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THE EVOLUTION OF CIRCULATION, HOMEOSTASIS AND IMMUNITY: AN HISTORICAL ACCOUNT FROM A LYMPHOLOGIST/HEMATOLOGIST VIEWPOINT

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CIRCULATORY BACKGROUND

In 1578, Hieronymous Fabricius of Aquapendente, sometimes recognized as the father of Embryology and Comparative Anatomy, discovered the valves in veins (1). Later, he became the Professor of Surgery and Anatomy at the University of Padua in Italy. In 1602-1604, he precepted William Harvey from England, along with many others from all parts of Europe during the height of the Italian Renaissance. While Fabricius was precepting Harvey, he was sponsored by Ferdinand II, the doge of Firenze to improve the breeding of Leghorn chickens in Livorno, and to put the finishing touches on his book on the venous valves. (1,2) His book, *De Venarum Osteolis,* was published in 1603 by G. Fitzeri in Frankfort, Germany. While dissecting the Leghorn chickens, Fabricius discovered a cloacal pouch, still called the bursa of Fabricius and showed this to William Harvey, along with his studies on venous valves. Harvey continued to study the valves and the bursa, after returning to England. By putting finger pressure over the valves and observing the direction of blood flow in between in forearm veins after release of pressure, Harvey discovered that blood flow in veins is toward the heart, and not away from the heart as believed by Fabricius, after Galen (A.D. 150). Harvey's Lumlean lecture before the British Royal Society in 1618 and his book, *Exercitatio Anatomic de Motu Cordis et Sanguinis in Animalibus,* was published in 1628 by G. Fitzeri in Frankfort. This proved to be a major event in the history of medicine. It is important to note that the publisher in Frankfort used the original drawings of Fabricius with added tiny gloved hands for applying the finger pressure over the venous valves. Historically, more important is that during 300 years of the Italian Renaissance, various members of the Medici family used money they made from the manufacture of pills and banking to directly foster the arts of medicine, painting, sculpture, construction and music. The terms medicine and medications still stand after the Medici today. Michelangelo and Fabricius persuaded the doge of Venice to build the oval anatomic dissection amphitheater that still stands today in the medical school at Padua.

Harvey is less well remembered for his continuing studies on embryogenesis and comparative anatomy during which he observed that blood could not flow without water and that red blood cells are generated in the gut and general body mesenchyme of eukaryotes long before the liver develops. (See *De Generatione Animalium*, London 1651.)

Marcello Malpighi (1628-1695) of Bologna discovered with the help of Anton Van Leeuwenhoek's microscopes the

connection of arteries to veins via capillaries in the lungs (1). Subsequently, with the help of back-lit transmission light microcopy in living tissues, Krogh, Zweifach, Knisely, Mackenzie, Whipple, and many others described the subdivision of capillaries into thoroughfares, true capillaries, sinuses and sinusoids, each with varying rates of flow, varying endothelial morphology depending on intraluminal hydrostatic pressure and varying permeability depending on various endothelial characteristics, as described in detail in (2).

HOMEOSTATIC BACKGROUND

Gaspar Aselli from Milan, commonly known as Asellius, while less well known, is the father of lymphology. In 1622 he discovered the lacteals and intestinal lymphatics that normally feed the body with water and food each time a meal is assimilated in the gut of animalian eukaryotes (1). This nutritive concept is embodied in the frontispiece of his Book, *De Lactibus Sine Lacteis Venis, Quarto Vasorum Mesaraicorum Genere, Novo Invento,* Mediolani, J.B Bidellum, 1627. The frontispiece showing young angels with large breasts on each side of the title inscription was published posthumously by Nicholas Peiresc of Aix-en-Provence after repeating Aselli's studies on a condemned human criminal. Aselli's original observations, before microscopes were invented and modern physiologic observations came to the front, probably provided the key to at least half of homeostasis, as we perceive it currently (1).

After Asellius, in quick succession between 1661 and 1663, Jean Pecquet in Montpelier, Olaus Rudbeck in Upsala, and Thomas Bartholin in Copenhagen independently discovered that intestinal lymph and lymph from body parts below the diaphragm in mammalian adults drains via the valved left thoracic duct into the left subclavian vein to circulate in the blood stream (1,2). Later, others discovered in mammals that a smaller right thoracic duct drains lymph from the right lung, and paired cervical ducts (usually without valves) drain lymph formed above the clavicles into central veins. Usually, the thoracic and cervical duct lymph drains into central veins in spurts that occur at the height of pulmonary inspiration when central venous pressure becomes less than the hydrostatic pressure in the valved left thoracic duct (3). In healthy human adults, the total quantity of lymph drained from all central lymph ducts into central veins is on the order of 2 L. daily (2). Because the adult human heart normally pumps ± 7800 L. of whole blood daily around and through the lungs and arteriovenous circulation, mixing during circulation normally allows the cells in each body region equal opportunity to partake of the molecular and cellular particles in lymph emanating from each living cell in the body, along with other molecular particles and cells which do not normally enter the lymph circulation, such as red blood cells (2).

After Carl Scheele and Joseph Priestly discovered oxygen, in 1777 Antoine Lavoisier, a French chemist, established that oxygen combines with many elements or compounds to generate energy in the form of heat, water and other oxidized compounds, all in accordance with the laws of mass/energy conservation which he promulgated (4). Subsequently, it was recognized that oxidation of many different elements or compounds creates energy not entirely dissipated in the form of heat, but variably stored along with water in secondary compounds. having differing configurations and chemical bonds, especially high energy phosphate bonds in adenine mono-, di- and tri-phosphates and other acid phosphates linked to sugars and other nucleotide bases (5). Simply, without oxygen and oxidation there would be no life as we know it on this earth.

Much later, following the work of Barclay et al (6), it became obvious that a gradient in oxygen concentration becomes established during embryogenesis in the arterio-venous circulation such that

periarteriolar lymphoid organs derived from mesenchyme normally develop a spectrum of mononuclear lymphoid cells in a microenvironment of high oxygen tension, while mesenchyme-derived myeloid organs and their precursors normally develop polymorphonuclear granulocytes, thrombocytes that lose their nuclei, and red blood cells that lose their nuclei before circulating in blood after birth (7). In the organized lymphoid tissues and central lymphatics of mammals after birth, the oxygen concentration is kept high, e.g 100 mm Hg vs 40 mm Hg in bone marrow (2,7,8).

In the late 1700s, William Hewson of London, sometimes called the Father of Hematology (9), in collaboration with William Cruikshank, John and William Hunter, after dissection of many species of animals in progressive stages of development concluded: 1. Lymphatics not veins are the primary means of absorption of cell products throughout the body in all animal species; and 2. Thymus and lymph glands develop to produce lymph rich in globular products essential to the normal growth of the body and repair of the constitution. Five years after Hewson died (1788), his colleagues published *Experimental Inquiries into the Properties of Blood. Part 3,* wherein much of their combined work was published by T. Cadell in the Strand.

In 1787, Paolo Mascagni of Sienna published his magnificent drawings of the visceral and cervical branches of the lymphatic system (10). From 1858 to 1891, Karl Ludwig from Leipzig and Rudolph Heidenhain from Breslau argued whether lymph is formed by transudation from blood capillaries or oxidation of sugars in cells. In retrospect, each was partially correct because the intracellular oxidation of glucose not only produces water, but also power in the form of colloid osmotic pressure in solutes (11,12).

With help of better microscopes and methods of tissue sectioning, Claude Bernard was the founder of modern concepts of the milieu extérieure and intérieure in animals and plants, as well as current concepts of homeostasis. In his book, *Leçons sur les Phénomènes de la Vie Communsaux Aaninaux et Vegetaux* published in 1878 by Bailliere in Paris (13), Bernard concluded that all body cells contribute to the formation of lymph and that lymph constitutes the plasma in blood, as well as the common means of nutrient exchange sustaining homeostasis throughout the milieu intérieur of all animals (more or less like the sap in plants and trees). Bernard's profound concepts remain to be appreciated currently, partly because the same advances in microscopy and other new technologies led to a wide spectrum of observations by a host of intensive investigators on every kind of plant or animal, organ system, cell, cell organelle, molecular structure and molecule each was privileged to study before or after death of the study subject.

During the period 1850 to 1918, the microscopic observation of bacteria by Louis Pasteur (14) and Robert Koch (15), on macrophages by Elie Metchnikoff (16), on clasmatocytes by Louis Ranvier (17) were all cogent. Progressive cytoplasmic fragmentation without nucleolysis by all kinds of mesenchymal mononuclear cells including macrophages, plasmacytes, lymphocytes and reticulum cells by Ranvier (17), Downey and Weidenreich (18) , Florence Sabin (19) and Otto Kampmeier (20) all led to advances in understanding secretory mechanisms and vasculogenesis still not appreciated. It should be interjected here that the fixation and staining methods vary with respect to the amount of water removed from cells and tissues. Immediate formalin fixation with staining later removes ± 30% of the water. Air drying before use of Wright's stain removes much less, and 2% glutaraldehyde less than formalin. In addition, death or tissue excision, by eliminating the hydrostatic pressure generated by the pumping heart, results in redistribution of water, solutes and suspended elements into receptive capillaries, sinuses, sinusoids and veins.

J. Aug Hammar (21) and Justin Jolly's (22) studies on thymus glands and other lympho-epithelial organs including the adenoids, tonsils and avian cloacal bursa during this period still remain classic, but not yet well appreciated, because these organs are partly epithelial with endocrine output, and are organized lymphoid tissues with differing sources of regional afferent lymphatic supply (2,23). At the same time, other cogent advances were made on sepsis, antiseptics and use of sterile gloves in surgery by Ignaz Semmelweis, Joseph Lister, William Halsted and H. Hunter Robb (24). These made surgery possible as we see it now. In retrospect, the observations of Ranvier (17) seem essential, because he conceived of the cytoplasm extruded by clasmatosis from macrophages, after ingestion and digestion of foreign and endogenous particles, as entities useful for normal growth and homeostasis, as well as immunity.

The year 1901 witnessed issue of the first Nobel Prizes. After discovery of dynamite in 1867, Alfred Nobel made several million dollars manufacturing and selling it. He had medical problems and felt sorry about his discovery, especially when used for bellicose purposes. At the time of his death on 10 December 1896, he donated the proceeds to be distributed annually among persons selected for having done the most for the good of humanity in the fields of physics, chemistry, physiology and medicine, literature and peace. Like the favors of the Medici, the prizes have fostered much interest and effort.

The years 1918 to 1935 after World War I witnessed the emergence of the United States as a medical power with the discovery of the effects of liver extracts on bone marrow in pernicious anemia in 1922 and the ease with which cells in marrow could be studied simply by means of needle biopsy and standard trichrome straining of aspirated smears. However, the sequential discovery of sulfonamides by Domagk in Germany, penicillin by Fleming and Florey (a distinguished lymphologist as well and future mentor to JR Casley-Smith) in England, and streptomycin by Waxman in the USA during the 1920s and 1930s, coupled with advances in surgical anesthetics and technologies, ushered in a Golden Age of Medicine that lasted from the 1940s until the 1990's, when the evolution of bacterial resistance and incurable retroviral infections became serious problems.

In 1942-1944, James Kindred (25) of Virginia published the results of his meticulous studies of lymphocytes in lymphoid organs, blood and sundry tissues of healthy well-fed mammals. He estimated that lymphocytes constitute 1/5 to 1/4 of body cells, but only 2% of body mass, owing to their small size in fully differentiated forms. On the basis of mitotic indices in lymphoid organs, he estimated the average lymphocyte span to be ± 2 days – a span later confirmed by Andreason and Ottesen (26) on the basis of radiophosphate turnover. Although it was long known that starvation and other forms of stress rapidly deplete the lymphoid tissue mass, especially among small lymphocytes, in 1946 Abraham White and Tom Dougherty (27) in Utah proved such effects are mediated by adrenal glucocorticoids that increase the rate of cytoplasmic shedding and lymphocytolysis for the benefit of other body cells.

With respect to homeostasis and lymphocytes, in 1924 Alexis Carrel (28) found in tissue cultures that peripheral blood lymphocytes (PBL) enhance the growth of other kinds of cells. In 1931 McCutcheon (29) found that small PBL migrate rapidly and randomly in tissue culture at rates up to 40μ per minute. In 1954, Humble, Jayne and Pulvertaft (30) described emperipolesis (Gr. em – in, peri – around, polesis – roundabout wandering) on the part of small PBL when displaced into tissue cultures of other kinds of cells. The lymphocytes were prone to wander round and round the other cells, actually migrate through some, and disintegrate within some, especially just before mitosis in the other cells. In 1969, we reported our

quantitative studies on small PBL disposition in the epithelial cells of healthy humans (31).We studied the jejunal, bronchial, endometrial, exocervical and corneal epithelium in each of 10 persons without known disease by capsule or surgical biopsy. The biopsies were fresh-fixed in 10% formalin, stained with hematoxylin and eosin, sliced at 5µ depths, and examined at a magnification of 1000x under oil immersion microscopy. In each of 40 specimens, we counted 1,000 epithelial cells sequentially encountered and differentially counted how many intra-epithelial lymphocytes were encountered above basement membrane within or between the cytoplasmic boundaries of each 1,000 epithelial cells. Differential counting subdivided the small lymphocytes into: A-B intact; C-D appreciable cloudy swelling in cytoplasm; and E-F nuclear pyknosis or karyorrhexis. Statistically averaged results per 1,000 epithelial cells were: jejunum total 74 with A-B 34, C-D 8, E-F- 33; bronchial total 47 with A-B 22. C-D 7. E-F- 18; endometrium total 35 with A-B 13, C-D- 6, E-F 26; and cornea (done later) total 25 with A-B- 15%, C-D 23%, E-F 63%. In the jejunum, ±95% of the intraepithelial lymphocytes were located between the nucleus of the epithelial cell and basal lamina. In the multi-layered exocervix and cornea, most of the intraepithelial lymphocytes were located in the basal germinative layers and few were found in superficial flattened layers. On the other hand, in the single layered female endocervix, many small lymphocytes appeared to migrate all the way through. The cornea was easiest to study because while no lymphocytes appeared to migrate through Descemet's membrane, they do migrate some distance from vessels in the corneal limbus.

Still later, under transmission electron microscopy, I studied the emperipoletic migration and disposition of small lymphocytes in thymic epithelium derived from the third gill pouches and in the large macrophages derived from mesenchyme in the germinal centers of organized lymphoid tissues. Although the numbers of small lymphocytes undergoing disintegration varied widely from 0-15 in each location, the manner of disintegration appeared different from that described by White and Dougherty (27) as a consequence of stress or cortisol administration. In the reticular thymic epithelium and in large germinal center macrophages, the small lymphocytes appear to disintegrate with a loss of water and more or less uniform condensation of nuclear and cytoplasmic organelles into hyaline structures that shrink and disappear without obvious separation into definitive parts. In the thymus, this form of lymphocyte disintegration has great physiologic importance because the output of thymic hormones that catalyze sequential oxidative chain phosphorylations resulting in the synthesis of lymphocytic DNA appears inversely proportional to the numbers of small lymphocytes which circulate back to the thymus gland normally lacking germinal centers (32). In the germinal centers of other organized lymphoid tissues, it appears to be the reutilization of small lymphocyte DNA that perpetuates immunologic, as well as inherited memory in mammals throughout the course of a life time, as originally proposed by Trowell in 1957 (33).

In 1973, Sherwin and Richters (34) confirmed and extended the foregoing observations on small lymphocyte emperipolesis by making movies of the lymphocytes migrating among and interacting with other kinds of cells in tissue cultures. The lymphocytes usually wandered randomly, but often circled round and round some cells they appeared to recognize; actually lysed some on surface contact; entered some and disintegrated therein; or often passed through without apparent interactions. The lymphocytes sometimes passed through single layers of cells, but seldom through multiple layers.

Thus, from a homeostatic point of view, it appears that many kinds of mesenchymal cells are sequentially and continually

involved, as depicted in organized lymphoid tissues in Fig. 2 in reference 8. Monocytes, macrophages, and granulocytes within and extruded from the bone marrow reduce noxious endogenous particles into units that can be reutilized for orderly growth and homeostasis with or without the help of opsonins or specific antibodies from the time of embryonic gastrulation (35) until death from whatever cause.

In addition, after injury, infection, or excision in many tissues, the DNA from small circulating and thymic lymphocytes is incorporated into fibroblasts to speed repair, as shown by Fichtelius, Dumont, Bryant and others using isotopic markers for tracing during the 1960's and 1970s (2).

In higher orders of eukaryotes with cartilagenous or stronger bony skeletons and red blood cells, livers with huge collections of hepatocytes and clasmatocytes develop from gut epithelium and mesenchyme to further process and excrete the remains of red blood cells, along with other molecules derived from cellular metabolism throughout the body (35). However, lower forms of eukaryotes lacking skeletons and red blood cells, still depend on the gut epithelium and gut-associated-lymphoid-tissue (GALT) to absorb food and water essential to life. The absorptive process involves increased oxygen consumption on the part of gut epithelium and GALT by a process called specific dynamic action, especially when proteins are broken down by gut enzymes and being absorbed (2,5,35,36).

HOMEOSTASIS AND IMMUNITY

Based on Lavoisier's theories on oxygen and principles of mass/energy conservation, all derivatives of mesenchyme, including endothelial cells, fibrobasts, macrophages (or clasmatocytes), lymphoid tissues, and myeloid tissues normally work cooperatively in the service of remaining tissues to sustain homeostasis almost from the time of conception until death in all animals. However, ever since Paul Ehrlich, Louis Pasteur and Robert Koch, concepts of the cells responsible for immunity have undergone more or less separate evolution with time. If one considers immunity as a process whereby noxious agents are not only destroyed, but also transformed into forms which can be reutilized for continual healthy growth or be excreted in non-toxic forms (2,4), immunity becomes an essential form of homeostasis.

In adult humans, we are talking about a total of ~100 trillion living cells (5), out of which: 25 trillion are circulating red cells without nuclei, but increasingly well adapted to transport oxygen and carbon dioxide via hemoglobin; 20 trillion are lymphocytes adapted to carry on various homeostatic functions during progressive stages of differentiation and disintegration; and 55 trillion other kinds of entodermal, mesodermal and ectodermal cells, each of which may contain organelles specialized to functions helpful to the sustenance of life and activities of the body as a whole.

From 1900-1935, the macrophage reigned as a means for destroying endogenous and exogenous agents, but not as sources of antibodies capable of destroying their circulating extracellular products. From 1935-1970, the plasmacytes reigned as the source of antibodies which later proved to be high molecular weight monoclonal products helpful for scientific purposes, but not very specific for treating people. with known infections.

In 1956, poultry scientists discovered that testosterone injections into the incubating eggs of Leghorn chicks impairs development of the cloacal bursa of Fabricius and results in impaired ability of newly hatched chicks to form antibodies toward Salmonella infections (37). This insight modified modern concepts of immunology and split concepts of the immune system into B-cell components responsible for humoral immunity and T-cell components responsible for cellular immunity.

After Fitzsimmons et al reported on

surgical bursectomy in 1973 (38), we studied the cloacal bursa in precocial birds, altricial birds, and two species of submerging turtles (39). It was found that: (a) surgical bursectomy during avian egg incubation does not alter immunity in the chicks that survive (38); (b) testosterone bursectomy involutes the thymus glands in chicks, just like testosterones and estrogens do after puberty in birds and mammals (2,21,32); (c) in altricial birds that feed their offspring via the tonsillar mucus, saliva, and foodstuffs regurgitated from their cropsacs, bursal development is retarded until fledging (39); (d) in submerging turtles, such as Cyclemis dentata and Fitzroy tortoises, the homologous cloacal bursae do not become heavily invested in mesenchyme-derived lymphoid tissue, retain a gill-like structure like the 3rd gill pouches of all eukaryotes, and are used like gills during respiration under ice or feeding in cold turbulent streams (32,39); (e) cloacae and cloacal bursae do not develop in mammals because urogenital septae develop early to separate colon output from output of the genitals and outflow tracts of the kidneys from the cloacae (40); and (f) although the bone marrow of mammals is currently considered bursa equivalent with respect to formation of B-cells, marrow does not develop in embryos until after the spleen, the formation of lymph and blood in general body mesenchyme and the thymus glands develop with sequential metamorphosis of the paired 5 gill pouches into the adenoids, tonsils, thymus, parathyroid and thyro-calcitonin producing glands (2,35,40).

Thus, it is unlikely that the cloacal bursa or marrow is the primordial source of B-cells or of many differing kinds of blood cells in mammals. In birds with long necks containing ± 5 pairs of thymus glands on both sides, big crop sacs, short transit times of ingested food between the cropsac opening into the cloaca, lined by pinocytic epithelial cells and filled in synchrony with pulmonary respiration, it seems the bursa of Fabricius is an important bio-energetic adjunct to their cervical thymus glands during flight, as well as an important means for limiting the numbers of pathogenic bacteria excreted from the cloaca onto the ground or surface water during flocking (2).

COMMENT

Although none disproven, many of such old observations on the role of organized lymphoid tissues in circulation and homeostasis have been forgotten in favor of immunologic functions of different kinds of mononuclear lymphoid cells and sundry soluble lymphokines derived therefrom. Actually, however, the evolution and current status of each plant and animal living on this planet depends on food available for growth and energy constantly provided by our sun more or less as summarized by Hickman et al (41) before recognition of the detrimental circulatory, homeostatic and immune effects of retroviruses lacking DNA were generally recognized. This subject will be the topic of a future manuscript.

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