Lymphology 25 (1992) 147-153

LYMPHSPIRATION

LYMPH, LYMPH GLANDS, AND HOMEOSTASIS

J.W. Shields

Department of Medicine and Hematology, Santa Barbara Medical Foundation Clinic, Santa Barbara, California, USA

ABSTRACT

Under aerobic conditions every respiring cell in the human body normally consumes oxygen to burn food and produce stoichiometric quantities of water which dissolves carbon dioxide and less soluble cell products. The effluent water and solutes appear in the form of lymph in the interstices between cells. The lymph effluent from all respiring cells flows to become the circulating lymph and blood plasma which coordinately sustain a steady state of homeostasis throughout the internal milieu. As a result, every living cell served by the vascular system has equal opportunity to partake of water and solutes emanating from or absorbed by remaining cells. Solute quantities available depend on cell location, regional plasma flow, local vascular permeability, molecular size, configuration, solubility and concentration, as well as avid cell receptors.

Proportional to oxygen consumption, organized lymph glands develop in environments of relatively high oxygen tension around regional arteries to filter and process lymph coming from regional cells, and to produce effluent lymph rich in soluble globulins extruded by local mononuclear cells (especially macrophages, plasmacytes, lymphocytes), along with suspended small cytoplasm-poor lymphocytes. In turn, such dissolved globulins and remarkably motile small lymphocytes help feed, regulate growth and provide immunity to remaining cells. The lymph effluent from lymph glands and residua from capillary filtrates, along with newly absorbed solvent water, join the blood circulation during pulmonary inspiration in volumes proportional to the volume of air inspired with each breath.

Under aerobic conditions respiring animal cells normally consume oxygen to burn soluble food, especially glucose and, in turn, produce stoichiometric quanta of energy and effluent water which dissolves carbon dioxide and other cell products. In multicellular creatures, such as a healthy 70kg human daily consuming 1000L of oxygen and 2500 Cal of food to pursue moderate activity, theoretically 150-300ml of effluent water will be produced by 75 trillion respiring cells to dissolve 800L of carbon dioxide and extruded cell products (1). The effluent water and soluble products appear in the form of lymph in the interstices between cells (2). The formation of fluid lymph is prerequisite to the formation of blood and lymph vessels (2), as originally shown by Sabin (3) and Kampmeier (4). Claude Bernard (5) noted that lymph effluent from all cells becomes the circulating lymph and blood plasma which helps maintain a steady state of *homeostasis* throughout the *milieu intérieur*. As a result, every living cell served by the entire vascular system has more or less equal

opportunity to partake of water and solutes continuing to emanate from remaining respiring cells. However, solute quantities available depend on regional plasma flow, local vascular permeability, molecular size, configuration, solubility and concentration, as well as receptors avid for relatively insoluble macromolecules (1,2,6).

Under the micro-aerophilic conditions existent during embryogenesis, the plasma- or lymph-filled tissue spaces originally described by Sabin (3) and Kampmeier (4) gradually coalesce with the local establishment of flow in progressions first proceeding toward and, later, from the heart, thus outlining the definitive vascular systems of birds and mammals (1-4). Subsequently, with progressive development of the heart, the pulsatile cardiac propulsion of lymph plasma containing diverse dissolved molecules, along with suspended red and white cells, varies with overall body needs for oxygen, as well as the functional requirements of each body part. Normally flow waxes and wanes, but never stops in arteries, arteriovenous thoroughfares and large veins. However, in true arteriovenous capillaries and sinusoids side-branching from the former, flow remains intermittent (1,2). As a result, the exchange of dissolved gases and soluble micromolecules takes place throughout the vascular system; while the exchange of soluble macromolecules and cells mostly takes place in the true arteriovenous capillaries and sinusoids, especially during ebbs between flow cycles when local oxygen tension becomes low, pH increases and local endothelial, as well as ground substance permeability become high (2). Thus, the respiring cells outside of vessels are constantly supplied with essential gases and micromolecules, and intermittently supplied with macromolecules essential to their growth and function. The water, gases and dissolved molecules which these cells produce during individual activity may be secreted into special ducts, into the lymphatic system, or be returned to the lungs through veins. Because lymphatics seldom connect directly with peripheral blood vessels in mammals (2), the heart does not propel lymph directly. Therefore, the endothelia lining lymphatic tributaries and lymph sinuses, lacking constant intraluminal hydrostatic pressure, remain nonexistent or primitive, as well as permeable to macromolecules and some kinds of circulating cells (2). This arrangement assures the recycling of macromolecules emanating from all body cells, after efficient mixing of their individually secreted metabolic products within the cardiopulmonary circuit (1,2).

Proportional to the basal metabolic rate (BMR), lymph glands develop in micro-environments of relatively high oxygen tension and redox potential around regional arteries to filter and process lymph coming from regional cells, and produce effluent lymph rich in soluble globulins extruded by local mononuclear cells (e.g., macrophages, plasmacytes, lymphocytes) and suspended small cytoplasm-poor lymphocytes (1,2). Such soluble globulins and suspended *emperipoletic* lymphocytes help feed, regulate growth and provide immunity to remaining body cells (2). The effluent from lymph glands and residua from capillary filtrates join blood circulation during pulmonary inspiration in volumes proportional to the depth of inspiration (7).

OVERVIEW

Under basal conditions, when a healthy 70Kg human has fasted 12 hours, is recumbent, awake and using only muscles involved in respiration and blood propulsion, his or her 75 trillion cells consume approximately 15L of oxygen/hour to produce 5ml of water/hour to dissolve carbon dioxide and other cell products (6). However, eating, standing and exercising substantially increased intracellular oxygen consumption in assorted cells and total cellular production of water by factors up to 15 fold (6,8). Thus, in a 70kg human sleeping 8 hours, working 8 hours and relaxing or exercising 8 hours per day, a factor of 2.5 times basal rates of oxygen consumption/water and carbon dioxide production can be expected (1). Therefore,

in a moderately active person during a usual day, we can expect the consumption of 1000L of inspired oxygen, and a net intracellular production of 150-300ml of water, along with 800L of soluble carbon dioxide (1). This 150-300ml of solvent water is 5-10% the volume of lymph daily delivered via the paired cervical and thoracic lymph ducts, i.e., 100-120ml/hour or 2.4-2.8L daily under the same moderately active conditions (6). The remaining water in central lymph undoubtedly derives from that volume which has not been directly resorbed into arterio-venous capillaries, after filtering through interstices and cells so that water and solutes can be sampled, used or discarded by intervening parenchymal cells before accumulation into lymphatics (1,2).

One may conclude, therefore, that 90-95% of the water in central lymph is derived from capillary filtration, along with water absorbed from the external milieu (±2300 ml/day) (6). The remaining 5-10% of water and solutes delivered to the bloodstream during each pulmonary inspiration comes from aerobic intracellular combustion of soluble, endocytized or phagocytized food in some 75 million cells, especially those cells consuming more oxygen at a given time to perform work or produce solutes essential to vital activities. Because the tributaries and sinusoids of the lymphatic system are relatively permeable to colloids, the bulk of proteins which exude or extrude from respiring cells gain access to blood circulation via the lymphatic system, along with circulating proteins which exude from capillaries but are not used immediately for nourishment of the respiring cells.

The Lymph Glands and Lymphocytes in Homeostasis

The precise feeding, regulatory, immune and other *homeostatic* roles of lymph glands and their products, including water, soluble globulins and *emperipoletic* lymphocytes, largely depend on the nature of respiring cells toward which each definitive gland becomes oriented when arterial circulation becomes established (2). For instance:

1. The diffuse and nodular intestinal lymphatic tissue develops around mesenteric arteries which course on to serve intestinal epithelial cells originally laden with egg yolk. With increased arterial flow and oxygen consumption during the absorption of each ingested meal, hypertrophic epithelial cells and hyperplastic lymphoid cells cooperatively produce effluent lymph rich in chylomicrons, soluble globulins and suspended lymphocytes to transport a variety of essential foods to remaining receptive body cells (2,9).
2. The spleen splits from intestinal lymphatic tissue to surround a branch of the celiac axis when the liver and pancreas develop by budding from the gut, and the hepatic mesenchyme becomes the major source of erythrocytes in the body (2). The spleen, then, becomes a major site of storage, as well as destruction of erythrocytes, other circulating cells and complex elements in blood (10,11). Normally the breakdown products are stored in the substance of splenic lymphoid cells, or transported via the portal vein to be re-shuffled by co-operating Kupffer cells and hepatocytes; and, then, are transported to blood or lymph, or selectively excreted into bile (2).
3. Lymph nodes develop along the course of systemic arteries to filter, process, and add lymph to that which emanates from every peripherally located respiring cell and organ in the body (1), excepting the spleen and bone marrow (2). So located, the nodes become instrumental in regional cell surveillance, as well as in immunity (2).
4. The adenoids and tonsils develop around cervical arteries coursing onward to supply pinocytic epithelial cells derived from the first and second gill pouches. Thus, the adenoids and tonsils respectively develop to trap, filter and process undigested molecules inhaled and swallowed, and to produce lymph rich in corresponding soluble antibodies, as well as suspended reactive lymphocytes generated from lymph follicles pointing toward hypertrophic pinocytes (2,12).
5. The thymus glands are closely

allied to the lungs, and develop only in animals obliged to survive through pulmonary respiration. During thyroxin-induced metamorphosis, these lymph glands develop around third gill arch arteries which course onward to supply pinocytic epithelial cells stranded from the third or fourth gill pouches (13). Stimulated by pituitary somatotrophins and thyroxin, the thymic epithelial reticular cells produce thymosins which link oxidative chain phosphorylations essential to sequential synthesis of nucleotides forming lymphocyte DNA (13,14). As a result, the thymus glands become the largest lymph glands, the most concentrated supply of small lymphocytes and the most concentrated store of DNA-linked phosphate in the body of humans and other mammals at the time of birth (2). At birth, with the onset of pulmonary respiration which compresses the thymus during expiration and decompresses the thymus during inspiration, the thymus glands commence characteristic patterns of involution with aging and stress (2). Age involution is accelerated after puberty by influx of estrogens or androgens (13) and feedback of *emperipoletic* lymphocytes to the pinocytic epithelial reticular cells (2,15). Stress involution, especially during anoxia, starvation and severe infection, is accelerated by the influx of adrenal glucocorticoids which induce cytoplasmic shedding and nucleolysis in intra- and extra-thymic lymphocytes (2,16). As a result, thymic effluent lymph is especially rich in "numberless globular particles" (i.e., small lymphocytes) essential to "normal growth of the body and repair of the constitution," especially during infancy (as originally deduced by William Hewson) (17); as well as lymphocyte breakdown products whose nutritive and bio-energetic attributes with respect to the maintenance of *homeostasis* at birth and following many forms of stress remain to be appreciated (2).

6. Although all the aforementioned "lymph glands" are not considered to be hemopoietic organs in human adults, they all produce erythrocytes along with lymph during embryogenesis; and may resume the production of myeloid elements (erythrocytes, granulocytes, platelets) under abnormal conditions (2). Normally during embryogenesis, after definitive arterio-venous oxygen tension gradients are established with growth of the placenta and functional development of the heart into four chambers (18) along with further evolution of the 4th and 5th gill pouches into parathormone and calcitonin-producing glands which accelerate the calcification of skeletal cartilages to form bone (14), myelopoiesis (the formation of erythrocytes, granulocytes and thrombocytes) shifts to the bone marrow (2). Thereafter, myelopoiesis flourishes in a microenvironment of low oxygen tension surrounding the venous sinusoids in the marrow (e.g., 40mmHg) (19); while lymphocytopoiesis continues in a microenvironment of relative high oxygen tension surrounding the arterioles in marrow and in all such "lymph glands" (e.g., 100mmHg) (20,21). Normally, each kind of "formed element," including lymphocytes, plasmacytes, monocytes, macrophages, granulocytes, thrombocytes and erythrocytes appears to grow best, and in this order, in relation to decreasing oxygen concentration, increasing carbon dioxide tension, increasing pH and suitable redox potentials in the environs of arteries and veins linked by capillaries or sinusoids (2,18). Adequate *homeostasis*, then, involves orderly cooperation of all such "formed elements," along with the fluid lymph wherein they become suspended in order to circulate and carry adequate oxygenation, nutrition, cell regulation and immunity to remaining body cells (1,2).

In a healthy 70kg human, the remaining body cells add up to 50 trillion served by 25 trillion circulating erythrocytes lacking nuclei and lacking mitochondria, and therefore, not counted as respiring cells (6); and a roughly equal number of small cytoplasm-poor lymphocytes whose aggregate mass is 1-2% of total body mass, the bulk of which is occupied by nuclei, ribosomes and mitochondria normally receptive to soluble oxygen influx in micro-environments of high redox potential (2).

The Bursa of Fabricius

Birds are unique in that they develop lymph-epithelial cloacal bursae homologous with epithelial cloacal pouches which serve as gills in some species of aquatic turtles; along with lymph-epithelial adenoids, tonsils and thymus glands derived from the 1st, 2nd, 3rd, and 4th cervical gill pouches after thyroxin-induced metamorphosis (14). At birth in mammals, with the onset of lung-breathing and stress involution of the thymus, colostrum appears functionally equivalent to the effluent from the cloacal bursae of precocial birds, such as newly-hatched chicks, ducklings and shore-birds (12). In altricial hatchlings, such as pigeons, song-birds and hawks, cropsac milk regurgitated from the tonsillar crypts and pharynx of both parents appear equivalent to the output from hypertrophic lympho-epithelial cloacal bursae in newly-hatched chicks (12). However, by the time of fledgling in altricial birds, bursal development is comparable with that in precocial birds (12). Subsequently, during puberty, adult life and senescence in all species of birds, their lymphoepithelial cloacal bursae involute with stress and aging parallel with their cervical thymus glands, and like the thymus glands of mammals (12-14).

Great immunologic significance is currently accorded to the avian bursae of fabricius, especially as sources of B-cells. However, true homologous counterparts remain to be identified in mammals, partly because neither cloacae nor cloacal bursae persist beyond early embryogenesis. Moreover, throughout phylogenesis and ontogenesis, the formation of lymph and various kinds of lymphocytes sequentially proceeds from the mesenchyme of the gut, liver, spleen, thymus and nodes before bone marrow develops to produce any kind of blood cells, including stem cells (1-4,12). During embryogenesis and subsequently, the generation of lymphocytes from mesenchymal reticular cells in all organized lymph glands involves progressive shedding of surface cytoplasm during which a variety of surface markers may be expressed or depleted during maturation into small cytoplasm-depleted lymphocytes which normally comprise the bulk of mononuclear cells normally migrating through lymph, blood, most body tissues and most body secretions (1,2,12,16,22).

With special respect to inspiration and lymph effluents, submerging aquatic turtles use their cloacal bursae to inspire dissolved oxygen with tail swishing, especially during hibernation under ice in northern climes. Birds anatomically differ from turtles primarily in that their bodies and leading extremities are covered with feathers well adapted to migration above water, while their feathered tails direct flight attitude. As opposed to the counterparts in aquatic turtles, the cloacal bursae of birds are lymphoepithelial, like the adenoids, tonsils and thymus glands. During flight, birds respire primarily by decreasing intrathoracic pressure through elevation of their well-developed clavicles which are pushed up by air resistance between wing thrusts. Their five paired cervical thymus glands, originally derived from the 3rd and 4th vestigial gill pouches, remain aligned alongside their long tracheae and are not compressed or decompressed by changes in intrathoracic pressure, as is common in mammals endowed with muscular diaphragms and paired, fused intrathoracic thymus glands. Instead, the 5-6 paired avian thymi depend on gravity and backward wing-thrusts to discharge their effluent lymph with forward or upward motion of the body. At rest and when not flying, birds depend on relatively weak intercostal muscles to respire. However, changes in intra-abdominal pressure generated by dorsal intercostal muscles undampened by membranous diaphragms assure filling and emptying of the Fabrician cloacal bursae whose lumina fill with inspiration and whose effluent lymph is expelled with expiration (12). Whether this effluent is primarily for immunologic purposes, trophic functions, or both during the magnificently long life-span of a given bird remains a *bursal* question.

Basically, the avian cloacal bursa fills

with liquid urine and feces, as well as blood, with inspiration; whereas the completely stranded cervical thymus glands of birds and other air-breathing vertebrates fill only with blood. With expiration, these "inspirational" lymphoepithelial organs expel their lymphatic effluents rich in small lymphocytes, energy-rich phosphorylated nucleotides derived from lymphocytolysis and lymphocytotropic hormones derived from their remarkably *pinocytic* epithelial reticular cells. Herein lies a *bursal* key!

Homeostasis and Lymphotropic Retroviruses

Since 1981 it has become painfully apparent that dreadful derangements in *homeostasis* in cats, mice, cattle and humans progressively involving failure to grow normally or body wasting, poor regulation of cell growth and immunologic failure, are caused by lymphocytopathic retroviruses. Human immunodeficiency virus, type I (HIV-1), is a prime example. Through reverse transcription of lymphocyte DNA, HIV-1 is prone to cause fatal lymphocytic hypoplasia or depletion with lympho-cytopenia (as in acquired immunodeficiency syndrome or AIDS), hyperplasia with auto-immune disorders (as in AIDS-related conditions), or neoplasia with massive tumors, often in the brain (as in AIDS-related lymphoma). Throughout the body, through the placenta, and through the blood/brain barrier such dread disorders may be spread by *provirus-infected* lymphocytes in body secretions commonly shared, such as blood, semen, colostrum or endocervical mucus, each of which normally contains $1\text{-}3x10^{5\text{-}6}$ small *emperipoletic* lymphocytes/ml; and few, if any, demonstrable HIV-1 cell-free retrovirons in healthy asymptomatic disease carriers (22). Therefore, it is important to recognize the nutritive and regulatory as well as immunologic roles of lymph glands and lymphocytes in the body economy. Moreover, it is *crucial* in AIDS prevention to minimize the sharing of *provirus-infected emperipoletic* small lymphocytes in such body secretions ordinarily essential to *homeostasis*, as well as the preservation and propagation of healthy human life within an ecosystem full of competing micro- and macro-organisms wherein the fittest are the most likely to thrive and survive.

ACKNOWLEDGMENTS

I thank the Santa Barbara Medical Clinic Lymphology Fund, and many benevolent patients for continuing help.

REFERENCES

1. Shields, JW: Intracellular combustion and ergonics in the production and propulsion of lymph. In: *Advances in Lymphology*. Bartos, V, JW Davidson (Eds.), Avicenum, Prague (1982), 14-22.
2. Shields, JW: *The Trophic Function of Lymphoid Elements.* Thomas, Springfield, 1972.
3. Sabin, FR: Preliminary notes on the differentiation of angioblasts and methods by which they produce vessels, blood plasma and red blood cells as seen in living chicks. Anat. Rec. 13 (1917), 199-204.
4. Kampmeier, OF: *Evolution and Comparative Morphology of the Lymphatic System.* Thomas, Springfield, 1969.
5. Bernard, C.: *Leçons sur les Phénomènes de la Vie Communs aux Animaux et aux Vegetaux.* Bailliere, Paris, 1878.
6. Guyton, AC: *Textbook of Medical Physiology.* Saunders, Philadelphia, 1991.
7. Riemenschneider, P, JW Shields: Human central lymph propulsion. JAMA 246 (1981), 2066-2067.
8. Fox, SM, JP Naughton, PA Gorman: Physical activity and cardiovascular health. II. The exercise prescription: frequency and type of activity. Modern Concepts Cardiovasc. Dis. 41 (1972), 42-43.
9. Shields, JW: Intestinal lymphoid tissue activity during absorption. Am. J. Gastroenterol. 50 (1968), 30-36.
10. Klemperer, P: The spleen. In: *Handbook of Hematology.* Downey, H (Ed.), Hoeber, New York (1938), 1587-1754.
11. Mackenzie, DW, AO Whipple, MP Wintersteiner: Studies on the microscopic anatomy and physiology of the living transilluminated human spleen. Am. J. Anat. 68 (1941), 397-456.
12. Shields, JW, DR Dickson, J Devlin, et al:

Thymic, bursal and lymphoreticular evolution. Developmental and Comparat. Immunol. 3 (1979), 5-22.
13. Marine, D: The thyroid, parathyroids and thymus. In: *Special Cytology*. Dowdry, EV (Ed.), Hoeber, New York (1932), 797-868.
14. Shields, JW: Bursal dissections and gill pouch hormones. Nature 259 (1976), 373-376.
15. Nagaya, H, HO Sieker: Feedback mechanisms of thymic lymphocyte production. Proc. Soc. Exp. Biol. Med. 126 (1967), 131-135.
16. White, A, TF Dougherty: The role of lymphocytes in normal and immune globulin production and the mode of release of globulin from lymphocytes. Ann. NY Acad. Sci. 46 (1946), 859-882.
17. Dameshek, W: William Hewson, thymicologist: Father of hematology? Blood 21 (1963), 513-516.
18. Shields, JW: On the relationship between growing blood cells and blood vessels. Acta Haematol. (Basel) 24 (1960), 319-329.
19. Grant, JL, B Smith: Bone marrow gas tensions, bone marrow blood flow and erythropoiesis in man. Ann. Int. Med. 58 (1963), 801-809.
20. Trowell, OA: Experiments on lymph nodes cultured *in vitro*. Ann. N.Y. Acad. Sci. 59 (1955), 1066-1069.
21. Witte, CL, RH Clauss, AE Dumont: Respiratory gas tensions of thoracic duct lymph. An index of gas exchange in splanchnic tissues. Ann. Surg. 166 (1976), 254-262.
22. Shields, JW: Lymphocyte emperipolesis in AIDS. Lymphology 22 (1989), 62-66.

Jack W. Shields, M.D., M.S., F.A.C.P.
Department of Medicine and Hematology
Santa Barbara Medical Foundation Clinic
Santa Barbara, CA 93102-1200 USA