Since Gross's original demonstration that a viral agent could induce lymphoma in mice (4), several new viral agents have been isolated which also induce murine leukemia with great regularity. The relation of these agents to each other is at present not clear. Each, however, induces a leukemia in suitable mice (11).

With the exception of the myeloid leukemias, two groups of murine viral leukemias may be recognized: those which begin abruptly (within days) after virus inoculation, and those which appear only after a prolonged latent period of weeks or months. Those leukemias which begin within a few days after virus inoculation are splenic in origin, and are represented by the Friend and Rauscher diseases (3, 12). Those which begin after a prolonged latency are thymic in origin and may be represented by the virus-associated AKR lymphoma and the lymphoma induced by the viruses described by Gross (5), Dunn (2), and Rich (13, 14).

In preliminary pathogenesis studies of the Rauscher disease, Rauscher noted that a high proportion of animals with splenic erythroblastosis developed a superimposed lymphatic leukemia (12). He emphasized the dual nature of the host response to the virus. The origin of the late-occurring lymphatic leukemia in the Rauscher disease was recently studied by Dunn (1), who concluded that, although the early erythroblastosis began in the spleen, the later-developing lymphatic leukemia began in the thymuses. The close similarity of the splenic erythroblastosis in the Friend and Rauscher diseases has been noted by Rauscher. Friend virus is known to induce a high incidence of thymic lymphoma in rats (9), but it has not as yet been reported to do so in mice. In this study we will document the fact that thymic lymphoma may occur late in the course of the Friend disease, in a manner analogous to that of the Rauscher thymic lymphoma.

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In previous studies of thymic lymphoma we noted that the tumor evolved unilaterally, in only one of the two thymuses (15, 16). We therefore examined those specimens of thymic lymphoma occurring in the course of the Friend and Rauscher diseases and compared the pathogenesis of these thymic lymphomas with those of the AKR and Rich lymphomas.

MATERIALS AND METHODS

Friend virus—Fifty-four female 5-week-old Swiss ICR mice, obtained from the colony maintained in our laboratory, were given inoculations intraperitoneally of 0.25 ml. of a cell-free supernate of a 20 per cent homogenate of spleens from mice infected with Friend virus. The viral stock was originally obtained from Dr. C. Friend and has been passaged in Swiss mice in our laboratory for 2 years. Eighteen uninoculated mice served as controls.

Rauscher virus.—Two hundred female 4- to 6-week-old Swiss ICR mice were given inoculations intraperitoneally of Rauscher virus prepared as described above for the Friend virus. The original virus was obtained from Dr. F. J. Rauscher, of the National Cancer Institute.

Protocol.—Animals were selected at random at regular intervals after inoculation, bled through the retro-orbital plexus for enumeration of the peripheral blood cells, and sacrificed with ether. The left and right thymuses were gently separated, weighed, and fixed separately. Wet touch impressions of spleen and liver were prepared after weighing those organs. Lymph nodes and all vital organs were also included. Tissues were fixed and stained by methods described previously (15).

RESULTS

FRIEND VIRUS

Two host responses to inoculation with the Friend virus were observed. These two responses were morphologically...
and chronologically distinct. The early response occurred in all inoculated mice and consisted of an intense proliferation of reticulum cells of the spleen, many of which differentiated to form erythroblasts. The late response occurred only in some surviving mice and consisted of a thymic lymphoma. The early splenic reticulum-cell proliferation was observed histologically as early as 3 days after inoculation and reached a maximum at about 30–50 days. The late evolution of lymphoma was first noted about 80 days after inoculation.

**Early splenic response.**—The increase in the weight and size of the spleen began at about 7–14 days after inoculation and reached a plateau at about 30 days. At that time many animals died from a characteristic type of splenic rupture associated with necrosis of the reticulum cells. The liver enlargement, which developed several days later than the spleen enlargement, was much less pronounced than that of the spleen. The histologic appearance of the spleen during this period of early host response was one of generalized proliferation of cells of the red pulp (Figs. 7, 9). The cells of the sinus reticuloendothelium and their derivative hematocytoblast precursor cells began proliferation as early as 3–7 days after inoculation and formed sheets of uniform immature reticulum cells, many of which differentiated to form erythroblasts.

In contrast to the intense proliferation of the cells of the red pulp, the cells of the lymphoid follicles of the spleen did not take part in the proliferation. The lymphocytes in the follicles were somewhat reduced in number, were of normal size, and showed no mitoses. Figure 7 shows a spleen 30 days after Friend virus inoculation, to illustrate the lack of proliferation of the lymphoid cells in the splenic follicles (Fig. 8) in spite of the intense proliferation of the reticulum cells of the red pulp (Fig. 9).

The immature, rapidly proliferating reticulum and erythroblast cells of the early response were observed as free emboli in the intrasplenic portions of the splenic veins (Fig. 1) and could be observed in the intrahepatic portions of the portal vein and sinusoids of the liver (Fig. 2). These cells were characteristically distributed in the sinusoids of the peripheral portions of the liver lobules and did not grow in the periportal connective tissue. At the time of rapid splenic enlargement (days 14–30) a transient, clear, straw-colored ascites was observed, which coincided with the massive embolization of the intrahepatic branches of the portal vein (Fig. 2).

The other organs, with the exception of the liver, remained relatively free of the embolic reticulum and erythroblast-like cells from the spleen. Foci of hyperplastic reticulum cells similar to the splenic cells did develop in the perivenule cuffs of some lymph nodes, but generalized lymph node involvement was not observed. After the 70th day many animals surviving the period of splenic enlargement showed considerable hemorrhage in the spleen; for this reason splenic weight after this period was no longer used as an index of the course of the disease. Nucleated cells in the peripheral circulation ranged as high as 50,000 per cubic millimeter.

**Late evolution of thymic lymphoma in Friend disease.**—Of the 54 virus-inoculated animals 31 were killed within 80 days after inoculation, before the time when thymic changes might be expected. The thymuses of these animals showed only slight bilateral lymphocyte depletion.

**Rauscher Virus**

Following inoculation of the Rauscher agent, two separate host responses, previously described by Rauscher (12), were observed. These responses were similar in nature to those observed following the Friend agent. An initial, early phase of splenic proliferation developed within a few days after inoculation. The second, or late, host response...
was the unilateral evolution of thymic lymphoma 80 days after inoculation.

Early splenic response.—Within a few days after inoculation of the Rauscher agent, focal areas of reticulum-cell proliferation were observed in the spleen. By the end of 2 weeks a marked increase in the size and weight of the spleens was evident. The rate of increase of splenic weight was qualitatively similar to that observed in the Friend disease. Histologically, large numbers of reticulum cells proliferated in the red pulp of the spleen, and many differentiated to form erythroblasts. As observed in the Friend disease, the splenic lymphoid follicles in all cases remained intact during this early phase. Necrosis and hemorrhage of the newly proliferated cells in the spleen were more pronounced than in the Friend disease, and splenic rupture occurred more frequently. A few weeks after inoculation many necrotic reticulum cells were observed lying free in the hemorrhagic pools of the enlarged spleens. These free cell emboli could be observed in the intrasplenic portions of the splenic vein and in the intrathepatic portions of the portal vein. Considerable numbers of these cells in the portal circulation passed through the liver and appeared in the peripheral blood, and nucleated peripheral cell counts as high as 700,000 per cu. mm. were observed.

During the preleukemic period, while the erythroblastosis of the spleen was at its maximum, both thymuses lost weight and became small. They declined from an average weight of 40 mg. per thymus (80 mg. combined) to 5–10 mg. per thymus (10–20 mg. combined). Histologically, there was moderate to marked loss of cortical lymphocytes in both thymuses. The lymph nodes during this preleukemic period were variable in size, often slightly enlarged, and histologically showed hyperplasia of the reticulum cells. The axillary nodes each weighed between 10 and 12 mg. compared with a control value of 5–8 mg.

Late thymic response.—Five of the sixteen animals (31 per cent) killed after day 78 had, in addition to the splenic erythroblastosis, a thymic lymphoma. In three of the five cases the tumor was limited to one of the two thymuses, whereas in the other two cases the tumor cells had already disseminated, and the mediastinum of those animals was filled with a tumor mass. As in the Friend study, the majority of animals surviving the period of splenic rupture were sacrificed early in the disease so that early thymic changes might be observed. Most of the animals were sacrificed, therefore, before the lymphoma had developed, as seen in the small number of cases of thymic lymphoma observed. In each of the three cases of unilateral lymphoma the opposite thymus was free of tumor cells and showed only lymphocyte depletion. Because of the marked degree of bilateral thymic lymphocyte depletion accompanying the early splenic hyperplasia, significant unilateral thymic lymphocyte depletion was not observed. Figures 5 and 6 illustrate the histologic appearance of the unilateral lymphoma and compare the histology of the tumor-free opposite thymus.

DISCUSSION

Before the data are discussed, a clarification of terminology may be of value. The term leukemia derives from transliteration into Greek of Virchow's descriptive phrase weisses Blut (white blood). Virchow used the term weisses Blut to describe the appearance of the peripheral blood at autopsy in a case report (19). The subsequent use of the term refers specifically to the presence of abnormal cells in the peripheral circulation. The term leukemia may thus be contrasted with sarcoma (i.e., lymphosarcoma, reticulum-cell sarcoma, etc.), which refers to a proliferation of cells in solid tissue masses. Modern workers generally recognize that the presence of the abnormal cells in the blood stream is but a reflection of the excessive proliferation of these cells in some organ in the body. Thus, any process of cell proliferation which results in a continuous unrestrained release of cells into the blood stream produces a “leukemia.” Once these cells enter the blood stream, they may or may not continue to proliferate.

In both the Friend and Rauscher diseases there is an early, intense proliferation of primitive splenic cells, some of which mature to erythroblasts. As Mirand and Grace (10) have demonstrated for the Friend disease, the cells in the peripheral blood stream are splenic in origin. Many of these cells enter the portal vein and thus appear in the liver and peripheral circulation. The presence of these immature cells of splenic origin in large numbers in the peripheral circulation may be considered to constitute a “leukemia.” These cells have been characterized by Zajdela (20) for the Friend disease, and by Rauscher (12) for the Rauscher disease, as hemopoietic cells. Later in the course of these diseases, a second phase of cell proliferation may begin in one of the two paired thymuses. These cells are thymic in origin and usually are designated as lymphoblasts because they resemble large, immature lymphocytes and are thought to be precursor cells of lymphocytes. Such thymic tumors composed of lymphoblast-like cells are generally designated “lymphosarcomas” or, simply, lymphomas. When the cells of these thymic tumors leave the thymus of origin and are found in large numbers in the blood stream, then this condition also is referred to as a “leukemia.” The two proliferative processes, splenic and thymic, both introduce large numbers of nucleated cells into the peripheral circulation, and therefore, both may be properly called “leukemic” processes.

The splenic and thymic processes originate in different organs, presumably in different cells, and possibly from different causes. Mirand and Grace (9) noted a high incidence of thymic lymphoma following inoculation with the Friend virus in rats, which do not develop the splenic changes. In this connection it may be noted that the virus isolated in our laboratory, by the inoculation of nucleoprotein extracts of the Friend virus, induced thymic lymphoma but no splenic proliferation (13). Rats given inoculations of the Rauscher virus also developed thymic lymphoma without the splenic erythroblastic proliferation; the rat-passaged Rauscher virus, while still capable of inducing thymic lymphoma, would no longer produce the splenic erythroblast response when reinoculated into mice.¹ These results may be contrasted with those of Mirand and Grace (9), who found that virus harvested

¹ F. J. Rauscher, personal communication.
from rats with thymic lymphoma from Friend virus was still capable of producing the early splenic changes when reinoculated back into mice. The fact that the two responses (splenic and thymic) may be separated by appropriate animal passage suggests either that the two host responses are induced by separate viruses, or, alternately, that the splenic response is induced by some labile factor of a single virus which may be lost during animal passage. In a comparative study of chromosomal aberrations in the Friend and Rauscher diseases (18) we observed that the chromosomal alterations during the early period of splenic proliferation could be readily differentiated from those which occurred during the later, thymic lymphoma.

The pathogenesis of the early, splenic phase, with its attendant peripheral dissemination of splenic erythroblasts, has been described for the Friend disease by Friend (3) and later by Metcalf et al. (8). A comparable description has been made of the Rauscher disease by Rauscher (12). In the study by Rauscher, the late-occurring disseminated lymphoma was clearly differentiated from the early, splenic phase. In further studies of the Rauscher disease, Dunn (2) noted that the lymphoma began in the thymuses, whereas the early, erythroblast phase was splenic in origin. Although the Friend virus is known to produce thymic lymphoma in rats (9), it has not been previously reported to produce such lesions in mice. As Mirand and Grace noted, rats do not develop the early, splenic lesions. Possibly because there is no mortality from the splenic lesions, large numbers of inoculated rats live long enough to develop the thymic lymphoma. In the absence of reports of thymic lymphoma in mice from other laboratories studying the Friend virus, other possibilities must also be considered. Despite the fact that the incidence of lymphoma in uninoculated Swiss ICR mice in this and other experiments kept in our laboratory for periods up to 2 years was extremely low (less than 3 per cent), we cannot rigorously exclude the possibility that another leukemogenic virus was present. At first appraisal the incidence of thymic lymphoma in this study following the Rauscher virus is lower than that reported by Rauscher (12), who noted a 70 per cent incidence of lymphatic leukemia. Since the mice in our study were killed before they would have died from the lymphoma, no comparative incidence data may be obtained from our study.

The sequence of events in the pathogenesis of the Friend and Rauscher diseases, suggested by our data, is diagrammatically summarized in Chart 1. Since many workers today use the rapidly proliferating splenic reticulum cells of the early Friend and Rauscher host responses
as a source of neoplastic cells for biochemical studies, it is important to draw attention to the difference between these cells and those of the later-developing thymic lymphoma. Since both types of cells may eventually be found in the spleen, derived data may be misleading if the dual nature of the host responses is not appreciated.

Both the splenic proliferation, with its attendant “leukemia,” and the subsequent thymic proliferation, with its “leukemia,” have features in common. In both, a massive proliferation of primitive cells occurs which enlarges the organ of origin many times. From both, a large number of immature cells are introduced into the circulating blood stream.

Although the two responses (splenic and thymic) have similarities, they also have differences. The splenic phase in both Friend and Rauscher diseases begins within a few days after virus inoculation. The thymic phase occurs only after a considerably longer latency. The splenic phase originates in cells of the red-cell precursor system and does not involve the lymphoid follicles of the spleen (Fig. 7); the thymic phase is primarily a lymphoid proliferation (Figs. 3–6). It may be noted that, whereas both phases of proliferation introduce large numbers of immature cells into the blood stream, only those of thymic origin form solid tumors at distant sites (metastases). The data suggest the possibility that the splenic phase, since it follows so rapidly after virus inoculation, may be a direct, cellular response to the virus. In this respect it is similar to the direct, rapid, cellular response of the mesenchymal cells of chickens infected with the Rous virus (6). The thymic response, however, is delayed and, when it occurs, evolves in only one of the two thymuses. This longer and more selective evolution of the thymic tumor suggests that some intermediate mechanisms are interposed between the virus and the thymic cells. The possible indirect action of the virus in thymic lymphomas has been recently discussed in another publication (15).

When the pathogenesis or sequence of events in the evolution of the various thymic lymphomas are compared, striking similarities are observed. In each of the four systems reported by us, AKR (15), Rich (16), and Friend and Rauscher (17), the evolution of the thymic lymphoma was unilateral, and the appearance of tumor was preceded by a distinct unilateral loss of lymphocytes. The frequency of the unilateral tumor changes was quantitated in our study of AKR mice (16), in which thymic lymphoma evolved unilaterally in at least 95 per cent of the cases. The incidence of unilateral lymphoma following inoculation with the Rich agent (15) was at least 90 per cent. In this study those thymic lymphomas which evolved after inoculation with the Friend or Rauscher virus were all strictly unilateral, following the pattern previously reported for the AKR and Rich lymphomas. With the exception of the extensive perivascular thymic hemorrhages so characteristic for the preleukemic period in the AKR mice (16), the evolution of the thymic lymphoma in all four viral systems studied appeared to follow the same regular, sequential, process. The data indicate that the nature of this neoplastic change follows a precise, unwavering, sequential, and orderly pattern. Successful interpretation of the biologic meaning of this process may offer a clue to its control.

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FIG. 7.—Spleen in Friend disease before lymphoma evolves in one thymus (compare with Fig. 10). Arrows indicate borders of a lymphoid follicle. The tissue surrounding the follicle is the red pulp, filled with proliferated reticulum cells and erythroblasts (see Fig. 9). The lymphoid follicle is normal in size and architecture (see Fig. 8). 30 days post-inoculation. × 100, H. & E. stain.

FIG. 8.—Lymphoid follicle from Figure 7, identified by central arteriole. Note normal appearance of lymphocytes (compare with Fig. 11). × 440.

FIG. 9.—Red pulp from Figure 7. Note large reticulum cells and smaller, darker, erythroblasts. × 440.
Fig. 10.—Spleen in Friend disease after thymic lymphoma has disseminated (compare with Fig. 7). Arrows outline vague borders of tumor-filled lymphoid follicle identified by central arteriole (outline of follicle more easily discerned by staining differences not reproduced in photograph). Note absence of megakaryocytes in central area. 112 days post-inoculation. × 100, H. & E. stain.

Fig. 11.—Lymphoma cells in lymphoid follicle. The difference between these tumor cells and the sheets of proliferated reticulum cells in the red pulp may be observed by comparison with Figure 12. × 440.

Fig. 12.—Reticulum cells and erythroblasts of red pulp, identified by megakaryocyte. Nuclei of reticulum cells are larger and paler than the lymphoma cells. × 440.


Comparative Pathogenesis of Murine Viral Lymphoma

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