OPINION

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What is Lymphology – Prospects in Human Studies*

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If we asked a medical graduate what is the lymphatic system, the answer would be: The lymphatic system is an anatomical structure composed of channels, with the principal function to maintain the blood volume by returning fluid and protein molecules which leak from the blood capillaries to the interstitial space, to the general circulation. In addition, there are circulating lymphocytes and lymphoid organs playing an important role in the process of defense against infection and tumor growth.

Using this commonly accepted approach our image of the lymphatic system is limited to its structural elements like cells, vessels, organs and to the function of these elements within the system, e.g. lymph protein transport, cooperation of various lymph cell populations in the lymph node, etc. However, thinking teleologically the mission of the lymphatic system should certainly be more wide-reaching. The lymphatic system serves the whole living organism in the process of homeostasis. In what way? By maintaining a physiological environment of each individual cell in the non-lymphoid tissues of the body. This microenvironment includes the fluid and ground substance surrounding the cell, as well as the neighbouring cells with their genetically defined self. One could, th define lymphology as the biomedical discipl dealing with *problems of regulation of the lular microenvironment.* Then, the principal tasks of the lymphatic system are:

a) maintaining of a most favorable compos tion of the mobile intercellular fluid and th ground substance for the tissue cells, their tegrity and function,
b) transporting away and processing chemic products released from cells, as well as thei subcellular shedded structures, e.g. membra receptors,
c) eliminating of dying or mutant cells,
d) removing of non-self organic (e.g. bacte viruses) and inorganic (e.g. carbon, silica) p ticles entering the intercellular space from environment.

A. This is the individual cell in a living co text of the neighbouring cells and the sur ing, amorphous ground substance which is source of signals setting the lymphatic sys into motion. Let us look at the lymphatic tem as subordinate to the tissue non-lymp cells and standing under their orders. This proach has many important implications. of all it allows to study the function of th lymphatic system in the context of the li non-lymphoid cells and not as a system w ing for itself. Here are some examples:

a) Studying the process of regulation of t interstitial volume we take into considera the physical factors responsible for the ca

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lary and lymphatic transport of water and macromolecules, but disregard the possible role of the nonlymphoid cells bathing in the interstitial fluid in the regulation of its volume and composition. Beyond doubt, cells themselves can mediate chemically the capillary permeability, regulate their water uptake from the interstitial fluid, control the chemical composition of the ground substance. The regulation of the interstitial volume cannot be a one-way, *vessels-intercellular space-cells*, process, without any feedback signals from the cells.

b) There is a continuous process of leukocyte migration through the non-lymphoid tissues. These cells presumably play a principal role in recognizing and removing exogenous and mutant-self antigens and transmitting informationg about their appearance in the tissue to the higher regulating centers like lymph nodes, spleen, bone marrow. The signals for the lymphocytes to leave blood circulation and enter the tissue space originate from the parenchymatous cells or their chemical products. Thus, the regulation of lymphocyte and monocyte traffic across the non-lymphoic cells is dictated primarily by the tissue events.

c) Does the concentration of the free moving proteins of different molecular weight and their biological activity in the interstitial space depend only on the capacity of the capillary and lymphatic transport or can the parenchymatous cells also influence protein concentration around them? Over billions of years of evolution cells have been organizing their environment. Today, there is a different quantitative composition of free-moving proteins in the interstitial space compared to the blood. It is likely that cells can to a large extent control the local protein concentration not only by excreting their proteinaceous products but also by increasing influx from blood circulation by enhancing capillary permeability, e.g. in an enhanced metabolic state. These examples have been presented to substantiate the rightness of reasoning that the functions of the lymphatic system are regulated primarily by signals originating from the parenchymatous cells of the non-lymphoid tissues.

B. There is another problem concerning the understanding of the function of the lymphatic system. The anatomical territory of the lymphatic system comprises the interstitial space, lymph vessels, organized lymphoid tissue and its locomotive messengers- the migratory cells. All of these elements are functional ly interrelated. Dissociation of the lymphatic transport pathways from the lymphoid tissue, while analyzing the function of the lymphatic system, is no longer tenable.
Two examples support this notion. One, the lymphatic endothelial cells line the channels serving fluid transport, but at the same time possess the phagocytic activity, a function ascribed to the immune system. Another, the capacity of fluid transport of lymph vessels restricts the kinetics of antigen transport away from the interstitial space and its delivery to the sites of elimination – lymph nodes.

C. The lymphatic system is one of the elements of the entire organization of body homeostasis. It can not work independently of the nervous and endocrine system. It has been documented recently, that the peripheral, as well as the central nervous system, play a distinct but still largely undefined role in the immune regulation. However, the operation of afferent and efferent pathways to and from the neuroendocrine structures has thus far not received any serious consideration in relation to the function of the lymphatic system.
There are indications that the primary immune response of rats to a particular antigen, SRBC or soluble antigens is followed by several-fold increase in corticosterone levels and also temporal changes in thyroxine levels. An evident increase in firing rates of neurones of ventromedial neuclei in the rat hypothalamus after intraperitoneal injection of SRBC was observed. Presumably, this neuroendocrine mechanism functions when a critical threshold of lymphoic tissue activation is reached, sufficient to elaborate products serving as a signal to the hypothalamus. Further knowledge of the profile of hormonal responses and the neuroendocrine-lymphoid tissue and lymph transport pathways interrelation will be required.

Summarizing what was said above, the lymphatic system is an organization: a) composed of the functionally interrelated lymphoid tissue and transportation pathways, b) operating for maintaining of a proper environment of the non-lymphoid cells in the organized tissues and securing their genetically restricted self, especially those tissues having contact with the outer world like skin, gut and lungs, and c) integrated with the nervous and endocrine systems.

Which are the main problems we should work on in order extend our knowledge of the *in vivo* function of the lymphatic system? These are:

1) maintaining of an appropriate chemical and physical environment for cells in the non-lymphoid tissues with special emphasis on the regulatory signals released from these cells,
2) function of the immune cells migrating through the non-lymphoid tissues,
3) cooperation of the immune cells at various regulatory levels *in vivo* (lymph nodes, spleen, bone marrow, etc.),
4) integration of the regulatory processes of the lymphatic, nervous and endocrine systems,
5) more emphasis on studies in humans with the non-invasive techniques.

In order to go into more details and ask mor specific questions I show a trivial picture illu trating the functional anatomy of the lymph tic system (Fig. 1). Several physiological que tions may be asked with regard to the event in its various anatomical regions. The entire system has been divided into four interrelate functional areas. The basic one named "com munity" illustrates a normal tissue with a blood capillary and an initial lymphatic and a group of parenchymatous cells. The cells lead an intensive life restricted by supplies and transport away of waste products and co trolled by immigrant immune cells. There ar proteins of various molecular weight in plas and interstitial fluid presented as suitcases a bags and mononuclear cells and some poly morphs also carrying on their surface specifi proteins. The protein molecules are transpo ed through the capillary membrane which a as a molecular sieve. What is the biological a tivity of these proteins in the interstitial sp which is, no doubt, influenced both quantit tively and qualitatively by the restricted tra port across the capillary membrane? How does the capillary membrane and the groun substance organization affect the transport antibiotics and chemotherapeutics to the ti sue fluid? Which are the subcellular organe and membrane fragments from the disinteg

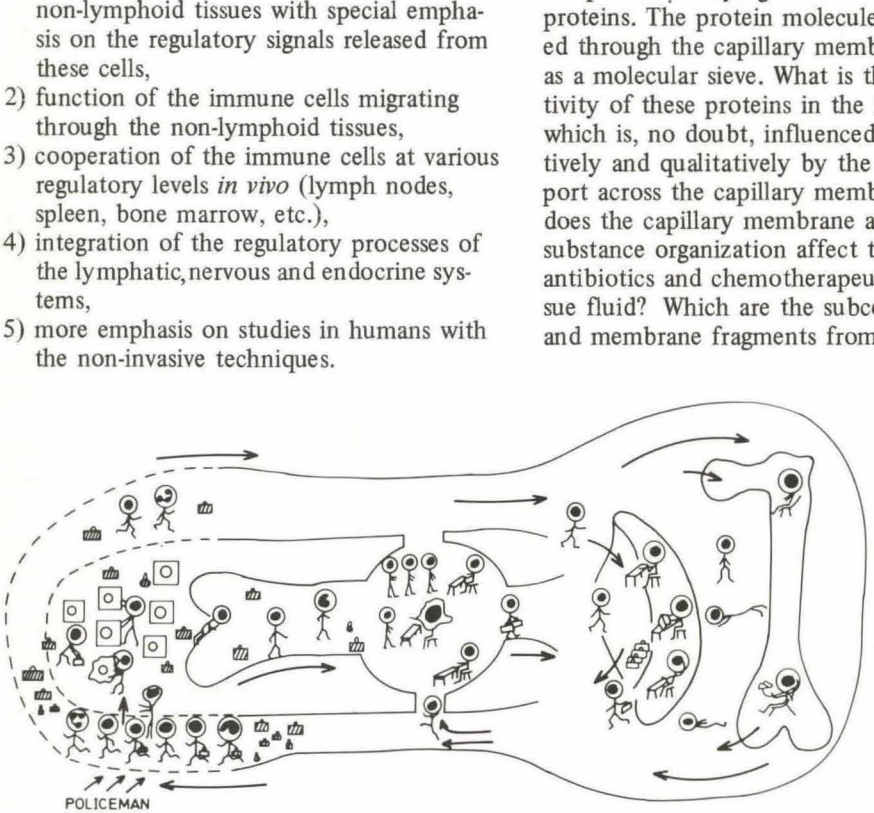

Fig. 1

ed dead cells released into the tissue fluid and lymph? Do these cellular elements, as well as the chemical substances released from cells act as signals informing the higher regulatory centers via lymph stream about the tissue integrity?

There are leukocytes in blood capillaries which look morphologically much alike. However, they are at various stages of maturity and possess different functional capacities. Howe could we characterize them functionally? Which cells leave blood circulation heading for the interstitial space and initial lymphatics? What is their function? Do they eliminate or limit spread of neoplastic cells? What are the forces moving the free interstitial fluid into initial lymphatics and further along the prenodal lymph vessels. The speed of lymph flow does not only mean the speed of fluid movement, it is also the quickness of transmitting of immune information with shedded antigen, primed phagocytes and lymphocytes from the tissue to the regional lymph node and other lymphoid organs.

Proteins and cells travel along lymphatics to the lymph nodes (district council), and once they reached blood circulation, also to the spleen (ministry) and bone marrow (parliament) (Fig. 1). One of the principal events occurring in the lymphatic organs is communication between lymphocytes. The cell communication events in the immune system is to control the correct antigen specificity and the appropriateness of the response. What do we know about the kinetics of cell communication in the lymph nodes, spleen and bone marrow? Is the spleen the principal site for elimination of particles and cells with non-self antigens or altered surfaces or rather the institution educating lymphocytes and monocytes? What is the role of the liver in lymphocyte recirculation? Does this organ only eliminate damaged cells or are there also subsets specifically migrating to the liver? Why do the lymphocytes assembly in shock or after steroid administration in the bone marrow?

We approached some of these problems in our human studies. The excerpts from our works will be presented.

Studies in humans on the function of the lymphatic system give us the most useful practical informations. The insight into the interstitial space and prenodal lymph in man can be obtained by drainage of the superficial lymphatics of the calf, the technique worked out by *A. Engeset* et al. (1973). This technique has undergone, in our hands, some refinements so that now we can obtain 10–20 ml of lymph from one vessel per day (*Olszewski* 1977a). The method of a continuous drainage of lymph from the limb has enabled the studies of the influence of physical factors like gravity, temperature and venous congestion and of local immune stimulation on lymph flow, pressures, chemical and cellular composition.

A. Immune and other biologically active proteins in the interstitial fluid and lymph. The concentration of proteins in serum is relatively stable. Also, the proportions of proteins of various molecular weight remain in serum rather constant. In the interstitial fluid and lymph the situation is different. Both, the total protein concentration and concentrations of single proteins undergo fluctuations dependent on the changing conditions of the capillary filtration, volume of the interstitial space, lymphatic transport away of tissue fluid, etc. These, in turn, depend on the capillary permeability coefficient, filtration pressures, filtration surface area, changes of interstitial fluid pressures generated by muscular contractions, contractility force of lymphatics, etc. Thus, theprotein concentration in the mobile interstitial fluid surrounding cells is not only significantly lower than in serum but also the levels of individual proteins are inversely proportional to their molecular weight (*Engeset* et al. 1977a, *Olszewski* et al. 1977b, 1977c). E.g. the average level of IgG in lymph obtained from human leg skin is 15–20%, of IgM 6–10% of that of serum. There might be also a difference in immune protein activity in tissue fluid and lymph compared with serum as for example with the C3 component (*Olszewski* et al. 1977d, 1978). The concentraion and activity of complement inhibitors in tissue fluid is higher than of the components they are acting upon (*Olszewski* et al. 1982a). Gravitional forces influence directly

transcapillary protein transport in the most dependent parts of the body. However, we have found that the regulatory mechanisms of microcirculation are extremely efficient and the change from the lying to the upright position does not increase the capillary permeability for large molecular weight proteins (*Olszewski* et al. 1977c, 1979).

In order to properly understand the events developing in the tissue space we should be constantly aware of the levels of proteins and other substances in the interstitial fluid. However, the estimation of an actual concentration of protein in the interstitial fluid based on their measurement in lymph is rather complex. This is due to the time lag necessary for the tissue fluid to reach the drained lymph vessel and collecting cannula This in turn is dependent on the rate of tissue fluid formation, tissue compliance – stress relaxation, and forces promoting tissue fluid and lymph flow. *Engeset* et al. (1979) measured in the human leg the time period necessary for the i.v. injected labelled protein to appear in lymph and the time of equilibration of labelled albumin between serum and lymph. The first appearance of labelled protein in sampled lymph was observed in less than 2h. The equilibrium between serum and lymph was reached after 26h. The patients were studied during their normal daily activities and night rest. Had we studied them lying in bed for several days, the time for equilibration would have probably been even longer. The results of this study have evident implications for understanding of the kinetics of extravascular distribution of drugs and substances bound to proteins.

B. Antibiotic penetration into tissue fluid and lymph. Prenodal lymph may be an extremely useful source of information on tissue fluid concentration of antibiotics and chemotherapeutics. Monitoring of antibiotic concentration in serum does not give reliable information on the level of drug in foci of infection. The widely accepted use of the concentration obtainable in serum by standard dosage of an antimicrobial agent as a guideline for setting of breakpoints in sensitivity and resistance of a pathogen to a drug may be unreliable. In the studies of *Bergan* et al. (1979) ampicillin showed a delayed appearance, lower peak concentrations and protracted decay in lymph as compared with serum. Thus, the breakpoint between sensitivity and resistance of bacteria to antimicrobial agents should be set at a much higher peak concentration than that obtainable in serum.

C. Cells in human prenodal lymph.
As mentioned before human prenodal lymph contains lymphocytes, macrophages and erythrocytes (*Engeset* et al. 1977b). Which are the leucocyte populations which leave blood circulation and enter the tissue space in the non-lymphoid tissues? What is their function? Can they control cancer cell metastases?

Lymphocytes migrate actively across the non-lymphoid tissues. The cell movement is accomplished by three closely correlated mechanisms: generation of driving force, adaption of cell shape, and formation of attachment sites. There are changes in direction of locomotion of locomotive cells, short tracks and reduction of speed imposed by the texture of their environment. But the main question remains in what tissue compartment the cells actually locomote. There are no empty spaces and what appears as such contains a network of proteoglycans associated with collagen. Is propulsion by mere physical pressure generated by locomoting cells sufficient, or lysis of host constituents is necessary. The tempo of migration of lymphocytes through the tissue to lymphatics is different from that of red. There is a relatively high concentration of lymphocytes in lymph when the lymph flow is low, quite opposite to red blood cells. This indicates that lymphocytes have their own tempo of active migration, and as we know from more detailed studies is relatively constant throughout the 24 hrs (*Engeset* et al. 1977b).

There appears to be some selection of migrating cells at the level of the capillary bed, which certain cell types are selected to enter the tissue space and lymphatics (*Engeset* et al. 1974, 1977c, *Godal and Engeset* 1978). Cell populations in afferent lymph of human

leg were defined by surface characteristics and cytotoxic activity in normal men (*Lukomska* et al. 1981). A higher percentage of E-rosette forming cells was found in lymph (78.5) than in blood (60.0). The percentage of lymph EA-RFC was 10.3 of EAC-RFC 13.1 and of surface immuno-globulin carrying cells 3.0. In blood 20.6 percent of cells formed EA-rosettes, 23.0 EAC-rosettes, 5 contained surface immunoglobulins. The differences between lymph and blood EA- and EAC-RFC were statistically significant ($p < 0.05$). The natural cytotoxicity against K 562 cells was 6 times lower in lymph as compared to blood ($p < 0.05$). 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.

We made also use of the monoclonal antibodies, characterizing the cell populations which migrate into the normal human skin and which having traversed the tissue, could be recovered from the afferent lymph vessels (*Olszewski* et al. 1982b). Significant differences were apparent between the types and proportions of cell populations in lymph and blood. The percentage of $OKM1^+$ cells (monocytes, null cells) in lymph was low when compared to that of blood. It may be noted that the OKM1 antibody did label only about 40 percent of the large mononuclear macrophage-like lymph cells supposed to be Langerhans cells. The percentage of $OKT3^+$ (T cells) in lymph was higher than in blood as was that of the $OKT4^+$ (inducer/helper) subset, while cells of the $OKT8^+$ (suppressor/cytotoxic) subset were found to be more numerous in blood. The $OKI\ a1^+$ cell population consisted of large veiled mononuclear cells and only very few small cells. The former were not detected in blood. Surprisingly, the large mononuclear cells present in lymph reacted with OKT6 antibody specific for cortical thymocytes. The discovery of high proportions of T cells, cells bearing Ia-like antigens, and a high inducer/suppressor ratio in normal human prenodal lymph reflects the intensity of "physiological" immune processes in the skin.

In another project we investigated the responsiveness to polyclonal mitogens of cell populations which could be recovered from the afferent lymph vessels (*Olszewski* et al. 1982c). A high spontaneous transformation rate of lymph mononuclear cells was observed after they have been shortly cultured *in vitro*. The ^{3}H-TdR incorporation was after a 24 h culture 2-times, and after a 72 h culture 5-times higher than by the PBM cells of the same subject. The lymph cells responded actively to very low concentrations of PHA, ConA and PWM. These concentrations were too low to activate the PBM cells. The mitogen concentration response curves of lymph cells were significantly higher and reaching peaks at other concentrations than of PBM cells. The population revealing the high autotransformation rate was found to belong to the $OKT4^+$ – enriched subset (inducer/helper). This subset had also the highest rate of incorporation of ^{3}H-TdR after stimulation with PHA. The findings of a high spontaneous activation of lymphocytes which trafficked through the skin and classification of this highly reactive subset as an OKT4 positive and highly responsive to mitogens, reflect the *in vivo* immune events in the normal skin. The highly reactive subset may belong to the autoreactive cells active in the autologous MLR.

Another problem of our interest in the studies on the physiology of the human lymphatic system has been the active transport of lymph in collecting vessels. We have found (*Olszewski* et al. 1968; *Olszewski and Engeset,* 1979a, b) that intrinsic contractions of human leg lymphatics generate pressure necessary for propelling lymph.

To study the efficiency of intrinsic contractions of lymphatics in propelling lymph in human legs, the end and lateral pressures and lymph flow were measured in leg subcutaneous lymph vessels in 25 normal men in a horizontal and upright position, during rest and during contractions of the foot and calf muscles (*Olszewski and Engeset* 1980). Systolic lymph end pressures generated by intrinsic contractions of lymphatics ranged between 12 and 70 mmHg, but in some cases reached values above 100 mmHg. Systolic lymph lateral pressures with free lymph flow were lower and

ranged between 5 and 30 mmHg ($p < 0.01$). There was almost no hydrostatic component of lymph pressure. Contractions of foot and calf muscles in the horizontal as well as in the upright position did not significantly effect systolic end pressures, but slightly increased lateral pressures ($p < 0.01$). There was an increase in the frequency of pulse waves ($p < 0.01$), but pulse amplitudes did not change. Lymph flow occurred in the resting human limbs and during limb muscle contractions only during the lymphatic pulse waves. There was no flow in the periods between the waves. However, the mean lymph flow was higher during muscular contractions than during the resting period ($p < 0.01$). External massaging of the foot did not produce any rise in lymph pressure, but the frequency of pulse waves and lymph flow increase. Filling of the lymphatics with lymph by external massaging or injection of fluid evoked intrinsic contractions. The pressure threshold for evoking contractions varied in different subjects from 5 to 25 mmHg. We have drawn the conclusion that intrinsic contractions of lymphatic collectors in the human leg is the main factor responsible for lymph flow during rest of the extremity.

I have presented excerpt from some works on the physiology of human lymphatic system, not mentioning any of the fascinating studies on the radiology, surgery and pathology or experimental investigations in animals carried out by the participants of our convention. This has been done on purpose. Whereas a spectacular progress has been made in the radiological diagnostic procedures, radiotherapy, *in vitro* pathophysiological studies and development of surgical techniques, our knowledge of the *in vivo* function of the lymphatic system in a normal man remains still very limited. More efforts in this direction are necessary.

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