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

Receptor Mediated Murine Leukemogenesis: Monoclonal Antibody Induced Lymphoma Cell Growth Arrest

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A. Introduction

We have proposed a receptor-mediated leukemogenesis hypothesis wherein T lymphomas would be clones of T cells bearing mitogen-linked surface receptors specific for the envelope determinants of the inducing MuLV (Weissman and Baird 1977; McGrath and Weissman 1978, 1979). We have tested a wide range of murine thymic lymphomas induced by thymotropic retroviruses and have shown that these cells indeed bear surface receptors highly specific for the retrovirus envelope glycoproteins produced by these cells (McGrath et al. 1978a,b). Even closely related recombinant retroviruses bound less well than the autologous retroviruses (McGrath et al. 1978b, McGrath and Weissman 1979). That these retroviruses and their interactions with T lymphoma cell receptors might be involved in the pathogenesis of murine leukemia was suggested by two kinds of experiments: First, only the leukemogenic viral isolates bound specifically to lymphoma cell receptors, while nonleukemogenic but closely related viral preparations bound less well or not at all (McGrath et al. 1978b, McGrath and Weissman 1979). Second, if one examined the distribution of receptor bearing cells in the thymus of a preleukemic AKR mouse, only T cells bearing cell surface receptors recognizing the inducing MuLV were capable of adoptive transfer of the leukemic state, whereas morphologically similar cells within the same thymus were not capable of leukemic proliferation in syngeneic hosts (McGrath and Weissman 1978, 1979).

While the above described experimental results are consistent with the receptor-mediated leukemogenesis hypothesis – and in fact were necessary postulates of the hypothesis – they do not constitute proof of the hypothesis. The central postulate of the hypothesis is that continued leukemic proliferation is the result of continued antigenic stimulation of these T cell lymphomas. The purpose of this study was to design experiments aimed at testing that postulate. To do so, we raised monoclonal antibody reagents to leukemia cell surface determinants and tested the effect of these antibodies on leukemic cell proliferation in vitro as well as on specific virus binding to these cells. Several antibodies were found which inhibited lymphoma cell proliferation in vitro. Evidence is presented that this may be due to a blockade in virus binding to these cells.

B. Results

Rat monoclonal antibodies raised against the spontaneous AKR/J thymic lymphoma cell line KKT-2 were added to exponentially growing KKT-2 cells in culture for 16 h, and ^{3}H-thymidine incorporation of these cells was quantitated as in Fig. 1. Antibodies recognizing the Thy-1 or T-80 molecules inhibited thymidine uptake by these cells, whereas anti-MuLV and antitumor-specific (43–17, 43–13) antibodies showed no effect. The inhibitory antibodies were effective at dilutions approaching 1:10,000 and KKT-2 growth inhibition was directly related to the level of cell surface antibody bound and the degree of interference with MuLV binding (McGrath et al. 1979, 1980a,b). Inhibitiory antibodies result in cell stasis at cell cycle phase G_1. In the initial set of experiments there was a linear fall to background in KKT-2 cell DNA synthesis, with a shift in DNA content from 4n

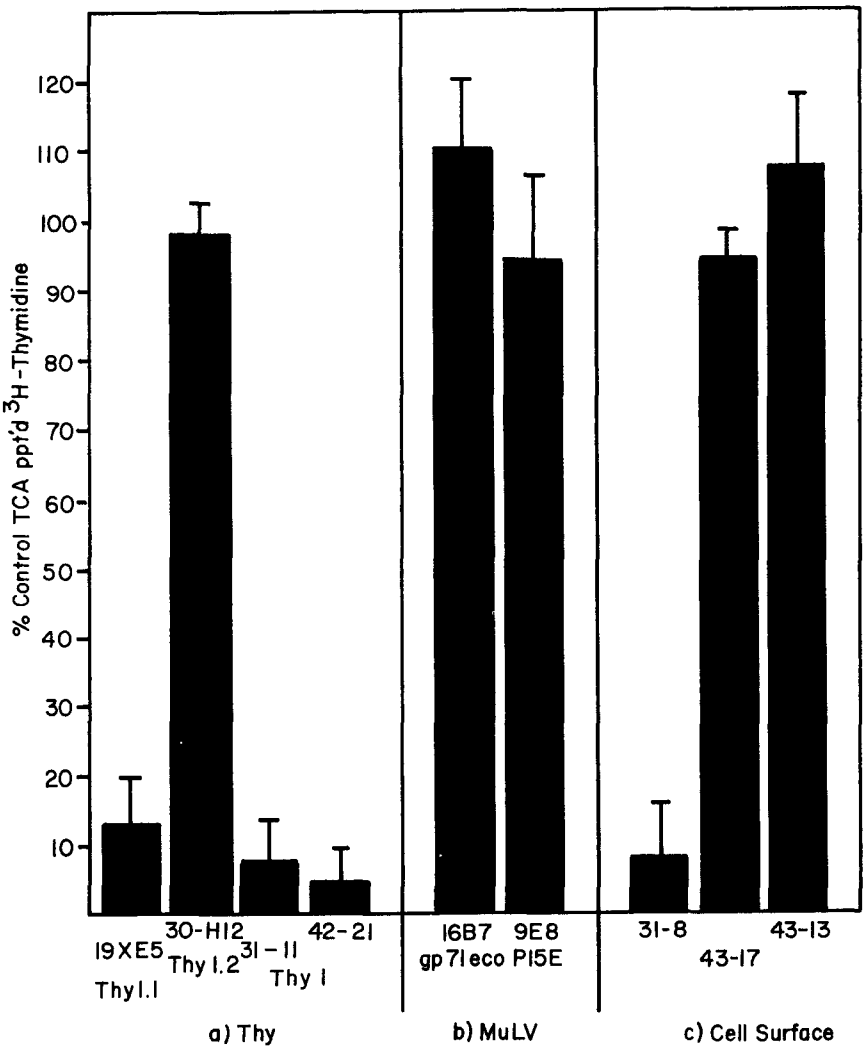

Fig. 1. Lymphoma cell growth inhibition assay. KKT-2 lymphoma cells were pelleted from subconfluent cultures and were resuspended in serum-free grwoth medium (MEM, GIBCO) for 2 h at 37°C at a density of 10^5 cells/ml. After repelleting, the cells were resuspended in cold tissue culture medium (MEM, 5% FCS) at a density of 2×10^4 cells/ml. Two and one-half milliliters (5×10^4 cells) were placed in the bottom of 25-cm^2 Corning tissue culture flasks and monoclonal antibody was added for a 1:12 final dilution. After growing for 16 h at 37°C, 0.1 ml medium containing 10 μCi [^{3}H] thymidine (NEN) was added to each culture for 2 h. Labeled cells were washed, 5% TCA precipitated, and percent growth inhibition was calculated using cells without antibody as equal to 100% growth. KKT-2 cells were tested for growth inhibition with (**a**) anti-Thy-1 (19XE5, 30-H12, 31–11, 42–21), (**b**) anti-MuLV (16B7,9E8), and (**c**) anticell surface antibodies (31-8, 43-17, 43-13). The above data represent five experiments ± standard deviations

towards 2n copies within 20 h (McGrath et al. 1979). This corresponded with a cellular size shift from large to small within 24 h culminating in noncomplement mediated cell death within 48 h (McGrath et al. 1979, 1980b).

Figure 2 shows that prebinding of KKT-2 receptors with KKT-2-SL virus protects the cell from inhibition whereas even closely related heterologous viruses do not. The level of cell surface bound antibody is not decreased by prebound KKT-2-SL virus, and preabsorption of antibody with KKT-2-SL virus does not remove inhibitory activity (McGrath et al. 1979, 1980a,b).

Prior to January 1980 antibody inhibition of KKT-2 cell growth by the inhibitory antibodies occurred rapidly in the first cell cycle following addition of the antibodies. Since that time and concomitant with a required change in tissue culture serum sources, inhibitory antibodies (42-21, 31-11, 31-8) act on KKT-2 cells only on the 2nd–4th day following addition of the antibodies. The same distribution of inhibitory and noninhibitory antibodies is seen as previously reported (McGrath et al. 1980a). The

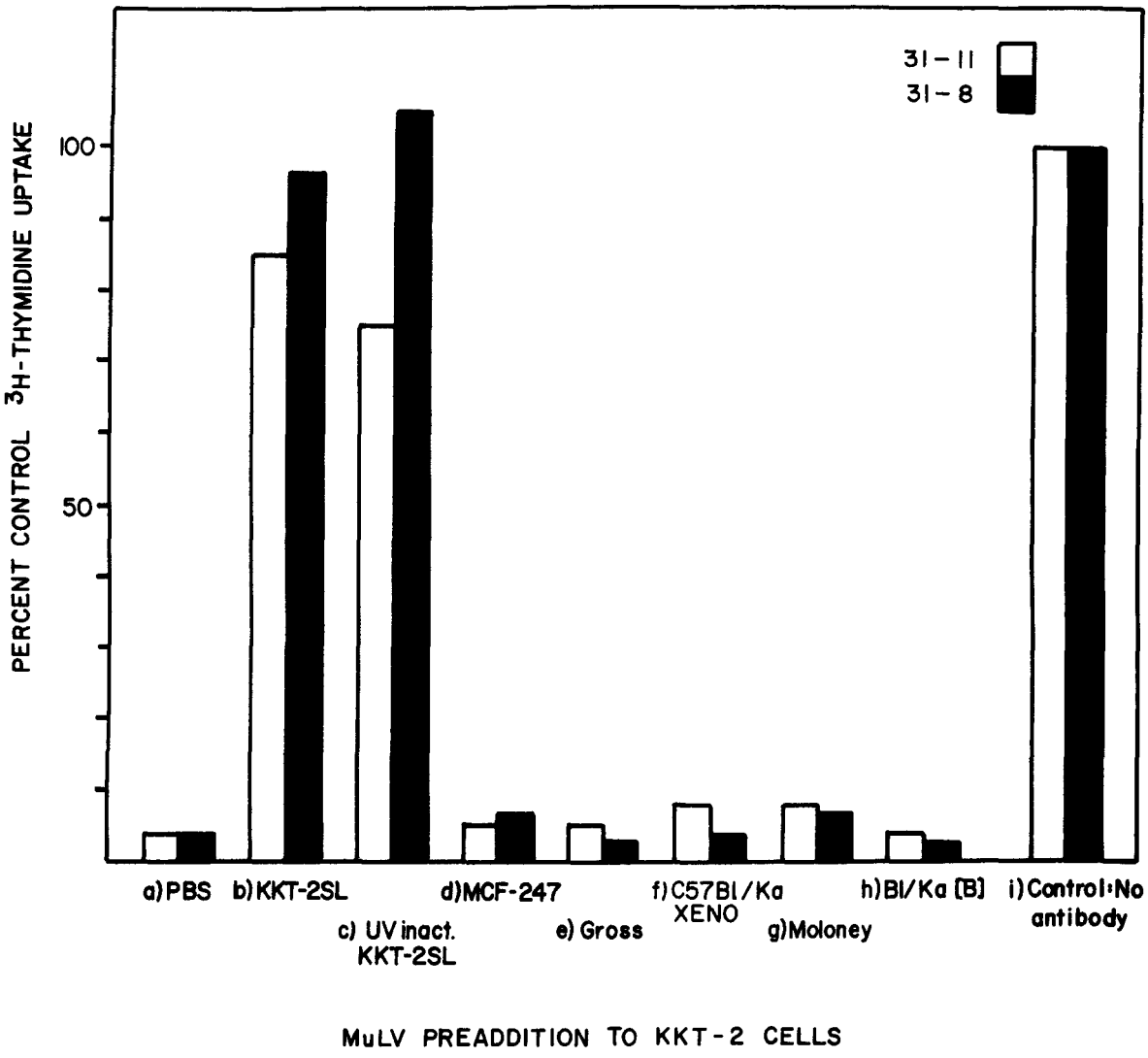

Fig. 2. The standard KKT-2 cell growth inhibition assay as outlined in Fig. 1 was carried out with 31–11 and 31–8 antibodies at a 1:250 dilution on 5×10^4 KKT-2 cells after preincubation with purified retroviruses. 0.01 a_{260} unit of Sepharose-4B-purified virus (McGrath et al. 1978c) in 0.3 ml PBS was incubated with KKT-2 cells for 60 min at room temperature prior to addition of inhibitory antibodies. This amount of virus represents a receptor saturation level as previously determined (McGrath et al. 1978b). The origin of each retrovirus population has also been previously described (McGrath et al. 1978b; McGrath and Weissman 1979). Sepharose-4B-purified KKT-2-SL virus was also UV inactivated and used to inhibit antibody-induced KKT-2 cell growth inhibition. Five milliliters of virus in PBS (1 A_{260} unit/ml) was irradiated for 145 sec at 4000 ergs/mm^2 prior to use (Niwa et al. 1976)

inhibitory antibodies (αThy-1 and αT80) act similarly on several other T-MuLV-induced T lymphomas. However, S49 cells are still inhibited in the first cell cycle.

Because anti-Thy-1 arrested lymphoma cell growth, we tested several mutant lymphoma cell lines for MuLV binding to determine more precisely the nature of the MuLV receptor. Table 1 shows that both Thy 1^- and H-2^- cell lines have equivalent MuLV receptor levels to those of their parental cell lines. We have not yet tested whether there is some correlation between S49 MuLV binding, growth regulation, and level of antibody bound per cell.

Because T lymphoma cells have been shown to be susceptible to lectin, dexamethasone (Ralph 1973), and cAMP-induced (Coffino et al. 1975a) cell death, we tested several S49 mutant cell lines for susceptibility to antibody mediated growth arrest. Table 2 shows that cAMP is probably not involved in the antibody-induced growth arrest. Similarities between dexamethasone and lectin induced cell killing are currently still under investigation.

	Fluoresceinated MuLV preparations		
	MCF-247	RadLV/VL_3	S49-SL
1. KKT-2	100	68	46
2. BL/VL_3	62	100	57
3. S49 (Clone 24–32)	48	62	100
4. S49 (Thy 1^-)	51	52	110
5. L691/M.E12 (Clone E12 H-2^-)	250	250	N.D.
6. L691/M (H-2^-)	160	160	N.D.
7. Balb/c thymocytes	5	5	8

Table 1. MuLV binding specificities: maximal percent age MuLV bound per cell[a]

[a] Maximal binding respresented by the mean fluoresceinated homologous virus bound per cell, i.e., RadLV/VL_3 binding to BL/VL_3 cells. N.D. = not done. Each entry represents the percentage of maximal fluorescence for the cell population as calculated by the following formula: % = mean fluorescence of test population/mean fluorescence of standard population × 100 where the standard population is the homologous virus-cell interaction. Fluoresceinated MuLV preparations were tested for binding specificity to four different cell populations. Binding was analyzed as described in experimental procedures and the legend to Fig. 1, and the mean fluorescence of virus bound per cell above cellular background was calculated. 100% binding was defined as virus binding to a homologous lymphoma (that is, MCF-247 to KKT-2 to RadLV/VL_3 to BL/VL_3, S49-SL to S49). The cell lines tested were: (1) spontaneous AKR/J thymic lymphoma, KKT-2; (2) RadLV-induced C57BL/Ka thymic lymphoma, BL/VL_3; (3) wild type spontaneous Balb/c thymic lymphoma S49, clone 24.32 (from Coffino, UCSF); (4) S49 Thy-1^-a mutant (Salk Institute); (5) C57/Leaden x-ray-induced T lymphoma L691, clone E12 selected for absence of H-2 (McGrath et al. 1980a and unpublished work in preparation); (6) Moloney-infected L691 producer cells, non-H-2 expressing from clone E12; and (7) normal 4-week-old Balb/c thymocytes

C. Discussion

Growth control of normal T cells and T lymphomas is extremely complex. The relationships between antigen-induced (Fathman and Weissman 1980) and growth factor induced (i.e., TCGF) (Morgan et al. 1976; Gillis et al. 1980) T cell proliferation are currently under intense investigation and the variables involved are multiple and poorly understood. As noted above, KKT-2 cells are now not inhibited in the 24 h growth assay; anti-Thy-1 antibodies no longer inhibit MuLV binding to those cells in a short-term assay. Yet this antibody does inhibit proliferation of KKT-2 cells in a 3–4 day incubation period. These findings may implicate several pathways to mitogenesis in this cell line, one of which is directly related to anti-Thy-1 and anti-T-80-induced inhibition. No experiment which we have carried out yet demonstrates the identity of the viral receptor. While αThy-1 antibodies may block T-MuLV binding and T lymphoma proliferation, the former effect must be via steric hindrance, as Thy-1 molecules need not be expressed for specific T-MuLV binding. It now becomes even more important to identify the T-MuLV receptors on T-lymphoma cells, to establish their relatedness between lymphomas, to establish whether they are products of cellular or viral genes, and to identify more precisely the viral determinants they recognize.

Table 2. Growth inhibition of S49 enzyme mutants after exposure to monoclonal antibodies[a]

S49 cell type	Treatment of cells				
	a) Control	b) Bt_2cAMP	c) 31–11 (1:80) Anti-Thy 1	d) 31–8 (1:80) Anti-T-80	e) PHA (10 μg/ml)
1. Wild type Clone 24.32	−	++++	++++	++++	++++
2. Protein kinase mutant	−	−	++++	++++	++++
3. Adenylate cyclase mutant	−	++++	++++	++++	++++
4. Deathless mutant	−	++	++++	++++	++++

[a] S49 cell lines were suspended at 10^5 cells per ml for 3 days in the growth inhibition assay outlined in Fig. 1. Cell growth and inhibition was quantitated by live and dead cell counting after treatment as shown above. a = control cells; b = 3.3×10^{-4} *M* final concentration of dibutyrl cAMP (Sigma); c = 1:80 final dilution of 31–11 anti-Thy-1; d = 1:80 final dilution of 31–8 anti-T-80; e = 10 μg/ml final concentration of phytohemagglutinin (Welcome, purified). The S49 mutant cell lines and their properties have been previously described: wild-type clone 24.32 and protein kinase mutant by Coffino et al. (1975b); adenylate cyclase mutant by Bourne et al. (1975); deathless mutant, which is arrested by cAMP but not killed, by Lemaire et al. (1977). ++++ = all cells dead; ++ = cell alive; no growth; and − = no effect.

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