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The regulation of hemopoiesis in the spleen

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1. Introduction

Bone marrow in humans and in adult rodents is recognized as the primary site for hemopoiesis. This prominence, however, has not always been enjoyed phylogenetically or even ontogenetically. This paper reviews: 1) the relative contribution and regulation of splenic hemopoiesis in health and specific experimental and disease states where reversion to its embryonic hemopoietic capacity occurs, 2) the role of the stromal

environment within hemopoietic tissue and its contribution to hemopoietic stem cell function and 3) the coregulatory roles of stem cells and microenvironment relative to hemopoiesis in health and disease. In each instance changes in splenic hemopoiesis are reviewed and the implications of these observations for regulation of splenic hemopoiesis are explored.

2. *Hematologic functions of the spleen*

The mammalian spleen is a multifunctional organ that has undergone radical structural and functional changes through vertebrate evolution. From the lowest fishes where 'disperse' or 'diffuse' spleens consist of loose or moderately compact lymphoid tissue surrounding venous channels within the gut, the spleen has developed in higher vertebrates into a compact structure located outside the gastrointestinal tract attached to the mesentery[30,31].

The contribution of the spleen to the immune system during evolution is directly related to the degree of splenic development and the existence of other organized lymphoid tissues[44]. Thus, in fishes and amphibians the spleen plays an insignificant role in the immune response since other lymphoid organs serve as prime sites of antibody synthesis. Among certain reptiles and in higher vertebrates the spleen plays a greater role immunologically.

In adult mammals, including man, the contributions of the spleen to hematologic function are not trivial and include serving as a repository for platelets which are released into the circulation during severe hemorrhage and monitoring red blood cell integrity to permit passage of healthy, competent cells while removing aged or damaged ones[61]. Another major and important function of the spleen throughout evolution and one which may occur transiently during fetal development in man is blood cell formation[23,60,63].

a) Phylogeny and ontogeny of hemopoiesis

In the phylogeny of hemopoiesis the bone marrow is a 'late arrival'. In lower vertebrates, including the cyclostomes, bony fishes, amphibia and certain reptiles, blood formation is exclusively extramedullary occurring variously in the submucosa of the gastrointestinal tract, gonads, kidney, liver and in amphibia and reptiles primarily in the spleen[31,60]. In higher vertebrates, birds and mammals, the major site of blood cell formation in the adult is the bone marrow though, with the exception of primates, foci of organized hemopoiesis are also seen in the spleen.

Mammalian embryonic development of the hemopoietic system involves a sequential movement of primary centers of hemopoiesis similar to that which occurred during evolutionary development (table, adapted from Ward and Block[60]). In man, the sequence and onset of appearance of hemopoietic foci have been reported as[7]: 1) yolk sac (4th week); 2) body mesenchyme and blood vessels (5th week); 3) liver (6th week); 4) spleen, thymus and lymph glands (2nd–4th month); and 5) bone marrow (5th month). By the 5th month of gestation the bone marrow becomes the major site for hemopoiesis. There is no evidence that cessation of hemopoiesis at any site causes a permanent loss of the potential to recover hemopoietic capacity[60]. In instances where bone marrow has been ablated, damaged or has failed to develop, these embryonic sites of blood formation retain their hemopoietic potential and become active in reverse ontogenetic order, i.e. to spleen, then liver, etc. In these conditions it is not known whether mesenchymal or stem cells already present in these tissues retain their embryonic capacity to produce blood forming elements and are activated or whether these sites merely provide the appropriate signal and microenvironment for colonization by circulating stem cells.

The relative contribution of the spleen to hemopoiesis in mammals appears to be species- and age-dependent. In rodents such as rats and mice, splenic hemopoiesis persists more or less markedly until adulthood[5,10,11,25]. In man, splenic hemopoiesis ceases after birth[23] and appears only in certain pathological conditions[19,60].

b) Regulation of hemopoiesis in the spleen

The normal mouse spleen, in contrast to the human spleen, is a major site of hemopoietic activity both neonatally and in adulthood[10,12] (figs 1 and 2). It plays an important physiological role in hemopoiesis in neonatal rodents at a time when they lack active marrow hemopoiesis[26,37]. This neonatal stage of development is similar to that described in the human fetus during the fourth month of gestation.

Pluripotential hemopoietic stem cells (CFU-S) and committed stem cells (CFU-C) first appear in the murine spleen in late gestation and initiate splenic hemopoiesis[43]. These stem cell populations increase and reach their peak numbers in the 3rd week after birth and begin to decline thereafter in association with rapid marrow development and hemopoietic activity[5,43]. In contrast with other mammals, this decrease in the splenic contribution to hemopoiesis is incomplete. A permanent excess of pluripotent stem cells relative to committed stem cells is retained in the murine spleen providing hemopoietic activity throughout the life of the mouse[5,11,43].

A similar but less pronounced pattern exists in the rat[59]. Here, however, the shift from spleen to bone marrow is more dramatic than in mice. In adult rats less than 5% of hemopoiesis occurs in the spleen[22]. Thus, a higher fraction of total body hemopoietic activity occurs in the spleens of mice than in spleens of rats of the same age. When the need exists for increased hemopoietic activity in these adult animals, the spleen is the first organ to respond[11]. The significant numbers of CFU retained in spleens of rodents provides the basis for their rapid hemopoietic response under pathological or experimental conditions, e.g. hemorrhage[10,36], hypoxia[11], endotoxin injections[51,52], and bone marrow ablation[4,21].

The degree of splenic contribution to blood cell formation has been convincingly demonstrated in splenectomized mice which have been bled[10] or in whom marrow spaces have been ablated[33]. Boggs et al.[10] showed that the relative decrease in erythrocyte production in splenectomized-bled animals was due to the loss of erythropoiesis within the spleen and estimated that approximately one-half of the increase in erythrocyte number following bleeding occurred in the spleen.

When bone marrow is experimentally ablated in mice using strontium-89 (^{89}Sr) there is a compensatory hemopoietic hyperplasia of the spleen[4,21]. Splenectomy in ^{89}Sr-treated animals results in eventual death due to hemopoietic insufficiency[33].

Splenic erythropoiesis differs from bone marrow erythropoiesis under conditions where red blood cell production is stimulated or depressed[11]. Following hypertransfusion of red blood cells into mice there is a fractionally greater decrease in radioiron uptake and in erythroid precursor cell numbers in the spleen compared to bone marrow. Following bleeding no increase in radioiron uptake or erythroid precursors was noted in the marrow but a marked increase in these parameters was found in the spleen. Similar results have been obtained in mice exposed to hypoxia. When hypoxic mice are splenectomized they are incapable of maintaining a normal hematocrit. From these data it has been suggested that in the mouse, bone marrow production of erythrocytes is maximal but falls short of the animal's normal needs. Therefore, splenic assistance is necessary for proper erythropoietic homeostasis. A similar but less dramatic parallel exists in the rat[35].

These data suggest that splenic hemopoietic function in rodents is linked in some way to that of bone marrow. The threshold of splenic hemopoietic activity appears to be at or slightly below the maximal level in bone marrow, activated considerably in adulthood as in mice, or minimally as in rats. This association places the spleen in an important position relative to regulation of hemopoiesis in health and disease.

The regulation of an orderly transfer of hemopoietic responsibility from spleen to bone marrow is unknown. What signals the spleen to reduce its hemopoietic contribution as the bone marrow becomes more active? In this regard it is of interest that this decline does not occur if fetal bone marrow development is prevented by injection of pregnant mice with ^{89}Sr [32]. Extramedullary hemopoiesis in these offspring produces normal numbers of blood cells for several weeks after birth. What is the nature (if any) of the communications between bone marrow and spleen? Are the factors which regulate bone marrow cell activity the same, qualitatively and quantitatively, as those which act on hemopoietic cells in the spleen?

3. Model systems to explore the regulation of hematologic function in the spleen

In primates and in most adult mammalian species bone marrow is the principal site for blood cell formation. When marrow function is compromised, however, as in osteopetrosis, myeloproliferative disorders, certain leukemias and lymphomas or experimental ablations, the spleen and other organs are able to compensate by reverting to their embryonic role[4,19,54,58,60]. Questions to consider in these situations include: 1) What signals are received by the spleen to stimulate stem cell proliferation and blood cell production? 2) What is the fate of the stem cells in the bone marrow during its collapse? 3) What specific cellular or functional changes occur in the spleen during this renewed hemopoietic activity? At least three models – congenital, pathological, and experimental – have provided information on the role and regulation of the spleen in compensatory hematologic function.

a) Splenic hemopoiesis secondary to congenital absence of bone marrow spaces. Example: osteopetrosis

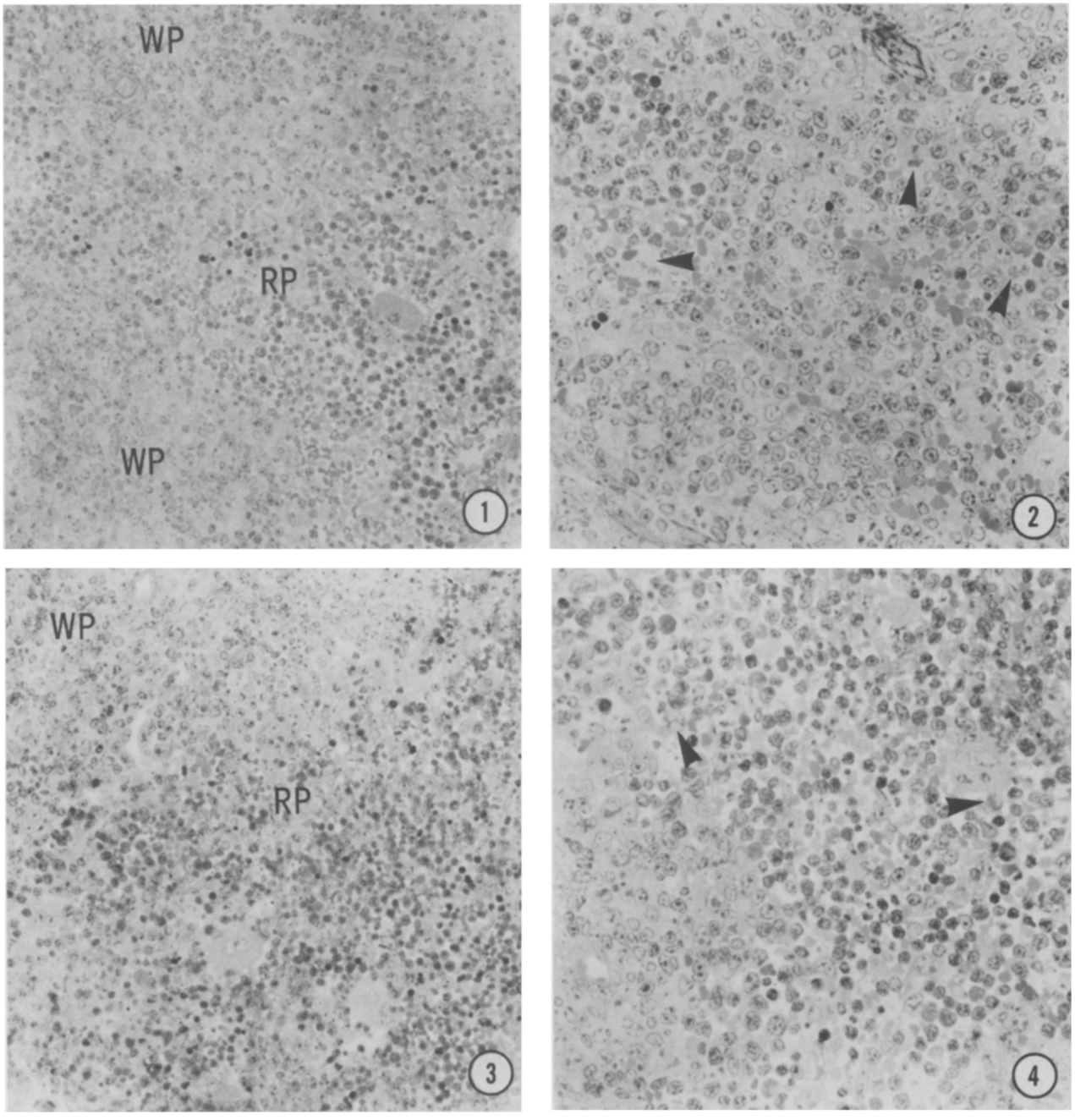
Infantile malignant osteopetrosis is a congenital skeletal disease characterized by an absence of bone marrow cavities due to severe reduction in bone resorptive capacity[38]. Resorption within developing long bones is inadequate to develop marrow spaces, precluding development of the bone marrow. The associated hematologic abnormalities, i.e. pancytopenia, are significant because anemia, hemorrhage or overwhelming infection are the primary causes of death of affected individuals[46]. Infants with the disease typically present with hepatosplenomegaly which upon microscopic examination show numerous well organized hemopoietic foci[19,50]. It has further been observed that spleen and peripheral blood from osteopetrotic infants contain substantially higher numbers of committed hemopoietic progenitor cells than age-matched controls or other patients without extramedullary hemopoiesis[19,45]. Though affected individuals' spleens show extensive hemopoietic activity such infants are typically pancytopenic suggesting that the loss of the marrow microenvironment cannot be adequately compensated by hemopoiesis in the spleen or liver. Because the differentiation and proliferation of marrow hemopoietic stem cells is dependent upon the establishment of bone marrow cavities[8], it is presumed that the extramedullary hemopoiesis seen in osteopetrosis, albeit insufficient, is due to continued embryonic function.

In addition to human osteopetrosis this disease is also genetically transmitted in a number of different animal mutations[38]. Splenic function and the regulation of extramedullary hemopoiesis have not been explored in these various animal models and deserve investigation. The hematologic status of a number of these osteopetrotic mutations has been recently described[62] and, like several other aspects of the disease, reveals marked differences between mutations with respect to abnormalities in several parameters such as hematocrit and spleen weight, cellularity and leukocyte concentrations. Spleens and livers, however, from each mutation tested showed significantly greater numbers of stem cells than those found in normal animals indicating an attempt to compensate for the loss of marrow function. A similar increase in splenic hemopoiesis[16] and stem cell numbers has been observed in mice with estrogen-induced osteosclerosis[42]. Examination of splenic tissue from young osteopetrotic microphthalmic mice (figs 1–4) reveals extensive hemopoietic activity within the red pulp of mutant animals compared to normal littermates. Additional studies of the hematologic manifestations in congenital osteopetrosis might be expected to provide significant insights into the regulation of splenic hemopoiesis.

b) Splenic hemopoiesis during marrow dysplasia. Example: myeloproliferative disorders

The term 'myeloproliferative disorders or syndromes' represents a group of diverse and poorly understood

blood diseases and includes chronic (CML) and acute myeloid leukemia (AML), polycythemia vera (PV), essential thrombocythemia (ET) and agnogenic myeloid metaplasia (AMM), also called myelofibrosis. In spite of the varied clinical manifestations of each disease, their etiologies have recently been appreciated and they are now termed 'clonal hemopathies'[41]. Clonal hemopathies occur when one or more abnormal hemopoietic population can be shown to be derived from a single cell. Cytogenetic techniques and glucose-6-phosphate dehydrogenase (G-6-PD) isoenzyme analyses have been used to demonstrate the presence of the stem cell abnormality (either as a chromosomal defect or absence of a G-6-PD isoenzyme) in granulopoietic, erythropoietic and megakaryocytic descendants in each of these diseases[1,29,41]. Though similar in origin from single pluripo-



Figures 1–4. Photomicrographs of splenic tissue from 10-day-old normal mice and their osteopetrotic (microphthalmic) littermates illustrate variations in extramedullary hemopoiesis. Figure 1. Photomicrograph of spleen from a normal mouse illustrating areas of white pulp (WP) and red pulp (RP). The red pulp is active in hemopoiesis as evidenced by the density of nucleated cells. Toluidine blue, ×260. Figure 2. Higher magnification of an area of red pulp from a normal mouse. Several hemopoietic cells can be observed in mitosis (arrowheads). Toluidine blue, ×400. Figure 3. Photomicrograph of spleen from an osteopetrotic (microphthalmic) mouse. In this condition the congenital absence of marrow cavities results in extensive extramedullary hemopoiesis seen here as an expansion of the red pulp for hemopoiesis. Note the extent of the red pulp in the mutant compared to that in a normal littermate (fig. 1). Toluidine blue, ×260. Figure 4. Higher magnification of an area of red pulp from a microphthalmic mouse. Several mitotic figures can be observed in hemopoietic cells (arrowheads). Toluidine blue, ×400.

tent hemopoietic stem cells, each of these diseases differs in the particular responses made by their abnormal stem cells to regulatory factors[2]. It appears that chromosomal rearrangement is necessary for initiation of AML[2], but what initiates the disease in the other disorders is not known.

Extramedullary hemopoiesis is a well recognized feature of the myeloproliferative disorders[54,58,60]. Chervenick[15] reported markedly increased numbers of colony-forming cells in blood of seven patients with myelofibrosis. All patients had hepatosplenomegaly and marrow fibrosis, another characteristic but variable feature of the myeloproliferative disorders. Though it was suggested[15] that the reduction in marrow volume due to fibrosis caused the emigration of marrow stem cells into the circulation resulting in their lodgement in liver and spleen, other evidence[60] suggests that this is not the case. It has been a consistent observation that in myelofibrosis splenomegaly precedes significant marrow fibrosis[60]. In contrast to blood cells from patients with myelofibrosis, fibroblasts do not have cytogenetic or isoenzyme abnormalities, suggesting that they are derived from a normal stem cell population. Thus, the marrow fibrosis that results from fibroblastic activity in myeloproliferative disorders is apparently a secondary and reactive process. The cause of this marrow fibrosis is unknown, but it may be related to factors produced by abnormal hemopoietic cells or to some marrow insult.

Knowledge of hemopoiesis in the fetal human spleen has been based largely on inferences derived from studies of human fetal tissue using conventional morphological techniques or from studies of nonhuman mammals[18,23,60]. Recent evidence[64] suggests that hemopoiesis in the fetal human spleen is limited or nonexistent. This conclusion is based on cytological and immunocytochemical data. According to these investigators[64] the very limited hemopoiesis observed in fetal human spleens results from trapping of stem and differentiating cells, known to circulate during fetal development, and their phagocytosis by the numerous active macrophages present in the cords of the red pulp. Thus, the splenic contribution to fetal hemopoiesis in humans appears to be much less than that in other mammals. The implications of these observations for the pathogenesis of hemopathies have yet to be formulated.

These data[64] imply that in myelofibrosis stem cells leave the bone marrow, settle in the spleen, and their development is frustrated by the reactive phagocytosis by macrophages. Engraftment is eventually established but at the cost of reduced capacity. These interactions may explain why splenic hemopoiesis is different qualitatively and quantitatively from that observed in the marrow.

What accounts for this presumed resistance of the spleen to hemopoietic colonization? Could it be a change in the stroma with maturation (see below) or a limited fetal human splenic capacity for hemopoiesis?

c) Splenic hemopoiesis secondary to experimental bone marrow ablation. Example: ^{89}strontium

Classic studies by Jacobson et al.[27,28] demonstrated that the mouse spleen, protected during whole body X-irradiation, could produce new peripheral blood cells as well as repopulate irradiated sites with stem cells. Anemia did not occur in these animals because of the compensatory increase in erythropoietic tissue in their spleens.

More detailed evidence of hemopoietic function and capacity in extraskeletal sites, i.e. liver, spleen, has come from experimental studies using ^{89}strontium (^{89}Sr), a bone-seeking radionuclide which rapidly and selectively ablates marrow hemopoiesis[4,21,27]. Remaining marrow hemopoietic activity in ^{89}Sr-treated mice has been shown to be insufficient to sustain the life of splenectomized mice even 37 days after isotope (4–6 μCi/g b.wt) injection[33].

Studying the role of irradiation-induced depletion of stem cells in bone marrow on proliferation of stem cells in the spleen, Fried et al.[21] found a marked reduction in numbers of femoral marrow stem cells within 3 days following ^{89}Sr injection, their numbers remaining low for more than a month. Stem cells in the spleen, however, more than doubled by day 8 following isotope injection and remained high for at least a month. Similar results have been reported by Adler et al.[4]. Thus, spleens of ^{89}Sr injected mice undergo compensatory hemopoietic hyperplasia following marrow ablation.

The hepatic contribution to hemopoiesis is negligible. ^{89}Sr-treated splenectomized mice were found incapable of sustaining hemopoietic function[3] with CFU-S virtually undetectable in either blood or liver. Similarly, estrogen-induced osteosclerotic mice do not demonstrate hepatic hemopoiesis in intact or splenectomized mice[16]. Thus, even under severe and sustained hemopoietic stress the liver does not appear to support any detectable proliferation of CFU-S in this species.

What then are the possible mechanisms for transfer of CFU capacities between bone marrow and spleen? What is the fate of marrow stem cells during marrow collapse and what controls the number of CFU-S? The increase in stem cells in the spleen in response to reduction in the stem cell compartment of the bone marrow induced by ^{89}Sr [21] was thought to be due to some undefined humoral factor activated as a result of destruction of the stem cells in bone marrow. However, Adler et al[4] have presented indirect evidence that the size of the granulocyte pool (i.e. committed granulocytic precursor cells and/or end cells) rather than the total CFU-S compartment regulates CFU-S proliferation. A significant granulocytopenia developed on the 10th day in ^{89}Sr-treated mice and returned to normal levels on day 21 and thereafter. This granulocytopenia was inversely correlated with the concentration of CFU-S in the spleen, which was at its highest level on day 10 before declining and stabilizing by day 21. These observations were interpreted to indicate that once the granulocytopenia was reversed a new stem cell: granulocyte pool equilibrium was established with the remaining stem cells being sufficient to maintain normal peripheral blood cell concentrations. These data, implicating a feedback regulation by stem cell progeny on CFU-S proliferation, are supported by the work of Tyler et al.[57]. Using diffusion chambers containing normal bone marrow cell suspensions implanted i.p. into mice made neutropenic by increasing concentrations of cyclophosphamide, they demonstrated a dose-related increase in

myelopoiesis and in pluripotent stem cell numbers compared to untreated controls. The origin and identity of this humoral factor remain unknown.

4. The relative importance and coregulatory roles of stem cells and the splenic microenvironment in hemopoiesis

That the spleen provides a suitable and adequate microenvironment for hemopoietic stem cell proliferation is well illustrated by the now standard technique[55] for assessing pluripotent stem cell concentration in a given population of cells injected into lethally irradiated mice. Examination of the spleen surface 7–9 days after transplantation reveals colonies of cells that consist histologically of differentiated cells of only one cell line (erythrocytic, granulocytic, megakaryocytic). The number of colonies formed is related in a dose-dependent fashion to the number of stem cells injected. The cell lineage expressed by the stem cell depends on characteristics of the particular microenvironment in which it finds itself in the spleen. Furthermore, colony-forming stem cells lodging in the spleen produce three times as many erythrocytic colonies as granulocytic (E/G ratio = 3) whereas in bone marrow of these mice there are about half as many E colonies as G (E/G ratio = 0.5)[65]. The basis for this difference, as suggested by the work of Curry and Trentin[17], lies in the stroma itself. In the spleen the stroma supports principally the development of erythropoietic cells while the stromal microenvironment in the bone marrow preferentially supports granulopoietic development.

Much has been learned, from studies using the anemic mouse mutations W/W^v and Sl/Sl^d, to support this concept of a relationship between hemopoietic stem cells and microenvironment. Genetically anemic W/W^v mice have defective and deficient numbers of hemopoietic stem cells, as measured by the spleen colony assay[48]. While Sl/Sl^d mice possess normal numbers of stem cells the defect in this mutation lies within the hemopoietic microenvironment into which these cells migrate[40,48]. Attempts to cure the anemia in Sl/Sl^d mice by implantation of stem cells from normal animals have been unsuccessful[9]. Reciprocal transplants of stem cells from Sl/Sl^d mice into irradiated normal recipients or W/W^v mice, however, rescue these animals and cure the anemia in the W/W^v mutants[9,40]. These results suggested that the hemopoietic microenvironment in the Sl/Sl^d mouse could not support stem cell growth and differentiation even though these cells were capable of normal differentiation when transplanted into a competent microenvironment. When a hemopoietically competent microenvironment is provided, the anemia improves, as evidenced by experiments where +/+ or W/W^v spleens or femora implanted into Sl/Sl^d recipients[9,20] provided a hemopoietic microenvironment into which Sl stem cells could migrate, grow and differentiate. By counting the numbers of spleen colonies formed following injection of normal marrow cells into irradiated Sl/Sl^d recipients having two spleens, their own and that of another genotype, Altus et al.[6] found normal numbers of colonies in +/+ and W/W^v spleens whereas few or no colonies in Sl/Sl^d spleens.

These studies provide elegant demonstrations of an interdependance of hemopoietic stem cells and their microenvironment and further research is required to define these roles on a molecular basis. This information may find useful application by serving as a basis for comparing similar parameters in various disease states, e.g. leukemias, myeloproliferative disorders.

5. Implications and conclusions

The last two decades have provided evidence for the interdependence of hemopoietic and nonhemopoietic (stromal) elements during blood cell formation. The increased recognition of the role of the stromal environment in hemopoietic cell function has, in large part, resulted from studies of the Sl/Sl^d and W/W^v mutations[47] and from studies in which stromal damage of bone marrow and spleen was found to cause defective hemopoietic cell regeneration[13,39]. Little is known clinically about hemopoietic microenvironments and stem cells in disease[65]. Certain data, however, have emerged supporting these earlier findings. Certain mouse leukemic stem cells have been found to respond variously to regulatory factors. Mouse erythroleukemic cells do not respond to erythropoietin[34] while myeloid leukemic cells are responsive to colony stimulating activity (CSA)[49]. Myeloid leukemia, in mice and humans, is presumed to be initiated as a result of chromosomal translocation[2,49]. These cells are malignant not because they cannot be induced by CSA to produce macrophages and granulocytes but because they no longer, unlike normal committed stem cells, require this protein for viability and growth. Further studies have shown that marrow granulopoiesis is associated predominantly with intramedullary production of CSA by an adherent cell population[14,24]. Greenberg et al.[24] found that marrow stromal cells in some patients with acute myeloid leukemia produced decreased amounts of CSA. Alteration of this parameter correlated well with the patients' clinical status and prognoses. Low marrow CSA was a negative prognostic indicator while normal CSA production typically accompanied complete remission.

Recent evidence of the very limited capacity of the fetal human spleen to support hemopoiesis[64] suggests that the splenic stroma may be quite different from that in bone marrow. Is this due to a change in the stroma, as occurs in many tissues during their development[56], or is the splenic stroma different from that of the bone marrow throughout development? These questions can be approached in vitro by cultures of stem cells and stroma from each site. Answers to these questions using direct assays of human fetal tissues rather than implications from studies of other species, will provide important

The ontogeny and phylogeny of hemopoiesis

Ontogeny	Phylogeny
1) Yolk sac	Cyclostomes (wall of intestine)
2) Connective tissue (gonads, meninges and mesentery)	Elasmobranchs, lungfish
3) Kidney	Teleosts
4) Liver	Teleosts, Urodeles
5) Spleen	Fishes, amphibia, reptiles
6) Bone marrow	Amphibia (Anura), reptiles, birds, mammals

clues to the differences in initial and compensatory splenic hemopoietic capacities between species and add to our knowledge of the regulation of hemopoiesis in health and disease.

In addition to these data, little is known regarding the effects of cytotoxic chemotherapeutic drugs on hemopoietic microenvironments. The hypothesis by Tavassoli[53] that damage to the microcirculation within hemopoietic tissues by such drugs had to be repaired before normal hemopoietic function could return is supported by other studies[13, 39] which have demonstrated that damaged stroma required regeneration before being capable of supporting hemopoiesis.

These investigations have provided opportunities to begin to: 1) understand how transformed stem cells respond to regulatory factors, 2) characterize and quantitate the ability of stromal elements in certain leukemic states to synthesize and elaborate substances involved in hemopoietic control and 3) identify the degree and nature of structural damage to hemopoietic microenvironments by chemotherapeutic drugs. Much remains to be learned, however, and pursuit of this topic in the model systems described is warranted. We have only a rudimentary understanding of the splenic capacity for hemopoiesis under conditions of hemopoietic stress. What is the effective hemopoietic capacity of the spleen in these various disease states compared to bone marrow? Are the disease mechanisms which affect a certain hemopoietic tissue, e.g. bone marrow, the same qualitatively and quantitatively as those in another hemopoietic tissue, e.g. spleen? If not, therapy should attempt to maximize the production of blood cells in the healthier site. An understanding of the associations, both in morphological and molecular terms, between hemopoietic stem cells and their microenvironment in bone marrow and spleen of normal individuals and those afflicted by these diseases is a requisite for improvements in their treatment.

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Compartments, domains and migration pathways of lymphoid cells in the splenic pulp

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The spleen is a highly vascularized hemopoietic organ, which can be considered as an encapsulated filter connected to the blood stream. Blood enters the spleen at the hilus through the splenic artery. The splenic artery ramifies into trabecular arteries which become gradually surrounded by lymphatic tissue. This lymphatic tissue forms a compartment known as the white pulp of the spleen. Small terminal arterioles open into the marginal zone, a diffuse area which surrounds the lymphoid white pulp and forms the border between the white pulp and the hemopoietic compartment in the spleen, the splenic red pulp. Alternatively, terminal arterioles extend via capillaries to splenic sinusses and venules. This 'closed' circulation opposes the open circulation where blood cells can freely enter the splenic parenchyma through the marginal zone.

The white pulp constitutes the immunologically active compartment in the spleen. It is a highly organized compartment in which three major domains can be distinguished: a) the peri-arteriolar-lymphoid sheath (PALS), b) lymphoid follicles inserted at the periphery of the PALS and c) a marginal zone which forms the border of the white pulp with the red pulp.

Lymphoid cells in the white pulp are not randomly distributed through the stroma; several types of experiments have shown that the two major subclasses of lymphoid cells, i.e. T lymphocytes and B lymphocytes, localize in distinct domains in the white pulp.