Chapter 4
Alterations in the Blood System During Myelosuppression Induced by Cytostatic and Radiation Treatment

The hematopoietic tissue belongs to the structures extremely sensitive to the action of antiblastomic drugs and ionizing radiation, which is explained mostly by a high proliferative activity of its constituent elements, so this system is the gold standard model to examine the regularities in the development of biological effects and the modes of action of the myeloinhibitory agents [9, 15, 21, 22, 143, 153, 207, 262, 267, 309]. However, the search for most rational and efficient ways to correct the hypoplastic manifestations should be based on more detailed knowledge about the mechanisms of suppression and recovery of hematopoiesis in patients subjected to radiation and cytostatic therapy.

Alterations in the blood system observed during the development of cytostatic and radiation diseases were examined in a great number of experimental works. Modern science accumulated numerous data on the state of the major subdivisions of hematopoietic tissue in experimental animals subjected to radiation and administered with different doses of antitumor drugs [6, 24, 58, 127, 208, 212, 224, 242, 263, 306]. However, interpretation of these data in respect to deciphering the mechanisms of recovery of the suppressed hemopoiesis does not encompass all the important features of regenerative processes in the hematopoietic tissue. Specifically, replenishment of the morphologically identified cells in the bone marrow can be effected either by reproduction of the hematopoietic elements that survived after the cytostatic therapy or by employing the less mature progenitor cells [112, 118, 205, 278]. It should be stressed that such important avenue of hemopoietic reparation as accelerated differentiation of the hemopoietic precursor cells under the conditions of suppressed cell proliferation received little attention.

Analysis of the present data revealed significant differences in the depth and duration of hemopoietic depression after the use of various cytostatic drugs and radiation therapy in the doses that are equivalent in the matter of general biological effect [12, 17, 58, 207, 212, 263, 267, 306]. Usually, these paradoxical differences are explained by unequal damage to the hematopoietic cells exerted by the myeloinhibitory agents with diverse modes of action. The toxic effect towards the hematopoietic elements is surely the most important feature, which

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determines the character of myelosuppressive effect in any particular case. However, little attention was focused to the changes in the functional state of the regulatory apparatus of the hematopoietic tissue provoked by the antiblastomic drugs, which most surely contribute to the specific manifestations of the cytostatic and radiation diseases.

The most important role in the control of hematopoiesis in the norm and diverse extreme influences is played by HIM composed of various types of the cell elements and extracellular matrix. HIM exerts the local control over proliferation and differentiation of the hemopoietic cells by releasing the humoral factors and by transmitting the signals during the direct cell-cell interactions [90, 95, 96, 123, 198, 229, 266, 313, 320, 359, 370, 371]. At the same time, the cells of hemopoietic microenvironment are rather sensitive to the cytostatic drugs and ionizing radiation [52, 93, 137, 200, 206, 211, 232, 285, 342, 364, 383].

In addition, the plasmatic membrane of hematopoietic progenitor cells at different maturation levels as well as the morphologically identified hemopoietic and stromal cells can expose the receptors to different transmitters (such as acetylcholine, catecholamines, serotonin, substance P, opioids, *etc*.). The studies revealed direct (receptive) and indirect (mediated via HIM cell elements) regulatory effects of neurotransmitters on proliferation and differentiation of the committed precursors of hemopoiesis [96, 215, 323, 352, 374]. Most probably, these data indicate existence of monoaminergic control over the processes of damage and regeneration of the hematopoietic tissue during myeloinhibitory influences.

Overall, the study of the roles played by HIM with its elements and by central and peripheral monoamines in sustaining the hemopoietic regenerative processes can help to develop the pathogenically reasonable ways to correct the disturbances in the blood system provoked by the antiblastomic therapy.

Under extreme influences leading to hypoplasia of the hematopoietic tissue (radiation, the use of cytostatic drugs in the doses that are equivalent by the general biological effect), hemopoietic recovery develops in various ways. Along with the direct suppressive effects of the toxic agents on hematopoietic cells, the recovery dynamics of hematopoiesis is mostly determined by the character of hematopoietic disorders. First of all, one should bear in mind the changes in functional activity of individual HIM elements [2, 40, 53, 179, 250]. For example, a single total irradiation of mice at a dose of 2.0 Gy provoked the development of the bone marrow variant of acute radiation sickness. The total cellularity of the bone marrow decreased to the end of day 1 after irradiation due to dramatic drop in the content of erythroid elements, immature neutrophils, and the lymphoid cells (on the average, by 70 % initial level). In 2 days, the bone marrow displayed the first signs of hemopoietic regeneration manifested by appearance of the immature forms of myeloid cells such as myeloblasts and promyelocytes. To the end of experiment day 4, the complete recovery of the total number of myelocaryocytes and cellularity of all hematopoietic lineages took place. The peripheral blood indices attained the initial values on experiment day 7 [2, 69].

A rather gradual and not especially intensive reparation of granulocytic and erythroid medullar lineages after exposure to ionizing radiation is explained by the

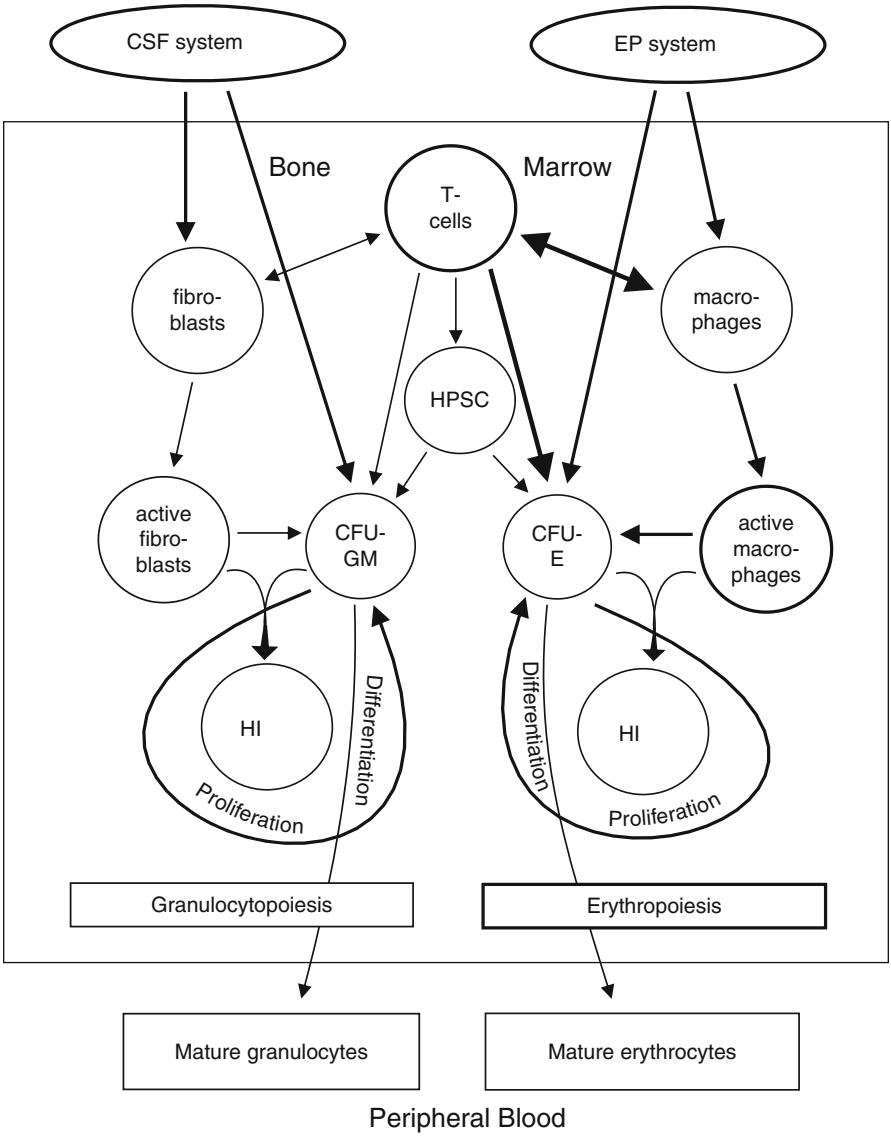

Fig. 4.1 Control of hematopoiesis during myelosuppression caused by total irradiation. Absence of any significant changes is marked with fine continuous lines, while the *dash* and *thick solid lines* indicate inhibition and activation, correspondingly

fact that the total irradiation produces no significant changes in the functional activity of HIM elements (Fig. 4.1).

Elevation of serum CSA and EPA accelerates proliferation of the corresponding precursors. The absence of any drastic changes in maturation of hemopoietic

elements results from a rather stable structure-functional state of the hematopoietic tissue. Stimulation of direct and indirect (mediated via macrophage system) mechanisms of the erythropoietic control exerted by T-cells results in acceleration of erythron recovery [2].

When administered at MTD, the most cytostatic drugs produce pronounced myeloinhibitory effect manifested by decreased cellularity of bone marrow and some hematopoietic lineages. In mice, the most pronounced and long-lasting depletion of the hematopoietic tissue was observed after injection of **5-fluorouracil**. In our studies, the maximum of depression was observed on experiment day 5 when the total count of myelocaryocytes was merely 6.3 % initial level. Slow recovery of hematopoiesis finished only to experiment day 14. Chronic inhibition of granulocytic and erythroid medullar lineages by 5-fluorouracil was accompanied by elevation of the content of the committed precursors in hematopoietic tissue resulting from the disturbances in the processes of their maturation [37, 53, 384] (Fig. 4.2).

This phenomenon resulted from stimulation of proliferation of the hemopoietic precursor cells against the background of unrestored (due to pronounced dissociation of the progenitors and the stromal elements of the microenvironment) structure-functional organization of the bone marrow. Under these conditions, up-regulation of proliferative activity of the clonogenic cells results from an increase in secretion of the hemopoietic growth factors by T-cells during the late terms of experiment due to their accumulation in the hematopoietic region and interaction with the adherent elements.

Under these conditions, the adrenergic system plays a positive role in the processes of hemopoietic tissue regeneration due to activation of HI formation, up-regulation of EPA production by HIM non-adherent cells, and elevation of serum CSA. On the one hand, the dopaminergic system increases the rate of erythron regeneration by raising the level of serum EPA and by increasing the erythropoietin-dependent activation of CFU-E proliferation, and on the other hand, it delays the recovery of granulocytic hemopoietic lineage due to down-regulation of CSA production by the adherent cells in the hemopoietic microenvironment. In its turn, the serotoninergic system augments the development of erythropoiesis depression due to down-regulation of EPA secretion by the adherent cells in HIM, although it increases the recovery rate of the granulocytic hemopoietic lineage by up-regulating formation of the granulocyte and mixt HI and by stimulating division and maturation of the granulomonocytic precursors mediated via the system of the colony-stimulating factors [84, 128, 129, 163, 165].

The changes in the content of the medullar hemopoietic cells in animals treated with anthracycline antibiotic **adriamycin** were biphasic in character. On postinjection days 2–4, the count of medullar nucleated cells decreased, which was followed by a transient rise to initial level and subsequent drop on days 9–11. The study of the mechanisms of hematopoietic recovery showed that activation of differentiation of the committed progenitors triggered by adriamycin and the consequential rapid regeneration of the hemopoietic tissue (Fig. 4.3) resulted from the leading recovery of medullar HI and especially from their intensive formation by the mature macrophages.

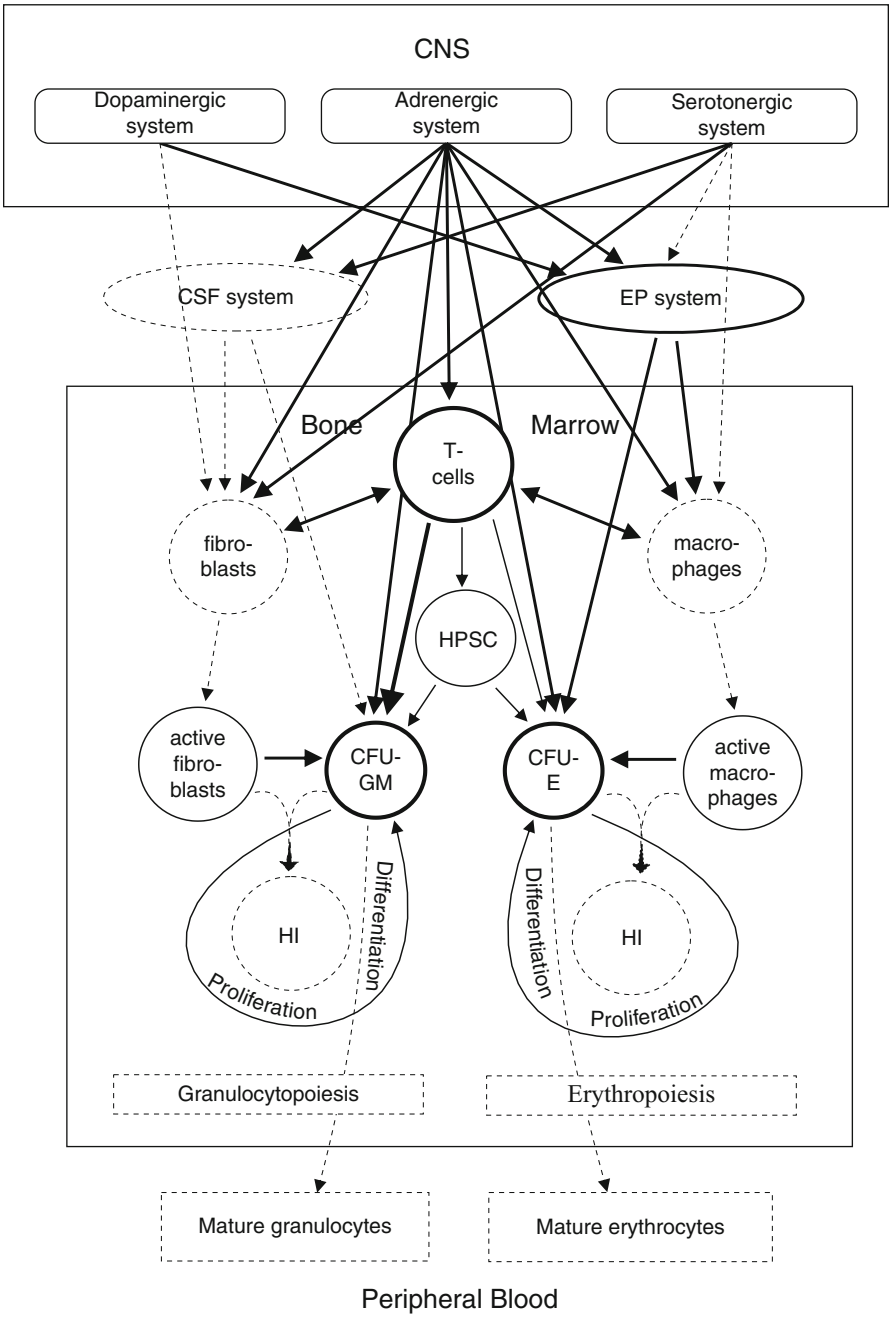

Fig. 4.2 Control of hematopoiesis during myelosuppression caused by injection of fluoropyrimidine antimetabolite 5-fluorouracil. Absence of any significant changes is marked with fine continuous lines, while the *dash* and *thick solid lines* indicate inhibition and activation, correspondingly

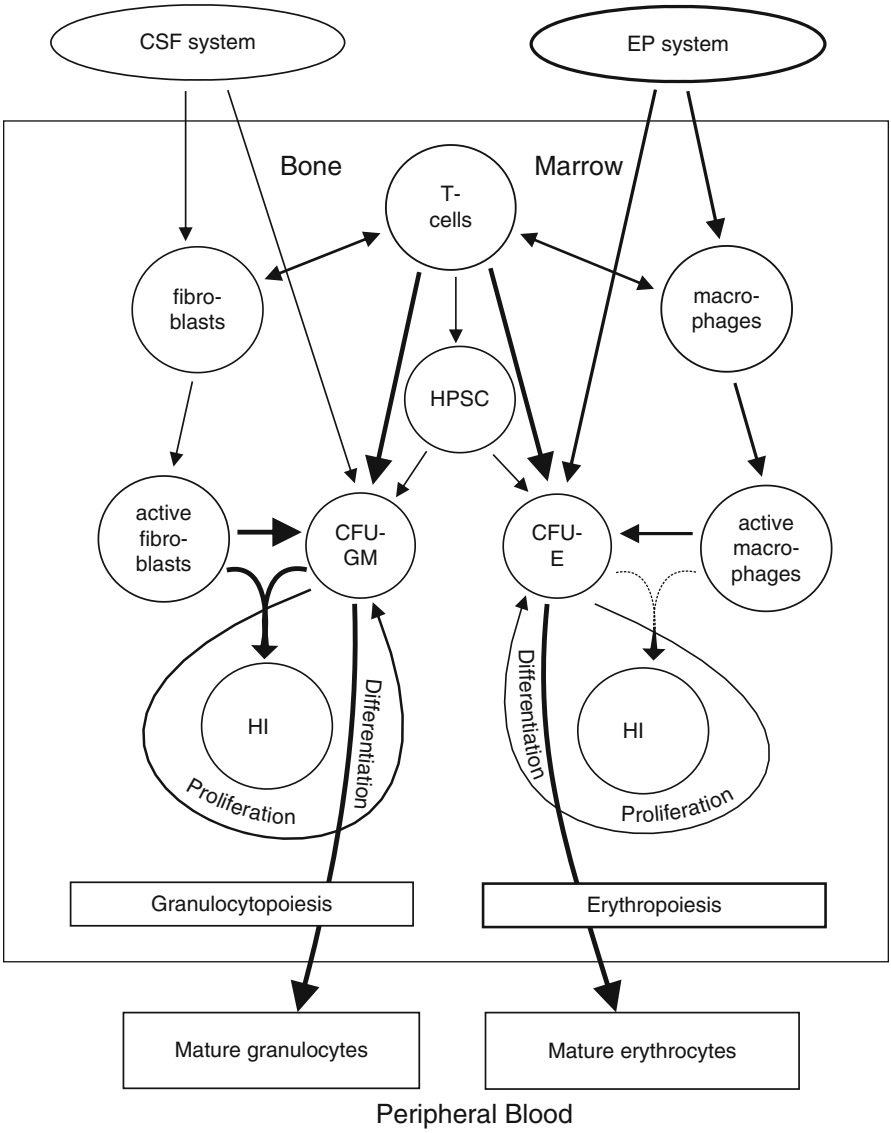

Fig. 4.3 Control of hematopoiesis during myelosuppression caused by injection of anthracycline antibiotic adriamycin. Absence of any significant changes is marked with fine continuous lines, while the *dash* and *thick solid lines* indicate inhibition and activation, correspondingly

In addition, an important role is played by stimulation of the coupling between the stromal mechanocytes and hemopoietic precursor cells [37]. At this, the high level of proliferative activity of hemopoietic precursors results from up-regulation of production of the hemopoiesis-stimulating activities by microenvironmental

elements at the early terms of examination promoted by vigorous recovery of their population.

Cyclophosphane also induced rapid reparation of the structure-functional organization of the bone marrow and early up-regulation of secretory activity of the adherent myelocaryocytes in cooperation with T-cells, which provided accelerated transition of the granulocyte-macrophage precursor cells to differentiation phase [37]. As a result, the total score of medullar cell rapidly restored mostly due to active regeneration of the granulocytic hemopoietic lineage. The content of immature forms of the neutrophilic granulocytes assessed on day 2 after injection of the alkylating agent was as low as 3.5 % initial level, but on day 5, the number of these cells increased almost 3-fold in comparison with initial level, and their count remained elevated to the end of experiment. In a natural way, the content of mature medullar neutrophils attained maximum to day 8, and it normalized during subsequent 4 days. At the same time, the long-term decrease in the content of erythrocaryocyte is probably explained by pronounced damaging effect of the alkylating agent exerted directly to the committed precursors of the erythroid hemopoietic lineage [37, 112].

The dopaminergic system predominantly augments the disturbances in the structure-functional organization of the erythroid compartment of hemopoiesis caused by the alkylating agent, which results in additional delay in regeneration of erythropoiesis and in more pronounced reticulocytopenia in the peripheral blood [128, 165]. These processes are accompanied by stimulation of G-CSF- and dopamine-dependent control mechanisms over proliferation of granulomonocytic progenitors impeding the development of neutrophilic leukopenia [84, 163].

The inhibitory effect of adrenergic system on granulocytic and erythroid hemopoietic lineages under the action of cyclophosphane (Fig. 4.4) is mediated via inhibition of functional activity of the HIM adherent cells (i.e., by down-regulating HI formation and EPA production) as well as via decrease in the division rate of granulomonocytic progenitors related to G-CSF and peripheral adrenergic mechanisms.

Additional down-regulating effect of the serotoninergic system on the erythron is related to inhibition of formation of the erythroid HI, moderation of functional activity of the erythroid progenitors (mediated by the erythropoietin system), and decrease in the level of EPA produced by auxiliary bone marrow cells [129, 165, 166].

Etoposide (a derivative of podophyllotoxin) decreased the total score of myelocaryocytes on postinjection days 1–7, but on day 8, this score elevated to 121.5 % initial value (Fig. 4.5). In the following, this parameter decreased again attaining the initial value to postinjection day 12. Examination of myelogram showed that the drop in the total bone marrow cellularity resulted from a decrease in the content of mature forms of neutrophilic granulocytes, nucleated erythroid cells, lymphoid elements, and monocyte-macrophages.

Accumulation of the progenitors of erythro- and granulomonocytopoiesis in the bone marrow due to differentiation of less mature cells developed in parallel with intensive recovery of the corresponding hemopoietic lineages [89, 179, 110]. At this, elevation in cellularity of the hematopoietic tissue was mostly provided by activation of maturation of the committed precursors. This process was caused by

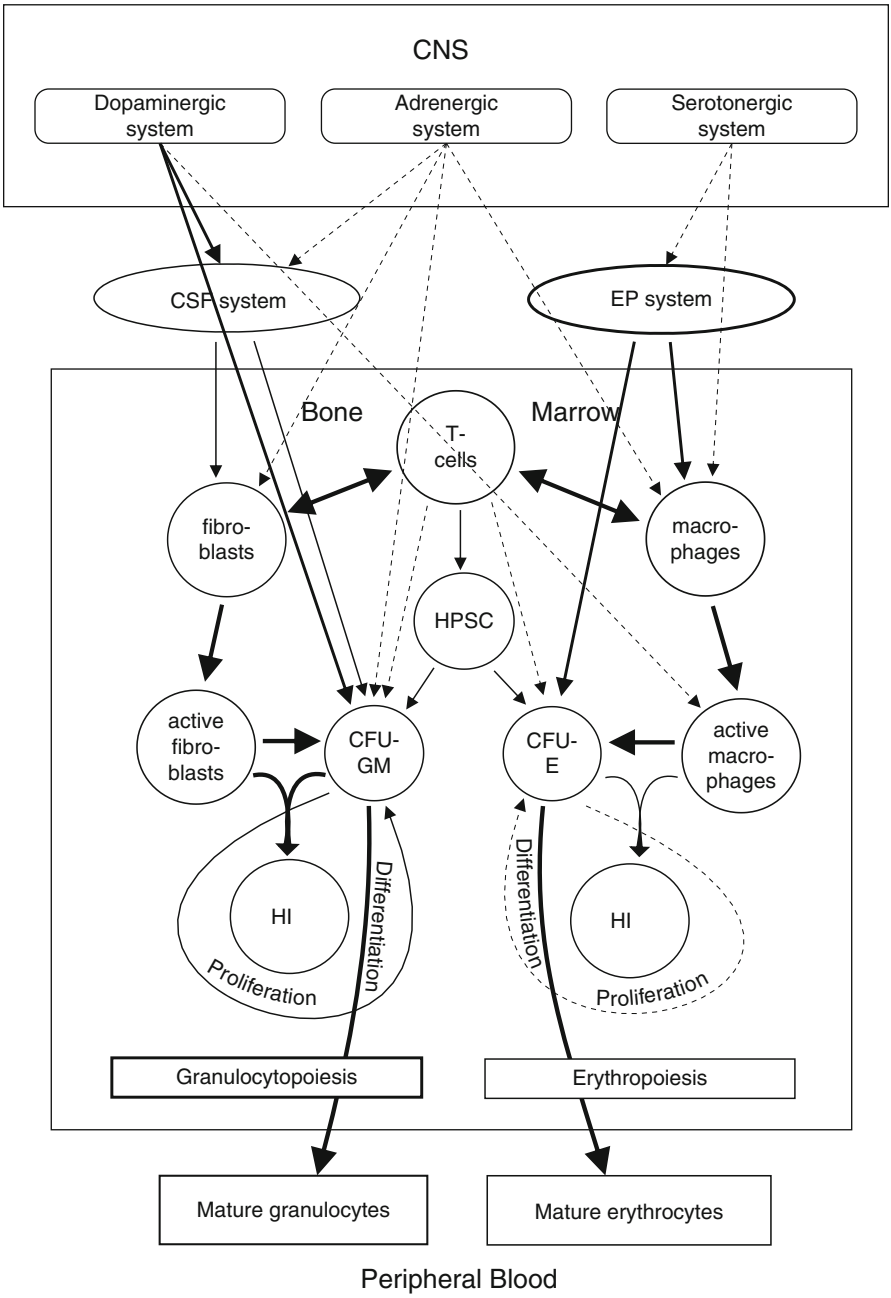

Fig. 4.4 Control of hematopoiesis during myelosuppression caused by alkylating agent cyclophosphane. Absence of any significant changes is marked with fine continuous lines, while the *dash* and *thick solid lines* indicate inhibition and activation, correspondingly

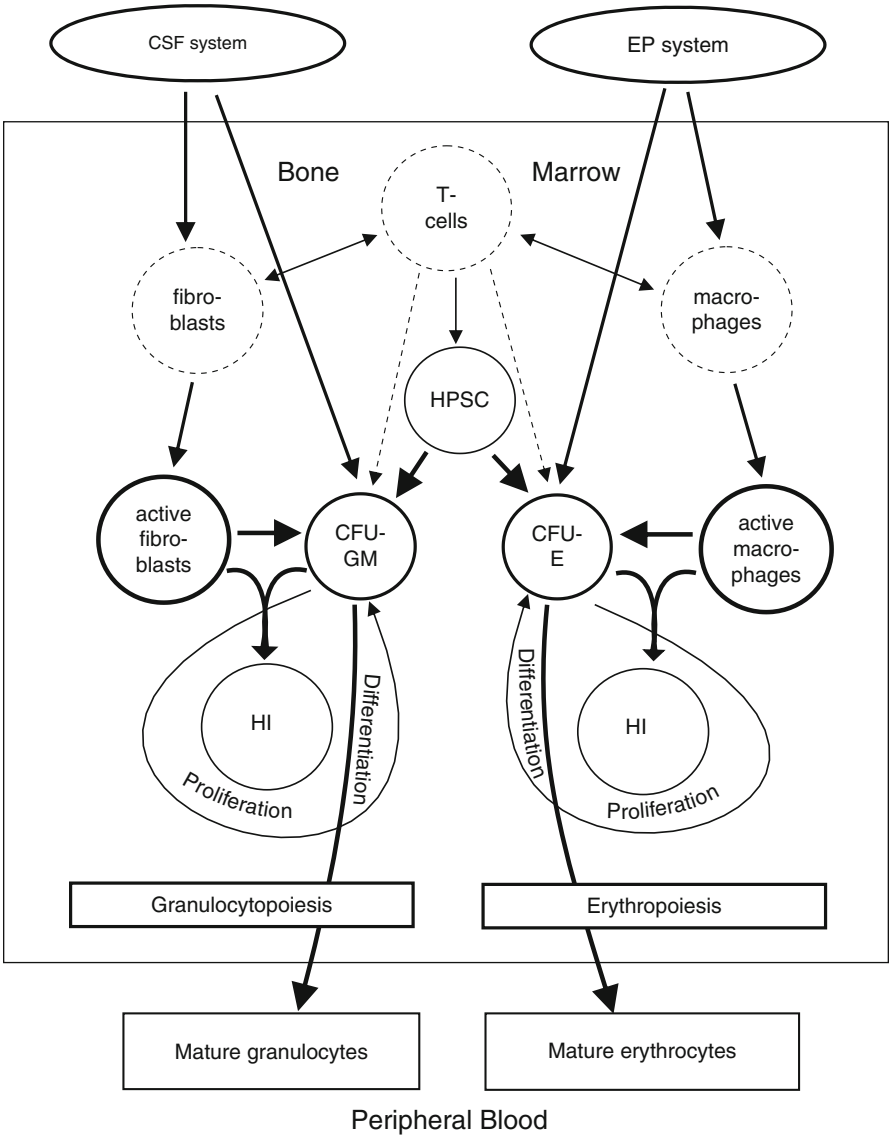

Fig. 4.5 Control of hematopoiesis during myelosuppression caused by etoposide, a derivative of podophyllotoxin. Absence of any significant changes is marked with fine continuous lines, while the *dash* and *thick solid lines* indicate inhibition and activation, correspondingly

augmented adhesive potency of the elements in the hematopoietic microenvironment towards the colony-forming cells accompanied by elevated score of the growth factors in the blood serum and by active production of the colony-stimulating factors by the adherent cells in the bone marrow [179].

Carboplatin provoked anemia and thrombocytopenia even at the early terms after injection. Decrease in the content of erythrocytes and hemoglobin in the peripheral blood resulted from a pronounced inhibition of medullar hemopoiesis on postinjection days 5–12 [194]. At this, leucopoiesis was damaged to a small degree, the alterations being observed only at the later terms of examination. Under the absence of adequate reaction of the distant humoral system (erythropoietin) to cytostatic damage produced by this nephrotoxic agent, there was an up-regulation in secretion of the erythropoiesis-stimulating humoral factors by the adherent cells in HIM. Such activation of the local mechanisms resulted in accumulation of erythroid progenitors in the bone marrow, but it could not ensure efficient recovery of the erythron (Fig. 4.6).

Thus, the character of hematopoietic recovery under the action of various myeloinhibitory agents significantly depends on peculiarities of the disturbances in the hemopoietic control provoked by applied agent. It is explained by the fact that intensity of growth and maturation of the hematopoietic cells during regeneration are determined by the damage of specific elements in HIM. For example, the use of 5-fluorouracil is characterized with disturbance of functional activity of the cells in the system of mononuclear phagocytes with relative functional integrity of the T-lymphocyte system [40, 53, 385]. In contrast, cyclophosphane is notorious for pronounced toxicity against T-cells [37, 57, 386]. In both cases, dysregulation of hematopoiesis results from uncoupling of the cooperative interaction between T-lymphocytes and macrophages in regulation of the cell cycle in the pool of progenitor cells, which is accompanied by prevalence of the corresponding processes according to proliferation and differentiation of these cells. Under the conditions of perturbed cell-cell cooperative interactions between various HIM elements, the activity of hematopoietic tissue is shaped by the spectrum of humoral agents (cytokines and GAG) secreted by these elements. In other words, in similar situations T-lymphocytes can control the proliferative processes (specifically, via IL-3 production), while the adherent cell can regulate differentiation of the cells (for example, via production of IL-1 and the lineage-restricted hemopoietins).

Disturbances in the hematopoietic mechanisms are also caused by radiation, although they are expressed to a far smaller degree than those produced by 5-fluorouracil and cyclophosphane [2, 387]. Correspondingly, the radiation-induced changes in the control of hematopoiesis do not significantly impede regeneration of the hematopoietic tissue.

Under the action of adriamycin or etoposide, the overall changes in hemopoietic microenvironment induced by cytostatic drugs promote the hemopoietic recovery processes. Thus, in these particular cases, these changes play the positive adaptive role.

In hypoplastic states resulting from the use of cytostatic drugs, the adrenergic, dopaminergic, and serotoninergic pathways control (1) proliferation and differentiation of the committed hemopoietic progenitors in HIM, (2) functional activity of HIM cell elements, (3) the system of colony-stimulating factors, and (4) the erythropoietin system. At this, the serotoninergic system is mostly responsible for alterations in erythroid hemopoietic lineage, while the adrenergic and dopaminergic systems predominantly affect the granulocytic hemopoietic lineage [165, 167, 172].

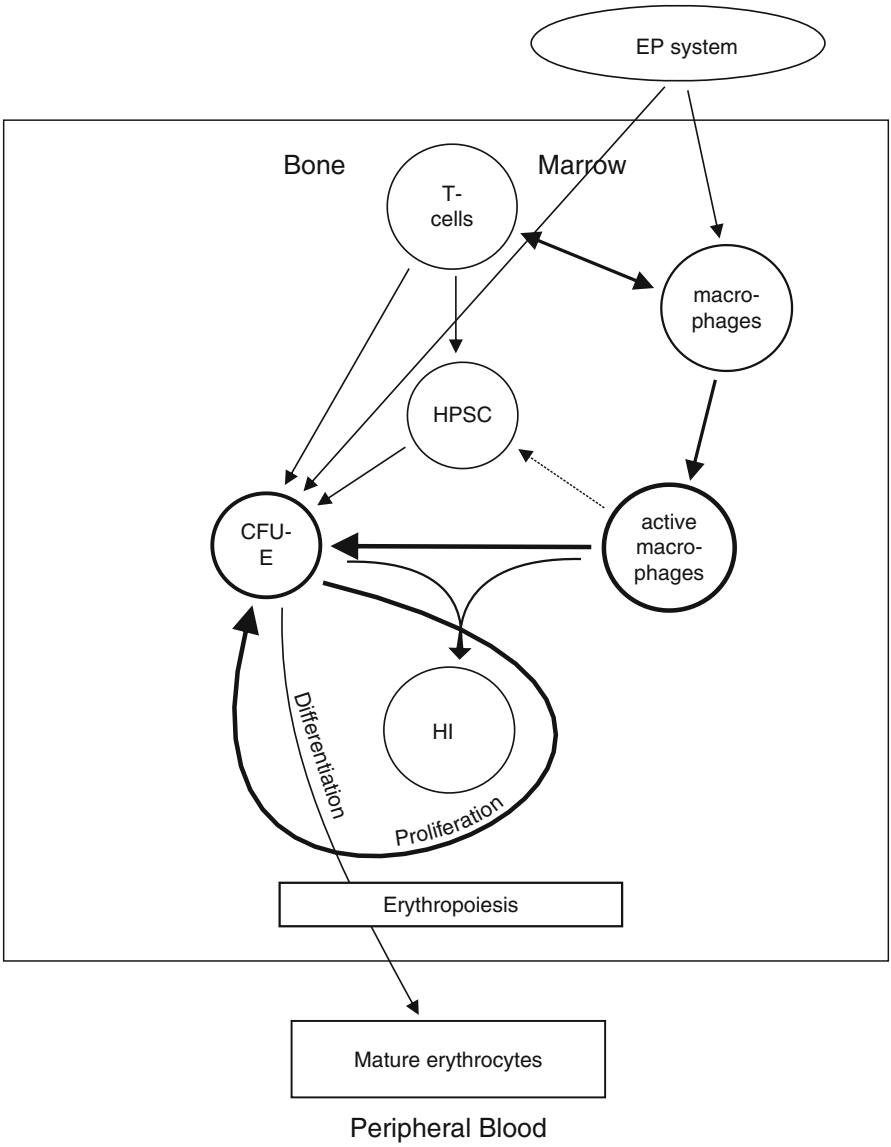

Fig. 4.6 Control of erythropoiesis during myelosuppression caused by carboplatin. Absence of any significant changes is marked with fine continuous lines, while the *dash* and *thick solid lines* indicate inhibition and activation, correspondingly

Examination of these control mechanisms showed that the ligands of monoaminergic nature (predominantly, α- and β-adrenomimetics) accelerate differentiation of the pluripotent hemopoietic cells into progenitors of granulomonocytopoiesis (induced by G-CSF *in vitro*) and increase the rate of division of the newly formed

CFU-GM. At the same time, the monoaminergic transmitters (typically, serotonin) enhance the feeder activity of the fibroblastic elements for CFU-G [172].

Reserpine-induced potentiation of granulocytopoiesis-stimulating activity of G-CSF under the action of cyclophosphane is explained by elevation of the content of the pluripotent hemopoietic cells, by increased rate of their differentiation towards the progenitors of granulomonocytopoiesis, and by the stimulating effect of the stromal elements and Thy-1.2$^+$-cells on the hemopoietic progenitors (predominantly, on CFU-GEMM) [82, 83].

In view of progressive improvements of the methods employed in treatment of the malignant neoplasms and increase in the number of the long-living patients, one of the central problems in tumor chemotherapy can be the long-term side effects of the toxic action of cytostatic drugs on the normal (not affected by the tumor) and actively proliferating cell systems. While the disturbances in nervous, cardiovascular, and endocrine systems can be rather easily detected and diagnosticated, the changes in the blood system can be latent with the manifestations observed only during additional hematopoiesis-disturbing influences [58, 325].

For example, exposure of mice to immobilization stress in 1, 3, and 6 months after injection of **doxorubicin** and **vinblastine** in MTD induced pronounced changes in the parameters of hemopoietic control mechanisms. Injection of antitumor drugs enhanced responsiveness of the hematopoietic tissue in 1 month. In 6 months postinjection, the compensatory and adaptive reactions of the bone marrow to immobilization stress were little expressed or entirely absent. A single injection of cytostatic drugs in MTD depleted the pool of the committed progenitor cells at the late terms of this study. While in 1 month after injection of cytostatic drugs, the adequate reaction of the precursors to the additional hemopoiesis-perturbing influences was still observed, the reaction of the committed progenitors to immobilization stress was disturbed 5 months later. The long-term side effects of the damaging action of vinblastine and doxorubicin on hematopoiesis included diminished feeder activity of the adherent elements of the bone marrow. The characteristic abnormalities revealed in 1, 3, and 6 months after injection of vinblastine and doxorubicin were the changes in the content of medullar Thy-1,2$^+$-cells and disturbance of their migration into the bone marrow during additional hematopoiesis-perturbing influences.

The long-term side effects of toxic action of the antitumor drugs also include rearrangement of the short-range hemopoietic control mechanisms. In 1 month after injection of such drugs, an enhanced level of production of IL-3 activity is observed, while in 6 months, synthesis of IL-3 is pronouncedly down-regulated in contrast to up-regulated production of IL-1. The major reasons of hematopoietic disturbances observed at the later terms after injection of the antitumor preparations are the disturbances in functional activity of the stromal elements in the hemopoietic organs and consequential involvement of the interaction mechanisms between the hematopoietic cells and HIM cell elements into these pathologic changes.

The common unspecific features such as migration of T-lymphocytes into the bone marrow and activation of HIM and hemopoietic precursors which are

characteristic of the stress reaction and hemopoiesis-suppressing extreme influences [2, 40, 44, 78, 86, 90], raise a problem of existence of certain universal neuroendocrine mechanisms of physiological and reparative regeneration in the hematopoietic tissue.

Really, in addition to significant disorganization in the structure-functional integrity of the hematopoietic tissue, the disturbances in the hematopoietic control provoked by hemopoiesis-inhibitory influences (cytostatic drugs, ionizing radiation, *etc*.) include a pronounced neuroendocrine component related to activation of the stress-mediating systems of an organism [51, 53, 233]. However, in contrast to 'pure' stress reaction, these disturbances are characterized with elimination of cause-and-effect interrelations between the sympathoadrenal and blood systems. Moreover, the adrenergic transmitters aggravate uncoupling of the hematopoietic mechanisms, which is characteristic of the hematopoiesis-suppressive stimulants, because in contrast to stimulation of relatively resistant cells of the blood system, they inhibit reparation of the hemopoietic precursors and HIM elements damaged by the extreme influences. Finally, uncoupling of the hematopoietic mechanisms decreases the rate of regeneration of the hematopoietic tissue.

In particular, the catecholamines administered to the animals pre-treated with the high doses of 5-fluorouracil up-regulate proliferation and differentiation of CFU-E and CFU-GM (to a smaller degree) which have been suppressed by the cytostatic drug [53]. Moreover, while augmenting homing and functional activity of T-lymphocyte that are relatively resistant against the antimetabolite, they simultaneously inhibit recovery of the damaged cells in HIM (assessed by the content and secretory activity of the adherent cells and their ability to form HI based on cell-cell interactions) which disturbs cooperation between the elements of microenvironment in the control of proliferation and differentiation of the progenitor cells resulting in the long-term period of hematopoietic inhibition observed under the action of 5-fluorouracil [40, 53]. Similar uncoupling effect of catecholamines on hematopoiesis was observed under cytostatic treatment with the high doses of cyclophosphane. Such directivity of the influences on the hematopoietic processes was characteristic of glucocorticoids (other family of the stress-mediating hormones) in the animals treated with cytostatic drugs.

By way of conclusion, it should be noted that the changes in hematopoietic control under myelosuppressive influences can be both adaptive and damaging depending on their nature. The accumulated data on the damage exerted by the myeloinhibitory factors of diverse nature at various levels of the blood system and its control apparatus attest to possibility to correct the hematologic pathologies by different ways. The first avenue is based on direct stimulation of proliferation and differentiation of the hematopoietic cells, which is now successfully implemented with various preparations of the hemopoietic growth factors. The second way is to affect the central neuroendocrine hemopoietic control mechanisms with the substances possessing (among other features) the nootropic properties. The third method is to block the peripheral structures responsible for deregulation of the action of the stress-mediating mechanisms on hemopoiesis. Finally, it is visible to

modulate the functional activity and structural organization of the local control mechanisms encompassed by the concept of HIM.

Evidently, to implement the above ways to treat the hemopoietic depressions of diverse genesis, it is vitally important to develop the differentiated therapeutic methods.