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THE FUNCTIONAL EVOLUTION OF GALT: A REVIEW

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ABSTRACT

This synopsis of the evolution of gut-associated lymphoid tissue (GALT) in increasingly complex animals suggests that GALT plays an essential role in cellular nutrition and energy metabolism as well as in immunity. Throughout phylogeny, the mediation of immunity as well as cell nutrition depends on the normal capacity of GALT to produce lymphocytes which customarily generate an evolving variety of soluble globulins during maturation and proceed to migrate throughout the body as emperipoletic cytoplasm-depleted cells which donate their residual constituents to genetically compatible cells; and destroy living matter recognized as genetically incompatible. A dividend is that the lymphocytes not only destroy the genetically foreign but also render the remains innocuous or useful as food.

Gut associated lymphoid tissue (GALT) is the only lymph producing tissue common to all forms of animals comprised of entodermal, mesodermal and ectodermal layers (1-3). In all phyla, species and subspecies, GALT arises initially from mesenchyme supporting yolk-laden entodermal cells in the fertilized egg during the process of gastrulation; and evolves to produce liquid lymph rich in globulins and lymphocytes which feed, regulate growth and provide immunity in remaining body cells during organization into definitive tissues and organs (1,3). The complexity of GALT increases more or less in proportion to the amount of food and oxygen consumed in order to support coordinate cell growth, life and customary activities in each subspecies (3).

GALT in Invertebrates

In animals without backbones, such as sponges, worms and insects, GALT immediately supporting entodermal cells lining the alimentary tract is the only definitive lymphoid tissue which persists throughout life in adult as well as larval stages (3). During alimentation, the GALT grows and transforms ingested food into liquid lymph rich in globulins, suspended lymphocytes and other kinds of leukocytes, which circulates via primitive vessels from the alimentary tract to carry nutrients to outlying tissues and return oxygen from superficial layers back to the gut (4). Flying insects are of special interest, because the GALT also produces soluble pigments, such as green chlorocrurins, blue hemocyanins, or brown heme, which increase the oxygen-carrying capacity of circulating lymph between ectodermal spiracles and the gut (4).

GALT in Lower Vertebrate Orders

The development of a cartilaginous backbone for body support and gills for breathing at the cranial end of the alimentary tract is a signal evolutionary event wherein the GALT commences to produce nucleated red blood cells, called erythrocytes, to

increase the oxygen transporting capacity of lymph (1,3,4). At this stage of evolution, the liver, pancreas, stomach and spleen develop from the mid-portion of the alimentary tract by evagination of entodermal cells into mesenchyme to form the bile ducts, pancreatic ducts and, then, the parenchyme of the liver and pancreas (1). The stomach develops as a widened pouch and the spleen develops by stranding of GALT toward the celiac axis which supplies lymph rich in red blood cells to the stomach, pancreas and liver (1,5). The net result is a division of labor on the part of the gastrointestinal tract wherein the mesenchyme of the liver and spleen become the principal erythropoietic sites in the body and the parenchymal cells of the liver become specialized to breakdown and convert the pigmented components of red cell hemoglobin into bile acids (3). The parenchymal cells of the stomach specialize to produce pepsins and acids which convert proteins into absorbable amino acids. The parenchymal cells of the pancreas become specialized to produce and excrete enzymes into the gut which break down carbohydrates and fats, along with endocrine cells which secrete insulin internally to help control carbohydrate metabolism in the liver and remaining body cells. The spleen, then, assumes a triple role: (a) as a major site of red cell production in its red pulp; (b) as a major site of red cell storage and destruction in the sinusoids of its red pulp, such that the breakdown of hemoglobin is initiated before further processing in the liver; and (c) as the major site of lymphocyte and lymph production apart from GALT in its periarteriolar white pulp (5).

Meanwhile, separate urogenital tracts arise from mesoderm and mesenchyme to connect with the caudal end of the alimentary tract via ureters and oviducts or vasa deferens (1). The parenchymal cells of the kidneys, then, become specialized to excrete urine rich in urea formed by hepatic parenchymal cells. Simultaneously, along with hepatic and splenic mesenchyme, the renal mesenchyme becomes a major erthrocytopoietic organ close to cellular sources of erythropoietin in animals whose skeletons consist of cartilage, instead of calcified bones (3,4).

GALT in Middle Vertebrate Orders

In middle vertebrate orders requiring more oxygen, food and stronger skeletons to crawl, walk or fly over land and sea, the cranial and cervical end of GALT undergoes metamorphosis wherein the gill pouches derived from gut entoderm sequentially transform into other structures in conjunction with the development of lung buds from the leading end of the alimentary tract (1,3). In brief, under the influence of thyroxin secreted from the thyroid gland which develops from entoderm in the ventral midline of the cervical alimentary tract, the paired gill pouches transform as follows (1,3,5,8).

1. Entoderm of the first gill pouches transforms into pinocytic epithelial cells which line portions of the nasopharynx to constitute adenoids densely invested with organized lymphoid tissue. The pinocytes appear oriented to trap, ingest and partially digest air-borne particles toward which the associated periarteriolar lymphoid tissue is reactive in order to produce lymph relatively rich in lymphocytes and immunoglobulins (3).
2. Entoderm from the second gill pouches invaginates into the mesenchyme to form deep crypts lined by epithelial pinocytes which entrap orally ingested and inhaled particles. The adjacent periarteriolar mesenchyme develops into dense reactive lymphoid tissue to constitute pharyngeal and lingual tonsils which, in conjunction with the adenoids, constitute Waldeyer's ring surrounding the entrance to the alimentary tract. The tonsils and adenoids not only drain effluent lymph into cervical lymphatics, but also form many motile small cytoplasm-depleted lymphocytes (SCDL) which migrate into the pharyngeal lumen and onward into the cropsac of birds and esophagus of other species (3,7). The functional significance is

relevant to immunologic protection of the pharyngeal, cervical and intrathoracic portions of the alimentary tract, as well as the body as a whole (3).

3. Entoderm from the third gill pouches invaginates into the cervical mesenchyme and becomes completely separated from the alimentary tract to constitute pinocytic epithelial reticular cells which, in conjunction with dense surrounding lymphocytopoietic tissue, constitute thymus glands. These develop alongside the course of carotid arteries and lymphatics in birds or fuse to form a single intrathoracic thymus gland in other species (6,8). The thymic epithelial reticular cells produce thymic hormones, called thymosins, which accelerate the sequential synthesis of nucleotides into the DNA of lymphocytes and, thus make the thymus glands relatively large and important lymphocytopoietic centers at the time of birth in most lung-breathing species (3,6,8). Thymosin output from the epithelial reticular cells is influenced positively by circulating concentrations of pituitary growth hormones, thyroxin, parathyroid hormones and thyrocalcitonin (3,8). Thymosin output is influenced negatively by estrogen, testosterone and the numbers of circulating SCDL returning to undergo disintegration in the epithelial pinocytes (3,8,9). Under the influence of adrenal glucocorticoid hormones during starvation, stress and some kinds of infections, the thymus undergoes extremely rapid involution by lymphocytolysis and shedding of lymphocyte cytoplasm to release relatively large quantities of circulating lymphocyte nuclear DNA and cytoplasmic products for the bioenergetic as well as nutritive benefit of remaining body cells (3,10).

4. The entoderm of the fourth gill pouches invaginates and becomes isolated to constitute the endocrine epithelium of parathyroid glands which produce parathormones.

5. The entoderm of the fifth gill pouches also invaginates and ultimately constitutes the thyrocalcitonin-producing cells within or apart from the parathyroid glands. The combined effects of these hormones is to turn cartilage into bone through calcification, such that the rib cage becomes an effective bellows and the skeleton and limbs can bear weight and tolerate stress when walking on land or in flight (3).

In conjunction with the calcification of avascular cartilage to produce cancellous bone, mesenchyme grows into the osseous medulla to produce arteries, veins and arterio-venous sinusoids surrounded by mesenchymal cells specialized to produce erythrocytes, granulocytes and megakaryocytes in a microenvironment of low oxygen tension (1-4,11) The progression of cancellous bone formation and medullary hemopoiesis usually starts in the clavicles, progresses into the sternum and ribs, and successively onward into the spinal column, pelvic girdle and bones of the extremities, depending mostly on the need for oxygenated arterial blood in the body of a given species (3,4). Reciprocally, the formation of red blood cells, granulocytes and blood platelets subsides in the mesenchyme of the liver, spleen and kidneys, such that bone marrow becomes the principal source of myeloid cells in the circulating lymph of all species of lung-breathing vertebrates (3,4). Exceptions to this general rule are found in cold-blooded subspecies, such as reptiles which hibernate during winter, and wherein the hepatic, splenic and renal mesenchyme become the principal sources of red cells under conditions of low oxygen demand for performing customary activities in the continual search for food (12).

In the mid-gut, we see increasing development of diffuse periarteriolar lymphoid tissue forming the core of villi in the small intestine and in the intestinal submucosa. The specialized villi produce and centrally propel milky intestinal lymph, called chyle, from ingested food altered by gastric, hepatic and pancreatic secretions (3). In the ileum periarteriolar lymph follicles become prominent. At the junction of the small and large intestine, we see variable

development of cecal appendages with dense lymphocytopoietic tissue, pointing toward epithelial pinocytes in crypts (3). At the junction of the ileum and colon, cecal appendages become large and well developed, especially in herbivores.

In the hindgut, diffuse lymphoid tissue surrounding crypts and underlying columnar entoderm predominates in the upper colon. The colon terminates in a dilated portion, called the cloaca, which receives the output of the ureters and oviducts or vasa deferens, before opening to the exterior via the anus (1). In conjunction with the underlying lymphoid tissue, the colon mucosal cells perform at least two vital functions: (a) the resorption of water which accumulates from ingestion, bile ducts, pancreatic ducts, exudation in the small intestine and from the ureters; and (b) the further breakdown of food, mostly highly polymerized carbohydrates in the form of cellulose, not digested by gastric, bile, pancreatic or intestinal enzymes. The latter colon function is influenced variably by saprophytic bacteria whose species are probably regulated to some extent by the output of GALT in the cecal appendages and terminal ileum.

Thus, from mesenchyme and entodermal cells lining the primitive gut, in craniocaudad order, we see the evolution of five definitive kinds of GALT: adenoids, tonsils, thymus, spleen and that which remains closely associated with intestinal mucosa. Each evolves into periarteriolar lymphopoietic tissue in a microenvironment of relative high oxygen tension to produce lymph relatively rich in dissolved globulins and migrating lymphocytes of differing specificities, depending on orientation and evolution in nearby entodermal cells (3,11).

GALT in Highest Vertebrate Orders

In mammals, the evolution of GALT is the same during embryogenesis, with ontogeny recapitulating phylogeny (1,7). However, mammals differ in that their embryos develop in the uterus wherein the temperature is ±37°C., and the placenta develops to bypass the primitive gut soon after gill development (1,3). Also, maternal colostrum rich in antibodies and maternal lymphocytes and, later, milk rich in carbohydrate, proteins and fat secreted from the mammary glands protect the GALT and supply nourishment relatively easy to digest (3). The formation of red blood cells, along with lymph plasma, lymphocytes and primitive vessels from mesenchyme supporting the yolk sac entoderm and, later, general body mesenchyme, starts early, probably owing to relatively great metabolic rate and fetal need for oxygen (1,3). However, as soon as primitive vessels and the mesodermal heart are demarcated in the body, the placenta extended from the gut via the umbilical cord becomes the principal source of nutrients essential for growth, along with oxygen (1,3). The mesenchymal (sinusoidal) components of the liver and spleen, then, become the principal centers of red blood cell formation throughout most of fetal life (1,3,4). Most of the GALT, except the thymus glands, remains atrophic and poorly developed (3). However, when the lungs become well-developed and the ductus arteriosus connecting the pulmonary artery with the proximal part of the aorta starts to close thereby creating a definitive arteriovenous oxygen gradient (3,11), the formation of erythrocytes, granulocytes and thrombocytes shifts to the bone marrow in a microenvironment of low oxygen tension. Lymphocytopoeisis starts to flourish in a microenvironment of relatively high oxygen tension in the periarteriolar mesenchymal tissue of the spleen and in regional lymph nodes which develop to filter and process lymph emanating from respiring parenchymal cells in all regions of the body (3).

In association with these events, the red cells developing in marrow resorb or extrude their nuclei to become biconcave discs, still called erythrocytes, wherein contained hemoglobin transports oxygen and carbon

dioxide more efficiently (3,4). More or less at the same time, many of the lymphocytes produced in the spleen, nodes and GALT progressively shed most of their cytoplasm to become small cytoplasm-depleted cells which, by virtue of unique emperipoletic motility (13,14), become more efficient in carrying DNA which can be reutilized for regulated growth in remaining body cells (3). In the thymus, where gill-derived epithelial reticular epithelial cells produce thymosins to accelerate the rate of lymphocyte DNA synthesis, most of the lymphocytes divide so rapidly that few nuclei become large and relatively little cytoplasm is produced (3,8). As a result the thymus becomes the largest concentration of DNA in the body, with 270 micrograms of desoxyribose acid linked to phosphorous (DNA-P) per 100 mg. of fresh tissue; and grows to constitute a mass of thymocytes approximately equal to the size of the heart at birth (3). However, with the onset of pulmonary respiration at birth and the changing intrathoracic pressure during inspiration and expiration, the thymus commences its characteristic pattern of involution through progressive shrinkage with discharge of lymph rich in thymocytes or their disintegration products for the bioenergetic as well as nutritive and immunologic benefit of remaining body cells (3,8). If the thymus fails to develop or is removed shortly after birth, the remainder of the GALT fails to grow normally, and the newborn animal fails to thrive, unless fed and maintained in a germ-free environment (1,5).

Under normal conditions in newborn mammals with intact thymus glands and breast-fed, the GALT in the adenoids, tonsils, and alimentary tract grows rapidly to attain a mass equal to one third of total lymphoid tissue mass in the body of a healthy well-fed individual (16-18). During the alimentation of a meal containing carbohydrate, protein and fat, the lymphocytes in the submucosa of the jejunum show increased mitoses within 30 minutes of ingestion, the intestinal mucosal cells in the villi and underlying submucosa undergo hypertrophy and hyperplasia to produce chyle which drains into the cisterna chyli of the thoracic duct for 2-3 hours, body oxygen consumption increases 30% (partly owing to the specific dynamic action of proteins and increased splanchnic blood flow) and the mass of small intestine increases appreciably (18). During starvation with water ad lib for 48 hours, the small intestine shrinks up to 58%, that of the spleen 38% and that of the thymus up to 90% (3,18), all largely owing to loss of mass in the organized lymphoid tissue supporting the entodermal cells (18) and specifically owing partly to cortisol-induced lymphocytolysis and cytoplasmic shedding (3,10). The cell mass in the liver and rest of the body shrinks less than 10% at the same time (3,18). The rate of mass restitution with realimentation is unknown in each compartment of the GALT, but normally occurs within a few days with adequate food. Nevertheless, it is reasonable to surmise from such studies that the GALT evolves to perform important roles in alimentation, as well as in mucosal and general immunity in mammals, as well as in less complex lower phylogenetic orders.

After weaning, mammals develop unique polarized germinal centers in GALT and lymph nodes (3,7). These develop from primary lymph follicles pointing toward pinocytic epithelial cells in the adenoids, tonsils, Peyer_s patches and cecal appendages; and point toward lymph sinuses or sinusoids in the regional nodes and spleen (3,7). Their sequential evolution begins in the Peyer's patches of the small intestine and proceeds into the spleen, regional nodes, adenoids and tonsils (7). They do not normally develop in the thymus or bone marrow. Their cellular organization and orientation is summarized elsewhere, especially in relation to increasing production of reactive antibodies, sensitized small cytoplasm depleted lymphocytes essential to maintenance of homeostasis in humans with high cell metabolic rates (9,20); and the devastation which occurs when retroviruses,

such as HIV-1, insert their RNA at random into the genes of large dividing germinal center lymphocytes of infected humans (20).

Unique Features of GALT in Reptiles and Birds

Some species of turtles develop paired pouches which evaginate from the cloaca near the ureteral orifices and oviducts (6,12). Such pouches are lined by branching strands of pseudo-stratified columnar entodermal cells resembling gill epithelium (6,8) and serve as gills when the turtles are submerged and survive via cloacal respiration (12). Some subspecies use these pouches to survive winter through hibernation under ice (21). Other species in Australia actually respire mostly via enormous cloacal pouches when feeding in rapidly flowing rivers (22).

In all species of birds, homologous pouches evaginate from the cloaca into mesenchyme during embryogenesis, and become densely invested in organized lymphopoietic tissue to constitute a fused single pouch called the bursa of Fabricius (6-8). These pouches in birds were first identified in Leghorn chicks by Hieronymous Fabricius in ±1602 at the same time that Fabricius, Professor of Anatomy and Surgery in Padua, Italy, was precepting William Harvey from England in Anatomy and Surgery (19). Neither could fathom the actual function of these pouches. However, after Fabricius demonstrated the valves in veins to his student, Harvey went on to show in 1617 and in 1628 how blood actually circulates with help from many valves in veins and a single valve in the proximal aorta, all which prevent back-flow. Harvey's demonstrations revolutionized the Science of Medicine (19). "Rediscovery" of the bursa of Fabricius in 1956 (23) revolutionized but complicated modern concepts of immunology (19).

The bursa of Fabricius really has no true anatomic or evolutionary counterpart in mammals, because the cloaca becomes divided during embryogenesis from the ureters and oviducts or vasa deferens by a urgogenital septum which normally persists in adults (1,19). In 1915 Justin Jolly (8) showed that this bursa, when invested with lymphopoietic tissue, becomes an organ appearing hybrid from pharyngeal tonsils and thymus glands of birds and mammals. Jolly called the avian bursa a cloacal thymus, partly because its evolution and involution with age and stress closely parallels and follows that of cervical thymus glands in birds and intrathoracic thymus glands in mammals. He noted that the entodermal cells which invade mesenchyme form crypts similar to those in tonsils, and epithelial reticulum similar to that in thymus glands. Also, he noted that the epithelial cells do not become completely separated from the lumen of the bursa; do not form Hassell's corpuscles, and that the bursal epithelium in contact with the bursal lumen is pinocytic, like gill epithelium. So located and constituted, the avian bursa of Fabricius, lies in position to entrap and process particles or macromolecules not yet eliminated by upstream parts of the GALT, renal nephrons and secretions emanating from the ovaries or testes. How the bursa further processes such influents for the nutritive as well as immunologic benefit of birds remains a complex question worthy of further exploration.

Exploring in terms of the GALT, a few observations on the life of birds (24) and peculiarities in cloacal GALT are cogent:

• In their magnificent adaptations to flight, foraging on the ground and in air, and migrating extreme distances to escape winter, birds develop a long rigid sternum and rigid upper rib cage, and ventilate primarily by elevation and depression of the clavicles during flight (24). The lungs are compressed with each downward thrust of the wings and decompressed when the wings are swept upward, partly by air resistance. Airflow patterns through the pulmonary alveoli are more efficient than those in mammals, and there is practically no dead air space. Blood oxygenation and exhalation of carbon dioxide

is geared almost directly to energy expenditure during flight. At rest, expansion and contraction of the lower posterior portion of the rib cage supplies adequate ventilation for lesser orders of energy expenditure.

• The esophagus of birds becomes dilated to form a pouch, called the crop or cropsac, wherein ingested food is accumulated during foraging and variably digested later. Onward passage is through a stomach, called the gizzard, with thick muscular walls which mechanically help grind up food formerly stored in the cropsac. After passage through the gizzard, the transit time of food through the intestines is extremely short, e.g one-half hour, as compared with that in mammals, e.g., 2-4 hours. The absorption and reutilization of carbohydrates for energy production in birds is more efficient than that in mammals (24).

• The oropharyngeal tonsils developed from the GALT of birds are compounded with mucus-producing glands which secrete mucus, immune globulins and small lymphocytes which help lubricate and protect the cropsac from infection (7).

• Birds are subdivided into two categories, precocial and altricial. Precocial birds, such as chickens, ducks and shorebirds, are well-developed with feathers, sight and ability to forage almost immediately after hatching. Altricial birds, such as pigeons, hawks and song-birds, are poorly developed without these characteristics. Until they are strong and well-feathered, altricial hatchlings are fed from the cropsac of their parents. In pigeons and other species, the crop contents are sometimes called cropsac milk, because of resemblance to mammalian breast milk in consistency, color, antibody and lymphocyte content (7,24).

• In altricial hatchlings, the bursa of Fabricius, thymus and remaining parts of the GALT are atrophic, compared with counterparts in precocial birds (7). However, by the time of fledging with ability to fly, usually 2-3 weeks after hatching in many bird species, the bursa, thymus glands and remaining parts of the GALT are comparable in development and mass in both categories (7), even though their flying, foraging, flocking, and migratory habits differ widely (24). During the interim between hatching and flight, the cropsac milk in altricial birds, like colostrum in mammals, is the main source of food, as well as maternal antibodies and sensitized lymphocytes in the young birds until they are able to forage on their own (7,24).

• Meanwhile, whether flying or not, both categories of birds must breathe air containing oxygen in order to grow and mature. The avian bursae and thymus glands, originating from invaginated gill epithelium, remain closely linked to respiration after the lungs develop. The invaginated epithelial reticular cells produce hormones, called thymosins or bursopoietins, which accelerate the rate of aerobic oxidative chain reactions involved in the proper sequential linkage of high-energy phosphorylated nucleotides into lymphocyte DNA (3,6,19). As a result, the paired cervical thymus glands and cloacal bursae become extremely large lymphocytopoietic organs at the time of fledging (7). The lymphocyte-rich lymph formed in the thymus glands (6,7,17,25-27) drains by gravity into the cervical lymph and thoracic ducts during pulmonary inspiration at rest; and with acceleration during flight owing to downward wing thrusts which elevate the avian body and increase gravitational forces (19). Lymphocyte-rich bursal lymph is pumped centrally by changes in intrabdominal pressure which are synchronous with pulmonary respiration (19,26,29) [birds generally lack muscular diaphragms, are endowed with long rigid sterna essential to attachment of muscles enabling lift essential to flight, and have relatively little abdominal musculature (24)]. Also, in synchrony with pulmonary inspiration, the bursa characteristically lacking intrinsic musculature, is filled with liquid from the cloaca and, then, emptied during expiration (29). Although anterior and posterior lymph hearts with striated muscle

cells respectively develop in close relation with the thymus glands and cloacal bursae of birds during embryogenesis, the lymph hearts usually disappear by the time of hatching (28).

• When the altricial and precocial birds age and pass through various forms of "stress," including starvation, hypothermia, hypoxia and infection, their thymus glands and cloacal bursae normally evolute and involute in unison (8). These glands achieve maximal mass compared to body mass at the time of fledging and greatest actual mass at the time of puberty (7,8). Gradual shrinkage, mostly in the lymphocytopoietic components, ensues until senescence (7,8). With stress, the thymus and bursa usually involute suddenly in unison, partly as a result of cortisol-induced lymphocytolysis (3,10) to release lymph rich in sequenced nucleotides whose high-energy phosphate bonds, as well as carbohydrate and amino acid contents are of great bioenergetic, as well as nutritive and immunologic import (3,10).

Avian Related to Mammalian Immunology

In 1956 Glick, Chang and Jaap (23) reported that testosterone injected into the eggs of Leghorn chicks seven days before hatching causes atrophy of the bursa of Fabricius and results in impaired antibody production. Subsequently, through comparison of effects of surgical neonatal thymectomy in mammals with the effects of testosterone bursectomy in Leghorn chicks (30), the focus has been on B-(bursa derived) and T-(thymus-derived cells) in birds and in mammals as the sources of humoral and cellular immunity, respectively.

In brief, criticism of experimental data leading to current concepts of B-lymphocytes and T-lymphocytes and their separate functions, the following points are cogent.

• All phylogenetic orders of animals ascending from worms to humans possess effective humoral and cellular immunity mediated via cellular components of the GALT whether or not they develop thymus glands or a cloacal bursa of Fabricius (31).

• In 1973 Fitzsimmons (32) and associates found that testosterone injections before hatching in Leghorn chicks produce thymic as well as bursal atrophy; and that surgical bursectomy during early embryogenesis does not impair antibody production in surviving chicks.

• Birds are renowned for their frugality with DNA, and the chicken is no exception. While the human HLA (human leukocyte antigen) complex comprises 3,600 kilobases embracing 128 functional genes and 96 pseudogenes, the chicken MHC (called the B-locus) has only 19 genes in 92 kilobases and no pseudogenes (33). The genetic mechanisms involved in coding for shedding of specific antibodies from B-cells and displaying specific surface receptors on T-cells appear remarkably similar (34). Contrary to Burnett's clonal selection theory of antibody production (34), the formation of new specific antibodies or surface receptors appears to be triggered by antigen-specific interactions, instead of selection from mutating clones (34). Recently, it was demonstrated in tissue culture that depending on specific cell growth factors, added lymphokines and duration of culture, bone marrow and splenic germinal center precursors of B-cells may transform into T-cells, natural killer cells, macrophages or dendritic cells (35).

• As originally emphasized by Trowell (36), a problem with studying lymphocytes apart from the body in a microenvironment of low oxygen tension and apart from adjacent arterioles and organized stroma, is that the lymphocytes soon die and disintegrate within 2-4 days. This problem was circumvented by Nowell (37), who introduced use of mitogenic phytohemmagglutinin (PHA) in tissue cultures. PHA was found to cause small circulating cytoplasm-poor lymphocytes, identified as T-cells, to dedifferentiate and transform large-blast-like cells resembling germinal center lymphocytes which produce immunoglobulins reactive with antigens previously encountered in the

body of the lymphocyte donor (38). However, antibodies toward antigens not previously encountered are not produced unless sensitized macrophages are added (39). The numbers of small lymphocytes which are transformed is directly proportional to the numbers which disintegrate in the medium, and those transformed cells which survive continue to do so via anaerobic glycolysis, more or less like malignant lymphoma cells (40,41). Subsequently, Gallo et al (42) found that, by adding supernates obtained from PHA-transformed healthy lymphocytes to the surviving lymphoblastoid cells or to established tumor cell lines, such cells could be sustained in tissue culture for weeks or longer. This discovery led to purification of a lymphocyte growth factor or lymphokine, now called IL-2, which enabled demonstration of HTLV-1 and HIV-1 retrovirus shedding from the plasmalemma in the lymphoblastoid cells, after introduction of provirus infected lymphocytes to PHA-stimulated cultures (42,43). A variety of growth-promoting factors, called interleukins with designated numbers, have been identified as soluble products of macrophages, lymphocytes and other kinds of mononuclear cells; and found to promote growth as well as differentiation of selected cells in tissue culture. The point that should be remembered from such studies is that with dedifferentiation, human T-cells can transform into antibodyproducing B-cells, at least in tissue culture. However, it is not certain that such transformed cells surviving through anaerobic glycolysis truly represent their counterparts in humans, or in birds.

• In the body of living birds and mammals the transformation of B-cells producing and shedding specific antibodies into small-cytoplasm depleted T-cells with specific cell surface receptors, can be explained simply by shedding cytoplasm and progressive nuclear condensation (3,16-20,26,27). Such decrease in lymphocyte size by cytoplasmic loss with an increasing nucleus/cytoplasm ratio is characteristic of the organized lymphopoietic tissues of all animals (26), including the bursa of Fabricius (6,7,19). However, birds with a paucity of genes dedicated toward immunologic protection (33), fail to develop definitive regional lymph nodes and polarized germinal centers in lymph follicles pointing toward adjacent pinocytic epithelial cells in the adenoids, tonsils, terminal ileum and cloacal bursa of Fabricius.

• The current concept that mammalian B- cells and thymic T-cells are originally and perpetually derived from pluripotential marrow stem cells seems questionable because lymphocytopoiesis in the marrow embryologically follows lymphocyte development in the cloacal bursa, thymus and spleen in birds, as well as lymphocyte formation in the thymus, spleen and lymph nodes of mammals (1,3). Moreover, owing to partition of the cloaca by a urogenital septum during mammalian embryogenesis, humans lack a true bursal equivalent open to the terminal output of the gastrointestinal, urinary and genital tracts (1,7).

• Whereas common retroviral diseases of humans, such as HIV/AIDS and adult T-cell leukemia primarily involve the polarized germinal centers in the GALT and regional lymph nodes (19,20): the retroviral diseases of chickens, quail and turkeys, such as lymphoid leukosis, reticuloendotheliosis, and lymphoproliferative disease manifest with malignant lymphomas, immune deficiency or "runt" syndromes appear indiscriminate with respect to involvement of the bursa, thymus or other components of avian GALT (44,45).

• Finally, it should be noted that in humans, as well as in birds, that inherited genes (70 to 100 thousand in human lymphocytes) are expressed differently during progressive stages of differentiation from mesenchymal precursors. Differences in expression can be appreciated simply by studying the structure of nucleoli, nuclei, nuclear membranes, cytoplasm, cytoplasmic organelles and plasmalemma during successive stages of development. Numbering

some 200 trillion, having an average life span of ±42 hours, a sojourn in blood circulation of 30-60 minutes and emperipoletic migration patterns which pervade almost all organs, tissues and cells of a 70 Kg. healthy human adult (3,46), lymphocytes are cells derived from many parts. Nevertheless, when developing from periarteriolar mesenchymal cells and growing in organized lymphoid tissues, they require relatively large quantities of appropriate substrates, as well as oxygen, to synthesize characteristic nuclear and cytoplasmic organelles (16). Under normal aerobic conditions, the oxygen diffusing from red cells through lymph and mesenchyme is utilized to phosphorylate and link intracellular nucleotides to form DNA which, in turn, codes for the production of RNA and sundry organelles which are dissolved, loosely held in sols or bound in gels whose water is derived from the intracellular combustion of glucose (16,19). The cell specific proteins formed by the ribosomes appear to be dispersed in cytosols between ribosomes. When cell proteins, such as normal globulins, immune globulins or sundry lymphokines are secreted into the surrounding mesenchymal ground substance, they are released in the form of plasmalemma-encased globules wherein depolymerizaton of the contents yields colloidal hydrosols capable of flowing to carry dissolved proteins away from individual cells. Careful observation of the myriad globules shed from developing lymphocytes reveals that most appear hyaline, indicating depolymerization under basophilic stains in light microscopy; and some of the globules still contain degenerating mitochondria and ribosomes under transmission electron microscopy. Under scanning electron microscopy, smooth plasmalemma initially encasing shedding globules is easy to see. (See Figures in references 18,19,20,26,27,41). The point to remember is that individual lymphocytes normally shed a variety of soluble or dissolved cytoplasmic proteins during differentiation in organized lymphoid tissues–not a single antibody or lymphokine without water.

SUMMARY

Gut-associated lymphoid tissue progressively evolves during and after embryogenesis in ascending phylogenetic orders of animals to help feed, control coordinate growth and supply immunity to remaining body tissues in proportion to the rate oxygen and food are consumed to support life and customary activities.

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