IV-5 Blood Cells

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The origin and the control mechanism of differentiation and proliferation of hemopoietic cells have been studied with the development of new experimental methods on the clonal growth of their precursor cells these past fifteen years. Among the many contributions by Japanese investigators, the following have been selected for review because of their originality.

Colony forming cells in vitro and its applications

Hara and Ogawa (1978) established a method of forming of colonies consisting of erythroblasts, granulocytes, macrophages and megakaryocytes from murine hemopoietic tissues and, later, from human bone marrow and peripheral blood. The clonal nature of these colonies was confirmed by evidence that an isolated single cell could form a mixed colony in culture medium (Hara and Noguchi, 1981). Characteristics of the precursors were similar to murine spleen colony forming units (CFU-S), a class of multipotent hemopoietic stem cells, in cell size, proliferative behavior and kinetics in response to anemia and transfused polycythemia (Hara, 1980; Hara and Ogawa, 1978; Hara and Noguchi, 1981). In spite of the absence of CFU-S in W-anemic mice, the precursors, the CFU-mix, were detected in hemopoietic tissues from W-anemic mice, suggesting that the CFU-S and the CFU-mix may include populations that differ (Hara et al., 1982). They also showed that the CFU-mix differentiates into not only three homopoietic cell lineages but the B-cell lineage as well (Hara, 1983).

Humoral factors that stimulate hemopoiesis

High grade leukocytosis is observed in some patients who bear various kinds of tumors without definite sign of infection. These tumors are supposed to produce the colony stimulating factor (CSF) which stimulates granulocyte-macrophage colony formation in semisolid culture media. Asano et al. (1978) first succeeded in transplanting pieces of metastatic lung cancer tumor into nude mice. Extreme granulocytosis developed in tumor-bearing mice, and disappeared after tumor removal (Asano et al., 1978; Suda et al., 1980). More than twenty human tumors have been serially transplanted into nude mice by Japanese investigators (Asano, 1980). Changes in hemopoietic stem cells in the tumor-bearing mice have been investigated from various aspects. Both granulocytes and cells from other cell lineages were significantly increased (Miura et al., 1981; Mizoguchi et al., 1982). CSF as well as other hemopoietic factors may be secreted from the tumor. Several cell lines were established from CSF-producing tumors (Okabe et al., 1978). They came from the original tumor cells or from tumors transplanted into nude mice. The latter had been treated with antimouse spleen antibody to eliminate mouse cells. Large scale culture of CSF-producing cells has been established, which will be expected for...
the further analysis of the nature of CSF (Okabe et al., 1982).

CSF from collected human urine materials has been purified by Motoyoshi et al. (1978). The purified material, shown as a single band by polyacrylamide gel electrophoresis, acts as a stimulator of human macrophages which produce real CSF active on human granulopoiesis. This idea was first described by Furusawa et al. (1978). Phase I and II studies of this CSF are being performed for the purpose of treatment of leukopenic patients (Motoyoshi, Kusumoto and Miura, 1982). CSF also was obtained and purified from various sources of murine and human cells. Fine purification studies have been published by Shikita et al. (1981) and his colleagues (Tsuneoka and Shikita, 1980, 1981; Tsuneoka et al., 1981). Mori and his co-workers (Tsurusawa et al., 1983; Izumi et al., 1983) showed the existence of a factor, with colony promoting activity, which may act on the early granulocyte/macrophage precursors.

**Differentiation of tissue mast cells**

Kitamura et al. (Kitamura et al., 1979; Kitamura, 1983) investigated the origin and the mode of differentiation of mast cells using his intriguing and unique mutant mouse system. Giant granules of bgj/bgj mice were used as the marker for the origin of the cells. W/Wr and Sl/Slr mice were used because they lack tissue mast cells. Injection of bone marrow cells from bgj/bgj mice showed the depletion of mast cells in W/Wr mice to be due to a defect in precursor cells and that in Sl/Slr mice to a defect in microenvironment (Kitamura and Go, 1979; Kitamura et al., 1979). Using these mice, he demonstrated that the mast cell is a progeny of the multipotent hematopoietic stem cell. Most blood cells such as erythrocytes, granulocytes and platelets leave hematopoietic tissue after final differentiation. In contrast, he clearly showed that the committed precursors of mast cells leave hematopoietic tissues, migrate in the blood stream, and finally differentiate in connective tissues such as the dermis and the peritoneal cavity (Kitamura and Go, 1979; Kitamura et al., 1979). The number of committed precursors of mast cells can be estimated by limiting dilution analysis. Some descendants of the migrating precursors retain the ability to proliferate and differentiate into mast cells even after lodging in the skin or peritoneal cavity. Differentiation of tissue mast cells provides a model for the differentiation of tissue cells that originate from hematopoietic tissues.

**Hemopoietic microenvironment**

The existence of a microenvironment that maintains hemopoiesis has long been believed. Seki (1973) reported an experimental method to form hemopoietic colonies on the surface of pieces of cellulose acetate membrane inserted in the peritoneal cavities of irradiated mice. The surface of the membrane was covered with macrophages and fibroblasts and provided a favorable microenvironment for the growth of hematopoietic colonies from multipotent stem cells and for leukemic cells. The previously treated membrane also could be cultured in vitro (Yoshida, 1979). The progenitor of the colonies formed on the membrane (CFU-ML) and the multipotent stem cell (CFU-S) do not have identical populations. This method has been widely used by investigators throughout the world and has contributed much to the analysis of microenvironments.

**Parabiotic chimeras**

Seno (1979) developed an experimental system for the aortic parabiosis of two rats.
These chimera animals provide an interesting tool with which to investigate the origin of hemopoietic cells by changing the hematological status in each partner animal. They reported that thenormal erythroid and myeloid as well as lymphoid stem cells migrated from peripheral blood. Further applications of this system should yield useful findings.

Radiation-induced myeloid leukemogenesis

Hirashima and his co-workers (Bessho and Hirashima, 1982) showed that after a single whole-body X-irradiation of 3 Gy to male REM/MsNrs strain mice, myeloid leukemia occurred from 4 to 11 months in about 1/4 of the mice. Of particular note is that deletion of the 2 chromosome was detected in more than 90% of the myeloid leukemic mice (Hayata et al., 1979).

Potentially leukemic cells (PLC) were analyzed by transplantation of spleen cells from irradiated male animals to female animals. The peak of PLC was apparently higher and three months earlier than that of overt leukemia. This result suggests that a fraction of the mice with PLC developed overt leukemia later. During the pre-leukemic period, the immediate decrease in CFU-S in the femur returned to the pre-irradiation value within one month, whereas the recovery of CFU-C required 205 days. This prolonged depression of the CFU-C compartment was clearly related to the presence of PLC. The transformation was strongly associated with the proliferative state of the CFU-C compartment induced by radiation.

Differentiation of leukemic cells

Differentiation of leukemic cells has been one of the central topics among the cancer investigators in relation to its therapeutic application. The cell line M1 first was established by Ichikawa (1969). He first detected that M1 cells differentiated into mature granulocytes or macrophages after treatment with the D factor, obtained from media conditioned by embryonic tissues and, later, with various kinds of other inducers. The most important phenomenon was that the “differentiated” leukemic cells lost their leukemogenicity when injected into the host SL mice.

The D factor was characterized as a glycoprotein with a molecular weight of 40,000 to 50,000 (Ichikawa, 1969), and it was also isolated from media conditioned by various kinds of cell line cells, or from body fluids. Much evidence has been shown that the D factor is distinct from the colony stimulating factor (CSF). Various known chemical substances also have been shown to be inducers of M1 cells. They are arginase, histones, lipids, glucocorticoid hormones, prostaglandins, cyclic AMP, vitamins and many other substances including Friend leukemia inducers (Hozumi, 1983).

Similar studies on cell differentiation have been extended to the human leukemic cell lines HL60, K562 and others (Tomida, Yamamoto and Hozumi, 1982; Honma et al., 1980a, 1982b; Hozumi, 1983).

Biochemical phenotypic changes associated with myeloid leukemia cell differentiation have been intensively studied. They include changes in membrane compounds, enzymes, and so on (Hozumi, 1983).

Ichikawa and his colleagues described in detail the changes in the cytoskelton and the development of actomyosin system during M1 cell differentiation (Nagata and Ichikawa, 1979; Nagata, Sagara and Ichikawa, 1980).

These investigations recently have developed into the studies on the effects of tumor promotors and on natural occurring vitamins that are known as biological
response modifiers. They have become widely known as candidate substances to "cure" leukemias through cell differentiation. Trials of sensitization of myeloid leukemia cells resistant to inducers of cell differentiation also has been an important topic (Hozumi, 1983; Honma et al., 1980b, 1982a; Tomida Yamamoto and Hozumi, 1982; Miyaura et al., 1981).

The differentiation of Friend leukemia cell line cells were first described by Friend and Scher (Ann. N.Y. Acad. 243, 155, 1975), and Ikawa et al. (1974). Since then many valuable papers have been published from Japan. Due to the limitation of space they will be referred to elsewhere.

**Hemopoiesis in early embryos**

Hemopoiesis in early embryos was investigated by Miura et al. (Miura, 1980; Miura et al., 1979; Miura, Terasawa and Sawatani, 1976), using organ culture explants consisting of dispersed- and reaggregated-avian yolk sac cells. Blood islands developed in these "artificial" explants, and the heme synthesis in each of them was measured. Addition of various kinds of Friend leukemia inducers, including DMSO and dimethylacetamide, stimulated heme synthesis in the explants (Miura et al., 1976, 1979). The maximum heme synthesis rate was positively correlated with that observed in Friend leukemia cell line cells. Stimulation seemed to occur in the early period of culture. As a comparison to the embryonic system, murine teratocarcinoma cells (PCCs/A1) were studied (Kajigaya and Miura, 1982). This cell line has the tendency to produce erythrocytes in organ cultures. The addition of DMSO or erythropoietin stimulated the incidence of blood island formation. There was an additional stimulatory effect by DMSO and erythropoietin. In all cases, denucleated erythrocytes were observed in the explants. Erythropoiesis in early embryos seems to be influenced more intensively by cellular microenvironments than by the humoral regulators active in adults.

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