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Rapid Immunological Induction of Murine Lymphomas: Evidence for a Viral Etiology

Abstract. *A graft-versus-host reaction induced in (SJL/J × C57BL/1)F_1 hybrid mice by injection of SJL/J spleen cells resulted in 100 percent incidence of tumors at 40 days. Transplantation studies revealed that the tumors were antigenically C57BL/1. Since both SJL/J and C57BL/1 mice carry tumorigenic virus, the evidence suggests a viral etiology.*

Numerous experiments in different laboratories have established that the graft-versus-host reaction (GVHR) can eventuate in lymphomas (*1–3*). The etiologic mechanisms, however, were obscure. Although most tumors have been of host genotype, several experimenters have also observed the induction of tumors which, on the basis of transplantation characteristics, were presumed to be of donor type—that is, of the same genotype as the parental cells utilized for the induction of the GVHR (*2, 3*). This was considered evidence supporting the hypothesis of Tyler (*4*) that excessive immunological stimulation of the donor parental cells could result in unrestrained growth of these cells. Recently, however, by selecting mouse strains with known viral leukemogenic potentialities for such experiments, results have been obtained which provide strong evidence for a viral etiology of GVHR-induced tumors.

The GVHR was induced in 16 (8 male and 8 female) 44-day-old (SJL/J × C57BL/1)F_1 [abbreviated (SB)F_1] hybrid mice by five weekly intraperitoneal injections of 6×10^6 to 8×10^6 spleen cells from 4- to 5-month-old male SJL/J mice. Total cell dosage was 37×10^6 cells in 4 weeks. A control group of 15 (SB)F_1 hybrids was given a similar dosage of spleen cells from 4- to 5-month-old male (SB)F_1 donors. Cell suspensions were prepared by a standard technique (*3*). On day 33 (after the initial cell injection), two mice in the experimental group died of acute allogeneic disease. There was no evidence of tumor at autopsy. On day 40, the remaining 14 mice were found to have large spleens; autopsy and transplantation studies were carried out.

The tumors in the experimental group of mice involved the spleen, lymph nodes, liver, and lungs. Spleen weight varied from 0.8 to 2.5 g. The mesenteric, retroperitoneal, mediastinal, and cervical lymph nodes were most frequently involved. Histologically, the tumors were reticulum cell sarcomas, with numerous mitoses. The thymus was not involved. No tumors were present in the control group.

Tumors from eight mice were transplanted initially into syngeneic (SB)F_1 mice. All were accepted. The tumors were then transplanted into syngeneic (SB)F_1 mice; parental strain mice, SJL/J and C57BL/1; mice containing a parental component, (NZB × SJL/J)F_1; and into an unrelated strain, NZB. A tumor cell suspension, prepared by a standard technique (*3*), was injected intraperitoneally into six to eight weanling mice in each group. During a 5-month observation period, tumor growth was observed only in (SB)F_1 and C57BL/1 mice, but 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 fourth week after transplantation. The clinically observable rate of tumor growth was also uniform. Tumor growth was confirmed by autopsy and further transplantation.

Various theories, not mutually exclusive, have been proposed to explain the induction of lymphomas by the GVHR (*1*). The experiments reported here provide evidence for a viral etiology because of (i) the characteristics of the strains of mice utilized for the GVHR, (ii) the short latent period required for tumor induction, and (iii) the induction of tumors which differ antigenically from either donor or host.

Studies of the mouse strains used in these experiments, SJL/J and C57BL/1, have shown conclusively that both strains carry tumorigenic virus; tumor inducibility, however, is quite different. The SJL/J strain exhibits an increasing incidence of spontaneous reticulum cell sarcoma with age, reaching 90 percent by 13 months (*5*). Histologically, the tumors resemble Hodgkin's disease of humans. Dmochowski and co-workers have proved the viral origin of such tumors (*6, 7*). Vertical transmission of the virus from mother to embryo was also demonstrated. In contrast, the C57BL strain exhibits a very low incidence of spontaneous lymphoma (*8*). Kaplan and his associates have established that the C57BL strain is a carrier of a latent leukemogenic virus that is readily activated by x-irradiation (*9*). [A viral etiology of spontaneous murine lymphomas was first demonstrated by Gross (*10*). He subsequently confirmed the principle of radiation activation of latent leukemogenic virus in another low-leukemia strain, C3H (*11*)]. Recent experiments in my laboratory have provided evidence that subline 1 of the C57BL strain is also a carrier of a latent leukemogenic virus.

First, parabiosis of (C57BL/1 × A)F_1 hybrid mice with syngeneic partners, followed by supralethal irradiation of one partner, has resulted in a significantly increased incidence of lymphomas, compared to that in normal control mice, in the shielded nonirradiated partner (unpublished observations). Second, it has been noted that neonatally thymectomized (C57BL/1 × A)F_1 hybrid mice develop a significantly higher incidence of spontaneously occurring lymphomas (as well as autoimmune changes) than do normal controls (*12*). Third, when (SB)F_1 mice were injected with massive doses of C57BL/1 spleen cells, all of the F_1 recipients developed reticulum cell sarcomas by 120 days. On transplantation, these tumors were accepted by both the F_1 and donor strain parent, but not by the SJL/J parent, and were presumed to be of donor genotype (*3*). All these findings can be best explained if it is assumed that the C57BL/1

strain (as well as its hybrids) is a carrier of a latent leukemogenic virus that can be released or activated by various disturbances of the cellular environment —for example, following irradiation of a parabiotic partner, neonatal thymectomy, or the induction of a GVHR.

From these considerations, it appears that the (SB)F_1 hybrid mouse, the host animal in the above experiments, is a carrier of tumor-inducing virus or viruses derived from both parents. Furthermore, it seems likely that both viruses are involved in lymphoma induction by the GVHR. Unlike the SJL/J strain, (SB)F_1 mice have a low incidence of spontaneously occurring lymphomas. However, the very fact that tumors were induced in 40 days after injection of small numbers of SJL/J spleen cells into (SB)F_1 mice, but only after 120 days after injection of massive doses of C57BL/1 cells, may indicate that the part played by the virus of SJL/J mice is more important in terms of tumor inducibility than that played by the virus of C57BL/1 mice. The latter virus may, on the other hand, be more important in the determination of the histocompatibility characteristics of the induced tumors (as indicated below).

Comparative experiments, in which various murine strain combinations were used, have demonstrated the immunological vigor of SJL/J spleen cells in the GVHR, with respect to F_1 hybrid morbidity and mortality (*13*). While this may be an expression of cellular immunity peculiar to this strain, it is quite possible that the virus of the SJL/J strain (*7*) released or activated, as a result of the cell damage associated with the GVHR, may at least be partly responsible. Thus the short latent period for tumor induction, 40 days after the initial injection of SJL/J parental spleen cells into (SB)F_1 mice, as well as the small number of cells required for tumor induction, could well be a reflection of the quantity of oncogenic virus in the spleen cell inoculum. This time interval is far shorter than that observed for GVHR induction of tumor with other strain combinations (*1–3*).

The induction of tumors in (SB)F_1 hybrid mice—which on transplantation grew in syngeneic (SB)F_1 and C57BL/1 mice, but not in SJL/J mice, the parental strain used for induction of the GVHR—is an interesting problem in the relation between viral oncogenesis and cell surface antigenicity. (It must be conceded that tumor acceptance in the SJL/J hosts might be demonstrable with a period of observation longer than 5 months; if this were so, the longer interval for tumor acceptance in this group would still pose questions relating to tumor histocompatibility.) At present, little is known about the exact relation between murine leukemia virus particles and the expression of virus-induced cell surface antigens (*14*). Antigenic changes in tumor cells may be considered in terms of antigenic loss or gain (*15*). Mitcheson (*16*) and Klein and Klein (*17*) have provided evidence of uniparental preference of heterozygous F_1 lymphomas and sarcomas which was interpreted as being due to loss of antigens derived from the other parent. In the experiments reported here, 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 C57BL/1 $\rightarrow$ (SB)F_1-induced tumors were also accepted by C57BL/1 mice, a common mechanism in the determination of the tumor cell surface composition may be operative in both GVHR's involving the same F_1 hybrid. Cell culture studies have demonstrated that the C57BL radiation leukemia virus could rescue a defective murine sarcoma virus genome; the resultant infectious virus particle carried the envelope of the C57BL virus (*18*). Thus in GVHR-induced tumors in (SB)F_1 mice, the C57BL/1 virus may be acting as a helper to the SJL/J virus.

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Concanavalin A Agglutination of Intestinal Cells from the Human Fetus

Abstract. *Concanavalin A markedly agglutinated isolated epithelial cells from the intestine of the human fetus but not from the intestine of the adult. Wheat germ agglutinin only moderately agglutinated cells from the intestine of an adult. These results extend the studies of concanavalin A agglutination of embryonic cells to human tissue, and they suggest that concanavalin A may be reacting with a common antigen on the fetal cell membrane.*

A major new approach in the study of cell surface membranes has been the use of phytoagglutinins, which bind to specific carbohydrate-containing sites on the cell surface, and which cause some cells to agglutinate (*1*). Although the exact role of these binding sites in the agglutination process is not understood, there appears to be a strong association between cell agglutination and changes in the cell surface properties associated with malignancy, such as decreased cell adhesion and contact inhibition (*2*, *3*). Fundamental changes in glycoproteins and glycolipids on the cell surface membrane accompany the changes in cell behavior that are involved in malignant transformation (*4*), and it has been suggested that cell surface glycoproteins serve as determinants of cell behavior (*5*). Moscona (*6*) extended these observations to embryonic differentiation by demonstrating agglutination of chick embryonic tissue cells by concanavalin A (Con A), and suggested that the unmasking of agglutinating sites on neoplastic cell membranes might reflect a return to the embryonic state with resultant increased cell mobility. This theory is consistent with recent clinical studies showing the appearance of fetal cell membrane factors in the serum of