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EDITORIAL

LYMPH AND LYMPHANGIOGENESIS: FUNCTIONAL ASPECTS

The studies of Wilting and cohorts in this issue of the Journal (1) on "Avian Models in Studies of Embryonic Lymphangiogenesis" are superb, timely and worthy of comment in relation to human, as well as avian physiology. Thus:

1. Their descriptive studies show that both intra- and extra-embryonic blood vessels of chick and quail embryos are accompanied by lymphatics. This observation may not be a new generic one, but it signals the importance of lymphatics in relation to the component cells of extra-embryonic tissues upon which developing embryos of all vertebrates depend for nutrition, as well as tissue oxygenation during natural embryonic development (2).

2. The authors stress that lymphatics of the chorioallantoic membrane (CAM) are drained by lymphatic trunks of the umbilicus, and are connected to the posterior avian lymph hearts. The posterior lymph hearts, like the anterior lymph hearts in the lower neck, speed access of lymph effluent from the CAM toward the developing blood vascular system at a crucial time in early embryonic development before other means of lymph propulsion become established with differential tissue growth and customary physical activity (2,3).

3. They found that lymphatic endothelial cells are characterized by the expression of Vascular Endothelial Growth Factor Receptors (VEGFR) -2 and -3; and that the local application of VEGF-C, the ligand of these two receptors, on the differentiated CAM induces proliferation of endothelial cells and formation of huge lymphatic sinuses. Several of these sinuses later become, or become confluent with, afferent and/or afferent lymphatics of well-organized lymphopoietic tissues, such as the thymus, avian bursa of Fabricius, gut-associated lymphatic tissues (GALT), as well as the definitive regional lymph nodes in most species of mammals (4,5).

4. They noted that exceptions to the differential histochemical identification of lymphatic endothelium from common blood vascular endothelium by means of receptors for VEGFR-2 and -3 occur during the early development of blood vessels in the extra-embryonic membranes and blood vessels in the lungs just before hatching in chick embryos. This might be explained by the considerations that development of an arteriovenous (A-V) circulation normally precedes the development of lymphatics in most, if not all, species of lung-breathing vertebrates; and that intraluminal hydrostatic pressure in pulmonary blood vessels is normally less than that in systemic blood vessels of each species.

5. By combining modern histochemical technologies for identifying receptors for differing vascular endothelial growth factors with methods for identifying the differing genetic constitutions of chick and quail endothelial cells, they showed by grafting the wing buds of 3-5 day old chick embryos to the stumps of excised counterparts in 3-5 day old

quail embryos that the wing lymph vessels extend from the body trunk by derivation from mesencyhme of new lymph vessels of quail origin, as well as by replication of vascular endothelial cells in both species.

6. They therefore conclude that: (a) in the wing buds of embryonic quail, lymphatics grow and extend by formation of new "lymphangioblasts" of mesenchymal origin, as well as by replication of differentiated lymphatic endothelial cells–more or less as suggested by Kampmeier (5) as opposed to the classic teachings of Sabin (6), who advocated that lymphatics arose primarily by sprouting from endothelium of veins. (b) vascular endothelial growth factors, such as VEGF-C, might prove useful in the treatment of patients suffering from lymphatic aplasia or hypoplasia.

In critique and extension of this study, the following points are cogent:

• As observed by Sabin (7) and Kampmeier (5), development of vascular endothelium is predicated on the formation of liquid lymph by dissolution of mononuclear mesenchymal cell cytoplasm to form isolated "lakes" or "puddles" containing formed elements. The microscopically visible "formed elements" are mostly nucleated red blood cells and lymphocytes in embryonic blood vessels and puddles devoid of formed structures except small lymphocytes in embryonic lymphatics.

• The formation of lymph, lymphocytes, erythrocytes and other "formed" elements such as granulocytes and thrombocytes, in warm-blooded birds and mammals requires substrates absorbed into the mesenchyme from the entodermal cells lining the yolk sac and quantities of oxygen carried by erythrocytes from points of diffusion through the egg-shell in birds or through the placenta in mammals (2). The puddles of blood which develop, first, in the mesenchyme supporting the yolk-laden entodermal cells and, later, throughout the general body mesenchyme of birds and mammals are formed and coalesce in a progression radiating from the yolk sac into the body mesenchyme until the heart, gills and gill arch circulation are demarcated (2,8). With the establishment of A-V circulation of lymph containing red blood cells in the embryo and vitelline circulation, the mesenchyme lining the blood puddles flattens to form endothelium, basement membrane and surrounding smooth muscle more or less in proportion to the rate of blood flow established in each defined blood vessel (2,9). Notable exceptions to this general rule are found in capillary beds where A-V blood flow remains intermittent, as in the sinusoids of the liver and spleen where blood vessels remain lined by macrophages, as well as thickened endothelium supported by fibrils intermittently permeable to objects as large as red blood cells (2,10). For lack of a better term, such endothelial cells were formerly designated as reticuloendothelial cells with an extraordinary capacity to engulf and digest acid colloids, as well as foreign organisms noxious to a given species (2,10,11).

In invertebrates, a blood vascular system does not develop, because the mesenchyme does not produce red blood cells (2,12). In warm-blooded birds and mammals, unable to carry enough dissolved oxygen in circulating lymph, the development of the lymphatic system awaits arterial circulation of blood cells through the gill arches (2,8,12). Lymphatic development then proceeds by formation of separate puddles of lymph whose formation from mesenchyme and progressive coalescence closely parallels the growth and extension of the aorta and its intra-embryonic and extra-embryonic branches (5). The first lymph puddles are formed as jugular lymph sacs in close relation to the jugular or subelavian veins below the gills in the lower neck, and they establish a lumen-to-lumen connection with these veins (5). The subsequent extension of lymphatics continues by formation of progressive coalescence of puddles in peri-aortic and

periarterial mesenchyme to form the central lymph ducts and branches which accompany development of the arterial system throughout the body (2,5). Progressive lymphatic development depends primarily on diffusion of oxygen and essential substrates from nearby arterioles, and partly on the output of cells in diverse segmental A-V capillary beds (2,9). As in the blood vascular system, the flattening of mesenchyme to form vascular endothelial cells, basal membrane and smooth muscle coats is more or less proportional to local flow rate (2,4,9). However, in the periphery and in the organized lymphopoeitic tissues, the most distal rami of the lymphatic system do not become completely lined by endothelium, but rather remain open into the ground substance formed by mesenchymal connective tissue cells (2,4,9,13-15) As a result, relatively large dissolved molecules absorbed from local parenchymal cells or emanating from regional cells during oxidative metabolism essential to cellular nucleotide synthesis and neoformation of water after aerobic oxidation of glucose are collected without passage by diffusion or pinocytosis through relatively impermeable endothelium (2,16).

• Although the lymphatic system gradually develops in this manner paralleling and adjacent to arteries and transports lymph via paired cervical and thoracic ducts into central veins, the peripheral to central flow of lymph is interrupted by organized lymphoid tissue. Here a variety of mesenchymal mononuclear cells, including reticulum cells, macrophages, plasmacytes and lymphocytes, cooperatively add or subtract dissolved macromolecules, add soluble globulins and small cytoplasm-depleted lymphocytes to the effluent (2,16). The organized lymphoid tissues is diffuse under some kinds of parenchymal cells or nodular and bulky under others, as described in detail elsewhere (12).

• An additional feature of lymphatic development in birds and mammals is the formation of extremely large lymph sinuses as extensions from the central lymph (5,8). The mesothelial cells lining these sinuses are more differentiated than reticuloendothelium lining A-V sinusoids or lymph nodal sinuses, and less differentiated than common vascular endothelial cells lining arteries and veins. These lymph-filled sinuses surround the heart, lungs and abdominal digestive organs such that expansion, contraction and other movements of contained parts are minimally impeded by other body structures during customary homeostatic activities. Small collections of organized lymphoid tissues in the form of "milky spots" variably develop in the lining of the pericardial, pleural and peritoneal lymph sacs for engulfing and disposing of foreign material (17).

• Another essential feature of lymphatic development is the formation of myriad intralymphatic valves which normally prevent the backflow of lymph, especially in lymphatics gravitationally inferior to the outflow tracts of the heart (3).

• Birds differ from mammals in that well-organized regional lymph nodes do not develop to interrupt the flow of lymph effluent from the periphery before flow into the thoracic ducts in synchrony with pulmonary inspiration (3,13). Instead, all species of birds are unique in that they develop a cloacal pouch densely invested with organized lymphoid tissue in the trailing end of the gastrointestinal tract (18), This pouch, called the bursa of Fabricius, like the thymus, is derived from invaginated pinocytic vestigial gill epithelium (18-20). Its evolution and involution with age and stress parallels that of the thymus glands in birds (19). Although lacking in mammals which develop urogenital septae during embryogenesis (8), the bursa in birds is filled and emptied from a mixture of urine, feces and genital secretions in synchrony with pulmonary respiration (16,20). Characterizations of immune functions of the avian bursa revolutionized modern concepts of mammalian immunology since 1956. However, the spectrum of avian bursal functions remain to be fathomed almost 400 years after Hieronymous Fabricius discovered the bursa.

• Finally, the careful studies of Wilting et al support the teachings of Kampmeier (5) with respect to the embryonic development of the lymphatic system. Their studies do not answer the question whether lymphatic endothelium notably differs embryologically or functionally from blood vascular endothelium. Perhaps, some of the endothelial cell receptor differences they describe can be explained by the rate of lymph flow and hydrostatic pressure gradients which become established locally during embryonic and later life. The clinical administration of liganded vascular endothelial growth factors for correcting lymphatic aplasia or hypoplasia remains speculative but an exciting possibility for patients with lymphedema as we enter the new millennium.

REFERENCES

1. Wilting, J, M Schneider, M Papoutsi, et al: The avian model in studies of embryonic lymphangiogenesis. Lymphology 33 (2000), 81-94.
2. Shields, JW: The Trophic Functions of Lymphoid Elements. Springfield, Thomas, 1972.
3. Shields, JW.: Central lymph propulsion. Lymphology 13 (1980), 9-17.
4. Downey, H. The structure and origin of lymph sinuses of mammalian lymph nodes and their relation to endothelium and endothelium. Haematologica 3 (1922), 431-468.
5. Kampmeier, OF: Evolution and Comparative Morphology of the Lymphatic System. Springfield, Thomas, 1969.
6. Sabin, FR: Origin and Development of the Lymphatic System. Baltimore, John Hopkins Press, 1913.
7. Sabin FR. Preliminary notes on the differentiation of angioblasts and methods by which they produce blood vessels, blood plasma, and red blood cells as seen in living chicks. Anat Rec 13 (1917), 199-204.
8. Arey, LB: Developmental Anatomy. Philadelphia, W.B. Saunders, 1942.
9. Shields, JW: Normal and tumor angiogenesis in relation to flow. Lymphology 32 (1999), 118-122.
10. Klemperer P. The Spleen. In: *Handbook of Hematology*. Downey. H. (Ed.), New York, Hoeber, 1938, pp. 1587-1754.
11. Jaffé, RH: The reticulo-endothelial system. In: *Handbook of Hematology*. Downey. H. (Ed.), New York, Hoeber, 1938, pp.977-1271.
12. Shields, JW: The functional evolution of GALT. Lymphology 33 (2000), 47-57.
13. Shields, JW: Lymphspiration: Lymph, lymph glands and homeostasis. Lymphology 25 (1992), 1447-1453.
14. Földi, M: The brain and lymphatic system revisited. Lymphology 32 (1999), 40-44.
15. Castenholz, A: Functional microanatomy of initial lymphatics with special consideration of the extracellular matrix. Lymphology 31 (1998), 101-118.
16. Shields, JW: Lymphspiration: Lymph, lymphomania, lymphotrophy and HIV lymphocytopathy. An historical perspective. Lymphology 27 (1994), 21-40.
17. Shimotsuma M, T Hagiwara, M Takahashi, et al: Milky spots and local immune response. Lancet 339 (1992), 1232.
18. Shields, JW, DR Dickson, W Abbott, et al: J. Thymic, bursal and lymphoreticular evolution. Devel Comparat Immunol 3 (1979), 5-22.
19. Jolly, J: La bourse de Fabricius et les organes lymphoepitheliaux. Archs Anat Microsc 16 (1915), 363-547.
20. Shields, JW: Bursal dissections and gill pouch hormones. Nature 259 (1976), 373-376.

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