

CONTINUING DISCOVERY OF THE LYMPHATIC SYSTEM IN THE TWENTY-FIRST CENTURY: A BRIEF OVERVIEW OF THE PAST

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Knowledge of the lymphatic system and how it functions has fascinated investigators since Gaspar Aselli discovered in 1622 in Milan, Italy, the intestinal lymphatics of the dog. For more than two centuries, efforts were devoted to detailed studies of the morphology of lymphatic pathways. Although it was clear that lymph flowed from tissues through lymphatics to the bloodstream, the mechanism of lymph formation remained perplexing until Ernest Starling of London described in 1896 his filtration hypothesis with flow of net capillary filtrate (i.e., lymph) through the interstitium into lymphatics (1). Whereas it was recognized that lymph contained lymphocytes, it was not until Gowans of London documented in 1964 that these cells enter lymphatics via lymph nodes from blood perfusion and exit via efferent lymph vessels (2). Only 10% of the entire recirculating lymphocyte population extravasated into non-lymphoid tissues and flowed via efferent/afferent lymphatics. The function of these lymph lymphocytes remained largely unknown until their role in reaction to viruses and transplantation antigens was shown in the 1970s. Clinical interest in the lymphatic system exploded after Kinmonth of London in the 1950s consistently visualized human lymphatics and lymph nodes by means of conventional (direct) lymphography (3). An avalanche of

observations and concepts on morphology and physiology of the lymphatic system soon followed. Important ones included capillary sieving of macromolecules and plasma protein (4), transport from the extravascular compartment back to the bloodstream, intrinsic contractility of lymphatics as the primary force propelling lymph forward (5), ultrastructure of ground matrix and initial lymphatics (6), occurrence of immunoglobulins (7), cytokines and chemokines in lymph and their role in local inflammatory processes (8), origin of immune cells in lymph and the role of migrating Langerhans cells in elimination of non-self antigens (9), the filtering function of lymph nodes for antigens, nodal cellular structure and participation in total body lymphocyte recirculation (10), and dependence of lymph node cellularity on peripheral antigenic stimuli (11).

In summary, the lymphatic system was first recognized solely as a network for absorption of tissue byproducts. With elucidation of Starling's filtration hypothesis, it became clear that the lymph system serves to maintain the partition of extracellular fluid and to stabilize the cell chemical environment. Later observations on total body lymphocyte trafficking from blood to both lymphoid and non-lymphoid tissues and their return via lymphatics, documented the role of the lymphatic system in immune defense.

Contemporary Assessment of the Lymphatic System

Where do we stand nowadays and what do we know about the anatomy and function of the lymphatic system at the dawn of the XXI century?

The lymphatic system is an organized network composed of functionally inter-related lymphoid tissue and transportation pathways of tissue fluid/lymph and lymphoid cells. Its components include: 1) cells (macrophages and lymphocytes, organized lymphatic tissue such as lymph nodes, spleen, bone marrow, and well-defined follicles in gut and lungs, liver lymphocytes, and a dendritic cell network in both lymphoid and non-lymphoid organs); 2) vascular pathways (lymphatics, intercellular and perivascular spaces); 3) fluids (tissue fluid and lymph). The lymphatic system is integrated with the hematopoietic and neurohormonal systems and can be divided into 1) peripheral (from the interstitial space to and including the nearest lymph node), and 2) central (efferent lymphatics, cisterna chyli and thoracic ducts, and all lymphoid organs).

Organs or tissues with the most active afferent arm of the lymphatic system are skin, gut and lungs, or in effect, structures constantly exposed to the external environment. Other non-lymphoid tissues are also continuously percolated by tissue fluid/lymph, and contain a network of dendritic cells and macrophages. Slight differences with respect to extracellular fluid formation is seen only in the eye and central nervous system.

Lymphatic System Function

The main tasks of the lymphatic system are: 1) Maintaining the extravascular homeostasis of the body and specifically securing proper liquid/gel environment for parenchyma and stromal cell function by regulating the tissue fluid volume and chemical composition and transport to the blood circulation of macromolecular parenchyma cell byproducts.

2) Transport of antigens from tissues to lymphoid organs in a soluble form or by immune cells and inducing immune reaction to infiltrating foreign antigens but tolerance to one's own antigens. These two apparently distinct functions are inseparable.

Tissue Fluid/Afferent Lymph Composition

Tissue fluid and lymph create in man a compartment of approximately 12 L, containing 1) immune cells and cellular components, apoptotic cells or bodies, cell lysates, heat shock proteins, exosomes; 2) bacteria, viruses or viral-like antigens, intracellular pathogens; 3) proteins: soluble, particulate or complexed with immunoglobulins; 4) antigens encoded by RNA or DNA; 5) ectoenzymes. Chemical and cellular processes take place continuously in the slowly flowing tissue fluid and lymph before the latter reaches the bloodstream while passing through organized antigen-filtering lymphoid tissue. It is functionally integrated with the blood circulatory system at the level of capillary exchange vessels and the outlet of the thoracic duct at its central venous junction. Impaired fluid flow at either site or loss of intrinsic contractile forces propelling forward flow results in deranged tissue homeostasis. Lymph flow is now regarded not only as a process for evacuating excess capillary filtrate/tissue fluid, but also as a means to transport local metabolic and waste products and remove bacteria and viruses that have gained access to the interstitial space. The speed of transport of foreign antigens, but not necessarily tumor antigens, to lymph nodes is a prerequisite for rapid immune defense reactivity. The issue as to whether the lymphatic transport of tumor cells to regional nodes is part of a defense mechanism or whether lymph node cells are cytotoxic or "cooperate" with tumor cells remains unanswered.

Self-Renewal Capacity of the Peripheral Lymphatic System

A feature of the lymphatic system hardly recognized until recently has been its extraordinary regenerative capacity. Mechanical interruption of lymphatic trunks is followed on by ingrowth of small lymphatics connecting severed ends of transected collectors. Long-standing mechanical obstruction is followed by formation of lymphatic collaterals and a dense network of small lymphatics distal to the site of obstruction (recognized on lymphograms as “dermal backflow”). These lymph vessels sprout from already existent lymphatics. Neo-lymph nodes can even arise along afferent lymphatics in peripheral tissues with chronic antigenic stimulation (i.e., infection). Normally, the concentration of vascular endothelial growth factor (VEGF) is low in afferent lymph (8); thus far, however, no data are available on VEGF levels in stagnant lymph.

A XXI Century Perspective

Limited access to the intercellular space and lymphatics in the living organism continues to impede research on lymphatic dynamics (e.g., compared with the blood system). Nonetheless, recent developments in visualization technologies including Magnetic Resonance Imaging (MRI) with superparamagnetic agents and ultrasonography, microsurgical techniques for collecting tissue fluid, and immunological methods with biological reagents marking genes bode increasing promise for fast progress along these lines.

The areas where most progress is anticipated are: 1) regulation of tissue fluid/lymph formation, volume and chemical constituents and their pharmacological control; 2) immunity to foreign antigens but tolerance to self-antigens in tissue fluid with transport to regional lymph nodes; and 3) adaptive regeneration of lymphatics and organized lymphoid tissue.

Tissue Fluid/Lymph Flow

The time has been reached that we should start referring to tissue lymphoperfusion. Tissue fluid/lymph comprise around 12 L of continuously but relatively slowly moving liquid. Complete ligation of lymphatics, with total obstruction of lymph flow is followed by tissue necrosis and subsequent fibrosis (12). Such extensive lymph stasis leads to lymphischemia of tissues characterized by accumulation in the interstitial space of excess water, filtered plasma proteins, extravasated blood cells and parenchyma cell byproducts, proliferation of parenchyma and stromal cells, and deposition of surplus ground matrix substance.

Tissue Fluid/Lymph Creating Cellular Environment

Tissue fluid and lymph is a body compartment where intensive metabolic processes take place, such as exchange of structural proteins and lipids between cells and the surrounding fluid environment. Inactive parenchymal cell products are released into tissue fluid only to become activated in the bloodstream (13). However, few of these processes have been studied in detail. Other areas to be further investigated are the physico-chemical conditions in the interstitial space including PO₂, PCO₂, pH, hydrostatic and colloid osmotic pressures and their respective effect on resident and migrating circulating cells. The factors listed reflect a sudden change in the environment from plasma to tissues as cells must adjust to different physico-chemical conditions in the interstitium. The possible servo-signal from tissue fluid to capillary endothelium is another area worthy of study. A search for drugs specifically acting upon lymphatic endothelial and smooth muscle cells needs to be intensified.

Immune Function

Immunologists have recently focused on the “interstitial space-afferent lymphatic-

lymph node pathway,” as this site is where immune events in skin, gut and lungs are initiated. These studies include immune cell trafficking, cell cooperation within lymph and lymph nodes, and diversification of immune reaction into cytotoxicity of the perpetrator or tolerance toward one’s own antigens.

Immune cell migration from blood across non-lymphoid and lymphoid tissues is a prerequisite for immune surveillance. Specific selection exists at the blood capillary membrane for leukocytes to extravasate into non-lymphoid tissues (14) and the high-endothelial venules in lymph nodes (15). This area needs further scrutiny including better understanding of its controlling factors.

Immune reactivity occurs in flowing lymph. Clusters of Langerhans cells with lymphocytes in freshly sampled lymph, form an “immunological synapse” required for presentation of processed (both foreign and self-antigens), to T cells and their activation (16). Better pharmacological control of this synaptic process would allow either stimulation or downregulation of the immune response.

Tissue fluid/lymph is rich in locally produced pro- and antiinflammatory cytokines and in chemokines attracting immune cells (8). How they effect lymph nodal cells, and how they influence tissue cells during lymph stasis requires further elucidation.

A fascinating area is the mechanism of so called “cross-tolerance,” i.e., tolerance induced by one’s own cellular antigens from peripheral non-lymphoid tissues presented by bone marrow-derived antigen presenting cells (APC) (cross-priming) (17). This phenomenon is in sharp contrast to bacterial antigens that evoke intense intolerance (i.e., rejection). Tissue dendritic cells are the elements preventing immune reactivity against one’s own antigens. Whereas dendritic cells react to ingested bacteria, no response is seen to incorporated apoptotic cells. Senescent and mechanically damaged cells (trauma) are a

rich source of self-antigens. Peripheral lymph contains a high percentage of apoptotic lymphoid cells and free apoptotic DNA. Their role in the induction of tolerance as well as reutilization of DNA fragments by lymph node cells remains barely investigated. Studies here are likely to bear fruit regarding the workings of the peripheral component of the lymphatic system.

The immune (inflammatory) response is constitutively connected with activation of the coagulation pathway. Peripheral lymph contains all major coagulation factors; however, at lower concentrations (18). Of interest, fresh blood injected into lymphatics does not elicit thrombus formation. Other studies have shown levels of fibrinogen D-dimers higher in lymph than in blood suggesting “physiological” proteolysis of fibrinogen and fibrin. More studies in this area are needed

A search should also be intensified for identification of lymphococcus, a putative commensal microorganism with high affinity to molecular structures of ground matrix and probably lymphatic endothelium. It is likely responsible, after penetration of epidermis or gut epithelium, for occult destruction of lymphatic vessels.

Lymphangiogenesis

Adaptive regeneration (i.e., formation) of lymphatics (lymphangiogenesis) and nodes (lymphonodogenesis) is a promising research avenue that may help resolve lymph stasis and prevent spread of cancers. Vascular endothelial growth factor 3 (VEGF3) has emerged as a pivotal cytokine promoting proliferation of lymphatic endothelium, and there are specialized receptors for this cytokine on endothelial cells (19). Recent data have suggested that there are lymphangiogenic agents produced by tumor cells (20).

Spontaneous re-uniting of lymphatic collectors with afferent and efferent lymphatics of a transplanted autologous lymph node has been documented 6 months

after nodal transplantation (personal observation). This observation suggests a dormant potential of lymphatics for regeneration when mechanically interrupted. Apparently, when lymphatic endothelium is damaged by infection, regeneration cannot take place.

Formation of “new” lymph nodes is a recently recognized phenomenon based on lymphangiography in conditions of chronic localized skin inflammation (personal observations). Whether these “nodes” represent growth of preexisting lymphoid follicles remains to be clarified. Paradoxically, in conditions of persistent limb infection, regional lymph nodes undergo cellular depletion and fibrosis, as new lymph nodes appear at the same time along deep lymphatics of the extremity. Antigenic stimulation via afferent lymphatics together with as yet an unclear cytokine stimulation mechanism remains behind the process of lymphonodogenesis in adult hosts. Stem cells which may differentiate into lymphatic-tube-forming and lymph node stromal cells need to be identified.

Final Remarks

The morphological and molecular structure and function at the lymphatic system are still largely unknown. Accordingly, even minor discoveries into its inner workings plays a key role in furthering insight into body homeostasis. Fortunately, recognition and importance of this system in the body economy has gained considerable momentum. Transplantology, oncology, infectious diseases, clinical immunology are unlikely to progress without uncovering the mysterious dynamics of lymph fluid, lymphocytes, lymphatics, and lymph nodes.

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