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Lymphomas in C3H Mice Perinatally Inoculated with (C3H × T_6) F_1 or C3H Spleen Cells*

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Abstract—*Acute fatal Host vs Graft (HVG) reactions may be induced in several strains of inbred mice by the perinatal inoculations of related, histoincompatible F_1 hybrid spleen cells. Susceptible recipients die young with T-cell deficiency and hyperplasia of the B-cell system. It seemed likely that mice which survived or avoided acute HVG reactions would be predisposed to lymphoid malignancies. A doubling of the natural incidence of lymphomas was observed in long term studies of C3Hf/Bi (C3H) mice perinatally inoculated with (C3H × T_6)F_1 spleen cells. However, an increased incidence and earlier onset of lymphomas was also seen in the C3H recipients of isogenic C3H spleen cells. Virological studies revealed the presence of ecotropic murine leukemia virus (MuLV) in both (C3H × T_6)F_1, and C3H donor cell inocula, the earlier expression of MuLV in the recipients of such inocula, and the production of infectious virions by the lymphomas in all C3H/(C3H × T_6)F_1 mice tested. It is suggested that the inoculation of C-type virus producing cells into hosts which are immunologically or genetically incapable of rejecting them may be a factor favoring the association of ecotropic MuLV and lymphomas in HVG and some Graft vs Host systems.*

INTRODUCTION

EXPERIMENTAL Host vs Graft (HVG) syndrome is the fatal complex of lesions which has been observed in six strains of mice perinatally inoculated with related F_1 hybrid spleen cells [1–5].

Deaths are due to thrombocytopenia and intestinal hemorrhage [1,6], hepatic necrosis [6] and renal disease [3]. The principal pathogenetic factor is the rapid formation of immune complexes, which cause membranous glomerulonephropathy [3] and apparently initiate disseminated intravascular coagulation [6]. The primary lesions are thought to occur in the lymphoid organs. The normal histology of the spleen and lymph nodes are greatly altered by severe depletion of T-lymphocytes [4, 7, 8] and marked hyperplasia of the B-cell system [9]. Thymic atrophy [1] appears to be related to the inability of the host to replace peripheral T-cells lost in the HVG reaction. There is no obvious correlation of susceptibility with alleles of the major histocompatibility complex in the six strains of mice in which HVG disease has been described: A(H-2^a), C3Hf/Bi H-2^k), RFM(H-2^f), BALB/c (H-2^d), C57Bl/6(H-2^b) and C57Bl/1 (H-$2^{b?}$).

There were several reasons to expect that long lived survivors of HVG reactions would be predisposed to lymphoid malignancies. Perhaps most important is that the induction of the disease involves the inoculation of adult cells into hosts at the age of maximum susceptibility [10] to any oncogenic murine leukemia virus (MuLV) which might be produced by the donor cells. The morphological and functional signs of disruption of the lymphoid tissues suggested other reasons. Lack of T-cells might result in the impaired ability to detect and eliminate malignant mutants [11] or infectious oncogenic agents [12]. Transition from hyperplasia to malignancy could well occur in parental host B-cells as the result of chronic antigenic stimulation by the histoincompatible donor F_1 hybrid cells [13]. An increased incidence of lymphomas has been observed in Graft vs Host (GVH) systems,

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which also involve chronic parent-vs-F_1 hybrid reactivity [14]. And finally, there are two preliminary reports of lymphoreticular hyperplasia and lymphomas associated with ectropic MuLV expression in mice with chronic HVG disease [4, 15].

C3Hf/Bi mice were chosen for our long term studies, in part because they were known to be less susceptible to acute death from aberrant HVG reactions, and because they had been observed to develop hepatomas as well as lymphomas. It was hoped that studies on the incidence of these hepatocellular tumors in C3H mice which had received (C3H $\times T_6)F_1$ spleen cells [C3H/(C3H $\times T_6)F_1$ mice] might serve as an additional test of the hypothesis that the T-cell system is responsible for the elimination of transformed cells. Histopathological studies were complemented by sensitive XC plaque assays for the presence of ecotropic MuLV in donor and host cells.

MATERIALS AND METHODS

Mice

The C3Hf/Bi (C3H) mice used in these experiments have been inbred in our conventional colony since receipt from the late Dr. Carlos Martinez. The T_6T_6 (T_6) mice, homozygous for the characteristic chromosome markers have also been inbred for over 20 generations in our colony since receipt of these Harwell-derived mice from Dr. John Trentin. The latter mice accept reciprocal skin grafts, but the histocompatibility type is not known.

Formation of chimeras

Experimental C3H mice less than 24 hr old were inoculated intravenously with 15×10^6 pooled (C3H $\times T_6)F_1$ spleen cells. At 7 days of age a second inoculation of 100×10^6 pooled (C3H $\times T_6$) F_1 spleen cells was given i.p. Litter-mate controls were inoculated with C3H spleen cells on the same schedule (C3H/C3H mice) or were untreated.

Clinical studies

The mice were observed daily and checked monthly for tumors by abdominal palpation. Body weights and fecal blood content [16] were determined bi-weekly. At intervals of 3 months, routine hematological studies including hemoglobin levels and total and differential white blood cell counts were determined by standard methods [17] on samples drawn from the retro-orbital plexus. Urinary proteins were detected with Ames N-Multistix, also at 3-month intervals.

Skin grafts

A 3×2 cm, full thickness piece of abdominal skin from a (C3H $\times T_6)F_1$, C3H, T_6 or RFM donor was transferred to the dorsal site of the recipient and secured with wound clips.

Histopathology

Samples of tissue were removed and placed in acetate buffered formalin. After fixation, bones were decalcified in a saturated solution of ethylene diamine tetra-acetate. Tissue sections were stained routinely with hematoxylin and eosin (H & E).

Electron microscopy

Pieces of kidney were fixed in either 1% osmium tetroxide for 1 hr or in 4% buffered gluteraldehyde for 2 hr, both in the cold. Gluteraldehyde-fixed material was post-fixed in 1% osmium tetroxide for 1 hr. All material was eventually embedded in Maraglas/DER. Sections were studied in a Siemens Elmiskop IA at 60 kV.

Assay for ecotropic MuLV.

Infectious ecotropic MuLV was assayed by the XC plaque assay [18]. Sterile cell suspensions from spleen, lymph node, thymus or bone marrow were prepared, and 2×10^7 cells were added to 60×15 cm Petri dishes which contained SC-1 cells [19] plated at a concentration of 1×10^5 cells 24 hr earlier. At the time of inoculation, the medium contained 16 μg polybrene per ml. Fluid changes were made after 24 hr and every 2 days thereafter. On day 7 the SC-1 cell monolayer was irradiated with u.v. light (30 sec at 85–90 ergs/mm^2/sec) and overlaid with 10^6 XC cells. On day 10 the cells were fixed with methanol and stained with hematoxylin. Holes in the XC monolayer bordered by multinucleate giant cells were counted as plaques.

Statistical analyses

The incidence and ages of detection of the various diseases as they occurred in C3H/(C3H $\times T_6)F_1$ and C3H/C3H mice were compared [20] with the same variables in untreated C3H controls. The incidences of lymphomas, hepatomas, all tumors, infections and deaths without apparent cause were com-

pared by contingency table analyses. Fischer's exact test used to analyse the significance of the appearance of leukemia and membranous glomerulonephropathy in C3H/(C3H × T_6)F_1 mice. Analyses of the differences in ages of onset of the various lesions were done using the Mann–Whitney U-test.

RESULTS

Deaths due to lymphomas

Untreated C3H mice from our colony are predisposed to the development of non-thymic lymphomas in significant numbers (Table 1). The incidence of lymphomas was doubled in mice inoculated with (C3H × T_6)F_1 cells, and tripled in mice which received C3H cells. In addition, the lymphomas appeared earlier and shortened the life span of affected mice by 21% in the case of C3H/(C3H × T_6)F_1 mice and by 26% in the case of C3H/C3H mice. In all these groups, most of the tumors appeared to originate from Peyer's patches in the jejunum (Fig. 1) and ileum, or the mesenteric lymph node. Ulceration of lymphomatous bowel led to detectable intestinal bleeding and sometimes perforation with death from peritonitis. The thymus was never involved, although the adjacent nodes were. Enlargement of these nodes occasionally led to respiratory difficulty due to tracheal compression and pneumonia. Splenic involvement was uncommon and infiltration of bone marrow was found only once. The majority of lymphomas from all groups could be classified as large, non-cleaved lymphocytic lymphomas of the diffuse type and immunoblastic sarcomas by the criteria of Lukes and Collins [21], and as reticulum cell neoplasms, type B, according to Dunn [22]. Because lymphomas and leukemias have never been detected in C3H mice aged less than 184 days, this was used as the minimum age for defining the population at risk. Hepatomas were found in 60% of C3H/(C3H × T_6)F_1 and 67% of C3H males with lymphomas. There was no apparent association with other diseases including infections.

Deaths due to leukemia

Leukemia was seen only in host mice which had received (C3H × T_6)F_1 cells (Table 1). All five cases diagnosed antemortem were acute lymphoblastic in type. Giemsa-stained cells typically had immature large lymphoid nuclei with variable degrees of chromatin clumping, one to three prominent nucleoli, and dark blue cytoplasm. Once started, the progression of the disease was rapid. Mice appeared terminal or died 6–8 weeks after initial detection. Two cases were diagnosed as leukemia in post mortem histopathological examination. In four of the seven cases, discrete masses of malignant cells were found which indicated that lymphomas were also present. Total white blood cell counts ranged from 67,300 to 337,000 per μl (mean = 157,000). Lymphoid cells comprised 91–100% of these cells on differential counts. Leukemic mice were anemic (hemoglobins averaged 7.3 g/dl compared with 14.8 g/dl for healthy controls) and severely thrombocytopenic. Microscopic foci of tumor cells were detected in bone marrow in two of five cases.

Deaths due to hepatomas

Although there was a trend toward a greater incidence of primary type A and B hepatic tumors in experimental C3H/(C3H × T_6)F_1 males (89%) than in untreated control C3H male littermates (67%) the differences were not statistically significant (Table 1). An interesting qualitative difference was noted. Hepatomas, classified as type B (Figs. 2a and 2b) by the criteria of Walker *et al.* [23], were seen only in 5 C3H males which had received (C3H × T_6)F_1 cells and one control mouse which could be considered immunologically abnormal because of the presence of amyloidosis [24]. Type B hepatomas are thought by some to have greater malignant potential than those with an A pattern [25]. However, in this series, there were no metastases, and no correlation could be made between histopathological type and size or number of tumors. Because hepatomas have not been detected in C3H males less than 400 days of age, this was used as a minimum for defining the population at risk. The incidence of lymphomas in C3H/(C3H × T_6)F_1 males with hepatomas (56%) was over twice that seen (25%) in C3H control males with hepatomas.

Other tumors

Other tumors found included two pulmonary adenomas, and a uterine leiomyoma. The former were incidental findings at autopsy. The latter was thought clinically to be a lymphoma and caused the animal to be sacrificed.

Deaths due to infections

Pneumonia, often with abscess formation, was by far the most common cause of death

Table 1. Incidence and mean survival times (MST) of C3H mice inoculated with $(C3H \times T_6)F_1$ spleen cells [$C3H/(C3H \times T_6)F_1$ mice] or with C3H cells (C3H/C3H mice), or untreated (C3H mice), and dying with malignancies

Group	Lymphoma*		Leukemia*		Hepatoma†		
	Incidence	MST (range) (days)	Incidence	MST (Range) (days)	Incidence	MST (range) (days)	Type B‡
$C3H/(C3H \times T_6)F_1$	27/54(50%)§	462(184–735)	7/54(13%)¶	380(286–569)	16/18(89%)	545(427–702)	3/16
C3H/C3H	9/13(69%)‖	423‖(265–570)	0/13		2/4(50%)	488(406–569)	0/2
C3H	7/31(23%)	567(489–661)	0/31		8/12(67%)	561(436–661)	1/8**

*All mice of ages ≧184 days. ‖$P<0.004$.
†Males of ages ≧400 days. ¶$P<0.035$.
‡Classification of Walker *et al.* [23]. **Amyloidosis.
§$P=0.024$.

due to infectious disease. Of the 23 infectious deaths shown in Table 2, 16 were due to pyogenic pulmonary infections. Other infections identified as the causes of death included hepatitis, peritonitis associated with incarcerated testis, and abscesses of the spleen and uterus. Although the incidence of infections in the experimental group did not differ greatly from that seen in C3H controls (Table 2) most experimental mice which had received F_1 hybrid cells died much earlier. Thus, 6 of 12 (50%) $C3H/(C3H \times T_6)F_1$ mice were dead before 240 days of age, as compared with 1 of 9 (11%) of the C3H controls dying of infections over the same period of time.

Deaths due to membranous glomerulonephropathy

Various degrees of glomerulosclerosis were found on light microscopic examination of the kidneys from 6 experimental mice with proteinuria of 300 mg/dl or greater (Table 2). When examined by electron microscopy, the subepithelial pattern of deposits was seen (Fig. 3), which characterizes membranous glomerulonephropathy due to immune complexes [26]. Lymphocytic depletion in thymic dependent areas and plasmacytosis were seen in the spleens and lymph nodes of three mice, of which two had lung abscesses. Anemia and thrombocytopenia were seen in 4 of 4 mice bled immediately antemortem. Amyloid, histochemically proven by congo red stain, was found in the kidneys and ileum of two C3H controls and one experimental mouse, but was not accompanied by significant proteinuria.

Hematological deaths

One $C3H/(C3H \times T_6)F_1$ mouse (Table 3) had thrombocytopenia, intestinal hemorrhage and lymphosplenomegaly, all hallmarks of classical acute HVG disease. The other was sacrificed at 196 days because of severe pancytopenia, found due to marked hypoplasia of hematopoietic cells in marrow and spleen.

Deaths with no lethal lesions

This category (Table 3) included mice which died without a cause which could be detected despite thorough autopsy, and mice which were thought to have tumors or infections (usually pneumonia), but which at autopsy were found to be free of lesions with killing potential. While 11 of 31 untreated C3H mice ranging in age from 272 to 774

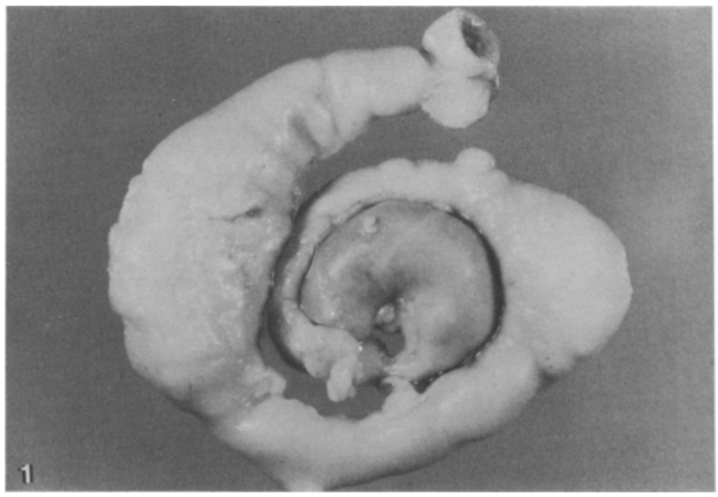

Fig. 1. Lymphomas are shown arising from two greatly enlarged Peyer's patches located in the jejunum not far distal from the centrally positioned stomach.

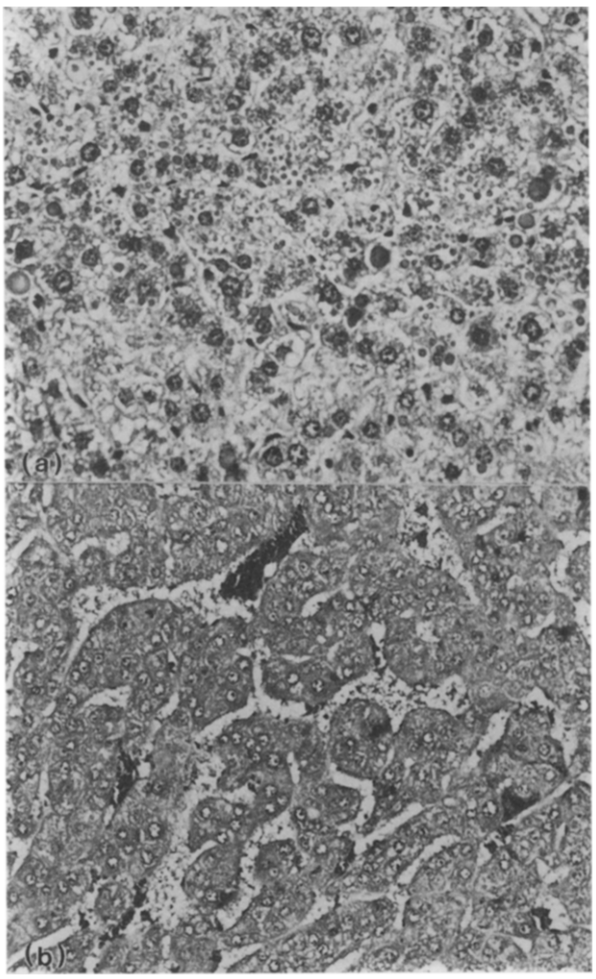

Fig. 2a. *Hepatoma, type A. The closely packed disordered neoplastic cells show extensive vacuolization and contain hyalin droplets of various sizes in their cytoplasm.* (*H & E* × 250).

Fig. 2b. *Hepatoma, type B. The irregular papillary arrangement of neoplastic liver cells outlines enlarged sinusoids.* (*H & E* × 140).

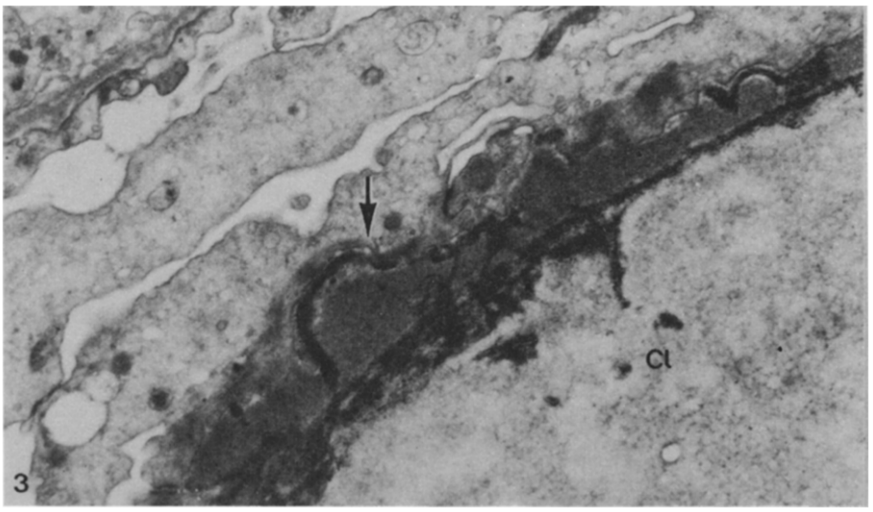

Fig. 3. Kidney. Electron dense deposits are seen external to the basement membrane and in the overlying cytoplasm of the epithelial cell (arrow). Fusion of the foot processes is also present (cl = capillary lumen) (× 10,380).

Table 2. Deaths due to infections and renal disease in C3H mice inoculated with (C3H × T_6)F_1 spleen cells [C3H/(C3H × T_6)F_1 mice], or with C3H cells (C3H/C3H mice) or untreated (C3H mice)

	Infections		Membranous glomerulonephropathy	
	Incidence	MST (range) (days)	Incidence	MST (range) (days)
C3H/(C3H × T_6)F_1	12/57(21%)	311*(149–702)	6/57(11%)*	342(210–608)
C3H/C3H	2/13(15%)	454(337–570)	0/13	
C3H	9/31(29%)	384(208–609)	0/31	

*$P<0.05$ for C3H/(C3H × T_6)F_1 versus C3H control groups.

Table 3. Summary of causes of death in untreated C3H mice and the C3H recipients of (C3H × T_6)F_1 spleen cells [C3H/(C3H × T_6)F_1 mice], or of C3H cells (C3H/C3H mice)

	C3H/(C3H × T_6)F_1		C3H/C3H		C3H	
Cause of death	Incidence	MST (days)	Incidence	MST (days)	Incidence	MST (days)
Infections	21%	311*	15%	454	29%	384
Renal	11%*	342	0		0	
Leukemia	13%*	380	0		0	
Lymphoma	50%*	462	69%*	423*	23%	567
Hepatoma in males	89%	545	50%	488	67%	561
All tumors	72%*	475	77%*	437	42%	588
Hematological	4%	152	0		0	
No lethal lesions	4%*	362	15%	393	32%	521

*Significantly different from control C3H values ($P<0.05$).

days were found free of significant disease at death, only 2 of 57 C3H/(C3H × T_6)F_1 littermates were found healthy at 272 and 425 days.

The causes of deaths in all groups are summarized in Table 3.

Expression of ecotropic MuLV

Tests of normal C3H mice and their F_1 hybrids showed that, with aging, there was a progressive increase in the numbers of mice with spontaneously expressed ecotropic MuLV, up to about a 75% incidence of positive mice (group I in Table 4).

The inference that donor C3H and (C3H × T_6)F_1 cells could serve as sources of infectious MuLV in cell transfer experiments was proven in the second set of experiments, where virus producing cells were quantitated in donor and host spleens (group II). The data show quite clearly that if virus were present in the donor cellular inocula, the C3H recipient was a susceptible host and was virus positive when tested at 3–6 weeks, an age much younger than that at which untreated C3H mice express MuLV spontaneously. The quantitative data showed good correlation between the number of oncornavirus producing cells present in the donor cellular inocula, and the number present in the host spleens after inoculation. However the identity of this virus with that of the donor cells has not yet been proven. Where ecotropic MuLV was isolated from most spleens, virus producing cells were also recovered in comparable numbers from the lymph nodes, but not the thymi of the recipients. The association of lymphocytic malignancy with ecotropic MuLV in 8/8 C3H/(C3H × T_6)F_1 mice (group III) was underscored by the discovery of two mice which were unusual on two counts. They were free of lymphoma and other major disease, and they did not express detectable amounts of ecotropic virus. We can only speculate that these mice were among those which were not expressing virus spontaneously at the time of sacrifice, and which had not received MuLV producing F_1 donor cells.

Survival of skin grafts

It was obvious that C3H mice, which had received perinatal inoculations of (C3H $\times T_6)F_1$ spleen cells had impaired ability to reject skin grafts bearing histocompatibility antigens of the T_6T_6 parent (Table 5). That there was not a generalized depression of immunologic reactivity can be inferred by the normal rejection of unrelated RFM skin grafts. In preliminary studies with phytohemagglutinin, no differences were seen in the reactions of control C3H and experimental mice. This provided further evidence against the existence of severe immunodeficiency in C3H/(C3H $\times T_6)F_1$ mice.

DISCUSSION

The most striking pathological effects of the perinatal inoculation of spleen cells into C3H mice were the greatly increased incidence of neoplasms in general, and of lymphomas in particular. Both semi-allogenic (C3H $\times T_6)F_1$ and isogenic C3H cells appeared capable of increasing the rate of lymphomagenesis. It was the high incidence of lymphomas in the C3H recipients of C3H cells which indicated that some factor other than a putative T-cell deficiency was of greater etiologic importance in the development of lymphoid malignancies in C3H/(C3H $\times T_6)F_1$ mice. The virological studies revealed the presence of ecotropic MuLV in both C3H and (C3H $\times T_6)F_1$ donor cell inocula, the earlier expression of an oncornavirus in the C3H recipients of such inocula and the production of infectious virions by the lymphomatous and leukemic tissues of all C3H/(C3H $\times T_6)F_1$ mice tested. It is pertinent that virus was never detected in the thymus, an organ never the primary site of the lymphomatous process. The consistency of these findings, taken together with the well established association of MuLV with lymphomagenesis [27–29] and the known susceptibility of neonates to oncornaviral infections [10] have forged links of circumstantial evidence implicating MuLV of donor cell origin in the development of lymphomas in C3H/(C3H $\times T_6)F_1$ mice and C3H/C3H littermates.

It was of interest to observe the occurrence of earlier deaths due to infections, membranous glomerulonephropathy, leukemia and type B hepatomas primarily or exclusively in C3H/(C3H $\times T_6)F_1$ mice. This clustering deserves further investigation, for the data provide no obvious explanation. Because of the high spontaneous incidence of hepatomas in untreated C3H males, and the failure to detect evidence of immunodeficiency in C3H/(C3H $\times T_6)F_1$ mice, it was not possible to attribute the appearance of these hepatocellular tumors in the C3H/(C3H $\times T_6)F_1$ mice to an impaired immune surveillance system. The prolonged survival of the (C3H $\times T_6)F_1$ skin grafts suggested the important possibility that the original F_1 spleen cell inocula had also been accepted for a prolonged interval.

The association of ecotropic MuLV with abnormal lympho-reticular hyperplasia was observed by Simpson *et al* [4] in three of the four HVG mice tested. The work of Cornelius [5] suggested that an oncornavirus had been inoculated with the donor cells, for an increased incidence of lymphomas developed in C57 Bl/1 mice inoculated with F_1 hybrid spleen cells within 24 hr of birth, but not after. In a recent preliminary report [15], he presented evidence that the lymphoid tumors were composed of transformed host cells which expressed ecotropic MuLV. We have recently isolated infectious N-tropic MuLV from the lymphomas which appeared within 6 months in 5 of 5 RFM mice given only a single neonatal inoculation of spleen cells from 12 month-old ($T_6 \times$ RFM)F_1 mice. The donors were free of tumor on histopathologic examination, and their spleens also expressed infectious N-tropic MuLV (Cross and Hard, unpublished). Thus, premalignant and malignant changes in the lymphoid system developed in association with ecotropic MuLV in all of the 5 parent/F_1 hybrid systems studied. The data presented in this report have provided the strongest evidence currently available, favoring a donor F_1 cell origin for the ecotropic MuLV found in association with the pre-malignant and malignant lymphoid tissue changes seen in HVG models.

The earlier onset and increased incidence of lymphomas observed in our experiments seemed strikingly similar to the results reported by the pioneers [30, 31] who inoculated AKR MuLV into newborn AKR mice which were already viremic [32]. How the neonatal inoculation of oncornavirus can lead to an increased incidence of lymphomas in mice in which an ecotropic MuLV is already endogenous is not well understood. Croker *et al.* [33] found increased amounts of C-type virus in the lymphoid tissues of mice which had been infected with MuLV as neonates. At the molecular level, work by Berns and Jaenisch [34] indicated that superinfection may cause an earlier increase in the number of copies of

Table 4. Ecotropic murine leukemia virus in normal C3H mice, (C3H × T_6)F_1 hybrid and C3H recipients of C3H or (C3H × T_6)F_1 hybrid spleen cells

Group	Test mice	Donor cells: Source	Donor cells: Age (weeks)	Host spleen cell cultures: Age (weeks) 3–6	8–12	14–18	25–36	66–110	No. spleen cells expressing MuLV/10^6 cells*: Donor	Recipient
I. Normal	C3H			0/11†	0/10	2/11	9/11	12/16		
	(C3H × T_6)F_1			1/8	1/6	3/4	18/22			
II. Spleen	C3H/C3H	C3H	16–20	0/2					1	
. Cell			24–60	4/4					55–2000	5, 42, 250, 350
. Transplant‡	C3H/(C3H × T_6)F_1	(C3H × T_6)F_1	7–10	0/2					0	
			20–24	3/7					1–8	0.1, 0.1, 0.3
			52–56	4/4					15–350	0.4, 1, 4, 20
III. Pathological	C3H/(C3H × T_6)F_1 with:									
	Lymphoma							7/7		
	Leukemia							1/1		
	Renal death						1/1			
	No lethal lesions							0/2		

*Detected by UV-XC plaque test.
†Number of mice positive/number of mice tested.
‡15×10^6 spleen cells inoculated into newborns and 100×10^6 cells given at 7 days of age.

Table 5. Fates of related and unrelated skin grafts on normal C3H mice, or C3H mice which had been perinatally inoculated with $(C3H \times T_6)F_1$ spleen cells [$C3H/(C3H \times T_6)F_1$ mice] or with C3H cells (C3H/C3H mice)

	Donor† skin						
	$(C3H \times T_6)F_1$		C3H	T_6T_6		RFM	
	Takes	MST‡(range)	Takes	Takes	MST(range)	Takes	MST(range)
Host*	Total	(days)	Total	Total	(days)	Total	(days)
$C3H/(C3H \times T_6)F_1$	1/13	46(24–75)	7/7	1/7	29(15–40)	0/6	13(11–14)
C3H/C3H	0/5	14(10–17)	2/2	0/3	14(10–16)	0/3	13(12–14)
C3H	0/4	14(11–17)	6/6	0/3	13(10–16)	0/3	12(10–14)

*Aged 125–360 days.
†Same sex as host.
‡Mean survival time of rejected grafts.

MuLV in the cell genome. Both teams were able to correlate evidence of increased amounts of virus with tumor formation. Inoculation of a second or recombinant MuLV with increased oncogenic potential [35] is another possible explanation for the increased incidence of lymphomas in the recipients of virus producing cells. Further testing may determine if either or both mechanisms are operative in the $C3H/(C3H \times T_6)F_1$, C3H/C3H and other systems.

Lymphomas are also one of the sequelae of chronic GVH reactions [14], although the association with ecotropic MuLV has not been so consistent as that seen in HVG models. In studies of a subline of C3H mice normally free of lymphoid tumors, Walford [36] found that lymphomas developed in hosts neonatally inoculated either with weakly histoincompatible C3H.K, or with isogenic C3H spleen cells. In the allogenic situation, lymphomagenesis was attributed to the proliferation of donor cells in response to host antigens. It was noted that this interpretation did not explain the increased incidence of lymphomas in the recipients of isogenic cells. The work of Hays [37] and Cornelius [38] showed clearly that the rapid appearances of tumors in their GVH systems were due to highly oncogenic viruses produced by the parental strain donor cells. In apparent contrast, cell free extracts of parental lymphoid cell inocula failed in some cases [14, 39] to induce tumors in young adult F_1 hybrid recipients, which regularly developed lymphomas following inoculations of intact cells. However, these observations did not rule out the possibility that infectious MuLV had been inoculated with the donor cells, because, to avoid death from acute GVH disease, these models required the use of F_1 hosts well past the age of susceptibility to all but the most highly oncogenic viral agents. More recently, it has been found that MuLV, endogenous but not expressed, can be activated by GVH reactions. When it was discovered that only xenotropic viruses were activated by allogenic reactions [40, 41] it was suggested that the ecotropic MuLV found in earlier studies [42] had probably been inoculated with the parental cells. Datta and Schwartz [43] initiated a large study of the oncogenic effects of inoculation of parental strain spleen cells expressing high or low titers of ecotropic MuLV into several varieties of related, young adult F_1 hybrid mice. They concluded that there was no correlation between the titers of either ecotropic or xenotropic MuLV's and the lymphomas which occurred within 7.5 months of the last inoculation of parental spleen cells. However, this is a relatively short observation period for detection of malignant transformation by most oncornaviruses. Also, quantitation of the amount of virus expressed may not have been an adequate measure of oncogenicity.

The increased incidence of lymphomas in two forms of parent-vs-F_1 hybrid allogenic disease suggests there may be factors in common which promote lymphoid tumor development. It is striking that for induction of disease, both GVH and HVG models require the inoculation of adult cells into recipients unable to reject them because of genetic make up or immunological immaturity. The prolonged residence of cells which continued to produce ecotropic MuLV would increase the total dose of the virus over an extended

period, and could thereby enhance the possibility of superinfection (or repeated reinfections) of host cells. The later development of immunodeficiency in some GVH [44] and HVG [4,8] systems could increase host susceptibility. Although Gleichman *et al.* [45] could find no correlation between immunodeficiency and lymphomagenesis in GVH mice, Varet *et al.* [39] noted the increased incidence of lymphomas following GVH reactions in mice already infected with ecotropic MuLV of low oncogenicity. In HVG mice, it would appear that three factors converge to favor the association of ecotropic MuLV with the premalignant and malignant lymphoid cell changes: (1) the age related susceptibility of the neonatal hosts to oncornaviruses; (2) tolerance of the donor, virus producing cells (as suggested by the skin graft data in Table 5); and (3) the later development of immune deficiency in some cases. Genetic susceptibility of the hosts to MuLV [29] and the oncogenicity of the inoculated MuLV would be additional variables of importance, especially in the GVH systems. It is not our intent to suggest that the ecotropic MuLV of donor cell origin is the only, or even the most important factor in the development of lymphomas in chronic allogenic diseases. It seems clear, however, that there is a definite association in some cases. Recent work has shown that some ecotropic viruses may increase the oncogenic potential of other MuLV's [46], and that certain isolates of ecotropes, which could not be detected by the XC plaque assay, may have high oncogenic activity [47].

Although work on oncogenesis in HVG systems is still in its very early stages, the consistency in the reports from different laboratories supports the idea that lymphomas associated with ecotropic MuLV expression can be expected in those hosts which avoid or survive lethal HVG disease. The determination of whether or not ecotropic MuLV of donor cell origin plays a pathogenic role will have to await further characterization of the virus(es) isolated from donor spleen cells and the tumors found in the hosts, and more studies to confirm that the tumours are of host origin.

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