PROGRESS IN HEMATOLOGY

VOLUME X

Edited by
Elmer B. Brown, M.D.

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Contributors

JOHN P. ATKINSON, M.D., Investigator, Howard Hughes Medical Institute and Head, Division of Rheumatology, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri.

JOHN B. CLEGG, Ph.D., Lecturer, Nuffield Department of Clinical Medicine, University of Oxford, Radcliffe Infirmary, Oxford, England.

MITCHELL P. FINK, M.D., The Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine and the Division of Allergy and Immunology, St. Louis Children's Hospital, St. Louis, Missouri.

MICHAEL M. FRANK, M.D., Head, Clinical Immunology Section, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

CHAIM HERSHKO, M.D., Department of Hematology, Hadassah University Hospital, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

BEULAH HOLMES, Ph.D., Associate Professor, Department of Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota.

G. IZAK, M.D., Professor of Medicine and Chief, Hematology Service and Hematology Research Laboratory, Hadassah University Hospital, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

CRAIG M. JACKSON, Ph.D., Established Investigator of the American Heart Association and Professor of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri.

YUET WAI KAN, M.B., B.S., Professor of Medicine and Laboratory Medicine, University of California, San Francisco; Investigator, Howard Hughes Medical Institute Laboratory for the Study of Human Genetic Diseases; and Chief, Hematology Service, San Francisco General Hospital, San Francisco, California.

ARTHUR S. LEVINE, M.D., Chief, Pediatric Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

PATRICIA A. McINTYRE, M.D., Associate Professor of Medicine, Radiology, and Radiological Sciences (Nuclear Medicine) and Environmental Health Sciences (Radiation Health Sciences), The Johns Hopkins Medical Institutions, Baltimore, Maryland.

WILLIAM V. MILLER, M.D., Director, Missouri-Illinois Regional Red Cross Blood Program; Associate Clinical Professor of Pathology and Medicine, Washington University School of Medicine; and Associate Clinical Professor of Pathology, St. Louis University School of Medicine, St. Louis, Missouri.

ELAINE L. MILLS, M.D., Fellow of the Minnesota Heart Association and Instructor, Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota.

PHILIP A. PIZZO, M.D., Senior Investigator, Pediatric Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

PAUL G. QUIE, M.D., American Legion Memorial Heart Research Professor and Professor of Pediatrics and Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota.

PAUL J. SCHMIDT, M.D., Director, Southwest Florida Blood Bank, Inc. and Clinical Professor of Pathology, University of South Florida, Tampa, Florida.

WILLIAM T. SHEARER, M.D., Ph.D., Associate Professor of Pediatrics, Washington University School of Medicine and Director, Division of Allergy and Immunology, St. Louis Children's Hospital, St. Louis, Missouri.

JOHN W. SUTTIE, Ph.D., Professor of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin.

DAVID J. WEATHERALL, M.D., F.R.S., Nuffield Professor of Clinical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Radcliffe Infirmary, Oxford, England.

WILLIAM G. WOOD, Ph.D., Lecturer, Nuffield Department of Clinical Medicine, University of Oxford, Radcliffe Infirmary, Oxford, England.

Introduction

PROGRESS IN HEMATOLOGY embodies the editor's continued conviction that a need exists for succinct, authoritative reviews covering the broad discipline of hematology. Some of these reviews are devoted to recent advances, others reassess the state of the art. To be effective in meeting their goals, the chapters must be written by authors who can convey to readers with diverse backgrounds and needs the excitement and the importance of advances in their field. I believe that this goal has been achieved by the twelve chapters in this volume. They are addressed to a wide audience—medical students, resident physicians, clinicians, teachers, and investigators—seeking both depth and breadth of information.

The editor is grateful to the contributors for their splendid cooperation in adhering to a tight schedule to assure timely publication as well as fulfilling his expectation of excellence of their chapters.

Elmer B. Brown

Contents

G. Izak, M.D.

Erythroid Cell Differentiation and Maturation

INTRODUCTION

Over 300 years have elapsed since Leeuwenhoek, in 1673, designated the erythrocytes as "small round globules." It took another 200 years until Hoppe-Seyler, in 1867, demonstrated that the hemoglobin constituting the bulk of the erythrocytes is able to take up and discharge oxygen readily. The discovery of the bone marrow as the source of the red corpuscles by Neuman in 1868 initiated a continuous, intense effort aimed at the clarification of the evolution of the erythrocyte, which remained the center of the probing interest of many investigators until the present time. The amount of the accumulated information on this subject is immense, and the present review cannot do justice to all the important contributions made to this area of scientific endeavor throughout the centuries. The following summary is devoted to the highlights of progress during the last two decades in the understanding of erythroid cell differentiation and maturation, and of the mechanisms responsible for their regulation. This review will reveal that, the large amount of information accumulated during the past two decades notwithstanding, substantial gaps remain in our understanding of erythroid cell differentiation and maturation, which may serve as a stimulus for further research efforts to fill these gaps.

The subject of this summary is erythroid cell differentiation and maturation. One could attempt to define the process of cell differentiation as the synthesis of specific product(s) that impart to the cell the potential ability to perform specific function(s) characteristic of a particular cell type. Cell maturation can be described as the chain of well-defined biochemical and morphologic events initiated by the differentiation which leads to the emergence of the final form of the mature element performing its predetermined tasks throughout its life span. In the case of the erythroid cell series differentiation could be conceived as the activation of the specific globin genes in the nuclear DNA, while maturation would start with the transcription of the code into the messenger RNA, the latter translating it via the protein-synthesizing system of the cell into the completed globin chains, which

would be the final step in the process of maturation. The above concept served as a guideline for the author in the construction of the discussion through the subsequent pages.

ERYTHROID CELL PRODUCTION—GENERAL REMARKS

During the past two decades increasing evidence has been produced suggesting that the recognizable erythroid precursor compartment in the bone marrow does not consist of a self-sustaining cell population, but is dependent upon the continuous entry of cells from an earlier progenitor source. The demonstration of the Ph_1 chromosome in patients with chronic myelocytic leukemia in the erythroid, megakaryocytic, and monocytic series, in addition to the myeloid elements [1], as well as the observations on transplantation chimeras [2], lent strong support to the existence of a pluripotent stem cell compartment. This latter stem cell compartment supplies individual cells which, as a result of appropriate stimuli, become committed to one of the series populating the hemopoietic tissue. Much information has been published recently supporting the concept that two functionally different, although morphologically indistinguishable stem cell compartments exist—namely, (1) the pluripotent and (2) the committed stem cell pools—which can be differentiated by a number of experimental procedures.

Since much of the evidence derived from experimental studies is based on in vivo and in vitro hemopoietic colony formation, it is appropriate to define briefly the terms that will be used throughout this discussion. Ever since Till and McCulloch reported in 1961 the formation of hemopoietic cell colonies in irradiated mouse spleen following the injection of normal hemopoietic tissue, it has been accepted that the single cell giving rise to each colony is the functional representative of a pluripotent stem cell (CFU-S) [3]. Since the first successful effort to clone hemopoietic cells in in vitro culture [4,5], these methods have been widely employed in the study of hemopoietic cell differentiation. The single cell being the progenitor of each colony is identified with the committed stem cell compartment and has been designated as CFU-C (for committed progenitors of granulopoiesis [4]) or CFU-E (for committed progenitors of erythropoiesis [6]). It is generally accepted that most of the stem cells of the pluripotent compartment are in resting (G_0) phase, while the committed cells are predominantly in cycle. The evidence for this contention has been furnished by the observation that hydroxyurea, a selectively lethal agent for hemopoietic cells in S phase, will substantially reduce the number of committed stem cells without affecting appreciably the number of pluripotent stem cells in hypertransfused animals [7–9]. Similar observations made by Iscove et al. [10] employing the "suicide technique" with high specific activity ^{3}H-thymidine lent further support to the above concept. While this situation prevails under physiologic steady-state conditions, there is evidence that both the CFU-S and CFU-C/E compartments can proliferate rapidly or slowly in response to the varying demands in the periphery for cell production [11]. The changes from resting to slowly or rapidly proliferating states are reversible, but once a pluripotent cell has been committed, it will follow its ordained pathway throughout the predetermined stages of its maturation (Fig. 1) [12,13]. While the above statement is true to date, recent evidence points to a possible oscillatory nature of hemopoiesis, for the detailed

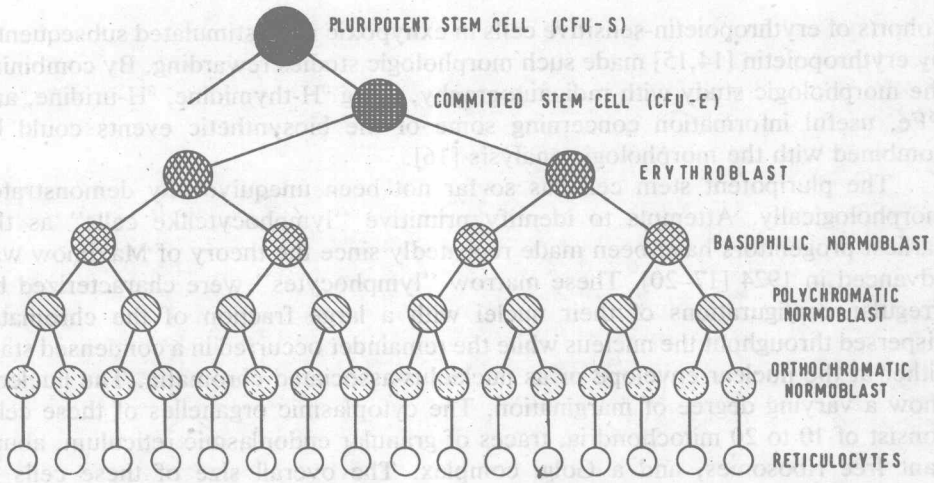

Fig. 1. A schematic illustration of the course of events starting with the pluripotent stem cell and terminating with the mature erythrocyte. The erythroid-oriented stem cell, as a result of appropriate stimuli, develops into the earliest, morphologically recognizable erythroid precursor (erythroblast). This cell passes through three arbitrarily defined stages of maturation—namely, basophilic, polychromatic, and orthochromatic normoblast—while it undergoes three or four mitotic divisions. The orthochromatic normoblast, after losing its pycnotic nucleus, becomes the reticulocyte, which matures in the circulating blood into the erythrocyte. The process from erythroblast to reticulocyte formation lasts about 72 hours, while an additional 48 hours are required for the maturation of the reticulocyte.

discussion of which the reader is referred to the lucid paper of Morley et al. [7] (see also p. 27).

Since we shall be dealing with erythroid cell differentiation and maturation, an attempt will be made to find answers to the following questions:

1. What are the intracellular events occurring during the transition from the pluripotent into the erythroid-committed stem cell?
2. What are the environmental and intracellular mechanisms responsible for the regulation of these events?
3. What is the sequence of intracellular changes characterizing the various stages of erythroid maturation?
4. What are the environmental and intracellular mechanisms responsible for the orderly sequence of events during erythroid cell maturation?

THE PLURIPOTENT STEM CELL AND ITS TRANSITION INTO THE ERYTHROID-COMMITTED LINE

Morphologic Identification of the Pluripotent Stem Cell

Since the extensive use of electron microscopy in the ultrastructural analysis of the hemopoietic tissues, it became possible to identify morphologically erythroid precursors very early in their development. The availability of methods to produce

cohorts of erythropoietin-sensitive cells in exhypoxic mice stimulated subsequently by erythropoietin [14,15] made such morphologic studies rewarding. By combining the morphologic study with radioautography, using ^{3}H-thymidine, ^{3}H-uridine, and ^{55}Fe, useful information concerning some of the biosynthetic events could be combined with the morphologic analysis [16].

The pluripotent stem cell has so far not been unequivocally demonstrated morphologically. Attempts to identify primitive "lymphocytelike cells" as the earliest progenitors have been made repeatedly since the theory of Maximow was advanced in 1924 [17–20]. These marrow "lymphocytes" were characterized by irregular configurations of their nuclei with a large fraction of the chromatin dispersed throughout the nucleus while the remainder occurred in a condensed state either at the nuclear envelope or as nucleolus-associated chromatin. The nucleoli show a varying degree of margination. The cytoplasmic organelles of these cells consist of 10 to 20 mitochondria, traces of granular endoplasmic reticulum, abundant free ribosomes, and a Golgi complex. The overall size of these cells is approximately 8 to 12 microns [21–24]. Many other investigators have described the enlargement of lymphocytes to become so-called "transitional cells," which were identified as hemohistioblasts [25–30]. It was suggested that the small lymphocyte represents the pluripotent stem cell in resting phase, which, following a stimulus for increased red cell production, enlarges to become the "transitional cell" [30]. A marked increase in the number of small lymphocytes was observed in the bone marrow following hypoxia and immediately after small doses of x-irradiation, before erythroid and myeloid regeneration occurred [20,29,31]. An increase of up to 80% in marrow lymphocytes was observed in guinea pigs irradiated with 150 to 200 R. These cells, when transplanted into lethally irradiated guinea pigs, were able to repopulate the bone marrow and protect the recipients against death [32]. Other authors also observed an increase in the number of small lymphocytes in hypertransfused mice with markedly decreased erythropoiesis [33]. These cells became labeled rapidly following erythropoietin administration, suggesting that they were the precursors of the erythroblasts that appeared later. It was further shown in experiments with serial transplantation of marrow into lethally irradiated mice that the percentage of small lymphocytes correlated best with proliferative capacity after transplantation [34,35]. These small lymphocytes avidly took up tritiated thymidine after they were transplanted [33]. Attempts to separate marrow cells confirmed the role of small lymphocytes in bone marrow regeneration. Thus, hemopoietic cell suspensions fractionated in glass wool columns yielded a small and medium-sized lymphocyte-rich fraction, which promoted cell proliferation in lethally irradiated mice [17]. This observation was confirmed by other investigators, who used in addition dextran gradient centrifugation to further enrich the lymphocyte–transitional cell fraction [36]. Combining morphologic and cytokinetic studies, Fliedner et al. [37–41] concluded that the bone marrow lymphocyte answers the criteria of the "resting stem cell," which when called upon by increased demand brought about by the administration of hydroxyurea, divides and gives rise to a "wave" of labeled blast cells that subsequently differentiated into myelocytic, erythropoietic, and megakaryocytic cell series.

Despite the voluminous evidence for the identification of the small and/or transitional bone marrow lymphocyte as the pluripotent stem cell, many published reports support evidence to the contrary. Using discontinuous albumin density gradient fractionation of rodent and primate bone marrow cell suspensions, Dicke et

al. were able to concentrate the pluripotent stem cell population 100-fold [42–44]. They characterized these stem cells by light- and electron-microscopic studies and found them to be different from lymphocytes by a large number of criteria. They then correlated the number of these cells identified morphologically with the number of colonies produced when the same cell suspension was cultured in agar.

These contradictory results suggest that not all lymphocytes can be identified with stem cells, but rather it is one of their distinct subpopulations. The recent observation of Perry et al. seems to be relevant to this point [45]. They reported that the so-called "null cells" obtained from peripheral blood, after the T and B lymphocytes were removed, possessed the functional characteristics of a pluripotent stem cell.

Other reports ascribed the role of pluripotent stem cell to an entirely different morphologic entity. Several investigators described a sequential morphologic evolution from primitive reticulum cells into erythroblasts [25,46–48]. These cells, however, had a very low uptake of labeled DNA precursors [29] even after irradiation [47], which made them poor candidates for being the pluripotent stem cells. Groups of small round cells, found in the spleens of irradiated mice, were thought to be hemohistioblasts [49], while other authors proposed primitive endothelial cells, derived from the avian yolk sac angioblasts, as the earliest hemopoietic progenitor cell [50]. None of these suggestions, however, gained further support by subsequent studies. Of interest are the studies with parabiotic rats, both of which were x-irradiated; both hind legs of one rat were shielded [51]. Twelve hours after irradiation tritiated thymidine was injected into the shielded animal while the parabiotic circulation was clamped. DNA labeling was interrupted by the subsequent administration of unlabeled thymidine, following which the cross-circulation was restored. The vast majority of the early-labeled cells in the nonshielded marrow were monocytoid in appearance, having a diameter of 8 to 10 μ with irregular nucleus, loose chromatin strands, and abundant cytoplasm with scanty azure granules. Many of these labeled cells were found in the peripheral blood of the unshielded rat and the same cells were the first to populate the marrow of the unprotected animal. Later some cells transitional between these monocytoid cells and early erythroblasts were also noted. Similar cells were observed by others when buffy coats from partially shielded, heavily irradiated rats were injected into lethally irradiated, unprotected recipients during the early phases of the latter's recovery [52]. The morphologic description of the cell identified by Bessis [53] as the earliest hemopoietic progenitor resembles closely the characteristics of the monocytoid cells discussed above.

A huge amount of valuable information has resulted from the work summarized above and from much additional data, which could not be dealt with here because of lack of space. Final acceptance of the pluripotent stem cell will depend upon the clear-cut isolation and ultrastructural analysis of functionally well-characterized populations of CFU-S and CFU-C/E. Until that time the morphologic identification of the pluripotent stem cell will remain equivocal.

Kinetic Studies on the Pluripotent Stem Cell

As noted in the previous section, the efforts to identify and characterize the pluripotent stem cell morphologically have not lead to uniformly accepted results. The need, however, to study hemopoietic progenitors stimulated the development

of a number of experimental systems that depended on function rather than structural appearance. These systems were designed to test certain functions required a priori from a pluripotential stem cell, if indeed this progenitor has the burden of maintaining hemopoiesis at the rate prescribed by the demand in the periphery. One of these functional prerequisites is the potential proliferative capacity of the stem cell sufficient to replenish its own pool, when the latter is depleted by injury or physiologic requirements. The second functional demand is that the stem cell must provide a specific target(s) through which appropriate control mechanisms can promote or suppress its rate of proliferation and/or its differentiation. A large number of assay procedures were developed over the past two decades to test the above functions of the stem cell, and a brief description of some of their important principles follows.

The radioprotection assay is based on the ability of bone marrow cell suspensions to protect supralethally irradiated mice against death by repopulating the hemopoietic tissue of the recipient animals [54–57].

The spleen colony assay is based on the formation of macroscopically visible hemopoietic cell colonies in the spleen of lethally irradiated mice following the injection of a suitable number of bone marrow cells [2,3]. There is a linear relationship between the number of cells injected and the number of colonies seen 10 days later. The proportion of stem cells seeding into the spleen is between 10 and 20% of that injected, and since each colony is derived from a single pluripotent cell [2], the absolute number of CFU-S in the suspensions can be calculated [56,58]. As these colonies differentiate into identifiable precursor cells, with the use of appropriate labels or stimuli, the efficiency of erythroid and myeloid repopulation can be assessed [59–61].

Erythropoietin assay measures the ability of erythropoietin to reinitiate red cell production in polycythemic mice and is quantitated by radioiron incorporation into newly formed red cells. This test can be used to assess the proportion of stem cells available for erythroid differentiation [62,63].

The autorepopulation assay measures repopulation of the hemopoietic tissue that is achieved from shielded femur or spleen after partial-body irradiation. The capacity of the shielded area to repopulate the irradiated animal can be assessed either by the number of splenic colonies formed (CFU-S) or by the rate of iron incorporation by newly formed red cells (measure of erythroid differentiation) [64,65,65a,65b,237].

The in vitro colony assay assesses the number of differentiating cell clones grown in suitable medium from bone marrow cell suspensions [4,5].

By the application of these methods (and some others not referred to above), it has become clear that the pluripotent stem cell does meet the functional prerequisites posed to its acceptance earlier. Spleen colonies have revealed that a single cell can generate a million cells or more in 10 days, which serves as an adequate proof of its proliferative capacity. The bulk of these cells may be readily recognized as differentiated blood cells, and their presence provide the evidence for the differentiation of the pluripotent stem cell. A varying number of the cells within the spleen colonies are themselves capable of colony formation [66], indicating that the pluripotent stem cell is capable of self-renewal. Finally, the colony-forming cells are subject to control mechanisms. That this is indeed the case follows from the observation that only a relatively small proportion of the pluripotent cells proliferate

and differentiate. Had all these cells realized their proliferative potential simultaneously, the great excess of differentiating cells flooding the system would have killed the animal [67].

Once one accepts the functional characteristics of the pluripotent stem cell as outlined above, the next question to be answered is related to the mechanism(s) governing the rate of proliferation of this stem cell pool. The information provided by McCulloch et al. [55] concerning the self-renewal of CFU-S is relevant to the analysis of this problem. These authors have shown that the number of CFU-S recovered from the spleens at varying time intervals after seeding grows exponentially from day 3 until day 10, with a doubling time of 20 to 25 hours, following which the growth curve becomes horizontal. This exponential growth with a modest overshoot, followed by maintenance of steady levels, could reflect an effective and precise regulating mechanism. When, however, similar growth curves are constructed from individual spleen colonies, a wide variation in the CFU-S is found among the individual clones [66]; many colonies contain a few pluripotent stem cells, while in others several thousands may be present. Analysis of the distribution of CFU-S among the clones leads to the suggestion that the self-renewal process may be stochastic in nature, rather than obeying a precise mechanism for its regulation [68].

Effects of Environment on the Pluripotent Stem Cell

Genetic factors play an important role in the ability of the pluripotent stem cells to respond to physiologic stimuli. Several such genetic regulators have been identified by employing the spleen colony technic to mice with genetically determined anemias. The mutations leading to severe macrocytic anemia have been characterized as being pleiotrophic, and mice of genotypes W/W^v and $S1/S1^d$ have similar phenotypes [69,70]. When marrow or spleen cells from W/W^v mice are injected into normal-irradiated recipients, no spleen colonies develop, although the normal number of splenic clones is found in the recipients of cells from normal littermates. When, however, hemopoietic stem cells of normal controls are injected into W/W^v recipients, colony-formation is observed even when the genetically defective host is not irradiated [71,84a]. Contrasting results are obtained when similar experiments are performed with mice of genotype $S1/S1^d$. Hemopoietic cells of these mice form normal spleen colonies in irradiated normal recipients. But when irradiated $S1/S1^d$ mice are used as recipients, normal hemopoietic cells fail to produce colonies [72]. These results suggest that in the W/W^v mice the pluripotent stem cell is defective and unable to respond to environmental stimuli, while in the $S1/S1^d$ animals the defect is an environmental factor extrinsic to colony-forming cells but obviously necessary for their normal function. One can conclude from these observations that there are at least two genetically determined hemopoietic regulators—one working within the cell, the other exerting its effect from without. These studies also provide useful information concerning the transition between resting and proliferating pluripotential stem cells.

Daily injections of endotoxin result in a 10-fold increase in the number of CFU-S in the spleen of normal mice [71], and this effect is inhibited by colchemid. Similar effects could be obtained by phytohemagglutinin [73], globulin [64], and antigen [74]. It is also generally accepted that the transfer of resting pluripotent stem cells

into irradiated hosts is associated with the induction of proliferation of these cells, probably resulting from unknown environmental factors. None of these stimuli, however, could induce colony-forming capacity in the mice with S1/S1^d genotype. It seems reasonable to assume that the S1 gene product is required for the transition from resting to proliferating stem cells. Attempts to identify these gene products have been unsuccessful. Parabiosis established between a normal mouse and one with genotype S1/S1^d failed to reveal any effect of the S1 gene product in the normal parabiont [75]. This result could be explained by an extremely short life span of the gene product, or by its not entering the circulation. One could speculate that the S1 gene product may require cell-cell contact to be effective. Evidence has been put forward that such short-range interactions do in fact operate through specific cell-surface components that can be characterized genetically and by their response to alloantisera, but as these studies deal with cells of the immune system, they will not be dealt with here [76,76a,178,180,191].

A further observation reported recently on the CFU-S seeding and differentiation in W/W^v anemic mice seems relevant. Bone marrow cells, as mentioned above, but not thymocytes from hematologically normal littermates of W/W^v mice, were able to form spleen colonies and cure the macrocytic anemia of their genetically defective littermates. Pretreatment with anti-θ serum and complement (C′) abolished the ability of the normal mouse bone marrow cells to cure the anemia of the genetically defective mice without, however, affecting the number of CFU-S formed in the recipients. The addition of normal thymocytes to the anti-θ-treated normal bone marrow suspension restored the ability of the injected marrow to cure the anemia of the genetically determined anemic recipients. The injection of T-lymphoid cells derived from the thymus of normal W/W^v mice, together with their bone marrow suspension, was associated with a replacement of the macrocytes in the recipients' peripheral blood by normocytes. These data suggested a requirement for θ-sensitive cells, not derived from CFU-S cells, but present in normal thymus, bone marrow, and spleen for the promotion of differentiation of CFU-S cells to mature erythrocytes in the W/W^v mice [77,77a].

Several investigators observed that parental thymocytes—and to a much more restricted degree, F_1 thymocytes—when transplanted into F_1 hybrids, accelerated the recovery of both CFU-S and erythropoiesis in the recipient spleens [78–80]. No effect was detectable in the bone marrow or in splenectomized recipients, suggesting a short-range interaction between the seeded thymocytes and the splenic tissue. An enhanced recovery of CFU-S was observed when cyclophosphamide was administered shortly before irradiation and transplantation of parental marrow into F_1 hybrids [80]. These experiments suggested that recognition at a cellular level played an important role in the initiation of cell division of the pluripotent stem cell [78] and that modification of cell surfaces by drugs might also prove to be a stimulus to growth of CFU-S [80].

In considering mechanisms regulating pluripotent stem cell proliferation and differentiation, we deal briefly with the concept of hemopoietic inductive microenvironment (HIM), introduced by Curry and Trentin [80a,81]. According to their concept, the spleen in which pluripotent stem cells settle must consist of different microenvironments, each dictating a given line of hemopoietic differentiation. Only when the still-uncommitted progeny of the pluripotent stem cell spill out of their original microenvironment and enter an adjacent one with different effects, do they

undergo a second line of differentiation dictated by the new microenvironment. In the spleen the microenvironment seems to favor erythroid differentiation [82], while in the bone marrow the conditions favor the granulocytic line [83]. To support further the above hypothesis it was shown that the erythroid-granuloid colony ratio both in the spleen and the marrow produced by retransplanted "homed" cells remained the same as the primary colonies formed by normal bone marrow cells [82]. When the heavily irradiated bone marrow plugs were implanted into the spleen prior to the infusion of the bone marrow suspension, the majority of the colonies evolving in the bone marrow stroma were granulocytic, while those in the surrounding splenic tissue were predominantly erythroid [83]. The nature of HIM is not clear, but it was proposed that it consists of foci of one or more radio-resistant reticuloendothelial cells that have the capacity to trigger the pluripotent stem cell into functional differentiation to one of the hemopoietic cell lines without detectable morphologic alterations.

Early experiments on radioprotection in parabiotic animals showed that pluripotent stem cells circulate in the bloodstream [84,84a]. It was proposed that these circulating pluripotent stem cells are in dynamic equilibrium with tissue CFU-S and settle and grow where a favorable microenvironment presents itself [85].

Another approach to the problem of interrelation between the microenvironment and hemopoietic tissue evolution is represented by the series of studies reported by Patt and Maloney. They studied the reconstitution patterns of mechanically depleted femoral shaft medullary cavities, and observed that this process involves an initial period of reorganization that blends with a more prolonged period of hemic cell repopulation. The pattern was found to be similar whether marrow was removed surgically, [86], by perfusion [87], or by a combination of both methods when employed in rabbits [87a]. Immediately after the femur evacuation, the cavity is filled with coagulated blood, which is soon replaced by granulation tissue. This phase is associated with the activation of the connective tissue in the haversian canals. At the end of the first week a well-formed connective tissue matrix occupies the evacuated area. It requires several weeks before this tissue is replaced by regenerated hemopoietic tissue. Between days 2 and 8 after the evacuation, a substantial number of undifferentiated cells appear in the connective tissue framework; following this, there is a gradual evolution of differentiated hemopoietic precursors. A marked overlap was noted between these cell populations. In an effort to identify the stem cell responsible for the repopulation of the femoral cavity with hemopoietic tissue, they have ruled out the possibility that circulating multipotent stem cells seeded the coagulum produced in the evacuated cavity, as it was found that irradiating the animal 800 R with the femoral shaft shielded did not interfere with the regeneration, whereas irradiation of the emptied femoral shaft area markedly impaired the regenerating process [88–90]. Having observed the migration of undifferentiated cells from the osseous tissue into the area of regeneration, these authors suggested two possible mechanisms whereby regeneration may emanate from cells associated with osseous tissue: (1) either from one cell type (pluripotent stem cell?), which would differentiate into cells forming the matrix of the bone marrow and into others serving hemopoietic pluripotent stem cells [89], or (2) from two different stem cells serving as separate progenitors for each of the two evolving tissues. The authors reached the conclusion that the regeneration of the bone marrow under their experimental conditions "stems from

mesenchymal cells within the Haversian canals which are implanted in the organizing blood clot'' [89]. It seems that the development of the bone marrow structure is dependent upon intimate interactions between mesenchymal cells and their derivatives, the nature of which is not known. The stimulus responsible for the reconstitution of the marrow by connective tissue cells is not known either, but it was suggested that since the amount of bone marrow removed represents only a small part of the total hemopoietic tissue and does not lead to appreciable consequences in the peripheral blood, the observed process probably represents a local phenomenon, where both the stimulus and the cells originate and perform at the site of injury.

The relationship between the bone marrow stroma and the hemopoietic cells has also been explored by other investigators. Irradiation of the femurs of rats in doses of 4000 R led to the destruction of the hemopoietic tissue in the irradiated area within 2 to 4 days. However, the integrity of the supporting stroma was retained. During the subsequent 2 weeks the hemopoietic tissue gradually regenerated. Three months following irradiation the marrow was found to be aplastic again, with the disappearance of the sinusoidal structure [91]. This ''biphasic aplasia'' suggested that the marrow consists of two organs dependent upon each other: the hemopoietic tissue depending upon the supporting connective tissue stroma. These experiments also indicated that the delay in the stromal injury induced by radiation, as compared to the early destruction of the hemopoietic tissue, was due to the longer life span of the stromal cells. When the firmly established aplastic marrow cavity was thoroughly emptied and a block of unirradiated bone marrow from the contralateral bone was placed into the cavity, hemopoiesis was reestablished in the irradiated bone [92].

Autologous bone marrow implants are capable of establishing themselves in a variety of tissues, such as the spleen, kidney, liver, muscle, omentum, and subcutaneous tissue [93]. The transplanted marrow does not itself survive: it loses its hemopoietic cells soon after its transplantation. Within 2 days the implant becomes overgrown by primitive cells resembling reticulum cells originating from the adventitia of the remnants of the implanted tissue. This stage is followed by bone formation at day 8, and the first sinusoids appear at about the same time, followed by the evolution of several islands of hemopoietic cells. The implant develops finally into a bony shell within which actively proliferating hemopoietic tissue can be seen [93]. These data stress the importance of the microenvironment for the process of hemopoiesis, and they reiterate the demand for the unique sinusoidal structure with its characteristic microcirculation as a vital requirement for the proliferation and differentiation of the progenitor cells. A histologic study on locally curetted bone marrow of irradiated mice revealed an incomplete regeneration of the evacuated area 12 weeks after the procedure. Infusion of bone marrow cells with a chromosome marker into these animals was associated with a prompt seeding of the irradiated recipients' marrow by the cells with the marker chromosomes, except for the curetted area, which excluded these cells until the regeneration of the stromal structure was complete 2 weeks after the evacuation. By that time, however, the regeneration of the hemopoietic tissue was found to be well under way. A close parallelism was observed between the recovery of CFU-S and the hemopoietic cell recovery. These studies support the concept of a local origin of bone marrow regeneration within the curetted cavities, but do not indicate whether the hemo-