

Immunologic Characteristics of Human Peripheral Lymph Cell Populations

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Summary

Cell populations in afferent lymph of human leg were defined by surface characteristics and cytotoxic activity in 7 normal men and 9 patients with localized cancer. A higher percentage of E-rosette forming cells was found in lymph (78.5 and 83.0) than in blood (60.0 and 63.0, $p < 0.05$). The percentages of lymph EA-RFC were 10.3 and 18.0, of EAC-RFC 13.1 and 8.0, of surface immunoglobulin carrying cells 3.0 and 3.1. In blood 20.6 and 18.0 percent of cells formed EA-rosettes, 23.0 and 15.6 EAC-rosettes, 5 and 9.5 contained surface immunoglobulins. The differences between lymph and blood EA- and EAC-RFC in normals were statistically significant ($p < 0.05$). In cancer patients only lymph-blood differences for S Ig⁺ were significant ($p < 0.05$). No significant differences were found between normals and cancer patients. In both groups, the natural cytotoxicity against K 562 cells was 6 times lower in lymph as compared to blood ($p < 0.05$), the cytotoxicity in those with cancer was higher than in normals ($p < 0.05$). The study indicates that B cells have a limited tendency toward leaving the blood circulation and migrating through tissues. Moreover natural killer cells do not seem to belong to the recirculating pool of lymphocytes.

There are two types of traffic of lymphocytes from the blood vascular system into lymph. In one lymphocytes continuously leave the circulation through the postcapillary venules in lymph nodes and enter efferent lymph (12). In the other lymphocytes migrate through capillary wall into non-lymphoid tissues and enter first afferent lymphatics and then lymph nodes and efferent lymph (9, 13, 18, 21). Experiments in animals have previously established that afferent lymph contains fewer cells than

efferent lymph, because the latter fluid is enriched by lymphocytes from the lymph node. Besides lymphocytes afferent lymph also contains monocytes, macrophages, erythrocytes and granulocytes (2, 10, 18). The mechanism of migration of lymphocytes through the normal capillary wall and tissue space to lymphatics in non-lymphoid tissues remains poorly understood, as is the role of these cells in tissues.

We have previously shown the feasibility of studying the cellular composition of afferent lymph in normal men (7, 11, 19). The present study was carried out to define the lymphocyte populations which selectively cross the non-lymphoid tissue barrier based on their surface and cytotoxic characteristics. In addition to normal subjects a group of patients with localized cancer was investigated to determine whether this disorder also affects the process of selective migration of lymphocytes through non-lymphoid tissues.

Methods

Studies were carried out in 7 healthy individuals and in 9 patients with cancer. The age of normals were between 20 and 21 and of tumor patients between 46 and 60. Each patient with cancer had a solid localized tumor remote from the lower extremity. These included cancer of the hypopharynx, tongue and breast. No treatment was given during the period of lymph and blood sampling. Clinically detectable edema

or inflammatory changes of the lower extremities were absent.

Collection of lymph: Lymph was collected from a superficial leg lymphatic. This vessel drained skin, subcutaneous tissue and perimascular fascia of the foot and a part of the lower leg. The technique of cannulation has been described previously (4). Lymph was collected over periods of 12 hours. Lymph cells were obtained by spinning down samples at 1400 rpm. The whole cell population which contained 95 % small lymphocytes and 5 % monocytes and macrophages, was used in testing.

Isolation of blood mononuclear cells: At the end of each lymph collection period, 5 ml blood samples were obtained from the cubital vein into syringes with 100 U. of heparin. Mononuclear cells were separated from blood by centrifugation at 1600 rpm for 35 min on Lymphoprep gradient (Nyegard Co A/S, Oslo, Norway). Cells were washed three times in RPMI 1640 (Gibco Biocult, Glasgow) and resuspended to adjust the concentration to 10^6 cells/ml.

Sheep erythrocyte rosette forming cells (E-RFC). One hundred microliters of cell suspension were mixed with 100 μ l of 0.5 % (sheep red blood cells) SRBC and 25 μ l heat inactivated, absorbed with SRBC fetal calf serum. The mixture was incubated at 37 °C for 5 min, then spun down at 1000 rpm for 5 min and followed by incubation at 4 °C for 18 hr. The pellet was gently resuspended and 200 lymphocytes were counted for rosette forming cells.

Receptors for IgG Fc (Ea-RFC). One hundred microliters of cell suspension were mixed with 1 % trypsinized SRBC coated with anti-SRBC antibody (Cordis Lab., Miami, Fla). This mixture was spun down at 1000 rpm for 5 min and followed by incubation at 20 °C for 30 min. The pellet was resuspended and 200 lymphoid cells were counted for rosette formation.

Receptor for complement (EAC-RFC): One hundred microliters of cell suspension were mixed with 100 μ l of trypsinized SRBC coated with anti-SRBC IgM antibody (Cordis, Miami, Fla) and fresh AKR mouse serum as

a source of complement. The mixture was incubated at 37 °C for 30 min and then centrifuged at 1000 rpm for 5 min. After resuspension 200 cells were counted to determine the number of rosette forming cells.

Surface immunoglobulins (SIg⁺ cells). One ml of cell suspension was spun down at 1300 rpm for 10 min. The supernatant was discarded and 100 μ l of anti-human IgG, AgA, IgM, kappa, lambda fluorescein conjugated antibodies (DAKO, Copenhagen) were added to the pellet. After 30 min incubation at 20 °C cells were washed 3 times in Eagle MEM + 0.1 % NaN₃ and resuspended in 100 μ l of medium. Two hundred cells were counted with fluorescent microscope.

Natural killer cells cytotoxicity assay. The target cells used in this assay were K 562 human myeloid cell line (obtained from Karolinska Institutet, Stockholm) grown in suspension in RPMI 1640 + 10 % FCS. Target cells (3×10^6) were labelled for 60 min at 37 °C using 100 μ Ci of Sodium Cr⁵¹ chromate (Institute for Atomenergi, Kjeller, Norway) and then washed 4 times in RPMI 1640. Labelled cells were resuspended in RPMI 1640 + 10 % FCS to a concentration 2.5×10^4 /ml. 0.2 ml of lymphocytes were added to round bottom small culture tubes together with 0.2 ml of K 562 cells to make the effector to target ratio 40 : 1, 20 : 1, 10 : 1 and 5 : 1. Control incubation in the absence of effector gave the background isotope release. Maximal release was determined in the presence of Triton X100 (1/50 dilution). All tests were done in triplicate and incubated for 18 hr at 37 °C in an atmosphere of 5 % CO₂ in air. After incubation the tubes were centrifuged at 1600 rpm for 10 min and 0.2 ml samples of supernatant were transferred to the other tubes and both aliquots counted on Intertechnique gamma counter. The percentage of ⁵¹Cr release was determined for each tube and using the mean values of the triplicate tubes the percentage cytotoxicity was calculated as:

% cytotoxicity =

$$\frac{\%^{51}\text{Cr release in sample} - \%^{51}\text{Cr release in control}}{\%^{51}\text{Cr release in Triton} - \%^{51}\text{Cr release in control}}$$

The low numbers of cells obtained from lymph limited the number of tests in 4 cases.

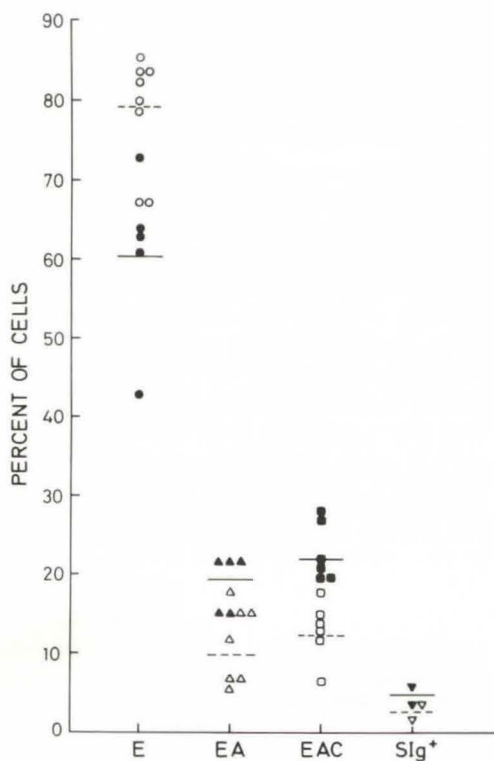


Fig. 1 Percentage of E-RFC, EA-RFC, EAC-RFC and Sig⁺ cells in afferent lymph (open signs) and blood (closed signs) in normal men. Horizontal lines represent mean values.

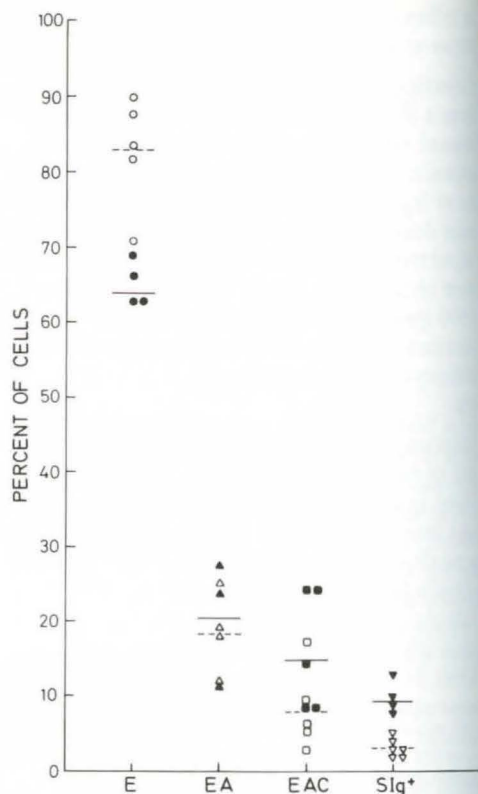


Fig. 2 Percentage of E-RFC, EA-RFC, EAC-RFC and Sig⁺ cells in afferent lymph (open signs) and blood (closed signs) in cancer patients. Horizontal lines represent mean values.

Results

Lymph flow and cell output. The mean lymph flow in normal subjects was 0.23 ± 0.11 (SD) ml/hr, in the cancer patients 0.47 ± 0.35 (SD) ml/hr. The mononuclear cell count in lymph was in the normal group 177 ± 137 (SD) cells/ μ l and the output $44.8 \pm 22.0 \times 10^3$ (SD) cells/hr, in the cancer group respectively 220 ± 157 (SD) cells/ μ l and 89 ± 90 (SD) $\times 10^3$ cells/hr. The differences were statistically insignificant.

Blood mononuclears. Blood mononuclear cell count was in normal men 3120 ± 842 (SD) cells/ μ l and in cancer patients 2848 ± 1246 cells/ μ l.

E, EA, EAC-RFC and Sig⁺ cells in lymph and blood. In lymph of normal individuals

$78.5 \pm 7.39\%$ of lymphocytes formed E-rosettes, in blood $60.0\% \pm 10.9$ ($p < 0.05$) (Fig. 1). EA-RFC were found in normal lymph in $10.2\% \pm 4.18$ and in blood in $20.6\% \pm 3.66$ ($p < 0.05$) and EAC-RFC in lymph in $13.16\% \pm 3.66$ and in blood in $23.0\% \pm 3.58$ ($p < 0.05$). The mean percentage of Sig⁺ cells in normal lymph was $3\% \pm 1.4$ and in blood $5\% \pm 1.14$ (NS). In cancer patients lymph lymphocytes formed $83\% \pm 7.42$ of E-rosettes, blood lymphocytes $63\% \pm 2.87$ ($p < 0.05$). EA-RFC were found in lymph in $18\% \pm 5.4$ and in blood in $20.6\% \pm 8.5$ (NS), EA-RFC in lymph in $8\% \pm 5.5$ and in blood in $15.6\% \pm 8.0$ (NS), and the Sig⁺ cells in lymph in $3.1\% \pm 1.2$ and in blood in $9.5\% \pm 2.65$ ($p < 0.05$) (Fig. 2).

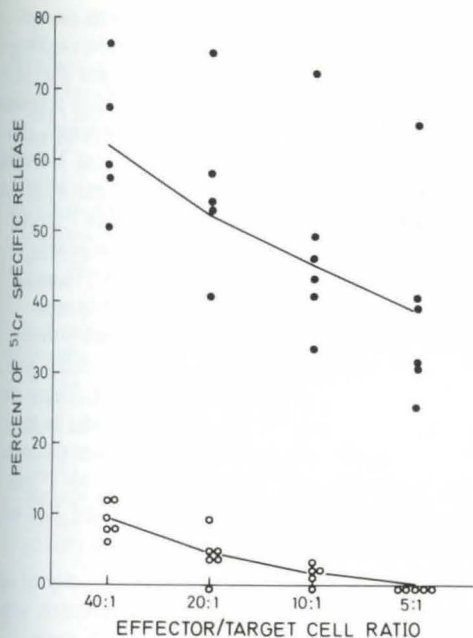


Fig. 3 Natural cytotoxicity of cells in afferent lymph (open signs) and blood (closed signs) against K 562 human myeloid line cells in normal men. The curve represents mean values at decreasing effector to target cell ratios.

There were no statistically significant differences in the measured parameters between the normal individuals and cancer patients, except of EA-RFC in lymph ($p < 0.05$).

Natural cytotoxicity. The natural cytotoxic activity of lymph lymphocytes of normal men, at the effector target ratio 40:1 was $9.8\% \pm 2.2$, of blood lymphocytes $63\% \pm 9.9$ ($p < 0.05$) (Fig. 3). In cancer patients the cytotoxicity of lymph lymphocytes was $16.4\% \pm 13.2$, of blood lymphocytes 79.7 ± 14.7 ($p < 0.05$) (Fig. 4). There was a linear fall in activity with the decrease in effector to target ratio in both groups. The mean lymph cell cytotoxicity was higher in cancer patients than in normals, especially at lower effector:target ratios ($p < 0.05$). The mean blood cell cytotoxicity was higher at all ratios ($p < 0.05$). In 2 patients the lymph lymphocyte cytotoxicity was evidently higher than in normals.

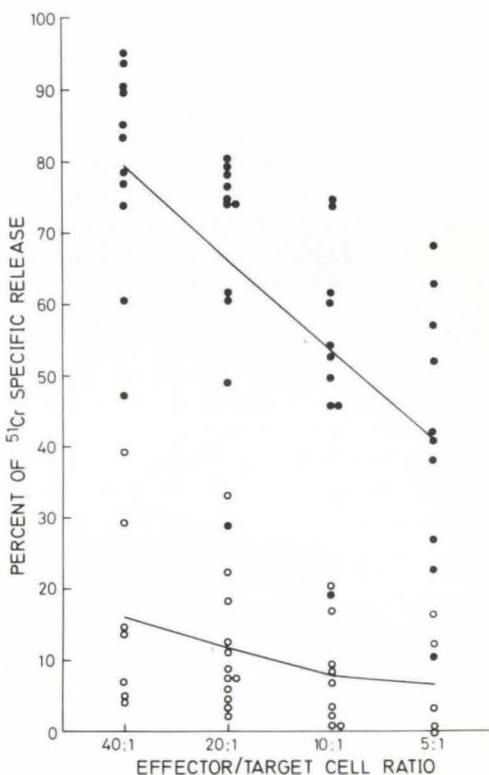


Fig. 4 Natural cytotoxicity of cells in afferent lymph (open signs) and blood (closed signs) against K 562 cells in cancer patients. The curve represents mean values at decreasing effector to target cell ratios.

Discussion

Both animal and human studies have suggested that lymphocyte migration through non-lymphoid tissues is a highly selective process. The question arises which subpopulations selectively cross the capillary membrane and enter lymphatics and what specific function do they perform in the tissues. In our previous work (11) we showed a reduced proportion of B cells, identified by immunofluorescent techniques, and an increased proportion of Ia positive lymphocytes in afferent lymph of normal men as compared with peripheral blood.

In this study we characterize further the lymphocyte population in afferent lymph by their surface markers and natural cytotoxic activity.

Because of a higher percentage of E-RFC in lymph than in blood and a lower percentage of EA-RFC, EAC-RFC and IgS⁺ cells in lymph than in blood it appears that B lymphocytes have decreased ability of escaping from the blood circulation and entering afferent lymph. Of course, it is possible that surface receptors of these cells undergo structural changes during passage through the tissues, and are no longer detectable by current techniques. These human are, however, in contrast to previous finding in animals. *Solley et al.* (20) for example found in sheep that afferent lymph contained the same percent of T cells in blood. While *Miller and Adams* (17) found that sheep afferent lymph contained approximately 6% of SIg⁺ cells, and in dogs 24% of afferent lymph mononuclear cells formed E-rosettes, 10% EA-rosettes and 12% EAC-rosettes; 7% of cells were SIg⁺, there were more E-RFC in lymph than in blood. Moreover the percentage of EA-RFC, EAC-RFC and SIg⁺ cells was equal in lymph and blood (10).

Our studies of lymph E-RFC in patients with solid tumors failed to reveal differences from healthy persons. The differences in percentages of EA-RFC and EAC-RFC between these 2 groups were also insignificant. There was also similar percentage of lymph. B cells as in normal group. These findings agree with our earlier observations of a high percentage of T cells and low percentage of B cells in patients with solid tumors (1, 3, 5, 6). The present studies revealed in addition, that in cancer patients, as well as normals, the percentage of cells possessing Fc receptor (EA-RFC) in lymph is lower than that in blood. It therefore appears that a localized tumor does not affect the process of lymphocyte recirculation in peripheral tissues distant from the tumor site. Although the natural cytotoxic activity of the whole population of lymph lymphocytes was lower than of blood lymphocytes in both normal persons and cancer patients and cytotoxicity against K 562 was slightly higher in blood and lymph of the cancer patients. We have no explanation for these differences. It is possible that age differential between the two groups is a factor. The natural killer (NK) cells are the non-adherent, non-B cells with low affini-

ty receptor for SRBC. Mot of them possess Fc receptor for Fc portion of immunoglobulin, but non for C3b (14). There are also indications that NK cells mediate in vivo resistance against tumors (15). On the other hand, conclusive evidence is still lacking for of NK cells in protecting against primary tumors of affecting the incidence of spontaneous or induced tumors (14). So far, no data are available on the natural cytotoxicity of lymph "floating cells". It is, however, known, that lymph node cell preparations exhibit notably lower natural cytotoxicity than corresponding peripheral blood preparations (15, 16). There are also findings suggesting that NK cells in human lymph nodes are different from peripheral blood NK (8). Thus, they may be T lymphocytes, Ig-negative without Fc and C3b receptors.

The finding of low natural cytotoxicity of cells in afferent human lymph in conjunction with similar findings by others in lymph nodes, tends to argue against a prominent role of NK cells in tumor surveillance. They also suggest that NK cells do not belong to the recirculating pool of lymphocytes. Unfortunately, low numbers of lymphocytes obtained from human afferent lymph limited our further characterization.

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