

Lymphoid Cell Migration Between Distant Lymph Nodes in Mice*

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Migration, circulation and recirculation of lymphocytes are important features of the immunologically active system (review: 1). While the existence of such a cell traffic between various lympho-reticular organs via the lymph and blood stream is well established the magnitude of this process with respect to 1) different types of lymphocytes, 2) readily and less readily mobilizable pools of lymphoid cells (2) and 3) inter-organ exchange, needs further clarification.

Lymphoid cell migration from the blood to lymph nodes and back to the blood by lymphatic vessels has been studied in mammals by different labeling techniques and thoracic duct drainage (review: 3). In larger animals cannulation of the efferent lymphatics of peripheral lymph nodes can be used successfully for similar purposes (4). Other methods suitable for studying cell migration include 1. extracorporeal irradiation of the circulating blood (5) or lymph (6); 2. shielding of lymphoreticular organs during ionizing whole body irradiation (7), or local irradiation; 3. temporary clamping of blood vessels during *in vivo* labeling with radioactive substances (review: 8); 4. parabiosis between labeled and unlabeled partners (9, 10); 5. injection of radioactively (11) or chromosomally (10) labeled lymphoid cells obtained from particular organs; and 6. regional labeling of lymphoreticular organs with thymidine-³H (12) or other tritiated nucleosides (13).

Each of these methods presents particular advantages and disadvantages. For instance, thoracic duct drainage in small animals may introduce complicating factors such as additional stress; handling of cells *in vitro* may be harmful for specially sensitive elements; injection of cells does not necessarily simulate natural circulation; labeling with thymidine-³H will mark only those cells which during availability of the radioactive precursor are in DNA synthesis; cells with marker chromosomes are only recognizable while in mitosis.

The present study was undertaken to evaluate in mice the magnitude of lymphocyte exchange between distant lymph nodes with as little interference with physiological conditions as possible. Cytidine-³H was injected into the foot pad of a hind leg in an attempt to obtain preferential labeling of the regional lymph nodes, and the appearance of more heavily labeled lymphoid cells in the contralateral axillary lymph node was

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followed as a function of time after application of the radioactive nucleoside. Although cytidine- ^3H may serve as a precursor of both DNA and RNA and, therefore, is not ideal for stable cell labeling, it was chosen because, unlike thymidine- ^3H (14), it can readily be incorporated into small lymphocytes.

Material and methods

Swiss albino mice (Hale-Stoner strain) were used in this study. All animals were weaned at 4 weeks of age. At the age of 5 months 22 females, weighing between 22.5 and 30 g, were given a single subcutaneous injection of 0.6 μc of cytidine- ^3H (Schwartz-Bio Research, specific activity 1 C/mM, concentration 20 $\mu\text{c}/\text{ml}$ 0.9% saline) into the foot pad of the left hind leg. Pairs of mice were sacrificed $3/4$, $1\frac{1}{2}$, 3, 6, 12 and 24 hours, 2, 3, 5, 7 and 10 days following injection of the radioactive precursor. The left popliteal and lumbar lymph nodes, the right axillary lymph node as well as the thymus, the spleen, Peyer's patches and additional lymph nodes were removed. Brush smears were prepared of the left popliteal and the right axillary lymph node of each animal. The air-dried preparations were fixed in absolute methyl alcohol for 15 minutes, dried, and covered with liquid film emulsion NTB-2 (Eastman Kodak Company). Autoradiographic exposure in a dark room at 4°C was carried out during 14 days. The developed, fixed and dried preparations were finally stained with Giemsa solution (1:100, pH 5.6). Additional animals were subjected to the same treatment and used for histological autoradiography.

In each smear preparation obtained from left popliteal and right axillary lymph nodes, 500 small lymphocytes were counted, and the number of silver grains over their nuclei and cytoplasm was determined. Large lymphoid cells were counted in addition. Background was corrected for by counting grains over cell-free areas of the smear which correspond approximately in size to small lymphocytes.

Results

The effectiveness of regional labeling is best documented by the difference, in mean grain counts, between large lymphoid cells in left popliteal lymph nodes and those in contralateral axillary lymph nodes. This is shown in Fig. 1 for the samples obtained $1\frac{1}{2}$ hours after injection of cytidine- ^3H . The mean labeling intensity of lymphoblasts in regional lymph nodes exceeds that of comparable cells in distant nodes by a factor of 24.4. It must be noted, however, that much of this label reflects incorporation of cytidine- ^3H into DNA since more than 50% of the type of large lymphoid cells considered for this comparison show initial labeling with thymidine- ^3H . Of particular interest is the fact that at this time interval no large lymphoid cell in the right axillary lymph node was found to be covered by more than 4 grains.

The total grain count per 1000 small lymphocytes is represented separately for the left popliteal and right axillary lymph nodes in Fig. 2, as a function of time after injection of cytidine- ^3H into the foot pad of the left hind leg. The initial total grain number per 1000 small lymphocytes of the regional lymph node exceeds that of the same cell type in the distant node by a factor of approximately 6 if the samples taken $1\frac{1}{2}$ hours after injection of the radioactive precursor are compared. With

increasing time intervals the total grain count per 1000 small lymphocytes of left popliteal nodes falls to half the initial values within 2-3 days while the corresponding curve for the right axillary node shows a very slight raise within the same period of time. The difference between the observed change, with time, of the total activity in small lymphocytes of the right axillary node and the hypothetical relative change if both curves were congruent, is visualized by the dotted area. It may be noted that the curve for the labeling intensity of small lymphocytes in the left popliteal lymph node shows a considerable degree of oscillation.

Since it was found that the maximum labeling intensity of large lymphoid cells in the right axillary lymph nodes $1\frac{1}{2}$ hours after administration of cytidine- ^3H did not exceed 4 grains (Fig. 1), the appearance of more heavily labeled lymphocytes in this localization was followed as a function of time after injection of cytidine- ^3H . The results are summarized in Fig. 3. Between 3 and 24 hours after injection of the radioactive precursor no more than 1-4 (average: 2.5) per thousand small lymphocytes in the right axillary lymph node show grain counts in excess of 5. At later time intervals the respective values vary from 0 to 4 (average: 2) per thousand.

Discussion

As shown by histological autoradiography, the subcutaneous injection of cytidine- ^3H into the foot pad of the hind leg leads to an intensive labeling of cells in the homolateral popliteal and lumbar lymph nodes while the initial labeling intensity per cell in other lymphoreticular organs remains weak (15). It appears that dilution of the injected labeled substance which is drained by the lymph occurs mainly when the thoracic duct lymph mixes with the venous blood. It may be assumed, therefore, that initially labeled cells with high grain counts, under the conditions of this experiment, were located in the regional popliteal and/or lumbar lymph nodes during availability of cytidine- ^3H . The evaluation of the present data is complicated by the fact that even in the lymph nodes regional to the site of injection of the radioactive nucleoside, many small lymphocytes show very weak initial labeling intensities, particularly in so-called primary follicles. The appearance with time, after the injection of cytidine- ^3H into the foot pad of one hind leg, of more heavily labeled

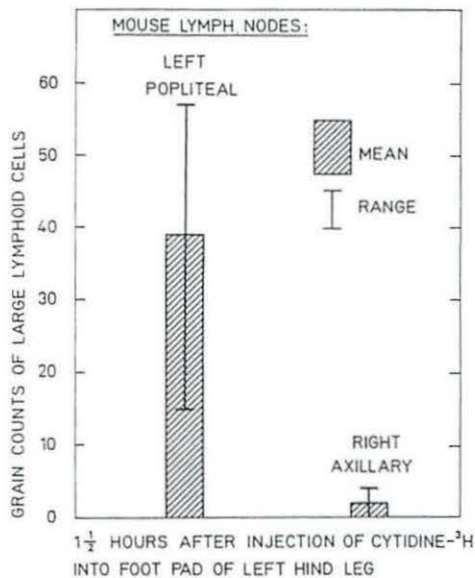


Fig. 1 Labeling intensity of large lymphoid cells in left popliteal as compared to right axillary lymph nodes $1\frac{1}{2}$ hours after a subcutaneous injection of cytidine- ^3H into the foot pad of the left hind leg.

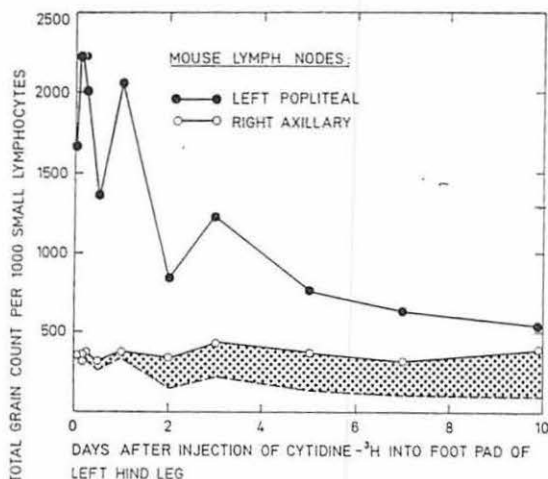


Fig. 2 Total grain counts (not corrected for background) per 1000 small lymphocytes in the left popliteal as opposed to the right axillary lymph nodes, as a function of time after injection of cytidine- ^3H into the foot pad of the left hind leg. Dotted area: difference between the observed grain counts in right axillary lymph nodes and hypothetical grain counts, if the lower curve were congruent to the upper.

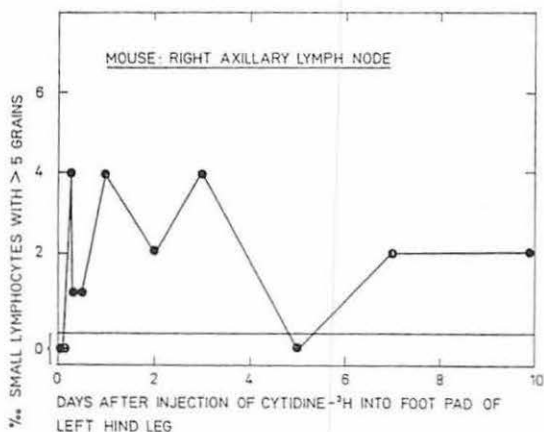


Fig. 3 Number of more heavily labeled small lymphocytes in right axillary lymph nodes as a function of time after a subcutaneous injection of cytidine- ^3H into the foot pad of the left hind leg.

thesis of the existence of a rapidly exchanging fraction of small lymphocytes. Time intervals later than 24 hours are of lesser value in this respect since already two days after injection of the radioactive precursor, the mean labeling intensity of small lymphocytes in the homolateral regional lymph node has fallen to approximately half its initial

small lymphocytes in the contralateral axillary lymph node must be considered with these restrictions in mind. The registered number of such cells most probably represents a *minimum value* and cannot be regarded as reflecting the actual magnitude of lymphocyte exchange between distant lymph nodes. It may be concluded from these observations that at any time interval from 3 to 24 hours after labeling with cytidine- ^3H , at least an average of 2.5% of small lymphocytes in contralateral axillary lymph nodes originated from lymph nodes regional to the site of injection of the radioactive precursor. One popliteal and one lumbar lymph node together are equal to approximately 4% of the total mass of extrathymic lymphoreticular tissues in mice (16). If one assumes an influx into the axillary lymph node of small lymphocytes from various extrathymic lymphoreticular organs according to the relative mass of these organs, at least 6.25% of the small lymphocytes contained in an axillary node would have reached this site via the blood stream within 3–24 hours prior to sampling. As stressed above this percentage must be accepted as a minimum value.

It may be pointed out that the relative number of more heavily labeled small lymphocytes found in the axillary lymph node between 3 and 24 hours after injection of cytidine- ^3H into the contralateral foot pad did not increase with time. This finding is consistent with the hypo-

value. Autoradiographic inefficiency will therefore become more important as time intervals are extended beyond 1 day.

An estimate of the *maximum values* for lymphocyte exchange between distant lymph nodes may be obtained by comparing the total uncorrected grain counts per 1000 small lymphocytes in the left popliteal and right axillary nodes, as a function of time after injection of cytidine-³H into the left hind leg. The respective curve for the left popliteal lymph node, as shown in Fig. 3, is the result of various processes such as 1) dilution of label by RNA metabolism, 2) reutilization of label, 3) appearance of more heavily labeled small lymphocytes originating from large lymphoid cells which were in DNA synthesis during availability of cytidine-³H, 4) emigration of labeled elements, and 5) influx of weakly labeled lymphocytes from the blood. For a number of reasons (17) the decrease of the mean grain count of lymphocytes with increasing time following injection of radioactively labeled pyrimidine nucleosides cannot be expected to represent a single exponential function. The results presented in Fig. 2 indicate that the observed grain numbers per 1000 small lymphocytes in the right axillary lymph node from 3 to 24 hours after injection of cytidine-³H into the left hind leg are slightly higher (by 40 grains or 12% on the average) than would be expected if the relative changes in total grain counts were the same in both lymph nodes. It is conceivable that this difference at least in part is due to influx of label from the more heavily labeled nodes. If this assumption were correct, the distant lymph nodes would contain, from 3 to 24 hours after injection of the labeled substance, approximately 1.8% of the initial radioactivity in the regional node in addition to its own initial activity. If we again extrapolate this value to all the extrathymic lymphoreticular organs, the latter could contribute by hematogenous influx as much as 45% of the small lymphocytes in the axillary node in less than 24 hours. As mentioned above, this value probably represents a maximum since the data are based on grain counts that were not corrected for background, and since label not contained in living cells may have reached the distant lymph nodes. Background will tend to produce a more pronounced relative supplement in grain counts of small lymphocytes in distant than in regional nodes. Further experimentation is necessary to clarify this point. Possible regional differences in the magnitude of lymphocyte exchange also remain to be examined. It is noteworthy in this context that both popliteal and axillary lymph nodes are subject to relatively little antigenic stimulation and, accordingly, belong to the so-called "oligosynthetic" lymphoreticular structures (18).

Summary

Cytidine-³H was injected into the foot pad of a hind leg in mice in an attempt to obtain preferential labeling of regional lymph nodes and to follow autoradiographically the appearance with time of more heavily labeled lymphocytes in distant lymph nodes. The results indicate that between 0.25 and 1.8% of the small lymphocytes contained in contralateral lymph nodes have reached this site within 3-24 hours from lymph nodes regional to the site of injection of the labeled nucleoside. The findings are consistent with the existence of a fraction of rapidly recirculating lymphocytes.

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Lymphatics of Blood Vessels

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Lymphatics of blood vessels

In recent years a number of investigations of the anatomy of the vasa vasorum of major blood vessels have been made (1, 2, 3, 4), but few observations of the lymphatic component of vasa vasorum have been recorded. Investigations of lymphatics of blood vessel walls were reported nearly one hundred years ago (5), and the anatomical findings apparently were unclear which resulted in considerable debate among contemporary investigators regarding what had been observed. Most of the controversy