Induction of Leukemia in Weanling C3H/Bi Mice by Means of Skin Transplantation from Preleukemic Donors[1] (35610)

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The induction of leukemia in weanling or adult mice has been a major problem which all investigators dealing with leukemogenesis have faced. The literature is full of more or less unsuccessful attempts to induce leukemia in weanling or adult mice. An early experiment reported by Dr. L. Gross is representative of these attempts (1). He initially showed that leukemic filtrates inoculated intraperitoneally or intracranially into young adult C3H mice (average age of 51 days) induced leukemia in only 37% of the animals (average age at onset of leukemia was 8.8 months). By contrast, injection of virus into neonatal mice regularly produces leukemia.

In our laboratory, a series of experiments exploring syngeneic skin graft rejection with the Gross passage A virus system were being carried out (2–4). The hypothesis of viral-induced antigenic alteration of normal tissues as the mechanism causing syngeneic graft rejection was being tested. One of the experiments involved grafting skin taken from preleukemic animals onto weanling mice. Most of the animals accepted the graft. But a very significant and totally unexpected result also came out of this experiment: All the weanling animals which had been grafted developed and died of leukemia.

Materials and Methods. The inbred mice of the C3H/Bi strain used in this study were originally obtained from the colonies of the late Drs. John J. Bittner and Carlos Martinez. The animals have been maintained by rigorous inbreeding procedures. Mice of this strain were initially foster-nursed on C57BL to eliminate the mammary carcinoma milk-borne agent. The occurrence of spontaneous lymphoid malignancy in the C3H/Bi strain is relatively low. However, if newborn or suckling mice are inoculated with Gross passage A (GPA) virus, lymphoid malignancy regularly develops.

The Gross passage A virus, originally obtained from Dr. L. Gross in 1962, has been maintained by serial passage in C3H/Bi strain mice. Virus is routinely harvested from tissues of mice in which a primary malignancy has been induced by injection of the virus into 2–5-day-old animals according to procedures described by Gross (5). The 2–5-day-old mice are lightly anesthetized with ether and then inoculated intrathymically with 0.05 ml of GPA virus. After weaning, not more than five of these inoculated animals were housed per cage. The mice were examined weekly for signs of malignancy; lymphoma development was confirmed by gross and microscopic observation. A detailed autopsy was performed at sacrifice or upon spontaneous death of the animal; samples of organs are fixed in 10% formalin processed by routine histologic methods and stained with hematoxylin and eosin. The animals were given water and Purina Laboratory Chow pellets *ad libitum* throughout the experiments.

For this study, animals inoculated as newborns with GPA virus were carefully exam-

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TABLE I. Incidence of Gross Leukemia and Bilateral Thymus Enlargement in Weanling C3H/Bi Inbred Mice by Preleukemic Skin Transplantation.

Group	Development of leukemia (days)			Thymus enlargement
	Range	Mean	%	Total number
I[a]	95–319	174	100	$\frac{41}{49}$ (84%)
II[b]	66–179	87	100	$\frac{19}{20}$ (95%)
III[c]			0	$\frac{0}{10}$ (0%)
IV[d]			0	$\frac{0}{15}$ (0%)

[a] C3H/Bi mice (4 week old) transplanted with skin taken from 4-week-old preleukemic syngeneic mice.

[b] C3H/Bi mice inoculated intrathymically with 0.05 ml of 20% cell-free filtrates from leukemic tissues when 2–5 days old.

[c] C3H/Bi mice (4 week old) transplanted with skin taken from 4-week-old normal syngeneic mice.

[d] C3H/Bi mice inoculated intraperitoneally with 0.1 ml of 20% cell-free filtrates from leukemic tissues when 4–8 weeks old.

ined by gross and microscopic procedures for evidence of malignancy at approximately 4 weeks of age. No signs of malignancy were found. The animals were considered to be preleukemic. Skin was taken from these 4-week-old preleukemic animals and transplanted onto normal 4-week-old syngeneic recipients of the same sex according to procedures previously described (3). Grafted mice were always housed in individual plastic cages and examined daily. The criteria for graft rejection was little or no hair growth or complete sloughing of the graft; luxuriant hair growth indicated graft acceptance.

A control group of weanling animals (4–8 weeks old) was inoculated intraperitoneally with 0.1 ml of GPA virus prepared from leukemic tissues according to procedures described by Gross (5).

Results. As indicated in Table I, skin was taken from weanling preleukemic C3H/Bi mice (4 week old) and grafted on normal syngeneic hosts of the same age (Group I). In the series of 49 transplants, all 49 mice that were skin grafted eventually died of leukemia. In addition, 4 normal syngeneic mice rejected the skin graft from the preleukemic mice and 45 mice accepted the transplant. None of the recipients of skin from preleukemic donors developed tumors at the graft site. The control group of 10 recipients of normal skin had 100% acceptance (Group III) and developed no leukemias. The latent period in the development of leukemia introduced by skin transplant was long—between 95–319 days (mean 174 days). Previously we reported (6) a latent period of 66–179 days (mean 87 days) when 2–5-day-old C3H/Bi mice were injected intrathymically with cell-free filtrates from leukemic tissues (Group II).

In the GPA system, thymus involvement is one of the standard parameters used to determine whether or not malignancy has been induced. In the same experiment mentioned earlier, in which GPA virus was injected intrathymically into 2–5-day-old C3H/Bi mice, a bilateral enlargement of the thymus was observed in 95% of the animals (Group II). Following the death of the weanling animals, a careful examination of tissues was carried out. It was found that 84% of the grafted weanling animals had bilaterally enlarged thymus. In addition, a disseminated lymphoma was found in all 49 of the animals regardless of the outcome of the skin graft.

This high incidence of thymus enlargement, in addition to the other evidence ob-

tained from the careful autopsy examinations, adds validity to the assertion that malignancy was induced in all 49 (100%) of the grafted weanling mice by the skin grafting procedures.

Of the 15 weanling or adult mice in control Group IV inoculated with virus prepared from leukemic tissues, none developed leukemia (Table I).

Discussion. From the experiments, it is clear that transplantation of skin from preleukemic mice to syngeneic recipients regularly establishes leukemia in the recipients. Several possible explanations for this challenging observation must be considered.

The most attractive explanation is that virus transferred with the skin transplant induces leukemia rather than an effective immunity in the recipient. The malignancy could be a result of either the virus concentration in the skin graft or the route of its introduction in the host. Either of these could favor the escape of the virus from immunologic surveillance or produce an immunodeviation (tolerance or enhancement) in which antibody produced was ineffective in viral neutralization.

Another possibility is that the virus in the skin of preleukemic animals exists in a form more invasive or oncogenic than that of virus from other sources. Hence a malignancy is developed instead of the usual immunity when the route of skin transplantation is used.

Other explanations focus on the role of the tumor cell. That the tumor cell itself may be involved in this unusual finding is suggested by the four instances in which syngeneic skin was rejected and is reinforced by other experimental findings in our laboratory which have shown that rejection depends on the presence of tumor cell in the skin (4). In this particular case, it could be that the number of cells present in the skin was so small or that the antigenic characteristics of the cells were such that the immune system did not respond to the cells or was inhibited. Or it might be that the role of introduction of the cells somehow inhibited the immune response. Because the immune response for one reason or another failed, the development of a disseminated malignancy might then result simply from the introduction of the tumor cell into the recipient. But if the malignancy were only the result of the introduction of tumor, it would be expected that tumor would first appear at the graft site. Such development was not observed. Probably the tumor cell is involved in the phenomenon, but its role is unclear.

Another possible explanation is that the oncogenic agent in the skin is in some intermediate, more virulent "processed" stage. As a result the agent is able to overpower the immune system and induce leukemia. If the route of introduction of the agent is the basis for the increased incidence of leukemia development, a thorough analysis of the process seems indicated because it might have bearing on the introduction and pathogenesis of malignancy during adult life in man and other mammals.

The mechanism involved seems to be a highly complex one. Further study will be carried out in order to evaluate these various explanations and to clarify the oncogenic process involved in this unusual induction of leukemia in weanling mice.

Summary. C3H/Bi weanling mice were grafted with skin taken from 4-week-old preleukemic, syngeneic mice (GPA virus system) which had been inoculated at birth with Gross passage A virus. Careful analysis of the tissues of the grafted weanlings (carried out after natural death of the animals) revealed a high incidence of thymus enlargement and a 100% incidence of disseminated lymphoma. This indicates that leukemogenesis was induced by means of skin transplantation in 100% of the weanling animals by the skin grafting of preleukemic skin.

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