Lymphocyte Traffic

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MILESTONES

Gowans JL, Knight J: **The route of circulation of lymphocytes in the rat.** *Proceedings of the Royal Society* (London) Series B 1964, 159:257

Stamper HB, Woodruff J: **Lymphocyte homing into lymph nodes: *In vitro* demonstration of the selective affinity of recirculating lymphocytes for high-endothelial venules.** *Journal of Experimental Medicine 1976,* 144:828, 833

Gallatin WM, Weissman IL, Butcher EC: **A cell surface molecule involved in organ-specific homing of lymphocytes.** *Nature* 1983, 304:30

James L Gowans

In a landmark publication[1] Gowans and Knight at the Dunn School of Pathology in Oxford reported that the output of lymphocytes from the thoracic duct of rats (about 10^9/day) is normally maintained by a large scale recirculation of cells from blood to lymph. The studies were made possible by skilled investigators who mastered the technique of thoracic duct cannulation. The thoracic duct, the terminal lymphatic draining conduit that connects the lymphatic and blood vascular systems, empties its lymphoid cells and protein-rich fluid into the superior vena cava. It was known that chronic cannulation of the thoracic duct created a lymphopenic state, suggesting that after entering the blood, the cells from the thoracic duct reenter the lymphatic system.

Tracing the fate of RNA-labeled small lymphocytes collected from the thoracic duct and transfused into the blood of normal rats, Gowans and Knight found that the main channel from blood to lymph was within lymph nodes, and that small lymphocytes entered lymph nodes by crossing the walls of a specialized set of blood vessels, the post-capillary venules (PCV).

Autoradiographs showed that after being transfused into the blood, the labeled small lymphocytes homed rapidly and in large numbers into lymph nodes, the lymphoid follicles of the spleen and the Peyer's Patches of the intestine, but not to thymus or bone marrow. Labeled small lymphocytes were seen penetrating the high endothelial cells lining the PCVs of lymph nodes within 15 minutes of being transfused. When the minor fraction of large lymphocytes from the thoracic duct were traced, most were found to emigrate to the intestine where they localized in the stroma of villi and assumed the appearance of plasma cell precursors. This finding established that different populations of lymphocytes emigrated to different anatomical sites.

In a companion study[4] using electron microscopy, Vincent Marchesi, a post-sophomore Pathology Fellow in Gowan's laboratory, showed that the migration across PCVs was a normal physiologic process in which the recirculating small lymphocytes exited the blood vascular compartment and reached the lymphatic compartment by physically entering the endothelial cells of lymph node PCVs and traversing their cytoplasm. This contrasted with the migration of granulocytes and monocytes in inflamed lymph nodes, where those leukocytes emigrated through the PCVs by passing between the intercellular junctions of high endothelial cells.

The studies from Gowan's laboratory established that the traffic of lymphocytes between the lymphatic and blood vascular systems was highly selective, not random. This implied that the process involved specific recognition and interaction between molecules on the surface of small lymphocytes and complementary molecules expressed on the surface of the endothelial cells of high endothelial venules (HEVs) in lymph nodes. The finding that large lymphocytes from the thoracic duct emigrated to the intestine and not to lymph nodes suggested that different populations of lymphocytes emigrated to different anatomical sites, a concept that today is well established.

The tool that revolutionized the study of lymphocyte migration was described in the sentinel publication of Stamper and Woodruff[2]. They incubated thoracic duct lymphocytes (TDL) with fixed histological sections of rat lymph nodes and observed highly selective adherence of small lymphocytes to the endothelium of HEVs. This *in vitro* assay correlated precisely with earlier *in vivo* studies that showed small lymphocytes migrated from blood into lymph nodes only via HCVs, and that the small lymphocytes from rat thymus and bone marrow rarely bound to lymph node HEVs.

With the specific and reproducible *in vitro* assay developed in the Woodruff laboratory it became possible to investigate lymphocyte binding to

HEVs at a mechanistic level. In a breakthrough set of studies, Gallatin and Butcher and their mentor Irving Weissman at Stanford developed a monoclonal antibody that recognized a surface molecule on lymphocytes involved in organ-specific homing[3]. By immunizing rats with a clone of mouse lymphoma cells that bound to lymph node HEVs they produced a monoclonal antibody (MEL-14) that blocked the *in vitro* binding of small lymphocytes to lymph node HEVs but not to Peyer's Patch HEVs, and blocked the homing of normal lymphocytes *in vivo*. MEL-14 allowed for preliminary isolation and characterization of the lymphocyte surface molecules involved in binding to lymph node HEVs.

The Stanford investigators blazed the pathways of lymphoid cell traffic, an area of investigation that attracted numerous other investigators, broadened its focus and has been extraordinarily productive of new knowledge. A growing list of chemokines, chemokine receptors and tissue-specific adhesion molecules have been discovered, characterized and shown to coordinate cell migration. The expression of unique combinations of adhesion molecules and chemokines have been shown to underlie tissue-specific migration, such as the homing of IgA+ B cells to the mammary gland late in pregnancy and during lactation[5]. The cytokines and other factors that regulate the expression of these combinations continue to be identified[6].

When the studies of Knight and Gowans were published in 1964 the field of immunology was still focused at the level of humoral immunity: the field of cellular immunology had just started to emerge from the dark ages. The heterogeneity of lymphoid cell lineages and functions were just being discovered. Previously it was thought by some that the function of small lymphocytes was trophic. It was reasoned that since lymphocytes circulated widely, had almost no cytoplasm, and were loaded with DNA, they functioned to bring DNA to cells throughout the body, like mobile fueling stations delivering DNA to cells in need of a fill up.

The milestone publication by Knight and Gowans was a pivotal step in the emergence of cellular immunology, and by the time the publication of Stamper and Woodruff appeared, the field was moving in high gear. Today we know a great deal about the genes, molecules, regulatory systems and mechanisms involved in the targeted movement of lymphocytes. We know that it is a mechanism for dispersing the immunologic repertoire, for directing lymphocyte subsets to the specialized microenvironments that control their differentiation, for regulating lymphocyte survival, and for targeting immune effector cells to the sites of antigenic or microbial invasion. Characteristic patterns of lymphocyte traffic underlie the organization of regional immunity, such as mucosal, cutaneous and reproductive immune responses.

As the molecular basis of lymphocyte traffic expanded, it became evident that similar systems and families of molecules operate in the traffic of other leukocytes, and might function in the movements of neoplastic and embryonic cells. As the complex molecular mechanisms involved in cellular traffic become fully understood, it could provide the knowledge to construct strategies that therapeutically manipulate normal and pathologic cellular migration.

References

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