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COMMENTARY

NORMAL AND TUMOR ANGIOGENESIS RELATED TO FLOW

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Most students of phylogeny will agree that invertebrates lack blood vessels and red blood cells, but produce lymph or hemolymph, along with vessels more or less in accordance with body size, along with customary feeding and locomotor activities (1). Students of embryology will agree, after Arey (2) and many of his predecessors, that lymph, blood, lymph vessels and blood vessels in vertebrates are primarily derived from mesenchyme in orderly functional sequences. Thoma (3) was one of the first to recognize that the endothelium and surrounding muscularis of vertebrate blood and lymph vessels develop proportional to the volume of fluid contained and the rate of flow.

Florence Sabin, in her classic descriptions of blood vessel development in chick and pig embryos (4-6), found that the embryonal mesenchyme (Mesen, middle + chyme, juice) nearest the yolk sac hypertrophies and dissolves to produce separate puddles of plasma in which red blood cells and leucocytes, also derived from mesenchyme, become suspended. The separate blood cell-containing puddles coalesce in a progression radiating from the yolk sac toward mediastinal mesenchyme where the heart develops to pump this newly formed blood. With continued body growth at the expense of the egg yolk and in accordance with major flow patterns established, the cardiovascular system extends by coalescence of similar puddles throughout the general body mesenchyme to form definitive arteries and veins linked by capillaries or sinusoids wherein flow remains intermittent. Here, endothelium, basement membrane and muscularis remain primitive, or absent, in portions (reviewed in 7). Sabin maintained that the lymph vessels in vertebrates arise by progressive sprouting from veins (6).

Contrarily, Otto Kampmeier (8,9) showed that the lymphatic system of vertebrates does not arise by sprouting from small or large veins. Instead, he found that lymphatics arise first in the cervical region near the outflow from the heart in the form of separate puddles of lymph devoid of red blood cells. These lymph puddles coalesce in a progression which follows and parallels the progressive growth of arteries toward the periphery. In the periphery, lymphatics end blindly in the mesenchymal connective tissue supporting arteriovenous (A-V) capillaries serving all parts of the developing body, except the bone marrow, spleen and ectodermal substance folded and invaginated to form the brain. The first puddles arising coalesce to form paired jugular lymph sacs which, in turn open into the central venous system to drain the paired cervical and thoracic ducts which extend craniad and caudad alongside large arteries. Characteristically, the organized lymphatic tissues, including the thymus, lymph nodes, adenoids, tonsils, diffuse lymphatic tissue of the gut and Peyer's patches, develop in this sequential order, also alongside arteries, such that the lymph emanating from each capillary bed

serving a given epithelium or given body region is filtered through sinuses and organized lymphatic tissue before access to the central venous system.

Partly owing to a small volume and rate of flow via the lymphatic system, compared with that in the cardiovascular system (e.g., 2 L daily vs.7200 L. daily in healthy, active 70 Kg. human), the lymph capillaries, peripheral lymphatics, central lymphatics and central lymph ducts show lower orders of development of endothelium, basal lamina and muscularis compared respectively with A-V capillaries, precapillary arterioles, small arteries or veins, and major arteries or veins. In fact, as emphasized by Földi (10) with respect to brain and many capillary beds; and by Downey (11) with respect to the nodes, flattened capillary endothelium and basement membrane are lacking in "pre-lymphatics" and in nodal sinuses. As results, the periphery of the lymphatic system and nodes remain relatively permeable to dissolved proteins, as well as smaller molecules which filter from the cardiovascular system (12,13) or which emanate from ectodermal, mesodermal and entodermal cells the cardiovascular and lymphatic system coordinately develop to serve (7).

In order to coordinately and effectively serve a variety of capillary beds, each of which varies greatly in functional activity during the course of a day in the life of a vertebrate, the separate cardiovascular and lymphatic systems normally develop volume capacities far exceeding volume of blood and lymph actually contained (7). A state of homeostasis throughout is sustained by the capacity of many parts of both systems to accommodate flow 10-20 times resting rates; and to shunt contained fluids appropriately in accordance with local tissue needs for water, oxygen, glucose, electrolytes, amino acids and proteins (7). In the cardiovascular system, as shown by Krogh (14), Zweifach (15) and others, throroughfare arteriovenous channels develop in all capillary beds to normally maintain a constant pressure gradient between the arterial outflow tracts of the heart and the venous inflow tracts returning blood from the body and lungs. True A-V capillaries, side-branching from A-V thoroughfares, open and close intermittently to sustain homeostasis, depending on the functional activity of given tissues at given times. The aortic valve develops to prevent back-flow into the anterior mediastinal heart which generates the A-V pressure gradient, and hundreds of venous valves develop in the extremities to prevent back-flow, as first emphasized by William Harvey (16). The venous valves prevent backflow, especially when the limbs are dependent, and when surrounding skeletal muscles intermittently contract to expel blood toward the heart.

Lacking a means of propulsion generated directly by powerful rhythmic contractions of the cardiac left ventricle, the entire lymphatic system, except that in the head and neck of vertebrates whose heads are always maintained upright or horizontal, develops myriad valves throughout in order to prevent back-flow toward the periphery, and use gravity, intermittent contractions of surrounding skeletal muscles, tonic and peristaltic contractions of surrounding smooth muscles, and pulsations of neighboring arteries to propel lymph centrally (17). Negative intrathoracic pressure (with respect to atmospheric pressure) created by muscles surrounding the thoracic cavity in order to inflate the lungs, normally enables the venous connections of the paired cervical and thoracic lymph ducts under the clavicles to expel central lymph into large central veins at a rate more or less proportional to the rate oxygen is inspired by the lungs (17,18). Although lymph hearts with striated muscles develop from the mesenchyme in invertebrates and in vertebrate embryos to propel lymph centrally, these usually disappear before birth in mammals (9,17).

Thus, the lymphatic system becomes organized during embryogenesis to return to the heart and lungs many molecules and cells

which filter from A-V capillaries and from arteries in lymph glands into the interstices, along with many molecules and products produced by living cells which the cardio-vascular and lymphatic systems developed coordinately in order to sustain a state of homeostasis throughout the milieu interieur (19-21). Moreover, as emphasized by Claude Bernard in 1878 (19,20), the lymph which arises from all living tissue cells and flows centrally to become plasma in the circulating blood is the essence which sustains this steady state. A century later, it should be added that in vertebrates the lymphatic system and the lymphatic apparatus (including the organized lymphatic tissues), as defined by Yoffey and Courtice (12) normally develop directly proportional to the basal metabolic rate or rate at which each vertebrate consumes oxygen to survive; and always develops alongside arteries in microenvironments of highest tissue oxygen tension available in the body (7,20). The lymphomyeloid complex (12), on the other hand, normally develops around veins in micro-environments of lowest tissue oxygen tension available to produce the bulk of blood cells which normally circulate via the cardiovascular system suspended in lymph (7).

That which is especially important to recall from embryology is that each artery supplying a given region of the mammalian body develops a periarterial lymphangion (22) whose primitive peripheral parts intermittently drain or fuse with fluid-filled interstitial spaces between living cells in order to collect water produced by the intracellular aerobic oxidation of glucose, along with other cell products, including diverse proteins; and whose highly developed central parts commonly convey this fluid, called lymph, mostly into paired cervical and thoracic lymph ducts which drain into central veins during pulmonary inspiration. After efficient mixing in the pulmonary circulation of the composite draining from all the central lymph ducts , the arterial system distributes each and all of the constituents rapidly throughout the body, along with sufficient dissolved and hemoglobin-bound oxygen acquired during inspiration, to sustain cellular utilization of selected molecular products for growth and function in each living body cell (21). Although the dissolved protein content differs in lymph accumulated by lymphangions from differing fluid-filled interstitial spaces, such as the spaces of Disse in the liver, the pre-lymphatics in the skin, the perivascular spaces in the brain, and the sinusoids of organized lymphatic tissues, the cumulative colloid osmotic effect is normally sufficient to offset a significant net loss of water under the heart-generated gradient of hydrostatic pressue normally sustained in the cardiovacular system.

Embryonal Angiogenesis Applied to Inflammation and Tumors

During vertebrate embryogenesis many provisional structures are formed, develop vessels and, then, disappear to become transformed into other structures with characteristic blood and lymph vessels. This is especially obvious in the first to fifth gill pouches during progressive transformations to generate the adenoids, tonsils, thymus, parathyroid and calcitonin-producing glands, respectively, during thyroxin-induced metamorphosis (7,20,23). After birth, many organs change, grow, or cyclically evolute and involute, especially in the female genital system, each time with ingrowth of arteries and veins generated from pre-established capillaries with increasing blood flow. The separate lymphatics extend by extension of lymphatic capillaries or fusion of pre-lymphatic lymphatic spaces to form lymph capillaries with increased lymph formation by locally growing cells, as well as molecular filtration from A-V capillaries (7,12,13,20). The process probably continues throughout the life span of the individual, depending on the turnover rates of all mesenchymal and parenchymal cells involved, especially as exemplified in the lymphatic and

parenchymal cells in gut with starvation and feeding (7,24). Angiogenic responses during tissue injury and inflammation differ primarily in that many local cells become injured simultaneously. As a result, many cells of myeloid, as well as lymphoid derivation become suddenly involved. Usually, polymorphonuclear leucocytes, monocytes and macrophages are involved first, ostensibly to clean up necrotic debris. Later, with reconstitution of the mesenchymal connective tissue framework, partly as a result of lymphocyte exudation (25), A-V capillaries grow in to re-establish blood supply. As local blood supply becomes established, lymphatics extend in or fuse with prelymphatic spaces to reconstitute lymph drainage. Usually, with establishment of drainage to regional nodes, orderly reconstitution of the damage ensues within 6-14 days, unless injury is repeated, foreign material was not sufficiently cleared from the site of injury, or the lymphocyte output from regional and remote lymph glands is quantitatively or qualitatively deficient, as sometimes seen in allograft recipients receiving combinations of adrenal glucocorticoids and nucleic acid inhibitors (7).

With respect to lymphangiogenesis in tumors, Folkman's "lymphagenesis," as criticized in (26) might have been otherwise termed "lymph hypogenesis" "lymphopenia," i.e., too little lymph coming from tumors with a paucity of hemangiogenesis or tumors consisting of cells which have resorted to anaerobic respiration, such that they produce very little carbon dioxide and water which flows away to help fill pre-lymphatics and lymphatics. Finally, with respect to Kaposi sarcomas (KS), these tumors look like embryogenic angiogenesis gone awry. Hemangiogenesis and lymphangiogenesis both appear dysplastic. Pre-lymphatics don't appear to link with lymphatics; basal lamina and flattened endothelium are inconsistent and often incongruous; the spaces containing erythrocytes don't appear to link properly with A-V capillaries, veins or arteries; and the relation of stroma to both kinds of vasculoid structures is weird. It looks like blood flow through and lymph flow out of these tumors are both impaired. Yet, in persons with KS as a side-effect of iatrogenic "immunosuppression," most, if not all, of these dysplastic phenomena disappear as soon as enough "immunocompetent" lymphocytes start circulating from regional and remote organized lymphatic tissues. How herpes simplex virus interacts with human immunodeficiency virus to disrupt emperipoletic lymphocyte communications with the periphery remains to be elucidated.

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