Translated from French to English - www.onlinedoctranslator.com

HEMATOPOIESIS AND REGULATION OF L'MYELOID HEMATOPOIESIS

Dr Nicolas Chapuis (Cochin) & Dr Ludovic Lhermitte (Necker)

Blood is a suspension of cells in a complex liquid (plasma) which belong to three categories:

- red blood cells (or erythrocytes, or red blood cells);
- white blood cells (or leukocytes) which are themselves composed of several distinct populations: lymphoids on the one hand (lymphocytes), myeloids (neutrophils, eosinophils, basophils and monocytes);
- platelets (or thrombocytes).

Although closely mixed in the blood, these cells have very different functions:

- red blood cells and platelets carry them in the blood, for the transport of gases, notably oxygen and hemostasis respectively;
- leukocytes of myeloid origin are only in transit in the blood. They pass into the tissues as needed to contribute to defense reactions;
- lymphocytes have a more complex circulation, passing through lymphoid organs and tissues, then recirculating. These are the supports of specific immunity.

All these cells originally come from the bone marrow, either directly (called myeloid cells) or indirectly (called lymphoid cells).**Hematopoiesis**is the process which allows the production of all blood cells from a contingent of**hematopoietic stem cells (HSC)**. This production is constant and finely regulated in order to be able to respond quickly and as efficiently as possible to the needs for blood cells. Thus, the bone marrow produces approximately 200 x 10^9 every dayred blood cells, 100 x 10^9pads, 50 x 10^9neutrophils.

1. THE HEMATOPOIETIC SYSTEM

To ensure the renewal of myeloid and lymphoid cells, there are so-called “stem” cells in the body. These stem cells are characterized by a double potential ensuring on the one hand **their own renewal**, on the other hand the**production of differentiated cells.**

1.1. Ontogenesis of the hematopoietic system

In humans, the first hematopoietic cells appear in the blood islands of the**yolk sac**. This early hematopoietic wave is ephemeral since it drops from the 5ththweek of gestation.Intra-embryonic and then fetal hematopoiesis is then established in the aortegonad-mesonephros region (**AGM**) or the hemogenic endothelial cells of the ventral endothelium of the aorta give rise to the HSCs of the AGM. These cells then successively colonize the hematopoietic organs (the liver, thymus, spleen and bone marrow) following a very precise sequence.

1.2. The hematopoietic niche

Hematopoietic stem cells are distinct from**mesenchymal stem cells**, also present in the bone marrow, and which are the origin of marrow stromal cells, osteoblasts, adipocytes and fibroblasts. Along with endothelial cells, mesenchymal stem cells constitute an anatomical and functional structure within the bone marrow in adults, ensuring the maintenance and regulation of stem cell functions:**the hematopoietic niche**. HSCs maintain a constant dialogue with their niche through direct contacts with different stromal cells, and diffusible and environmental factors. A certain number of ligand/receptor pairs and adhesion molecules are involved in the interactions between osteoblasts and HSCs and regulate the quiescence/self-renewal balance of the latter. Diffusible factors are represented by cytokines and chemokines.

1.3. The hematopoietic compartments

1.3.1. Hematopoietic stem cells

Primordial stem cells are**multipotent**. They are rarely in mitosis, which makes them relatively protected from certain attacks such as ionizing radiation and antimitotic chemotherapies. These undifferentiated, still multipotent cells will give rise to stem cells with more restricted potential, but still at the origin of several lineages and capable of self-renewal and differentiation. We thus identify a common myeloid stem cell, a common lymphoid stem cell. A phenotypic marker is found on HSCs, the molecule**CD34**, which is also expressed on more mature myeloid cells. The expression of the CD34 molecule is used in practice to estimate the number of HSCs and possibly to separate them from more mature myeloid and lymphoid cells. However, HSCs represent only a minimal fraction of CD34+ cells.

Thus, in the absence of specific morphological or immunological characteristics, HSCs can only be identified based on their functional characteristics, namely their ability to induce the reconstitution of a complete hematopoietic tissue after xenogeneic transplantations in immunodeficient mice (NOD-SCID for Non Obese Diabetic-Severe Combined Immunodeficiency). Experimental models have therefore been developed to enable the analysis of the reconstitution potential*in vivo*of human hematopoietic cells having stem cell characteristics. The cells are injected into mice and the analysis of their development in murine tissues several weeks after transplantation makes it possible to confirm the existence of HSCs (**or SRC for Scid Repopulating Cell**) within the pool of injected hematopoietic cells.

1.3.2. Hematopoietic progenitors

At the following stages of the hematopoiesis process, cells that are still incompletely differentiated will appear, but which are no longer capable of self-renewal: they are referred to as " **hematopoietic progenitors**".The process that takes place over several generations of cells ultimately results in progenitors differentiating toward a single lineage. Because of their ability to form these colonies*in vitro*, they are called CFU (Colony Forming Unit), CFU-E, CFU-G or CFU-GM, for example, for erythroid, granular or granulo-monocytic progenitors, respectively. Specialization only occurs gradually with progenitors which no longer give rise to all the myeloid lineages but only to some of them.

Too few in number, compared to the differentiated cells at the end of the lineages (the hematopoietic precursors), and without morphological characteristics allowing their formal identification, progenitors like HSCs are not recognized by examination of the myelogram.**The clonogenic test**is a test used*in vitro*to highlight the presence of mature hematopoietic progenitors within a given cell population. The different hematopoietic colonies observable*via*this test are characteristic for each of them of a given hematopoietic progenitor. This test consists of culturing the study population in a semi-solid methylcellulose medium supplemented with recombinant cytokines. After 7 to 14 days of culture, it is possible to visualize distinct hematopoietic colonies originating from myeloid clonogenic cells (or Colony Forming Cell CFC) restricted to a single hematopoietic lineage: BFU-E or Burst Forming Unit-Erythroid (more large in size and more immature than CFU-E, or Colony Forming Unit-Erythroid), CFU-Mk for Megakaryocyte, CFU-G for Granulocyte and CFU-M for Monocyte/Macrophage. Colonies arising from bipotent clonogenic myeloid cells, such as CFU-GM and BFU-E/Mk, can also be observed.

To highlight the presence of more immature hematopoietic progenitors, it is necessary to use experimental models using a stromal support, mimicking the natural microenvironment to allow immature hematopoietic cells to nest there. In order to simplify these co-cultures, stromal lines derived from adult mouse bone marrow were established. Thus, after 28 to 35 days of co-culture, the cells of this layer as well as those supernatant can be recovered and cultured in methylcellulose medium. If 7 to 14 days later, CFCs representative of all myeloid lineages are observable, it is possible to conclude retrospectively that the study population initially seeded contained immature hematopoietic progenitors also called**LTC-IC (Long Term Culture Initiating Cell).**

clonogenique LTC IFC

1.3.3. Hematopoietic precursors

Hematopoietic precursors, along with mature cells in the process of leaving the bone marrow for the bloodstream, constitute the vast majority of bone marrow cells (approximately 99%). All these cells are identifiable by their morphology and by the profile of the antigens expressed on their surface.

This pattern of hematopoiesis with the succession of HSCs, progenitors and hematopoietic precursors is certainly in reality much more complex. Indeed, recent studies have highlighted a new, more complex and evolving model of hematopoiesis during development from fetal life to adulthood with the presence of multipotent stem cells at the origin of unipotent progenitors.

1.4. Clinical applications: exploration of hematopoiesis and bone marrow transplantation.

The exploration of hematopoiesis is carried out firstly by carrying out a complete blood count and then possibly by studying the bone marrow via carrying out a myelogram or an osteomedullary biopsy (BOM).

1.4.1. Exploration of the figured elements of the blood by the hemogram

quantitative + morphology

The complete blood count is carried out by taking an anticoagulant blood sample, venous in adults or capillary in small children. It involves the quantitative analysis of red blood cells, leukocytes and platelets and the morphological examination of these cells. The quantitative part consists of measuring the absolute number of cells per unit volume of blood. The NFS machines measure red blood cells, leukocytes, platelets, but also the characteristics of red blood cells and the leukocyte formula. Microscopic examination of a colored blood smear is therefore not used systematically to produce the blood count but remains essential in various pathological situations to highlight morphological particularities that automatic machines cannot detect.

1.4.2. Exploration of the bone marrow by myelogram

anesthésie superficielle

In current practice, cytological examination of the bone marrow, or**myelogram,**provides information on the state of this hematopoietic organ. The examination is carried out by trocar puncture of a fertile epiphysis, generally the sternum at the level of the manubrium or a posterior iliac spine. A superficial anesthesia of the skin is performed. The myelogram is a quick and generally painless examination. The marrow is aspirated with a syringe, then a smear is prepared and colored as for blood. This examination does not give any absolute figures, but only percentages reflecting the relative proportions of the various normal marrow cells. It is very important to know whether the smear is more or less rich in cells, which gives a rough assessment of the cellular richness of the marrow, and thus makes it possible to better use the percentage data. Ex.: a high level of lymphocytes on a very poor smear suggests bone marrow aplasia and above all reflects the rarefaction of other cells. On the other hand, the same level on a rich smear suggests lymphocytic leukemia with invasion of the marrow by lymphocytes.

The cells of the marrow are grouped into “lineages” which are the set of precursors of a type of circulating cells. In practice, variations in the percentage of all cells in a lineage are important to appreciate, because they give indications of which ones are enriched or depleted. Within lineages, the more mature the cells, the more numerous they are. The disruption of this balance, with an excess of immature forms, suggests a maturation disorder. Qualitative abnormalities (cellular dysplasia) can also be observed.

1.4.3. Exploration of the bone marrow by osteomedullary biopsy (BOM)

Anesthésie locale

BOM is carried out under local anesthesia of the periosteum which must be very careful, more and more often assisted by the inhalation of a sedative gas (Kalinox), with a special trocar which allows a small bone fragment to be “cored” into the the posterosuperior iliac spine. It allows a histological study of the non-discerated marrow, as it is on the myelogram by aspiration and making the smear. It allows us to better appreciate the cellular richness of the marrow, but less well the cellular morphology. In practice, it is a specialized examination, complementary to the myelogram which is often carried out as a second intention. It also makes it possible, with a much greater probability than the myelogram, to detect

Hemogramme → Myelogramme → BOM

nodular marrow invasions, whether cancer metastases or lymphoma locations.

	Myélogramme (= cytologie)	Biopsie ostéo-médullaire (= histologie)
Prélèvement	Ponction médullaire et frottis	Carotte osseuse, coupes anatomo-pathologiques
Facilité	+++	+
Visualisation des cellules	+++	+
Délai de rendu	+++	+
Appréciation de la richesse	+	+++
Appréciation de la fibrose	-	+++
Architecture	-	+++

1.4.4. Hematopoietic stem cell transplant

The ability of HSCs to reconstitute the entire hematopoietic tissue is used in the context of HSC transplantation. Indeed, HSCs circulate from the marrow to the blood and vice versa, which explains why blood precursors can be used instead of the marrow to reconstitute the myeloid tissue in certain transplant indications to allow the cure of certain hematological diseases. malignancies (leukemia, lymphoma, myeloma) and other hematological disorders (primary immunodeficiency, bone marrow aplasia, myelodysplasia).

HSC transplantation can be autologous (using the patient's own cells) or allogeneic (using cells from a donor). Stem cells can be harvested from bone marrow, peripheral blood, or umbilical cord blood. Peripheral blood has largely replaced bone marrow as a source of stem cells. Umbilical cord HSC transplantation is essentially limited to children because the number of umbilical cord HSCs is too low for an adult. For peripheral blood collection, the donor is treated with recombinant growth factors to stimulate the proliferation and mobilization of stem cells; a standard apheresis is then performed 4 to 6 days later. The transplant (which is done by a simple transfusion) is preceded by treatment aimed at eradicating the disease and immunosuppression so that the graft can be accepted.

2. REGULATION OF MYELOPOIESIS

The progressive differentiation of hematopoietic cells and the preferential orientation towards this or that lineage, depending on needs, result from complex phenomena which are still incompletely elucidated. The regulation of hematopoietic cell differentiation occurs both through factors external to hematopoietic cells produced by the bone marrow microenvironment (growth factors, cytokines) but also through intrinsic factors specific to hematopoietic cells such as activation or repression of certain transcription factors, epigenetic modifications or metabolic adaptations.

2.1. Regulation by intrinsic factors

2.1.1. Transcription factors

THE ***transcription factors*** more or less specific to a stage and a type of differentiation, expressed in stem cells and progenitors, play a crucial role. Their role is highlighted by the inactivation of the corresponding gene in mice. Thus the inactivation of GATA-1 inhibits erythroid differentiation, that of PU-1 granulo-monocytic differentiation. More subtly, it is the interactions between different transcription factors which will allow the activation of the transcription of genes involved in differentiation towards a specific lineage and the concomitant repression of other specific transcription factor(s).) for differentiation towards one or more other lineages.

2.1.2. Epigenetic factors

GATA1 => ↘ erythroïde

PU1 => ↘ granulo monocytaire

Epigenetic modifications also influence hematopoietic differentiation. Indeed, the expression of a gene can be regulated by a modification of DNA methylation since low methylation most often results in high expression of the gene, while a high level of methylation inactivates the embarrassed.
Overall, the study of DNA methylation in murine hematopoietic cells shows an increased level of methylation in cells committed to the lymphoid lineage and decreased in cells committed to the myeloid lineage. Promoters of genes involved in maintaining the stem cell pool have also been shown to become methylated during differentiation. Finally, it was also observed in mice that a defect in the expression of DNMT1 (a DNA methyltransferase involved in DNA methylation) in hematopoietic cells led to a defect in lymphoid differentiation and an increase in myeloid hematopoiesis. with increased expression of target genes of the transcription factors GATA1 and CEBPα.
Thus, the **epigenetic factors** *via* the modification of the expression of genes necessary for the differentiation of hematopoietic cells play a key role in the regulation of hematopoiesis.

2.2. Regulation by extrinsic factors

Hematopoietic growth factors have been well known since their genes were cloned. We very schematically distinguish two categories:

- Terminal differentiation factors, some of which are specific to a lineage o
    - L'*erythropoietin (EPO)* for the erythroid lineage;
    - There *thrombopoietin (TPO)* for megakaryocytes and platelets;
    - THE *G-CSF* for the granular lineage; THE *M-CSF* for monocytes;
    - o
    - L'*IL5* (interleukin 5) for eosinophils;
    - THE *GM-CSF* which acts on granules and monocytes.
- Active factors upstream: o
    - THE *Stem Cell Factor* (SCF) (or Kit-ligand),
    - GM-CSF but also thrombopoietin are not only active at the terminal stages but also upstream.
    - Many interleukins (IL3, IL6), chemokines like SDF1 which attract cells to the marrow, also intervene

Hematopoietic progenitors express receptors for several growth factors. During differentiation, expression becomes restricted and finally limited to the receptor for a given factor which then becomes very abundant, which favors orientation towards the corresponding lineage. There are 2 main families of hematopoietic growth factor receptors

- **Receptors with intrinsic kinase activity**: c-Kit, M-CSFR, FLT3...
- **Cytokine receptors** coupled to a protein carrying Tyrosine kinase activity: EPO receptor, TPO receptor, interleukin receptors, etc.

Receptors with intrinsic tyrosine kinase activity are activated after ligand binding. Thanks to their intrinsic kinase activity, Tyrosine residues are phosphorylated in the intra-cytoplasmic part of the receptor, thus allowing the recruitment of a large number of proteins and the induction of intracellular signaling.
Cytokine receptors are sometimes homodimeric (EPO receptor, TPO receptor) or more often heterodimeric. They consist of an α subunit responsible for the interaction with the ligand and a transmembrane β subunit responsible for the transmission of cellular signaling after ligand binding. Being devoid of intrinsic tyrosine kinase activity, these receptors are associated in their intra-cytoplasmic part with tyrosine kinases of the JAK family which are activated after binding of the ligand. The β subunit is frequently shared by several cytokine receptors, which has led to their grouping into families (IL3, IL6 and IL2 receptor family).
Thus, among the receptors expressed by myeloid cells, the IL3, IL5 and GM-CSF receptors include an α chain specific for each of these cytokines and a common c chain. The α subunit binds the ligand even in the absence of the βc subunit, but the latter is necessary for signal transduction.

3. MEGAKARYOPOIESIS

Platelets arise from the fragmentation of the cytoplasm of megakaryocytes in the bone marrow. These themselves come from the differentiation of a stem cell, then from specific progenitors, according to a unique mode of division and maturation: endomitosis. The nucleus multiplies without the cell dividing: 2N being the number of chromosomes of the precursor cell, the megakaryocytes of successive generations will contain 4N, 8N, 16N, 32N, 64N. At the same time, the cytoplasm enlarges and platelets are formed. Megakaryocytes that release platelets are usually at the 32N stage, but 16N and 64N forms also release platelets.

Regulation of megakaryopoiesis:

Thrombopoiesis is regulated by **thrombopoietin (TPO).** TPO is produced mainly by the liver and kidney. It is passively regulated by the level of its receptor (Mpl), therefore by the megakaryocytoplatelet mass:

- if the circulating platelet mass is high (thrombocytosis), the majority of TPO binds to the Mpl receptor on platelets, is internalized and degraded, which reduces the quantity of free TPO to stimulate megakaryocytes;
- if there are few platelets (thrombocytopenia) little TPO binds to the platelets and a greater quantity is free to act on the megakaryocytes.

Other hematopoietic growth factors (IL3, IL6) also play a role, but more incidentally. The lifespan of platelets in humans is 7 to 10 days.

4. ERYTHROPOIESIS

It is the set of mechanisms that lead to the formation of red blood cells. It is a permanent and adaptive phenomenon which can, in case of increased need, be multiplied by 7 or 8. The erythroblastic lineage includes all the cells which differentiate by synthesizing hemoglobin resulting in red blood cells. We distinguish in order of maturity of growth: the proerythroblast, the basophilic erythroblast, the polychromatophilic erythroblast, the acidophilic erythroblast, the reticulocyte, the red blood cell or red blood cell. The different categories of erythroblasts are recognized based on the characteristics of the nucleus and the cytoplasm. The more the cells are advanced in the lineage, the more their size decreases, the more the initially basophilic and RNA-rich cytoplasm becomes acidophilic and rich in hemoglobin and the more the nucleus condenses until its expulsion which transforms the acidophilic erythroblast into a reticulocyte.

Between these multipotent cells and the erythroblasts are several generations of precursors. The last pre-erythroblastic cells, CFU-E, have only two possible futures:

- differentiate into a proerythroblast, under the effect of erythropoietin which triggers the terminal differentiation program, including the synthesis of hemoglobin
- or die by apoptosis, if no erythropoietin molecules reach them.

In the erythroblastic lineage there are four mitoses between the proerythroblast and the polychromatophilic erythroblast. The acidophilic erythroblast does not divide and its nucleus is eventually expelled, giving rise to anucleate reticulocytes. The expulsion of the nucleus has the effect of reducing the energy expenditure of the cell and increasing its plasticity. Protein synthesis in the cytoplasm is very specialized, with hemoglobin being by far the main protein synthesized. The increase in hemoglobin concentration in the cytoplasm explains the increasing acidophilia with maturation. Little by little, all the cytoplasmic organelles disappear and, at the reticulocyte stage, only vestiges remain. Everything has disappeared in the adult red blood cell which can no longer synthesize, but only conserve hemoglobin. DNA synthesis and cytoplasmic differentiation are synchronized, so that the same stage of nuclear evolution morphologically corresponds to the same stage of cytoplasmic differentiation. The breakdown of this synchronism is always pathological. The mechanism of mitosis-differentiation synchronization is poorly understood, but the cessation of DNA synthesis occurs when the hemoglobin concentration in the cytoplasm approaches normal.

4.1. Regulation of erythropoiesis:

The regulation of erythropoiesis is essentially achieved through **erythropoietin (EPO).** It is a glycoprotein whose gene was cloned in 1984. It is produced industrially by genetic engineering and used therapeutically since 1988. It is produced by peritubular renal endothelial cells and incidentally by liver cells. Tissue oxygenation regulates the synthesis of erythropoietin. This is stimulated by tissue hypoxia, and repressed by hyperoxygenation or the increase in circulating globular mass (for example, by transfusion). The essential role of EPO is to trigger the differentiation of stem cells into proerythroblasts, but it is also to increase the rate of hemoglobin synthesis in erythroblasts and to accelerate the exit of reticulocytes from the marrow.

4.2. Activation of signaling pathways downstream of the EPO receptor:

The EPO receptor (R-EPO) is a homodimeric receptor lacking intrinsic tyrosine kinase activity. Each of its 2 chains are therefore associated in their intra-cytoplasmic part with a protein tyrosine kinase JAK2.

JAK2 proteins have a kinase domain (JH1 domain) which is preceded by a JH2 domain which has inhibitory activity on the kinase domain. JAK family proteins (JAK1, JAK2, JAK3) are associated with a large number of cytokine receptors and allow signal transduction after ligand binding *via* the phosphorylation of Tyrosine residues at the intra-cytoplasmic part of these receptors. These phosphorylated Tyrosine residues allow the recruitment of a large number of intracellular signaling proteins which will promote cell proliferation and survival. These signaling pathways are common to a large number of receptors.

In the absence of its ligand, R-EPO is in a conformation in which the JAK2 tyrosine kinases are far apart and inactive. The binding of EPO to its receptor will lead to a conformational modification of the R-EPO and the bringing together of the JAK2 kinases which will then activate and phosphorylate the tyrosine residues present in the intra-cytoplasmic part of the R-EPO. The phosphorylation of Tyrosine residues allows the recruitment of signaling proteins with an SH2 domain (domain having a high affinity for phosphorylated Tyrosine residues) and the activation of signaling pathways including the JAK/STAT5 pathway, the ERK/MAPK pathway. or the PI3K/AKT pathway.

4.3. The JAK/STAT5 pathway

STAT proteins are transcription factors (TFs) whose structure includes, among others, an SH2 domain and a DNA binding domain. In the absence of EPO, STAT5 TFs are inactive and localized in the cytoplasm of erythroid cells. After binding of EPO to its receptor and phosphorylation of Tyrosine residues by JAK2 kinases, STAT5 TFs are recruited to the intra-cytoplasmic part of R-EPO by their SH2 domain. STAT5 is then phosphorylated by JAK2 kinases at a Tyrosine residue, leading to a modification of the SH2 domain of STAT5. The SH2 domain of STAT5 will then lose its affinity for the phosphorylated Tyrosine residues at the intra-cytoplasmic part of the R-EPO in favor of its own phosphorylated Tyrosine residue. STAT5 dimers form and translocate into the nucleus to attach to the promoter of their target genes including the gene encoding the antiapoptotic protein BCL-XL.

The induction of this signaling pathway after binding of EPO to its receptor is essential to allow the survival of erythroid cells during differentiation. Indeed, it has been shown in mice that inactivation of the STAT5 gene leads to a defect in BCL-XL production and apoptosis of erythroid cells. Likewise, inactivation of the BCL-XL gene is lethal in mice and leads to a sharp reduction in fetal erythropoiesis.

5. GRANULOPOIESIS

The polynuclear neutrophil (PN) is a nucleated cell “at the end of its life”. Its essential function is the phagocytosis of foreign bodies and especially bacteria which it is capable of destroying. The polynuclear therefore essentially has an antibacterial activity which it ensures thanks to a series of remarkable properties.

It is a very mobile cell which can thus infiltrate between the cells of the endothelium to pass from the vessels into the tissues (diapedesis). Certain substances attract neutrophils, notably bacterial products and certain serum constituents involved in antigen-antibody reactions. The activated complement fractions C3, C5 and, more incidentally, C6 and C7 activate enzymes present on the membrane, triggering movement. Neutrophils move from areas of low concentration to areas of high concentration of the activator. This is formed wherever an antigen-antibody reaction takes place and in foci of attrition. It diffuses from a distance and attracts polynuclear cells towards the focus. The degranulation of the PR then contributes to accentuate the phenomenon, certain products of the granules released into the environment themselves having a chemotactic power.

Having left the vessels, the polynuclear cell arrives in the tissues. It performs the function of phagocytosis, it ingests foreign bodies which it includes in intracytoplasmic vacuoles. Phagocytosis is most effective when particles are coated with antibodies or complement ("opsonized"). Included in the vacuoles, the bacteria are then destroyed during the next two stages. This is the first step in killing bacteria. It results from the accumulation in the phagocytosis vacuole of various substances produced by the PN and capable of lysing the bacterial membrane. These are oxygen peroxide (H2O2) (which is produced during phagocytosis by the activation of the pentose "shunt"), myeloperoxidase contained in the primary granulations, iodine, bromine or chlorine. The parallel release of part of these substances from the cell explains that the polynuclear plays an important role in inflammation, the environment was altered by these substances. Once the membrane of the bacteria is damaged, it can be attacked by hydrolases, contained in the lysosomes, which flow into the phagocytosis vacuole ("degranulation" of the PN). It is thus finally completely destroyed. The PNs emptied of their granulation by the destruction of the bacteria die, forming "pus".

Granulopoiesis constitutes the process allowing the production of PN. It successively includes:

- the hemoblast, a cell that is still poorly differentiated;
- the myeloblast which differs from the previous one by the formation of the first azurophilic grains
- the promyelocyte which is distinguished from the previous one by the significant accumulation of azurophilic grains actively synthesized by the Golgi apparatus;
- the myelocyte which is characterized by the appearance of the second type of granulations, called "neutrophils" while the azurophilic granules are no longer synthesized;
- the metamyelocyte, final stage of maturation of the previous one, incapable of mitosis. The nucleus begins its lobulation, favorable to cellular movements. It gradually evolves into polynuclear.

In a normal marrow, the elements are more numerous as they are more mature. In the normal state, all the elements of the lineage remain in the marrow except the PN which passes into the blood. The total duration of granulopoiesis is approximately 10 days. The cells of the lineage are capable of mitosis up to the myelocytes. The cells which no longer divide, that is to say the metamyelocytes and the polymorphonuclear cells, remain in the marrow for another 4 or 5 days in total to finish maturing there before passing into the blood. In the blood, not all PNs are in circulation; they are divided into two approximately equal sectors:

- the circulating sector; (the one who is evaluated by the blood count),
- the marginated sector which is made of PN stuck to the vascular walls. They immediately return to circulation or to the tissues as needed. Exercise and adrenaline also mobilize the PN of the marginal sector by sending them back to the circulating sector.

In the event of increased PN needs, the organization has two resources.

- It can immediately mobilize bone marrow reserves. The polymorphonuclear cells which have completed their maturation in the marrow, which can be recognized by a still poorly segmented nucleus, pass prematurely into the blood. In case of very high needs, during an infection or in the regeneration phase after profound neutropenia, metamyelocytes and even myelocytes can pass into the blood, this is myelemia.

- If the need is prolonged, production increases, which results from the increase in the number of progenitors that enter into neutrophil differentiation. The duration of differentiation can also be reduced to one week. The marrow then has an appearance of granular hyperplasia due to the increase in elements of the PN lineage. This regulation depends on the increased production of growth factors specific to this lineage. These are mainly GM-CSF and G-CSF. G-CSF is most specific to the neutrophil granule lineage.

The PN stays very little in the blood which is only a place of passage. In approximately 12 hours, 50% of the PN in the blood have already left it to pass into the tissues. From there, the PN never return to the blood: they carry out their phagocytosis functions and are destroyed by macrophage cells, on site or in the lymph nodes.

6. CLINICAL APPLICATIONS

6.1. Clinical use of hematopoietic growth factors

The hematopoietic growth factors seen previously are regularly used in clinical practice to stimulate the production of myeloid cells.

This is particularly the case for the collection of circulating HSCs with a view to hematopoietic cell transplantation. Patients will be treated with growth factors such as G-CSF in order to mobilize stem cells from the marrow before collecting CD34+ cells by leukopheresis. This treatment increases the number of CD34+ cells collected.

G-CSF is also used in the management of aplasia secondary to chemotherapy to reduce the duration of neutropenia and limit the risk of infection.

Finally, growth factors such as G-CSF or EPO are used for the treatment of primary bone marrow failure (G-CSF in severe congenital neutropenia, EPO in MDS with low serum EPO levels)

6.2. Dysregulation of myeloid hematopoiesis: example of polycythemia vera

In certain circumstances, a deregulation of the mechanisms normally controlling myeloid hematopoiesis is observed and leads to the development of hemopathy. This is for example the case in the **Vaquez polycythemia.**

Polycythemia is characterized by an increase in the total volume occupied by red blood cells, the most pathogenic consequence of which is an increase in blood viscosity and the risk of thrombosis.

Polycythemia may be secondary to hypoxia or non-physiological hyperproduction of EPO (secreting tumors of the kidney, hemangioblastoma of the cerebellum) or may be primary; This is a pathology called polycythemia Vaquez.

Polycythemia vera is a myeloproliferative syndrome, that is to say a clonal proliferation affecting a stem cell, therefore all myeloid lineages but predominating on erythropoietic precursors which are hypersensitive to several growth factors, but especially to EPO. This pathology is characterized in more than 95% of cases by the acquisition of a mutation in the gene coding for tyrosine kinase. **JAK2 (V617F mutation)**. This mutation affects the JH2 domain of the gene coding for JAK2 and induces the loss of the inhibitory effect of the JH2 domain with respect to the JH1 kinase domain. This results in a constitutive activation of signaling downstream of R-EPO even in the absence of EPO which stimulates proliferation and promotes the survival of erythroid cells during differentiation. The presence of EPO is no longer necessary to stimulate erythroblasts.

The risk of thrombosis requires, if the polycythemia is significant, treatment, possibly urgently through bloodletting. In certain cases (depending on age and/or the presence of thrombocytosis) myelosuppressive treatment is used (Interferon, Hydroxyurea). Given the presence of the activating mutation of JAK2, molecules targeting the tyrosine kinase activity of JAK2 have been developed (Ruxolitinib-Jakavi®) and are also used in the treatment of Vaquez's disease.