CELL-SURFACE ANTIGENS ASSOCIATED WITH DUALTROPIC AND THYMOTROPIC MURINE LEUKEMIA VIRUSES INDUCING THYMIC AND NONTHYMIC LYMPHOMAS*

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The murine leukemia viruses (MuLV)[1] that are endogenous in mice and are inherited as integrated mouse genes can be classified according to their host range as ecotropic (infecting only mouse cells), xenotropic (infecting cells other than mouse), and amphotropic viruses (infecting both mouse and non-mouse cells). Of late, a class of viruses has been described that possess the host range of both ecotropic and xenotropic viruses (1, 2), and, unlike the amphotropic isolates, are serologically related to them. These dualtropic (eco-xeno) viruses have been isolated from Moloney MuLV stocks (3, 4) and from preleukemic and leukemic AKR thymus tissue (2) and have been implicated in the leukemogenic process (2, 4, 5). Evidence indicates that they may be intragenic recombinants in the gene coding for the virus envelope glycoprotein gp70 (2, 6–8). Dualtropic viruses may induce cytopathic foci in mink lung cells and were therefore designated mink cell focus-inducing viruses (2).

X-ray-induced lymphomas of C57BL/6 mice have given rise to a group of indigenous viruses (radiation leukemia virus [RadLV]) that include ecotropic; xenotropic, and thymotropic MuLV. The latter class of viruses have affinity for thymus lymphoid cells (9–11) and probably have a recombinant envelope glycoprotein (12). Viruses of the thymotropic class have been shown to be potent inducers of thymic lymphomas in C57BL/6 mice (13, 14). More recently, we have isolated dualtropic MuLV from the epithelial reticulum cells of RadLV-induced thymic lymphomas (M. Haas. Unpublished observations.). Like the thymotropic class of viruses, these cloned dualtropic isolates induced thymic lymphomas in young adult C57BL/6 mice with high efficiency and short latency.

X-ray-treated C57BL/6 mice have also given rise to splenic tumor extracts that, after serial in vivo inoculation (15), induced nonthymic lymphomas in the strain of origin. We have isolated viruses from stromal bone marrow cell lines grown from lymphoma-bearing mice (16) and could show that virus isolates cloned from the bone

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[1] *Abbreviations used in this paper:* BCL, B cell lymphoma(s); FA, fluorescent antibody; MuLV, murine leukemia virus(es); PBS, phosphate-buffered saline; RadLV, radiation leukemia virus; TCL, T cell lymphoma.

marrow cultures exclusively induced nonthymic lymphomas[2] in 100% of inoculated adult mice with a latency of only 3 wk. Significantly, these lymphomagenic virus isolates possessed the dualtropic host range, and, presumably, they were recombinant in the *env* gene (17).

Thus, whole-body x-irradiation of C57BL/6 mice followed by serial inoculation of cell-free tumor extracts has given rise to a range of viruses indigenous to the mice: (*a*) thymotropic T cell lymphoma-inducing virus; (*b*) dualtropic T cell lymphoma-inducing virus; (*c*) dualtropic nonthymic B cell lymphoma-inducing virus; (*d*) dualtropic nonlymphomagenic virus; as well as (*e*) ecotropic and (*f*) xenotropic virus classes. Because the virus classes appear to breed true in culture and the lymphomagenic isolates each induce one specific type of lymphoma in vivo (16, 17), we wished to study the differences among the virus isolates and thus prepared virus-specific serological probes to use them as reagents in the detection and identification of similar viruses in natural systems. Moreover, our reagents established the specificity of cell-surface antigens associated with the different viruses and revealed the serological cross-relatedness of the lymphomagenic virus classes among themselves, and with the ecotropic and xenotropic virus isolates. With antisera made in rabbits and qualitative absorptions with live virus-producing cells, we could show that the dualtropic T cell lymphoma (TCL)-inducing virus, the thymotropic TCL-inducing class, and the dualtropic B cell lymphoma (BCL)-inducing class, each determined specific cell surface-associated antigens. Moreover, these specificities were different from the ones demarcated by either ecotropic or xenotropic viruses isolated from the same mouse strain and thus may each be serologically unique and, by inference, genetically distinct.

Materials and Methods

Cells and Viruses. The following cell lines were used: mouse fibroblast line SC-1 (18); mink lung cell line ATCC CCL64 (19); the rabbit cornea cell line SIRC (20); as well as stromal cells and cell lines derived from the thymus or bone marrow of healthy or lymphoma-bearing B6 mice (16, 21). The virus isolates used in this study were all derived from C57BL/6 cells and/or tumors and are listed in Table I. They include ecotropic, xenotropic, and thymotropic isolates, as well as dualtropic isolates, some of which induce thymic or nonthymic lymphomas in the strain of origin. All (cloned) dualtropic and ecotropic viruses used in this study were B-tropic on mouse cells. Cultured cells and viruses were grown in the Dulbecco-Vogt modification of Eagle's minimum essential medium, supplemented with 10% fetal bovine serum. Virus titrations were done with the fluorescent antibody (FA) center procedure (16) with a broadly reactive anti-MuLV serum raised in Lewis rats against a regressor lymphoma line induced with RadLV (22). Virus titration by the FA procedure works equally well for ecotropic, dualtropic, and xenotropic virus isolates that are growing in mouse, rabbit, or mink cells, respectively.

Antisera and Serological Assays. Antisera were raised in rabbits by three or four subcutaneous injections of 10^7 SIRC cells chronically infected with dualtropic or xenotropic virus. For each biweekly injection, SIRC cells were reinfected with cloned virus to minimize the hypothetical possibility that chronically infected SIRC cells would select for the expression of a particular virus-associated cell-surface antigen. The rabbits were bled 2–3 wk after the last injection. Serum aliquots were kept frozen at −70°C.

Indirect live-cell immunofluorescence was done as follows (23): Trypsinized cells were washed with phosphate-buffered saline (PBS), and 2×10^5 cells in 50 μl were suspended in wells of

[2] The nonthymic lymphomas that were induced by viruses described in this paper were immunoglobulin-secreting B cell malignant lymphomas (P. K. Pattengale, M. Haas, T. Beardsly, C. Taylor, and J. R. Lukes. Immunopathology of virus-induced B cell and T cell malignant lymphomas in C57BL/6 mice. Manuscript in preparation.).

TABLE I
*List of MuLV Used**

Virus	Class	Description	Grown in	Reference
RCN-BM5	Dualtropic plus ecotropic	Mixture of dualtropic and ecotropic viruses produced by stromal cell line grown from bone marrow. Virus induces B cell-malignant lymphomas.	Fibroblastoid bone marrow cell line derived from C57BL/6 mouse bearing a nonthymic-malignant lymphoma.	16, 17
S(FA2)	Ecotropic	Cloned virus isolate from RCN-BM5 tissue culture fluid. Not lymphomagenic.	SC-1	17
S(FA4)	Dualtropic	Cloned virus derived from the RCN-BM5 cell line. Not lymphomagenic.	SC-1	17
M(BC4)	Dualtropic	Cloned virus derived from RCN-BM5 cell line. Potent inducer of B cell-malignant lymphomas.	Mink	17
136.5 136.7	Thymotropic	Viruses produced by cloned thymoma cell lines, induced by virus inoculation in C57BL/6 mice. Potent inducers of thymic-malignant lymphomas.	C57BL/6 thymoma cells	13, 14, 16
136DT-2	Dualtropic	Dualtropic virus clone isolated from thymus epithelial reticulum cultures grown from a 136.7 virus-induced thymic lymphoma. Potent inducer of thymic-malignant lymphomas.	SC-1 or mink	21
54-1-5X	Xenotropic	Cloned virus isolated by cocultivation of C57BL/6 virus-induced thymoma cells with mink lung cells. Cloned. Virus is innocuous in vivo.	Mink	14

* All viruses originated from the C57BL/6 mouse strain. All dualtropic and ecotropic isolates were B tropic on mouse cells.

round-bottom microtiter plates. 20 μl (or other volumes in some cases) of diluted antiserum was added and incubated for 30 min at room temperature. After three washing cycles with 200 μl of PBS, 50 μl of fluorescein-conjugated anti-rabbit IgG (heavy plus light chain) $F(ab')_2$ made in goats (N. L. Cappel Laboratories, Inc., Cochranville, Pa.) at a dilution of 1:20 was added, and the cells were again incubated for 30 min. Three more washings followed, and the cells were left in 50 μl of PBS in the cold. Fluorescent cells were observed in a Zeiss UV microscope (Epi-illumination, Carl Zeiss, Inc., New York) at a magnification of 10 × 25 or 10 × 63, and the percent fluorescent cells showing sharp surface delineation was determined. The resulting fluorescence showed up as very strong, bright surface fluorescence at the circumference of the cells plus spotted staining over the rest of the cell membrane. FA-negative cells could not be seen even in vague outline. Thus, qualitative absorption of the antisera essentially resulted in an all or none fluorescent staining pattern in the UV microscope. Internal (positive and negative) controls were carried in each experiment, and multiple antisera made against each virus were prepared and analyzed even though in each instance the results of only one are presented.

Absorptions. Absorptions were carried out essentially as described by Cloyd et al. (23). The rabbit antisera had titers of 1,000–3,000 for the 50% staining point on specific target cells. The antisera (at dilutions of 1:25 or 1:40) were absorbed (overnight in the cold) twice in succession with 5×10^7 live trypsinized cells per milliliter of antiserum. Each antiserum was absorbed with a set of antigens (cells or virus-infected cells) after which it was tested by microimmunofluorescence on a battery of cells or virus-infected cells. Double absorptions were clearly necessary; however, triple absorptions were not necessary to completely absorb out cross-reactive antigenic determinants. Therefore, the absorbed antisera reacted with cell-surface antigens that were present on the target cells and that presumably were absent from the absorbing cells. In this fashion one can construct a map depicting the serological cross-reactivities among cell-surface antigens associated with the different lymphoma-inducing and other indigenous viruses of the B6 mouse.

Upon titration of an antiserum by the microimmunofluorescence procedure, one observes that with increasing antiserum dilution both the percent stained cells and the intensity of surface staining decrease. Unfortunately, upon reaching the vicinity of 50% stained cells, membrane staining becomes weak and the assay less reliable (Table II). Thus, the 50% staining point of a specific antiserum is subject to much experimental error, though useful as an indication of relative serological activity. Therefore, we used absorbed antisera at low dilutions (1:25 or 1:40), which yielded bright, clear-cut cell-surface fluorescence or a lack of fluorescence,

TABLE II

Activity of Nonabsorbed Antisera for Various Infected and Noninfected Cell Lines

Test cell‡	Virus properties§	Antiserum prepared against virus*				
		RCN-BM5 (DT; BCL)	M(BC4) (DT; BCL)	136.5* (Thymo; TCL)	M(136DT-2) (DT; TCL)	54-1-5X (X; I)
Mink	—	2‖	5	5	2	<2
SC-1	—	2	2	200	2	<2
S(FA2)	E; I	200	30	800	70	<2
S(FA4)	DT; I	1,200	900	300	200	150
M(BC4)	DT; BCL	1,200	900	300	200	150
136.7	Thymo; TCL	200	100	3,600	150	<5
M(136DT-2)	DT; TCL	—	—	200	3,000	150
MinkX (54-1-5X)	X; I	140	200	—	100	1,200

* Antisera were prepared against virus-infected SIRC cells except 136.5 virus, which was injected into rabbits after purification by banding through two sucrose gradients.

‡ Virus isolates that were initially cloned in SC-1 cells bear an "S" prefix, those cloned in mink cells bear the prefix "M."

§ E, ecotropic; DT, dualtropic; I, innocuous upon injection into B6 mice; X, xenotropic; thymo, thymotropic virus.

‖ Reciprocal of antiserum dilution at which 50% of the cells were stained in the microimmunofluorescence test.

respectively. In our experiments we recorded both the percentage of cells that were stained and their relative staining intensity. However, in time we concluded that the immunofluorescence procedure as used essentially yields absolute results, i.e., a staining combination (absorbed serum plus particular infected cell) is either strongly positive for most (90–100%) of the cells or is completely negative for >99.9% of the cells. Thus, the data presented are subject to little error.

All antisera used were tested by microimmunofluorescence on rabbit SIRC cells. None showed any reactivity with the SIRC cells, and, therefore, these data were omitted from the tables.

Results

Activity of Antisera Prepared Against Lymphomagenic and Xenotropic Virus Isolates. Antisera were prepared in rabbits against SIRC cells infected with (*a*) noncloned virus produced by RCN-BM5 bone marrow-adherent cells, virus that induced B cell-malignant lymphomas; (*b*) the BCL-inducing cloned virus isolate M(BC4); (*c*) the TCL-inducing dualtropic virus isolate 136DT-2; and (*d*) the xenotropic virus-isolate 54-1-5X, in addition to sucrose-banded TCL-inducing thymotropic virus 136.5. Table II shows the activity of these antisera for various uninfected cells and virus-infected cells in the microimmunofluorescence test. It appeared that the antisera had little if any activity on noninfected mink, SC-1, or SIRC cells, except the antiserum to 136.5 (sucrose gradient-purified) virus, which displayed some anti-mouse cell activity. The antisera made against dualtropic BCL-inducing virus showed activity on ecotropic, xenotropic, and thymotropic virus-infected cells; however, this activity was of lower titer (1:30 to 1:200) than the titer found on cells infected with dualtropic virus of the BCL-inducing series (1:900 to 1:1,200). A corresponding, though opposite, specificity was found for the activity of the other antisera (Table II). One noteworthy result was the serological difference between the two TCL-inducing viruses, the thymotropic virus 136.7 and the dualtropic isolate M(136DT-2), which

was isolated from the thymic epithelium of a thymoma induced by the thymotropic isolate 136.7. Antisera raised against either virus displayed high titers on cells infected with the respective homologous viruses, but low titers with the respective heterologous TCL-inducing viruses. These antiserum titration results suggested that one might succeed in separating the different virus-associated serological activities and prepare type-specific viral reagents by proper absorption of the antisera.

Serological Specificity After Qualitative Absorptions of the Antisera

ANTISERA MADE AGAINST BCL-INDUCING DUALTROPIC VIRUS. Rabbit antisera prepared against the dualtropic BCL-inducing virus clone M(BC4) were absorbed with cells replicating ecotropic, dualtropic, xenotropic, and thymotropic viruses. Microimmunofluorescence determinations were then performed on a set of test cells. Table III shows the two-dimensional serologic cross-reactivity of antiserum raised against the BCL-inducing virus clone M(BC4). The nonabsorbed serum was equally active on (mink) cells infected with lymphomagenic dualtropic virus M(BC4) and on SC-1 cells infected with the innocuous dualtropic virus isolate S(FA4), which were both cloned from the same BCL-derived bone marrow culture (Table III, line 8). Absorption of the antiserum with dualtropic virus isolated from BCL entirely abrogated the activity on these viruses and on all other viruses tested. Thus, this antiserum contained predominantly anti- (BCL-derived) dualtropic specificities. The activity on BCL-derived dualtropic virus could not be absorbed out with either ecotropic, xenotropic, or thymotropic virus-infected cells.

Reactivity was lower on cells replicating xenotropic virus and weak on 136.7 cells, but no reactivity was found on uninfected SC-1, mink, or SIRC cells or on ecotropic [S(FA2)] virus-infected SC-1 cells. The serum reactivity on (TCL-inducing) 136.7 cells could be adsorbed with these cells themselves and with ecotropic virus, but not with xenotropic virus, which indicates a relative serologic likeness of 136.7-type thymotropic virus to ecotropic virus. Similarly, the antiserum activity detected with xenotropic virus could be absorbed out by dualtropic but not by 136.7 virus, which indicates a relative lack of serologic relatedness between xenotropic and thymotropic virus.

TABLE III

Serological Activity of Antiserum to Cloned BCL-inducing Dualtropic Virus M(BC4)*

Antiserum absorption with MuLV-infected cells		Antiserum activity for cells infected with MuLV								
Cells‡	Virus type§	Cells:‡ Virus type:	SC-1 —	Mink —	S(FA2) E; I	S(FA4) DT; I	M(BC4) DT; BCL	MinkX X; I	136.7 Thymo; TCL	M(136DT-2) DT; TCL
							%			
SC-1	—		0‖	0	0	100	100	50	30–50	100
Mink	—		0	0	0	90	100	50	30–50	100
S(FA2)	E; I		0	0	0	90	100	0	0	—
S(FA4)	DT; I		0	0	0	0	0	0	0	0
M(BC4)	DT; BCL		0	0	0	0	0	0	0	0
MinkX	X; I		0	0	0	90	100	0	30	0
136.7	Thymo; TCL		0	0	0	90	100	50	0	70
None	—		0	0	0	100	100	60	50	100

* Antiserum was absorbed at a dilution of 1:25 and used in microimmunofluorescence tests at a final dilution of 1:90.

‡ The prefix "S" means virus-infected SC-1 cells; prefix "M" indicates that virus was grown in mink cells.

§ E, Ecotropic; X, xenotropic; DT, dualtropic; thymo, thymotropic; I, innocuous.

‖ Percentage of cells with membrane fluorescence.

However, xenotropic virus did not absorb out the activity detected on (BCL-derived) dualtropic viruses S(FA4) and M(BC4). These serologic relationships show that cell-surface antigens associated with BCL-inducing or BCL-derived dualtropic viruses are unique and different from those of either thymic leukemia-inducing, ecotropic, or xenotropic virus isolated from the same inbred mice. Moreover, absorption of anti-BCL serum with cells infected with ecotropic virus produced an antiserum reagent that was specifically active only with BCL-derived dualtropic viruses (Table III, line 3).

ANTISERA MADE AGAINST THYMOTROPIC, TCL-INDUCING VIRUS. Rabbit antisera prepared by inoculation of sucrose-banded thymotropic virus (inducing T cell [thymic] lymphomas) was absorbed with a group of virus-producing cells, and each absorbed serum was tested on the set of test cells as shown in Table IV. Antisera to thymotropic virus showed strong reactivity on cells infected with thymotropic virus, activity that was abrogated by absorbing the serum with the homologous virus-infected thymoblasts but not with normal thymocytes and in part with ecotropic virus, but not by absorption with BCL-inducing dualtropic or with xenotropic viruses. This establishes the relative closeness of the thymotropic virus cell-surface-associated antigenicity to ecotropic virus and its remoteness from either xenotropic and (BCL-derived) dualtropic antigens.

The anti-thymotropic virus antisera did not react with either xenotropic or ecotropic virus; however, it reacted to a considerable degree with BCL-dualtropic virus, activity that could not be absorbed out with thymotropic virus 136.7, thus indicating that this serum contained at least a second reactivity besides the anti-thymotropic one. This reactivity was found on BCL-inducing dualtropic viruses. However, absorption of the anti-thymotropic virus serum with BCL dualtropic virus growing in either SC-1 or mink cells, yielded a serum reagent that was reactive only with thymotropic virus, and thus defined a cell-surface-associated antigen that was unique for thymotropic virus (Table IV, line 4).

Table IV also shows that the rabbit serum raised against thymotropic (TCL-

TABLE IV

Serological Activity of Antiserum to Gradient-purified TCL-inducing Thymotropic Virus 136.5*

Antiserum absorption with MuLV-infected cells		Antiserum activity for cells infected with MuLV							
Cells‡	Virus type§	Cells:‡ Virus type:	SC-1 —	Mink —	S(FA2) E; I	S(FA4) DT; I	M(BC4) DT; BCL	MinkX X; I	136.7 Thymo; TCL	M(136DT-2) DT; TCL
							%			
SC-1	—		0	0	0–50‖	80¶	70	0	100	—
Mink	—		80[w]	0	—	80	—	0	100	—
S(FA2)	E; I		0	0	0	0	0	0	50	0
S(FA4)	DT; I		0	0	0	0	0	0	80–90	0
M(BC4)	DT; BCL		80[w]	0	0	0	0	0	100	0
MinkX	X; I		80[w]	0	0–50[w]	80	50[w]	0	100	0
136.7	Thymo; TCL		0	0	0	80	70	0	0	0
None	—		80[w]	0	0–50[w]	80	70	0	100	80[w]

* Antiserum was absorbed at a dilution of 1:40 and used in microimmunofluorescence tests at a final dilution of 1:140

‡ The prefix "S" means virus-infected SC-1 cells; prefix "M" indicates that virus was grown in mink cells.

§ E, ecotropic; X, xenotropic; DT, dualtropic; thymo, thymotropic; I, innocuous; w, weak.

‖ Several anti-thymotropic sera varied in their activity with ecotropic virus, being either nonreactive or weakly reactive on 50% of ecotropic virus-infected cells.

¶ Percentage of cells with membrane fluorescence.

inducing) virus reacted poorly with cell-surface antigens associated with dualtropic (TCL-inducing) virus M(136DT-2). The reactivity that was detected on these cells with unabsorbed anti-thymotropic virus serum (Table IV, last column) was completely absorbed out by ecotropic, xenotropic, and dualtropic virus, thus indicating that the observed reactivity was weak and nonspecific. Thus, the thymotropic and dualtropic TCL-inducing virus isolates were serologically different, even though we isolated the dualtropic virus 136DT-2 from the thymus epithelial moiety of thymomas induced by the thymotropic virus 136.7. The serological difference between these two classes of TCL-inducing virus isolates is also evident from the experiments with sera raised against the dualtropic isolate 136DT-2, as described in the following section.

ANTISERA MADE AGAINST TCL-INDUCING DUALTROPIC VIRUS. Rabbit antisera raised against SIRC cells infected with the TCL-inducing dualtropic virus clone 136DT-2 were absorbed with a series of antigens (virus-infected or noninfected cells) and used in microimmunofluorescence tests on a group of virus-infected cells (Table V). The nonabsorbed antisera showed very strong reactivity with cell-surface antigens associated with homologous 136DT-2 virus, and weaker reactivities with the eco-, thymo-, and BCL-inducing dualtropic viruses. This was also evident from the antiserum titers shown in Table II. Some heterologous reactivities were absorbed out by noninfected mouse SC-1 cells; thus these probably did not represent virus-associated reactivities.

The other substantial reactivity of this antiserum was detected on cells infected with xenotropic virus. This activity could be absorbed with both xenotropic virus or with the homologous dualtropic virus 136DT-2, which indicates that antiserum raised against 136DT-2 dualtropic virus possessed a genuine anti-xenotropic virus reactivity. The reactivity for xenotropic virus that was detected with the antiserum to 136DT-2 virus differed from the reactivity found on homologous 136DT-2 virus-infected cells, because antiserum absorption with xenotropic virus did not remove the homologous activity on 136DT-2 virus-infected cells. Absorption of this serum with xenotropic virus thus gave rise to a reagent specific for TCL-inducing dualtropic virus 136DT-2 (Table V, line 6). The xenotropic cross-reactivity associated with TCL-inducing virus 136DT-2 was not found in the reverse experiment in which anti-xenotropic virus sera

TABLE V

Serological Activity of Antiserum to Cloned TCL-inducing Dualtropic Virus 136DT-2*

Antiserum absorption with MuLV-infected cells		Antiserum activity for cells infected with MuLV						
Cells‡	Virus type§	Cells:‡ Virus type:	S(FA2) E; I	S(FA4) DT; I	M(BC4) DT; BCL	MinkX X; I	136.7 Thymo; TCL	M(136DT-2) DT; TCL
					%			
SC-1	—		0‖	—	—	50	0	100
MLC	—		50	70	70	50	70	100
S(FA2)	E; I		0	70	50	50	0	100
S(FA4)	DT; I		10	0	0	50	10	100
M(BC4)	DT; BCL		0	0	0	50	70	70
MinkX	X; I		0	0	0	0	0	80
136.7	Thymo; TCL		10	70	70	50	0	100
M(136DT-2)	DT; TCL		0	0	0	0	0	0
None	—		50	70	70	50	70	100

* Antiserum was absorbed at dilution of 1:25 and used in microimmunofluorescence tests at a final dilution of 1:90.

‡ The prefix "S" means virus-infected SC-1 cells; prefix "M" indicates that virus was grown in mink cells.

§ E, ecotropic; X, xenotropic; DT, dualtropic; thymo, thymotropic; I, innocuous.

‖ Percentage of cells with membrane fluorescence.

were analyzed (Table VI). In this case, absorbing the anti-X serum with dualtropic virus 136DT-2 removed all of the reactivity associated with xenotropic virus.

Table V shows that TCL-inducing viruses of the thymotropic class, 136.7, and those of the dualtropic class, 136DT-2, are serologically unrelated. Data shown in Table V confirm this lack of serological relatedness as already shown in Table IV. Absorption of anti- (TCL-inducing) 136DT-2 antiserum with thymotropic virus 136.7 removed the reactivity for the thymotropic as well as ecotropic virus (Table V, line 7), but removed none of the major anti-dualtropic virus reactivity nor its minor xenotropic virus reactivity. Thus the thymotropic and dualtropic TCL-inducing viruses induce distinctly different cell-surface-associated antigens.

ANTISERA MADE AGAINST XENOTROPIC VIRUS DERIVED FROM C57BL/6 MICE. Rabbit antisera raised against SIRC cells infected with strain C57BL/6-derived xenotropic virus possessed reactivity against the homologous virus, reactivity that could be removed by absorption with xenotropic virus-infected cells (Table VI). This antiserum showed no reactivity with either eco- or thymotropic viruses, nor did either of these viruses absorb out the xenotropic reactivity from the serum. Serologic cross-reactivity was observed between the xenotropic virus and the three dualtropic virus isolates tested. Whereas the BCL-derived dualtropic viruses S(FA4) and M(BC4) in part absorbed the anti-xenotropic reactivity, antiserum absorption with the TCL-inducing isolate 136DT-2 completely removed the xenotropic reactivity from this antiserum.

The anti-xenotropic virus-associated reactivity was not effected by absorption with noninfected cells (Table VI, lines 1 and 2). This, and the other data shown in Table VI, suggests that the reactivity associated with xenotropic virus is more monospecific compared with that directed against the presumed recombinant dualtropic and thymotropic virus classes. However, xenotropic reactivity as was associated with the dualtropic viruses derived from TCL was not identical to that associated with BCL-derived dualtropics as the latter only partially removed the serum reactivity present

TABLE VI

Serological Activity of Antiserum to Cloned Xenotropic Virus of C57BL/6 Mice*

Antiserum absorption with MuLV-infected cells		Antiserum activity for cells infected with MuLV						
Cells‡	Virus type§	Cells:‡ Virus type:	S(FA2) E; I	S(FA4) DT; I	M(BC4) DT; BCL	MinkX X; I	136.7 Thymo; TCL	M(136DT-2) DT; TCL
					%			
SC-1	—		0	70‖	—	100	0¶	80
MLC	—		0	70	70	100	0	80
S(FA2)	E; I		0	50	50	100	0	80
S(FA4)	DT; I		0	0	0	50"	0	30
M(BC4)	DT; BCL		0	0	0	50"	0	40
MinkX	X; I		0	0	0	0	0	0
136.7	Thymo; TCL		0	70	70	100	0	80
M(136DT-2)	DT; TCL		0	0	0	0	0	0
None	—		0	70	70	100	0	80

* Antiserum was absorbed at dilution of 1:25 and used in microimmunofluorescence tests at a final dilution of 1:90.

‡ The prefix "S" means virus-infected SC-1 cells; prefix "M" indicates that virus was grown in mink cells.

§ E, ecotropic; X, xenotropic; DT, dualtropic; thymo, thymotropic; I, innocuous.

‖ Percentage of cells with membrane fluorescence.

¶ There was a consistent, very weak (w) activity of 136-type thymoma cells for anti-xenotropic sera. This reactivity could be absorbed out by any of the viruses used and was thus nonspecific.

on TCL-derived dualtropic isolate 136DT-2 (Table VI, compare lines 4 and 5 with line 8).

Type-Specific Antiserum as a Probe of Dualtropic Virus Cell-Surface-associated Antigens. Type-specific antisera to virus-associated cell-surface antigens have been used to demonstrate the presence of serologically different dualtropic viruses in B cell lymphomagenic mice. Viruses were cloned from the supernatant fluid of a noncloned bone marrow-adherent cell line RCN-BM5 (16, 17). In neutralization experiments with anti-AKR virus gp70 serum and in interference experiments (17) not all dualtropic isolates behaved similarly. Thus we argued that the observed neutralization and interference differences may be a result of differences among the dualtropic viruses, presumably heterogeneity among the recombinant viral neutralizing glycoproteins gp70.

Antiserum made against the BCL-inducing MuLV clone M(BC4) was shown to possess type-specific properties (Table III, line 3) after absorption with ecotropic virus clone S(FA2). We checked the affinity of this reagent for BCL-derived dualtropic virus clones by microimmunofluorescent staining of virus-infected cells. Two kinds of controls were included in the experiments (Table VII). Ecotropic virus isolates that were cloned from the same RCN-BM5 cell line were added as negative controls. In addition we used virus isolates that were recloned from the BCL-inducing clone M(BC4), against which the test antiserum was made. This control would suggest whether serologic differences among the cloned viruses were frequently induced by the in vitro cloning procedure or whether the variants were present in the infected bone marrow cell line RCN-BM5, and possibly in vivo. Table VII shows that the BCL-specific antiserum strongly stained the M(BC4) virus isolate and five of its recloned virus progeny, but a significantly higher serum concentration was needed to stain virus clones FC2 or BD1, even though the latter viruses replicated with a comparable infectious titer (17) as is also suggested by their microimmunofluorescent

TABLE VII

*Microimmunofluorescence Test with a Type-Specific Anti-Viral Serum Reagent Demonstrating Differences Among Dualtropic Virus Clones Isolated from BCL Bone Marrow Cell Line RCN-BM5**

Virus		Reciprocal of serum dilution at which 50% of cells were stained	
Isolate	Type	BCL-type-specific serum‡	Broadly reactive α-MuLV serum§
S(FA2)	E¶	<2	2,500
S(FA6)	E	<2	1,280
M(BC4)	DT	900	2,500
M(BC4)-m_{1-5}‖	DT	900	2,500
S(FC2)	DT	25	2,500
M(BD1)	DT	40	1,780

* Dualtropic virus was cloned on SC-1 (S) or mink lung cells (M) from the supernatant medium of the BCL-derived cell line RCN-BM5.

‡ Rabbit antiserum against clone M(BC4), absorbed twice with cloned ecotropic virus S(FA2) (Table III, line 3).

§ Rat anti-MuLV-induced tumor cells (22), a broadly reactive anti-MuLV serum (Materials and Methods).

‖ This entry refers to five separate isolates recloned on mink cells from M(BC4) virus.

¶ E, ecotropic; DT, dualtropic.

staining with a broadly reactive anti-MuLV serum. As expected, the ecotropic virus control did not stain with the anti-BCL virus-specific reagent at any dilution.

Discussion

The aims of this work were (*a*) to characterize the antigens associated with different dualtropic and lymphomagenic viruses isolated from irradiated C57BL/6 mice to establish their serological cross-reactivities; (*b*) to prepare type-specific antisera to the virus-associated antigens for use as reagents in experiments designed to trace the etiology of natural, virus-free lymphomas; and (*c*) to establish a serological fingerprint methodology by which future dualtropic virus isolates may be related to the ones previously encountered.

Our results show that unique serologic reactivities were associated with dualtropic and thymotropic lymphoma-inducing viruses isolated from x-ray-induced lymphomagenic C57BL/6 mice. Thus, the TCL-inducing dualtropic viruses were serologically different from the BCL-inducing isolates and from the thymotropic (TCL-inducing) virus isolates. With few exceptions, the different dualtropic and thymotropic virus classes each induced reactivities against ecotropic and/or xenotropic virus, in addition to distinct reactivities directed only against the homologous (immunizing) virus. This was shown by the absorption experiments in which different cross-reactivities were removed from the antisera. Specific absorption schemes then revealed monospecific reactivities when tested by microimmunofluorescence tests. Moreover, proper absorption of the antisera with cross-reacting virus-associated antigens gave rise to antisera reacting only with the homologous (dualtropic) viral antigen. Thus, we could produce antisera that were monospecific for TCL-inducing viruses (thymotropic or dualtropic) or for BCL-inducing dualtropic viruses. The new virus-associated antigenic specificities thus defined provide evidence for the recombinant nature of the dualtropic and thymotropic virus isolates. This presumed recombinant status of the dualtropic viruses appears to have occurred in the viral *env* gene (1, 2, 6, 7). The new monospecific antigenicities that we found suggest that within one inbred mouse strain (C57BL/6) new antigenic specificities may arise as a result of recombination. Whether these new antigens are a result of major informational sequence substitutions or of minor gene sequence differences that result in conformational changes of the antigens is not now known. However, the number of new antigenic specificities seems to be large; preliminary serologic mapping of viruses isolated from C57BL/6 mice by us or by other investigators reveals a considerable lack of identity among the dualtropic viruses isolated (M. Haas. Unpublished observations.). The extent of antigenic variability that may be generated by *env* gene recombination may thus be large, and may play a crucial role in the response of the host to the virus and to virus-infected cells, and in the target cell specificity of recombinant viruses (16).

Microimmunofluorescence tests with a group of xenogeneic antisera absorbed by ecotropic or xenotropic antigens of the types shown in Tables III–VI qualitatively characterizes the dualtropic virus isolates with respect to their antigenic relatedness to ecotropic or xenotropic viruses. For example, antiserum to the (BCL-inducing) virus clone M(BC4) did not react with ecotropic virus-associated antigens but reacted with xenotropic virus (Table III); the reactivity of anti-thymotropic (BCL-inducing) virus 136.5 with the homologous virus could be weakened significantly by absorption with ecotropic but not xenotropic virus-associated antigens (Table IV); cross-reactivity of

antiserum to dualtropic (TCL-inducing) virus 136DT-2 with dualtropic viruses S(FA4) M(BC4) was absorbed out by xenotropic virus but not by ecotropic virus (Table V); anti-xenotropic virus serum did not react with ecotropic virus or with thymotropic virus 136.7, whereas its cross-reactivity with three dualtropic viruses could not be absorbed out with ecotropic virus (Table VI). Thus, in a relative sense, the thymotropic viruses were less xenotropic than the associated (TCL-inducing) or the nonassociated (BCL-inducing) dualtropic isolates. Whether these relative characteristics influence the natural immune responses of the murine host to the virus remains to be determined.

The monospecific reactivities that are associated with TCL- and BCL-inducing viruses now warrant the considered conclusion that different recombinant retroviruses induce different hematologic diseases in C57BL/6 mice. However, there seems to exist a rather large number of discrete antigenic variants among the dualtropic (recombinant) viruses that can be isolated from x-ray-treated mice, many of which have not been observed to induce any disease in vivo. Is TCL- and BCL-induction thus limited to one specific recombinant species each, i.e., is there a direct relationship between the specific serologic characteristic of a recombinant isolate and its lymphomagenic potential?

Previously we have observed considerable organotropism of lymphomagenic viruses (16); BCL-inducing virus predominantly replicated in spleen and lymph node tissue, whereas TCL-inducing thymotropic virus could be extracted only from thymic tissue. It is conceivable that this organotropism was a result of an inherent cytotropism of each virus. Virus cytotropism is presumably a result of the presence of specific cell-surface receptors on classes of lymphoid cells (24), which would have affinity for specific viral envelope glycoproteins (gp70). The serologic specificities of lymphomagenic virus isolates that we have described here also may be determined by viral gp70. Thus, it follows that the unique virus-associated serologic reactivities that we have studied may be correlated with the disease induced by the viruses. However, to answer this question, more lymphomagenic viruses must be isolated and tested. The preparation of monoclonal reagents to monospecific dualtropic virus reactivities may well be able to decisively settle this issue.

Summary

Unique type-specific antigens were detected on cells infected with dualtropic and thymotropic viruses isolated and x-ray-induced T cell- and B cell-malignant lymphomas of C57BL/6 mice. These antigens were defined by membrane fluorescence with antisera made in rabbits against rabbit cells chronically infected with cloned virus. The antisera were qualitatively absorbed with a group of cells chronically infected with related dualtropic, ecotropic, and xenotropic viruses. The absorbed antisera detected type-specific, virus-related cell-surface antigens that were unique for different dualtropic virus isolates. The unabsorbed sera also reacted with antigens found specifically on ecotropic and xenotropic virus-infected cells. These findings support the contention that T cell lymphoma (TCL)-inducing and B cell lymphoma (BCL)-inducing viruses isolated from x-irradiated C57BL/6 mice are *env* gene recombinants in which ecotropic gene sequences have been substituted by xenotropic sequences. We found that unique antigenicities are associated with each TCL-inducing and BCL-inducing dualtropic virus, and that the thymotropic TCL-inducing virus isolates (e.g.,

136.5 and 136.7 viruses) represent a separate serologic group, different from the dualtropic TCL-inducing viruses. By using a series of absorbed antisera in microimmunofluorescence tests we could perform serologic virus mapping of dualtropic clones isolated by us or by others and relate them serologically to previously isolated clones. These virus mapping experiments indicated that many serologically different recombinant viruses can be isolated from C57BL/6 mice. It is suggested that many distinct recombinant viruses may exist in lymphomagenic C57BL/6 mice, some of which are associated with specific lymphoma induction.

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