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Specificity of Cell Surface Virus Receptors on Radiation Leukemia Virus and Radiation-Induced Thymic Lymphomas

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We have developed a system for analysis of murine leukemia virus (MuLV) receptors on the surface of thymic lymphoma cells utilizing the fluorescence-activated cell sorter. The binding of fluoresceinated or rhodaminated MuLV to target cells showed saturation kinetics and was blocked by homologous MuLV, and bound MuLV had a polypeptide profile identical to that of input MuLV. Thymic lymphomas bound specifically the MuLV which induced them, whereas only 0.5 to 2% of normal thymocytes showed equivalent MuLV binding. Simultaneous binding of excess fluoresceinated RadLV and rhodaminated MCF-247 AKR virus to radiation leukemia virus-induced or spontaneous AKR thymic lymphomas demonstrated that even in the presence of both viruses the cells bound preferentially the inducing MuLV. Examination of the C57BL/Ka endogenous viruses showed that radiation leukemia virus-induced thymic lymphomas bind only thymotropic-leukemogenic radiation leukemia virus and not eco- or xenofibrotropic MuLV's. Thus, virus binding in this system involves only leukemogenic isolates of these retroviruses and implies a central role of this receptor-ligand interaction in the processes of leukemic transformation.

Murine lymphomas and lymphatic leukemias are usually thymic in origin, and are induced by a class of type C RNA viruses known collectively as the murine leukemia viruses (MuLV's) (11, 12). Initially, these viruses were divided into two classes by virtue of their respective host range: ecotropic retroviruses, which infect mouse (and perhaps rat) cells, and xenotropic retroviruses, which infect a diverse range of non-mouse cells. Ecotropic retroviruses have been further divided according to their specific tissue tropisms, i.e., thymotropic retroviruses infect thymocytes, whereas fibrotropic retroviruses infect fibroblasts (3).

The C57BL/Ka mouse produces retroviruses of widely varying host ranges. Of these, only radiation leukemia virus (RadLV) is thymotropic and leukemogenic (4). RadLV is not fibrotropic and is serologically distinct from the fibrotropic C57BL/Ka retrovirus isolates [BL/Ka(B), BL/Ka(N), and BL/Ka(X)] (2). These fibrotropic retroviruses cannot productively infect thymocytes in vivo or induce lymphomas and lymphatic leukemias (3).

Virus adsorption to a target cell surface is a necessary antecedent to viral infection. MuLV infection, however, does not guarantee cellular transformation, i.e., thymotropic RadLV infects fibroblasts inefficiently, whereas RadLV injected intrathymically into a susceptible mouse will cause a productive infection and may ultimately lead to the development of thymic lymphomas (4, 11, 12). Therefore, investigation of MuLV-cell surface interactions with fibroblasts may not elucidate the role that these interactions play in leukemogenesis.

It has previously been demonstrated that each thymic lymphoma bears receptors for the MuLV which induced it (15, 16). These observations prompted the formulation of the "receptor mediated leukemogenesis" model of MuLV-induced thymic lymphomas (20). It was proposed that a virus-cell surface interaction might be important in terms of selecting a subpopulation of "transformable" cells from the thymus cell population and further, that this interaction might be a necessary event for the induction (and perhaps maintenance) of leukemic transformation. Central to that hypothesis is the postulate that each thymic lymphoma would be made up of the clonal progeny of a normal T cell bearing highly specific receptors for a particular MuLV glycoprotein.

In this communication we utilize the fluorescence activated cell sorter (FACS) (9, 10, 13-16) to quantitate the binding of fluorescent C57BL/Ka retrovirus isolates to mouse lymphoid cells. We show that RadLV-induced T-lymphomas bear receptors specific for leukemogenic RadLV's, but do not bind fibrotropic C57BL/Ka

retroviral isolates. RadLV-induced lymphomas are distinguishable from radiation-induced lymphomas that have receptors which bind several, but not all, murine retroviruses.

MATERIALS AND METHODS

Cells. BL/VL_3 and BL/AQR are established cell lines derived from radiation leukemia virus-induced thymic lymphomas. BL/RL_{12}-NP is an established nonproducer cell line derived from a C57BL/Ka radiation-induced thymic lymphoma (M. Lieberman et al., manuscript in preparation). MFT-2 is a Moloney virus-induced thymic lymphoma from (C57BL/6J × BALB/c)F_1, and LSTRA is a Moloney lymphoma from BALB/c. The KKT-1 cell line is derived from a spontaneous AKR/J thymic lymphoma. XP-63-AG(NP) is a nonsecretor BALB/c myeloma cell line obtained from R. Levy, Stanford University. All cell lines were grown in minimal essential medium (Gibco) supplemented with 10% fetal calf serum (FCS) in 5% CO_2-95% air. All thymocyte suspensions were prepared as described previously (1).

Virus. Supernatant fluids containing virus were harvested from recombinant AKR MuLV (MCF-247) virus-producing mink lung fibroblasts (from Janet Hartley, National Institutes of Health), from BL/Ka(B) (clone I_3F)-producing C57BL/Ka mouse embryo fibroblasts, from BL/Ka(X) (clone MNT)-producing mink lung fibroblasts, and two RadLV-LTC preparations: RadLV/VL_3 and RadLV/AQR from the BL/VL_3 and BL/AQR thymic lymphoma cell lines (Lieberman et al., manuscript in preparation). (It has been shown that the RadLV-LTC [i.e., RadLV from lymphoma cell culture] RadLV/VL_3 is similar to wild-type RadLV [Decléve et al., Virology, in press].) All virus was purified by Sepharose 4B chromatography in a phosphate buffer (PBS) (pH 7.4) before use (17). RadLV/AQR virus was labeled by incorporation of [^{3}H]- or [^{14}C]leucine, and virion polypeptides were analyzed by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) as previously described (21).

Virus fluoresceination and rhodamination. Sepharose 4B-purified virus in PBS (pH 7.4) at an absorbancy at 260 nm (A_{260}) of 1.5 to 3.0 U/ml was incubated with 1 mg of fluorescein isothiocyanate (FITC) lot E8HYS (BBL) per ml or with 0.025 mg of tetramethylrhodamine isothiocyanate (tRITC) lot F7EKOB (BBL) per ml at room temperature for 1 h. One-tenth volume of 1 M Tris buffer (pH 7.4) was then added to stop the fluoresceination reaction. The virus was rechromatographed through Sepharose 4B to remove free FITC and tRITC prior to use. Fluoresceinated virus consistently had A_{495}/A_{260} ratios of 0.2 to 0.35 and rhodaminated virus had A_{515}/A_{260} ratios of 0.1 to 0.2. All lots of FITC and tRITC were carefully screened for detrimental effects on final fluorescent MuLV binding to receptor-positive (BL/VL_3) and receptor-negative (normal thymocytes) cell preparations (e.g., see Fig. 1). Not all batches of FITC were usable. With usable lots of FITC and tRITC, a 40 to 60% decrease in specific fluorescent MuLV binding occurred with a 1:1 unlabeled MuLV blockade (as in Fig. 5), whereas binding to neonatal C57BL/Ka thymocytes never exceeded 3% of all cells above the FACS background.

Virus binding assay and FACS analysis. The FACS is an automated fluorimeter which is capable of measuring the light-scattering properties and associated fluorescence of individual cells at a rate of up to 3,000 cells per s (9, 10, 13). In this study the associated fluorescent ligands were fluoresceinated and rhodaminated MuLV's. FACS data are expressed as the fraction of input cells belonging to a particular light-scattering class (relative cell size) or bearing a particular amount of fluorescence per cell on the Y-axis versus increasing cell size or fluorescence intensity on the X-axis (9, 10). Simultaneous analysis of two fluorochromes can be carried out, with a frequency distribution of cells expressing particular levels of the fluorochromes plotted in three dimensions, again with fractional distribution on the vertical (Z) axis versus fluorochrome intensities on the horizontal axes (X, rhodamine; Y, fluorescein) (9, 14).

The binding of fluoresceinated and rhodaminated virus to target cells at binding site saturation was carried out as previously described (14, 15). Briefly, 0.1 U at A_{260} of labeled virus in PBS was incubated with 5×10^5 target (lymphoma or thymocyte) cells in 0.2 ml of PBS supplemented with 1% FCS at 4°C for 75 min and agitated every 15 min. This mixture was then pelleted through 1 ml of FCS, suspended in PBS containing 10^{-3} M NaN_3, and filtered through a nylon screen before FACS analysis. Simultaneous estimation of binding of fluorescein- and rhodamine-labeled viruses to live cells was carried out on the FACS, using the analytical techniques developed by Loken, Parks, and Herzenberg (9, 14). Computer programs for analysis and presentation of three-dimensional, two-color fluorescence were developed by Wayne Moore (Stanford University School of Medicine).

RESULTS

Virus binding assay. Fluorescence-activated cell sorter analysis was used to quantitate binding of fluoresceinated virus to specific target cells. In these and subsequent experiments, simultaneous analysis of live and dead cells was carried out (by using a light-scattering parameter), and only fluorescence signals from live cells were recorded. Figure 1 illustrates characteristic binding curves of RadLV/VL_3 to the RadLV-induced BL/VL_3 lymphoma cells and to normal C57BL/Ka thymocytes. The frequency of cells in each fluorescence category is plotted on the Y-axis versus the FACS-detected amount of fluorescence per cell on the X-axis. For each cell population analyzed, the percentage of viable cells showing above-background fluorescence (that is, greater than the fluorescence of cells analyzed in the absence of fluoresceinated virus) and mean and median fluorescence bound per cell has been computed. Thus, 92% of viable BL/VL_3 cells bound RadLV/VL_3 above BL/VL_3 background, and 2.5% of viable normal thymo-

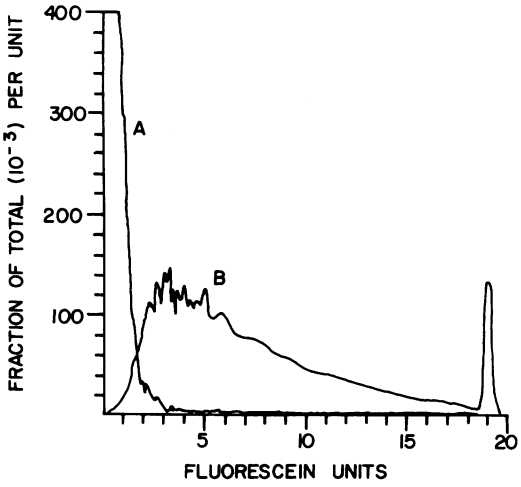

FIG. 1. *FACS analysis of RadLV/VL$_3$ binding. One-tenth A$_{260}$ unit of fluoresceinated RadLV/VL$_3$ virus in 0.2 ml of PBS (pH 7.4) was added to an equal volume of PBS supplemented with 1% FCS (PBS-FCS) containing either 5 × 10^5 BL/VL$_3$ cells or 1-week-old normal C57BL/Ka thymocytes. These suspensions were incubated on ice with periodic shaking for 75 min, layered over 1 ml of FCS, spun at 350 × g for 10 min, suspended in 1 ml of PBS, and filtered through a screen before FACS analysis. The two curves show frequency of cells binding increasing amounts of fluoresceinated virus. (A) Fluoresceinated RadLV/VL$_3$ bound to 1-week-old normal C57BL/Ka thymocytes. (B) Fluoresceinated RadLV/VL$_3$ bound to BL/VL$_3$ cells. Cells which have a FACS-detected fluorescence level greater than 19 units are collected and combined in the highest (approximately 19 fluorescein units) fluorescence channel. These cells represent less than 5% of all cells analyzed.*

cytes bound the same virus above normal thymocyte fluorescent background.

Live versus dead cell virus binding. It is extremely important to analyze virus binding to live cell populations only, since dead cells (identified by their smaller FACS-detected size and inability to take up the vital dye fluorescein diacetate [10, 13]), especially thymocytes, bind MuLV to a significant degree. Figure 2 shows BL/Ka(B) fibrotropic virus binding to live versus dead C57BL/Ka thymocytes. Note that the mean level of fluoresceinated BL/Ka(B) virus binding to dead thymocytes is much higher and that the percentage of cells binding that virus is much greater than the levels bound to live thymocytes. To test the possibility that dead cells accounted for the fraction of thymocytes binding RadLV/VL$_3$ in Fig. 1, a FACS scatter analysis of this subset of cells was carried out; greater than 90% of these cells possessed scatter profiles characteristic of viable thymocytes.

Polypeptide analysis of cellular bound virus. To show that material associating with these target cells is indeed virus, 0.1 A$_{260}$ unit of [^{3}H]leucine-labeled RadLV/AQR was incubated with 5 × 10^5 BL/AQR cells as in the previous experiments. These cells were washed through FCS and prepared for SDS-PAGE. The cell-bound material was then coelectrophoresed with [^{14}C]leucine-labeled RadLV/AQR. Figure 3 shows that the bound RadLV/AQR polypeptides are indistinguishable from the polypeptide pattern of input virus.

Binding site saturation and blockade. If the binding of exogenous RadLV is due to RadLV-specific receptor sites, and is not due to virus-virus aggregation at the cell surface, one should be able to saturate these binding sites at virus excess, and to inhibit binding with non-fluoresceinated virus. Saturation of RadLV/AQR binding to two RadLV-induced lymphomas (BL/VL$_3$ and BL/AQR) is shown in Fig. 4. Inhibition by non-fluoresceinated RadLV/VL$_3$ virus was shown (Fig. 5) by simultaneous incubation of the same cell number with an equal amount of either RadLV/AQR or RadLV/VL$_3$ viruses. Similar experiments have demonstrated specific unlabeled RadLV/VL$_3$ inhibition of binding of fluoresceinated RadLV/VL$_3$ by those normal thymocytes which bind RadLV.

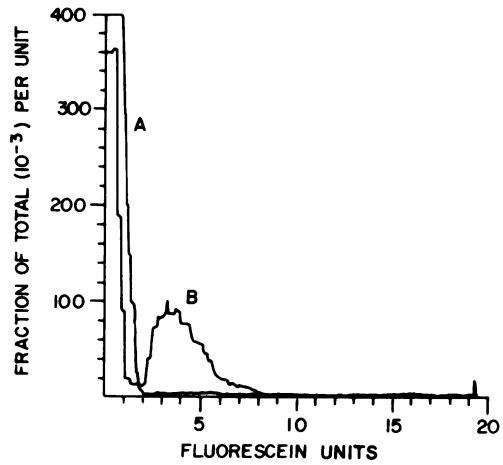

FIG. 2. *FACS analysis of dead thymocyte virus binding. One-tenth A$_{260}$ unit of fluoresceinated BL/Ka(B) virus in 0.2 ml of PBS (pH 7.4) was added to an equal volume of PBS-FCS containing 10^6 2-week-old C57BL/Ka thymocytes. This suspension was incubated on ice for 75 min, pelleted through FCS, and suspended in PBS for FACS analysis. The two curves show frequency of cells binding increasing amounts of fluoresceinated virus. (A) Fluoresceinated BL/Ka(B) binding to normal live thymocytes (scatter range 90 to 230 U). (B) Fluoresceinated BL/Ka(B) binding to dead normal thymocytes (scatter range <90 U).*

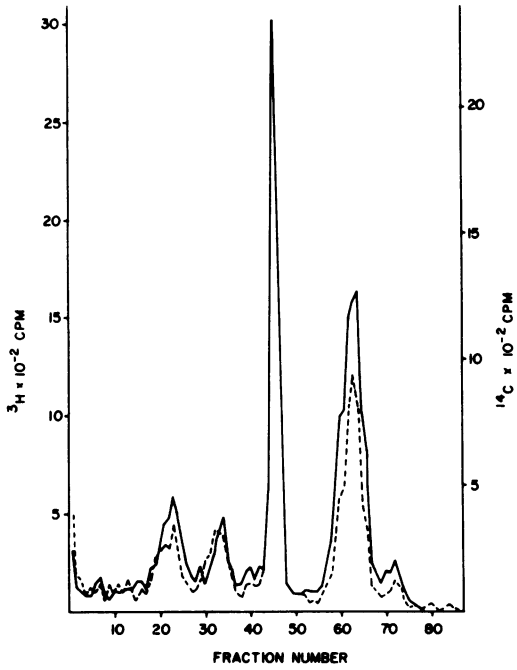

FIG. 3. *SDS-PAGE analysis of bound versus input RadLV/AQR virus. One-tenth A_{260} unit of internally [^{3}H]leucine-labeled Sepharose 4B-purified RadLV/AQR virus containing 1 × 10^6 cpm was bound to 5 × 10^5 BL/AQR cells as in the text. The cells were washed through FCS, and the pellet containing the bound virus was prepared for SDS-PAGE (21). The polypeptide profiles represent [^{3}H]leucine-labeled cellular bound RadLV/AQR virus coelectrophoresed with purified [^{14}C]leucine-labeled RadLV/AQR virus on a 7% polyacrylamide gel. ^3H, -----; ^{14}C, ——.*

Specificity of MuLV binding. We have shown that RadLV/VL_3 binds to RadLV-induced thymic (T)-lymphomas and to a minor population of normal thymocytes. We have previously shown that thymotropic MuLV's bind to T-lymphomas and not to non-T-lymphomas (16), and that T-lymphomas bind preferentially the MuLV which induced them (15, 16).

Simultaneous two-color FACS analysis with two virus populations, one fluoresceinated and the other one rhodaminated, was used to define further the specificity of RadLV binding to T-lymphoma cell lines. We have demonstrated previously that fluoresceination and/or rhodamination does not alter the specificity of viral binding to a defined lymphoma (15). Fluoresceinated RadLV/VL_3 and rhodaminated MCF-247 viruses were analyzed for binding to BL/VL_3 and KKT-1 cell lines. This allowed simultaneous binding analysis with two viruses of distinctly different specificities. The binding of these paired virus preparations to these two different cell populations is shown in Fig. 6. When BL/VL_3 cells were analyzed alone (Fig. 6A) or intermixed with KKT-1 cells (Fig. 6C), most BL/VL_3 lymphoma cells bound the RadLV/VL_3 virus, but did not bind the MCF-247 virus. Similarly, most KKT-1 lymphoma cells bound MCF-247 (Fig. 6B and C), but did not bind the RadLV/VL_3 virus. Thus, each virus-induced lymphoma possesses receptors which can discriminate between the two viruses.

Radiation-induced T-lymphoma binding. Although thymic lymphomas induced by X irradiation were the first sources of the thymotropic leukemogenic virus RadLV, most radiogenic lymphomas from C57BL are initially virus negative and remain so for a time after establishment in culture (Lieberman et al., manuscript in preparation). One of these lymphomas established in culture, the primary radiation-induced thymic lymphoma-derived BL/RL_{12}-NP cell line, expressed no detectable virus after more than 50 subcultivations. We therefore initiated a series of experiments to test whether these lymphoma cells have receptors for any MuLV's. As shown in Fig. 7, BL/RL_{12}-NP binds MCF-247 and RadLV/VL_3 equally well (we have previously reported similar dual binding specificities to the C57 Leaden radiation-induced thymic lymphoma L691 [15]). Binding of either virus (MCF-247 or RadLV/VL_3) could be completely inhibited by preincubation with excess unlabeled virus of either type, indicating that the simultaneous binding of both viruses to the same cell is most likely mediated by common determinants.

In collaboration with P. Besmer, N. Rosenberg, and D. Baltimore (Massachusetts Institute of Technology Center for Cancer Research) we have shown that the L691 radiation-induced thymic lymphoma cell line can bind and become infected with Gross, Moloney, and MCF-247 viruses, and that these superinfected cells have endogenously blocked receptors for these MuLV's (P. Besmer, N. Rosenberg, M. S. McGrath, I. L. Weissman, and D. Baltimore, manuscript in preparation). To test whether a similar phenomenon exists in the RadLV system, we tested the virus-binding capacity of BL/RL_{12}-NP cells which had been infected with RadLV. The results (Fig. 8) confirmed that such superinfected cells no longer express free receptors and that endogenously produced RadLV blocks binding of both homologous RadLV/VL_3 and the MCF-247 viruses.

Thymotropic-leukemogenic versus fibrotropic retrovirus binding. We wished to test whether the virus receptors on BL/VL_3 are spe-

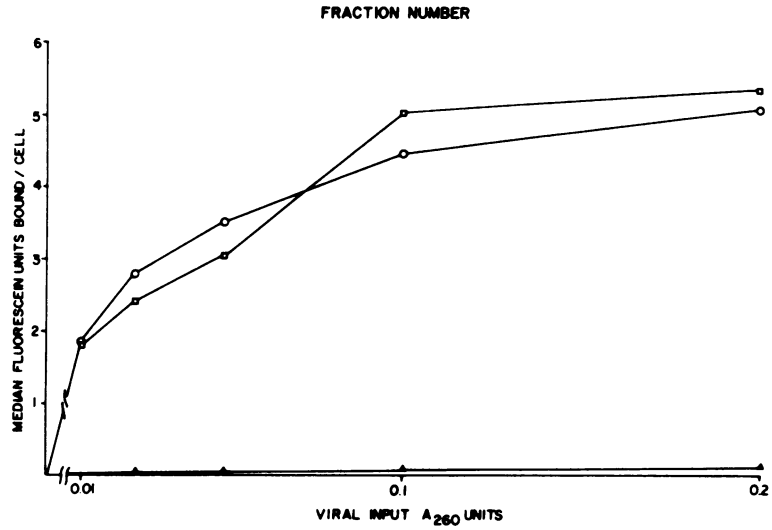

FIG. 4. *RadLV/AQR saturation of lymphoma binding sites. Fluoresceinated RadLV/AQR was incubated with 5 × 10⁵ BL/VL₃ cells, BL/AQR cells, and normal C57BL/Ka thymocytes at concentrations at A_{260} from 0.01 to 0.2 unit in the standard binding assay. The above curves show median fluorescence bound to BL/VL₃ cells (□), BL/AQR cells (○), and normal C57BL/Ka thymocytes (▲) at an increasing viral input.*

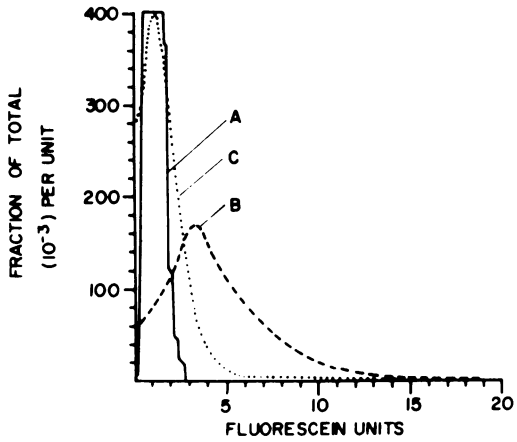

FIG. 5. *Blocking of fluoresceinated RadLV/VL₃ binding with unlabeled RadLV/VL₃. One-tenth A_{260} unit of fluoresceinated RadLV/VL₃ was incubated with 5 × 10⁵ RadLV/VL₃ cells in the presence or absence of 0.1 A_{260} U of RadLV/VL₃ and binding assessed as in the text. (A) Normal BL/VL₃ cell fluorescence. (B) RadLV/VL₃ binding to BL/VL₃ cells. (C) Ratio of 1:1 fluoresceinated RadLV/VL₃ to RadLV/VL₃ blocking of BL/VL₃ cell binding. Fluoresceinated RadLV/AQR binding is blocked in a similar manner by either RadLV/VL₃ or RadLV/AQR.*

cific for the leukemogenic isolates of C57BL/Ka retroviruses, or whether they recognize all C57BL/Ka retrovirus isolates. Thus we initiated an analysis of the comparative binding of the fibrotropic- [ecotropic BL/Ka(B) and xenotropic BL/Ka(X)] and thymotropic-leukemogenic RadLV/VL₃ viruses to the BL/VL₃ cells. These three classes of viruses have been shown to possess similar core proteins, but they have distinct envelope glycoproteins (Decléve et al., Virology, in press). All three viruses were fluoresceinated to identical degrees, and virus binding was analyzed at RadLV/VL₃ saturation levels (0.1 A_{260} unit per 5 × 10⁵ cells). As Table 1 shows, RadLV/VL₃ bound to RadLV and radiation-induced C57BL/Ka thymic lymphomas, but not to MFT-2, LSTRA, or the mouse myeloma cell line. In contrast, BL/Ka(B) bound only to the BL/RL₁₂-NP lymphoma cell line, whereas BL/Ka(X) bound only to LSTRA cells. Therefore, RadLV specific receptor expression is greatest on RadLV-induced lymphomas and is specific for thymotropic-leukemogenic RadLV. Although BL/RL₁₂-NP cells bound RadLV/VL₃ and BL/Ka(B) to similar degrees, binding of either virus was insignificant on the BL/RL₁₂-NP RadLV-infected cells, which indicates that radiation-induced lymphoma receptors recognize common or similar determinants on these two virions, or that distinct receptors, if they exist, are in close apposition to each other.

To analyze further the specificity of binding manifested by the C57BL/Ka isolates, homologous and heterologous cold virus blocking of RadLV/VL₃ binding to BL/VL₃ and BL/RL₁₂-NP cells was investigated. The results of these blocking experiments (Fig. 9) demonstrate that

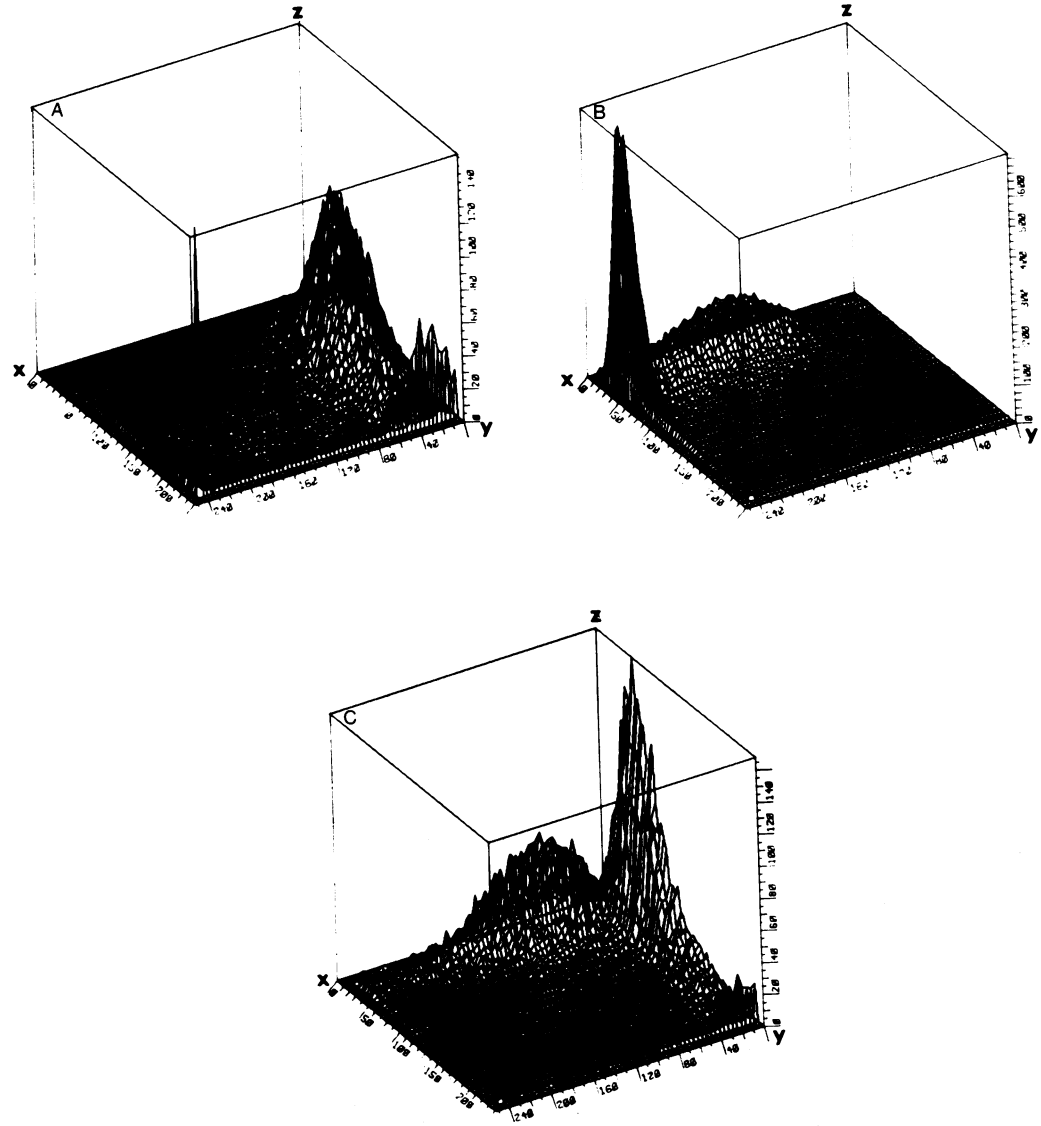

FIG. 6. *Two-color FACS analysis: RadLV/VL$_3$ and MCF-247 binding to BL/VL$_3$ and KKT-1 lymphomas. These three-dimensional perspective plots show rhodaminated MCF-247 and fluoresceinated RadLV/VL$_3$ binding to two different target cells, KKT-1 and BL/VL$_3$. Rhodaminated MCF-247 virus binding to cells increased along the X-axis, fluoresceinated RadLV/VL$_3$ increased along the Y-axis, and the frequency of cells binding a particular level of fluorescence increased along the Z-axis. The fluorescein and rhodamine backgrounds are both equal to 30 units. Each plot represents analysis of 5 × 10^4 cells. (A) 10^6 BL/VL$_3$ cells were incubated simultaneously with 0.2 A$_{260}$ unit of fluoresceinated RadLV/VL$_3$ and 0.2 A$_{260}$ unit of rhodaminated MCF-247 virus, and two-color binding was assessed by the FACS. (B) 10^6 KKT-1 cells were assayed for simultaneous binding of fluoresceinated RadLV/VL$_3$ and rhodaminated MCF-247 as in (A). (C) 5 × 10^5 BL/VL$_3$ cells were mixed with 5 × 10^5 KKT-1 cells, and this population was analyzed for simultaneous binding of fluoresceinated RadLV/VL$_3$ and rhodaminated MCF-247 as in (A).*

BL/VL$_3$ cells possess receptors specific for homologous virus only, whereas BL/RL$_{12}$-NP cells possess RadLV/VL$_3$ receptors that can be blocked by either homologous or B-tropic, but not X-tropic, isolates.

DISCUSSION

These experiments demonstrate that RadLV-induced thymic lymphoma cells bear receptors specific for leukemogenic RadLV, but do not

bear receptors for the nonleukemogenic C57BL/Ka retroviruses or for the AKR MCF-247 virus. In contrast to RadLV-induced lymphomas, two radiation-induced thymic lymphomas appear to bear receptors recognizing determinants common to several (but not all) murine type C viruses. In this study and companion studies, these radiation-induced lymphomas have been shown to be infectible by binding MuLV's (Lieberman et al., Besmer et al., manuscripts in preparation). Such infected lines demonstrate a generalized receptor blockade subsequent to productive virus infection similar to viral interference seen in other virus systems (19). The levels of virus binding and viral interference are related to the proportion of virus receptors which are bound (presumably by endogenous virus production; Besmer et al., manuscript in preparation). It would appear that most T-lymphoma cells examined in this study are in significant receptor excess.

Essential to the analysis of receptor binding specificity by individual cells within a lymphoma population has been the adaptation of the FACS for these systems. FACS analysis of murine type C virus binding measures several parameters unavailable to radiolabeled virus and gp71 binding assays (5, 7). These are as follows: (i) quan-

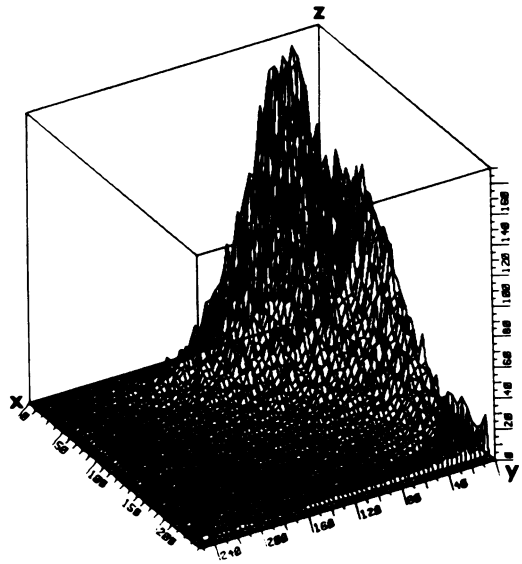

FIG. 7. *Two-color FACS analysis: MuLV binding to the radiation-induced thymic lymphoma BL/RL$_{12}$-NP. Two-tenths A$_{260}$ unit of rhodaminated MCF-247 (X-axis) and 0.2 A$_{260}$ unit of fluoresceinated RadLV/VL$_3$ (Y-axis) were co-incubated with 10^6 BL/RL$_{12}$-NP cells, and binding was analyzed and plotted as in Fig. 6.*

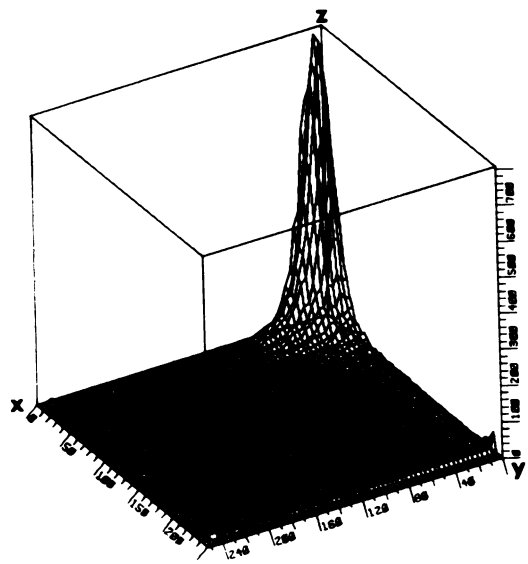

FIG. 8. *Two-color FACS analysis: MuLV binding of RadLV-infected BL/RL$_{12}$-NP 10^6 BL/RL$_{12}$-NP cells which had been productively infected with RadLV were analyzed for simultaneous binding of rhodaminated MCF-247 and fluoresceinated RadLV/VL$_3$ viruses as in Fig. 6. Normal cellular fluorescent background: X = 30, Y = 30.*

TABLE 1. *Binding of C57BL/Ka mouse retrovirus isolates to target cells*[a]

MuLV bound	Target cells						
	BL/VL$_3$	BL/AQR	BL/RL$_{12}$-NP	RadLV-infected BL/RL$_{12}$-NP	MFT-2	XP-63-AG(NP) (Mouse myeloma)	LSTRA
RadLV/VL$_3$	100	100	8 ± 7	12 ± 8	12 ± 8	10 ± 5	14
BL/Ka (B)	8 ± 5	20 ± 11	67 ± 18	3 ± 10	12 ± 14	15 ± 8	ND[b]
BL/Ka (X)	11 ± 4	15 ± 7	12 ± 4	10	11 ± 7	12	100

[a] Binding of each of the above C57BL/Ka viruses was carried out as described in the text. The numbers represent percent of mean fluorescence bound per cell compared with the standard 100% represented by RadLV/VL$_3$ binding to BL/VL$_3$, BL/AQR, and BL/RL$_{12}$-NP thymic lymphoma cells. Where the experiment was done more than three times, the percent binding has the standard deviation given to the right.

[b] ND, Not determined.

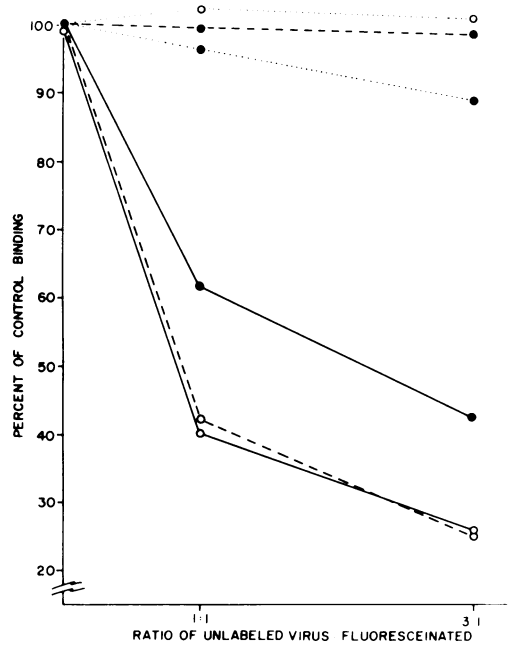

FIG. 9. *Receptor specificity of BL/RL$_{12}$-NP cells compared to BL/VL$_3$ cells. Fluoresceinated RadLV/VL$_3$ binding to BL/VL$_3$ and BL/RL$_{12}$-NP lymphoma cells was blocked by co-incubation with unlabeled MuLV's. One-tenth A$_{260}$ unit of fluoresceinated RadLV/VL$_3$ was incubated in 0.6 ml of PBS with 5 × 10^5 BL/VL$_3$ or BL/RL$_{12}$-NP cells alone, with 0.1 A$_{260}$ unit of unlabeled MuLV, or with 0.3 A$_{260}$ unit of unlabeled MuLV and analyzed for binding as in the text. Fluoresceinated RadLV/VL$_3$ binding to BL/VL$_3$ (●) and BL/RL$_{12}$-NP (○) in absence of blocking MuLV was considered to be 100% binding. Unlabeled blocking viruses in 1:1 and 3:1 ratios of unlabeled to fluoresceinated RadLV/VL$_3$: BL/Ka(B), -----; BL/Ka(X), ·····; RadLV/VL$_3$, ——.*

titation of the amount of virus bound per cell and the range of these amounts in a defined population of cells; (ii) determination of the frequency of cells binding virus above background, i.e., 2% of normal thymocytes; (iii) the ability to exclude nonspecific binding by dead cells; (iv) the ability to assess binding characteristics of a single cell population by using two different MuLV's and (v) the ability to sort out populations of cells which possess viral receptors from those which do not have receptors for measurement of biological activity.

There are several aspects of these studies which may be important in understanding the role of lymphoma cell receptors in the process of leukemogenesis, and perhaps in the development of highly leukemogenic retrovirus isolates. First, if these receptors are related only to the process of retrovirus infection, one would have expected the RadLV-induced tumors to bind C57BL/Ka retroviruses in general. Since the two RadLV-induced tumors studied here (and others tested independently) bind only the leukemogenic isolates, we propose that receptor recognition of oncogenic retroviruses may play a role in the process of leukemogenic transformation or in the selection of malignant subpopulations during leukemogenesis in vivo (15, 20).

Second, several studies of these thymotropic MuLV's indicate that a common event preceding the emergence of leukemic cells is the appearance of putative recombinant MuLV's. Thus, MCF-247 and other MCF AKR viruses all appear to have ecotypic core determinants and recombinant eco-xeno gp71 determinants, as demonstrated by both peptide analysis of virion proteins (6) and RNA oligonucleotide mapping (18). Recent analysis of RadLV isolates reportedly indicate that this class of virus also expresses ecotypic and xenotypic gp71 antigens with ecotypic core proteins (Decléve et al., Virology, in press). If recombinational events give rise to leukemogenic RadLV isolates, these studies demonstrate that the RadLV-induced lymphoma bear receptors which recognize only these leukemogenic retroviruses, and not their ecotropic or xenotropic parents. In addition, these studies demonstrate that RadLV is distinct from the AKR recombinant virus MCF-247. This latter observation is in agreement with the work of Decléve et al. (Virology, in press).

A possible clue as to a potential site of these recombinational events may derive from the study of radiation-induced lymphomas. These cells (unlike normal thymocytes) appear to express receptors enabling binding and infection by several leukemogenic and ecofibrotropic isolates. Such cells might act as culture vessels for the replication of any endogenous viruses which can superinfect them, allowing intracellular recombination with endogenous xenotropic *env* determinants during the life of the host, or upon subsequent passage of these tumors to other hosts. We wish to test whether all radiation-induced T-lymphomas bear such generalized receptors, or whether L691 and BL/RL$_{12}$-NP are special cases. Thus, more extensive analysis of the virus binding properties of several independent radiation-induced and RadLV-induced lymphomas is obviously now an important priority, as is the search for cells with multispecific receptors during the preleukemic period.

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