. More recently, using frozen sections [Woodruff assay (65)], an array of molecules, some termed vascular addressins, have been characterized which appear to be highly relevant to lymphocyte-endothelial interactions (66).

In our laboratory, Abernethy (67) has devised a procedure to isolate and grow microvascular endothelium from lymphoid tissue. In addition, endothelial cells derived from large vessels and lymphatic vessels from sheep are being cultivated. The exposure of these endothelial cells to TNFα *in vitro* enhances their

Table 1
Conclusions From Lymphocyte Traffic Experiments Involving Sheep Lymph

1. Most lymphocytes are rapidly cleared from the blood (11,12).
2. The highest concentration of intravenously-injected labeled cells reappears in lymph near 21 hours later (11).
3. The kinetics of blood to lymph traffic of lymph cells are similar for: efferent and afferent lymph, large and small lymph nodes, normal compared with chronically inflamed skin (5-7,11).
4. Following antigenic stimulation of a lymph node, specific antigen-reactive lymphocytes temporarily disappear in lymph and then exit in greatly increased numbers at the end of an immune response (68).
5. The output of antibody-forming cells in lymph can surpass the numbers of antibody-forming cells found in the stimulated lymph node (38,39).
6. Potential mediators like lymphokines, interferons and arachidonic acid metabolites can be recovered in lymph following appropriate stimulation (10,69,70).
7. The proportions of T and B cells are different in efferent and afferent lymph (44).
8. There are selective patterns of migration through different tissues (5-7).
9. T cell sub-sets are unequally distributed between blood, afferent and efferent lymph (44-46).
10. $CD4^+$ cells differ from $CD8^+$ cells in their migratory capacity (7).
11. Blood vascular and lymphatic endothelial cells can be cultivated *in vitro* and the lymphocyte binding to such cells can be enhanced by treating the endothelium with tumor necrosis factor alpha (67).
12. TNFα and IFNγ promote the extravasation of lymphocytes (3).
13. IFNα inhibits the exit of lymphocyte from lymph nodes but not the entry into the nodes (4,59).
14. Blood to lymph traffic of lymphocytes is significant in the fetus (71,72).
15. New cell tracking labels permit the tracking of lymphocytes *in vivo* for 2 months or longer (Teare and Hay, see text).
16. Most cells in efferent lymph are derived from the blood via post-capillary venules within the lymph node (28).
17. In normal efferent lymph very few lymphocytes are synthesizing DNA (28).
18. Macrophages in afferent lymph do not transverse the lymph node to appear in efferent lymph (1).
19. Cells responsible for immunological memory exit lymph node via efferent lymph and not via the blood (36).
20. Lymphocytes in lymph nodes do not gain access to the blood except via the lymphatic system (36,37).
21. A proportion of afferent lymph macrophages have high levels of surface MHC Class II, are effective antigen presenting cells and are the Langerhans' cells observed in connective tissues and skin (30).
22. The macrophages in afferent lymph from the liver are probably mobile Kupffer cells (15).
23. T cells bearing the γ/δ T cell receptor constitute greater proportions of the T cells in subcutaneous afferent compared with efferent lymph (44-46).
24. Peyer's patches are the primary source of B cells in sheep (13,73).
25. During the rejection of a renal allograft 5 liters of lymph and a mass of lymph cells approximately ½ the weight of the kidney can be recovered in afferent renal lymph (24).
26. Post capillary venular endothelium need not be "high" in order to accommodate significant lymphocyte traffic (74).
27. Fetal thymectomy produces immunological deficits including the incomplete development of Peyer's patches (75,76).
28. Lymph nodes extract about 25% of blood-borne lymphocytes which enter (77).
29. Local injections of cyclosporin A can suppress lymphocyte traffic in delayed hypersensitivity lesions but not in TNFα traffic sites [Kalaaji and Hay; O'Hara, Phillipson, Hunter, et al (see text)].
30. There are vast numbers of lymphocytes entering cell cycle immediately after birth (13,78).

lymphocyte-binding capacity (40, Borron, P, unpublished observations). Although it is attractive to hypothesize that this is the *mechanism of action of TNFα in vivo*, direct evidence of this is not available.

New directions

It is reasonable to conclude that our understanding of the mechanisms regulating lymphocyte traffic are clearer now than they were prior to performing the experiments summarized in *Table 1*. One senses progress and an expansion of relevant knowledge even though there are many examples of intriguing but, as yet, poorly understood biological phenomena. However, many fundamental aspects concerning the migratory behavior of lymphocytes remains unknown. For example, how long do these sub-sets of lymphocytes live? Are there lymphocytes which remain in the blood and do not move freely between blood and lymph? What are the consequences of viral infection on the migratory capacity of lymphocytes? Can therapeutic benefits be derived from promoting or suppressing lymphocyte traffic in well-defined regions or tissues?

One new development which may promote new experimentation and the approach to such questions in humans is the availability of new cell-tracking labels. Over the years we have followed lymphocytes labeled with 51-Cr, 111-In, fluorescein isothiocyanate and substituted rhodamine isothiocyanate. By standardizing the labeling conditions all of these labels yield comparable results (12). However, these labels are essentially protein-binding compounds and since proteins associated with lymphocytes turn over, the cells lose intensity after a few days. Recent data obtained in our laboratory using dyes from Zynaxis Cell Science which incorporate into the plasma membrane indicates that lymphocytes can be tracked in sheep *in vivo* for months (Teare, GF, JB Hay, unpublished observations). Chronic experiments involving the steady state-analysis of lymphocytes could be very important in immunodeficiency states as well as in a variety of immune-related diseases.

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