The role of the thymus in Friend virus-induced leukemia in mice
by Nicola Mitri Kouttab

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements of the degree of DOCTOR OF PHILOSOPHY in Microbiology
Montana State University

Abstract:
The purpose of these studies was to examine the role of the thymus in acute lymphocytic leukemia by Friend virus and to understand more clearly the nature of the target cell for the virus. For these studies, congenitally athymic (nude) mice, their phenotypically normal littermates, CBA and Balb/c mice were used. Nude mice implanted with either Balb/c thymuses or littermate thymuses were injected with Friend virus. The leukemic process was compared between these two groups, and also compared to the leukemic process in littermates and Balb/c mice. It was found that nude mice without thymus implants, although dying rapidly following virus-challenge, had only developed a mild leukemic process as judged by hematologic and pathologic data, and may have died from a generalized virus infection. In contrast, nude mice implanted with thymuses developed a typical leukemic process similar to that seen in littermate and Balb/c mice.

The results of experiments designed to ascertain the existence of cellular immunity against the leukemic cells, and humoral immunity in the form of virus neutralizing antibodies in nude mice implanted with CBA thymuses (the CBA mouse being totally refractory to Friend virus) or Balb/c thymuses showed that by the method employed no immunity was produced in these mice. Since the results of a long-range experiment using nude mice implanted with CBA thymuses showed that these mice eventually develop typical leukemia, it was concluded that a likely role of the thymus relates to the activation and proliferation of bone-marrow cells which serve as target cells for the virus. This conclusion was strengthened by the use of endotoxin in nudes and littermates. Nude and littermate mice given endotoxin (a bone-marrow cell mitogen) and Friend virus developed a more severe leukemia.

To determine the nature of the target cell, Balb/c mice were immunosuppressed with anti-μ, a known B lymphocyte immunosuppressant, from the day of birth and on alternate days until termination of the experiment. These mice were injected with Friend virus at day 38 of age. For comparison Balb/c mice were injected with either normal rabbit serum or phosphate buffered saline. Results of preliminary experiments showed that the leukemic process was abrogated in Balb/c mice immunosuppressed with anti-μ but not in control mice. Therefore, it was concluded that the target cell is an μ-bearing cell of an antibody-producing lineage.

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ABSTRACT

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INTRODUCTION

The murine leukemia viruses have been classified morphologically into "splenic" and "thymic" viruses (1) depending on the nature of the target cell. Although some viruses infect thymus cells to yield a leukemic process (2,3,4), Friend virus (FV) has been shown to infect bone-marrow-derived cells (2,5), whereas, little or no evidence exists that thymus-derived cells are infected by the virus.

In a previous study (6), it was shown that the plaque forming cell (PFC) response of Balb/c mice to sheep red blood cells was inhibited if these mice were treated with FV. This study agrees with other studies (7,8) which used electron microscopy that the virus affects cells of antibody-producing lineage. Other studies (9,10) showed that virus-like particles are present in lymphoid cells presumed to contain or secrete antibodies. Virus particles were identified in immature blast-like lymphoid cells and not in plasma cells, suggesting that there was a specific effect on early progenitors of antibody-producing cells (11). Later studies (12) showed the presence of virus-like particles in PFC, however, there is evidence (13) that these particles are non-infectious, and therefore, their exact role is not known. In their studies, Koo and coworkers (14,12) showed that the PFC response to SRBC was diminished or inhibited depending on the time of virus injection prior to immunization. They postulated that the mechanism of immunosuppression was due to the

competition between the virus and SRBC antigen for the same cell (potential antibody-producing cell). These same workers further postulated that only cells that are infected by the virus at an early stage are arrested in their function and maturation, whereas mature antibody-forming cells may be infected but are not affected, and thus maintain their normal functions. However, it has not been shown at what stage of development or maturation the potential antibody-forming cell may be infected by the virus.

There is little evidence that FV, like the Gross virus (2), infects thymus-derived cells (T cells) or that the virus even requires the presence of T cells for the leukemic process. There is convincing evidence that T cells may function to inhibit or even destroy lymphoid cells having undergone malignant transformation. Thus, Haran-Ghera (15) has shown that interaction of radiation leukemia virus with thymuses produces a resistance in the host to lymphoid leukemias induced by the same virus. The author demonstrated that resistance was associated with a thymus-derived lymphocyte population.

Under these circumstances, it is presumed that the leukemia virus induced an antigenic change in the infected cell which triggered, in turn, a T cell mediated immune response. Adoptive immunity to transplanted, viral and spontaneous leukemia in mice has been also demonstrated by several workers (16,17,18) who used allogeneic

cells obtained from lymph nodes, and spleen. Mathe and coworkers (17) also showed that adoptive immunotherapy may be successful in humans with acute lymphoblastic leukemia.

Studies with thymectomized animals are subject to objections unless the contribution of the thymus prior to birth is assessed (19). It has been suggested (20) that unless the animals involved are known to lack an in utero epithelial component of the thymus, the distinction between thymus-independent and thymus-dependent antigens is meaningless. Therefore, in order to overcome difficulties in interpretation of data from experiments employing thymectomized mice we have used the nude mouse, described by Flanagan (21) and shown to be congenitally athymic (22), to study the effect of the thymus on Friend virus-induced leukemia in mice.

Thus, these studies were designed to ascertain (a) the effect of Friend virus (FV) on nude mice with or without thymus implants, and on their phenotypically normal littermates, and Balb/c mice (the usual host animal for the virus), (b) the mechanism by which the thymus exerts its effect (if any) on the leukemic process, and (c) the target cell for the virus. The results show that the thymus influences the development of a typical leukemic process in nude mice through its effect on a μ-bearing target cell.

MATERIALS AND METHODS

Mice

Inbred conventionally reared CBA and Balb/c male and female mice ranging in age from 4-6 weeks were used. The CBA mice were originally obtained from Jackson Memorial Laboratories, and have since been maintained in our laboratory by brother-sister mating. These CBA mice were found to be resistant to FV. The Balb/c mice were originally obtained in 1966 from the National Cancer Institute in the germfree state, and were conventionalized in 1967. The Balb/c mice have since been maintained by random mating in our laboratory.

The homozygous nude (nu/nu) mice and their phenotypically normal littermates (+/nu and +/+) were the offspring of heterozygous (nu/+) animals obtained by crossing nu/nu males with females from our Balb/c colony. The nude mice and littermates were housed in a clean environment. All mice received sterilized Purina 5010C and acidified-chlorinated water (23) ad libitum.

Production of Leukemia in Mice

Friend leukemia virus (FV) was obtained from Dr. Fieldsteel of the Stanford Research Institute in 1968 and has been maintained in our laboratory by frequent passage in adult Balb/c mice. For experimental work, a virus stock was prepared as has been previously described (6).

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Four to six week old mice were divided into designated groups and injected intraperitoneally (IP) with 0.2 ml of various doses of virus stock. The mice were allowed approximately 2-3 weeks to develop the leukemic and then were assayed for leukemia.

The criteria used to follow the leukemic process have been described (6). Briefly, these consisted of splenomegaly, total and absolute white blood cell (WBC) counts and differential counts of the peripheral blood smears to check for abnormalities of blood cells, and to derive the absolute WBC counts. The counts were initially obtained at weekly intervals, then at two-week intervals. In addition, bone-marrow (BM) smears from the tibia and femur of nude, littermate and Balb/c mice injected with virus were made at 30 days post-virus injection. The bones were placed in Hank's balanced salt solution, pH 7.2, in an ice bath, the ends cut, and the marrow aspirated from the lumen. Cell suspensions of the extracted marrow were made by gently passing it through a syringe. Smears were then made, stained with Wright's blood stain and differential counts performed. Body weights and tissue sections from liver, spleen, kidney, intestines and lungs were also used for evaluation of the leukemic process.

Thymus Implantation

The two thymuses from one donor were implanted into one recipient mouse, one gland in each axillary region of the recipient. The

implants were allowed three weeks to become established in the mice before the mice were used for experiments. The status of the implants in experimental mice was determined by histological sections.

Electron Microscopy

Sections from liver, spleen, kidney, lung and nudes were studied with the electron microscope. Tissues were fixed in 2.5% glutaraldehyde for 1 hour at 4°C., followed by 1% osmium tetroxide for 1 hour at 4°C. The cells were then processed through the epoxy resin technique according to Spurr (24), thin sectioned, stained with uranyl acetate and lead citrate, and examined in a Zeiss EM-9A electron microscope.

Titration of Virus Obtained from Nudes

Spleens of nude mice injected with FV were aseptically removed and a 20% suspension made in a sucrose stabilizer described by Bovarnick et al. (25). The suspension was homogenized in a Sorvall Omni-Mixer type)M-1150 at 4°C, and the homogenate centrifuged at 2,000 rpm for 10 minutes at 4°C. The LD_{50} of the virus recovered in the supernatant was estimated in Balb/c mice according to the Reed-Muench method (26).

Mitogens

Three different mitogens were used, phytohemagglutinin-M (PHA, GIBCO), pokeweed mitogen (PWM, GIBCO), and endotoxin (LPS). The LPS from Escherichia coli 0113, extracted by the phenol-water method (27) was supplied by Dr. J. A. Rudbach (University of Montana). The PHA and PWM were reconstituted with 10 and 5 ml of sterile distilled water, respectively, dispensed into 1 ml aliquots and frozen until needed. The LPS was dissolved in PBS at a concentration of 1 mg/ml, divided into 1 ml aliquots and stored frozen until needed. Since nude mice have been found to have a strong sensitivity to LPS (Jutila, personal communication) and died within a short period after administration, nude mice were given an aqueous solution composed of bacitracin (Commercial Solvents Corp., Indiana), neomycin sulfate (Biosol, The Upjohn Co., Michigan), and vitamins (Syr-vite, Wolins Pharmacal Corp., New York), in amounts of 4 gms/liter, 29 ml/liter, and 3.3 ml/liter, respectively, all dissolved in 1 liter of distilled water. The mice were placed on antibiotics for 1 week prior to injection of LPS.

Preparation of Immunoglobulins and Antisera

The immunoglobulins and the antisera have been prepared as previously described (28).

RESULTS

The Leukemic Process in Nudes

The absolute lymphocyte counts for nudes, littermates and Balb/c mice given either 2.24 LD_{50} doses (10^{-4} dilution of stock virus) or 224 LD_{50} doses (10^{-2} dilution of stock virus) of FV are compared in Figures 1 and 2 over an 80 day period. At the onset of the experiment, normal values for Figure 1 were 1008 cells/mm^3 for nudes, 2300 cells/mm^3 for littermates, and 1533 cells/mm^3 for Balb/c mice. For Figure 2, the normal values were 2412 cells/mm^3 for nudes, 2120 cells/mm^3 for littermates, and 1777 cells/mm^3 for Balb/c mice. With both doses the lowest counts were obtained in nudes and littermate mice, as contrasted to higher counts in Balb/c mice indicating that the latter were more susceptible to the virus. With a higher virus concentration (224 LD_{50} doses), Balb/c mice exhibited counts in excess of 80,000 cells/mm^3, as contrasted to nudes which yielded counts not exceeding 15,000 cells/mm^3 at death. Although the absolute lymphocyte count for nudes was low in comparison to littermates and Balb/c mice, an elevated lymphocyte count at death suggested that FV produced a low grade leukemia. That this elevated count in FV injected nudes is due to the virus can be seen in Table 1 which compares the absolute lymphocyte counts of various groups of nude mice, including a group which was untreated in any way and used to test the effect of the conventional environment on nude mice. This group was designated

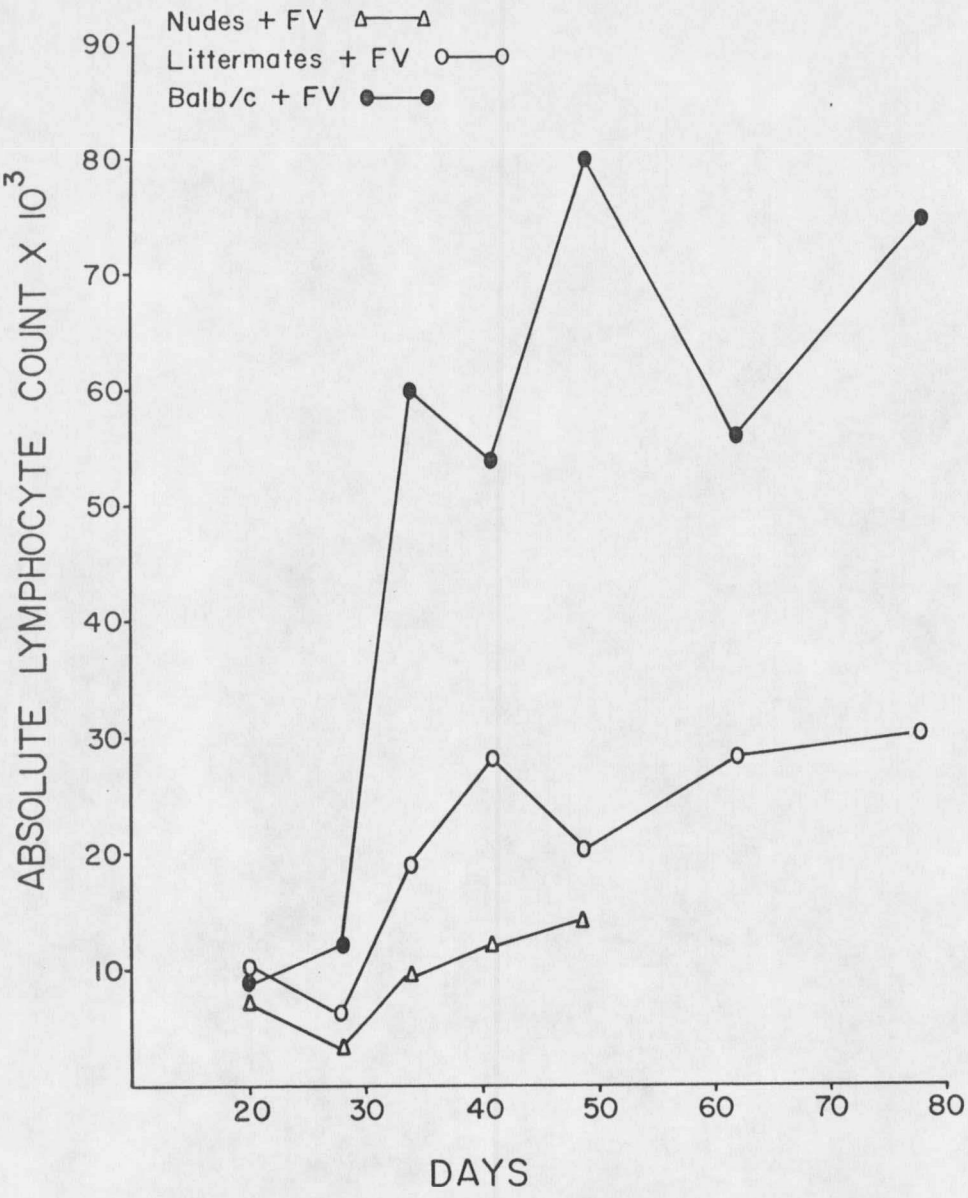

Figure 1. Absolute lymphocyte counts of nude, littermate, and Balb/c mice injected with 224 LD_{50} doses of Friend virus (FV).

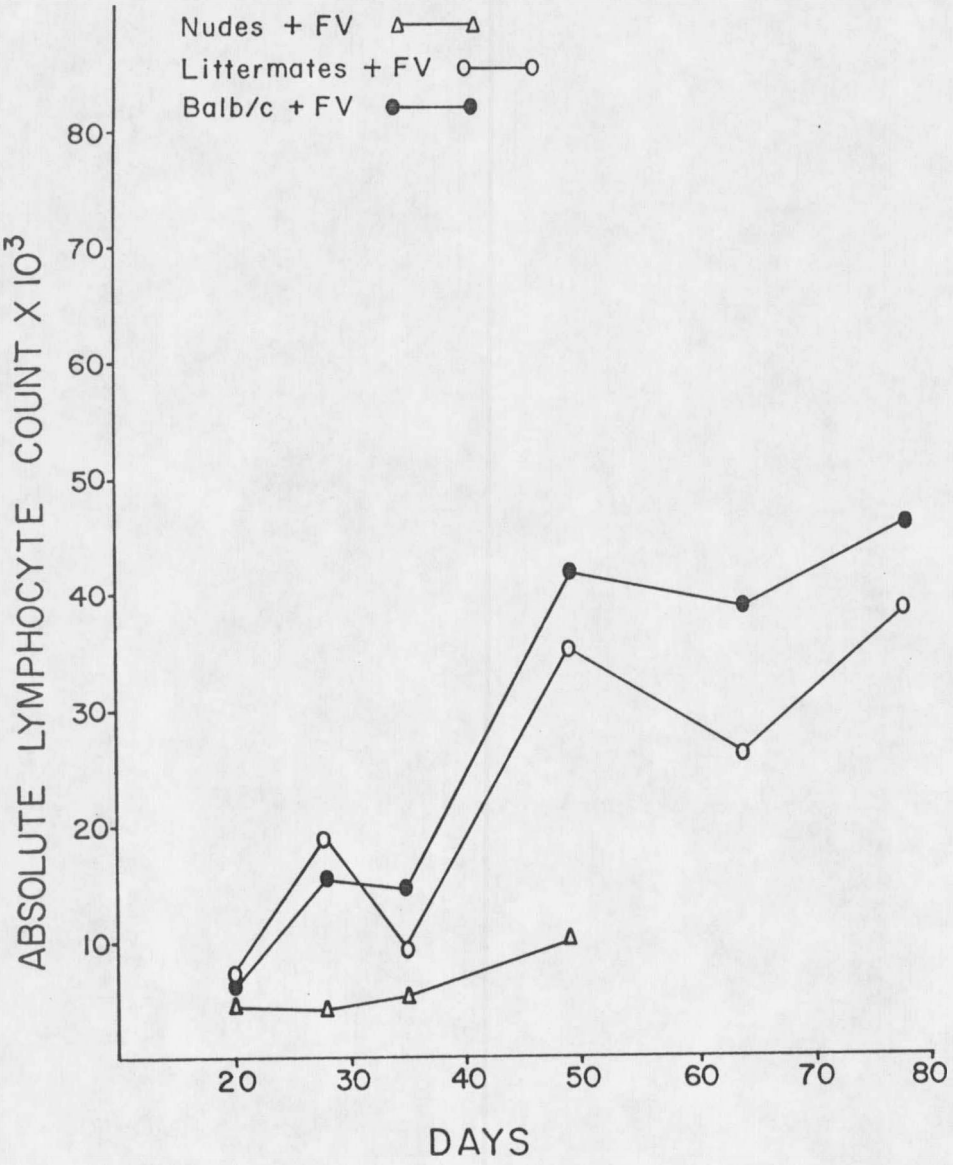

Figure 2. Absolute lymphocyte counts of nude, littermate, and Balb/c mice injected with 2.24 LD_{50} doses of Friend virus (FV).

Table 1. Absolute lymphocyte counts of nude mice challenged or not challenged with Friend virus (FV).

Days post virus injection	Nude* controls	Nudes[a] + FV 10^{-2}	Nudes[b] + FV 10^{-4}
20	1505	7414	4771
34	4881	9880	5349
48	3252	14492	10461
62	2505	--	--
78	3976	--	--

a - FV 10^{-2} = 224 LD_{50} doses of Friend virus.

b - FV 10^{-4} = 2.24 LD_{50} doses of Friend virus.

* - These nude mice, untreated in any way, were used to check the effect of the conventional environment on nude mice.

"nude controls". The table shows that over a 48 day period nude mice injected with either 10^{-2} (224 LD_{50} doses) or 10^{-4} (2.24 LD_{50} doses) dilution of FV stock attained higher counts than "nude controls". Table 2 shows that the absolute neutrophil count of "nude controls" was significantly higher than the other groups. This increase in neutrophils could be a compensatory action due to their depressed lymphocyte count or reflects an infection process.

Effect of Allogeneic Thymus on Leukemia in Nudes

Since the most important difference between nudes and their phenotypically normal littermates is the absence of the thymus in the former, the above study suggested that the thymus may play a role in the enhancement of leukemia. In order to explore this possibility, nude mice were implanted with littermate or Balb/c thymuses. After allowing time for establishment of the thymuses in nude mice, FV was given at a dilution of 10^{-4} of FV stock (2.24 LD_{50} doses). The leukemic process in these mice was then compared with sham-operated nudes, littermates and Balb/c mice, all injected with the same dose of virus. Figures 3 and 4 compare the absolute lymphocyte counts for these groups of mice. The data illustrated that thymus-grafted nudes attained higher counts than sham-operated nudes and reached levels that are comparable to those observed in mice from which the thymuses were taken.

Table 2. Absolute neutrophil counts of nude mice challenged or not challenged with Friend virus (FV).

Days post virus injection	Nude* controls	Nudes[a] + FV 10^{-2}	Nudes[b] + FV 10^{-4}
20	1278	3214	451
34	6904	1130	1006
48	6504	4789	1231
62	8882	--	--
78	10456	--	--

a - FV 10^{-2} = 224 LD_{50} doses of Friend virus.

b - FV 10^{-4} = 2.24 LD_{50} doses of Friend virus.

* - These nude mice, untreated in any way, were used to check the effect of the conventional environment on nude mice.

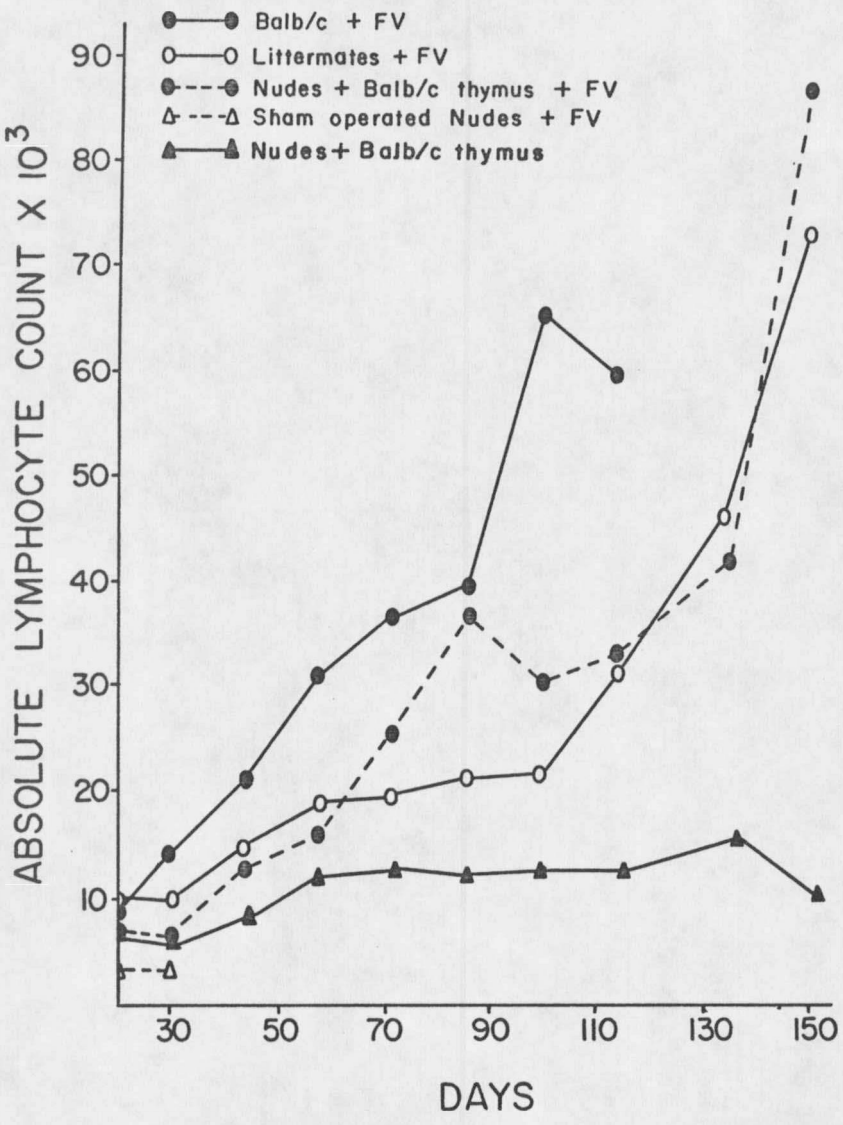

Figure 3. Absolute lymphocyte counts of nudes receiving Balb/c thymus and of sham operated nudes, littermates, and Balb/c mice injected with 2.24 LD doses of Friend virus (FV)

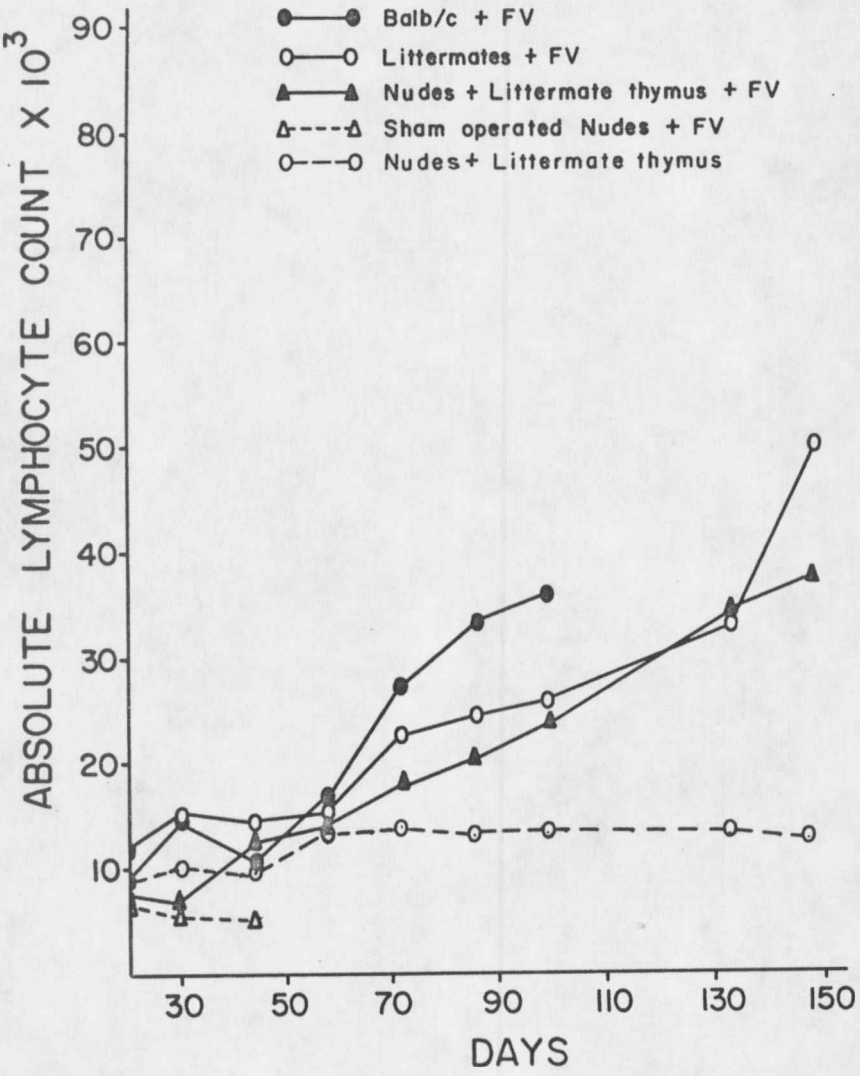

Figure 4. Absolute lymphocyte counts of nudes receiving littermate thymus and of sham operated nudes, littermates, and Balb/c mice injected with 2.24 LD_{50} doses of Friend virus.

Cytologic observations. Both Balb/c and littermate mice infected with FV showed changes in the nuclei of peripheral lymphocytes. These were characterized by lobulation, increase in size and indentations to yield nuclei resembling those of monocytes. The blood smears showed large numbers of degenerated cells which were classed as basket or smudge cells. Abnormalities were also seen in red blood cells (RBC), most prominently polychromatophilia and nucleated RBC. These changes, although present in nude mice, were not observed as frequently as in the other mice. However, this may well be due to the fact that the nude mice had died within a short period (30-46 days) following virus injection.

A possibility to be considered is that the death of the nude mice given FV is due to erythroleukemia. Yomoto and co-workers (29) using FV in different dilutions have shown that in both Balb/c and AKR mice, erythroid leukemia was present in short term survivors (30-75 days post virus injection, with the exception of two mice which showed erythroid leukemia in 110 days), whereas lymphatic leukemia was present in long term survivors. However, data from bone-marrow smears, hematocrits and peripheral RBC counts showed no difference between normal and FV injected mice. Therefore, the possibility of erythroleukemia was dismissed.

Pathologic studies. Table 3 compares body weights, mortality and survival time for nudes, thymus grafted nudes and Balb/c mice in

Table 3. Mortality among thymus-transplanted nudes, sham-operated nudes, Balb/c and littermate mice injected with 2.24 LD_{50} doses of Friend virus (FV).

Group	Average Body wt.*	Death/ No. in group	Mean survival Time (days)
Normal nudes	20.22/15.97	7/ 7	88
Exp. 1#			
Sham-operated Nudes + FV	15.00/10.55	10/10	43
Nude + Littermate Thymus + FV	14.00/18.07	9/ 9	155
Nude + Littermate** Thymus	18.14/22.98	0/ 4	--
Littermate + FV	20.91/30.64	10/12	188
Balb/c + FV	17.50/24.55	11/11	121
Exp. 2#			
Sham-operated Nudes + FV	14.19/11.22	10/10	29
Nude + Balb/c Thymus + FV	19.42/29.22	9/10	167
Nude + Balb/c** Thymus	20.35/26.03	0/ 5	--
Littermate + FV	22.74/29.12	6/10	188
Balb/c + FV	23.20/24.94	10/10	118

* - The numerator is body weight at start of the experiment, the denominator is body weight at death or sacrifice during the experiment.

** - The body weights of these mice were taken at the start and termination of the experiment.

two experiments. In both experiments loss of body weight was seen only in sham-operated nudes. The similarity between the two experiments in terms of mortality and survival time is striking. Both experiments indicate that the leukemic process in Balb/c mice is more severe (100% death) than in littermates (60-80% death) which is also indicated by the difference in survival time. Also noticeable is the prolongation of survival time of nude mice transplanted with either Balb/c or littermate thymus, as compared to sham-operated nudes. Thus, the thymus seems to have an effect on the general health of the nude mouse; protection of the immunodepressed nudes from environmental insults. Untreated nudes usually failed to survive beyond 90 days and died from a syndrome which could be designated as wasting (30).

Tissue sections from all groups were examined microscopically after death or sacrifice. In Balb/c mice, littermates and thymus-transplanted nudes there were significant changes in the spleen and liver. There was loss of normal architecture of the spleen due to massive accumulation of leukemic cells. In the liver there was extensive destruction of parenchymal cells and separation of lobules by the leukemic cells. It is of interest that livers in nudes transplanted with Balb/c thymus and given FV became very enlarged and most weighed in excess of 8 gms which was about one-third of the body weight of the animal. Enlargement of the liver was also seen in nude

mice transplanted with littermate thymus and injected with FV. There was also evidence of infiltration of leukemic cells into the alveolar septae and autolysis of the lung. The kidney and intestines appeared normal.

This pathologic picture was slightly different in sham-operated nudes given FV. The spleen exhibited many megakaryocytes, hemorrhage, and necrosis, and many reticular cells. The liver was infiltrated with neutrophils and many degenerated cells were present. Other organs appeared normal.

An examination of electron micrographs of organ thin sections from sham-operated nudes or normal nudes injected with FV revealed large numbers of C-type particles in the lymph nodes, lungs, spleen, liver and kidney which suggested that FV persists in nude tissue following challenge. Furthermore, the pathology of the spleen and liver indicated that the virus was active and may have multiplied in these organs.

Titration of virus. Nudes implanted with Balb/c thymuses and sham-operated nudes were challenged with 2.24 LD_{50} doses of FV stock. After the leukemic process had progressed for 30 days, the mice were sacrificed and their spleens aseptically removed. Virus was recovered from the spleens as described in Materials and Methods. Balb/c mice in groups of 5 were injected with 0.2 ml IP of each dilution of virus

ranging from undiluted to a dilution of 10^{-6}. For comparison, Balb/c mice were also injected with stock virus diluted in the same manner. The leukemic process in Balb/c mice was left to progress for 90 days after which the virus titer or each group was obtained by the Reed-Muench method (26). The mortality for each group was also recorded. The results of such a titration are shown in Table 4. The table indicates that the stock virus was the most active, followed by virus extracted from spleens of thymus-implanted nudes, and finally sham-operated nudes. Therefore, although virus multiplies in sham-operated nudes, it does so to a lesser extent than in thymus-implanted nudes.

The Role of the Thymus

The preceding experiments with thymus grafts suggested a role for the thymus in the production of a typical leukemic process in mice. However, the role of the thymus has not as yet been clearly defined. Two mechanisms may be operative: first, it is possible that the thymus provides immunity to the virus or leukemic cell, thus allowing the thymus-implanted nudes to survive for long periods. This immunity is eventually overcome by the leukemic process resulting in death of the animals from leukemia. Second, it is probable that the thymus synergizes with BM cells to cause the production of an increased number of target cells for the virus.

Table 4. Titration in Balb/c mice of stock Friend virus (FV), and FV obtained from spleen-homogenates of nude mice with or without Balb/c-thymuses, and given 2.24 LD_{50} doses of FV.

	Group providing virus and mortality in Balb/c mice					
	Stock FV		Nudes + FV		Nudes + Balb/c thymuses + FV	
FV dil.	No. deaths/No. injected	Survival# time days	No. deaths/No. injected	Survival time days	No. deaths/No. injected	Survival time days
Undil.	5/5	85	4/5	41	5/5	33
10^{-1}	5/5	62	3/5	41	5/5	53
10^{-2}	5/5	49	2/5	41	4/5	66
10^{-3}	5/5	69	1/5	74	3/5	70
10^{-4}	3/5	86	0/5	--	1/5	60
10^{-5}	1/5	78	0/5	--	0/5	--
10^{-6}	0/5	--	0/5	--	0/5	--
LD_{50}	$10^{-4.32}$		$10^{-1.42}$		$10^{-3.16}$	

- Where no survival time is given, the mice were all alive at termination of the experiment.

To test the above hypotheses, the effect of the thymus on the leukemic process in thymus-implanted nudes was evaluated in two ways. First, thymus-implanted nudes were studied for the production of humoral immunity in the form of virus neutralizing antibodies. Second, cellular immunity, if present, would be expected in thymus-implanted nudes challenged with virus, and not in unchallenged thymus-implanted nudes since these latter mice have not been exposed to the virus. Thus, a group of thymus-implanted nudes challenged or unchallenged with FV were followed over a long period to assess the progress of the leukemic process. For these experiments, nude mice implanted with CBA thymuses were used since the CBA mouse is refractory to the virus. It was expected that the CBA resistance to FV or the leukemic cells may be conferred to nudes by CBA T cells. For comparison, nudes implanted with Balb/c thymuses were also used.

Hematologic findings. Figure 5 compares the absolute lymphocyte counts of nudes treated in various ways, CBA, and Balb/c mice over a 164 day period. The pretreatment mean absolute lymphocyte counts for nudes, CBA and Balb/c mice were 2133 cells/cu mm, 5254 cells/cu mm and 9325 cells/cu mm, respectively. Sham-operated nudes died shortly after virus injection without exhibiting marked symptoms of leukemia. It was evident throughout the experimental period that nude mice transplanted with CBA thymus exhibited higher counts than

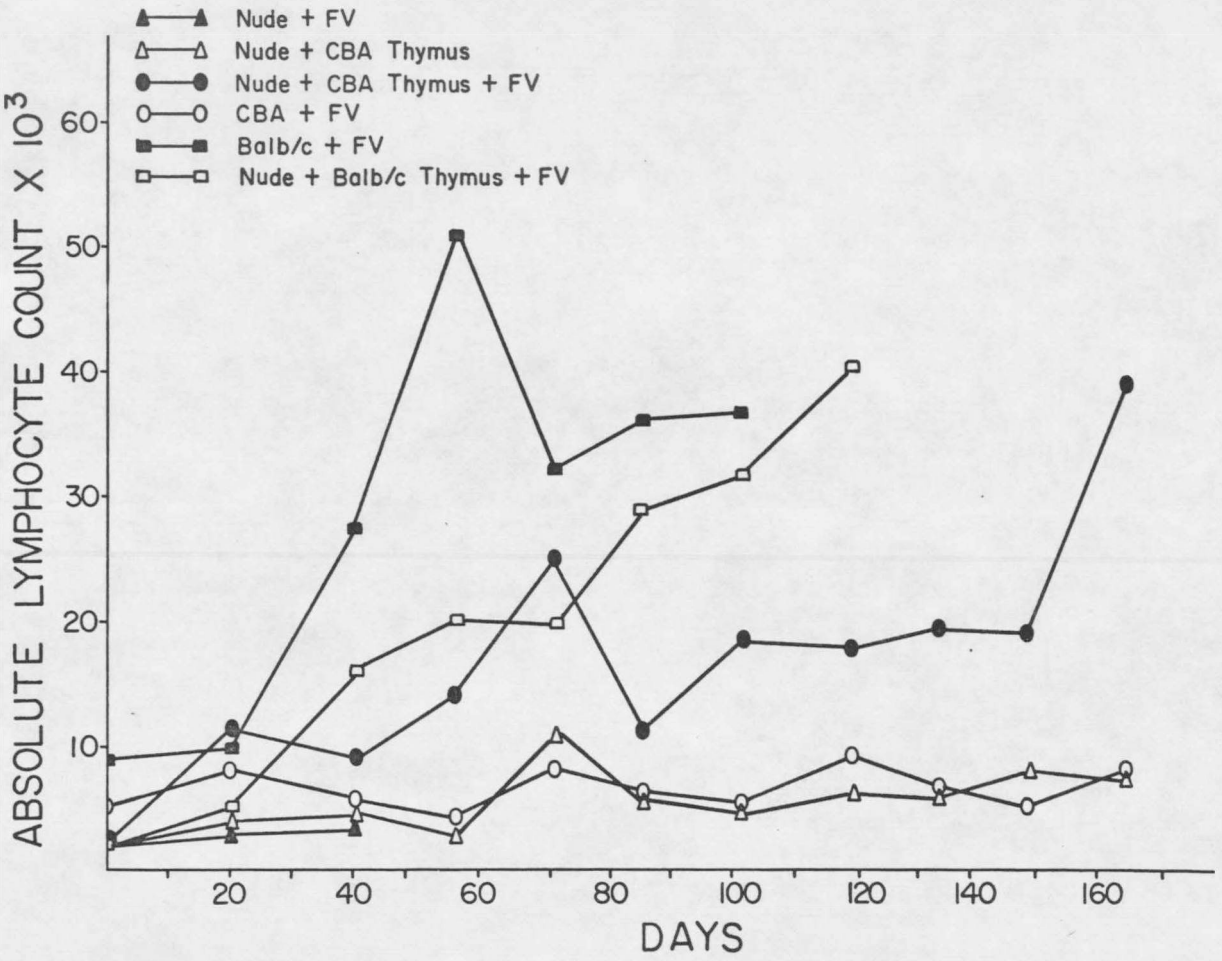

Figure 5. Absolute lymphocyte counts of nudes receiving CBA thymus, or Balb/c thymus, and of sham-operated nudes and Balb/c mice injected with 2.24 LD doses of Friend virus (FV).

either sham-operated nudes or nudes transplanted with CBA thymus but not injected with virus. This indicates that the CBA thymus in FV injected nudes was responsible for the higher count, and this becomes more important when it is noted that CBA mice are totally refractory to FV. As expected, Balb/c mice were the most susceptible to the virus. Also of interest is the observation that in nude mice transplanted with Balb/c thymus the leukemia developed rapidly, and spleen enlargement occurred early after virus injection. Abnormalities in peripheral lymphocytes and red blood cells were most prevalent in Balb/c mice, followed by nudes transplanted with either Balb/c or CBA thymus. Few abnormalities were seen in sham-operated nudes challenged with virus, probably due to their early death after virus injection. These abnormalities have been previously described (6). In contrast, CBA mice exhibited normal peripheral blood smears, indicative of their resistance to FV.

Pathologic findings. Table 5 compares body weights, mortality, spleen and liver weights, and survival time for the different groups of mice. Loss of body weight was seen only in sham-operated nudes. The data show that the leukemic process was most severe in Balb/c mice where all the mice died. The severity of the leukemic process can also be seen in thymus-transplanted nudes injected with FV. These mice had enlarged livers and spleens which were comparable to those

Table 5. Mortality among thymus-transplanted nudes, sham-operated nudes, Balb/c and CBA mice, injected with 2.24 LD_{50} doses of Friend virus (FV).

Group	Average body wt.* (gms)	Spleen# wt. (gms)	Liver# wt. (gms)	Deaths/ No. injected	Survival time (days)
Sham-operated nudes + FV	14.50/11.02	0.13	0.81	7/7	46
Nudes + CBA thymus + FV	17.46/22.04	1.29	4.60	8/9	171
Nudes + CBA thymus	15.60/21.34	0.07	1.39	1/6	--
Nudes + Balb/c thymus + FV	14.06/19.93	1.67	1.57	5/5	135
Balb/c + FV	23.21/28.07	2.30	3.60	8/8	109
CBA + FV	18.11/26.20	0.10	1.27	0/9	--

* - The numerator is body weight at initiation of the experiment, the denominator is body weight at death or sacrifice during the experiment.

- The weights were taken at death or sacrifice of the animals.

of Balb/c mice. However, the livers of nudes transplanted with CBA thymuses and injected with virus were much larger than those of nudes transplanted with Balb/c thymuses and injected with virus. This latter group showed a rapid development of leukemia as is evidenced by hematologic studies and also by the enlargement of the spleen early post virus injection. The thymus-grafted nudes survived for a long period when compared to other groups, and this survival is attributed to the thymus since we have shown previously that normal or sham-operated nudes not injected with virus did not survive beyond 90 days. As can also be seen from the table, there were no deaths in the CBA mice, and the spleen and liver weights of these mice taken upon termination of the experiment were normal.

Tissue sections from all groups of mice were examined microscopically upon death or sacrifice. In the sham-operated nudes with virus, the spleens exhibited hermorrhage, necrosis and abundance of megakaryocytes. There was little disturbance of the architecture of the spleen. The liver in these mice was mostly normal in architecture, but was infiltrated with neutrophils and many degenerated cells. Other organs appeared normal. Tissue sections from thymus-transplanted nudes that were not challenged with virus did not show any pathological changes. This was also true for CBA mice injected with virus, substantiating the thesis that CBA mice are refractory to FV. Pathology of tissue was abundant in Balb/c mice, and

thymus-transplanted nudes challenged with virus. The main organs involved in these mice were the spleen and liver. In the spleen there was massive infiltration of leukemic cells resulting in the total loss of normal architecture. Extensive destruction of parenchymal cells was seen in the liver, and the lobules were separated by the leukemic cells. This involvement of the liver was much more evident in Balb/c mice and in nudes transplanted with CBA thymuses and given FV, than in nudes transplanted with Balb/c thymus and given FV. In the latter group, the spleen was the primary target. However, in all 3 groups above there was evidence of infiltration of leukemic cells into the alveolar septae and autolysis of the lung. The kidneys and intestines appeared normal.

Graft versus leukemia. This experiment was modified from the experiment described by Bortin and co-workers (16). It was designed to test for the presence of cellular immunity in transplanted nudes, described above, against the leukemic cells produced by FV. Four to six weeks old Balb/c mice serving as secondary hosts were irradiated with 600 r of whole body irradiation with cobalt 60 from a Picker unit. The irradiation field size was 18 x 18 cm, the distance from source to target was 60 cm, and the delivery rate was approximately 87 r per minute. Irradiation was given to prevent the rejection of the donor cells, and to eliminate or minimize host immunity against

Table 6. Effect of nude spleen cells on lethally irradiated Balb/c mice that were given 1 x 10^4 or 64 x 10^4 nucleated spleen from a leukemic Balb/c mouse.

	Survival of Bioassay Mile Over a 60 day Period								
	1 x 10^4			8 x 10^4			64 x 10^4		
** Spleen cell donors	No. dead/ No. injected	Survival time	Average spleen weight (gms) of survivors	No. dead/ No. injected	Survival time	Average spleen weight (gms) of survivors	No. dead/ No. injected	Survival time	Average spleen weight (gms) of survivors
Nudes + FV*	1/3	16	(0.89)	2/5	15.40	(0.55)	1/5	16	(0.94)
Nudes + Balb/c-thymus	NT	NT		2/3	22.17	(0.83)	1/4	42	(1.12)
Nudes + Balb/c-thymus + FV	NT	NT		1/5	40	(0.78)	0/4	--	(0.91)
Nudes + CBA-thymus	2/4	17.23	(0.71)	2/5	16.24	(0.55)	2/3	17.16	(0.71)
Nudes + CBA-thymus + FV	2/4	25.53	(1.24)	3/5	16.24	(1.13)	1/5	15	(0.81)

* - Mice were given 2.24 LD_{50} doses of Friend virus (FV). NT = not tested.
** - The number of spleen cells obtained from donors = 1 x 10^7 cells.

irradiation control experiment showed that mice died up to 12 days post irradiation. However, it would be difficult to ascertain that mice in the various groups with a survival time of 15-17 days have actually died from the leukemic process rather than from irradiation. Furthermore, it is more likely than not that some mice died from a graft-versus-host reaction. Therefore, although some groups, when compared with their controls (e.g., nudes + Balb/c thymus + FV compared with nudes + Balb/c thymus), may indicate that immunity is evident, the above reasons would pose a serious question that adoptive immunity was present. Furthermore, spleen weights from survivors were similar in all groups, again strengthening the conclusion that no immunity was present. It should also be mentioned that enlargement of spleens could be due to radiation followed by infection, rather than to leukemia.

Humoral immunity. To test for the production of virus neutralizing antibodies, we performed experiments similar to those performed by Bendinelli and Nardini (31). Sera were obtained from the same 5 groups above (a-e) used for the graft versus leukemia (GVL) experiment, then diluted 1:2, 1:8, and 1:32. The sera were then incubated at 56°C for 1 hour to inactivate any FV that may be present. A separate vial containing FV was incubated with the sera and then injected into Balb/c mice to insure that inactivation of the virus does occur

at 56°C for 1 hour. Equal amounts of sera and FV (diluted to 22.40 LD_{50} doses) were mixed and incubated at 4°C overnight. Balb/c mice, 4-6 weeks old, were then injected IP with this mixture, such that each mouse received a full dose of FV, i.e., 22.40 LD_{50} doses. In order to affirm that FV was not inactivated by incubation overnight, a separate vial containing FV at 22.40 LD_{50} doses was incubated overnight and then injected into Balb/c mice in a similar manner as the mixture. The experiment was allowed to proceed for 4 weeks after which time the mice were sacrificed and spleen weights were recorded.

Table 7 describes the effect of incubation of FV with various sera. As can be observed from the spleen weights of the various groups, no neutralizing antibodies to the virus at all sera dilutions were detected. If antibodies were present, they were not in sufficient quantities to neutralize the FV.

Effect of Mitogens

The preceding experiments of thymus grafting suggested that a thymus is required for the enhancement of the leukemic process in nude mice. This was true whether the thymus was obtained from a mouse that is genetically related to the nude mouse, i.e., Balb/c or littermates, or obtained from a mouse that is genetically distant to the nude mouse, i.e., CBA. This in turn points towards a mechanism whereby the thymus-derived cell (T cell) is required to interact

Table 7. Effect of incubation of 22.40 LD_{50} doses of Friend virus (FV) with sera obtained from various groups of nude mice challenged with 2.24 LD_{50} doses of FV.

Groups providing sera[a]	Average spleen weight of Balb/c mice (gms) 4 weeks post challenge		
	1:2 (Range)	1:8	1:32
Nudes	1.21 (0.47-2.21)	0.89 (0.35-1.37)	1.04 (0.64-1.62)
Nudes + Balb/c-thymus	0.75 (0.47-1.21)	0.88 (0.30-1.13)	0.84 (0.19-1.40)
Nudes + Balb/c-thymus + FV	0.85 (0.51-0.92)	0.78 (0.35-1.60)	0.57 (0.21-0.82)
Nudes + CBA-thymus	1.12 (0.61-1.60)	0.96 (0.87-1.28)	0.83 (0.42-1.29)
Nudes + CBA-thymus + FV	1.53 (1.22-1.77)	1.25 (0.11-1.97)	1.19 (0.51-1.69)

a - Two other control groups were used: 1) Balb/c mice injected with 22.40 LD_{50} doses of FV left at 4°C overnight, gave an average spleen weight of 0.74 gms.

(2) - Balb/c mice injected with 22.40 LD_{50} doses of FV incubated at 56°C for 1 hour gave an average spleen weight of 0.11 gms.

with a BM cell causing proliferation and maturation of these latter cells which not serve as target cells for the virus. The consequence of this proliferation is the production of a large pool of target cells. If this is the case, then it should be possible, by the use of nonspecific mitogens, to duplicate the effect of the thymus graft. To this end then, we tested the effects of PHA, PWM and LPS on the leukemic process.

PHA and PWM. These mitogens were further dissolved in sterile distilled water to give a final dilution of 1:50 for PHA and 1:25 for PWM. Littermates and nudes were injected IP with either PHA or PWM at 10 day intervals, starting with 0.2 ml and doubling the amount at the next injection time until termination of the experiment. Friend virus at 2.24 LD_{50} doses was given to some groups at 3 days following the first injection of either PHA or PWM. Figures 6 and 7 show the absolute lymphocyte counts for mitogen-treated mice. It is evident that no effect on the leukemic process was seen in either littermates or nudes. No difference in differential counts was observed between groups given mitogens and FV, or FV only, with the exception that neutrophils were more abundant in the former group. The blood picture of nudes and littermates injected with FV with or without mitogens showed the usual leukemic cells as has been previously described in this thesis.

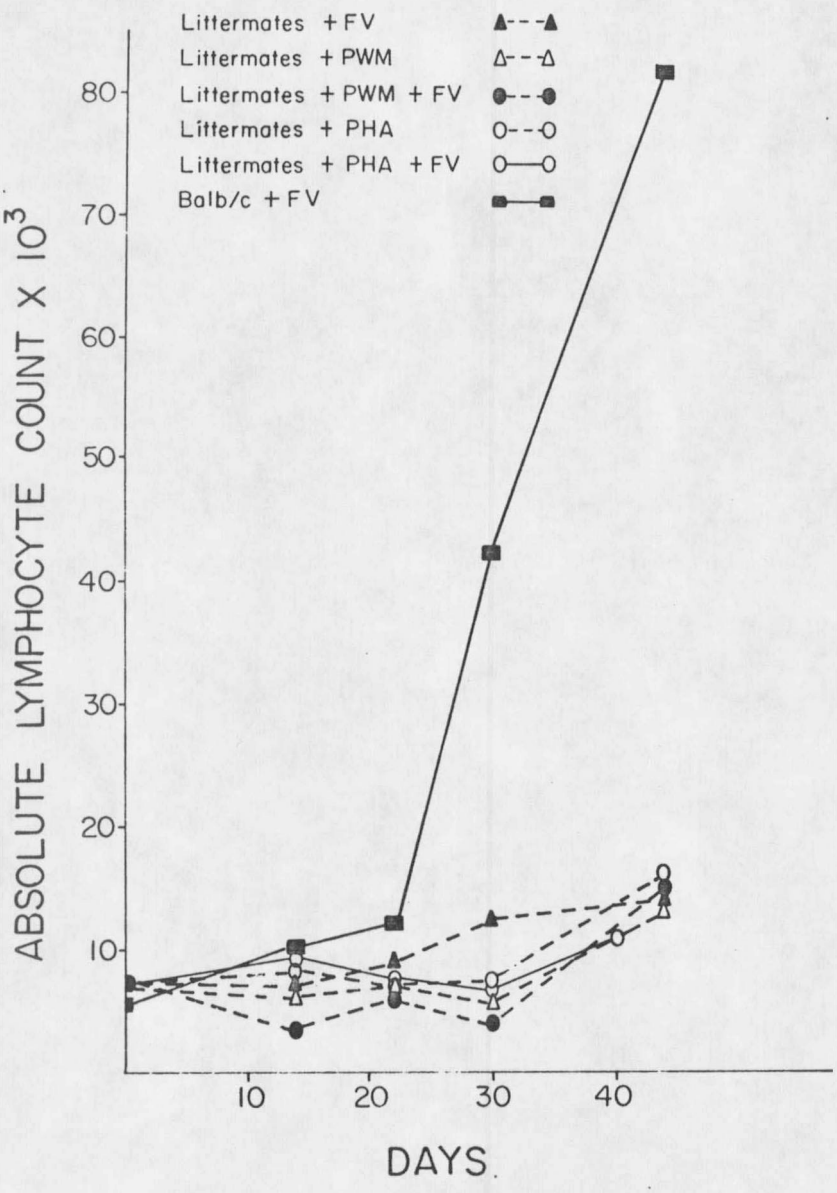

Figure 6. Absolute lymphocyte counts demonstrating the effects of phytohemagglutinin-M (PHA) and pokeweed mitogen (PWM) on Friend virus (FV, 2.24 LD_{50} doses) leukemia in littermate mice.

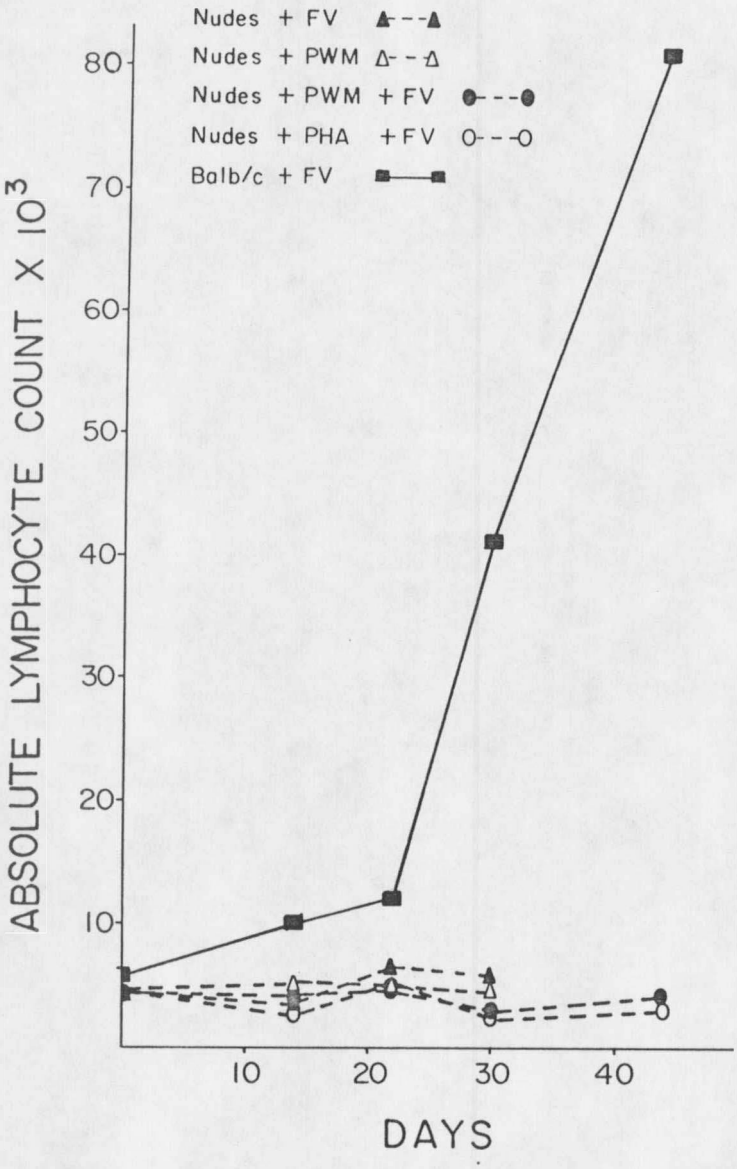

Figure 7. Absolute lymphocyte counts demonstrating the effects of phytohemagglutinin-M (PHA) and pokeweed mitogen (PWM) on Friend virus (FV, 2.24 LD_{50} doses) leukemia in nude mice.

LPS. The LPS was diluted in PBS in yield a final dose of 20 ug/0.25. Littermate and nude mice were injected IP at 3 day intervals for one week, and then at 10 day intervals until termination of the experiment. Friend virus at 224 LD_{50} doses was injected into designated groups 3 days after the first injection of LPS. Figures 8 and 9 show the effect of LPS on the absolute lymphocyte counts of these mice over a 40 day period. The pretreatment values for these mice were 2370 cells/mm^3 for nudes, and 3301 cells/mm^3 for littermates. In contrast to PHA and PWM, LPS had a marked effect on the leukemic process in both littermates and nudes. This was also seen in the peripheral blood where leukemic abnormalities were much more frequent in mice treated with LPS. These abnormalities were previously described in this thesis.

Pathologic findings in treated mice.

A. PHA and PWM. Table 8 describes the effect of PHA and PWM on the leukemic process in mice. It is evident that both these compounds had an adverse effect on nude mice without showing much effect on the leukemic process. This is seen when spleen and liver weights of the various groups are compared which did not show much variation. In littermates there is a slight effect produced by PHA as can be judged from the spleen and liver weights which were higher than in

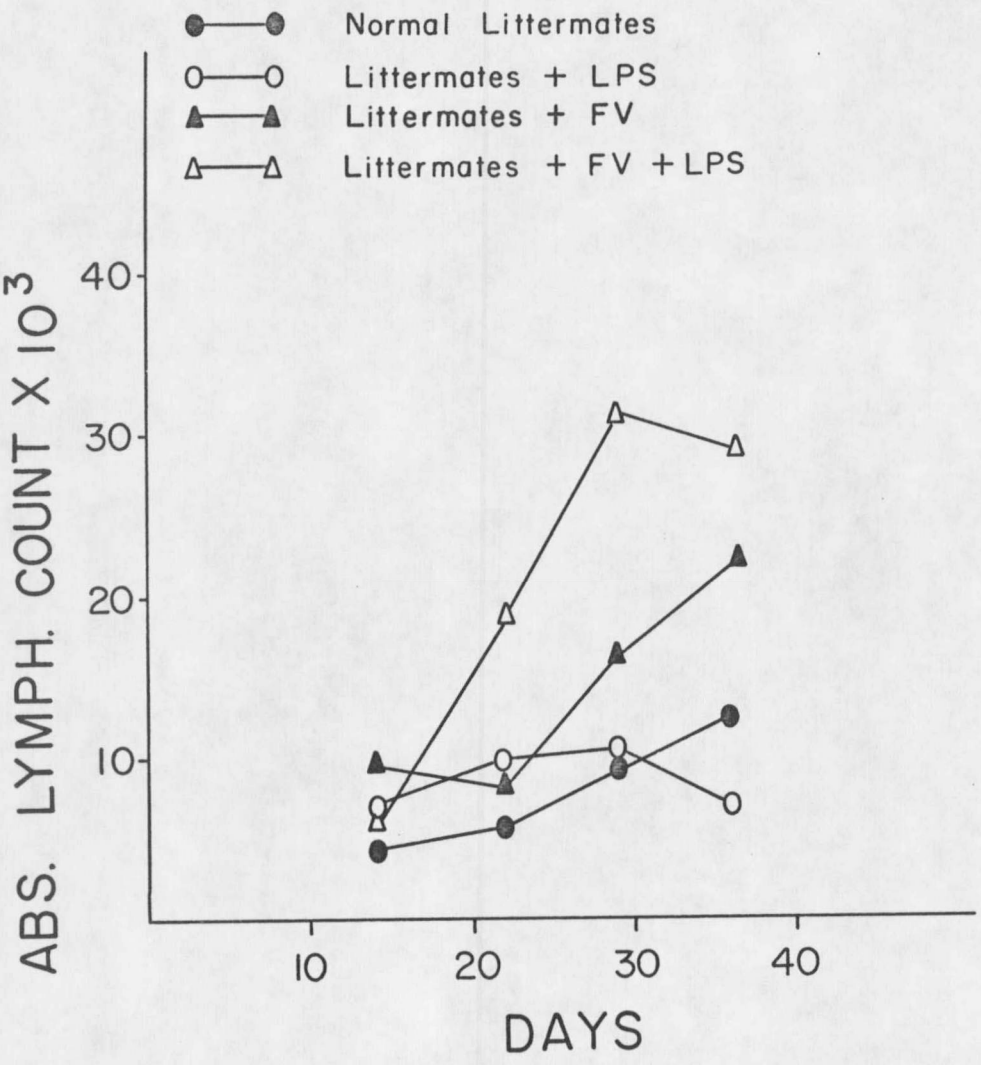

Figure 8. Absolute lymphocyte counts demonstrating the effect of endotoxin (LPS) on Friend virus (FV, 224 LD_{50} doses) leukemia in littermate mice.

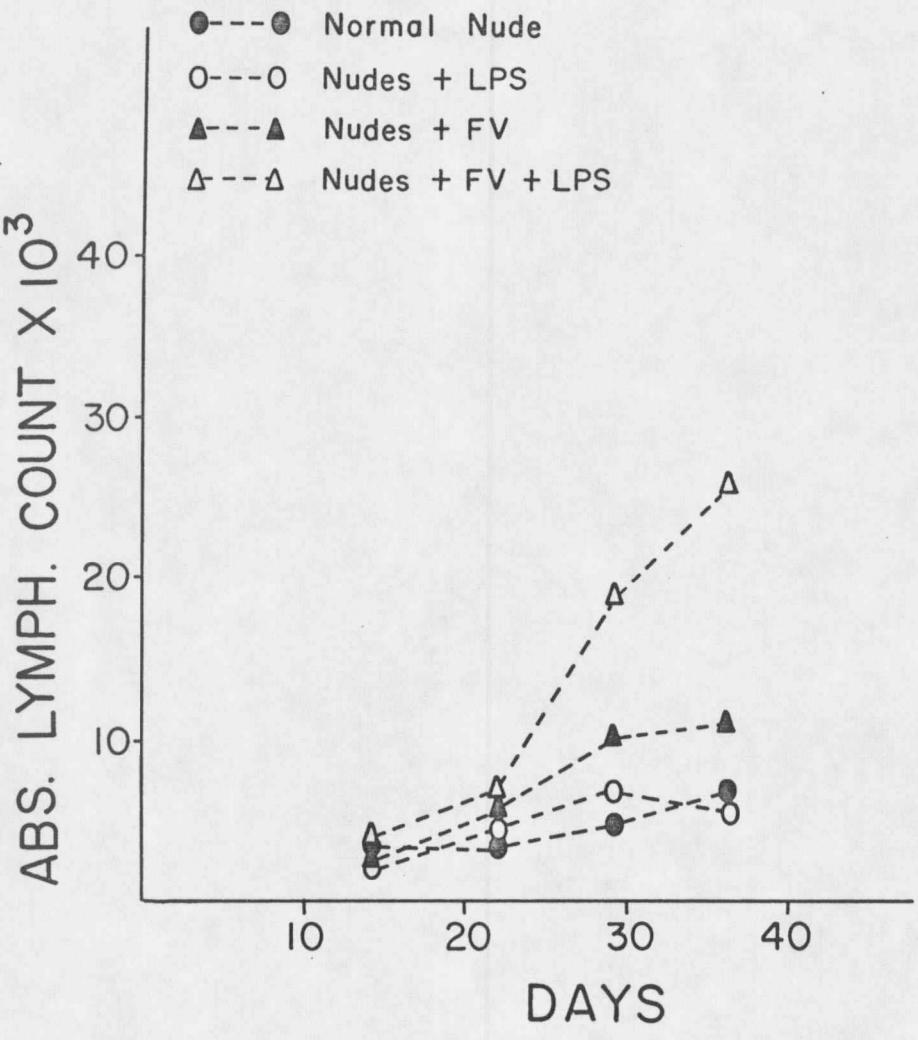

Figure 9. Absolute lymphocyte counts demonstrating the effect of endotoxin (LPS) on Friend virus (FV, 224 LD_{50} doses) leukemia in nude mice.

Table 8. The effect of phytohemagglutinin (PHA), and pokeweed mitogen (PWM) on nudes and littermates with or without 2.24 LD_{50} doses of Friend virus (FV).

	Body** weight gms	Spleen weight gms	Liver weight gms	Survival# time days	Death/ No. injected
Balb/c + FV	12.84/13.69	0.92	1.55	53	5/12
Littermates + PWM	26.34/28.78	0.14	1.66	--	0/ 6
Littermates + PWM + FV	21.85/23.97	0.53	1.74	--	0/ 8
Littermates + PHA	20.57/24.73	0.20	1.69	--	0/ 7
Littermates + FV	25/61/30.12	0.88	1.80	--	0/ 6
Nudes + PWM	16.31/15/73	0.19	0.95	44	6/ 8
Nudes + PWM + FV	16.22/16/92	0.20	1.05	44	6/ 9
Nudes + PHA + FV	15.82/15/38	0.21	0.94	28	5/ 7
Nudes + FV	14.37/17.64	0.19	0.86	35	6/ 6

** - The numerator is body weight at initiation of the experiment, the denominator is body weight at death or sacrifice during the experiment.

- Where no survival time is given, the mice were all alive at the termination of the experiment.

any other group. However, this did not seem to affect the absolute lymphocyte counts.

B. Pathologic findings in LPS treated mice. Table 9 describes the effect of LPS on the leukemic process in nudes and littermates. In contrast to PHA and PWM, LPS has enhanced the leukemic process in both groups of mice. Although this may not be evident from the survival times, since in littermates there were no deaths as yet at termination of the experiment, and in nudes the differences in survival times are small, spleen weights were definitely enlarged in mice treated with LPS and injected with FV. Liver weights did not enlarge in mice treated with endotoxin and FV over mice treated with FV, however, there was an increase over the normal. Enlargement of the spleen, which is the main target organ for the FV, coupled with the rise in absolute lymphocyte counts, indicate that LPS had an enhancing effect on the leukemic process in both littermates and nudes.

Nature of the Target Cell

Since we have established that LPS enhances the leukemic process, it became clear that LPS in nude mice causes the stimulation of BM cells in the nude mouse thus providing a large compartment of target cells. Moreover, it is presumed that these cells are BM cells that belong to the antibody-producing lineage.

Table 9. The effect of endotoxin (LPS) on nudes and littermates with or without 224 LD_{50} doses of Friend virus (FV).

	Body* weight gms	Spleen weight gms	Liver weight gms	Survival# time days	Death/ No. injected
Littermates (normal)	19.94/25.27	0.08	1.32	--	0/4
Littermates + LPS	20.40/22.04	0.21	1.30	--	0/7
Littermates + LPS + FV	20.75/25.72	1.34	1.57	--	0/8
Littermates + FV	23.21/25.85	0.98	1.91	--	0/5
Nudes (normal)	21.35/19/95	0.10	0.84	--	0/5
Nudes + LPS	17.99/14.18	0.14	0.83	39	2/6
Nudes + LPS + FV	19.34/17.13	0.24	0.90	41	6/8
Nudes + FV	18.32/15.14	0.15	1.05	43	5/6

* - The numerator is body weight at initiation of the experiment, the denominator is body weight at death or sacrifice during the experiment.

- Where no survival time is given, the mice were all alive at termination of the experiment.

It has been shown by Manning and Jutila (28,32) that neonatal injection of mice with rabbit anti-μ caused a total suppression of all serum immunoglobulins and responsiveness to immunization with sheep erythrocytes. Their data demonstrate that precursors to antibody-producing cells bear μ heavy chain receptors and immunospecific anti-μ serum prevents maturation and differentiation of these cells in vivo. Because FV has been shown to infect these precursoral cells, the use of an anti-μ serum should prevent the differentiation or transformation of infected cells into malignant cells.

Effect of suppressive treatment on leukemia. Litters of Balb/c mice were dived into groups injected either with anti-μ, PBS or normal rabbit serum (NRS). Immunosuppression with anti-μ was started at day 0 (day of birth) when the mice received 0.05 ml IP. The size of subsequent injections given on alternate days was increased until the desired dose size (0.20 ml) was reached. Injections were continued until day 94 of age. Friend virus at 2.24 LD_{50} doses was injected at day 38 of age.

Hematologic data. Figure 10 compares the absolute lymphocyte counts of Balb/c mice treated with anti-μ or PBS or NRS over an 85 day period. The absolute lymphocyte counts of these mice before virus was injected were 12022 cells/mm^3 for the anti-μ group,

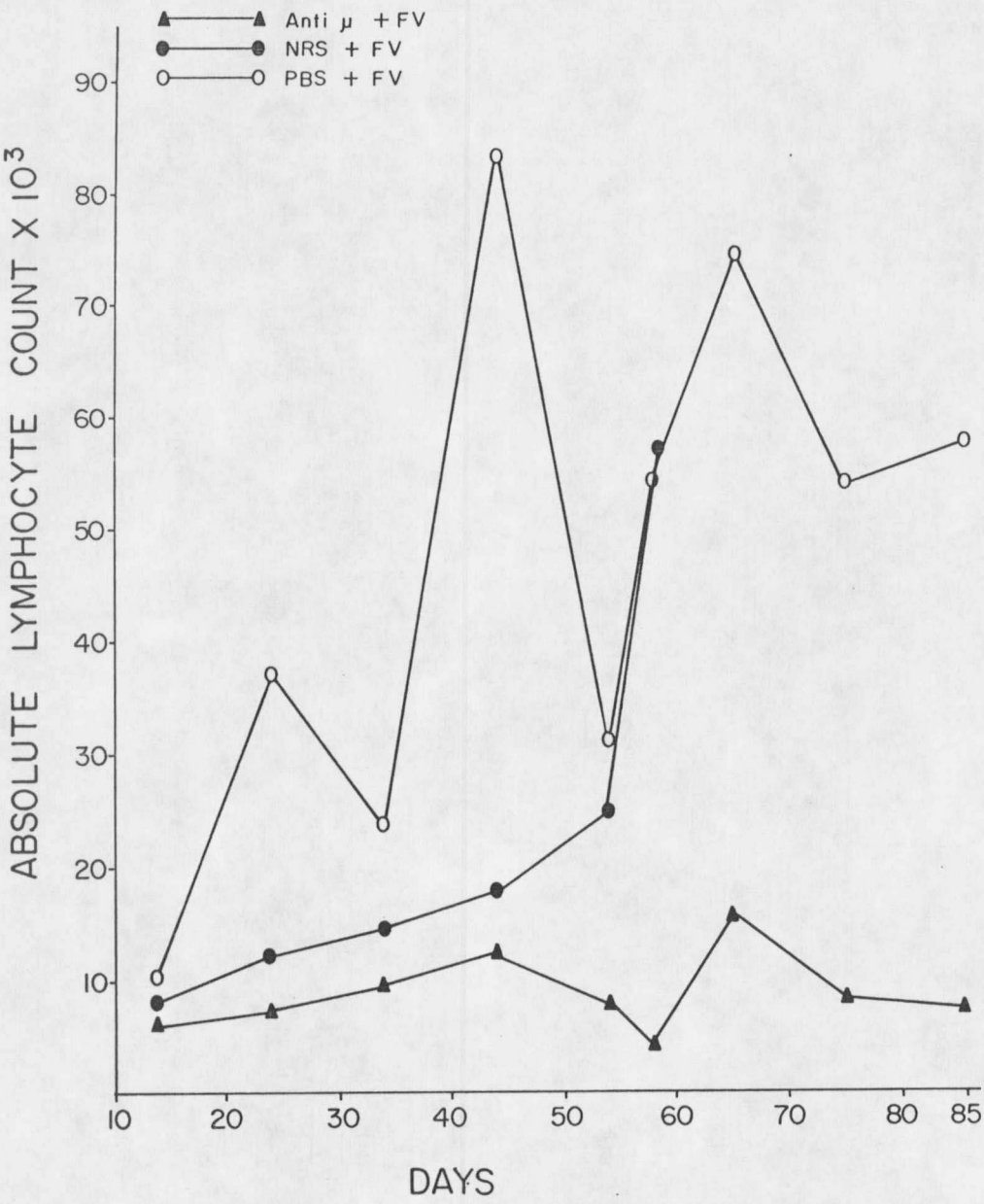

Figure 10. Absolute lymphocyte counts of Balb/c mice given anti-μ, or normal rabbit serum (NRS), or phosphate buffered saline (PBS), and challenged with 2.24 LD doses of Friend virus (FV).

10036 cells/mm^3 for those treated with PBS, and 8476 cells/mm^3 for the NRS mouse. Virus was injected at 38 days of age. Although the data for the figure were obtained from a small number of mice, there are distinct differences between the anti-μ group and the other two groups to merit reporting. It can be seen that the absolute lymphocyte count for the anti-μ group did not rise above 16,000 cells/mm^3 and then only after immunosuppression was stopped. The count rose to its highest peak 10 days after immunosuppression was stopped, but then again declined and remained at a normal level, even up to 45 days after cessation of immunosuppression. On the other hand, mice treated with PBS showed a rapid increase in the absolute lymphocyte count and reached in excess of 80,000 cells/mm^3 at day 45. Mice treated with NRS initially showed a gradual rise in the absolute lymphocyte count, but then a dramatic increase after day 44 to attain counts in excess of 55,000 cells/mm^3. This mouse was accidentally killed while being bled to test for the amount of immunoglobulins in the serum, however, it is evident that leukemia was present.

Leukemic cells were abundant in the peripheral blood of mice injected with PBS or NRS, but sparse in mice treated with anti-μ. Whereas mice treated with PBS or NRS showed large numbers of abnormal lymphocytes in the peripheral blood, the mice treated with anti-μ showed large numbers of neutrophils which appeared normal. This

excess of neutrophils could be due to a compensation mechanism since most of their lymphocytes are non-functional due to immunosuppression. Red blood cell abnormalities as previously described in this thesis were prominent in mice treated with PBS or NRS, but rare in anti-μ mice.

Pathologic findings. Although the anti-μ mice were severely suppressed, they did not show any signs of infection or wasting. Thus, in physical appearance they did not differ from other mice. This was true until about 90 days post virus challenge when one of the mice exhibited severe symptoms of wasting. Mice in all groups either maintained their weight or gained weight by the time of death or sacrifice. This was true even for the anti-μ mouse which eventually wasted. The initial weight considered here was that taken at 38 days of age just before challenge with virus. Of the three groups of mice, those treated with PBS died as a result of the leukemic process during the experiment. One of these mice died at 30 days and another at 45 days post challenge with virus.

The spleen and liver weights of mice treated with PBS or NRS indicated that leukemia was present. Upon death (55 days post challenge with virus), the mouse treated with NRS had a spleen weight of 1.47 gms and liver weight of 1.23 gms. Also upon death one mouse with PBS had a spleen weight of 1.51 gms and a liver weight of

1.39 gms, whereas upon sacrifice of another mouse with PBS, the spleen weight was 0.84 gms and liver weight was 4.21 gms. All the above spleens and livers together with the lung showed involvement with leukemia, as has been previously described. On the other hand, one mouse treated with anti-μ when sacrificed had a spleen weight of 0.06 gms and liver weight of 0.58 gms. The spleen and liver appeared normal and indicated that this mouse did not have signs of a visible leukemia, but rather would have died from a wasting syndrome. The other mouse with anti-μ is still surviving with apparent normal health.

DISCUSSION

These studies demonstrate a difference in the course of the leukemic process in Balb/c, nude mice and their normal littermates. Thus, the disease was most severe in Balb/c mice, as evidenced by hematologic and pathologic data and failed to develop in a typical fashion in nude mice.

In general, normal or sham-operated nudes died within 50 days after virus injection. This is in contrast to either sham-operated or normal nudes untreated with FV which survived approximately 90 days. This points to the fact that the early death of nudes injected with virus may be due to the virus. Although these mice exhibited a modest rise in absolute lymphocyte count, they did not exhibit typical symptoms of leukemia. The early death may be due to a generalized viral infection rather than to a typical leukemic process. The electron microscopy study lends credence to this notion since large numbers to C-type particles were seen in the lung, spleen, kidney, liver and lymph nodes of nudes.

That nude mice transplanted with thymus develop a typical leukemic process is extremely interesting and strongly implicates the thymus in the disease process. At least two mechanisms may be considered for the enhancement of leukemia by the thymus. First, the thymus may provide a large compartment of stem cells or target cells for the virus, or expand the pool of virus infected cells through the

release of mitogenic factors. Second, it is possible that the thymus provides some form of immunity against the leukemia or challenge from environmental organisms which allows the mice to survive for long periods and thus allowing time for development of a typical leukemia process.

The results of experiments to test for immunity showed that by our method no immune response was detectable in thymus grafted nudes against either the FV or the leukemic cells. However, the methods used in other studies employed larger numbers of effector cells (16), presensitization of the animals to the leukemic cells (18) or the virus (31), or direct interaction of the virus with the thymus cells (15).

It has been previously shown that in germfree (GF) mice infected with either FV (6) or Rauscher virus (33), the leukemic process was enhanced by antigenic stimulation. This suggested that an expansion of a pool of susceptible cells may accelerate following antigenation. In a study using Rauscher virus, Siegel and Morton (34) have shown that the leukemic process is accelerated by a single IP injection of Bovine serum albumin (BSA) in complete Freund's adjuvant when given up to 18 days before virus inoculation. Larson et al. (35) have shown that antilymphocyte serum increased the susceptibility of mice to infection with FV. This enhancement was attributed to

stimulation of primitive precursor cells. Larson et al. (35) are in agreement with Bennett and Steeves (36) that FV affects precursors of humoral antibody-producing cells (B-lymphocytes) but not thymus lymphocytes (T-lymphocytes). Thomson (37) has also shown that the thymus did not provide target cells for FV. Therefore, it would appear that the availability of cells of the antibody-producing lineage is a prerequisite for the production of a typical leukemic process.

Although PHA has been found to stimulate thymus-derived cells (38,39,40) and PWM has been shown to stimulate both BM cells and T cells (39,40), these studies failed to demonstrate enhancement of leukemia with these mitogens. This failure, either in normal littermates or nudes, may be due to a dose which may not have been sufficient to cause stimulation in vivo. On the other hand, at least for PHA, it may be possible that the dose used was too large. It has been shown (41,42,43), for example, that large doses of PHA can inhibit the immune reaction to various antigens. This may due to the fact that mice given PHA have a decreased number of plaque-forming cells (44). For the success of such a study in our system, it would be first necessary to establish the optimal doses of PHA and PWM for stimulation of BM and T cells in vivo.

Studies have shown that the effect of LPS is principally upon BM cells (38,45). Smith and co-workers (46) have shown that in

endotoxin-treated mice, the average number of cells per spleen is 4 times that in untreated mice. The BM cells of nude mice have been shown to repond mitogenically to LPS (47). By using LPS in nude mice, the leukemic process was enhanced in these mice and, in effect, duplicated the enhancement by the thymus graft. Steeves and co-workers (48) have shown that Salmonella typhosa endotoxin enhances the spleen focus formation induced by a member of (spleen focus-forming virus (SFFV)) the FV-complex. Our FV-complex also contains SFFV (unpublished results).

One other aspect should be considered. It has been shown that activation of a leukemia virus occurs in long-term graft-versus-host (GVH) disease (49,50,51). In a recent study, Hirsch and co-workers (52) have demonstrated the activation of leukemia virus after a homograft transplantation. These workers also showed that immunologic reactions to histocompatibility antigens, whether in GVH or host-versus-graft (HVG) cause the activation of leukemia viruses. By homograft transplantation and antilyphocyte serum treatment of mice, Hirsch and co-workers (52) postulate that the immune reaction activates a leukemia virus, and the virus is amplified by the inability of the recipient to immunologically eliminate it due to immunosuppression. Our experiments with CBA-thymus implants in Balb/c-derived nudes may be considered as similar to a GVH mechanism. Thus,

it is probable that the enhancement of leukemia in nude mice implanted with CBA thymuses is due to a mild GVH reaction which stimulates the production of target cells for the virus.

In the last part of these studies we have shown that the leukemic process, as judged by hematologic and pathologic criteria, can be minimized, if not completely abrogated, by treatment with anti-μ . This would indicate that the target cell for FV is very probably an early cell in the antibody-producing lineage which is in agreement with less definitive studies (6 14). Whether the virus infects an early progenitor cell lacking receptors or one bearing μ chain receptors could not be ascertained by these studies. On the other hand, it can be concluded that normal differentiation of target cells includes a μ chain bearing stage which can be destroyed or impaired.

Since we are using highly purified anti-μ, it would appear very likely that this cell bearing μ-receptors is in its first differentiation step after antigen stimulation (28). It would be of great interest to check as to whether treatment of mice with anti-IgG or anti-A would have any effect on the leukemic process, and whether the leukemic process can be arrested or retarded by anti-μ after the symptoms have become evident.

The mechanism by which leukemia is suppressed in mice treated with anti-μ is not clear. It is possible that the anti-μ kills the

target cells for the virus, or that if the cells are not killed, the virus is somehow prevented from infecting or transforming the cell. Of significance, however, is the fact that these mice are free of leukemia nearly two months after cessation of immunosuppression. If immunosuppressed mice develop leukemia after treatment with anti-μ is stopped, it would mean that the virus may be harbored by cells other than antibody-producing cells such as the macrophage (53), or progenitor cells are simply arrested transiently only to resume maturation after the anti-μ antibodies disappear.

In summary, our studies have shown that the thymus is required for the production of a typical leukemic process in congenitally athymic mice, that the mechanism by which the thymus enhances leukemia may include the production of activated BM cells as target cells for the virus. Finally, the target cells for the virus bear μ chain receptors at some stage in their development.

SUMMARY

The role and mechanism of the thymus in virus-induced leukemia was investigated in congenitally thymusless (nude) mice and their phenotypically normal littermates. Nude mice were implanted with thymuses obtained from Balb/c, littermate or CBA mice. After allowing 2-3 weeks for the thymuses to become established, leukemia was produced in these mice by the IP injection of various doses of Friend leukemia virus. The leukemic process was left to progress for 2-3 weeks, and then was assessed by hematologic and pathologic criteria. The nature of the target cell for the virus was also investigated.

The results indicated that the thymus is required for the production of a typical leukemic process in mice injected with FV. Nude mice without thymus implants developed only a mild leukemia, but died rapidly (within 50 days) after challenge with virus. On the other hand, nudes with thymus grafts developed a typical leukemia. The results also showed that the mode of section of the thymus may relate to the activation and/or proliferation of bone-marrow cells, thus an enlargement of a compartment of target cells for the virus.

Results of preliminary experiments indicated that the target cell for Friend virus is a μ-bearing cell belonging to the antibody-producing lineage.

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