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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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

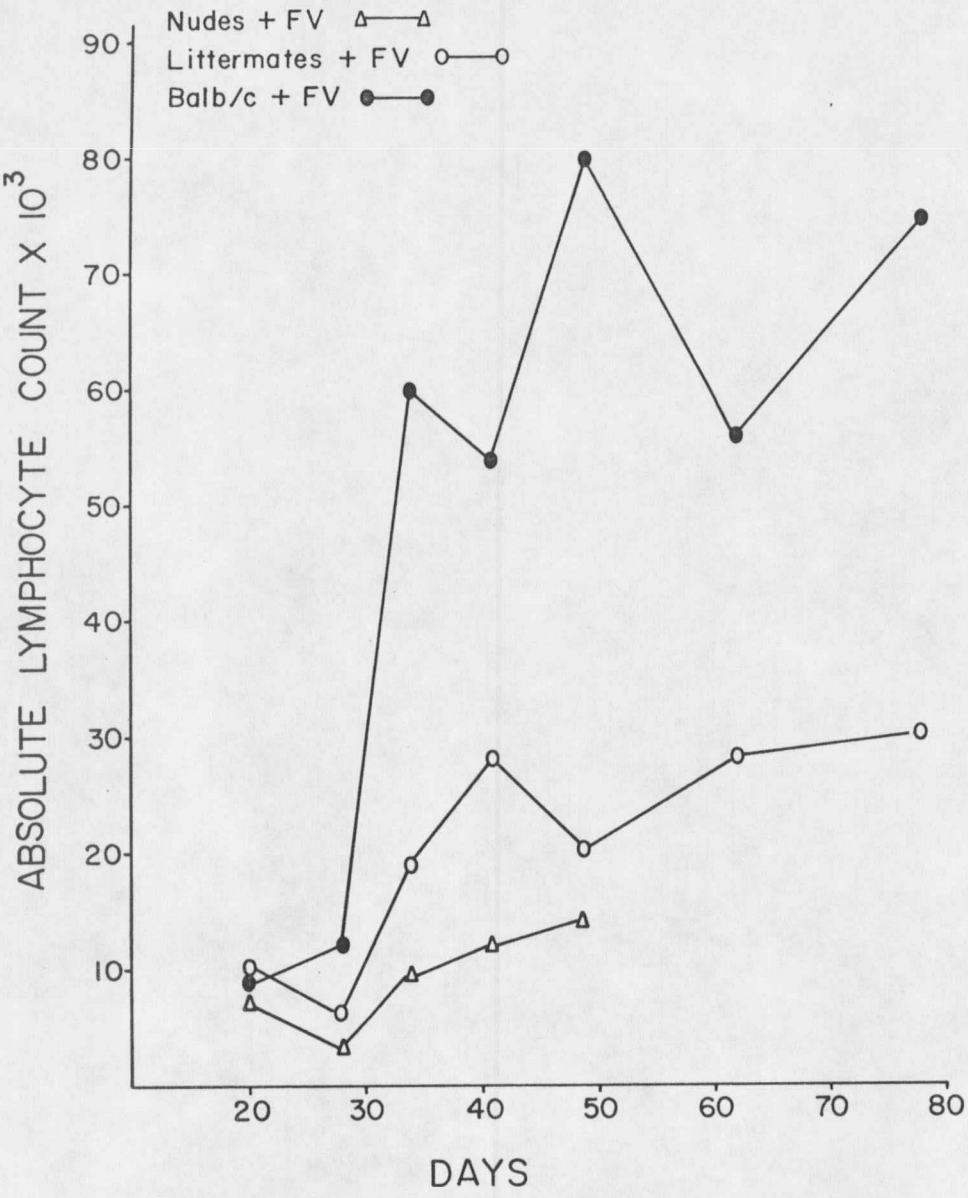

Figure 1. Absolute lymphocyte counts of nude, littermate, and Balb/c mice injected with 224 LD_{50} doses of Friend virus (FV).

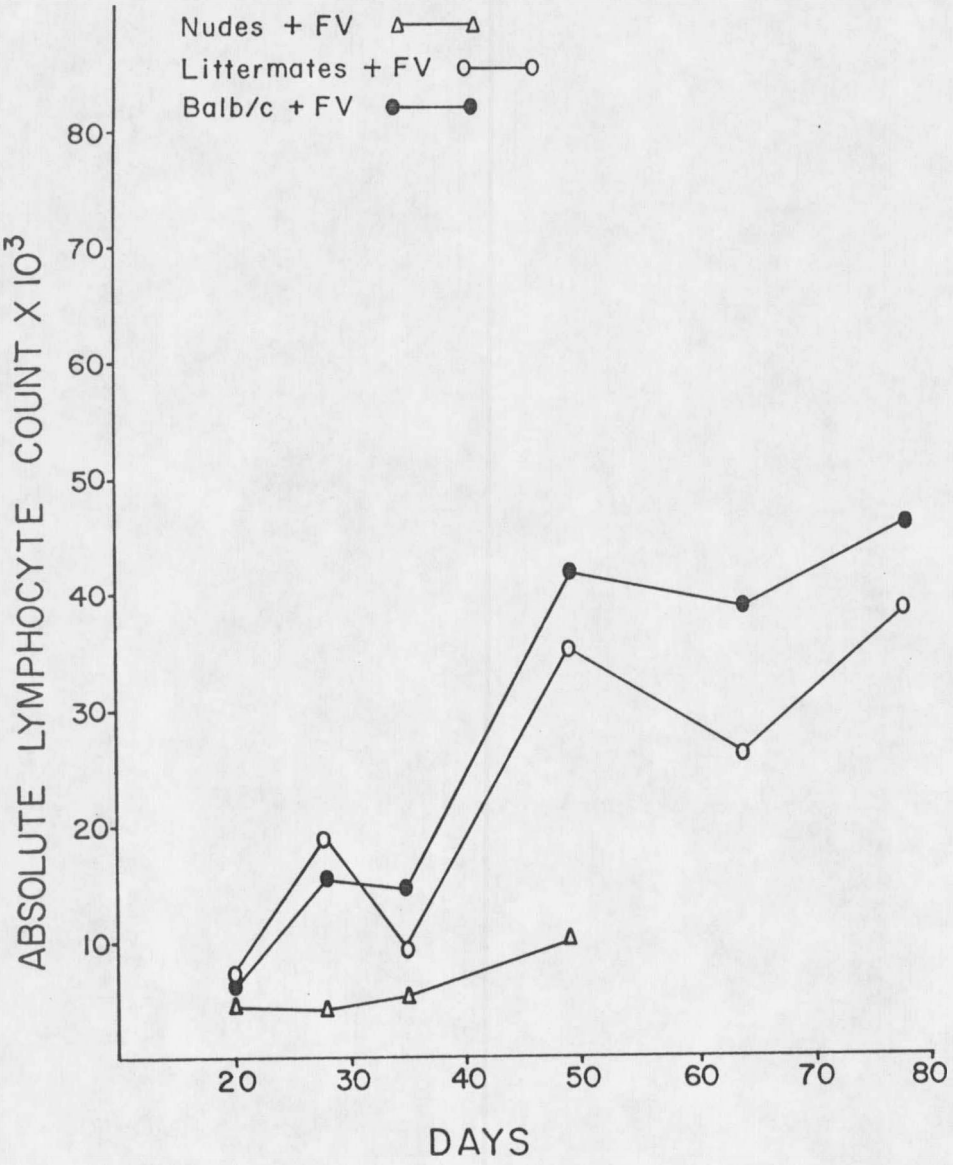

Figure 2. Absolute lymphocyte counts of nude, littermate, and Balb/c mice injected with 2.24 LD_{50} doses of Friend virus (FV).