RAPID VIRAL INDUCTION OF MURINE LYMPHOMAS IN THE GRAFT-*VERSUS*-HOST REACTION

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Recent reports from different laboratories have provided conclusive evidence that the graft-*versus*-host reaction (GVHR)[1] can induce lymphomas (1–4). The etiologic mechanisms, however, were not well defined. Although most tumors induced by this reaction have been found by transplantation studies to be of host genotype, several experimenters have observed the induction of tumors which were presumed to be of the same genotype as the parental cells utilized for induction of the GVHR (3, 4) and hence of donor origin. This was considered evidence supporting the hypothesis of Tyler (5) that excessive immunological stimulation of the donor parental cells by the foreign component of the F_1 hybrid could result in unrestrained growth of these cells. Hays (6) observed that when mice which had previously received intrathymic injection of Gross leukemia virus were used as donor mice in the GVHR, the induction of lymphomas was facilitated; the virus could also be isolated from the induced tumors. In a separate experiment, she observed that cell-free preparations of donor spleen cells could induce lymphomas (7). Recently, by selecting mouse strains with known viral leukemogenic potentialities for such experiments, results have been obtained which provide concrete evidence for a viral etiology of GVHR-induced tumors.

Materials and Methods

Mice.—C57BL/1 (B), SJL/J (S), A, (SJL/J × C57BL/1)F_1 ([SB]F_1), and (C57BL/1 × A)F_1 ([BA]F_1) mice used in these experiments were obtained from the author's colony. The B and A strains were obtained in 1967 from the University of Minnesota, Minneapolis, Minn., and are descended from the colony of the late Dr. J. J. Bittner. The S mice were obtained from Dr. E. Murphy, Jackson Laboratory, Bar Harbor, Maine, in 1968. The inbred strains are maintained by strict brother-sister mating.

Viable Spleen Cell Suspensions.—These were prepared by a standard technique (4).

Frozen and Thawed Spleen Cell Suspensions.—After preparation as a viable cell suspension (and a cell count) the inoculum was frozen at −76°C, then thawed in a water bath at 38°C.

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[1] *Abbreviations used in this paper*: B, C57BL/1; (BA)F_1, (C57BL/1 × A)F_1; GVHR, graft-*versus*-host reaction; S, SJL/J; (SB)F_1, (SJL/J × C57BL/1)F_1.

This was carried out a total of four times. The volume injected was equivalent to that for a viable suspension with a similar cell count.

Liver Suspensions.—These were prepared in a similar manner to the spleen cell suspensions (4). The dose given was equivalent, in milligrams, to that of the viable spleen suspension.

Preparation of Viable Tumor Cell Suspensions.—These were prepared by a standard technique (4) so as to obtain a 33% (w/v) suspension. 0.3–0.6 ml (approximately 0.1 ml/3 g body weight) of the suspension was injected intraperitoneally into 25–35-day old (weanling) mice.

Preparation of Cell-Free Tumor Extract.—A combination of the techniques of Gross (8) and Jasmin (9) were used. The tumor was removed rapidly while the mouse was under ether anesthesia and plunged into lactated Ringer's solution at 0°C. (All additional manipulations were carried out at 0°C.) It was then weighed, cut into small pieces with scissors, and suspended in lactated Ringer's solution (20% w/v). The tumor fragments were then broken down with a Potter-Elvehjem homogenizer (Corning Glass Works, Corning, N.Y.) with a loosely fitting stem; thereafter the cell suspension was ground for 5 min in the same homogenizer using a tightly fitting stem. The suspension was centrifuged for 15 min at 1400 *g*; the supernatant was removed and recentrifuged at 7000 *g* for 15 min. To the supernatant of the second centrifugation, polyethylene glycol (Carbowax 6000), was added to a 5% (w/v) concentration. The mixture was incubated for 3 hr at 0°C. The precipitate was resuspended in sufficient lactated Ringer's solution to permit ready passage through a No. 30 needle. 0.1 ml of this suspension was injected intraperitoneally into newborn (less than 24 hr old) (BA)F_1 and B mice. The polyethylene glycol technique permitted a four- to sixfold concentration of the cell-free extract.

Experimental Design and Observations.—The following groups of mice were used: (*a*) (SB)F_1 mice given five weekly intraperitoneal injections of $6–8 \times 10^6$ spleen cells from 4–5-month old male S donors. Total cell dosage was 37×10^6 cells in 4 wk; (*b*) (SB)F_1 mice given frozen and thawed S spleen cells; (*c*) (SB)F_1 mice given S liver; (*d*) (SB)F_1 mice given viable (SB)F_1 spleen cells from 4–5-month old (SB)F_1 donors; and (*e*) normal (SB)F_1 mice. Groups *b*, *c*, and *d* were injected according to the dosage schedule for group *a*. Host (SB)F_1 mice were 30–44 days old; each group consisted of approximately equal numbers of males and females. During the first 40 days, the experimental and control groups were observed daily, weighed twice weekly, and examined twice weekly for splenic and lymph node enlargement. At 40 days all animals in groups *a* and *b* were autopsied and tumor transplantation was carried out. Thereafter, observation and examination of the remaining groups were carried out weekly.

After transplantation of viable tumor or cell-free tumor preparation, the groups of host animals (see Results) were examined twice weekly. Tumor growth was confirmed by autopsy and histologic studies. Additional transplantation of some of these tumors was carried out.

RESULTS

The results are summarized in Table I. 16 (SB)F_1 mice received viable S spleen cells (group *a*). During the 4th wk after the initial injection of cells, weight loss as a result of the GVHR was noted in 7 of the 16 mice. On the 33rd day, two mice died of acute allogeneic disease, with no evidence of tumor on autopsy. On day 40, the remaining 14 mice were found to have large spleens; autopsy and transplantation studies were carried out. Spleen weight varied from 0.8 to 2.5 g. The mesenteric, retroperitoneal, mediastinal, and cervical lymph nodes were most frequently enlarged. Histologically, the tumors were reticulum cell sarcomas, with numerous mitoses, and with fairly uniform cytology (Dunn's type A reticulum cell sarcoma [10]). Some areas of lymphosarcoma were present. The normal cytology of spleen and lymph nodes was almost completely replaced by tumor; in many lymph nodes there was evidence of extension into the sur-

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TABLE I

Lymphoma Induction in $(SB)F_1$ Mice by the GVHR

Group	Inoculum	Observation period	Tumors*
		days	
a	Viable S spleen cells	40	14/14
b	Frozen and thawed S spleen cells	40	11/11
c	S liver	120	0/17
d	Viable $(SB)F_1$ spleen cells	120	0/15
e	—	120	0/30‡

* No. mice with histologically verified tumors/No. mice in group.

‡ Normal $(SB)F_1$ mice have an incidence of lymphoreticular tumors of less than 5% at 1 yr of age.

rounding connective tissue. The liver and lungs were heavily infiltrated. Kidney involvement was common and consisted of tumor cell deposits in the glomeruli or under the renal capsule. The thymus was atrophic, weighing from 2 to 10 mg, with no histological evidence of tumor. Mice with tumor bore no evidence of histological lesions which have been associated with acute or chronic GVHRs (11, 12), except for slight plasma cellular reaction in the loose connective tissue about the lymph nodes and other organs. Skin, bowel lining, blood vessels, kidneys, and ureters appeared undamaged.

All of the $(SB)F_1$ mice which had received frozen and thawed S spleen cells developed tumors by 40 days. These results were identical with those obtained in $(SB)F_1$ mice injected with viable S spleen cells (Table I). A tumor was first evident in one mouse in this group 28 days after the initial injection of frozen and thawed spleen cells. There was no weight loss in any of the mice in this group. The tumors were identical, in their gross and microscopic pathology, with those observed in $(SB)F_1$ mice receiving viable S spleen cells. During a 4-month observation period, tumors did not develop in $(SB)F_1$ mice receiving S liver, $(SB)F_1$ spleen cells, or in normal $(SB)F_1$ mice.

Tumors from eight $(SB)F_1$ mice which had been injected with viable S spleen cells (group *a*) were transplanted initially into syngeneic $(SB)F_1$ mice. All were accepted. These tumors were then used for two purposes: (*a*) preparation of cell-free extracts (see below), and (*b*) transplantation into syngeneic $(SB)F_1$ mice, parental strain mice, S and B, mice containing a parental component, $(NZB \times S)F_1$, and into an unrelated strain, NZB (Table II). During a 6-month observation period, tumor growth was observed only in $(SB)F_1$ and B mice, and in none of the other groups; this was uniformly true for all of the eight original tumors. In those groups in which tumor acceptance occurred, this was clearly evident by the 4th wk after transplantation. The clinically observable rate of tumor growth was also uniform among the mice in each group.

Tumors from eight $(SB)F_1$ mice which had been injected with frozen and thawed S spleen cells (group *b*) were transplanted in a manner similar to the

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tumors arising in $(SB)F_1$ mice injected with viable S spleen cells (previous paragraph). The results were also similar; that is, the tumors were accepted by $(SB)F_1$ and B, but not by S, $(NZB \times S)F_1$, or NZB hosts.

Cell-free preparations, derived from tumors originating in $(SB)F_1$ mice injected with viable S spleen cells, were injected into newborn B or $(BA)F_1$ mice. Cell-free extracts of seven of eight tumors resulted in tumor growth in such B or $(BA)F_1$ hosts (Table III). The latent period from the time of injection to clinical evidence of tumor varied from 33 to 49 days. The tumors involved lymph nodes, spleen, liver, lung, and kidney. The thymus was not involved except late in the disease by direct extension from the adjoining mediastinal nodes. Histologically the tumors were predominantly lymphosarcomas with occasional areas of reticulum cell sarcoma. Four of the seven tumors induced in newborn mice by means of cell-free preparations were transplanted as a viable tumor cell suspension into weanling mice. Two tumors arising in B mice grew after transplantation into syngeneic B hosts, confirming the histologic diagnosis of a malignant tumor. Two tumors (of a total of three) which had developed in $(BA)F_1$ newborns were successfully transplanted into $(BA)F_1$, B, and $(SB)F_1$ hosts. Growth in $(BA)F_1$ mice confirmed the histological diagnosis of a malignant tumor and

TABLE II

Transplantation Characteristics of GVHR-Induced Lymphomas of $(SB)F_1$ Mice

Host strain*	Tumor acceptance‡
$(SB)F_1$	8/8
S	0/8
B	8/8
NZB	0/8

* 6–8 weanling mice in each group.

‡ No. tumors accepted/No. tumors transplanted; 6 months observation period.

TABLE III

Tumor Induction by Cell-Free Preparations of GVHR-Induced Lymphomas

Tumor No.	Newborn hosts	Tumor incidence	Latent period
			days
1	B	10/10	49
2	$(BA)F_1$	6/6	47
3	$(BA)F_1$	5/5	47
4	B, $(BA)F_1$	4/5, 4/4	42, 42
5	B	3/3	44
6	B	2/2	48
7	$(BA)F_1$	0/4	72*
8	B	8/8	33

* Observation period.

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provided evidence of the viability of the tumor cell suspension; growth in B and $(SB)F_1$ hosts indicated that the tumors were B in phenotype.

DISCUSSION

Various theories have been proposed to explain the induction of lymphomas by the GVHR (1–5). These include the actual injection of malignant donor cells, excessive immunological stimulation of the donor cells by the foreign component of the F_1 hybrid host, proliferation of reticuloendothelial tissue in response to lymphoid tissue damage associated with this reaction, mutagenic effects of lymphocyte breakdown products, immunologic suppression, and release or activation of an oncogenic virus. In the present experiments, evidence for a viral etiology of such tumors is provided by the characteristics of the strains of mice used for GVHR tumor induction, the very short latent period required for tumor induction, the induction of tumors which differ markedly antigenically from either donor or host, and, most significantly, the induction of tumors in F_1 mice with frozen and thawed extracts of donor spleens, as well as the induction of tumors in newborn hosts with cell-free preparations of GVHR-induced lymphomas.

Intensive studies of the mouse strains used in these experiments, S and B, have shown conclusively that both strains carry tumorigenic virus; however, tumor inducibility, histology, and organ distribution differ markedly. The S strain exhibits an incidence of spontaneous reticulum cell sarcoma increasing with age, reaching 90% by 13 months (13). Histologically the tumors resemble Hodgkin's disease of humans. Dmochowski and coworkers have proved the viral origin of such tumors (14, 15). Vertical transmission of the virus from mother to embryo was also demonstrated. In contrast to S mice, the B strain exhibits a very low incidence of spontaneous lymphoma (16). In an extensive series of ingenious experiments, Kaplan and his associates established that the B strain is a carrier of a latent leukemogenic virus which is readily activated by X-irradiation (17). The induced tumor, a lymphatic leukemia, originated in the thymus with subsequent involvement of the lymphoid tissues. Gross, who first demonstrated a viral etiology of spontaneous murine lymphomas (18), confirmed the principle of radiation activation of latent leukemogenic virus in another low-leukemia strain, C3H (19). Recent experiments in the author's laboratory have provided evidence that subline 1 of the B strain is also a carrier of a latent leukemogenic virus.

Firstly, parabiosis of $(BA)F_1$ hybrid mice with syngeneic partners, followed by supralethal irradiation of one partner and separation of the partners 1 month later, has resulted in a significantly increased incidence of lymphomas, compared with normal control mice, in the shielded, nonirradiated partner (unpublished observations). Secondly, it has been noted that neonatally thymectomized $(BA)F_1$ hybrid mice develop a significantly higher incidence of spontaneously occurring lymphomas (as well as autoimmune changes) than do normal controls

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(20). Thirdly, when (SB)F_1 mice were injected with massive doses of B spleen cells, all of the F_1 recipients developed reticulum cell sarcomas by 120 days. After transplantation, these tumors were accepted by both the F_1 and the B parent (i.e., the donor strain parent) but not by the S parent, and were presumed to be of donor genotype (4). All of these findings can be explained best if it is assumed that subline 1 of the B strain (and its hybrids) is a carrier of a latent leukemogenic virus which can be released or activated by various disturbances of the cellular environment, such as after irradiation of a parabiotic partner, neonatal thymectomy, or the induction of a graft-*versus*-host reaction.

From these considerations, it appears that the (SB)F_1 hybrid mouse, the host animal in the present experiments, is a carrier of tumor-inducing virus(es) derived from both parents. However, the very fact that tumors were induced in 40 days after injection of small numbers of S spleen cells into (SB)F_1 mice but only after 120 days after massive doses of B cells may indicate that the part played by the virus of S mice is more important in terms of tumor inducibility in the GVHR involving (SB)F_1 hybrid mice than that played by the virus of B mice. The latter virus may, on the other hand, be more important in the determination of the histocompatibility characteristics of the induced tumors (see below).

The latent period for tumor induction, 40 days after the initial injection of S parental spleen cells into (SB)F_1 mice, is far shorter than that observed for GVHR induction of tumor utilizing other strain combinations (1–4, 6, 7). This, as well as the small number of cells required for tumor induction, is probably a reflection of the quantity of oncogenic virus in the spleen cell inoculum, and of an unusual degree of host susceptibility. (Comparative experiments in this laboratory, utilizing various murine strain combinations, have amply demonstrated the apparent immunological vigor of S spleen cells in the GVHR, in terms of F_1 hybrid morbidity and mortality [21]. While this may be an exaggerated expression of cellular immunity peculiar to this strain, it is quite possible that the virus of the S strain, released or activated as a result of the cell damage associated with the GVHR, may at least be partly responsible.)

The induction of tumors in (SB)F_1 hybrid mice after injection of viable S spleen cells, which after transplantation grew in syngeneic (SB)F_1 and B mice, but not in S mice, the parental strain used for induction of the GVHR, is an interesting problem in the relationship of viral oncogenesis and cell surface composition. Antigenic changes in tumor cells may be considered in terms of antigenic loss or gain (22). Mitcheson (23) and Klein and Klein (24) have provided evidence of uniparental preference of heterozygous F_1 lymphomas and sarcomas which was interpreted as being caused by loss of antigens derived from the other parent. In the present experiments, all tumors induced in F_1 mice were uniformly accepted by only one parental strain. This contrasts with the variable results in the above studies, and implies a highly specific mechanism in the determination of their antigenic composition. Since B $\rightarrow$ (SB)F_1-induced tumors were also accepted by B mice, a common mechanism in the determination of the

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tumor cell surface composition may be operative in both GVHRs involving the same F_1 hybrid. Cell culture studies have demonstrated that the B radiation leukemia virus could rescue a defective murine sarcoma virus genome; the resultant infectious virus particle carried the envelope of the B virus (25). Thus in GVHR-induced tumors in $(SB)F_1$ mice, irrespective of the parental cell donor used for initiation of the reaction, the B virus may be acting as a helper to the S virus; the S virus is a more powerful oncogenic agent but the B virus is a more potent determinant of the tumor cell surface composition. The antigenic composition of the tumor cell membrane would be altered from that of the normal cells of the $(SB)F_1$ host to a sufficiently high concentration of B antigen, relative to S-derived antigen, to be accepted immunologically by B hosts. This suggests that the B virus, when activated, is capable of controlling the genome of the $(SB)F_1$ host cell or at least that portion derived from the B parent. At the present time, little is known about the exact relationship between murine leukemia virus particles and the expression of virus-induced cell surface antigens (26). (In this regard, the F_1 hybrid animal, containing tumor-inducing viruses from one or both parental strains, may be of particular value in the study of the relationship between oncogenic virus and tumor antigenicity. In pure strain mice, a virally induced tumor would arouse no particular interest if it were found to be acceptable to syngeneic pure strain hosts on transplantation; but the F_1 mouse, which is unable to reject parental strain tissue, would permit the development of tumors with parental antigenicity, and more significantly, the detection of their unique character by subsequent transplantation studies.)

Weanling $(SB)F_1$ mice injected with frozen and thawed spleen cells from S donors developed lymphomas with a latent period, distribution, and histology very similar to those after the injection of viable S spleen cells into similar hybrids. This most unexpected finding constitutes strong evidence for a viral etiology of GVHR-induced tumors. It indicates that the induction of tumors in this reaction does not depend specifically on an immunological mechanism but appears merely to depend on the liberation or activation of a sufficient quantity of oncogenic virus. The fact that tumors induced in $(SB)F_1$ mice with frozen and thawed S spleen cells were identical in their transplantation characteristics with those induced by viable S spleen cells supports this interpretation. This result is also of significance from the point of view of tumorigenesis in general, in that a cell-free extract of normal mouse spleen carries oncogenic information. This provides corroborative evidence for the finding that viruses isolated from normal spleens of BALB/c mice produce leukemia when inoculated into newborn syngeneic mice (R. J. Peters et al., manuscript in preparation) [27]. Additional support is thus provided for the Huebner-Todaro viral oncogene hypothesis, in that exogenously added viruses may transform cells by "switching on" the endogenous oncogenic machinery (the induced tumors were antigenically different from donor or host). Electron microscope studies of various organs of S mice have revealed numerous C-type virus particles in the spleen but few in

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the liver (14). The absence of tumor development in $(SB)F_1$ mice injected with intact S liver cells may therefore be due to the low concentration of virus within the liver or to preservation of the liver cells with relative absence of free virus.

Tumor induction in newborn mice after injection of cell-free preparations from GVHR-induced tumors is also strong evidence for a viral etiology of such tumors. In contrast to most such studies, the latent period was extremely short, as little as 33 days. This is probably due to a combination of several factors: (*a*) a high virus content in the tumor, as well as further concentration with polyethylene glycol; and (*b*) the use of $(BA)F_1$ and B recipients. The latent virus of these newborn hosts may be acting as a helper virus to that contained in the inoculum.

Transplantation of two of the three tumors induced in $(BA)F_1$ newborn mice revealed that in both instances the tumors were antigenically B tumors. Conceivably, viable cells were contained in the original cell-free preparation of the GVHR-induced tumors. The technique of preparation of the cell-free inoculum, however, was a standardized procedure. Also, cell-free extracts of spontaneous lymphomas of $(BA)F_1$ mice have induced lymphomas in B hosts, whereas viable suspensions of such $(BA)F_1$ tumors were not accepted by B mice (unpublished observations). Far more attractive is the concept of a helper role for the latent virus of the $(BA)F_1$ newborns, derived from the B parent, for the S virus of the cell-free extract, as in tumors induced in $(SB)F_1$ mice by frozen and thawed S spleen cells.

In his studies of the radiation induction of leukemia, Kaplan noted a paradoxical increase in leukemia incidence when the same total dose of radiation was given not as a single exposure but in several fractions separated by a few days (28). Maximal tumor incidence was achieved by using young adult (age 30–36 days) mice irradiated at 4- or 8-day intervals for a total of four equal doses. Since the mutagenic action of radiation is characterized by independence of mutation yield with respect to dose rate and fractionation, somatic mutation was clearly an untenable mechanism for the leukemogenic action of radiation. Previous experiments in this laboratory revealed that a single injection of 40×10^6 S spleen cells into weanling $(SB)F_1$ mice resulted in a 17% incidence of tumors in 8 months (21). By contrast, in the present experiments, when a similar total dosage of S spleen cells was divided into five equal doses given at weekly intervals, tumor incidence increased to 100% in only 40 days. It is therefore tempting to consider somatic mutation improbable as a cause of GVHR-induced tumors on these grounds alone. Further experiments by Kaplan, and by this author, have demonstrated a viral etiology of radiation- and GVHR-induced tumors, respectively. A detailed study of the influence of host age and the number and spacing of the parental cell injections on the incidence of GVHR-induced lymphomas has not yet been carried out. In this regard, the present experiments already show a maximal tumor incidence when these parameters approximate those in the radiation experiments, and suggest a common underlying mechanism, using both modalities, in the temporal aspects of viral leukemogenesis.

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There is no literature on the relationship of the viruses of the S and B strains of mice; however, the present experiments, as well as other investigations in this author's laboratory, provide some insight into their mutual oncogenic potential. Since $(SA)F_1$ mice also have a low incidence of spontaneously occurring lymphomas (personal observations) the infrequency of such tumors in $(SB)F_1$ mice as compared with that in the parental strain S cannot be attributed to a special strain-specific influence of the B component of the hybrid. In the GVHR model $A \rightarrow (BA)F_1$, tumors were induced only after many months, and were transplantable only to A and $(BA)F_1$ but not to B hosts (4). This contrasts with the present experiments in which $S \rightarrow (SB)F_1$ resulted in tumors which were phenotypically B. Also, the S virus does not appear to possess vigorous oncogenic powers universally in $S \rightarrow F_1$ GVHRs; current experiments, in which S spleen cells have been injected into $(NZB \times S)F_1$ hybrids in weekly doses, already indicate that the remarkably rapid oncogenesis of the $S \rightarrow (SB)F_1$ combination will not be duplicated. Thus, presently available data seem to indicate that the viruses of the S and B (subline 1) strains of mice are strongly and perhaps uniquely synergistic in tumor induction. These strains and their hybrid would therefore appear to possess exciting possibilities for further studies of tumor induction by cooperating viruses utilizing immunological and other means. Such investigations are important for further elucidation of the oncogene theory of tumorigenesis (27).

Control of tumor development can operate at various levels and is a complex and as yet poorly understood phenomenon (27). Tumor induction in any situation, including the GVHR, will occur when the combined factors for expression exceed a threshold. In the present experiments, it seems likely that high concentrations of oncogenic virus in a susceptible host have readily overcome inhibitory influences, resulting in "acute" induction of tumors. I suggest that tumor induction in more chonic GVHRs in mice is also basically viral in nature, with various factors facilitating the expression of viral oncogenic potential. Thus excessive immunological stimulation of donor cells might result in virus activation or liberation. (Donor-type tumors have not yet been investigated for a viral etiology.) Impaired immunological reactivity has been demonstrated in F_1 host mice during the GVHR (29, 30) and could permit the survival of clones of neoplastic cells which might otherwise be eliminated. This, together with the well-known phenomenon of declining thymus function with age, may be important in tumor induction in chronic GVHRs involving a major portion of the life-span of the host animal (4).

SUMMARY AND CONCLUSIONS

When weanling $(SJL/J \times C57BL/1)F_1$ hybrid mice were given five weekly injections of small doses of viable SJL/J spleen cells, so as to induce a graft-*versus*-host reaction (GVHR), reticulum cell sarcomas were induced in all of the host mice by the 40th day after the first cell injection. Such tumors, on transplantation, were accepted by syngeneic $(SJL/J \times C57BL/1)F_1$ and C57BL/1

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hosts, but not by SJL/J or NZB mice. Cell-free extracts of SJL/J spleens injected into similar hybrids resulted in identical tumors in all hosts within the same period; the transplantation characteristics were also similar. Normal (SJL/J × C57BL/1)F_1 hybrids as well as similar hybrids injected with SJL/J liver or syngeneic F_1 spleen cells did not develop tumors.

Cell-free preparations of eight tumors induced in F_1's by viable SLJ/J spleen cells were injected into newborn (C57BL/1 × A)F_1 and C57BL/1 mice: tumors were induced, with seven of eight tumor preparations, with a latent period of 33–49 days. Such tumors were lymphosarcomas, and, in the case of (C57BL/1 × A)F_1 hosts, further transplantation revealed that they were antigenically C57BL/1 tumors.

These experiments provide conclusive evidence for a viral etiology of GVHR-induced tumors. Furthermore, tumor induction in the GVHR does not appear to depend specifically on an immunological mechanism but is most probably due to release or activation of a sufficient quantity of oncogenic virus within a certain time period in a highly susceptible host. Comparison with radiation induction of viral leukemia in mice revealed similarities in regard to optimal host age and the spacing of administration of the tumor-inducing agent.

SJL/J mice carry a type C virus which causes a high incidence of spontaneous Hodgkin-like tumors by 1 yr of age; C57BL/1 mice do not develop lymphomas spontaneously but carry a latent leukemogenic virus. Their hybrid also has a low incidence of spontaneous lymphomas. Based on the results of these and previous experiments, the viruses of these strains of mice appear to be highly synergistic in tumor induction in the GVHR. The SJL/J virus is a powerful oncogenic agent. The C57BL/1 virus may be a helper virus to the SJL/J, but is a more powerful determinant of the antigenic composition of the induced tumors. This suggests that the virus of C57BL/1 mice, when activated, is capable of controlling the C57BL/1 genome. Because of the ease and rapidity of viral tumor induction, the SJL/J and C57BL/1 strains of mice, with their F_1 hybrid, should be useful for further study of the mechanisms controlling induction of such tumors.

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