Physiopathology of Human and Virus-induced Murine Leukemias

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Summary

The authors describe a coherent model for differentiated leukemias derived from physiopathological studies on Friend leukemia.

In Friend leukemia, Friend virus induces permanent differentiation of erythropoietin-responsive cells. This erythropoietic proliferation and maturation is accompanied by a marked cell loss and provokes enlargement of the stem cell compartment. The so-called leukemic cells have a limited proliferation capacity and may not be truly malignant as opposed to blasts in acute leukemias.

Clinical, hematological, and physiopathological data that are presently available in chronic granulocytic leukemia, polycythemia vera, and the erythroleukemic component of erythroleukemia are compatible with the Friend physiopathological model. It is suggested that these differentiated leukemias initiate from an uncontrolled differentiation of a committed cell compartment, which stimulates proliferation of the stem cell compartment. The disease would be due to a proliferation and accumulation of "subnormal" cells characterized by a shorter mean life-span than the normal differentiated cell population.

Although limited, the data available suggest that the physiopathology of acute leukemias is clearly distinguishable from that of differentiated leukemias; several immunological and therapeutic applications of this model are outlined.

Introduction

Many models for experimental leukemias are available: transplantable leukemias, leukemias induced by RNA and DNA viruses, radiation, and chemically induced leukemias.

Transplantable L1210 leukemia has been used in many laboratories to screen drugs for chemotherapeutic effects (11, 29) and more recently to screen adjuvants (15). L1210 leukemia is used extensively because of its sensitivity to most of the antitumor drugs that have been found to be active in human leukemias and cancers. As established by Skipper et al. (30), L1210 cells can be quantified in vivo, and the effects of drugs or immunotherapy can easily be detected and measured. Experimental conditions are highly reproducible; i.e., the LD<sub>50</sub> is usually stable for a given histocompatible strain of mice, and the proliferation kinetics of L1210 cells has been adequately studied. However, L1210 leukemia and other transplanted tumors do not constitute good models for acute human leukemias because of differences such as: leukemic cells are inoculated into healthy mice; all tumor cells are in the division cycle and proliferate during the exponential growth phase of the disease, and no cell loss is observed during this phase; L1210 leukemia represents an "in vivo" model of tumors but does not mimic true leukemia. These considerations have led some investigators to use virus-induced leukemias, particularly murine, such as Gross, Moloney, Friend, and Rauscher leukemias.

Some of the advantages viral leukemias offer over other models, include: (a) the possibility of predicting and modulating the incidence and latency of leukemias; (b) a physiopathology that may be comparable to certain human leukemias as shown by Furth et al. (10); and (c) a good model for elaborating a strategy of treatment combining chemotherapy, immunotherapy, and antiviral drugs. Many results concerning the prophylaxis and therapy of virus-induced leukemias have been published and others will be reported during this Symposium. Here, we shall discuss the question of which murine leukemias should be compared to human leukemias on a physiopathological basis.

Physiopathology of Experimental Model Systems

Is Friend Leukemia a Model for Differentiated Leukemia? Friend leukemia is characterized by erythropoietic proliferation that begins in the spleen and invades the liver and blood at a later stage. During the terminal phase of the disease, even nonhematopoietic organs like kidneys and lungs may be infiltrated by hematopoietic cells. The duration of the latency period and the mean survival time are proportional to the amount of virus inoculated. With high doses of virus, the disease kills susceptible mice within 3 weeks. The physiopathology of Friend leukemia has been extensively studied. The main features may be summarized as follows. The erythropoietic proliferation in the spleen begins very rapidly, within 72 hr after virus inoculation, as shown by Tambourin and Wendling (33). The target cell of the virus is very probably the ERC<sup>2</sup> or a closely related erythroid precursor (8, 32). The virus has the capacity to induce ERC differentiation but not to restore this compartment when depleted by polycythemia in myleran-treated mice (8). The constant flux from ERC to the more differentiated erythroid cells provokes an increase of stem cells

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2 The abbreviations used are: ERC, erythropoietin-responsive cell; FV, Friend virus; CFU-s, splenic colony-forming unit(s) (pluripotent hematopoietic stem cells); CFC, colony-forming cell; AML, acute myeloid leukemia; CGL, chronic granulocytic leukemia; CFU-c, granulopoietic committed stem cells.
in the spleen and blood but not in the bone marrow (5, 38). There are no changes in the bone marrow with the polycythemia-inducing strain of FV, whereas a 50% reduction of bone marrow CFU-s is found with the anemia-inducing strain (37) of FV. These stem cells have a limited proliferation capacity as shown by repeated transplantations in irradiated hosts (36). Autoradiographic studies of proliferation kinetics in the spleen and liver of leukemic mice inoculated with the polycythemia-inducing strain of FV have demonstrated that erythroblastic and "Friend" cells multiply rapidly, even at the terminal stage of the disease (31). One of the most striking conclusions of this study was the demonstration of a high cell loss (over 90%) even during the exponential growth phase of the disease. Several authors have also clearly demonstrated that "leukemic" cells are capable of differentiating into reticulocytes and erythrocytes. Friend leukemic spleen cells are not usually transplantable even when large inocula and several routes of inoculation are used (18). In rare instances, Friend tumors have been obtained by transplanting leukemic spleen fragments taken from animals during the terminal phase of the disease (9). The tumor cells have chromosomal markers (22) and are probably monoclonal. However, they are not representative of cells usually found in Friend leukemic spleens. To account for the above data, we propose the following model (Chart 1). Friend leukemia is a disease that begins by permanent FV-induced differentiation of the ERC compartment. This erythropoiesis is characterized by the marked cell loss that occurs in the proerythroblastic and erythroblastic compartments. This permanent cell loss provokes a compensatory increase of CFU-s in the blood and spleen. The accumulation of cells in the spleen, liver, and blood is due to the positive balance between a marked hyperproduction and a surprisingly high rate of cell death.

According to this model, there are no truly malignant cells in Friend leukemia. The disease is due to a proliferation and accumulation of "subnormal" cells characterized by a shorter mean life-span than the normal erythroid cell population. This model does not agree with the data of Thomson and Axelrad (34) and Rossi et al. (25), who isolated tumor cells in Friend leukemia; but it corresponds well with data obtained by Wendling et al. (36). A mathematical version of this model is presently being worked out in our laboratories.

Is AKR "Leukemia" a Model for Thymic Lymphosarcoma? AKR leukemia is closer to thymic lymphosarcoma than to acute lymphoid leukemia. The disease begins in the thymus and involves lymph nodes, spleen, and liver. Bone marrow and blood are usually invaded at later stages. The latent period of spontaneous leukemia is long and the earliest changes are seen in the thymus after 50 to 60 days. In other virus-induced lymphoid leukemias in the mouse, the latent period is also long, varying from 8 to more than 20 weeks, even with high doses of virus. This latency contrasts with the immediate proliferation observed in Friend and Rauscher leukemia after virus inoculation. An autoradiographic study of AKR leukemia by Metcalf and Wiadrowski (19) showed that labeling indices vary with the size of lymphoblasts and that higher indices are observed in large lymphoblasts as in human acute lymphoid leukemia (16). In murine lymphoid leukemia, cells are easily transplantable (28) and behave like malignant cells. CFC’s in agar have recently been studied (6), and it has been shown that in AKR mice the number of bone marrow CFC’s always decreases, whereas the number of splenic CFC’s is normal or increases. Such an increase does not compensate for bone marrow depletion. The long latency, transplantability of tumor cells, and decrease of normal CFC’s and probably also of hematopoietic stem cells make this model completely different from the previous one.

Murine AML’s. Few data concerning the physiopathology of murine AML’s are available. Serial transplantation of leukemic cells has been reported but the minimal tumor-inducing dose has not been calculated.

Graffi leukemia used to be a model for murine myeloid leukemia. It has not been extensively studied, perhaps be-

Chart 1. Hypothetical model of Friend leukemia. Top, early action of the virus. The FV acts mainly on the "2nd step" differentiation (Arrow 2). It provokes a pathological differentiation of ERC’s into proerythroblastic (PE) cells. This differentiation escapes from the normal controls: feedback mechanisms and erythropoietin action. Feedback mechanisms continue to control the "1st step" differentiation (Arrow 1). Bottom, pathological process of the disease (exponential growth phase). Friend disease is characterized by (a) an overproduction of proerythroblastic cells or hyperbasophilic cells (HB) which is mostly inefficient, since at least two-thirds of the proerythroblastic cells die rapidly and since RBC are short-lived. This overproduction results in (b) a continuous depletion of the ERC compartment and therefore the production of precursor cells, CFU-s, ECP, ERC, is stimulated, apparently, by feedback mechanisms of control. The balance between the production of proerythroblastic cells and their death (with or without maturation) is positive. CFU-s, pluripotent stem cells in G0, noncyling G0, state, and in G1-S-G2, cell cycle. They are probably regulated by their own proliferation control, but their rate of turnover seems to be influenced by feedback mechanisms. ECP, erythroid committed population. This population has a large amplification capacity and an age structure. ECP cells endow a maturation process, the last stage of which is the ERC stage, EB, basophilic erythroblasts (EB, early, EB, late); EP, polychromatophilic erythroblasts; HB, hyperbasophilic cells, which are pathological proerythroblasts or closely related precursors; Rtc, reticulocytes; ↓↓, "1st step" differentiation of pluripotent stem cells into erythroid committed cells. The factors controlling this step are unknown; feedback mechanisms and microenvironmental factors may influence it; ↓↓, "2nd step" differentiation. ERCS are differentiated into proerythroblasts. This step is induced by erythropoietin, which probably stimulates ECP proliferation also, and has a promoting effect on proerythroblastic maturation: ↓↓, stages probably under the control of feedback mechanisms.
cause of the difficulty of inducing myeloid leukemia in many strains. Recently, a stable myelogenous leukemia virus was isolated and purified from a stock of FV (17). This new virus isolate may provide a good model for AML.

**Comparison of Murine and Human Leukemias**

**Chronic Leukemias.** Most of the experiments performed on mice are not feasible in humans, for whom only limited information is available. Table 1 summarizes the known physiopathological features of human leukemias. Although many parameters have not been studied, CGL, polycythemia vera, and erythroleukemia (Di Guglielmo), seem to have many features that correspond to the Friend leukemia model; for instance, all stages of pathological cell line differentiation are found in the bone marrow, spleen, and blood of leukemic patients. Certain anomalies of the maturation process such as asynchrony of maturation of the nucleus and cytoplasm (1, 35) and the reduced level of leukocyte alkaline phosphatase in CGL (39) have been described, but there is no evidence of true malignant cells, characterized by an unlimited capacity for multiplication. In 95% of CGL a specific chromosomal marker was found in myelocytic, erythrocytic, and megakaryocytic cells. This marker does not exist in lymphoid and nonhematopoietic cells and is believed to be due to a somatic mutation affecting myeloid stem cells only. The presence of this chromosomal marker in megakaryoblastic and erythroblastic cells that do not have abnormal proliferation patterns raises the question of whether this feature signifies a neoplastic disease as is the case for aneuploidy. The absence of CGL induction after transfusion into leukopenic patients of large numbers of leukemic cells also gives evidence against the malignancy of these granulocytic cells. Only transient grafts have been observed under such circumstances (21).

In erythroleukemias, recent cytogenetic studies (13) have shown that, during the erythroid phase, aneuploidy was minimal or absent. In addition, many reports indicate that the proliferation of marrow erythroid cells is under physiological control (24, 27). Data concerning chronic lymphoid leukemias are not available but Salmon and Seligmann (26) recently suggested that, in most chronic lymphoid leukemias, the proliferating B-cells are arrested somewhere along the normal pathway of lymphoid differentiation.

Several other features also demonstrate the similarities between the Friend leukemia model and human differentiated leukemias. Autoradiographic studies after in vivo injection of \(^{3}H\)thymidine have shown a high labeling index in the spleen, which correlated well with the stage of the disease (3, 4). In CGL and polycythemia vera, hematopoietic stem cells differentiate normally in vitro and their total number, calculated by the agar colony technique (CFU-c) increases in the spleen and peripheral blood (12, 20). All these data are therefore compatible with the Friend leukemia model, where the main defect causing the disease consists of the permanent differentiation of committed precursor cells. Recently, Pedersen (23) also proposed a model for CGL considered as a perturbation of the inflow of pluripotent stem cells into the committed stem cell compartment.

**Acute Leukemias.** In contrast to chronic leukemias, it appears that experimental studies of the physiopathology of acute leukemia models are lacking. AKR leukemia resembles the thymic form of some of the lymphoid leukemias observed in young children. There is a need for a model for AML. Data on human acute leukemias thus far reported indicate major differences from chronic leukemias: i.e., defective differentiation in the pathological cell line; aneuploidy in AML (7); a normal or reduced number of CFU-c, a defective in vitro proliferation and maturation capacity despite continuing CFU-c dependence on the colony-stimulat-

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**Table 1**

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Labeling indices</th>
<th>CFU-c&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Proliferation</th>
<th>Maturation</th>
</tr>
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<tbody>
<tr>
<td>Chronic leukemia</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Granulocytic</td>
<td>Diploid: Philadelphia chromosome</td>
<td>Increased in the bone marrow and peripheral blood</td>
<td>CSA&lt;sup&gt;a&lt;/sup&gt; dependent</td>
<td>CSA dependent</td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>Diploid</td>
<td>ND</td>
<td>Normal or decreased; very increased in peripheral blood</td>
<td>CSA dependent</td>
</tr>
<tr>
<td>Monocytic</td>
<td>Usually diploid</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Erythroleukemia&lt;sup&gt;x&lt;/sup&gt;</td>
<td>Diploid for the erythrocytic line</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Acute leukemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloblastic</td>
<td>Aneuploid</td>
<td>Decreased</td>
<td>Abortive CSA dependent</td>
<td>Abortive</td>
</tr>
<tr>
<td>Myelomonoblastic</td>
<td>Usually diploid</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Diploid or aneuploid</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
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<sup>a</sup> In man, only CFU-c can be quantified by in vitro techniques.
<sup>x</sup> CSA: cell surface antigen; ND: not determined.
<sup>x</sup> The erythrocytic proliferation can often be controlled by blood transfusions.
ing factor (20), low labeling indices, and a prolonged cell cycle time in abnormal cells (14). The control of the disease requires aggressive chemotherapy as it is true for most generalized cancers.

Extensive physiopathological studies on murine models for acute leukemias will conceivably permit better understanding of the events leading to the malignant perturbation of normal differentiation in hematopoietic stem cells. The acute transformation of Rauscher leukemia studied by Bohrorn et al. (2) may represent a good experimental model.

Immunological and Therapeutic Applications

The viral origin of human leukemias has not yet been established. Nevertheless, the knowledge of the physiopathology of virus-induced murine leukemias already has many potential applications. According to the model presented here, interpretations of the immunological responses in Friend leukemia should be reconsidered. The presence of “tumor antigens” and antitumor antibodies is questionable.

If there are no malignant cells, if a continuous replication of Friend leukemia virus is necessary to maintain permanent ERC differentiation, neutralization of FV or efficient antiviral chemotherapy should suffice to block the development of the disease and the hyperproduction of erythroid cells, which have only a limited proliferation capacity. In human differentiated leukemias, after reduction of the increased pool of hematopoietic committed precursors by chemotherapeutic agents like hydroxyurea in CGL, partial control of pathological proliferation may be possible by physiological means, in the form of differentiated cell transfection. It is also possible to cryopreserve the large number of stem cells present in the peripheral blood of a patient taken during an early proliferative phase of the chronic stage of the disease and to use them for restorative purposes after the intensive myelosuppressive therapy necessary to obtain and maintain hematological remission during the blastic crisis of CGL. These few examples underline the importance of physiopathological studies of experimental and human leukemias.

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