Haematology and Blood Transfusion Vol. 26
Modern Trends in Human Leukemia IV
Edited by Neth, Gallo, Graf, Mannweiler, Winkler

Ontogeny of Abelson Murine Leukemia Virus Target Cells

G. L. Waneck and N. Rosenberg

A. Introduction

Abelson murine leukemia virus (A-MuLV) is a replication defective retrovirus that induces a thymus-independent lymphoma after a short, 3–5 week latent period (Abelson and Rabstein 1970). The virus is unique among murine retroviruses in its ability to transform lymphoid cells in vitro (Rosenberg and Baltimore 1976; Rosenberg et al. 1975). The lack of thymic involvement in the disease (Abelson and Rabstein 1970; Siegler et al. 1972) and the susceptibility of athymic nu/nu mice to the virus (Raschke et al. 1975) coupled with an inability to detect T lymphocyte markers on the tumor cells (Sklar et al. 1975) has suggested that this lymphoma is made up of malignant cells related to B lymphocytes. The ability to detect immunoglobulin (Ig) in some tumors further supports the involvement of cells of the B lymphocyte lineage in this malignancy (Potter et al. 1973; Prekumar et al. 1975).

A significant percentage of clonally derived in vitro transformants isolated from adult bone marrow also express Ig (Boss et al. 1979; Pratt and Strominger 1977; Rosenberg and Witte 1980; Siden et al. 1979). Like the in vivo derived tumor cells, these isolates lack phenotypic markers characteristic of mature T or B lymphocytes (Boss et al. 1979; Silverstone et al. 1978). A very large number of the isolates express terminal deoxynucleotidyl transferase (TdT) (Rosenberg and Witte 1980; Silverstone et al. 1978), an enzyme associated with many T lymphocytes and probably with immature B lymphocytes (Chang 1971; Kung et al. 1975; Silverstone et al. 1978).

The studies of the phenotypic markers expressed by A-MuLV-transformed cells suggest that they may be analogous to a normal cell present early in the B lymphocyte lineage. While this type of study may serve as a useful guide to predicting the nature of the cell(s) susceptible to A-MuLV, no conclusive evidence of a correlation between the phenotype of the transformed cell and the phenotype of the target cell(s) has been presented.

Direct examination of A-MuLV target cells is difficult because of the heterogeneity of hematopoietic cell preparations and the low frequency of these cells in the population. The observation that bone marrow contains the highest number of A-MuLV susceptible cells among adult hematopoietic tissues (Rosenberg and Baltimore 1976; unpublished work) is consistent with the hypothesis that A-MuLV transforms fairly undifferentiated cells. The ability to eliminate more than 90% of A-MuLV target cells from bone marrow with a monoclonal antibody that reacts with normal pre-B lymphocyte colony forming cells (Shinefeld et al. 1980; Kincade, and Sato, personal communication) also supports this notion.

Recently, we have examined the appearance of A-MuLV target cells during ontogeny by determining the frequency of these cells in fetal liver at various times of gestation. These experiments demonstrate that cells susceptible to A-MuLV-induced transformation arise within a defined 24 h period between day 12 and day 13 of gestation and increase in number until birth. Study of Ig and TdT expression in clonally derived lines arising from livers at different times during gestation demonstrates that the frequency of cell lines expressing these markers varies depending upon the gestation time. Because of the consistent frequency of expression of these markers in adult bone marrow derived cell lines, the variations observed with the cell lines of fetal origin may

indicate a shifting of target cell populations during ontogeny.

B. Results

I. Presence of A-MuLV Target Cells in Fetal liver

Fetal livers from embryos at various stages of gestation were examined for the frequency of cells susceptible to A-MuLV-induced transformation using the quantitative agar assay (Rosenberg and Baltimore 1976). Consistent with previous results (Rosenberg and Baltimore 1976; Rosenberg et al. 1975), infection of late gestation livers resulted in high numbers of transformants (Fig. 1). The maximum number of foci was found in 18–19 day fetal livers where the frequency was about twice that observed in adult bone marrow. Examination of earlier gestation livers revealed a gradual decrease in the number of transformants, with only 1.3 foci/ 10^6 nucleated white blood cells being observed at day 13. No foci were observed when livers from day 11 and day 12 of gestation were examined. After birth the number of foci observed after infection of liver cells had declined drastically and cells susceptible to A-MuLV could be detected in bone marrow (Fig. 1).

II. Lack of Toxicity in Fetal Liver Preparations

The failure to detect A-MuLV transformants in early fetal liver and the low frequency of foci in 13–14 day fetal liver could result from either a lack of target cells or from the presence of toxic factors or cells that suppress the expression of potential transformants. To distinguish

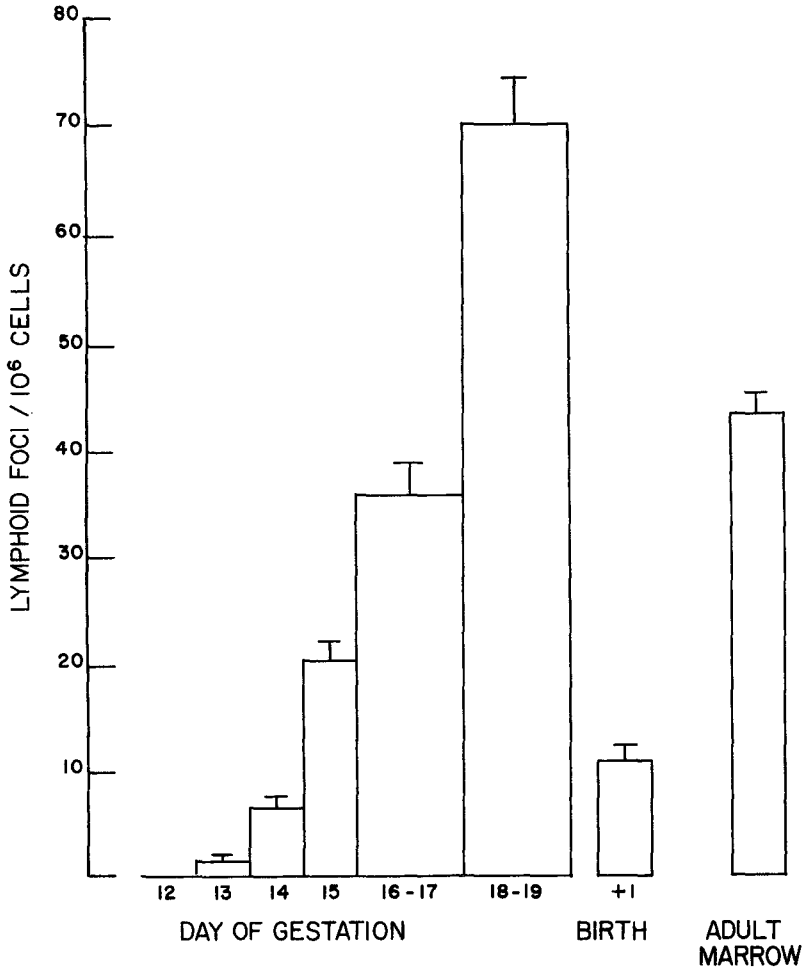

Fig. 1. Ontogeny of A-MuLV target cells. Fetal livers at various points in gestation were removed from embryos, dissociated, and infected with A-MuLV P160 (Rosenberg and Witte 1980). Gestation times were determined by vaginal plug with the morning of detection being considered day 0 of gestation. Bone marrow from 6–10-week-old adult mice was also examined. Foci were measured using the agar transformation assay. The results of 2–4 experiments have been pooled. The *error bars* indicate the standard error of the mean number of foci/well

between these two possibilities, fetal liver cells from gestation times when the number of transformants observed was low were infected and plated either alone or after dilution with an equal number of adult bone marrow cells (Table 1). The mixed cultures contained the same total number of cells as each of the cultures in which the two types were plated alone. Thus, the expected number of foci in the mixed plating was calculated by halving the sum of the foci observed when the two types of cells were plated separately. In all cases, the expected and observed frequency of transformants coincided closely (Table 1).

III. Phenotypic Markers Expressed by Fetal Liver Clones

Clones of A-MuLV-transformed cells were removed from agar with a pasteur pipette and either examined morphologically after Wright-Giemsa staining or adapted to grow as continuous cell lines. Study of over 50 colonies from various times of gestation revealed that all of these clones were composed of lymphoid cells characterized by a large nucleus with one or more nucleoli and a scant cytoplasm that lacked granules. No morphologic features distinguishing these clones from clones isolated from adult bone marrow were noted.

The expression of Ig and TdT was examined by SDS polyacrylamide gel analysis of ^{35}S-methionine-labeled immunoprecipitates. These two lymphocyte markers are expressed by a large number of A-MuLV transformants derived from adult bone marrow. For example, one survey of 22 such clonally derived isolates revealed that 68% of them were μ positive and 91% expressed TdT (Rosenberg and Witte 1980).

Nearly all the cells lines derived from foci of transformed neonatal bone marrow or liver cells expressed both Ig in the form of μ chain and TdT (Table 2). When a large number of late fetal liver-derived cell lines were examined, about the same frequency of μpositive clones was observed but TdT synthesis was not detected in any of the cell lines. A reversed pattern of TdT and μ expression was observed when foci from early fetal liver were examined. In this case, only 14% of the isolates expressed μ while 64% synthesized TdT (Table 2).

C. Discussion

Cells susceptible to A-MuLV-induced transformation appear in fetal liver within a defined, less than 24 h period between day 12 and day 13 of gestation. The inability to detect transformants in earlier fetal liver is due to a lack of detectable numbers of target cells and not the result of inhibitory cells or factors in these tissues.

Sensitive immunofluorescent staining techniques have detected Ig-positive cells in fetal liver at days 11–12 of gestation (Raff et al. 1976). However, precursors of functional mitogen- responsive B lymphocytes are first detected at day 13 of gestation (Melchers 1977). The kineties of appearance of this lymphocyte precursor cell parallels the appearance of A-MuLV target cells. The coincident appearance of cells with these two properties

Table 1. Detection of A-MuLV transformants in liver and bone marrow mixtures[a]

Day of Gestation	Foci/ 10^6 Cells			
	Plated separately		Plated together	
	Liver	Adult marrow	Observed	Expected
12	<0.5	44	21	22
13	3.0	28	20	16
14	13	32	24	23
15	21	54	36	37
newborn	22	32	28	27

[a] Liver and bone marrow cells were infected with virus separately or as 1:1 mixtures and then plated. The total cell number in both cases was the same. Transformation was examined using the agar assay. The expected number of foci was derived by halving the sum of the foci observed when each cell type was plated alone

Source of clones	No. clones tested	% Clones positive for marker	
		μ	TdT
Neonatal liver	4	100	100
Neonatal marrow	3	67	100
17–19 d fetal liver	16	75	<6
13–14 d fetal liver	14	14	64

Table 2. Expression of μ and TdT by A-MuLV-transformed cells[a]

[a] Cultures of cells were examined within 3–4 weeks after removal from agar. Expression of μ and TdT was detected by SDS polyacrylamide gel analysis of ^{35}S-methionine-labeled immunoprecipitates as previously described (Rosenberg and Witte 1980; Witte and Baltimore 1978). The goat anti-Moloney virus, polyspecific rabbit antimouse Ig and the rabbit anti-TdT sera used in these studies have been previously described (Rosenberg and Witte 1980; Silverstone et al. 1980; Witte and Baltimore 1978; Witte et al. 1978)

does not mean that the mitogen-responsive precursors and A-MuLV target cells are identical. However, the appearance of the mitogen-responsive precursor cells may signal the onset of active lymphopoiesis in the fetal liver, even though a few Ig-positive cells are present prior to day 13.

Results obtained with adult bone marrow indicate that actively cycling cells are most susceptible to A-MuLV-induced transformation (unpublished work). Thus, the time at which target cells appear in fetal liver could reflect both the appearance of appropriate lymphocytes and the entry of significant numbers of these cells into the cell cycle.

The frequency of clones expressing μ and TdT varies depending upon the age of gestation of the fetus. This variation is probably significant because the frequency of expression of the markers is quite constant among the large numbers (>75) of cell lines isolated from adult bone marrow. In addition, clones from neonatal animals are similar to the clones from adult tissue with respect to frequency of μ and TdT positive cells. Thus, changes in the frequencies of μ and TdT expression among fetal liver-derived cell lines probably reflect changes in the predominant target cell population during ontogeny. This shift may result from the different proportions of certain types of lymphoid precursors existing in rapidly cycling states at different times during gestation.

Acknowledgments

This work was supported by Public Health Service Grant CA-24220 and Program Project grant CA-24530 from the National Cancer Institute. N.R. is a recipient of an American Cancer Society Research Scholar Award (Massachusetts Division).

References

Abelson HI, Rabstein LS (1970) Lymphosarcoma: Virus-induced thymic-independent disease in mice. Cancer Res 30:3313–2222 – Boss M, Greaves M, Teich N (1979) Abelson virus transformed haemopoietic cell lines with pre-B cell characteristics. Nature 278:551–553 – Chang LMS (1971) Development of terminal deoxynucetidyl transferase activity in embryonic calf thymus gland. Biochem Biophs Res Commun 44:124–131 – Kung PC, Silverstone AE, McCaffrey RP, Baltimore D (1975) Murine terminal deoxynucleotidyl transferase: Cellular distribution and response to cortisone. J Exp Med 141:855–865 – Melchers F (1977) Blymphocyte development in fetal liver II. Frequencies of precursor B cells during gestation. Eur J Immunol 482–486 – Potter M, Sklar MD, Rose WP (1973) Rapid viral induction of plasmacytomas in pristane-primed BALB/c mice. Science 182:592–594 – Pratt DM, Strominger J Parkaman R, Kaplan D, Schwaber J, Rosenberg N, Scher CD (1977) Abelson virus – transformed lymphocytes: Null cells that modulate H-2. Cell 12:683–690 – Prekumar E, Potter M, Singer PA, Sklar MD (1975) Synthesis, surface deposition, and secretion of immunoglobulins by Abelson virus – Transformed lymphosarcoma cell lines. Cell 6:149–159 – Raff MC, Megson M, Owen JJT, Cooper MD (1976) Early production of intracellular IgM by B-lymphocyte precursors in mouse. Nature 259:224–226 – Raschke WC, Ralph P, Watson J, Sklar M, Coon H (1975) Brief communication: Oncogenic transformation of murine lymphoid cells by in vitra infection with Abelson leukemia virus. J Natl Cancer Inst 54:1249–1253 – Rosenberg N, Baltimore DA (1976) Quantitative assay for transformation of bone marrow cells by

Abelson murine leukemia virus. J Exp Med 143:1453–1463 – Rosenberg N, Witte ON (1980) Abelson murine leukemia virus mutants with alterations in A. MuLV specific P120 molecule. J Virol 3:340–348 – Rosenberg N, Baltimore DR, Scher CD (1975) In vitro transformation of lymphoid cells by Abelson murine leukemia virus. Proc Natl Acad Sci USA 72:1932–1936 – Shinefeld L, Sato V, Rosenberg N (1980) Monoclonal rat antimouse brain antibody detects Abelson murine leukemia virus target cells in mouse bone marrow. Cell 20:11–18 – Siden ET, Baltimore D, Clark D, Rosenberg N (1979) Immunoglobulin synthesis by lymphoid cells transformed in vitro by Abelson murine leukemia virus. Cell 16:389–396 – Siegler R, Fajdel S, Lane E (1972) Pothogenesis of Abelson-virus-induced murine leukemia. J Natl Cancer Inst 48:189–218 – Silverstone A. E, Rosenberg N, Sato VL, Scheid MP, Boyse EA, Baltimore D (1978) Correlating terminal deoxynuckotidyl transferase and cell surface makers in the pathway of lymphocyte ontogeny. In: Clarkson B, Till JE, Marks PA (eds) Cold Spring Harbor conferences on cell proliferation, vol 5. Cold Springs Harbor Press, Cold Spring Harbor, pp 432–452 – Silverstone AE, Sun L, Witte ON, Baltimore D (1980) Biosynthesis of murine terminal deoxynucleotidyl transferase. J Biol Chem 255:791–796 – Sklar MD, Shevach EM, Green I, Potter M (1975) Transplantation and preliminary characterization of lymphocyte surface markers of Abelson virus-induced lymphomas. Nature 253:550–552 – Witte ON, Baltimore D (1978) Relationship of retrovirus polyprotein cleavages to viron maturation studied with temperature – Sensitive murine leukemia virus mutants. J Virol 26:750–761 – Witte OM, Rosenberg N, Paskind M, Shields A, Baltimore D (1978) Identification of an Abelson murine leukemia virus-Encoded protein presnets in transformed fibroblasts and lymphoid cells. Proc Natl Acad Sci USA 75:2488–2492