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SINGLE CELL TRANSPLANTS: GROSS VIRUS-INDUCED MOUSE LYMPHOMAS¹

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California.

SUMMARY

Low doses of and single Gross virus-induced mouse lymphoma cells were isolated in a gelatin medium and transplanted surgically to syngeneic recipients. Transplantation characteristics of cells isolated from a primary lymphoma induced by neonatal injection of Gross virus were compared to characteristics of cells isolated from lymphomas which had been maintained by syngeneic cell passage after initial isolation from a similar virus-induced source. Single cells could produce lymphoma in syngeneic recipients only when donor cells were from the cell passage source. Primary virus-induced cells transplanted produced lymphoma only one time in 56 attempts at a transfer of 10 to 20 cells and never in 40 attempts at single cell transfer.

This study reports results that indicate that single mouse lymphoma cells can produce lymphomas, but that success of such transplants is dependent upon the cell source. The work was stimulated by the concept that lymphomas originate from one or more clones of neoplastic cells derived from single cells. Neoplastic transformation of the precursors of lymphoma cells may be an event which occurs quite frequently in the life of the host, but only certain types of transformed cells or certain conditions affecting the immunological reactivity of the host predispose to development of frank lymphoma by the transformed cells. Thus, the usual event may be elimination of the clone; the unusual event, the development of lymphoma.

The fact that lymphomas could be developed as clones from a single transformed cell is based on the work of Furth and Kahn (4). In 97 attempts at intravenous injection with a micromanipulator, 5 single mouse leukemia cells developed into frank disease with latent periods of 15-50 days post transfer. Donor cells were S2, an atypical leukemia, or Akf5, a lymphoid leukemia. Transplant recipients were stated to be inbred.

Skipper, et al. (9) showed that in 993 attempts at intraperitoneal transfers of single L1210 mouse cells with a micromanipulator, 165 (16.6%) resulted in widespread, lethal disease. The median day of host death following inoculation of the cell was 14.8 days. Range of latent period was 13-42 days; 99% of deaths occurred in 13-25 days.

Both of these studies utilized a micromanipulator and injection as the technique for transfer of the single cells. The technique has been criticized (8) in that cells can remain uninjected when they adhere to walls of the micropipette. The study reported here utilized a gelatin

transfer technique which resulted in minimum trauma to the transferred cell and insured that the cell had been placed in the recipient, thus making evaluation of the percentage of single cell takes more meaningful.

MATERIALS AND METHODS

Mice. AKR/J, CBA/H, CBA/T₆T₆ (donor for CBA/H recipient) and C3H/HeJ mice derived from strains from The Jackson Laboratory were maintained by continuous single-line, brother and sister mating. All animals were of the same sex for maintenance of lymphoma lines and experiments on the line. Mice were 2 to 4 months of age at initiation of the experiments.

Lymphomas. The original lymphoma cell source, obtained from Ludwik Gross, was a C3H_f/Bi mouse with primary lymphoma induced by passage A virus. This virus-induced lymphoma was maintained by passage of cell-free filtrates of the lymphoma to 3 to 5 day old AKR/J mice. Lymphomas are termed "virus-induced" (VI) when they result from injection of cell free filtrates into neonatal mice. Cell passaged lymphomas were initiated by transfer of suspensions of 1×10^8 cells derived from mesenteric, axillary, inguinal lymph nodes and a portion of the spleen of adult C3H/HeJ (for C3H lines) or adult CBA/T₆T₆ (for CBA lines) animals developing lymphoma after receiving virus as neonates. Passaged lymphomas were maintained by transfer of 10^6 to 10^8 cells intraperitoneally to syngeneic recipients, and are termed "long transplanted" (LT). Long transplanted lines were used as a source of donor cells for single cell isolation and syngeneic transplantation. Lymphomas derived from such single cells are termed "clonal lines" (CL).

Gelatin Technique for Single Cell Transfers. Single mouse lymphoma cells were isolated and transferred as detailed previously (10) utilizing

gelatin droplets. The gelatin concentration was modified to 12.5% for work here. Briefly, the technique consists of isolating single lymphoma cells in optically clear gelatin in Hanks' balanced salt solution. Cell suspensions are deposited in gelatin droplets in a dilution scheme on a large coverglass. Viability of the cell is assessed by the morphological analysis technique (11) utilizing the inverted phase contrast microscope. Droplets are removed from coverglass with a sterile spatula and transplanted surgically to recipients. The gelatin rapidly dissolves leaving the viable cell intraperitoneally.

T₆ Chromosome Assay. T₆ chromosome studies were done as detailed previously (5).

RESULTS

LD 90-100 Doses of Test Lymphoma Lines. Syngeneic mice were injected intraperitoneally with one of the following titrated doses of donor cells: 1×10^3 , 1×10^4 , 1×10^5 and 1×10^6 . These test doses were quantitated in solution and injected intraperitoneally. The LD 90-100 was defined as the minimum dose of cells tested which resulted in fatal lymphoma in 90 to 100% of recipients. Test doses ranging to 1×10^4 were within the range for the LD 90 - 100 of C3H lines LT1, LT 3, CL 3, CL 6 and CBA LT 1. It was necessary to add doses of 1×10^7 and 1×10^8 to find the LD 90 - 100 for C3H VI lymphoma cells. Test doses of 10 - 20 cells in gelatin were necessary to determine the LD 90 - 100 for C3H LT 2 and CL 1. Latent periods for lymphoma are reported as the number of days from injection of test doses to death of the recipient from generalized lymphoma confirmed at autopsy. Lines studied and LD 90 - 100 doses are reported in Table 1. It is interesting to note that the clonal lines,

derived from a single cell implant from the parent lines indicated, maintain the latent periods for lymphoma and LD 90 - 100 doses similar to those of the parent lines. The LD 90 - 100 of the C3H VI line is considerably higher than for any of the LT or CL lines.

Comparison of Route of Injection of 1×10^3 Lymphoma Cells. All LT lines were transferred to syngeneic recipients by two routes, subcutaneous and intraperitoneally, into two parallel series of animals. Incidence and latent periods of lymphoma were compared between the two routes for each line (Table 2). It can be seen that incidence of lymphoma did not vary for C3H lines LT 2 and LT 3 although latent periods were longer by the SC route. For the C3H LT 1 and CBA LT 1 lines latent periods were longer by the SC route only in the CBA strain and in both strains the incidence of lymphoma decreased when the cells were transferred SC.

Single Cell and Low Dose Implants in Gelatin. The gelatin cell transfer technique was used to transplant single cells or doses of 10 - 20 cells (Table 3). The interesting point here again is that the clonal lines behaved on transfer very much as their parent lines. Also, selection of a single cell for derivation of a clonal line did not result in the clonal line growing significantly better, i.e. with a higher incidence and/or shorter latent period of lymphoma in the recipient. All of the LT and CL lines could be transplanted at the 10 - 20 or single cell dose while only 1 in 56 attempts at transfer with 10 - 20 cells and no attempts at transfer of single cells were successful with the VI cell source. All of the CBA CL lines derived from single cells bear the donor T₆ chromosome marker indicating that recipient lymphomas were indeed of donor origin. (3 single cell recipients, Table 3).

DISCUSSION

The most striking result from this study is the significantly different LD 90 - 100 doses of lymphomas on primary transplant from a VI source as compared to lymphomas similarly derived originally from virus-induced lymphomas but maintained by syngeneic transplant before determination of the LD 90 - 100 dose. Future work will explore a number of possible reasons for these findings. It has already been shown (6, 12) that antigenic simplification, modification or loss of antigens occurs over prolonged cell passage of tumors. However the relationship of virus expression in the transplanted cell should be explored in relationship to success of syngeneic transplantation. For the present, one can speculate that the cell source from mice receiving virus as newborns may contain more virus and that the antigens of the virus result in a greater antigenicity of the cell manifested by the higher LD 90 - 100. Barbieri, et. al. (1) reported that when two C 57 BL/6 sarcomas were infected in vitro with Rauscher leukemia virus the tumor-producing capacity of the cells, which continued to multiply normally in vitro, dropped considerably when assayed in syngeneic mice. There was a great decrease in incidence of lymphoma and prolongation of latent periods in the recipients. The mechanism of the cell-virus interaction in this response is not yet understood.

It is also interesting that our most successful attempt at single cell transfer in gelatin with CL 1 resulted in a 24% incidence of lymphoma. Skipper working with L 1210 cells and a micromanipulator technique

obtained a 16.6% incidence of lymphoma. The results are comparable and may indicate that long transplanted lines cannot transplant with a 100% incidence with single cells, but that the 16 - 24% incidence represents the number of cells at a growth stage (2) critical to success to syngeneic implantation. The fact that one recipient of 10 - 20 virus-induced C3H lymphoma cells developed lymphoma may indicate that cells at this critical growth stage are scattered throughout the VI population, but are not as numerous as in populations of long transplanted lines. Cikes and Friberg (3) monitored in vitro the expression of H-2 and Moloney virus-determined surface antigens during one cell cycle by an indirect membrane-immunofluorescence technique. The highest proportion of antigen-positive cells occurred during the G1 period of the cell cycle. Fewer antigen-positive cells were found as the cells entered the S period and during the S period.

It is interesting that only C3H LT 1 and CBA LT 1 exhibited a decrease incidence of takes when injected subcutaneously as compared to intraperitoneally. Longer latent periods of lymphoma following subcutaneous as compared to intraperitoneal injections have been noted in the L 1210 mouse system (8). It also has been shown that antigenicity and malignancy of mouse tumor lines maintained in vitro and transplanted in vivo depended on the site of implantation in the recipient (7). While intraperitoneal grafts resulted in a high incidence of deaths, subcutaneous grafts frequently produced tumors which regressed and immunized the recipient to subsequent intraperitoneal challenge. Donor cells were obtained from several different tissues of adult mice grown in vitro which underwent a malignant transformation in the absence of any known carcinogen at 2 to 9 transfer generations. Our lines C3H LT 1 and CBA LT 1 may well be

different antigenically from the other LT and CL lines. This will be tested in the future.

From this work it is apparent that single cells do produce fatal lymphoma upon intraperitoneal transplant to syngeneic recipients, but that such lymphomas result more readily if the donor source has been maintained several generations by cell transfers.

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Table 1. Lymphoma lines

Line	Generation Number at Initiation of Study of Line	Lymphoma Latent Period (Days) in Recipients of 1×10^6 Cells IP ^a		LD 90 - 100 ^b
		Range	Median	
C3H LT 1	139	7 - 13	11	1×10^3
CL 6 ^c	24	7 - 13	9	1×10^4
LT 2	41	6 - 8	7.5	10 - 20
CL 1 ^d	33	6 - 13	8	10 - 20
LT 3	20	7 - 15	10	1×10^3
CL 3 ^e	41	5 - 11	9	1×10^4
VI	1	28 - 28	28	1×10^8
CBA LT 1	34	9 - 19	13	1×10^3

^aLatent periods were calculated for generation 3 post isolation from the virus-induced source to generation number listed in column 2 of table.

^bDone on generation number listed in column 2 of table.

^cDerived from a single cell from LT 1 at generation number 151.

^dDerived from a single cell from LT 2 at generation number 53.

^eDerived from a single cell from LT 3 at generation number 25.

Table 2. Comparison of IP and SC injection routes for 1000 lymphoma cells

Line	IP			SC		
	Incidence	Latent period (days)		Incidence	Latent period (days)	
		Range	Median		Range	Median
C3H LT 1	30/31	15-34	26	5/27	17-29	20
LT 2	50/50 ^a	15-17	17	16/16	18-41	25.5
LT 3	20/20	14-33	18	19/19	15-30	23
CBA LT 1	9/9	19-27	21	4/8	20-42	26

^a100 instead of 1000 cells transferred

Table 3. Low dose gelatin transplants from lymphoma lines

Line	Cell dose					
	10-20			1		
	Incidence	Latent period (days)		Incidence	Latent period (days)	
		Range	Median		Range	Median
C3H LT 1	13/20	16-34	20	2/52	19, 20	
CL 6	4/19	20-51	33	N.D.		
LT 2	45/45	15-30	19	3/4	17-21	20
CL 1	20/20	16-21	17	12/50	17-34	18
LT 3	8/20	14-18	15	7/52	13-27	18
CL 3	10/30	13-19	16	N. D.		
C3H VI	1/56	53	-	0/40		
CBA LT 1	13/20	22-46	27	3/39	21-34	27