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Development of the Spleen

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Summary

Embryogenesis and later development of the spleen's hematologic and immune functions involves ingrowth of vascular channels, influx of reticular cells and related byproducts to form a filtering network, migration of cells from other organs such as bone marrow and thymus, and final maturation of transient and resident cell populations.

While other animal organ systems subserve more than one biological function, the spleen is probably unique in that (1) both its hematologic and immune functions rely upon a sophisticated filtering system, (2) parenchymal cells do not "export" activity (i.e. exocrine function) and (3) its cellular secretions (e.g. immunoglobulins) arise from cells which have preferentially migrated there.

Based upon studies of non-human species not necessarily applicable to man, this treatise proposes that evolution of both hematologic and immune splenic functions depends upon:

1. development of mutual vascular channels;
2. influx of reticular cells and their products to form a filtering network;
3. further influx of cells from other organs (bone marrow and thymus) to form the bulk of the mature spleen; and
4. maturation of transient and resident cell populations.

Embryogenesis

A general description of embryonic development of the human spleen is difficult to find. Several well-known embryology texts do not even index the spleen, while others only briefly mention an outgrowth of the mesogastrium invaded by blood vessels and eventually organized into a loose collection of red and white blood cells. Examination of individual reports, however, reveals a far more sophisticated and highly organized series of events.

Barzanji and *Emery* (1) provide an overview of human splenic embryogenesis. The organ first appears in 8–10 mm embryos approximately 6 weeks into gestation as thickened areas in the coelomic epithelium of the dorsal mesogastrium, which after ingrowth of blood vessels later fuse. Lymphocytes appear by the third month when the organ takes on its characteristic shape. The spleen grows proportionately with the body to an appropriate size near term. Germinal centers, however, do not form until three weeks after birth even if in utero or perinatal infection supervenes (2).

One has to turn to studies in both man and other species to obtain details of blood vessel, reticular and lymphoid cell development and organization. At 8.7 weeks blood vessels in 38 mm crown-rump length (CRL) human fetuses appear as thin-walled vascular loops; true arteries, veins, and capillaries are not recognizable until after the embryo attains a length of 42 mm. Vasculogenesis is prominent in 8.9 – 9.3 week spleens and evolves from a closed loop system into distinct channels opening into sinuses formed by endothelial cells (3), an arrangement essential to the spleen's functions.

In early spleens, the bulk of the organ is formed from a spongework of branching connective tissue termed reticular cells. In

38 mm fetuses, the cells are bulky but branchless; short branches appear by 42 mm and by 57 mm, slender processes form an open meshwork, particularly in the organ's center. As these processes progressively enlarge, the interstitial space among cells widens (3).

Vessel maturation and reticular cell formation are not independent phenomena, but together set the stage for development of organized areas destined to become white and red pulp. As blood vessels proliferate, reticular cells and their fibers form a sheath around developing arterioles in a pattern similar to that seen in developing lymph nodes (4). During the twelfth through seventeenth week of human development, a variety of cells invades the periarteriolar sheath, including monocytes, lymphocytes and the dominant cell type, macrophages (5).

In the rat, these macrophages likely become the interdigitating cells (6) creating the network for attracting lymphoid cells primarily of T-lineage in the periarteriolar zone. In fetuses with a CRL greater than 98 mm the marginal zone, formed after macrophage and red cell influx, surrounds the periarteriolar zone and continues into the cords of red pulp.

The red pulp likewise consists of a meshwork of arteries, veins and dark reticular cells into which red cells migrate. Venous sinuses are mature by this time, separated from one another by cords of marginal zonal tissue. Fenestrations in the basement membrane of the sinus suggest that cells move easily from one compartment to another (see below and Circulatory Dynamics of the Spleen) (5).

Further differentiation into more specialized zones, such as germinal centers, does not take place until after birth even though surface immunoglobulin (sig) bearing cells (B cells) and erythrocyte rosette forming cells (T-cells) appear as early as 13 weeks of human gestation (7). Differentiation of immune reactive cells does not seem to depend upon antigen stimulation. Neither children with intrauterine infections nor infants born prematurely (8) display germinal centers in the spleen until approximately 3 weeks of age. The process may depend, however, on formation and maturation of reticular and interdigitating cells. For example, experiments in rats show that 5' nucleotidase-bearing reticular cells precede T-cells in the periarteriolar areas while acid phosphatase-containing cells arise primarily in germinal centers (9). Further organization of white pulp into nodular, transitional and periarteriolar sheaths is described but underlying regulating mechanisms are unknown (10).

Hematologic Function

Crosby (11) enumerates eight red blood cell (RBC) related functions of the spleen: erythropoiesis, production, cell surface maturation, storage, culling, iron metabolism, pitting, and destruction. Periods of development and mechanisms responsible for each function remain elusive, and data derived from both *in vivo* and *in vitro* animal experiments may not apply to man. For example, erythropoiesis is commonly studied using explants or suspensions of spleen cells cultured *in vitro* on chorioamniotic membranes (12) or injected into irradiated immunoincompetent subjects (13). Chicken spleen cells by day 15-17 after fertilization exhibit hematopoietic activity which disappears at hatching and depends upon cellular aggregates rather than single stem cells. Repopulation of irradiated mouse spleen or bone marrow by spleen cells varies with donor age. In untreated animals, spleen cells per gram body weight is greatest at 12 weeks while spleen colony-forming units per gram body weight is maximal at 10 days and plateaus by 70 weeks.

Uptake of radiolabelled iron is another tool for measuring erythropoiesis. In the rat, ratio of splenic to bone marrow erythropoiesis is greatest in the weanling and declines slowly thereafter. The reverse is true if the ratio of body to spleen weight is used suggesting that erythropoiesis is limited to a finite period during a stage of slow splenic growth and that mature cell storage or production of other cells accounts for differences in organism: organ weight (14).

In man, red cell precursors are found as early as 6-7 weeks of gestation (15). By 8-9 weeks,

maturation is reflected in red cell debris in newly immigrated macrophages and progenitor cells in sinusoids. RBCs in various stages of development as well as mature cells appear in sinusoids by 20 weeks, although the source of these precursor cells is still speculative. The presence of progenitor cells among mesenchymal cells removed from the vascular system suggests an extravascular origin. Whether and when true erythropoiesis occurs in man, however, remains unclear.

Other cells such as granulocytes may also be produced and undergo maturation in the spleen. Neonatal mice exhibit high levels of colony-forming cells ($180–270/10^5$) compared with adults ($4–28/10^5$ cells) (16). Lymphocyte precursors invariably arrive after macrophages (see below) and granulocyte precursors (16, 17). Phagocytic function measured by technetium sulfacolloid uptake in rodents shows the liver to be the chief site of uptake for the first 4 weeks of life. Splenic uptake is low at birth and reaches adult levels at ten weeks of age (18).

Studies of red blood cell dynamics and maturation in developing or neonatal animals are only rudimentary. Perfusion studies in the rat suggest that various populations of red cells exit the spleen at different times. Fast washout populations include mature cells (e.g. peripheral RBCs), while those that wash out slowly include young cells (58% reticulocytes) (19). Reticulocytes preferentially adhere to connective tissues, reticular or interdigitating cells and sinus walls after release from the bone marrow and eventually undergo continued differentiation within the spleen (20). This maturation process involves alteration of lipid constituents in the cell membrane and is impaired by splenectomy (21).

Immune function

Comparison of age-related events is hampered by routine use of spleen cells as effector cells *in vitro* rather than *in vivo* studies. In this system, degree of cellular maturation is unknown, and interactions among different cell types cannot be evaluated in their natural environment. Within these constraints, this section examines immune ontogeny from three arbitrary (and perhaps inseparable) viewpoints: cell identification and description (See also Immunoarchitecture of the Human Spleen), cell mediated immune function and antibody production.

T-cell areas in the mouse spleen are purportedly distinguishable from B-cell zones by texture of staining pattern in routinely fixed specimens (22). In two groups of young adult animals (11 weeks) raised in germ-free environment until 8 weeks, T-cell zones are comparable but B-cell areas are slightly larger in one of two seemingly matched groups, possibly from seasonal differences in the environment.

Surface properties distinguish the various splenic cell populations. Density gradient electrophoresis, a technique based on surface charges of cells, identifies a bimodal distribution of splenic lymphoid cells in 3.5 to 5.5 and 7.5 to 17 week Balb/c mice but a unimodal distribution at 6 weeks of age reflecting greater mobility of T-cells over B-cells. Thymectomy alters this pattern suggesting a thymic influence on surface charges (23).

Recently, identification of surface and cytoplasmic antigens or cell products provides a more precise identification of lymphoid cell lineages and maturation patterns in the spleen. Precursor (pre) T- and B-cells are distributed along with null cells in an age-dependent manner in the mouse spleen. Neonatal and 24-week mice display the greatest percentage of null cells while each of the three groups of cells is equal in 3 and 8 weeks old mice (24). Other workers trace the origin of pre B-cells to the fetal liver and hypothesize migration from that organ to the spleen, where development continues (25, 26).

Lymphocytes are noted in the human fetal spleen at 13 to 15 weeks of gestation or about 6 to 7 weeks after their appearance in the thymus and blood respectively (27). T-cells (E rosette-forming) constitute the majority of cells in the thymus and the minority in the spleen from 13 to 26 weeks of gestation. Although only 1 to 2% of these cells are spleen cells before 14 weeks, all immunoglobulin classes as measured by surface immunoglobulin

(sig) are present in fetuses older than 19 weeks. B-cells predominate over T-cells in the spleen throughout gestation, the greatest difference occurring at 20 weeks.

The term T-cells is somewhat misleading as multiple subsets of cells are able to form E-rosettes yet function differently. Monoclonal antibodies directed against antigens identify these subsets and allow further analysis of T-cell ontogeny in the mouse. Lyt-1 denotes helper function, while Lyt-2 and -3 denote cytotoxic-suppressor activity and Thy-1 cortical thymocytes. Use of these reagents, reveals that peripheral T-lymphocytes derived from immature thymus cells accumulate in the spleen by one day of age but account for only 1% of splenic cells (28). By 2 weeks, however, the adult frequency of Lyt-1, Thy-1 and Lyt-2 bearing cells is already attained. Lyt-1 cell number is only slightly greater than Thy-1, while Lyt-2 cells are the least frequent. Thus, despite continued growth of the spleen, the total population of T-cell subsets is reached by 2 weeks of age and remains constant.

Other surface antigens serving as important markers of lymphocytes are the histocompatibility antigens incorporated on the mouse H-2 and Ia loci. Newly formed bone marrow lymphoid cells in adult mice bear H-2 antigens, but develop Ia more slowly, the more immature cells expressing low density of the antigen. In young postnatal mice, cells bearing Ia are rare in both bone marrow and spleen, and antigen incidence and density do not attain adult levels in the spleen until 4 and 10 weeks respectively (17).

Enzymes associated with lymphoid cell function also fluctuate during ontogeny. Adenosine deaminase (ADA) and nucleoside phosphorylase (PNP), enzymes of the purine salvage pathway, are required for normal lymphocyte development. ADA levels are equivalent in the spleen of 2, 7 and 24 months old mice; on the other hand, PNP levels decline steadily from 2 months of age and by 24 months are only 63% of their earlier peak, possibly related to declining T-cell function with aging (29).

As the host matures, changes take place in both afferent and efferent lymphoid cell-mediated phenomena. Antigen processing must precede T- and B-cell effector reactions. Accessory cells, primarily macrophages, bearing Ia antigens (necessary for cellular communication) appear and by birth, function in the mouse thymus. Although they are evident in the spleen at 2 weeks of age, they do not function until weaning (approximately 3–4 weeks of age) and presumably therefore cannot stimulate proliferation, maturation and activation of resident splenic T-cells until that time (30).

Considerable species variation exists. Some workers find that murine lymphoid cells bind a variety of antigens even before birth, although efferent responses are undetectable until the second week of life (31). Fetal sheep splenic cells also bind antigens as early as 58 days gestation but make no antibodies until considerably later (32). Antigen binding cells are present in chick embryo spleen on days 14 and 20, associated with an increase in the number of receptors to some but not all antigens. The role of these unknown antigen binding cells in the embryo remains obscure (33).

Lymphocyte response to mitogen stimulation is a commonly used though poorly delineated measure of maturation. In one study, phytohemagglutin (PHA) and lipopolysaccharide provoke their greatest response in 4 month old compared to 11, 20 and 30 month old animals (34), as the number of lymphoid cells decreases although spleen weight increases.

Similarly, in young mice (3–4 months old) compared to aging ones (26–30 months), more spleen cells but not thymic cells exist in stage G1 of the cell cycle and incorporate ^{3}H after concanavalin A (CON A) or PHA stimulation, perhaps reflecting the decreasing number of immunoreactive cells in these organs with aging (35).

Post-thymic T-cells in neonatal mice are only one-tenth as responsive as adult splenic T-cells to PHA, graft-versus-host production, mixed-leukocyte reaction, and *in vitro* and *in vivo* B-cell helping, although T-cell cytotoxicity assays are comparable to adults. Thymectomy at 5 days has no influence on expansion and matura tion of post-thymic T-cells (36).

Lymphocytes mediate yet another function – antibody dependent cell cytotoxicity (ADCC) whereby T-cells reorganize antibody on a target and proceed to "kill" that target. This function is at a low level in murine spleens at birth, peaks at age 2 months, and then gradually declines to steady state level during the ensuing 10 months. Removal of phagocytic cells abrogates the ADCC effect only in Balb/$_c$ mice (37).

T-cells themselves are not necessary *in vitro* for several functions, and humoral substances such as T-cell replacing factor (TRF) facilitates B-cell response to sheep red blood cells (SRBC). While this substance is not present in mouse spleen cell preparations at birth, it emerges by 1–2 weeks of age (38).

One explanation for diminished immunocompetence of neonatal compared to adult mouse spleen cells is presence of suppressor cells. Neonatal spleen cells suppress MLC reactions between adult spleen cells and inhibit antibody production to SRBC (39). In several strains of mice, an increase in MLC reactivity develops as suppressor cell activity diminishes with age. In another mouse strain, however, this response does not occur suggesting other influences on maturation of this phenomenon (40, 41). Some evidence suggests that the suppressor population does not derive from T-cells, but rather from miniature macrophages left behind after adherence procedures and that T-lymphocytes are not inhibitors of antibody production (42). Nonetheless, in the cow, suppression is not even limited to neonates, and a soluble suppressor substance extracted from mesenteric lymph nodes of calves greater than four years of age inhibits production of antibody by mouse spleen cells in response to thymus dependent and independent antigens (43).

In vitro differences in B- and T-cell function between young and old in the mouse are substantiated *in vivo* by lack of antibody response as measured by uptake of ^{51}Cr and ^{125}I-5-iodo-2-deoxyuridine to intravenous SRBC injection in 24 month old in contrast to 3 month old mice. Decreases in both T- and B-cell dependent and independent function or an increase in suppressor activity may explain these results (44).

Production of antibody is one of the most intensely studied immune functions of the spleen (26, 45), and only the factors influencing development of ths phenomenon are addressed here. As previously mentioned, pre-B-cells are present in the spleen, and the number of immunoglobulin secreting cells varies with age and species (46). Some workers propose that pre-B-cells must mature within the splenic envirionment before they are able to respond to non-specific T-cell mediator effects (47).

Likewise, after T-cells appear in the spleen and then develop surface markers, their reactivity is curtailed by thymectomy in the immediate postnatal period. If neonatal spleen is grafted into syngeneic adult animals, cell number and suppressor activity are the same as *in situ*. Spleen graft growth or function in contrast to mitogen and MLC responses is not adversely affected by host thymectomy. If the recipient is thymectomized, however, graft spleen development is enhanced as are cell numbers and antibody production (48). These latter observations suggest that after thymectomy immature helper T-cells selectively increase, and suppressor cells are "suppressed", suggesting that splenic regulatory factors are necessary for normal growth *in vivo* (49). Additional observations in aging mice (50) support the contention that maturation of antibody formation by spleen cells differs from maturation of cell proliferation. Whether these differences are functional or due to changing cell populations remains unclear.

Conclusion

The spleen plays an important role in development and maintenance of hematologic and immunologic function in man and animals. Both functions depend upon channeling and filtering red and white blood cells as well as foreign particulate material through mutual and/or separate compartments of the spleen. The vascular network established during embryogenesis and the sequential influx of reticular and later macrophage derived cells lay the foundation for subsequent migration of lymphoid and red cells leading to further development and maturation of the spleen.

While the regulating mechanisms responsible for this sequence of events remain sketchy, molecular biology of cell-cell adhesiveness in embryonic nervous tissue as elucidated by *Edelman* et al. (51) may well apply to the spleen, not only providing an explanation for the initiation of events during embryonic life but also for the continued influx of new cells to specific sites throughout the functional lifetime of this organ.

The nature of mutual interactions among the spleen, bone marrow and thymus is another fruitful area for exploration. Is this interaction simply a matter of shifting cell populations or are there humoral mediators?

Studies of the parallel development of hematologic and immunologic function of the spleen may provide some needed answers.

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