2 Structure and Physiology of the Granulopoietic System

The delineation of the structure of the granulopoietic system and the measurement of its normal kinetics have seen major progress in the past two decades. Until not long ago, our knowledge of the granulopoietic system was based almost exclusively on morphological observations: The composition and morphology of the bone marrow cells were studied under normal conditions and in various diseases. Careful studies performed under certain clinical conditions, such as the recovery phase of acute agranulocytosis, have yielded an astonishing amount of information. However, major progress in this field has come from the introduction of techniques using radioisotopes, such as $^3$H-thymidine or disopropyl fluorophosphate (DFP$^{32}$), and the development of stem cell assays. These studies offer detailed information on the structure and physiology of the granulopoietic system. Excellent reviews of this subject have appeared previously [103, 134, 197, 203, 337, 488, 562, 563, 586]. Here, an admittedly superficial outline of current concepts will be given, since an understanding of the structure and physiology of the granulopoietic system is essential for a detailed discussion of the effects of cytotoxic drugs upon this system.

Schematically, the granulopoietic system is composed of a sequential series of cellular compartments whose constituent cells differ from each other by morphological, functional, and/or operational criteria. Most of these compartments are located within the bone marrow (and, in rodents, in the spleen). However, the most mature cells circulate in the peripheral blood (and migrate into the tissues), and a small proportion of stem cells also circulate in the peripheral blood.

The most immature compartment is that of the pluripotent stem cells, which by definition are capable of both replication and differentiation into the granulopoietic, erythropoietic, and megakaryocytic cell lines. This means that, to maintain the pluripotent stem cell pool size, one stem cell must form for each stem cell differentiating into one of the subsequent compartments. Morphologically, these pluripotent stem cells most likely resemble small lymphocytes [59, 252, 644]. Experimentally, pluripotent stem cells are assayed by the spleen colony technique, which has been worked out in rodents (see page 9). Pluripotent stem cells are responsible for bone marrow repopulation after marrow-ablative treatment. Thus, the behavior of pluripotent stem cells is of utmost importance for the overall reaction of the granulopoietic system to cytotoxic drugs. In rodents, pluripotent stem cells are found in bone marrow and spleen, whereas they are restricted to the marrow in higher mammals. In addition, there is evidence that pluripotent stem cells circulate in the peripheral blood in all mammalian species.

Pluripotent stem cells, as defined by the spleen colony assay, do not form a homogenous population. Rather, the pluripotent stem cell pool is composed of a heterogenous group of cells all fulfilling the definition of a pluripotent stem cell, but nevertheless differing in important aspects. Thus, recent work [56, 67, 281] indicates that pluripotent stem cells possess different degrees of “stemness”, i.e., they differ with respect to their replicative potential and proliferative activity (see also page 178). Overall, the pluripotent stem cells have a low proliferative activity under steady-state conditions.
conditions: The proportion of pluripotent stem cells in S-phase of the cell cycle (measured by the $^3$H-thymidine suicide technique or in vivo by hydroxyurea treatment) has been reported to be less than 20% [23, 44, 620]. However, radiation experiments or transplantation procedures have clearly documented that pluripotent stem cells can rapidly increase their proliferative activity, in response to a pool depletion, so as to normalize their compartment size [133, 155, 227, 420, 421, 466, 628].

It has been suggested that the bone marrow cell production is geared to the cellularity of discrete marrow areas [452]. Later, the same group of workers [366] has postulated that the mechanism for maintenance of a normal pluripotent stem cell compartment size in the bone marrow is mere population size control, where the density (i.e., the mean distance) of stem cells determines stem cell proliferation. Others [420] have postulated that the number of pluripotent stem cells in S-phase of the cell cycle controls the entry of $G_0$ stem cells into the active cycle. Experimental work has suggested the existence of short-range humoral factors involved in stem cell proliferation control [359, 360].

A minimum size of the pluripotent stem cell compartment appears to be required for differentiation of these cells into the subsequent cellular compartments. If the size of the pluripotent stem cell pool falls below a certain critical value (in mice, values around 10% have been determined experimentally [109]), then self-replication, without simultaneous differentiation of pluripotent stem cells, appears to occur until the threshold compartment size is reestablished. This shift from differentiation to self-replication may be considered a protective mechanism that prevents “burning-out” of the vital pluripotent stem cell pool.

“Committed” stem cells are considered to be the immediate progeny of pluripotent stem cells [379]. By present definition, committed stem cells have only a small capacity for self-replication. The major difference from the pluripotent stem cells is that commitment to one of the hematopoietic cell lines has taken place by mechanisms that are still poorly understood. Following commitment, a committed stem cell can give rise to cells of only one specific pathway of hematopoietic differentiation. For example, a stem cell committed to granulopoiesis can only produce granulocytic cells, but not cells of the erythropoietic or megakaryocytic series. Committed stem cells are assayed by in vitro culture methods: Stem cells committed to granulopoiesis have been defined operationally to be those cells giving rise to granulocytic/macrophage colonies in vitro (see page 10).

Committed stem cells are again located in the bone marrow and spleen of rodents, but are restricted to marrow in higher mammals. Furthermore, committed hematopoietic stem cells are found in the peripheral blood. Committed granulopoietic stem cells still resemble small lymphocytes morphologically, although they can be separated physically from pluripotent stem cells [266, 641]. Committed granulopoietic stem cells proliferate actively. Even under steady-state conditions, the majority of the committed granulocytic cells appear to be in an active cell cycle [298, 378]. Thus, values of 50% for the S-phase fraction of colony-forming units (culture) (CFU-C) have been reported for mice, while in humans, this value has been determined to be around 45% [348]. The difference in proliferative activity between CFU-spleen (CFU-S) and CFU-C is a very important point when cells are exposed to cytotoxic drugs in vivo, since the cytocidal action of these agents is often critically dependent upon the proliferative state of the cells upon which they act (see page 158).