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Binding Characteristics of Rauscher Leukemia Virus Envelope Glycoprotein gp71 to Murine Lymphoid Cells

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The major envelope glycoprotein (gp71) purified from Rauscher leukemia virus (R-MuLV) binds efficiently to murine lymphoid cells but not to either murine nonlymphoid cells or lymphoid cells from other species. Binding of ^{125}I-labeled R-MuLV gp71 was competitively inhibited by unlabeled glycoprotein, as well as by whole R-MuLV, but not by murine xenotropic viruses, R-MuLV p30, and several unrelated proteins. Polyacrylamide gel electrophoresis profiles of iodinated gp71 after binding to lymphoid cells were similar to prebound profiles. Antibody to R-MuLV gp71 prevented binding, whereas normal serum had no effect. Adsorption of the glycoprotein to murine lymphoid cells occurs rapidly and is time and temperature dependent. The procedure described is sensitive for detecting the binding activity of approximately 10^4 cells. Binding was proportional up to 2.5×10^5 cells per ml and plateaued above 10^7 cells per ml. In the presence of excess R-MuLV gp71, BALB/c thymocytes bound approximately 2.4×10^4 molecules per cell.

Oncornavirus infection of mice results in the depression of both humoral and cellular immune reactions in vivo (for review, see 5, 22). Furthermore, evidence that in vitro cellular immunity is impaired in virus-infected mice has been reported (10, 12). These observations have more recently been extended by the demonstration that freeze-thawed extracts of Rauscher leukemia virus (R-MuLV) suppress in vitro cell-mediated immune reactions of normal mouse lymphocytes (A. K. Fowler et al., in press). Similarly, UV-inactivated feline leukemia virus also suppresses in vitro blastogenic responses of normal cat lymphocytes (L. L. Hebebrand et al., in press). Since most, if not all, mammals contain genetic information for related type C viruses—xenotropic viruses—that are inducible in vivo in the host by hormonal (6, 7, 11) and immunological (14) stimulation, we have suggested that virion components endogenous to the host function as regulators in normal immunological processes (13). Indeed, xenotropic viruses are actively expressed in maternal (8) and fetal (15, 17) tissues during pregnancy, a period of intricate hormonal and immunological interaction.

The primary event in virus-cell interaction is the adsorption of the virion, presumably via a viral envelope component, to specific cellular receptors. Recent progress in identifying and isolating oncornaviral proteins now permits a more detailed elucidation of virus-cell interaction. Accordingly, we have undertaken studies to determine the effect of purified virion proteins on in vitro murine lymphocyte transformation and to examine their binding characteristics, the receptors involved, and the mechanisms of viral protein-cell membrane interaction that lead to modification of cell behavior. During these studies, we have observed that the major envelope glycoprotein (gp71) of R-MuLV, like AKR gp71 (18), induces transformation of normal lymphocytes, whereas a lower-molecular-weight virion protein(s) appears to depress T-cell function (Fowler et al., in press). In this communication, we extend our previous findings by characterizing the binding of R-MuLV gp71 to lymphocytes and other cells derived from various organs of several strains of mice.

MATERIALS AND METHODS

Cell preparation. Thymuses, spleens, or epididymides were excised and minced in 20 ml of RPMI 1640 medium and gently sieved through a 60-mesh stainless-steel filter. Thymic cell preparations were then further washed three times by repeated centrifugation ($250 \times g$) and resuspension with fresh medium. More than 90% of the thymocytes were viable by the trypan dye exclusion test. Splenic cell suspensions were washed once with fresh medium and then layered on a Ficoll-Hypaque gradient. The lymphocyte-enriched layer was collected and washed three additional times with fresh medium. The viability of the splenic lymphocytes was consistently above 90%. For sperm cell purification, the epididymal cell preparations were

initially low-speed centrifuged (200 × *g* for 3 min) to remove particulate material. The cells remaining in the supernatant were then concentrated by centrifugation (1,000 × *g* for 20 min) and further purified by discontinuous sucrose gradient (20:50:80%) centrifugation (approximately 80,000 × *g* for 60 min) at 4°C. The sperm cells collected at the 50:80% sucrose interface were washed an additional three times with fresh medium. The final sperm preparation contained fewer than 5% contaminating cells. No estimate of sperm viability was made. Peripheral lymphocytes were prepared from heparinized blood by Ficoll-Hypaque separation and were processed identically to the splenic lymphocyte preparations. Greater than 90% of the cells were viable. Blood used for the preparation of peripheral lymphocytes was collected from rodents by cardiac puncture and from primates by venipuncture. Erythrocytes used in these studies were also washed three times with fresh medium.

Glycoprotein purification and characterization. Banded R-MuLV (10^{12} virus particles per ml), produced in JLS-V9 cells and having an infectious titer of 2×10^6 focus-forming units per ml, was freeze-thawed twice and centrifuged at 105,000 × *g* for 90 min at 4°C. The supernatant was lyophilized and dialyzed against a solution containing 0.01 M *N,N*-bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) (pH 6.5), 0.001 M EDTA, and 1.0 M NaCl and applied to a Sephadex G-100 column (1.5 by 90 cm) equilibrated with the same buffer. Peak fractions containing envelope glycoprotein were identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis stained with either Coomassie blue or Schiff reagent and, if contaminated with bovine serum albumin, further purified by phosphocellulose chromatography as previously described (25). The purified glycoprotein was labeled with ^{125}I (1×10^4 to 5×10^4 cpm/ng of protein), using the chloramine-T method (9). After iodination, 95 to 98% of the acid (10% trichloroacetic acid)-precipitable glycoprotein was precipitable with specific antiserum prepared against purified R-MuLV gp71 and demonstrated less than a 2% immune precipitation with anti-bovine serum albumin. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the iodinated glycoprotein demonstrated a single band migrating in the 71,000-dalton region (see Fig. 3A) and hereafter will be referred to as gp71.

Binding assay. Cells were incubated at 37°C in the presence of [^{125}I]gp71 in 1.0 ml of medium containing 1% bovine serum albumin. Cell concentration was maintained at 10^6 cells per ml unless otherwise indicated. All binding assays were performed in polystyrene tubes (12 by 75 mm) that had been prewashed with medium containing bovine serum albumin. During incubation, cells were gently mixed on a roller drum (10 rpm). After incubation, the cells were centrifuged at 400 × *g* for 10 min at 4°C. The pellet was resuspended in 2.5 ml of cold medium containing bovine serum albumin and recentrifuged. This procedure was repeated two additional times. The radioactivity associated with the final cell pellet was measured directly in a Nuclear-Chicago gamma counter. To estimate nonspecific binding of the radiolabeled gp71 to reaction vessels, control tubes, to which no cells were added, were processed identically. This value, which was consistently less than 300 cpm per tube, was subtracted from the radioactivity associated with corresponding test cultures to derive a corrected specific binding value.

RESULTS

The binding of [^{125}I]gp71 to murine splenic and thymic cells was initially linear and temperature dependent (Fig. 1). At 37°C, binding occurred rapidly, and within 15 min 50% of the maximum bound level was attained. The rate of binding subsequently decreased, and after 100 min there was minimal increase in total binding level. After prolonged incubation (24 h) total cell-associated label decreased approximately 20%, presumably due to degradation of the glycoprotein. In comparison, at 0°C (wet ice) the rate of binding was much slower, with 50% saturation occurring at approximately 75 min. For maximum binding at 0°C, nearly 6 h was required, but the total level bound was similar to that observed at 37°C, 3,693 versus 3,607 cpm per 10^6 thymic cells, respectively.

To further investigate binding specificity, thymic cells were preincubated for 1 h at 37°C with varying levels of unlabeled R-MuLV gp71 and p30, as well as with several unrelated proteins (bovine serum albumin V, ovalbumin, myoglobin, and cytochrome *c*), before adding [^{125}I]gp71. After an additional 30 min of incubation, only the unlabeled gp71 competitively inhibited binding (Fig. 2). The preincubation of cells with as little as 3 ng of unlabeled gp71 reduced binding by 10%, and increasing this level to 6 and 13 ng reduced binding 20 and 50%, respectively. Total competition, however, required approximately a 50-fold excess of unlabeled to labeled glycoprotein (200 ng per 10^6

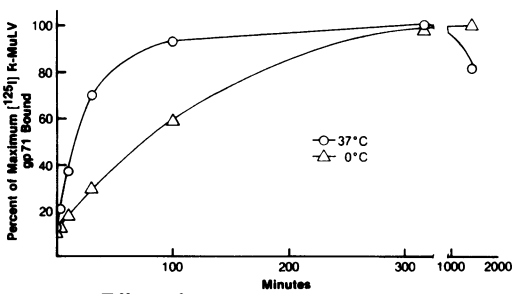

FIG. 1. *Effect of temperature on the binding rate of R-MuLV gp71 by BALB/c thymic cells. A 3.5-ng amount of ^{125}I-labeled R-MuLV gp71 (102,000 cpm) was added to 10^6 thymocytes and incubated at either 0 or 37°C. Cell-bound radiolabel was determined as described in the text. Maximum levels bound were 3,693 and 3,607 cpm at 0 and 37°C, respectively. Each point represents the mean value of quadruplicate cultures.*

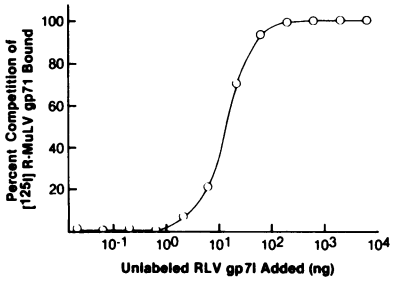

FIG. 2. *Competitive inhibition by unlabeled R-MuLV gp71 of ^{125}I-labeled R-MuLV gp71 binding by BALB/c thymic cells. Thymocytes (10^6/ml) were incubated for 60 min at 37°C with varying amounts of unlabeled gp71 before 3 ng of [^{125}I]gp71 (30,200 cpm) was added. After an additional 30 min of incubation, cell-bound radiolabel was determined as described in the text. Each point represents the mean value of duplicate cultures. The preincubation of thymocytes with 6 ng of unlabeled gp71 reduced binding 20%.*

cells). Similarly (data not shown), antibody to R-MuLV gp71 prevented binding, whereas normal serum and antimurine immunoglobulin G and immunoglobulin M sera had no effect. Additional evidence of specificity was also obtained by the pretreatment of thymic cells with infectious R-MuLV as well as two murine xenotropic viruses, NZB-C135 (21) and M-55 (1). At equivalent concentration, 10^{10} virus particles per ml, R-MuLV reduced binding 80%, whereas no effect was observed with the xenotropic viruses.

The observation that unlabeled gp71 competitively inhibited binding indicates that the iodinated and unlabeled R-MuLV gp71 molecules are biologically very similar and that the measured binding is not an artifact resulting from the nonspecific binding of by-products of iodinization or from the uptake of small levels of free ^{125}I. To further examine this possibility, the sodium dodecyl sulfate-polyacrylamide gel electrophoresis profile of [^{125}I]gp71 bound to splenic cells after a 60-min incubation period at 37°C was compared with nonreacted labeled glycoprotein (Fig. 3). Although several minor peaks of radioactivity were noted in the cellular extract, the major peak of activity corresponded to the standard labeled R-MuLV gp71, indicating that no significant change in molecular weight occurred during the binding assay procedures. The minor peaks undoubtedly represent aggregation and degradation products of the glycoprotein and are consistent with the interpretation that the observed reduction in cell-bound label after prolonged incubation is largely attributable to glycoprotein degradation.

The effect of cell density on binding was studied by adding 3 ng of [^{125}I]gp71 to BALB/c thymic cells. As shown in Fig. 4, the assay was sensitive for detecting the receptor activity of 10^4 cells, and binding was proportional up to a cell density of 10^5 cells per ml. At higher cell concentrations, the relative binding level gradually decreased and eventually plateaued above concentrations of 10^7/ml. A consistent observation made throughout these experiments was that only a portion of the input radiolabeled glycoprotein, ranging from 15 to 40%, depending on the probe preparation, was bound by murine lymphoid cells despite a large excess of cells (10^7). Although receptor site masking or interference may be involved, it is reasonable that a significant portion of the iodinated gp71 maintaining immunological reactivity is not biologically active based on its binding properties. Data favoring this interpretation have been obtained by multiple reincubation of reactant supernatants removed from thymus cells (10^7/ml) previously pulsed and incubated with [^{125}I]gp71 with fresh thymus cells. Fresh cells, during the second and third incubations of the reactant supernatants, bound only 6.7 and 3.1%, respectively, of the residual immunologically reactive [^{125}I]gp71. In comparison, 29.8% of the immunologically reactive glycoprotein was bound during the first incubation period. The total [^{125}I]-gp71 bound during four reincubation cycles represented only 41.7% of the immunologically reactive glycoprotein input. The observed variable binding efficiency probably reflects procedurally related degradation of the glycoprotein; however, a natural heterogeneity of the gp71 molecule must also be considered.

To estimate the number of receptor sites per cell, BALB/c thymic cells were incubated with an excess of ^{125}I-labeled glycoprotein. As may be noted in Fig. 5, the total iodinated gp71 bound per 10^6 cells increased up to approximately 2.7 ng (38.5×10^{-15} mol) as the level of supplemented glycoprotein was elevated to 107 ng/ml. Extrapolating from this value, the number of R-MuLV gp71 molecules bound per cell is approximately 2.5×10^4.

Cell specificity of R-MuLV gp71 binding was examined two ways and is summarized in Table 1. The first compared the binding level of murine lymphoid cells to nonlymphoid cells (erythrocytes and sperm), and the second determined the relative binding level of lymphoid cells from several species. In general, murine lymphoid cells exhibited the highest potential for binding R-MuLV gp71, and, in most strains studied, thymus-derived cells bound more viral envelope glycoprotein than splenic cells. A notable exception to the observed high relative binding level of lymphoid cells for R-MuLV gp71 was consistently noted in the AKR mouse, in which the binding level of both thymic and splenic cells

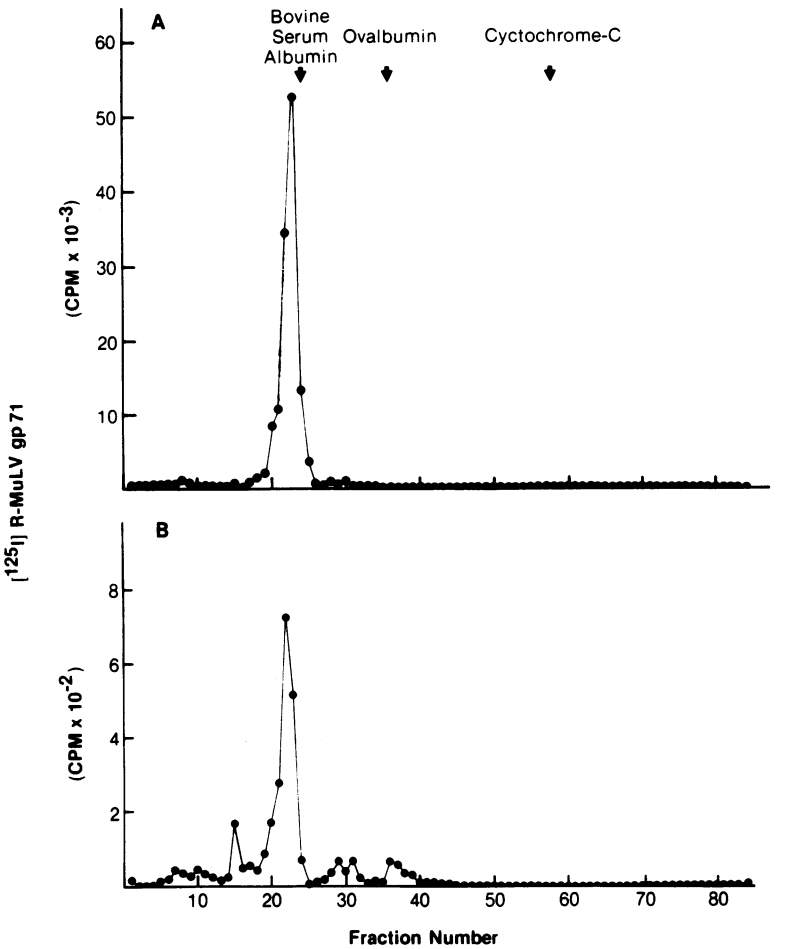

FIG. 3. *Polyacrylamide gel electrophoresis of ^{125}I-labeled R-MuLV gp71 run on 7.5 to 25% linear gradients (cylindrical gels 10 cm in length) in the presence of 0.1% sodium dodecyl sulfate. Samples were prepared by heating for 2 min at 100°C in a solution containing 50 mM Tris-hydrochloride (pH 6.7), 2% sodium dodecyl sulfate, 0.5% β-mercaptoethanol, and 0.01% bromophenol blue. After 16 h of electrophoresis (9 mA per gel, 75 pulses per s, 0.5 μF), gels were sliced into 1.3-mm fractions. Arrows indicate positions of marker proteins. (A) Control R-MuLV gp71; (B) BALB/c splenic cell extract after a 60-min incubation at 37°C with R-MuLV gp71. Before extraction, cells were washed as described in the text.*

was approximately 20% of that noted for the other strains. Of the murine nonlymphoid cells examined, erythrocytes exhibited the lowest binding level (<2% of reference control), whereas the binding level of sperm cells was slightly higher (10 to 15% of reference control).

Except for the binding level of the Sprague-Dawley rat (20% of reference control), the binding of R-MuLV gp71 to lymphoid cells from the other species tested (rabbit, baboon, human) was consistently low (<10%).

DISCUSSION

The results presented demonstrate that the major envelope glycoprotein purified from R-MuLV binds efficiently to murine lymphoid cells. The rate of R-MuLV gp71 binding to splenic and thymic cells is rapid and initially linear but, in contrast to that noted for fibroblastic cells (4), is temperature dependent. The rate of binding at 37°C was approximately fivefold that observed at 0°C (wet ice), although the maximum extents of binding were similar at both temperatures. These findings are consistent with earlier data showing that the rate of poliovirus adsorption to cells in vitro is decreased by a reduction in temperature (16) and may reflect a change in the random probability of virus-cell interaction.

The adsorption of gp71 to lymphoid cells is highly specific. This is indicated by the inhibition of binding by antiserum to R-MuLV gp71

and the competition for available membrane receptors by either murine ecotropic virus (R-MuLV) or its purified viral envelope glycoprotein (R-MuLV gp71). Murine xenotropic viruses

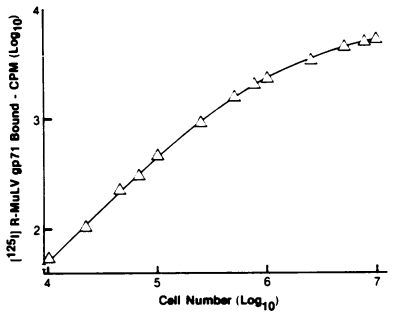

FIG. 4. *Effect of cell density on the binding of R-MuLV gp71 by BALB/c thymocytes. Thymocytes at varying concentration (10^4 to 10^7/ml) were incubated for 45 min at 37°C in the presence of 3 ng of ^{125}I-labeled R-MuLV gp71 (25,000 cpm). Cell-bound radiolabel was determined as described in the text. Each point represents the mean value of triplicate cultures.*

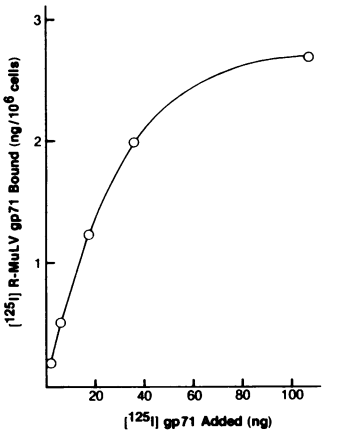

FIG. 5. *Effect of R-MuLV gp71 concentration on the level bound by BALB/c thymic cells. Thymocytes (10^6/ml) were incubated for 60 min at 37°C in the presence of increasing amounts of ^{125}I-labeled R-MuLV gp71. Maximum input (110 ng of gp71) contained 525,000 cpm. Cell-bound radiolabel was determined as described in the text. Each point represents the mean value of duplicate cultures.*

TABLE 1. *Relative binding of ^{125}I-labeled R-MuLV gp71 to different cells*

Species	Strain	Age	Cell type	No. of expts	Relative [^{125}I]gp71-binding efficiency[a] (mean %, range)
Mouse	BALB/c	21 days	Thymic	8	100.0
		65 days	Thymic	2	139.2 (130.5–143.2)
			Splenic	3	65.7 (61.7–69.9)
			Sperm	2	12.4 (10.8–14.0)
			RBC[b]	5	0.7 (0.5–1.2)
	NIH Swiss	65 days	Thymic	2	105.8 (93.8–118.5)
			Splenic	3	94.2 (85.0–107.0)
			RBC	2	1.2 (0.6–1.7)
	NZB	65 days	Thymic	2	158.3 (149.4–167.2)
			Splenic	3	88.1 (72.7–99.5)
			Sperm	2	8.2 (6.6–9.8)
			RBC	2	0.6 (0.5–0.8)
	AKR	65 days	Thymic	2	16.8 (15.1–18.6)
			Splenic	3	21.7 (14.4–30.0)
			Sperm	2	11.9 (11.0–12.8)
			RBC	2	1.2 (0.7–1.6)
Rat	Sprague-Dawley	6 mo	Thymic	2	20.2 (19.9–20.4)
			Splenic	2	28.3 (18.1–38.5)
Rabbit	NZW	7 days	Thymic	2	6.6 (4.6–8.7)
			Splenic	2	9.3 (9.2–9.4)
		6 mo	Peripheral	2	4.2 (3.2–5.2)
Baboon	Cynocephalus	5 yr	Peripheral	1	8.4
Man	Caucasian	20–30 yr	Peripheral	2	5.6 (3.6–7.7)

[a] All values are relative to the binding level of thymic cells from 21-day-old BALB/c mice assayed in parallel. Cells (10^6/ml) were incubated for 60 min at 37°C in the presence of 3 to 4 ng of R-MuLV[^{125}I]gp71. Cell-bound radiolabel was determined as described in the text. The number of replicate cultures per experiment ranged from two to four.

[b] RBC, Erythrocytes.

(NZB and M-55), on the other hand, failed to interfere with R-MuLV gp71 adsorption to lymphoid cells, indicating that mouse lymphoid cells, like mouse fibroblastic cells (4), contain a population of membrane receptors for ecotropic virus that have no demonstrable affinity for mouse xenotropic virus. This is further evidenced by the high binding affinity for R-MuLV gp71 exhibited by lymphoid cells from NZB mice, a strain known to produce high titers of xenotropic virus (20) and whose thymocytes express considerable endogenous xenotropic viral envelope glycoprotein on their cell surface (19). Similarly, thymocytes from BALB/c mice, recently reported to naturally express endogenous xenotropic viral envelope glycoprotein (3), also bind high levels of R-MuLV gp71.

Several differences were apparent in the relative binding capacity of cells from different organs and different strains of mice. Lymphoid cells derived from the spleen, the primary target organ of R-MuLV-induced erythrocytopoietic disease, consistently exhibited a much greater capacity for binding R-MuLV gp71 than did either sperm cells or erythrocytes. Thymic cells, by comparison, bound as much viral envelope glycoprotein as splenic cells, and thymic cells from some strains bound significantly more than splenic cells. The high degree of thymocyte binding of R-MuLV gp71 is best explained by a cross-reactivity of murine ecotropic viruses for the same cell surface receptors. Thus, thymocytes, the target cells for certain murine ecotropic viruses, such as Moloney leukemia virus, contain receptors in vivo that probably bind R-MuLV and other murine ecotropic viruses equally well. Indeed, murine ecotropic viruses have been shown to use the same receptors on mouse fibroblast cells in vitro (2, 4, 24). Furthermore, the observed reduced binding of R-MuLV gp71 by AKR lymphoid cells, a strain known to synthesize endogenous ecotropic virus, is consistent with this interpretation, since viral synthesis would result in in vivo saturation of the available cellular receptor sites for ecotropic viruses. In preliminary studies, we have also noted a significant reduction of R-MuLV gp71 binding to lymphoid cells of BALB/c mice after exogenous infection with either R-MuLV or Friend leukemia virus (unpublished data).

The existence of cross-reactive binding sites for R-MuLV envelope glycoprotein on cells from at least one other species, the rat, is also suggested from these data. The extent of R-MuLV gp71 bound by rat lymphoid cells, though low compared with mouse lymphoid cells (20 to 30%), was consistently observed. This binding appeared to be specific by competition experiments and is consistent with the earlier observation that newborn rats are highly susceptible to R-MuLV infection (23).

In the presence of excess iodinated glycoprotein, thymocytes from BALB/c mice bind approximately 2.7 ng (38.5×10^{-15} mol) of R-MuLV per 10^6 cells. This is equivalent to an estimated binding level of approximately 2.4×10^4 molecules per lymphoid cell. By comparison, murine fibroblasts—cells of different derivation and exhibiting morphological characteristics widely different from lymphoid cells, including a larger cell surface area—bind 5.3×10^5 molecules per cell (4), or approximately 20-fold that observed for thymocytes. Such estimates, however, are based on the assumption that all cells within the population examined contain the same family or families of receptors at equivalent numbers and that they bind the same amount of glycoprotein. These assumptions may be more valid with certain murine cell populations than with others; certainly, the data presented here do not rule out the possibility that the binding efficiencies of specific lymphoid cell subpopulations vary, that the numbers of receptors per cell differ, or that multiple receptor forms exist on individual cells. Indeed, the difference noted between the relative binding level of thymocytes and splenic cells from NZB and BALB/c mice suggests that one or more of these possibilities is likely in vivo. It should also be noted that, in addition to lymphoid cells serving as vehicles for virus adsorption and replication, immunocytes may also bind virus via antigen-specific receptors as part of the normal events leading to antiviral immunity. Although antigen-binding cells are present in only low numbers before immunization, we cannot completely rule out their involvement in the binding studies performed here; however, preliminary blocking experiments with anti-mouse immunoglobulins indicate that their participation is minor. Additional studies are needed to clarify these points and to determine the extent of cell type specificity and maturation level in the regulation of virus-cell receptor activity in vivo. Such information may provide insights into the mechanism of viral protein-cell membrane interaction that leads to the modification of cell behavior in immunity and oncogenesis.

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