Renewed granulocyte support practice and its alternatives

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Introduction

Granulocyte, i.e., polymorphonuclear (PMN) cell transfusions have been utilized in neutropenia with progressive infections or neutrophil dysfunction, with varying results and controversies concerning their therapeutic efficacy. By the use of antibiotics, recombinant hematopoietic growth factors, such as recombinant human granulocyte colony stimulating factor (rHuG–CSF), and immunoglobulins in neutropenic patients, the risk of life-threatening infections was reduced, but not completely eliminated. Despite the increasing use of platelet concentrates in the treatment of patients with bone marrow failure resulting in pancytopenia and/or quicker reconstitution of hematopoiesis promoted by rHuG–CSF, severe neutropenia might limit the intensity of myeloablative radio-chemotherapy (1–6). That was why intensified activities to provide methods of PMN collection for supportive therapy took place in the seventies of the 20th century. In the following two decades, PMN transfusions were rarely applied, primarily because of the difficulties related to the collection of the adequate number of viable cells. However, more recent advances in PMN cell transfusion, enabled by growth factors collection (e.g., rHuG–CSF) for cell mobilization (the enrichment of cell yield), produced renewed interest for this therapy – the so-called modern granulocyte transfusion therapy era (2–11). Therefore, PMN-collection is apheresis derived blood component, usually around 300 mL in volume, composed of a mixture of neutrophile PMN and other elements that are considered as contaminants, such as red blood cells, platelets, and citrated plasma. Both higher granulocyte count in donor’s circulation, and the processing of larger blood volume provide collection of markedly larger PMN doses (6–8×10^9/unit), compared with previous cell values (1–2×10^9/unit) (12–21).

PMNs have the most important role in the protection against bacterial or fungal invasion, and there is a direct association between their number in the circulation and the occurrence of infection and consequential systemic inflammation. Namely, the risk of infection is real when the PMN count is below 1×10^3/L (6), increasing significantly if PMN ≤ 0.5×10^3/L (22). Among the parameters important for the development of the infection, the kinetics of the event, as well as the degree and duration of neutropenia are worth mentioning. Febrile reaction, i.e., the increased temperature is often first and sometimes only indicator of the infection, which requires early recognition. Following the use of high-dose radio-chemotherapy, the infection could be confirmed in 60–90% of febrile patients with the decreased PMN count (5). Because of the simultaneous occurrence of neutrocytopenia and the effect of other interconnected factors (e.g., immunosuppression, long-term central vein catheter, etc.), affinity towards infections in patients with highly expressed neutropenia was even higher (2–5, 10–13).

It has already been mentioned that antibiotic therapy, combined when necessary with intravenous immunoglobulin preparations, represented the most effective therapy in

the treatment of neutropenia (acquired or idiopathic) patients and systemic inflammation. Positive therapeutic effect was achieved in around 80% of patients who were not considered as candidates for PMN support (5, 11). However, it was not always easy to predict which patient will react favorably to antibiotic therapy, or to evaluate the additional benefit gained by PMN transfusion. The application of rHuG-CSF and recombinant human granulocyte-macrophage or granulocyte-monocyte colony stimulating factor (rHuGM-CSF) could reduce the period of neutropenia, thus decreasing the infection risk (22–30). That is why the treatment of neutropenic patients included both PMN support and the stimulation of their production and activity by growth factors, i.e., corresponding cytokines (22–24, 31–34).

The aim of this paper was to present the mobilization protocols and apheresic collection (harvest) of the sufficient number of viable PMNs, as well as their therapeutic use in the treatment of neutropenic patients, with a short review of the adverse effects of PMN support. A brief description of cytokine stimulation of granulo-monocytopenesis, as a valuable alternative and/or the addition to PMN support, was also provided.

**PMN support – theory and practice**

During the sixties of the 20th century, it was demonstrated that $16.3 \times 10^6$ kg of PMNs was produced and released from bone marrow into the circulation daily, which amounted to around $110 \times 10^6$ cells/day for a person of average (70 kg) body mass (6, 22). Unlike other blood cells, PMNs remain shortly in the circulation after maturation (not longer than 6 hours), and create the so-called **circulating** and **marginal pools**. The number of PMN in the circulating pool is $31 \times 10^4$ kg bm, i.e., around $20 \times 10^8$ cells in a person of average body mass, that is around 20% of daily produced PMN quantity, which should be considered during their transfusion (5, 6). Finally, PMN count in the **marginal pool** was $39 \times 10^7$ kg (6). However, the antimicrobial effect of PMN is expressed just after the migration from the circulation to the tissue (Figure 1).

For supportive therapy, PMNs were initially collected from the patients with myeloid leukemia, and later on from healthy persons. By using an experimental model of sepsis, it was demonstrated that prevention of animal death or clinical improvement could be achieved by transfusion of $1.5–2 \times 10^9$ kg PMN (6). It corresponded to the PMN count of at least $1–1.4 \times 10^9$ for patients of the average body mass. It was also demonstrated that PMN yield could be achieved only by the collection of cells from the myeloid leukemia patients ($10–50 \times 10^6$ cells/unit), or persons primed by using mobilizing agents — so called rHuG-CSF mobilized donors (5, 10).

During the eighties of the last century, PMN transfusions were limited because of: a) unsatisfactory yield and viability of the collected PMNs and their short post-transfusion survival in the circulation; b) cell activation during collection, and c) complex procedures and costly preparation of the adequate PMN doses (24, 31–34).

So far, the PMN supportive protocols in the treatment of neutropenic patients have not been standardized. Yet, the majority of authors believe that PMN transfusions are justified in the cases of: a) sepsis caused primarily by gram-negative bacteria; b) resistance to the wide-spectrum antibiotics; c) diseases with favorable prognosis or with possibility of long-term remission, and d) decreased PMN count $\leq 0.5 \times 10^9$ /L of peripheral blood (Table 1) (10–13, 31–35).

As presented in Table 1, PMN transfusions had evident beneficial therapeutic effect in the following diseases and disorders: a) fungal or bacterial infections in immunosuppressed patients; b) sepsis in the newborns; c) severe pneumonia (possible benefit), and d) febrile conditions of unknown etiology, abscss, PMN dysfunction, AIDS (variable degree of beneficial effect) (10, 22–24).

PMN transfusions were most effective in life-threatening sepsis or fungal infection associated with neutropenia (PMN $\leq 0.5 \times 10^9$/L), when antibiotic therapy failed. Such conditions were most frequently found after myeloablative radio-chemotherapy (e.g., hematopoietic stem cell transplantation), and in the newborns. Patients suffering from the severe aplastic anemia and inherited relapsing neutropenia could also be successfully treated with PMN transfusions (10, 22–24, 36). Commonly, lethal outcome in around 7% of the patients suffering from acute leukemia was caused by bacterial infection following myeloablative chemotherapy aimed at achieving the first remission. The frequency of fungal infections in those patients was around 8%, with the lethality rate of 100% (2). Justification for PMN transfusions in patients with different genetic or immune disorders, benign hematological diseases, and some solid tumors, is disputable (5, 10–13).

Sufficient yield of PMN can be harvested from the circulation of an assigned apheresis donor, using blood cell separator (granulocytapheresis). During the apheresis, extracorporeal circulation is used, as well as additional or rouleaux forming agents as hydroxethyl starch (HES) and mobilizing agents (corticosteroids, rHuG-CSF). That is why PMN donors should be selected according to the specified clinical and laboratory investigations, rather than according to the criteria referring to blood donors. Repeated PMN collection from one donor is permitted after at least 48 hours interval, while the total volume of collected plasma must not exceed 1,000 mL weekly, i.e., 200 mL of red blood cells in the 8 weeks interval (2–5, 10, 24).

During one apheresis procedure, $1 \times 10^{10}$ or more PMNs should be collected (11–13, 35). In the USA, however, the smallest PMN count to be contained in one unit is not yet determined, since PMN concentrate is not registered by the Food and Drug Administration (FDA). On the other hand, according to the American Association of Blood Banks standards, PMN count should be $1 \times 10^{10}$ per unit following the processing of 10 L of blood in 75% of the prepa-
The use of different types of separators does not influence the PMN yield, although the use of rouleaux forming and/or mobilizing agents significantly alters its count. Namely, to obtain the adequate PMN yield, prior to the apheresis procedure the donors should be treated with corticosteroids and/or rHuG-CSF, and during the collection HES should be used (11, 24, 35). Since the PMN yield largely depends on their circulating count in the course of apheresis, as well as on the volume of the processed blood, it is clear that the increase of circulating PMNs count also increases their absolute number in the final blood product (10).

Corticosteroids as mobilizing agents act so as to mobilize PMN from bone marrow into peripheral blood, as well as from the marginal into the circulating pool (17–19, 22–24). The standard stimulation was achieved by dexamethasone (8–10 mg per rs at 12–15 hrs and 2–3 hrs before the apheresis). Such pretreatment of donors could double the circulating PMN count (24, 35), and resulted in 2-fold increase of PMN yield in the blood product (2–3×10^6 cells when approximately 10 L of blood was processed), which came to around 30% of the physiological daily production of PMN (6, 10, 17–19).

PMNs collected by apheresis procedure, stimulated by corticosteroids, are functionally well preserved. They express adequate in vitro bactericidal activity (unaltered phagocytosis and superoxide production) and have normal hemotaxis. Thus, the collected PMNs have a good in vivo recovery (i.e., the increase of the circulating PMN count after transfusion) and survival, with unchanged capacity of migration to the inflammation site (5, 17–19). Finally, it should be mentioned that systemic activity of corticosteroids in donors was very low, although there were data on the occurrence of cataract after the mobilization by dexamethasone (19).

More efficient separation of PMN from the red cell layer, obtained by HES (molecular mass of 450 kD) and caused by red cell rouleaux formation and sedimentation occurs during the apheresis procedure. That is important because the density of PMN and red blood cells is very similar. In addition, the PMN and red cell ratio in donor’s peripheral blood was around 1:1 000 (2–4, 24). The effect of the use of HES is not only the increased PMN yield, but also the reduced red cell content in the final product. The half-life of HES in the circulation is 24–29 hrs, thus if apheresis procedures are repeated in 48 hrs intervals, HES doses should be decreased progressively. Thus, the volume of 10% HES solution during the first apheresis should be around 500 mL, in the second one around 300 mL, and in the third apheresis around 200 mL (10, 24, 35, 36).

Until recently, PMNs have been prepared by the so-called adhesive filtration. PMNs were bound to plastic fiber filter (adhesion process), followed by the elution and centrifugation of cells. Although the cell yield was satisfactory, this method is no longer used because of frequent adverse effects in donors, due to complement system activation, leukostasis in pulmonary blood vessels, perineal pain in women, i.e., priapism in men (6, 11, 21, 22).

It has been mentioned that the number of reports on PMN mobilization by the use of rHuG-CSF and their apheresis collection is still increasing (7–9, 12, 14–18, 37). Initially, mobilization by rHuG-CSF (around 30 minutes after injection) caused a transient decrease of circulating PMN. That was most probably a consequence of their adhesion to endothelial cells, along with the expression and/or activation of the cell surface membrane molecules (5, 14–18). A significant increase of PMN count in blood occurs due to: a) release from the depot (around 12 hrs after the application of rHuG-CSF); b) shorter stay of PMN in the bone marrow, and c) the increase of their amplification department (around 24 hrs or more, following the injection of rHuG-CSF) (2–6, 23). Finally, a few days after the use of rHuG-CSF, the increase of PMN count was also noted in the extravascular space, i.e., in some tissues (22).

There are still relatively few data regarding the use of rHuG-CSF, so that its use in PMN apheresis cannot be considered as a routine procedure. Single subcutaneous application of 300 μg of rHuG-CSF (around 5 μg/kg) is efficient in achieving 5–6 fold increase of circulating PMN, without any adverse effects in donors. This kind of premedication enables the collection of around 2–6×10^10 PMN by processing 5–7 L of donor’s blood. Besides, in vivo recovery and functional capacity of PMN cells collected 12–16 hrs after rHuG-CSF injection were satisfactory (5–9, 22–24, 37).

Protocols of the simultaneous use of dexamethasone (8 mg 12 hrs prior to apheresis) and higher rHuG-CSF dosage (600 μg 8–12 hrs prior to apheresis) were published (17, 18, 37). After this kind of mobilization, an average 10-fold increase of granulocyte count was achieved in donors’ peripheral blood, as well as cell yield of around 8–10×10^10 per unit of PMN concentrate (12, 37).

The investigation demonstrated that phagocytosis capability, oxidation effect, bactericide potential, and the migration of PMN mobilized by rHuG–CSF were satisfactory (10–12). Besides, it was noted that PMN mobilized by corticosteroids and rHuG-CSF demonstrated a higher degree of adhesive molecules (e.g., CD11b, CD14, CD18) and Fc-receptor expression (e.g., CD32, CD64), with decreased expression of L-selectine. Half-life of mobilized PMN in circulation upon transfusion was longer, most probably as a consequence of the collection of higher percentage of younger cells (2). Regarding the adverse effects of HES, it was recently demonstrated that this agent could have the injurious effect on PMN function, i.e., it reduced hemotaxis and migration through endothelium into extravascular space (21). The important issue was whether the PMN was collected after a single mobilization or the multiple use of rHuG-CSF, when a higher percentage of the younger PMN was obtained (15, 16). The younger PMN exhibited more
intensive metabolic activity, stronger bactericide/fungicide
effect, expressing various surface molecules and showing
modified cell apheresic characteristics (altered density) (2,
15, 16).

Finally, there are reports on in vitro production of
PMN (similar to platelets and hematopoietic stem cells) by
ex vivo expansion method, although currently in experi-
mental conditions only (25). This procedure enabled 50-fold
increase of PMN count in the culture in relation to the num-
ber of cultivated progenitor cells (5, 25).

In practice, though, PMNs should be transfused to pa-
tients right after the preparation, since in in vitro conditions
they easily and quickly become susceptible to programmed
cell death (apoptosis) and/or cytolysis (necrosis), which
limits the duration of their therapeutic efficacy (4, 5, 24).

PMN storage in suspension diluted with autologous
plasma in 1:10 ratio did not demonstrate acceptable results
(14). Current investigations are directed towards the deter-
mination of different synthetic additive (preservative) solu-
tions efficiency, such as cell culturing media and espe-
cially enriched infusion solutions. So far, cell culturing
media have demonstrated the best additive solution charac-
teristics, although PMNs stored in them are not considered
for therapeutic use. Infusion solutions (e.g., Ringer lactate),
enriched with buffers and human serum albumin, seemed to
be convenient and efficient additive in the storage of PMNs
intended for clinical use (10, 14).

It has been demonstrated that PMNs stored at tem-
peratures of 4±2 °C maintain bactericide activity 1–3 days,
although their hemotaxis capacity is reduced for around
50% after only 24 hours. PMN storage at 20±2 °C for up to
8 hrs did not lead to in vivo reduction of recovery, survival,
and capacity of migration transfused PMN into the tissue
(22). However, the mentioned in vivo PMN functions were
significantly disturbed if they were stored for more than 8
hrs, independently of the storage temperature (24, 31–33).

Clinical effects of PMN transfusion are difficult to
characterize and confirm. Yet, some characteristic signs of
the efficacy of supportive therapy are well defined: a)
eliminination of the infection syndrome; b) conversion of
positive hemocultures to the negative ones; c) presence of
PMNs in exudates and orogranulocytes in the recipient's
oral cavity fluid; d) increase of the number of circulating
PMN one hour after transfusion, and e) reduction of the in-
creased body temperature and the improvement of patient's
general condition.

Cell recovery and survival after transfusion could be
investigated in vivo using PMNs labeled with isotopes (1,
33). However, in vivo recovery, i.e., the increase of PMN
count in patient's circulation after transfusion was difficult
to determine. Following transfusion of around 1–2×10^10
PMN, the expected count increment in the peripheral blood
of the patient weighing 70 kg was only 0.2–0.4×10^7/L (22,
24). It is complex to detect such minor changes of the cir-
culating PMN count because of the possibility of counting
errors, increased body temperature, sepsis, as well as dis-
seminated intravascular coagulation (DIC). General clinical
improvement should not be expected after transfusion of
one unit of PMN concentrate. On the other hand, there are
data confirming the survival of 100% of patients with sepsis
after transfusion of 4 units of PMN concentrate, while in the
control group the survival was only around 30% (2, 22, 23).

Finally, although successful in the majority of cases,
PMN transfusion, as well as any other transfusion, could be
associated with the occurrence of adverse effects (febrile
nonhemolytic transfusion reaction, transfusion related acute
lung injury, transfusion associated graft versus host disease,
hyper- or hypotensive crisis, virus transmission, etc.) (10,
38–41). However, these effects could be relatively success-
fully prevented and/or treated by adequate procedures and
with medications including: a) choosing the HLA-
compatible donor; b) selection of the most adequate process
of PMN preparation (apheresis); c) adequate transfusion
rate of PMN – less than 10*10^9 cells/hour, and d) antihis-
taminic, antipyretic, and corticosteroid premedication, if
necessary (11, 24, 33, 37).

Cytokines, stimulating factors of granulo-
monocytopenosis

Life-threatening severe neutropenia is a highly restric-
tive factor in myeloablative radio-chemotherapy in patients
with solid tumors, as well as a significant cause of morbidity
after hematopoietic stem cell transplantation (5). Therapeu-
ctic use of recombinant cytokines, i.e., hematopoietic
growth factors, significantly reduced duration and severity of
neutropenia by the stimulation of granulo-monocytopenosis.
These cytokines, termed also as colony-stimulating
factors (CSF) include previously mentioned G-CSF and
GM-CSF, as well as monocyte colony stimulating factor
(M-CSF) and interleukin–3 (IL-3 or multi-CSF) (4, 24–29).

CSFs are glycoproteins acting as regulators of prolif-
eration and differentiation of committed granulo-monocytopenotic progenitors. Likewise, they have an effect on
certain functions of mature granulocytes and monocytes.
With other hematopoietic cytokines, CSFs can have either
synergic or inhibitory relation/effect. Thus, IL-1 and IL-6
act as costimulating cytokines of certain CSFs, while in
certain functions they are inhibited by TNF-α, IFN-γ, and
TGF-β. Although CSFs are glycosylated, glycosylation is not
necessary for the expression of their biological activity in
vivo. After purification and cloning of certain CSFs, their
recombinant analogues (rHu-CSF) were first prepared and
then clinically evaluated in the eighties of the previous

Recombinant CSFs, primarily rHuG-CSF and recom-
binant human granulocyte macrophage-colony stimulating
factor (rHuGM-CSF), are used in the treatment of patients
with inherited or acquired leukocytopenias associated with
severe infections. The degree of cytopenia and PMN func-
tional, i.e., the capacity of adhesion to vascular endothe-
lum, migration through tissues, and bactericide effect, is
equally relevant. These cytokines enabled hematopoietic stem cell mobilization into peripheral blood (42–54). As already mentioned, there are reports on the use of rHuCSF in the apheresis of neutrophile PMN (12, 14–18).

**Granulocyte colony stimulating factor**

G-CSF is the essential regulator of granulocytopoiesis, whose synthesis is controlled by the gene localized at the long arm of chromosome 17 (q11–q12 regions). Human G-CSF is a glycoprotein of around 20 kD of molecular mass, produced by monocytes, fibroblasts, stromal bone marrow cells, endothelial and most probably epithelial cells in response to specific stimuli after the activation of IL-2 and TNF, i.e. IL-3 and TNF (25–29, 42).

Apart from its basic role in the regulation of the granulocytopoiesis, G-CSF acts as a stimulator of committed granulo-monocytopoietic progenitors, megakaryocytopoietic progenitors, and B lymphocytes. It has an effect on some functions of mature PMNs and macrophages, such as phagocytosis, synthesis, and the release of superoxide, antibody-mediated cytotoxicity, and cell migration. Namely, G-CSF also acts as endogenous mediator, synthesized during the inflammation process, stimulating PMN mobilization from bone marrow into the circulation (25–29, 42, 43).

Current recommendations for the use of G-CSF include its application in the prevention and/or treatment of neutropenia in patients after myelo suppressive or myelotoxic therapy. The purpose of the use of rHuG-CSF, as well as of rHuGM-CSF following hematopoietic stem cell transplantation was to accelerate the reconstitution of hematopoiesis, and, perhaps, increase the repopulating capacity of transplanted stem cells. Their use significantly reduced the period of neutropenia, thus reducing the frequency of infection, consumption of antibiotics, and duration of hospitalization (26–28, 42).

There are numerous protocols of therapeutic use of rHuCSF. Intravenous application (nowadays rarely performed) might be done by continuous or intermittent infusions of the preparation. The majority of recommendations included the application of rHuG-CSF in daily doses of 30–60 μg/kg, mostly as one-half hour infusions, with the application time limited to 28 days. Subcutaneous application implied the use of rHuG-CSF preparation in the dose of 5–20 μg/kg daily, with the gradual lowering of the dose when PMN values of 1–3 × 10⁹/L were reached (5, 42, 43).

The role of rHuG-CSF and rHuGM-CSF in combination with other hematopoietic cytokines and/or cytostatics if necessary, is essential in the processes of mobilization of hematopoietic stem and progenitor cells into peripheral blood (52–66). Most often administered rHuG-CSF doses were 5–10 μg/kg/day during 5 days (allogenic transplantation), or 10–16 μg/kg/day until the number of leukocytes reached 5–10 × 10⁹/L of peripheral blood (autologous transplantation) (5, 44–58).

Finally, there are data on the use of rHuG-CSF in order to obtain rapid PMN recovery in some other conditions or disorders, as well, such as congenital agranulocytosis, leukemias, myelodysplastic syndrome, AIDS-associated neutropenia, cyclic and idiopathic neutropenia (5, 43).

The use of rHuG-CSF is well-tolerated in most cases in patients/donors. Side effects may include bone pain, myalgia, diarrhea, and reactions at the injection site (5, 43, 54). The alterations of biochemical parameters (e.g. increased alkaline phosphatase, lactate dehydrogenase, alanine aminotransferase, and/or glutamyl transpeptidase) are possible. Transient granulocytopenia and thrombocytopenia, mobil liphorm skin eruption, splenomegaly, headache, influenza-like symptoms, insomnia, and extreme tiredness may also occur. There are no data about the delayed side effects of rHuG-CSF. It should be pointed out that the stimulation of granulocytopoiesis by rHuG-CSF does not cause deficiency of other hematopoietic lineages (5, 42).

**Granulomonocyte colony stimulating factor**

GM-CSF is a cytokine, which regulates proliferation and differentiation of PMN progenitors and macrophages. Its synthesis is under the control of a gene localized on chromosome 5 (region q23–q31) closely adjacent to the gene for M-CSF, IL-3, IL-4, IL-5, and IL-9. Molecular mass of biologically active GM-CSF is approximately 14–35 kD (depending on the degree of glycosylation) (25–29, 43, 67).

Cells that produce GM-CSF are lymphocytes T, monocytes, fibroblasts, endothelial cells, and stromal cells in bone marrow. The synthesis of this cytokine is initiated with some specific antigen and stimulated by IL-1 or IFN-γ. It was confirmed that the role of GM-CSF in the protection against different microorganisms (increased PMN and macrophage number) was very important. Besides pluripotent hematopoietic progenitors, colony-forming unit-granulocyte-erythroblast, megakaryocyte, and macrophage (CFU-GEMM), and committed progenitors of granulomonocytopoiesis, colony-forming unit granulocyte-macrophage (CFU-GM), GM-CSF had stimulating effect on mature PMN. It intensified phagocytosis and superoxides production in them after the exposure to chemotactic peptide (5, 25–29, 58).

Therapeutic indications for the use of HuGM-CSF are cytopenias primarily caused by intensive radiochemotherapy. Its use was also indicated after the transplantation of hematopoietic stem and progenitor cells (5, 63). The justification of the use of rHuGM-CSF in patients with myelodysplastic syndrome, AIDS, or congenital agranulocytosis is still object of investigations (5, 25–29, 42).

The most frequent side effects of rHuGM-CSF therapy were fever, myalgia, bone pain, anorexia, diarrhea, skin eruptions, headache, hypoxia, and hypotensive crisis. When higher rHuGM-CSF doses (15–20 mg/kg/day) were applied, fluid retention might occur together with edema and cardiopulmonary disorders, such as pericardial or pleural effusions, so-called capillary leak syndrome (5, 25–29).
Monocyte colony stimulating factor

M-CSF is the basic regulator of differentiation and proliferation of the committed monocyte progenitors. At the same time, these progenitors are capable to produce M-CSF by autocrine mechanism. In addition, M-CSF is produced by fibroblasts, endothelial cells, osteoblasts, keratinocytes, astrocytes, and epithelial cells of the uterus. Synthesis of M-CSF is under control of a gene localized on chromosome 5 (region q33). M-CSF is a glycoprotein whose molecules are present in the circulation as dimers with large molecular mass (47–90 kD) (25–29, 43).

There are limited data on the clinical use of rhuM-CSF preparation. Regarding that it is the potent stimulator of the production and the activity of mature monocytes, its therapeutic use would be justifiable in the treatment of patients with melanoma or fungal infections. It would also be of particular value in the treatment of patients who underwent myelosuppressive therapy. Namely, M-CSF increased monocyte production, i.e., the count of cells which produced other hematopoietic growth factors. There are no plentiful data on the side effects of rhuM-CSF, but among them bone pain and thrombocytopenia should be mentioned (5).

Interleukin 3

IL-3 is a multipoietin, which stimulates the differentiation and proliferation of committed progenitors of granulocyte lineage, as well as the progenitors of monocyte, megakaryocyte, mastocyte, and, in synergy with erythropoietin, partly erythocyte lineage. In addition, IL-3 regulates the proliferation and differentiation of pluripotent hematopoietic progenitors (CFU-GEMM). IL-3 stimulates the functions of different mature blood cells, as well as some immune effector functions. Regulatory gene for IL-3 is localized on chromosome 5 (region q23–31). It is also a glycoprotein with the molecular mass of 15–30 kD, depending of the degree of glycosylation. Most significant cells which produce IL-3 are the activated lymphocytes T (5, 25–29, 68).

Therapeutic use of rIL-3 (during the application of rhuG-CSF) can significantly accelerate the rate of hematologic reconstitution and clinical recovery of patients with bone marrow aplasia, providing more efficient mobilization of stem and progenitor cells into peripheral blood, as well. Clinical investigations of the role of IL-3 in patients with myelodysplasia and amegakaryocytic thrombocytopenia are still in progress (5, 68).

The best way of application and optimal dose of rIL-3 is not yet defined. However, most authors recommended subcutaneous application in the dose of 125–500 μg/m² of the patient’s body surface, daily. After the application of rIL-3, significant increase of neutrophilic PMN in the circulation was observed (130–700%). Finally, side effects of rIL-3 may occur, including fever, headache, congestion of conjunctiva and nasal epithelium, skin hyperemia, tiredness, and sometimes pleural effusion (5, 42, 43, 68).

PMN transfusion, particularly cytokine stimulation of granulo-monocytopenosis, represents relatively new research field, in which the number of the potential questions is larger than the number of definitive answers. Hence, the question should be raised if therapeutic use of recombinant growth factors could completely replace PMN transfusions in the treatment of patients with neutrocytopenia? Most probably not. It is more likely that further improvements of protocols of apheresic harvest of the sufficient number of viable PMNs shall be necessary. Moreover, further investigations of biology of committed progenitors and the effects of GM-CSF, as well as other hematopoietic growth factors shall also be required.

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