

EXPRESSION OF HYBRID Ia MOLECULES ON THE CELL SURFACE OF RETICULUM CELL SARCOMAS THAT ARE UNDETECTABLE ON HOST SJL/J LYMPHOCYTES*

BY STANLEY M. WILBUR‡ AND BENJAMIN BONAVIDA

From the Department of Microbiology and Immunology, School of Medicine, University of California, Los Angeles, California 90024

The reticulum cell sarcoma (RCS)¹ of SJL/J mice has been shown to express alien histocompatibility membrane antigens (1, 2). This form of tumor antigen is, by definition, present on cancerous, but not normal, syngeneic tissues. However, it differs from other forms of tumor-associated antigens because it is a normal membrane component of one or more strains allogeneic to the host (3). In the case of the RCS, both antibody and cytotoxic lymphocytes directed against syngeneic H-2^s or allogeneic H-2^d determinants react strongly with an in vitro cultured RCS. Weak reactivities with anti-H2^b cytotoxic lymphocytes have also been seen in these neoplasms. In contrast to the results found with the in vitro cultured RCS lines, in vivo passaged lines have previously shown no detectable alien histocompatibility antigens as recognized by specific cytotoxic lymphocytes. Furthermore, specific antibody could detect only specificities of the H-2^d haplotype and no H-2^b specificities (1).

An additional trait of the RCS is that it readily induces a syngeneic host immune response (4). Proliferation of Ly 1⁺23⁻ T lymphocytes can be induced in mixed lymphocyte-tumor in vitro cultures (5). Lymphocytes can also be induced to proliferation in vivo by immunization with irradiated tumors (6). Specific cytotoxicity against the tumor has been demonstrated when the in vitro RCS lines are used as stimulators in mixed lymphocyte-tumor cultures and as targets in cytotoxic assays (1). In addition, syngeneic T lymphocytes have been induced to cytotoxicity against in vivo passaged RCS by special hyperimmunization regimens (7). In primary mixed lymphocyte-tumor cultures, in vivo passaged RCS and spontaneous RCS have been shown to induce only T lymphocyte proliferation and no detectable cytotoxicity.

Little is known of the antigenic determinants on the RCS that are involved in the host anti-tumor immune response. Earlier work found that antisera directed against syngeneic IA-encoded products (i.e., anti-Ia.4) could inhibit the SJL/J anti-RCS proliferative response. This suggested that host responsiveness was induced via interaction with syngeneic Ia molecules (8). In that study, an in vivo passaged RCS was used, so there was no detectable cytotoxicity by the anti-tumor effector lymphocytes. Conversely, another investigation found that syngeneic anti-RCS cytotoxic lympho-

* Supported by grants CA 24314 and CA 19753 from the National Cancer Institute.

‡ Supported by the Cellular and Molecular Biology Training Grant GM07185 from the U. S. Public Health Service.

¹ Abbreviations used in this paper: FCS, fetal calf serum; HBSS, Hanks' balanced salt solution; RCS, reticulum cell sarcoma.

cytes, induced by an in vitro cultured RCS line, could lyse not only the tumor, but also targets expressing H-2^d alloantigens (1). The reasons for this apparent conflict have previously remained unresolved. Further studies of antigen recognition by the noncytotoxic effector lymphocytes, primed against syngeneic in vivo passaged or spontaneous RCS, have been severely hampered because good quantitative assays do not exist.

We have found, however, that noncytotoxic anti-RCS effector lymphocytes can recognize and specifically bind to the RCS, despite the absence of subsequent cytolysis. This assay of lymphocyte-target cell binding was used to identify the antigens recognized by the noncytotoxic syngeneic effectors. Evidence was obtained that indicated that the alien histocompatibility antigens found on the RCS of SJL/J mice are intimately involved in the syngeneic host immune recognition. Furthermore, a role for syngeneic Ia molecules was also found. Syngeneic lymphocytes primed against the RCS could bind not only to the RCS, but also to normal allogeneic spleen cells. Genetic mapping with congenic recombinant mice and monoclonal anti-Ia.7 antibody treatment further examined the nature of the alien molecules expressed on the RCS and recognized by the anti-tumor effectors. The results indicate that the alien molecule may be a hybrid Ia molecule consisting of a syngeneic normally unexpressed IA^a-encoded β -chain in association with an alien α -chain encoded within IE. Hybrid Ia molecules have been identified on normal cells of many haplotypes (9-11). However, expression of these molecules on the RCS was unexpected because they are not normal membrane constituents of SJL/J cells.

Materials and Methods

Mice. Animals of both sexes at 6-10 wk of age were used in all experiments. With a few exceptions, all strains were purchased from The Jackson Laboratory (Bar Harbor, Maine). Animals of strains B10.OL and B10.A were bred in our facility from stock kindly provided by Dr. Donald C. Shreffler (Washington University, St. Louis, Mo.). B10.S, D2.GD and (B10.A \times B10.GD)F₁ mice were a generous gift from Dr. Walter Urba and Dr. Linda Wicker in the laboratory of Dr. William Hildemann (University of California, Los Angeles). B10.S(9R) and B10.GD mice came from the laboratory of Dr. Eli Sercarz (University of California, Los Angeles).

Tumors. All RCS used in this work arose spontaneously from SJL/J mice of 6 mo-1 yr of age. The RCS-LA16 line was maintained in vivo by serial passage in young SJL/J animals. This line has been found to be derived solely from SJL/J mice by analysis of the polymorphic variants of both phosphoglucomutase and glutathione reductase. The in vitro line, RCS-LA6b, was maintained in continuous cell culture. It was grown as a single cell suspension in RPMI-1640 supplemented with 10% fetal calf serum (FCS) (Grand Island Biological Co., N. Y.) and 1.8×10^{-4} M reduced glutathione (Sigma Chemical Co., St. Louis, Mo.). A primary spontaneous RCS was removed from the mesenteric lymph node of an animal 11 mo of age. It was used immediately or cryogenically preserved. This line has been termed RCS-LA30.

Antisera. The monoclonal anti-Ia.7 antibody was a generous gift from Dr. U. Hammerling (Sloan-Kettering Institute for Cancer Research, New York) and was classified as number 13/18 in his laboratory. The hybridoma anti-Thy 1.2 antiserum was derived from ascitic fluid of pristane-pretreated BALB/c mice that had been injected with the cell line H022-1. This line was obtained from the Cell Distribution Center at The Salk Institute, La Jolla, Calif. Detailed methodology has been described elsewhere (12). All sera were stored at -70°C .

Preparation of Lymphocytes and RCS. Responding lymphocytes were derived from either SJL/J mesenteric lymph nodes or CBA spleens. All nontumor target cells were from the spleens of normal animals of the appropriate strain. The particular tissue (spleen, lymph node, or cancerous lymph node) was removed and minced in Hanks' balanced salt solution (HBSS; Grand Island Biological Co.) using tissue forceps. The tissue integrity was further disrupted by

repeated passages through a 12-ml syringe, and finally the preparation was filtered through a narrow meshed nylon screen. Erythrocytes in the spleen cell preparations were lysed by a 15-s treatment in distilled water, followed by the addition of an equal volume of 2- \times concentrated HBSS.

In Vitro Cultures. 96 well microtiter plates (Falcon 3042, Falcon Labware, Div. of Becton, Dickinson and Co., Oxnard, Calif.) were used throughout. To each well, the appropriate cell mixture was added in 0.2 ml RPMI-1640 that had been supplemented with 10% fetal calf serum (RPMI-1640/10% FCS), 5×10^{-5} M 2-mercaptoethanol, 1% nonessential amino acids, 1% sodium pyruvate, and 1% antibiotic-antimycotic. All cultures were incubated for 4 d at 37°C in a water-saturated atmosphere of 10% CO₂ in air. Proliferation was assessed by the addition of 1 μ Ci [³H]thymidine (10 Ci/mM sp ac) (ICN Pharmaceuticals, Inc., Irvine, Calif.) to triplicate test wells during the last 16–18 h of culture. Test wells were harvested on a MASH II (Microbiological Associates, Walkersville, Md.).

In mixed lymphocyte cultures, 5×10^5 responding lymphocytes were cultured with 5×10^5 irradiated (2,000 rad) allogeneic-stimulating spleen cells. For the mixed lymphocyte-tumor cultures, 5×10^5 SJL/J mesenteric lymph node cells and 5×10^4 irradiated (10,000 rad), syngeneic RCS were mixed. All cells were irradiated with a ⁶⁰Co source. In both culture systems, nonimmune lymphocytes were generated by incubating the appropriate responding cells with irradiated syngeneic lymphocytes in numbers equal to the added stimulator cells. Viability was determined by trypan blue exclusion.

Fluorescence. After two washes in HBSS, the in vitro primed responding lymphocytes (or, in some cases, RCS) were suspended in HBSS to 1×10^6 cells/ml. A 2.0% solution of fluorescein dissolved in distilled water was added to the cells. The cells were labeled in a final concentration of 0.027% wt/vol fluorescein for 15 min at 37°C. They were washed twice in HBSS and suspended in RPMI-1640/10% FCS to 1×10^6 cells/ml and used immediately. In some preliminary experiments, the particular target cell was fluoresceinated by incubation in 6×10^{-2} g/ml euchrysin for 45 min at 37°C. Both labeling procedures gave equivalent results.

Target-Lymphocyte Binding Assay. Targets at 1×10^7 cells/ml and in vitro primed lymphocytes at 1×10^6 cells/ml, both in RPMI-1640/10% FCS, were incubated separately at 30°C for 5 min. In most experiments the cultured lymphocytes were labeled with fluorescein. However, in some preliminary experiments, the target cell was labeled with euchrysin. 1 ml of cultured lymphocytes was added to 1 ml of the target cells, and this was allowed to incubate at 30°C for another 10 min. The cells were then centrifuged for 5 min at 160 g (maximum) and then gently resuspended in 0.5 ml of RPMI-1640/10% FCS. The percentage of cultured lymphocytes that bound to a particular target cell was counted under a fluorescent microscope using the appropriate barrier filters and excitation.

Competition of Tumor-associated Binding with Monoclonal Anti-Ia.7 Antibody. The RCS-LA16 was preincubated on ice in anti-Ia.7 antibody or a control serum for 2 h at a dilution of 1:1,000 in RPMI-1640/10% FCS. The cell concentration was at 1×10^7 cells/ml. The control sera were normal SJL/J serum and the hybridoma anti-Thy 1.2 antibody. After one wash in HBSS, the sera-pretreated RCS-LA16 were used in the standard lymphocyte-tumor binding assay. In this manner, the ability of monoclonal anti-Ia.7 serum to inhibit the lymphocyte-tumor interaction was assessed.

The anti-Ia.7 antibody was also absorbed on several normal cell populations or on the RCS-LA16 to correlate Ia.7-directed antibody activity with inhibition of binding. One population that expresses Ia.7, CBA, was used in absorption. SJL/J and C57Bl/6 spleen cells, which are both negative for Ia.7, were also used. In all cases, the absorption was at a serum dilution of 1:1,000 in RPMI-1640/10% FCS and a cell concentration of 5×10^7 lymphocytes/ml or 2×10^7 RCS-LA16/ml. After 2 h on ice, the cells were centrifuged and the supernate was stored at -70°C.

Calculation of Data. All data were expressed as the difference between the percentage of immune and nonimmune lymphocytes which bound to a particular target cell. The significance of binding above background levels was determined by the Student's *t* test and standard errors were calculated using standard formulas.

Results

Demonstration by the Lymphocyte Target-binding Assay That Both Alien H-2^d and Syngeneic H-2^b Specificities Are Expressed on the RCS of SJL/J mice. Alloprimed lymphocytes were

used to verify that both syngeneic and alien determinants are found on the in vivo passaged RCS. SJL/J lymph node cells were primed against H-2^d alloantigens by co-culturing for 4 d with irradiated DBA/2 spleen cells. Binding to normal spleen cells by these effectors can only be demonstrated against DBA/2 cells and not spleen cells of other haplotypes. In addition, these same anti-H2^d effector lymphocytes bind significantly to the in vivo passaged RCS. This result is not confined to SJL/J responding cells. When CBA spleen cells were primed with irradiated DBA/2 lymphocytes, equivalent results were obtained (Table I). The binding of primed lymphocytes to the RCS exhibits antigenic specificity because SJL/J anti-H2^k effector lymphocytes demonstrate significant binding against only CBA spleen cells, and in no instance can binding be found against the RCS or normal spleen cells of other haplotypes (Table I).

It was of interest to determine whether the RCS expressed its appropriate H-2^s determinants as well as those of the H-2^d haplotype. CBA spleen cells were co-cultured for 4 d with irradiated SJL/J lymphocytes. As expected, the anti-H2^s effector lymphocytes bound to normal SJL/J spleen cells. Furthermore, they recognized and bound effectively the in vivo passaged RCS (Table I). These results show that the in vivo passaged RCS expresses its own syngeneic H-2^s molecules and also specificities of the H-2^d haplotype.

Syngeneic Lymphocytes, Primed In Vitro against the RCS, Recognize the Tumor and Allogeneic Specificities. Normal SJL/J lymph node cells were co-cultured with irradiated RCS-LA16 for 4 d. Then their binding activity against the RCS or normal spleen cells was assessed. When tested against the stimulating RCS-LA16, the in vitro primed effector lymphocytes recognize and bind significantly to the tumor (Table II). In agreement with previous work (7), in no instance could cytolysis be detected, against either the RCS- or H-2^d-bearing cells, by these effector lymphocytes (data not shown). The binding to the tumor was highly reproducible. The percentage of primed lymphocytes which bind tumor is routinely more than two times the binding observed by nonimmune cultured lymphocytes. In addition, these same effector lymphocytes can recognize allogeneic DBA/2 specificities (Fig. 1). No demonstrable activity can be detected against C57Bl/10, CBA, or syngeneic SJL/J spleen cells. These data indi-

TABLE I
Demonstration of Alien and Syngeneic Histocompatibility Determinants Expressed on RCS-LA16 Using Alloreactive Lymphocytes

Mixed lymphocyte cultures*			Targets of effector lymphocytes‡				
Responder	Stimulator	Proliferation	RCS-LA16	DBA/2 (H2 ^s)	SJL/ (H2 ^d)	CBA (H2 ^b)	C57Bl/10b (H2 ^b)
SJL/J	DBA/2	332,440 ± 4,163	11.5 ± 1.5§	18.0 ± 1.8§	0	0	0.6 ± 3.0
CBA	DBA/2	68,004 ± 5,425	17.7 ± 2.6§	32.0 ± 1.1§	4.0 ± 2.5	0	2.7 ± 2.7
SJL/J	CBA	457,509 ± 26,209	0	0	0	16.0 ± 0.8§	0.7 ± 1.9
CBA	SJL/J	20,069 ± 2,682	21.0 ± 1.2§	0.6 ± 1.2	18.5 ± 1.3§	0.3 ± 1.2	0.3 ± 0.7

* Mixed lymphocyte cultures were incubated for 4 d with equal numbers of responding spleen cells and irradiated allogeneic spleen cell stimulators as described in Materials and Methods.

‡ All data represent percentage of binding ± SE as expressed as the percentage of immune lymphocytes minus the percentage of nonimmune lymphocytes which bound a particular target. Percentage of nonimmune binding for all SJL/J effectors was 9.4 ± 3.9% and for all CBA effectors was 10.4 ± 2.2%.

§ $P < 0.005$.

|| Not significant.

TABLE II
Recognition of the RCS-LA16 by Tumor-activated Syngeneic Lymphocytes

Mixed lymphocyte-tumor culture	Proliferation*	Binding‡
		%
Experiment 1	173,053 ± 36,030	15.3 ± 2.1§
Experiment 2	129,534 ± 12,808	18.0 ± 2.9§
Experiment 3	157,179 ± 14,961	19.5 ± 1.5

* Mixed lymphocyte-tumor cultures were prepared by co-culturing SJL/J lymph node cells with irradiated RCS-LA16, as described in Materials and Methods.

‡ The data are expressed as the percentage of binding of nonimmune lymphocytes subtracted from the percentage of binding by immune lymphocytes ± SE. Percentage of binding by nonimmune lymphocytes averaged $9.7 \pm 3.5\%$ in all three experiments.

§ $P < 0.005$.

|| $P < 0.0005$.

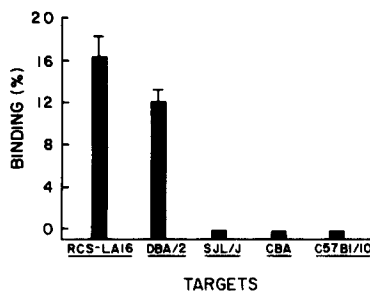


FIG. 1. Specificity of recognition by syngeneic tumor-activated lymphocytes. The percentage of nonimmune binding to all targets was $11.1 \pm 3.2\%$. All data is presented with background nonimmune binding subtracted from the percentage of binding by immune cells. The significance of binding to the RCS-LA16 and DBA/2 spleen cells was $P < 0.0005$ as determined by the Student's *t* test. These results are representative of three separate experiments.

cated that the alien H-2^d specificities present on the RCS are directly involved in syngeneic effector cell recognition of the tumor.

This alien histocompatibility-associated recognition by syngeneic anti-RCS effector lymphocytes is not confined to the *in vivo* passaged RCS-LA16. An *in vitro* cultured line, RCS-LA6b, and a primary spontaneous tumor, RCS-LA30, give equivalent results. As was the case with RCS-LA16, syngeneic lymphocytes primed against either of these tumor lines can recognize both the tumor and normal DBA/2 antigens. Again, no binding is evident against C57Bl/10, CBA, or SJL/J spleen cells (Table III).

Alien Specificities Detected by SJL/J Anti-RCS Effector Lymphocytes Mimic Hybrid Ia Determinants of the d Haplotype. Congenic recombinant mice were used to determine the regions of the H-2^d genome that encode the alien specificities recognized by SJL/J anti-RCS effector cells (see Table IV for haplotypes). B10.D2 spleen cells are significantly bound by the effector lymphocytes. No detectable activity can be demonstrated against B10.BR spleen cells (Fig. 2). These results were expected because previously H-2^d, yet not H-2^k, specificities were shown to be involved. The data also indicated that non-H2-encoded gene products do not play a role in the binding. The

TABLE III
Identification of Alien Determinants on Cultured and Spontaneous RCS

Mixed lymphocyte-tumor cultures*		Targets of effector lymphocytes‡					
Stimula- tor	Proliferation	RCS-LA6b	RCS-LA30	DBA/2 (H2 ^d)	SJL/J (H2 ^b)	CBA (H2 ^k)	C57Bl/10 (H2 ^b)
RCS-LA6b	38,128 ± 2,647	23.6 ± 1.9§	ND	13.7 ± 2.5§	0	0	0
RCS-LA30	27,423 ± 4,162	ND	10.2 ± 1.5§	12.8 ± 2.0§	0	0	0

* These cultures were set up as described in Materials and Methods.

‡ All data were expressed as percentage of binding of nonimmune cells subtracted from the percentage of binding with immune lymphocytes. Nonimmune binding averaged 12.1 ± 2.6%.

§ $P < 0.005$.

|| ND, not done.

TABLE IV
H-2 Regions of Recombinant Strains Used

Strain	K	I					D	Binding
		IA	IB	IJ	IE	IC		
C57Bl/10, C57Bl/6	b	b	b	b	b	b	b	-
DBA/2, B10.D2	d	d	d	d	d	d	d	+
CBA, B10.BR	k	k	k	k	k	k	k	-
SJL/J, B10.S	s	s	s	s	s	s	s	-
B10.OL	d	d	d	d	d	d	k	+
B10.A	k	k	k	k	k	d	d	-
D2.GD, B10.GD	d	d	b	b	b	b	b	-
(B10.A × B10.GD)F1	k/d	k/d	k/b	k/b	k/b	d/b	d/b	+
B10.S(9R)	s	s	ND*	k	k	d	d	+

* Not defined.

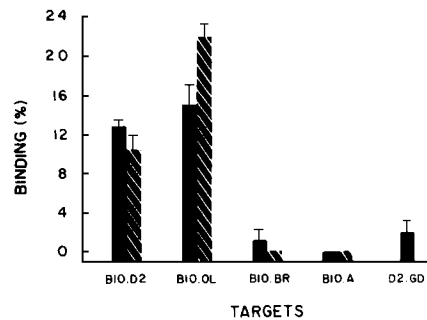


FIG. 2. Genetic mapping to the I region of alien determinants recognized by syngeneic tumor-activated lymphocytes. The percentage of nonimmune binding to all targets was $9.1 \pm 2.7\%$. All data are presented with percentage of nonimmune binding subtracted from the percentage of binding by immune cells. Two representative experiments are shown in solid histograms (experiment 1), and slashed histograms (experiment 2). In experiment 1, the significance of binding to B10.D2 targets was $P < 0.0005$ and to B10.OL targets it was $P < 0.005$. In experiment 2, the significance of binding to B10.D2 targets was $P < 0.005$ and to B10.OL targets it was $P < 0.0005$. No other targets showed significant binding.

effector cells can bind to spleen cells of the H-2^d haplotype, regardless of the non-H2 background genes of the particular strain. The data also show that antigen recognition of SJL/J anti-RCS lymphocytes does not exhibit the H-2-restricted recognition that is normally associated with syngeneic "modified self" responses. The recognitive process seems more allied to that seen by alloreactive lymphocytes.

B10.A spleen cells are not recognized by the SJL/J anti-RCS effector cells. Thus, IC^d through D^d region products are not involved in the binding process. Conversely, the effector cells do bind to B10.OL spleen cells. The molecules that are recognized, consequently, are encoded somewhere between the K^d and IE^d regions. On the other hand, D2.GD spleen cells are not recognized (Fig. 2). This finding eliminates those products encoded within K or IA as relevant. It does not, however exclude the possibility of a hybrid Ia molecule that contains an IA-encoded component. The D2.GD (or B10.GD) strain does not synthesize the complementary IE component (11). Thus, no hybrid Ia molecules are expressed.

The possibility of hybrid Ia molecules expressed on the RCS was examined. Both B10.A and B10.GD spleen cells were not recognized by the SJL/J anti-RCS effector lymphocytes. Nevertheless, the effector cells did recognize an F₁ hybrid of these two strains. (B10.A × B10.GD)_{F1} spleen cells are recognized to an extent equivalent to the B10.D2 strain (Fig. 3). These transcomplementation data indicate that the SJL/J anti-RCS effector cells recognize a hybrid Ia molecule. The F₁ animal derived an IA^d-encoded product from the B10.GD parent and an IE^k-encoded product from the B10.A parent. These two products associate on the cell membrane of the F₁ animal and cross-react in the binding assay with the IA^dIE^d hybrid found in mice of the H-2^d haplotype. These results also indicate that IA^kIE^k hybrid Ia molecules do not cross-react because B10.A, B10.BR, or CBA spleen cells are not recognized by the SJL/J anti-RCS effector lymphocytes.

Further evidence that the SJL/J anti-RCS effectors recognize a hybrid Ia molecule was obtained using a monoclonal anti-Ia.7 antibody. The Ia.7 specificity is present on the IE component of hybrid Ia molecules, regardless of their haplotype. Consequently, if the SJL/J anti-RCS effector cells recognize a hybrid Ia molecule on the tumor, this antibody should inhibit the binding. This was found to be the case (Table V). When RCS-LA16 was preincubated with anti-Ia.7, recognition of this tumor by SJL/J anti-

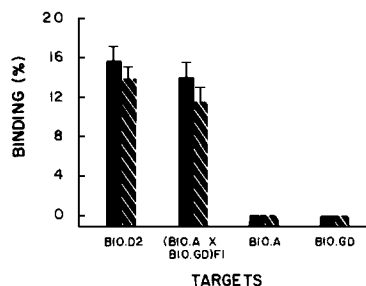


FIG. 3. Determination that hybrid Ia molecules are recognized by tumor-activated syngeneic lymphocytes. The percentage of nonimmune binding to all targets averaged $12.8 \pm 1.6\%$. In all data, the background nonimmune binding was subtracted from immune effector cell binding. Experiment 1 (solid histograms) and experiment 2 (slashed histograms) are two representative experiments. The significance of binding in both experiments to the B10.D2 and (B10.A × B10.GD)_{F1} spleen cell was $P < 0.0005$.

TABLE V
Inhibition of Lymphocyte to RCS Binding by Monoclonal Anti-Ia.7 Antibody

	Sera*	Absorbed on	Binding‡ %
Experiment 1	—	—	20.3 ± 2.7§
	Anti-Thy 1.2	—	22.7 ± 1.6§
	Normal mouse serum	—	17.0 ± 0.8§
	Anti-Ia.7	—	2.2 ± 1.5
Experiment 2	—	—	21.1 ± 1.5§
	Anti-Ia.7	—	0.6 ± 1.1
	Anti-Ia.7	CBA	16.8 ± 0.9§
	Anti-Ia.7	C57Bl/6	1.4 ± 1.4
	Anti-Ia.7	SJL/J	0
	Anti-Ia.7	RCS-LA16	18.4 ± 0.8§

* Antibody treatment and absorptions on normal lymphocytes or RCS were performed as described in Materials and Methods. Two representative experiments are shown.

‡ All data were expressed as the percentage of immune binding minus the percentage of nonimmune binding. Background nonimmune binding averaged 13.2 ± 2.8% in experiment 1 and 15.2 ± 2.3% in experiment 2.

§ $P < 0.0005$.

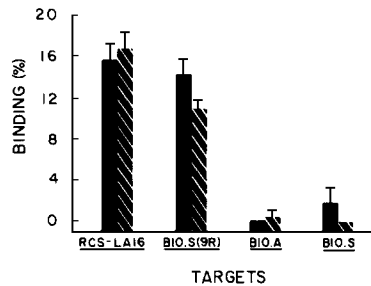


FIG. 4. Binding of SJL/J anti-RCS effector lymphocytes to B10.S(9R) spleen cells. Background percentage of nonimmune binding to all targets averaged 12.1 ± 2.5%. The percentage of nonimmune binding was subtracted from the percentage of binding by immune effector cells. Two representative experiments (solid and slashed histograms) are presented. Significance of binding over nonimmune levels was $P < 0.0005$ for the targets RCS-LA16 and B10.S(9R). No other target showed significant binding.

tumor effector cells is completely abrogated. Neither normal SJL/J serum nor a hybridoma anti-Thy 1.2 antibody is inhibitory as a control. The blocking activity is completely removed by preabsorbing the antibody on CBA spleen cells, which bear Ia.7. Conversely, C57Bl/10 spleen cells, which do not synthesize an IE product and thus do not express Ia.7, cannot remove the blocking activity. Normal SJL/J spleen cells also do not make an IE product, so they fail to absorb any blocking activity. However, the RCS-LA16 can absorb the blocking activity as well as CBA spleen cells. Thus, the RCS differs from the SJL/J host in its expression of an Ia.7-containing polypeptide that is probably encoded within IE and is the α -chain of a hybrid Ia molecule.

SJL/J cells can normally synthesize the IA-encoded product of the hybrid Ia molecule. However, the polypeptide is not expressed on the membrane because H-2^s animals do not synthesize the complementary IE-encoded component. Since the RCS

can synthesize an IE-like product, it would be expected that the IA^s component would also be expressed on the tumor. This hypothesis was tested using the congenic recombinant strain B10.S(9R). This animal expresses a hybrid Ia molecule in the form of IA^sIE^k. B10.S(9R) spleen cells are recognized by the SJL/J anti-RCS effector lymphocytes to an extent equal to the recognition of RCS-LA16 (Fig. 4). As controls, neither B10.S nor B10.A spleen cells were recognized. Thus, the alien hybrid Ia molecule expressed on the RCS that is recognized by the SJL/J anti-tumor effector lymphocytes probably consists of an IA^s-encoded β -chain in association with an IE-encoded α -chain of unknown, and probably irrelevant, haplotype.

Discussion

The results of this study reveal that RCS-primed syngeneic lymphocytes recognize the alien H-2 antigens expressed on the tumor. Furthermore, the anti-tumor immune response is directed against specificities found on hybrid Ia molecules of murine strains bearing H-2^d. In addition to H-2^d determinants, the host anti-tumor responding lymphocytes were also shown to recognize hybrid Ia molecules from mice bearing several other cross-reactive haplotypes. One such murine strain, B10.S(9R), synthesized a hybrid Ia molecule that consisted of a β -chain of the s haplotype and an α -chain of the k haplotype (Fig. 4). This last result was significant. Normal SJL/J cells synthesize the β -chain of the hybrid Ia molecule. However, it is not expressed because of their inability to synthesize a complementary α -chain (11). The data suggest that the RCS can synthesize an α -chain and consequently expresses both this α -chain and the β -chain in the form of a hybrid Ia molecule.

Since the SJL/J anti-RCS effector lymphocytes are not cytotoxic, our studies assessed antigen recognition by quantitating lymphocyte-target cell adhesions. Although it is well documented for cytotoxic T lymphocytes (14, 15), noncytotoxic T lymphocytes have rarely been shown to bind to cell membrane-associated antigens. In guinea pigs (16, 17) and mice (18), antigen-pulsed macrophages have been shown to bind to antigen-primed T lymphocytes. In both systems the binding has been associated with lymphocyte recognition of products of the I region of the major histocompatibility complex, in conjunction with antigen (18, 19). Other work has demonstrated binding of alloprimed Ly 1⁺23⁻ lymphocytes to liposomes bearing the stimulating allogeneic Ia antigens (20). These lymphocytes are presumably noncytotoxic, as has been indicated by other investigators (21, 22). T lymphocytes may not bind liposomes and intact cells identically. Consequently, lymphocyte-liposome interactions may not precisely represent interactions occurring between lymphocytes and target cells.

Recent data have demonstrated that at least two cell surface glycoproteins are encoded within the I region. Both proteins consist of two noncovalently associated polypeptides, a heavy α -chain and a light β -chain (9, 10, 13, 23, 24). One Ia molecule is a product exclusively of the IA subregion and is expressed in all murine strains. Each chain of the other Ia molecule is synthesized in different I subregions. The β -chain is synthesized within IA; however, the IE subregion encodes the α -chain. Unlike the other Ia polypeptides, this chain is not synthesized in all murine strains. Animals bearing the H-2^b or H-2^s haplotypes do not synthesize the α -chain (11). Consequently, they do not express the hybrid Ia molecule even though synthesis of the β -chain is normal.

Unlike normal SJL/J cells, the RCS does appear to synthesize an α -chain. The exact mechanism by which this chain is synthesized in the RCS is completely unknown. It may be that in normal SJL/J mice the genes encoding the α -chain are repressed. Upon oncogenesis, there is a loss of regulation which permits α -chain synthesis. Since all α -chains are nearly identical (10), it cannot be determined whether the derepressed genes encode a syngeneic H-2^s product or one that is allogeneic.

Synthesis of an α -chain by the RCS would permit expression of the IA^s-encoded β -chain in the form of a hybrid Ia molecule. This Ia molecule would be recognized as foreign by the SJL/J host and could induce an immune response analogous to one elicited in mixed lymphocyte cultures by Ia differences alone. In fact, both responses are similar in demonstrating only proliferation of Ly 1⁺23⁻ T lymphocytes, and no detectable cytotoxicity in primary cultures (5, 21, 22).

This mechanism of antigen presentation and host recognition assumes that the hybrid Ia molecules exhibit a relative extensive cross-reactivity. Peptide digest analysis of purified α -chain has revealed that there is little or no difference between polypeptides encoded from different haplotypes (10). Thus, antigenic determinants found on the α -chain would be shared by all haplotypes. Cross-reactivity between β -chains has also been found. In the context of the GL-Phe immune response, murine strains encoding β -chains of the d, s, or b haplotypes all show high responses. Conversely, animals that express a β -chain of the k haplotype or no hybrid Ia molecule are low responders (25–28). The serologically defined Ia. 22, specificity is expressed on animals encoding a β -chain of the k, b, or s haplotypes. In contrast, the d haplotype expresses the Ia.23 specificity (29, 30). Thus, it seems that the pattern of cross-reactivities observed among haplotypes is entirely dependent upon the assay system employed.

Previously, little had been known of the RCS tumor antigens to which the SJL/J host responds. Earlier work had indicated that Ia molecules of the RCS of the H-2^s haplotype (i.e., those expressing Ia.4) were implicated (8). However, interpretation of the data was difficult. It could not be determined whether the observed inhibition of the anti-RCS proliferative response by anti-Ia.4 antibody was due to blockage of antigen recognition or inhibition of the cooperation between responding cells. Thus, it is by no means conclusive that Ia molecules expressed on normal SJL/J cells are the antigenic determinants on the RCS to which the host responds. It is possible, however, that both IA-encoded β -chains contain the Ia.4 specificity. If this were the case, then it could be expected that anti-Ia.4 antisera would block the response via interaction with the β -chain component of the hybrid Ia molecule.

Recently, another approach has been used to analyze the antigens involved in the syngeneic anti-RCS response (31). In this study, the F₁ offspring of a mating between SJL/J and several other strains were assessed for their ability to exhibit an anti-RCS proliferative response. Although the role of hybrid Ia molecules was not directly mentioned in the text, there was a correlation between hybrid Ia expression in the F₁ animal and recognition of the RCS as "self" with no subsequent induction of proliferation. Conversely, in the F₁ animals that did not express hybrid Ia molecules, there was marked anti-RCS responsiveness, as would be expected, due to recognition of "foreign" Ia determinants. Consistent with our conclusion, their data strongly suggested that hybrid Ia molecules, which contained the IA^s-encoded β -chain component, were immunogenic and induced the host anti-RCS proliferative response.

A potential artifact when investigating alien tumor antigens on transplantable or

cultured lines is cross-contamination with cells allogeneic to the host. Conceivably, the RCS could be a mixture or fusion product of SJL/J sarcoma cells and allogeneic cells expressing H-2^d antigens. If one assumes that the SJL/J mice are from a pure strain, then primary tumors should be free of any contamination. The data presented evidence that primary tumors and transplantable lines gave equivalent results (see Table III). Further serological studies have shown the alien Ia.7 specificity on primary and transplantable RCS, but not on normal SJL/J lymphocytes (S. Wilbur et al., manuscript in preparation). RCS-LA16 was also analyzed biochemically for polymorphic variants of phosphoglucosyltransferase and glutathione reductase (32, 33). The results showed conclusively that RCS-LA16 was solely of SJL/J origin. There was no evidence of cross-contamination with any of the known murine strains which express H-2^d antigens.

In conclusion, our results demonstrate that the RCS expresses alien hybrid Ia molecules. Furthermore, we propose that the RCS, unlike the host, is capable of synthesizing the α -chain component of the hybrid Ia molecule. The α -chain then associates on the cell membrane with a syngeneic, but normally unexpressed, β -chain to form a molecule recognized as foreign by the host. Consequently, syngeneic lymphocytes respond to the tumor in a manner similar to that observed in mixed lymphocyte cultures against only I region differences. The hybrid Ia molecule expressed on the RCS is also sufficiently similar to the one encoded in mice expressing H-2^d, so that there is strong cross-reactivity both serologically and cellularly. This accounts for the observed alien H-2^d specificities previously reported to be expressed on the RCS cell surface (1, 2).

Summary

SJL/J (H-2^s) lymphocytes, primed in vitro against primary, cultured, and transplantable syngeneic reticulum cell sarcomas (RCS) were found to recognize and bind to the tumor without subsequent cytolysis. Additional data showed that the recognition was also directed against Ia molecules of the H-2^d, but not H-2^k, haplotype. Normal spleen cells of DBA/2, B10.D2, and B10.OL mice were bound, whereas those of CBA, B10.BR, B10.A, B10.GD, and D2.GD were not. Furthermore, the Ia molecules were in the form of a hybrid, because spleen cells from F₁ progeny of a B10.A and a B10.GD parent were recognized and bound as effectively as the RCS. Recognition was not restricted solely to the H-2^d haplotype. Spleen cells from B10.S(9R) mice were also significantly bound. This result suggested that the RCS expresses a hybrid Ia molecule containing a β -chain of the H-2^s haplotype. Recognition of this hybrid Ia molecule by the host resulted in a cross-reactive recognition of H-2^d specificities. Further analysis revealed that the RCS express on their cell surface an α -chain of the hybrid Ia molecule which is involved in host anti-tumor recognition. Preincubation of the RCS with monoclonal antibody directed against the Ia.7 specificity on the α -chain could block lymphocyte-to-tumor cell binding. The blocking activity could be removed by preabsorption of the antibody on the RCS, as well as normal Ia.7-bearing lymphocytes, but not on lymphocytes that do not express Ia.7, such as SJL/J.

The data suggest that the hybrid Ia molecules expressed on the RCS, and recognized by tumor-primed syngeneic lymphocytes, are composed of both a syngeneic and an alien chain. The component alien to the SJL/J host is the Ia.7-bearing α -chain. Normal SJL/J cells synthesize but do not express the β -chain. In the RCS, however,

alien α -chain synthesis permits expression of the syngeneic β -chain in the form of a hybrid Ia molecule.

The authors gratefully acknowledge the laboratory of Dr. R. Sparkes for the biochemical analysis of the tumors, Dr. U. Hammerling for his gift of monoclonal anti-Ia.7 antibody, and Dr. Ornella Marelli for expert assistance. We also thank Mr. John Fan, Ms. Susan Wright, Ms. Ann Kristy, and Ms. Donna Wilbur for their critique of the manuscript.

Received for publication 15 September 1980.

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