Cytology and Pathogenesis of Rauscher Virus Disease in Splenectomized Mice

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Summary

The modifying effect of splenectomy and/or thymectomy in mice susceptible to Rauscher virus was studied to elucidate the cytoto- and pathogenesis of the disease. Early mortality due to splenic rupture was completely eliminated by splenectomy prior to virus infection but not by thymectomy. However, the development of the disease as shown by the leukemic blood picture and hepatomegaly could not be prevented by either procedure, indicating the presence of susceptible cells other than in the spleen. Histologic observations suggested that the bone marrow is the most likely site for such cells. Thymectomy did not produce any noticeable change on the course of the disease. Microspectrophotometric analysis of proliferating leukemia cells in the liver showed that most of these cells contain variable amounts of hemoglobin in their cytoplasm. As judged from these analyses, a high degree of “maturation” was attained by many cells of undoubted leukemic origin.

Introduction

Since the original work of Gross (9) implicating viruses in the etiology of murine leukemia, several other agents capable of inducing neoplasms of the hematopoietic tissue of the mouse have been isolated from different sources (2, 3, 7, 8, 11, 12, 14, 20—22, 24). Most murine leukemias induced by virus or irradiation are lymphocytic, originating in the thymus. Thymectomy prevents the development of the disease or modifies the host response, eventually leading to the development of other types of leukemia (10, 15, 18). On the other hand, for Rauscher virus infections, which resemble Friend virus infections in many respects (6, 16), it has been claimed that there are dual phases of host response and that the target of viral action, at least during the early phase, is limited to the spleen (5, 19, 24, 25). The origin and nature of the affected cells following infection is not well understood. Siegler and Rich (25) suggested from their histologic observations that the proliferating cells in the splenic red pulp were reticulum cells, many of which differentiated to form erythroblasts. It has been demonstrated by microspectrophotometry that both human and fowl erythroleukemic cells contain hemoglobin in their cytoplasm (4), and the type of hemoglobin in fowl erythroleukemia has also been determined by electrophoretic analysis (1).

Materials and Methods

MICE. Approximately 150 female mice of NMRI strain, purchased from Anticimex Farm, Evelund, Sweden, were employed throughout the present experiments. NMRI mice are originally derived from an inbred strain maintained at Naval Medical Research Institute, Bethesda, Maryland, and are being randomly bred under specific pathogen-free conditions. In a preliminary experiment, it was found that young adult mice of this strain are highly susceptible to Rauscher virus. The age of mice at virus inoculation was standardized at 3–4 weeks. Mice were kept in plastic cages, 5–7 in each, and maintained on commercial pellets and water ad libitum.

RAUSCHER VIRUS. Deep-frozen virus preparation (P-940) was obtained through the courtesy of F. J. Rauscher, National Cancer Institute, Bethesda, Maryland. In our laboratory, the virus was maintained through serial passages in female NMRI mice. Standard virus material was prepared from pooled spleen of infected mice. A 15% spleen homogenate in normal saline was made in a chilled mortar. The homogenate was centrifuged at 2000 rpm and the resulting supernatant was recentrifuged at 3000 rpm, both for 15 min at 0°C in a MSE refrigerated centrifuge. The resulting supernatant was then spun down at 10,000 rpm (approximately 7000 × g) for 5 min at 0°C in an L model Spinco. The final supernatant in sealed ampules was kept at −70°C in a Revco deep freezer until used as standard virus material. Each mouse was inoculated i.p. with 0.2 ml of this material.

OPERATIONS. Splenectomy and thymectomy were performed by standard surgical procedures under i.p. Nembutal anesthesia. The virus was inoculated 2–3 days following operation.

HISTOLOGY. Mouse tissues for microscopic examination were fixed in 10% neutral formalin, and paraffin sections were stained with hematoxylin and eosin.
MICROSPECTROPHOTOMETRY. Continuous absorption spectra in the wavelength range 245–450 μm were taken of cytoplasmic areas, 1.5 μm in diameter, from neoplastic cells imprinted on quartz slides, fixed in methanol, and subsequently immersed in glycerol. The microspectrophotometer was essentially of the same construction as described by Cowles et al. (4), but equipped with Zeiss Ultrafluor objective numerical aperture 1.25 and condenser numerical aperture 0.8. For cytologic identification, the cells were stained with May-Grünwald Giemsa after the spectral absorption analyses.

Results

MORTALITY IN VARIOUSLY CONDITIONED MICE. The mortality curves following Rauscher virus infection in the various experimental groups are shown in Chart 1. It is clear that splenectomy abolished the early mortality almost completely. More than 60% of intact and thymectomized mice died from the splenic rupture by the 40th day following virus infection at which time no mortality was observed in splenectomized or splenectothymectomized infected mice. Thymectomy on the intact or splenectomized mice, on the other hand, was without effect in altering the mortality. After the 40th day, however, hepatosplenomegaly in the remaining intact or thymectomized mice and hepatomegaly in the splenectomized or splenectothymectomized mice became apparent, accompanied by progressive anemia and all affected mice died by the 115th day following infection.

HEMATOLOGIC FINDINGS. Hematologic changes were studied only in the intact and splenectomized mice at various intervals following virus infection, since it was noted during the mortality study that thymectomy did not produce any further blood change in infected mice. Charts 2–5 illustrate the findings. Blood samples were obtained from at least 6 mice randomly chosen in each group at every interval up to 50 days following infection and from 2-3 mice afterwards.

In general the hematologic findings were essentially similar in intact and splenectomized mice, indicating the failure of presplenectomy in abolishing the susceptibility of the host to Rauscher virus infection. As shown in Chart 2, a steady increase in nucleated blood cells was noted in both groups of mice. Before the 20th day of infections these cells were mostly leukocytes but after this time a progressive appearance of erythroblasts and numerous smudged cells made it difficult to perform accurate differential blood counts. Later on, nucleated cell counts of peripheral blood varied considerably and in some cases exceeded 500,000 in the terminal stage.

There was a progressive anemia which started at 15 days following infection in both groups (Chart 3). The degree of the anemia in splenectomized mice tended to be slightly more severe than in intact mice. In the terminal stage, peripheral red cell counts dropped to less than ½ the normal value, and the hematocrit to less than 20% in most cases (Chart 4). Abnormalities of red blood cells such as polychromatophilia, diffuse basophilia, anisocytosis, and poikilocytosis were common in advanced cases (Fig. 1). Development of a marked erythroblastosis was one of the characteristic features of the disease (Chart 5). A few mature erythroblasts were already observed at 7 days after infection in both groups which was followed by a sharp rise after 20 days. Although the erythroblastosis was accompanied by a progressive anemia, the association was not close and the former was usually much in excess of the latter.

In advanced stages, atypical erythroid cells of various degrees of maturity could be seen in peripheral blood. Most primitive cells varied in size from approximately 20 to 25 μm in diameter, possessing a rather intensely basophilic cytoplasm and a large nucleus in which the chromatin was finely dispersed and uniformly distributed. One or a few large nucleoli were present. There was a perinuclear clear zone in most of these primitive cells. These features indicate that they are most likely erythrogene elements and morphologically analogous to megaloblasts in man. Therefore, we arbitrarily designated them “megaloblastoid cells.” Occasional mitosis in megaloblastoid cells (Fig. 1), and denucleating processes in more mature forms were observed.

Although no accurate differential counts of peripheral leukocytes of individual cases were carried out, levels of lymphocytes and granulocytes were well maintained throughout the observa-
CHART 2. Nucleated cell counts in the peripheral blood. Each value represents the mean from 6 animals, randomly chosen from the different groups at the day of sampling. In the later stages of the experiment the counts were performed on all of the surviving animals. There are no significant differences between the splenectomized and the intact animals after inoculation with the virus.

CHART 3. Red blood cell counts in the peripheral blood. Sampling as in Chart 2.

Pathohistologic findings in splenectomized mice. The development of the disease in the intact hosts was quite similar to that described previously by others (5, 24, 25) with some exceptions which will be mentioned later.

In splenectomized mice, the 1st definite histologic evidence of the disease was seen in the bone marrow (femur and sternum) at the 10th day of infection, particularly along the sinus walls. Active, proliferating foci of megaloblastoid cells were intermingled with many erythrogenic cells of varying maturity (Fig. 2). These cell foci had increased in number and size by the 17th day. The livers of these mice had no such foci but exhibited active extramedullary hematopoiesis in the portal area (Fig. 3). This probably was due either to a compensatory response for splenectomy or merely a host reaction to a virus infection or both. On the 22nd day, however, a coexistence of the preexisting extramedullary hematopoiesis along portal veins and an accumulation of megaloblastoid and maturing erythrogenic cells in sinusoidal spaces was seen (Fig. 4). No histologic evidence of the disease was found in any other tissues up to this period after infection.

As the disease progressed, an enormous hepatomegaly de-
developed, attaining more than 10 gm in weight and occupying almost the entire abdominal cavity (Fig. 5). The surface of the liver was finely granular, lustrous, and dark red in color. The organ was diffusely infiltrated with megaloblastoid and erythrogenic cells of varying maturity. In advanced cases, tumor-like grayish nodules, 2–3 mm in diameter, were commonly observed at autopsy, and were composed of megaloblastoid cells with numerous mitoses. Most mice dying with hepatomegaly were slightly icteric.

The proliferation of megaloblastoid cells in the bone marrow was consistent but marrow was never completely replaced by them and the normal architecture was relatively well preserved even in the terminal stage.

In the kidney, interstitial accumulation of megaloblastoid cells was not uncommon among the advanced cases but never was prominent. In the lung, alveolar walls were somewhat thickened by the deposition of megaloblastoid cells. The 1st evidence of megaloblastoid cell infiltration in the lymph node was found at 25 days in 1 intact and at 36 days in 1 splenectomized mouse. The foci were located in the subcapsular region or in the medullary cord, but were not diffuse. Lymphopoiesis was well maintained throughout the course of the disease.

In the thymus, no evidence of the disease was noted in any case and, as a rule, the gland was involuted in advanced cases. The foregoing hematologic and pathohistologic descriptions of the splenectomized mice apply to the intact infected mice as well. Thus, the most likely explanation for the sequence of the disease in splenectomized mice is that leukemic cells develop in bone marrow as the initial response of the host to the virus infection, and migrate to the liver where an active proliferation and dif-

**Chart 4.** HCT values showing a similar progression of anemia in the splenectomized and intact animals after virus inoculation.

**Chart 5.** Nucleated erythroid or megaloblastoid cell counts in the peripheral blood. Sampling as in Chart 2. (See text.)
hemoglobin in their cytoplasm to practically fully hemoglobinized erythrocyte precursors. The latter were relatively scarce, significant shift of the ultraviolet band towards longer wavelengths of absorption spectra from cytoplasmic areas of imprinted cell preparations from the enlarged liver. Some of the analyzed cells are represented in Fig. 6. With very few exceptions, a Soret band around 410 mμ indicated the presence of hemoglobin derivatives (4). In the ultraviolet range there was an absorption band at 260 mμ, corresponding to a high content of ribose nucleic acids. The more hemoglobinized cytoplasms displayed a slight but significant shift of the ultraviolet band towards longer wavelengths (Chart 6).

Spectral absorption analyses of a fairly large number of cells from the discrete cell groups in the enlarged liver indicated a continuity from leukemia cells with very small amounts of hemoglobin in their cytoplasm to practically fully hemoglobinized erythrocyte precursors. The latter were relatively scarce, however.

Discussion

The disease elicited by Rauscher virus in susceptible mice is characterized by an abrupt stimulation of hemopoiesis with massive proliferation of primitive cells in the splenic red pulp and concomitant hemorrhage which often brings about rupture of the spleen and death of the host during the early phase of the disease (24, 25). Early death could be completely blocked and the life-span of the host prolonged by splenectomy prior to infection. Nevertheless, splenectomy did not prevent the disease. The neoplastic manifestations, such as high nucleated cell counts in peripheral blood and infiltration of leukemia cells in various tissues in splenectomized mice were essentially similar to those in intact infected mice, although the manifestations were slightly delayed in the former. This indicates that susceptible cell populations are present in the spleen and other tissues as well, and that the role of splenectomy in Rauscher virus infection is different from the effect of thymectomy on lymphoma induction by other leukemia-inducing viruses where the development of lymphoma is largely prevented (13, 17).

The fact that proliferating foci of atypical cells developed 1st in bone marrow strongly indicates that the marrow is the site of initial response of the splenectomized host to virus infection. The cell infiltrations observed in other tissues such as liver, kidney, lung, and lymph node most probably represent secondary foci developed through metastasis from the bone marrow.

According to other authors (5, 24, 25), the development of lymphoid leukemia originating in the thymus is common among survivors following the splenic phase of the disease. In the present studies, however, no lymphoid leukemia was observed in either intact or splenectomized infected mice throughout the experimental period. Various factors such as infecting dose of the virus, strain, and age of the recipients seem to be at play in modifying the host response with regard to the lymphoid tissue (24).

So far, no satisfactory evidence has identified the cell line from which the proliferating cells in Rauscher virus infection originate, although certain suggestions have been made (24, 25). The easily identifiable erythroblasts in the peripheral blood might represent either leukemia cells or else a release of normal erythrocyte precursors from the spleen after destruction of the sinusoids by the virus-induced malignant cell proliferation. The present microspectrophotometric analysis, however, demonstrates that the majority of atypical cells in the liver, the most active site of cell proliferation, contain cytoplasmic hemoglobin. This indicates that these cells are erythrogenic and that the disease might be comparable to erythroleukemia in man and fowl in which hemoglobin synthesis in the leukemia cells has been demonstrated (4).

In contrast with other murine leukemias, the minor involvement of the lymphoid tissues tends to support our interpretation of the results of the microoptical analysis. It is worth noting that the proliferation of erythrogenic cells in the livers of these infected adult mice is reminiscent of embryonic life during a period of which the liver appears to be the almost exclusive site for erythropoiesis (23).

The spectral analyses also suggested a sequence from leukemia cells with very small or no detectable amounts of hemoglobin in their cytoplasm to practically fully hemoglobinized erythrocyte precursors, eventually leading to the abnormal erythrocytes in the peripheral blood at advanced stages of the disease. Several possibilities can account for this phenomenon. The initial attack by the virus on the primitive erythrogenic stem cells might be followed by 2 different modes of development: a “horizontal” proliferation at the level of the immature “megablastoid” cells and a “vertical” differentiation of some of the cell population leading to the appearance of anucleated but defective erythrocytes (Fig. 1). The quantitative dominance of the immature,
atypical cells (Fig. 6) regularly seen in the liver foci suggests, however, that this “vertical” differentiation is limited to a relatively small number of leukemia cells or is a slow process. It might also be that in some cells the otherwise dominating influence of the leukemia virus genome is lost, for instance by elimination from a daughter cell by the preceding cell division.

References

11. ———. Attempt at Classification of Mouse Leukemia Virus.


Fig. 1. Peripheral blood picture of a splenectomized mouse at 74 days after virus infection. RBC, 396 × 10⁴; HCT, 21%; total nucleated cell count 110,800. Note the prominent poikilocytosis. One of 3 erythrogenic cells in the picture is in mitosis. X 1500.

Fig. 2. Sternal bone marrow of a splenectomized mouse at 10 days after virus infection. Active proliferation of erythrogenic cells with numerous mitoses. X 320.

Fig. 3. Liver of a splenectomized mouse at 17 days after virus infection. Active extramedullary hematopoiesis in the portal area. Two megakaryocytes are seen. X 128.

Fig. 4. Liver of a splenectomized mouse at 22 days after virus infection. Coexistence of extramedullary hematopoiesis in the portal area (lower) and proliferation of erythrogenic cells in the sinusoidal spaces (upper). X 128.
FIG. 5. A splenectomized mouse sacrificed at 60 days after virus infection. Development of enormous hepatomegaly. Note the minor involvement of lymph nodes and the involution of the thymus.

FIG. 6. Imprint smear from the enlarged liver in a splenectomized mouse, 76 days after inoculation of the virus, showing large, atypical leukemia cells, 2 of which are in mitotic division. There is also a maturation into normoblast-like red cell precursors with pyknotic nuclei, one of which has almost completed a cell division. X 1500.
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