MINIREVIEW

Physiologic Role of Heme and Cytochrome P-450 in Hematopoietic Cells (43186A)

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Heme (ferroprotoporphyrin IX) is a ubiquitous molecule that serves as the prosthetic group of a variety of important hemoproteins that are essential for hemopoietic processes. Heme is involved in oxygen transport as the prosthetic group of hemoglobin, in prostaglandin synthesis as the prosthetic group of cyclooxygenase, in the enzymatic decomposition of H_2O_2 as the prosthetic group of catalase and peroxidase, and in the inactivation of oxygen molecules, as the prosthetic group of mitochondrial and microsomal cytochrome P-450. The latter refers to a family of isozymes for which heme serves as the prosthetic group that oxidizes a wide variety of structurally unrelated compounds, inactivates leukotrienes (leukotriene B4), and metabolizes arachidonic acid (AA) to bioactive metabolites, some of which are involved in the signal transduction process for hematopoietic growth factors. However, the effect of heme on erythropoiesis appears to be distinct from its direct involvement as a prosthetic group.

The expression of specific heme metabolic enzymes determines the level of cellular heme that is necessary for proper erythropoiesis. This concept is supported by evidence that hereditary or experimental alterations in enzymatic or biosynthetic events are often accompanied by a disturbance in heme levels, and that treatment with drugs or inhibitors of heme synthesis may affect progenitor cells, resulting in altered growth and differentiation (1). It is becoming increasingly evident that the role of heme metabolic enzymes in the regulation of hematopoiesis has a dual nature, since metabolic enzymes appear to participate in the implementation of both stimulation and suppression of erythropoiesis (1, 2). Enhancement of erythropoiesis obtained with the growth factors interleukin 3 and erythropoietin (Epo) also results in increased levels of δ-aminolevulinic acid synthase (ALAS) and porphobilinogen deaminase (PBGD), the proposed rate-limiting enzymes in the heme biosynthetic pathway. Suppression of erythropoiesis may be observed by either insufficient levels of one of the heme biosynthetic enzymes, or by increased heme degradation by heme oxygenase (HO). Several potential regulatory phenomena are associated with the enzymatic steps involving erythroid-specific and nonerythroid genes for ALAS and PBGD. Abnormalities of heme metabolism and certain hematologic disorders may arise when these regulatory events are disturbed or by which HO is expressed. The significant role of HO in normal erythropoiesis and in the control of hematopoietic cellular heme is just beginning to emerge. For example, repression of HO is seen as a trigger of erythroid differentiation (3, 4).

Regulation of heme biosynthesis in mammals has been extensively studied, and the data indicate that there are two distinct regulatory systems, referred to as hepatic (nonerythroid) and erythroid types. In erythroid and bone marrow cell types, the regulatory mechanism is complex and may involve more than one rate-limiting step in the heme pathway. For hepatic cells, the regulatory mechanism is more clearly understood (5). In this review, we will discuss mainly erythroid and bone marrow heme.

Heme Biosynthesis and Catabolism

Heme metabolism and the incorporation of heme into cytochrome P-450 is schematized in Figure 1. There are eight major enzymatic steps for heme biosynthesis, of which the first step and the last three steps occur in the mitochondrion (5). Heme catabolism occurs in the microsomal cytoplasmic compartment. The first step of heme biosynthesis is the formation of δ-aminolevulinic acid (ALA), which is a mitochondrial process; its ultimate conversion into coproporphyrino-

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gen (coprogen III) occurs in the cytoplasm, and the last steps take place within the mitochondrion. The first step, condensation of glycine and succinyl-coenzyme A to form ALA is catalyzed by the rate-limiting enzyme ALAS. ALA then passes into the cytosol where ALA dehydratase (ALAD) catalyzes the condensation of two molecules of ALA to form the pyrrole porphobilinogen (PGB). Four molecules of PBG are then condensed to yield the first tetrapyrrole in the pathway, uroporphyrinogen III (URO III); this reaction is catalyzed by PBGD and URO III cosynthase resulting in reversal of one PBG molecule. Another enzyme, URO decarboxylase, converts the four acetic acid side chains on the molecule to methyl groups to yield coprogen III. The remaining biosyntehtic steps occur in the mitochondria. The final step in heme biosynthesis is the insertion of ferrous iron into proto IX to form protoheme (heme) by the mitochondrial enzyme, ferrochelatase (FC) or heme synthetase.

Heme degradation occurs mainly by oxidative cleavage of the α-methene bridge of the molecule, by HO leading to the formation of biliverdin IX. Biliverdin is further metabolized by the enzyme biliverdin reductase to yield bilirubin (5).

Gene Regulation

The genes for most of the heme pathway enzymes have been identified and cloned, and this has recently been reviewed in detail (6). Gene cloning for six biosynthetic enzymes and two for the catabolic pathway has been achieved (6).

Cloned cDNA for ALAS, ALAD, PBGD, URO III cosynthase, and URO decarboxylase have been obtained from mammalian sources (for reviews, see Refs. 6–8). cDNA clones for FC obtained from yeast indicate a 2.9-kb genome DNA and contain an open reading frame of 1179 nucleotides (9).

Two enzymes of the heme biosynthetic pathway, ALAS and PBGD, have special features and may have regulatory functions in heme synthesis by hematopoietic cells. ALAS exists as two isozymes that are encoded by nonerythroid and erythroid-specific genes, respectively. By contrast, PBGD, which also exists as two isozymes, arises from a single gene comprised of two overlapping transcriptional units, each with its own promoter. Transcription from one or the other of these promoters gives rise through differential splicing to two distinct mRNA species that encode the distinct nonerythroid and erythroid isoforms. On the other hand, only a single copy and one species of mRNA have been identified for each of the other heme synthetic enzymes.

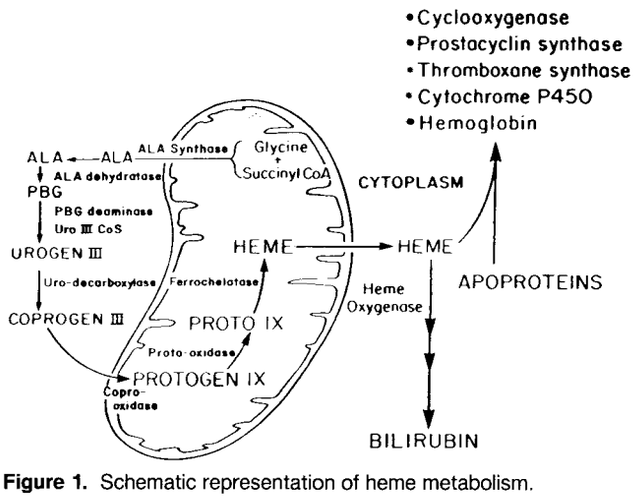

Figure 1. Schematic representation of heme metabolism.

cDNA for HO has been cloned from rat spleen (10) and human macrophages (11), and the primary structure of the protein deducted from the nucleotide sequence consists of 288–289 amino acids with a molecular weight of 32,800–33,000 (for review, see Ref. 12). There is a high degree of sequence homology between human and rat forms (~90%). Shibahara *et al.* (13) have shown that rat HO is transcriptionally induced by heat shock. This transcriptional relation has been observed in rat fetal liver (14) and brain (15). Furthermore, hemin does not induce the same effect and it is considered that heat and hemin induction of HO are by different mechanisms.

Hemopoietic Systems

Although the bone marrow is the primary site of hemotopoiesis in the adult mammal, the embryonic blood islands, liver and spleen, are hemopoietic sites during fetal development. In this regard, cell preparations from human fetal liver (13–22 weeks) gave rise to large numbers of Epo-dependent benzidine-positive erythroid colonies (16), and similar progenitors migrate from the liver to seed the bone marrow at a later stage of development.

Regulation of heme biosynthesis and degradation has been studied in bone marrow cells from a variety of species as well as cell lines which can be induced to differentiate, and especially erythroid cells.

Using reticulocytes, it was found that heme appears to inhibit its own synthesis mainly by direct repression of ALAS (17, 18). Furthermore, excess heme was found to act as a translational inhibitor of globin synthesis (19), and also prevented the uptake of transferrin-bound iron into cells (20). Experiments by Levere and Granick (21–23) revealed that cells of the blastoderm have a heme biosynthetic mechanism similar to that of reticulocytes. Studies showed that blastoderms incubated *in vitro* start synthesizing heme at about 48 hr after incubation and that addition of ALA to the medium causes heme to be synthesized much earlier (21–24). Exposure to ALA resulted in rapid synthesis of large amounts of porphyrins, heme and globin, and hemoglobin at about 12 hr earlier than nonexposed cultures.

In normal human bone marrow cultures induced

with Epo, there is also an induction of enzymes in the heme biosynthetic pathway (2, 25). ALAS, ALAD, and PBGD are significantly induced by Day 4 and hemoglobin first appears on Day 7. Levels of PBGD increase linearly with increasing Epo concentration and are proportional to increasing heme concentration (26).

Granulocyte macrophage colony-stimulating factor (GM-CSF) has also been shown to increase the activity of ALAS (27). In addition, no significant change in HO occurred with GM-CSF-exposed marrow cells. Since GM-CSF stimulates myelopoiesis, CSF stimulation of ALAS may be an important event in myeloid differentiation. Nevertheless, it is clear that heme is required for a multitude of functional processes in myeloid and erythroid cells.

Increases in several heme synthetic enzymes preceding cell differentiation and the appearance of hemoglobinized erythroid colonies (CFU-E, BFU-3) and accumulation of globin mRNA has been shown in several bone marrow colony culture systems (2, 28). It was found that as growth and differentiation of CFU-E occurs, the activity of HO drops, while the biosynthetic enzymes ALAS and ALAD increase in activity. These enzyme patterns were accompanied by an increase in heme synthesis, with maximal activity for human bone marrow cultures between 7 and 8 days (28). A similar developmental profile was seen with murine CFU-E development; however, maximal developmental activity occurs between 48 and 56 hr (2–4). Addition of cycloheximide, which is an inhibitor of cytoplasmic protein synthesis, was found to inhibit the activities (or synthesis) of the heme enzymes (4).

The heme biosynthetic enzyme PBGD has been measured in human BFU-E colonies (2). Results from experiments using BFU-E colonies demonstrated an activity of this enzyme to range between 100 and 200 pmol of URO III formed/10^7 cells/hr. Addition of hemin or conditioned media from phytohemagglutinin-stimulated lymphocytes were found to stimulate PBGD activity. Other studies demonstrated that the activity of PBGD was found to be increased by Epo prior to an increase in BFU-E colony growth (2, 26, 29).

Fluctuations in heme metabolism have been noted in senescent animals (30). In contrast to young animals, bone marrow cells from senescent rats were found to have depressed ALAS activity, whereas HO was elevated. Additionally, there were depressions in [^{14}C] glycine incorporation into heme, [^{14}C]leucine and [^{3}H] uridine incorporation into protein, and nucleic acid synthesis.

Regulation of Heme Biosynthetic Enzymes and Erythropoiesis

In erythroid tissue, where the largest amount of heme is produced, the control scheme for heme synthesis is not well defined. It differs from that found in the liver cells where heme feedback inhibition of ALAS is described. However, in erythroid cells, heme biosynthesis is linked to proliferation and maturational events following Epo's action. It is likely that enzymatic regulation is different in erythroid cells. In nonerythroid cells, the rate of heme synthesis is largely determined by the level of ALAS, the first enzyme of the heme biosynthetic pathway (1, 5), and the level of ALAS is controlled by the intracellular free heme concentration. Thus, heme serves as a negative regulatory of its own biosynthesis and the subsequent synthesis of apo hemoprotein in the liver. However, recent studies using a variety of erythroid model systems, such as the erythroleukemic cell lines K562 and HEL, and separated human marrow erythroid progenitor cells (25), suggest that heme does not feed back and inhibit ALAS as in the liver system. Rather, addition of hemin to K562 or HEL cells results in elevation of the activities of ALAS and other enzyme activities distal to ALAS (25, 31).

When bone marrow cells are exposed to Epo in suspension cultures, heme synthesis increases in terms of iron incorporation into heme (32), and this is necessary for induction of globin synthesis (33). When rats were exposed to hypoxia or given Epo, bone marrow PBGD activity increased 3.5-fold by *de novo* synthesis, whereas the steady-state activities of ALAS, 4,5-dioxovaleric acid transaminase, ALAD, and FC were not significantly affected (34). These studies suggested that PBGD may be a key enzyme in regulating the amount of heme produced. Exposure to hypoxia for 10 days revealed that bone marrow PBGD activity reached a 3-fold increase by Day 8, after which its activity rapidly decreased and was accompanied by an increase in PBG oxygenase, reaching a steady state within 2 days (35). It appears that either ALAS or PBGD may be a rate-limiting enzyme for bone marrow heme synthesis; however, this is not fully clarified.

Other investigators have suggested that FC is the rate-limiting enzyme in erythroid cells (36, 37). Based on studies in rabbit reticulocytes, Ponka *et al.* (38, 39) have demonstrated that heme regulates its own synthesis by inhibiting the uptake of iron from transferrin. Furthermore, Hradilek and Neuwirt (40) have shown that when the level of cellular heme is increased, the efficiency of iron released is decreased. Heme is a very efficient iron carrier (41). When cellular heme reaches high levels, it will result in induction of HO with concomitant heme degradation and iron release. Iron release may coordinately regulate ferritin and transferrin levels through the iron-responsive element (IRE) (42–44).

IRE regulatory sequences are in the 3′-untranslated region of the transferrin receptor mRNA and the 5′-untranslated region of ferritin mRNA (42, 43). IRE interact with a cytosolic binding protein that exists in either a low- or high-affinity state. Iron deprivation

results in a switch from the lower to higher affinity state of the IRE-binding protein. High affinity binding of the protein to the 3′-IRE increases the metabolic half-life of the transferrin receptor mRNA, thereby increasing its translation and enabling increased iron acquisition. In addition, binding of the protein to the 5′-IRE inhibits ferritin translation and subsequent depletion of the chelatable intracellular pool of iron. Recently, depression of ferritin mRNA translation by hemin has been demonstrated *in vitro* (45). This type of regulation by iron rather than heme may not be applicable to normal human bone erythroid progenitor cells since addition of iron does not potentiate BFU-E growth in the presence of Epo (2).

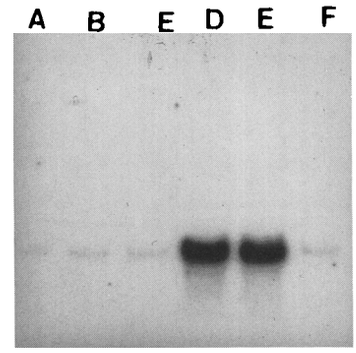

Figure 2. Northern analysis of heme oxygenase mRNA from HEL cells exposed to hemin. A, Control; B, 10 μM; C, 50 μM; D, 100 μM; E, 200 μM; F, 200 μM, plus 5 μg/ml actinomycin D. Ten micrograms of the total RNA from each sample were used.

Cell Receptors and Oncogenes

The binding of growth factors is mediated through specific cell surface receptors that initiate a cascade of biochemical events leading to proliferation and differentiation. In many instances, the action of hemopoietic growth factors has close association with the expression of oncogenes and their products (46). Expression of *src*-related oncogens is associated with tyrosine kinase activity, and this activity is also associated with growth factors such as platelet-derived growth factor and epidermal growth factor binding to receptors (47). The c-*fms* gene product appears to be identical to the cell membrane receptor for macrophage (CSF-1 (47, 48). HL60 cells induced to differentiate to macrophages with vitamin D_3 or 12-*O*-tetradecanoylphorbol-13-acetate express enhanced c-*fms* RNA levels and the presence of CSF-1 receptors (48).

We have recently demonstrated that hemin upregulates the expression of Epo receptors in murine and human (HEL) erythroleukemia cells (49). By employing ^{125}I-labeled bioactive recombinant Epo, results indicated that HEL and murine erythroleukemia cells expressed a low number of high-affinity surface receptors. Treatment of cells with hemin (50 μM) for 1–2 days revealed a progressive increase in Epo receptors. Dimethyl sulfoxide also increased receptor expression, but to a lesser degree. Induction of receptor expression was prevented by incubating cells with succinyl acetone (SA), which is an inhibitor of heme synthesis. Therefore, Epo receptor expression can be up- or down-modulated by the levels of heme and hence the cell's response to Epo. Hemin is also known to have effects on the induction of the enzyme HO. As mentioned, studies support the concept that a fall in HO is a prerequisite for the onset of erythroid differentiation. This is then followed by a rise in HO and may be a requirement for maximal growth and differentiation (3, 28, 30). Low levels of hemin (10–50 μM) have little effect on HO; however, higher levels (100–200 μM) induce HO.This can be seen by Northern analysis in Figure 2 as an induction of HO mRNA (Fig. 2, Lanes D and E) when HEL cells were induced to differentiate with hemin. The induction of HEL cells with hemin is also associated with an initial up-regulation of c-*myc* expression, followed by eventual increases in HO and PBGD (25, 46). Therefore, hemin may have multiple effects, and induction of HO may contribute to the differentiation process.

More recently, it has been suggested that heme's signal for differentiation may be coupled with specific changes in expression of c-*myc* and *myb* (46). Using HEL cells, we have also shown that hemin induced a transient increase in c-*myc* mRNA after 1–3 hr, which proceed that of PBGD and globin mRNA levels. Similarly, using Epo-exposed Raucher cells, it was found that there was a strong correlation between the magnitude of changes in c-*myc* and *myb* expression and the degree of Epo-induced hemoglobinization (50). This is consistent with the functional role for c-*myc* and *myb* gene products for the mechanisms of action involving induction of differentiation of Epo.

Protective Effect of Hemin on Hematopoiesis

Numerous investigations have demonstrated that hemin has beneficial effects on hematopoiesis and can overcome suppression of bone marrow growth induced by various agents. Initial bone marrow culture studies indicated that hemin-enhanced CFU-E growth (4, 51) and similar effects were then found for burst-forming units (BFU-E) and pluripotent stem cell colonies (52, 53).

SA, which is a specific inhibitor of heme synthesis, also has a potent inhibiting effect of CFU-E growth in the presence Epo (54). This inhibitory effect is overcome by the addition of hemin to the cultures.

Hemin exerts a protective role against certain toxic substances such as heavy metals and some drugs (55, 56). In this respect, 3-azido-3-deoxythymidine-induced bone marrow toxicity was found to be correlated with a marked suppression of ALAS, and this toxic effect was overcome by addition of exogenous hemin (57).

Other toxic agents such as Pb or Cd directly interfere or inhibit heme synthesis, and it is thought that exogenous hemin can bypass the steps in heme synthesis that the metals inhibit (56). It has also proved to have beneficial hematologic effects on cytopenias, myelodysplastic syndromes, porphyrias, and anemias (58). The antineoplastic drug doxorubicin produces a decrease in microsomal heme and cytochrome P-450 (55), and it has recently been found that hemin can overcome some of the drug hematotoxicity (55). Other studies show that hemin may also potentiate the action of growth factors such as Epo in sickle cell anemia bone marrow cells (59).

Specificity of Hemin

Exogenous hemin is not only incorporated into hemoglobin of erythroid cells, but also stimulates heme synthesis. In contrast, cobalt protoporphyrin (CoPP) increases heme biosynthesis, but is not incorporated into hemoglobin (60). Among eight other metalloporphyrins tested, only CoPP had this effect. In other studies, we have found that tin protoporphyrin or CoPP was not protective against human bone marrow 3-azido-3-deoxythymidine toxicity, whereas hemin had a significant effect (Table I). On the other hand, zinc 2,4-bisglycol protoporphyrin was without effect, but zinc protoporphyrin was a potent inhibitor of bone marrow CFU-E, BFU-E, and CFU-GM growth. Furthermore, the heme precursor protoporphyrin IX was found to offer a beneficial effect, so it appears that the protective effects are restricted to hemin or the heme precursor protoporphyrin IX (Table I).

Mechanism of Hemin Action

With respect to erythroid cells, experiments indicate that hemin potentiates Epo action, resulting in increases in bone marrow erythroid proliferation and a concomitant stimulation of ALAS and PBGD (25, 26). Furthermore, it was found the induction of ALAS occurred in the presence of SA, and that the regulatory role of hemin in erythroid progenitors was to exert negative feedback on ALAS in the presence of sA (26, 60, 61). Hemin effects may involve several mechanisms and hemin is an important regulator of globin synthesis, it promotes globin mRNA and increases its rate of translation (for review, see Ref 19) on commitment to erythropoiesis (5, 6) and on protein degradation (5). Additionally, hemin has been shown to be involved with the initiation of protein synthesis in several types of cells (62). Iron metabolism is also affected, and hemin may influence the levels of iron by ferritin storage proteins and transferrin receptors (41–45). The hemin effect on iron is very complicated, and there are several pathways that have been suggested. Heme may directly regulate ferritin mRNA translation (45, 63) and also detabilizes transferrin receptor mRNA. Heme may directly inhibit iron release from transferrin (45, 64). Heme acts as an iron carrier, and excess heme causes induction of HO and subsequent release of iron; iron then regulates its own synthesis via IRE as described earlier. However, whether heme regulates the proliferation and differentiation process in erythropoiesis is not clear.

Stenzel's group (65) has reported that hemin induces mitogenesis in human T lymphocytes. More recently, they found that heme and heme analogs, *Sn*-protoporphyrin, which is an inhibitor of heme oxygenase, synergize with interleukin-2 in stimulating thymidine incorporation by lymphocytes (66). Heme and *Sn*-protoporphyrin stimulate tumor necrosis factor and tumor necrosis factor-α production by peripheral blood macrophages in the presence of T lymphocytes (66).

The authors have studied the direct effect of heme and inhibitors of heme synthesis on the ability of adherent stromal cells to produce stimulatory and/or inhibitory factors. Stromal cells were incubated with and without inhibitors of heme synthesis, and then the conditioned media were recovered and tested on BFU-E and CFU-GM growth. Results indicated that conditioned media from adherent stromal cells exposed to inhibitors of heme had a significant inhibitory effect on BFU-E and CFU-GM growth. Formation of these inhibitory factors was prevented by supplementation of adherent stromal cells with heme (25). We suggested

Table I. Effect of Heme Analogs (Protoporphyrins; 50 μM) and AZT (0.1 μM) on Human Bone Marrow BFU-E Growth[a]

Heme analog	% Control growth						
	CoPP	PPIX[b]	SnPP	ZnPP	ZnDP	Hemato-PP	Hemin
Analog alone	115	165	100	30	110	5	167
+AZT	47	118[c]	38	—	39	—	86[c]
AZT alone				30			

[a] Human bone marrow BFU-E were grown in methylcellulose cultures in the presence of 2 units/ Epo/ml and 6×10^5 cells/ml and then BFU-E scored after 14 days of growth at 37°C. Heme analogs and AZT were prepared and added to cultures as described previously (2, 57, 60) and control cultures contained Epo alone without additions.

[b] PPIX, protoporphyrin IX; SnPP, tin protoporphyrin; ZnPP, zinc protoporphyrin; ZnDP, zinc 2,4-bisglycol protoporphyrin; Hemato-PP, hematoprotoporphyrin; AZT, 3-azido-3-deoxythymidine.

[c] $P < 0.001$.

that within the adherent cell complex (macrophages, stromal cells), heme may provide the central network for the elaboration of growth-promoting substance(s), which directly promotes hemopoietic colony formation and possibly the expression of growth factors and/or receptors. A defect in heme synthesis may be accompanied by an increase in negative regulation and suppression of pluripotent stem cells. For example, adherent cell macrophages deficient in heme may promote the release of excess E-type prostaglandin or other factors that counterbalance stimulatory factors. Adequate heme, on the other hand, may stimulate the synthesis and/or release of growth factors(s) from macrophages such as interleukins, which in turn signal the release of CSF from stromal cells (Fig. 3). These observations may lend support to the therapeutic potential of heme therapy for hematologic disorders in which heme biosynthesis is disturbed, e.g., in myelodysplastic syndrome and in sideroblastic syndromes (58).

The discovery that heme has a selective inhibitory action on cytoplasmic DNA polymerase from erythroid hyperplastic bone marrow cells and DNA polymerase and from human neuroblastoma cells suggests that heme may have direct effects on cellular proliferation and differentiation. More recently, hemin has been shown to have a selective inhibition of reverse transcriptase of various viruses (67). Hemin inhibits viron-associated reverse transcriptase of murine leukemia virus.

Other mechanisms may involve HO and AA. Hemin can induce HO, and this enzyme is also important in preventing oxidative damage. In this respect, Keyse and Tyrrell (68) have shown that this enzyme may constitute part of the defense mechanism against

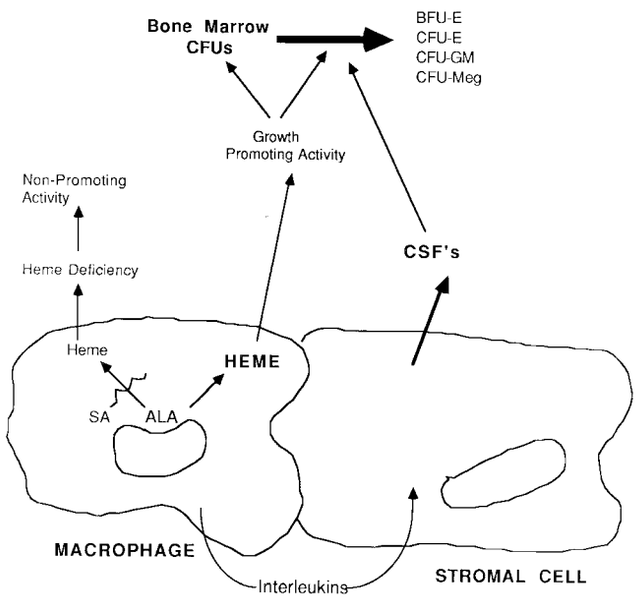

Figure 3. Schematic representation of the possible involvement of heme in positive and negative feedback control mechanisms of stem cell proliferation and differentiation.

oxidative damage produced by free radicals. Heme metabolites are thought to have scavenging activity toward oxygen-free radicals and their ability to prevent oxidative damage. It has been considered that the cell response to HO induction constitutes an important cellular defense mechanism against UV or cytochrome P-450-dependent free-radical formation (68). HO is a stress protein that is induced by heavy metals such as cobalt and iron, and plays another important role in degradation of incorrect hemoprotein(s) such as cytochrome P-420. Therefore, in addition to the proposed role of HO as a regulator of cellular growth and differentiation, it provides a protective role during oxidative stress and hemin may modulate this effect.

Finally, hemin is also essential for the enzymatic oxidation of AA to prostaglandins (69) and recently Lutton *et al.* (70) found that heme-dependent cytochrome P-450 epoxygenase metabolizes AA, and that products of this pathway stimulate *in vitro* erythropoiesis.

Role of Cytochrome P-450 in Hematopoiesis

It has been shown that the bone marrow possesses cytochrome P-450 and may function as an important drug-metabolizing organ (71).

Cytochrome P-450 represents a unique family of hemoproteins that serve as the terminal acceptor in the NADPH-dependent mixed function oxidase system. This system catalyzes the oxidative transformation of a large number of endogenous and exogenous substrates (71–73). Cytochrome P-450 exists in multiple forms that differ in substrate specificity, and a particular form may be detected only after its induction with a specific drug. Among the blood cells, monocytes, Kupffer cells, peritoneal macrophages, leukocytes, lymphocytes, and neutrophils have been shown to contain cytochrome P-450-dependent aryl hydrocarbon hydroxylase (AHH) (74, 75). Furthermore, macrophage AHH activity was found to be depressed following phagocytic activation with a concomitant increase in HO activity. These cells may in turn modulate cytochrome P-450 and the heme pool by the HO system. Other forms of cytochrome P-450 are involved with thrombin synthase prostaglandin synthase and leukotriene B4 hydroxylate. These various heme proteins have been shown to play important roles in the metabolism of endogenous substrates.

The cytochrome P-450 system has been characterized in murine hemopoietic colonies (76). Addition of 3-methylcolanthrene to Epo-dependent CFU-E was found to induce AHH activity above controls in a dose-dependent manner. CFU-E taken from different incubation periods (1–4 days) revealed that AHH was maximal in 2 ½-day cultures and thereafter declined. In contrast, maximal AHH activity was obtained from 6 to 7-day CFU-GM. These results demonstrated that AHH is present in developing hemopoietic colonies,

and that induction of AHH may indicate an important role for these cytochrome P-450 monooxygenases in cell development and differentiation as well as in the metabolism of various drugs.

It is of significance that developing CFU-E cultures are rich in erythroblasts and that these cultures demonstrated inducible AHH activity. Studies by Shiverick *et al.* (77) have also demonstrated inducible cytochrome P-450 activity in erythroblasts.

Exposure of bone marrow cells to carcinogens resulted in the induction of cytochrome P-450 drug metabolizing systems (78). The pattern of enzyme induction by 3-methylcholanthrene or benzene suggests that depression of hematopoietic cell function may depend, in certain instances, on the pharmacologic microenvironment of these cells and their ability to respond to various inducers and xenobiotics by biotransformation and detoxification (79).

Genetic localization for cytochrome P-450 AHH has been achieved. Brown *et al.* (80) prepared hybrid clones segregating human chromosomes by fusing mouse RAG cells to human bone marrow cells, and tested for the mixed-function monooxygenase system. Isozyme analysis showed the AHH activity (human bone marrow) was concordant with the expression of the human isozymes malate dehydrogenase and isocitrate dehydrogenase, which are known to be expressed by human chromosome 2. Therefore, these results indicated that the gene(s) required for AHH activity in the bone marrow are located on human chromosome 2.

Substantial evidence indicates that cytochrome P-450 is important not only in drug metabolism, but also in the metabolism of endogenous substrates such as lauric acid and AA. For example, the presence of cyclooxygenase and lipoxygenase pathways of AA metabolism in hemopoietic cells has been documented (81–84). Their metabolites, prostaglandins, hydroxyeicosatetraenoic acid (HETE), and leukotrienes, have been implicated in the modulation of cell proliferation and differentiation and have both enhancing and inhibitory effects on the hemopoietic system (81–84). However, it is becoming evident that compounds of a newly discovered branch of the AA cascade, the cytochrome P-450-dependent pathway or third pathway, are very important to the hemopoietic system (70, 85–87).

Studies over the past few years have revealed that this novel cytochrome P-450-dependent monooxygenase system for the metabolism of AA does exist in hemopoietic cells (85–87). The three pathways for AA metabolism are represented in Figure 4. The third pathway is a microsomal mixed function oxidase system, strictly dependent on molecular oxygen and NADPH. Three different types of reactions can operate in this system: (i) oxidation leading to the formation of mono-HETE; (ii) epoxidation leading to the formation

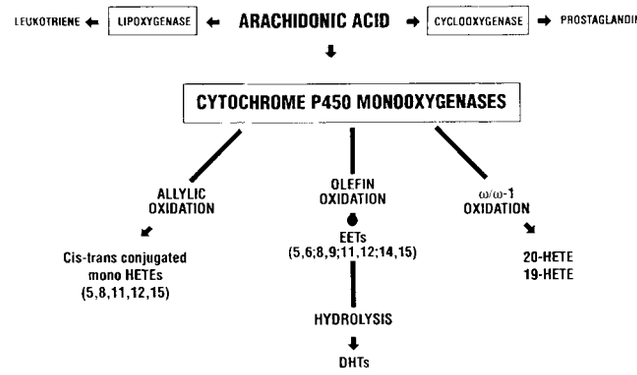

Figure 4. Schematic representation of the three major pathways of AA metabolism by lipoxygenase, cyclo-oxygenase and P-450 AA. EET, epoxyeicosaltrienoic acid; DHT, dihydroxytrienoic acid.

of epoxyeicosatrienoic acids; and (iii) oxidation at ω and ω-1 positions of AA to form the 20- and 19-HETE. It should be stressed that while three potential pathways for AA conversion exist, the specific pathway by which AA will be transformed depends on the tissue, kind of stimuli, co-factor availability, and so forth.

Metabolism of AA via cytochrome P-450 has been observed in several tissues including bone marrow cells, macrophages, endothelial cells, and neutrophils (85–87). Several studies have demonstrated that some of the cytochrome P-450-AA metabolites possess a wide range of biologic activities. These include stimulation of peptide hormone release, inhibition and stimulation of Na^+-K^+-ATPase (88), vasoreactivity (89), mobilization of microsomal Ca^{2+} (90), and inhibition of platelet aggregation (91). Furthermore, synthetic 19- and 20-HETE have now been produced and can be used as standards for comparisons.

Recently, it has been shown that human bone marrow and peripheral blood cells are capable of metabolizing AA by this pathway to form 19- and 20-HETE, both of which have marked effects of Epo-dependent erythropoiesis (CFU-E and BFU-E) (70, 87). Addition of 10^{-11} *M* 19- or 20-HETE to CFU-E cultures stimulated growth by as much as 3- to 4-fold, respectively. Similar effects were seen for BFU-E growth and little or no effect was seen for CFU-GM growth. In another study, the ability of adherent bone marrow stromal cells to metabolize AA by the third pathway was demonstrated (87). The adherent stromal cells were found to metabolize AA to cytochrome P-450 metabolites, the formation of which was inhibited by 7-ethoxyresorufin and SKF-525A, but not by indomethacin or BW755C. These stromal cytochrome P-450 AA metabolites potentiated CFU-E growth by 6- to 7-fold at pmolar concentrations. Furthermore, endothelial cells are part of the bone marrow stromal hemopoietic microenvironment and certainly an important constituent of the vascular network. Abraham *et al.* (92) and Pinto *et al.* (93) have characterized cytochrome P-450-

dependent mono-oxygenase and mixed function oxidase systems in vascular endothelial cells. Since this type of cell comprises an important component of the hemopoietic stroma, metabolites of this system may directly influence hemopoietic progenitor cells.

Depression in heme metabolism may lead to a variety of disturbances that can be overcome with a sufficient supply of heme. Heme and cytochrome P-450 play important roles not only in detoxification of various environmental agents, but also in metabolism of endogenous substrates such as AA. It is clear that cells of the bone marrow, bone marrow stroma, endothelial network, and peripheral blood are important for the generation of novel AA metabolites by the third pathway. These metabolites have powerful effects on hematopoiesis *in vitro* and their *in vivo* role remains to be elucidated. Products of heme and hemoprotein(s) such as cytochrome P-450 may be involved in the signal transduction network for growth and differentiation, and also as a free radical scavengering system. Although numerous metabolic changes have been described, the significance of these changes in triggering the onset of differentiation by heme is not known. It is clear that closer examination of the role of oncogene gene expression and erythroid heme metabolic enzymes needs to be evaluated.

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