PROPERTIES OF RETICULUM CELL SARCOMAS IN SJL/J MICE

V. Nature of Reticulum Cell Sarcoma Surface Antigen Which Induces Proliferation of Normal SJL/J T Cells*

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Recent studies on transplantable reticulum cell sarcomas (RCS)[1] in syngeneic SJL/J mice have demonstrated that these lymphoma cells induce a marked degree of proliferation in peripheral T and thymus cells from normal SJL/J mice (1) without any accompanying development of cytotoxic cells in vitro (2). The nature of the antigen inducing this proliferation is unclear. No presensitization to the antigen appears needed for the cells to respond since thymus and spleen cells from neonatal mice give a definite response even before reactivity to allogeneic spleen cells is detected.[2] Nevertheless, the possibility exists that virally induced antigens on the RCS cells are involved, particularly since SJL/J mice have been suggested to harbor leukemia virus which can induce RCS in BALB/c mice (3) and in F_1 mice of the SJL/J strain (4).

Antigens known to induce high proliferative responses without an apparent need for previous sensitization are Ia antigens of the *H-2* complex and antigens specified by the *Mls* locus in mouse strains. The response of SJL/J cells to RCS develops faster in vitro than the one to alloantigens and is higher in magnitude (1, 2). In other respects, responses to RCS cells resemble the primary responses to Ia antigens or to *Mls*-locus antigens which, in the absence of other antigenic differences, are also known to be low or negative for cytotoxic cell development (5–7). The nature of the antigen-inducing syngeneic responses, such as that of normal thymus cells to spleen (8) or lymph node (9) cells is unknown, but the proliferation induced in this situation is also unaccompanied by cytotoxic cell development. In all these cases, B lymphocytes (and macrophages) bear the antigens responsible for the stimulation of T cells, which possibly brings in another similarity since primary RCS is said to arise in germinal centers (10) and ^{3}H-labeled transplantable RCS cells localize in splenic and lymph node follicles (11) and thus may be B-cell derived. It has also been suggested that RCS cells are Thy 1− and Ia+ but do not exhibit cell surface Ig or any other B-cell surface marker (2). It is not known, however,

* Supported by grant nos. CA-14462, AI-12715, and AI-12734 from the U. S. Public Health Service.

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; LN, lymph nodes; LPS, lipopolysaccharide; MLC, mixed lymphocyte cultures; MLR, mixed lymphocyte reaction; MuLV, murine leukemia virus; PHA, phytohemagglutinin; RCS, reticulum cell sarcomas.

[2] Ponzio, N. M., and G. J. Thorbecke. Properties of RCS cells in SJL/J mice. VI. Characterization of lymphoid cells which proliferate in response to RCS cells. Manuscript in preparation.

whether primary RCS cells are already able to stimulate T cells into proliferation or whether this is a property acquired after several transplantations.

In the present studies it will be demonstrated that primary RCS cells also stimulate T cells and that transplantable RCS cells bear Ia antigens on their surface. Incorporation of antisera to Ia antigens of the SJL/J strain in the medium appears to block the ability of these cells to induce a proliferative response, without affecting the ability of SJL/J T cells to respond to allogeneic cells or nonspecific stimuli.

Materials and Methods

Mice and Tumors. Female SJL/J and male BALB/c mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. ASW, B.10S, A.TH, and A.TL mice were bred in the Washington University laboratories.

The RCS tumors utilized in these studies were derived from spontaneous RCS in SJL/J mice (12). The transplantable RCS tumors (RCS-5 and RCS-19) were maintained in serial passage by transfer of 10^7 tumorous lymph node cells into syngeneic mice at regular intervals.

Antisera. Anti-Thy 1.2 was a generous gift of Dr. L. J. Old and Ms. E. A. Carswell from the Sloan-Kettering Institute for Cancer Research, New York, and was prepared by immunization of (A/Thy 1.1 × AKR/*H-2*^{b})F_1 mice with the A strain leukemia ASL-1 (13). Rabbit anti-mouse Ig was prepared by immunization with 0.5 mg of Fab fragments of mouse Ig and complete Freund's adjuvant as previously described (14). Five anti-Ia sera and one anti-H-2.19 serum were employed in these studies. The specific strain combinations for antiserum production are listed in the respective tables. All six antisera were produced by the immunization procedure described previously by David et al. (15).

Cultures. Lymphocyte cultures were prepared in flat-bottomed Linbro tissue culture plates (IS-FB-96-TC; Linbro Scientific Co., New Haven, Conn.) in RPMI-1640 (Associated Biomedic Systems, Inc., Buffalo, N. Y.) with 10% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.) and 5×10^{-5} M 2-mercaptoethanol (Eastman Kodak Co., Rochester, N. Y.). Cells used for stimulation of a proliferative response were either irradiated (RCS cells, 6000 R; allogeneic lymphocytes, 1,500 R) from a ^{137}cesium source (Radiation International Inc., Parsippany, N. J.) or treated with 25 μg/ml mitomycin C (Drug Development Branch, National Cancer Institute, Bethesda, Md.) for 30 min at 37°C and washed three times before culture. Optimal stimulation was obtained using 2×10^5 responder cells and either 5×10^4 irradiated RCS tumor cells or 2×10^5 irradiated allogeneic lymphoid cells per well. In some experiments, nonspecific stimuli were utilized as a measure of lymphocyte reactivity. These included incubation with concanavalin A (5 μg/ml: Miles-Yeda, Ltd., Rehovot, Israel), phytohemagglutinin (PHA-P, 5 μg/ml; Difco Laboratories, Detroit, Mich.), and lipopolysaccharide (20 μg/ml: Difco Laboratories) for 72 h. One μCi ^{3}H-thymidine (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.; sp act 0.36 Ci/mmol) was added to each well 24 h before collection of cultures on glass fiber filters (Reeve Angel, Clifton, N. J.) with an automated cell harvester. Filters from five replicate cultures were counted in a Packard Tri-Carb Scintillation Counter (Packard Instrument Co., Inc., Downers Grove, Ill.) using Scintiverse liquid scintillation cocktail (Fisher Scientific Co., Fairlawn, N. J.).

Ficoll-Isopaque Gradients. Continuous Ficoll-Isopaque (Ficoll 400; Pharmacia Fine Chemicals Ltd., Uppsala, Sweden and Isopaque-Sodium Metrozoate; Gallard-Schlesinger Chemical Mfg. Corp., Carl Place, N. Y.) density gradients were prepared according to Loos and Roos (16). RCS cells derived from lymph nodes (LN) of tumorous SJL/J mice were layered on top of the gradient and tubes were spun for 20 min at 2,400 rpm at 4°C. 1-ml fractions were collected by inserting a long needle to the bottom of the gradient tube and pumping the contents through an automatic fraction collector (LKB Instruments, Inc., Rockville, Md.).

The optical density of each fraction was determined and found to be linear. Cell counts in each fraction were performed using a Coulter Counter Model B (Coulter Electronics Inc., Hialeah, Fla.), and a graph was prepared plotting cell number versus fraction number, which showed several peaks at various densities. Discontinuous gradients were then prepared corresponding to

the peak densities obtained from the graph, and RCS cells separated on such gradients were utilized in the studies reported here.

Antibody-Mediated Cytotoxicity. Antisera directed against various membrane antigens were tested for their ability to lyse target cells in the presence of complement (C) by a modification of the microcytotoxicity test developed by Mittal et al. (17). 2 μl of the antisera to be tested was serially diluted in wells of "Terasaki" microtest plates (no. 3034 Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) 2 μl of target cells were added to each well and the plates were incubated at 37°C for 20 min. For one-stage testing, C′ was added directly to wells, while in the two-stage test plates were "flicked," to remove unbound antisera, C was added to each well, and incubation continued for an additional 30 min at 37°C. In both types of testing, after incubation with C, plates were flicked, trypan blue added, and percent cytotoxicity determined by microscopic examination for uptake of dye into damaged cells.

Appropriate controls, i.e. cells incubated with antibody or C alone, showed levels of cytotoxicity <10%. In all cases the C was absorbed twice with agarose in the presence of EDTA to remove nonspecific cytotoxicity before use (18, 19).

In treating lymphoid cells and tumor cells with various antisera before culture, similar methods were employed in 17 × 100 mm tubes to accommodate larger numbers of cells. Killed cells were removed on a simple Ficoll-Hypaque gradient (densities 1.0750–1.0782) before use of the cells as stimulators or responders in cultures.

Results

Proliferative Response of SJL/J LN Cells to Primary and Transplantable RCS. A few primary RCS which spontaneously developed in LN of 8- to 12-mo-old SJL/J mice were tested for their ability to induce proliferation in normal syngeneic lymphoid cells. The results in Table I show a definite proliferative response to 6,000 R γ-irradiated RCS cells of primary tumor origin when the tumor cell to normal LN cell ratio in the cultures was 1:1 or 1:2 in both experiments. Far fewer stimulating RCS cells were needed when the transplantable RCS-5 tumor line was used, which actually stimulated better at ratios of 1:4 than at 1:1 and indeed even with ratios as low as 1:8 and 1:10 excellent stimulation was obtained.

Stimulation of normal SJL/J cells by RCS was not obtained when the tumor cell types were separated by a Millipore or dialysis membrane such as in Marbrook chambers. This indicates that stimulation is not due to a soluble mediator secreted by or liberated from tumor cells, as was also shown previously by the lack of stimulation with tumor cell supernates (1). It was concluded that cell contact appeared necessary for stimulation to occur.

To characterize further the stimulating cell population in RCS, with respect to cell density, Ficoll-Isopaque separation of the tumor cells was performed before culture. In preliminary experiments with continuous density gradients, it was noted that tumor cells peaked at densities of 1.0660 and 1.0694. Discontinuous gradients were then prepared to obtain fractions at these as well as higher densities and the proliferative response of normal LN cells to each fraction was determined (Table II).

Although stimulation was obtained even with the highest density fraction, the lowest density cells were clearly the most effective stimulators in both experiments. ^{3}H-thymidine incorporation in unirradiated cells from each fraction was also studied and found highest in the lowest density fraction over the first 24 h in culture (data not shown). When aliquots from each fraction (2×10^6) were injected intravenously into normal SJL/J mice, each caused approximately

TABLE I

Comparison of Response of Normal SJL/J LN Cells to γ-Irradiated Lymphoma Cells from Spontaneous (Primary) Syngeneic RCS and from Serially Transplanted RCS-5

Stimulator cells	Ratio* of RCS/LN	Δ cpm (SI)‡	
		Exp. 1	Exp. 2
Spontaneous RCS	2:1	–	20,261 (49.0)
	1:1	2,312 (36.6)	12,074 (101.6)
	1:2	649 (7.8)	5,232 (79.1)
	1:4	155 (4.3)	2,461 (39.4)
	1:8	44 (1.8)	1,015 (16.9)
Transplanted RCS-5	1:1	748 (1.3)	12,862 (13.3)
	1:2	4,986 (3.4)	13,812 (15.2)
	1:4	7,704 (5.5)	14,456 (16.8)
	1:8	–	12,857 (15.3)
	1:10	10,190 (8.5)	–

* RCS/LN ratio based on a constant number of responder SJL/J LN cells (2×10^5) and various numbers of RCS tumor cells cultured for 96 h in 0.2 ml in microplates. ^{3}H-thymidine (1 μCi) was added during the last 24 h of culture.

‡ Δ cpm, cpm in mixed cultures minus background cpm of responder LN cells and RCS stimulator cells cultured separately. SI, stimulation index, given in parentheses, calculated by dividing the cpm in mixed cultures by the addition of cpm in responder and stimulator cells cultured separately.

TABLE II

Response of SJL/J LN Cells to γ-Irradiated Syngeneic Ficoll-Isopaque Gradient Separated RCS-5 Cells

Fraction of γ-RCS	Density	Δ cpm (SI)*	
		Exp. 1	Exp. 2
Unfractionated	–	7,073 (17.6)	7,704 (5.5)
Fraction A	1.0660	9,275 (22.6)	8,868 (6.7)
B	1.0694	7,333 (14.8)	12,104 (8.1)
C	1.0750	5,949 (14.6)	6,997 (5.3)
D	1.0782	5,263 (14.6)	7,838 (5.7)
E	1.0820	3,207 (9.8)	5,419 (4.5)

* Δ cpm, cpm in mixed cell cultures minus background cpm of responder LN cells and RCS stimulator cells cultured separately. SI, stimulation index, given in parentheses, calculated by dividing the cpm in mixed cultures by the addition of cpm in responder and stimulator cells cultured separately.

the same degree of tumor growth as judged by enlargement of LN and spleen 7 days later.

Stimulation of Normal LN Cells from Other H-2^s Mouse Strains by RCS Cells of SJL/J Origin. It is known that strong proliferation may result from the mixing in culture of lymphoid cells from two mouse strains differing at the *Mls* locus (20). Mice of the B10 background (B10.S) have a nonstimulatory ("silent") *Mls*-locus antigen differing from the one in SJL/J and A.SW mice (21). Since mixed lymphocyte cultures (MLC) between different *H-2^s* strains have not been

TABLE III

Proliferative Response of LN Cells from Different H-2^s Strains to RCS of SJL/J Origin

Strain (responder LN cells)	Δ cpm ± SE (*n*) in cultures* stimulated by:				% SJL/J response to RCS
	SJL/J	RCS	BALB/c	C57BL/6‡	
SJL/J	–	13,244 ± 1,071 (24)	2,923 ± 1,508 (4)	–	100
ASW	276 (2)	5,355 (2)	3,770 (2)	–	40
B10.S	196 ± 107 (3)	2,516 ± 774 (4)	4,590 ± 780 (3)	–	19
A.TH	454 ± 1,772 (3)	6,197 ± 1,841 (4)	2,488 (1)	9,184 ± 4,407 (3)	47
A.TL	6,116 ± 3,207 (3)	666 ± 4,950 (4)	–	8,983 ± 7,572 (3)	5

* Microplate cultures consisted of 2 × 10^5 responder LN cells together with 5 × 10^4 RCS cells (6,000 R), or 2 × 10^5 SJL/J or BALB/c spleen cells (1,500 R) in 0.2 ml medium for 96 h, the last 24 of which were in the presence of 1 μCi ^{3}H-thymidine. Δ cpm, cpm in mixed cultures minus background cpm of responder LN cells and stimulator RCS or spleen cells cultured separately. Background cpm of responder cells varied from approximately 300–1,600.

‡ Responses to C57BL/6 spleen cells were probably somewhat higher than to other allogeneic cells because mitomycin-treated rather than γ-irradiated stimulator cells were used.

reported, it was of interest to study the three major *H-2^s* strains, A.SW, B10.S, and SJL/J in this regard. Results in Table III demonstrate very little if any stimulation of A.SW or B10.S LN cells by SJL/J spleen cells, even at ratios of 1:1; neither did A.SW or B10.S spleen cells stimulate SJL/J LN cells (data not shown). This did not suggest any great proliferation to *Mls*-locus antigens within the *H-2^s* strains and this topic was therefore not pursued further.

Although in both of the other *H-2^s* strains, RCS cells induced proliferation at ratios of 1:4 (RCS cells to normal LN cells), the response in SJL/J cells to RCS was invariably higher. This suggested an influence of the genetic background on the responsiveness of the LN cells to RCS, particularly in the B10.S mice. In further studies, LN cells from the recombinant strains A.TH and A.TL were also tested for responsiveness to RCS-5. It was found that A.TH cells responded approximately equally well as SJL/J cells, whereas A.TL showed lower responses to RCS even than to normal SJL/J spleen cells (Table III). It was also shown that RCS cells induced good responses in BALB/c LN cells, suggesting the presence of allogeneic mixed lymphocyte reaction (MLR)-inducing antigens on the lymphoma cell surface (data not shown).

Study of Surface Antigens of RCS Cells. In view of the fact that RCS cells induced good MLR in BALB/c cells, it was of interest to determine whether RCS cells expressed Ia antigens on their surface. Both the single-stage and two-stage cytotoxicity tests were performed on gradient-separated, low density RCS cells with serially diluted anti-Ia sera of various specificities and rabbit C. The antisera containing anti-Ia4 with or without anti-Ia5 and/or anti-Tla reactivity all caused approximately 60% lysis of RCS-5 (as well as RCS-19) cells with maximal effectiveness up to dilutions of 1:640. Under similar conditions, only about 32% of normal SJL/J LN cells were killed, while anti-Thy 1.2 killed an average of 18% RCS cells and 67% normal LN cells (Table IV). Rabbit anti-mouse Fab killed 11% RCS cells and an average of 36% normal LN cells.

Since many murine tumors show viral antigens on their surface and since it has been demonstrated recently that many anti-Ia sera contain antibody to leukemia virus antigens (22, 23), it seemed important to determine whether RCS-5 cells expressed these surface antigens. It was found that neither goat anti-murine leukemia virus (MuLV) gp69/70 nor rabbit anti-MuLV gs30 had any cytotoxic effect on RCS cells even in dilutions of <1:20 (Table IV). Rat anti-

TABLE IV
Presence of Ia Antigens on the Surface of RCS Cells of SJL/J Mice

Antisera* + C	Specificities detected	% Cytotoxicity‡ with: RCS cells	% Cytotoxicity‡ with: Normal SJL/J LN cells
Anti-Ia serum			
(A.TL × B10.M)F_1 anti-A.TH	Ia.4; Tla	61	37
A.TL anti-A.TH	Ia.4, 5; Tla	61	32
(A.TL × 5R)F_1 anti-A.TH	Ia. 4, 5	60	37
(A.TL × A.TFR3)F_1 anti-A.TH	Ia.4	66	55
(A.AL × B.10)F_1 anti-A.TL	H-2.19	69	71
(A.TH × B.10M)F_1 anti-A.TL	Ia.1, 2	20	
Anti-Thy 1.2	θC3H	18	67
Rabbit anti-mouse Ig	Fab	11	36
Goat anti-MULV	gp 69/70	10	11
Rabbit anti-MuLV	gs 30	9	11
Rat anti-MuLV	NTD Gross virus	14	10
Rabbit anti-xenotrophic virus	Anti-NZB virus	20	
Rabbit anti-xenotrophic virus	Anti-BALB/c virus	15	

* All normal sera, including SJL/J, A.TL, rabbit, and rat gave <11% cytotoxicity.
‡ Values given represent the mean of 3–10 separate determinations. Dilutions of anti-Ia and anti-Thy 1.2 sera for which results are given are 1:320 or 1:640. All negative sera were tested as low as 1:20 or 1:40 as well as at higher dilutions; values given for negative sera are with lowest dilution tested.

MuLV also failed to show significant cytotoxicity. At a dilution of 1:40, it killed 14%, and at 1:160 <10% of RCS. This relatively high background was, however, not due to the presence of MuLV on tumor cells since absorption of known rat anti-MuLV sera with RCS-5 cells failed to remove any antibody to MuLV proteins, whereas absorption with normal SJL/J thymus cells did (E. Stockert and N. M. Ponzio, unpublished observations).

Another group of viruses which is relatively ubiquitous in different mouse strains such as NZB and BALB/c consists of the so-called xenotropic viruses. Recently, Morse et al. found xenotropic viral protein on the surface of a small percentage of SJL/J spleen cells (H. C. Morse, III, T. Chused, B. Mathieson, T. Sharp, M. Boehm-Truipp, and J. Hartley, unpublished results). For this reason, cytotoxicity studies with antisera to xenotropic viruses were also performed. They failed to show significant cytotoxicity above background at dilutions as low as 1:20 (Table IV).

Pretreatment of RCS cells with anti-Ia sera and C effectively reduced their ability to induce a proliferative response in syngeneic LN cells. As shown in Table V, cells remaining after removal of killed cells induced only 23–51% of the original response, while cells surviving treatment with either anti-Thy 1.2 or with anti-Ig plus C retained full capacity to induce stimulation. In contrast, pretreatment of responding LN cells with anti-Ia antisera plus C had no effect on their ability to respond, whereas anti-Thy 1.2 plus C abolished their responsiveness (Table VI). Incubation of RCS cells or SJL/J LN cells with anti-Ia sera

TABLE V
Remaining Stimulatory Capacity to SJL/J LN Cells of RCS Cells Pretreated with Various Antisera and C

Pretreatment of stimulator X-RCS-5*	Δ cm‡		% of control response
	Exp. 1	Exp. 2	
Untreated	11,372	12,766	
Anti-Ia.4 (Tla) + RC′		2,516	23.0
Anti-Ia.4, 5 (Tla) + RC′		3,532	32.3
Anti-Ia.4 + RC′		5,562	50.9
Anti-Thy 1.2 + RC′		11,925	109.2
NMS + RC′		10,919	100.0
RC′		11,393	
Anti-Ig + GPC′	6,397		82.1
NRS + GPC′	7,790		100.0

* 2×10^5 LN cells plus 10^5 stimulator cells per well. Stimulator cells (6.6×10^6/ml) were incubated in the presence of anti-Ia (1:100), anti-Thy 1.2 (1:60), anti-Ig (1:30), normal SJL (NMS; 1:100), or normal rabbit (NRS; 1:30) sera and either rabbit C (RC′) (1:18) or guinea pig C (GPC′) (1:15). Anti-Ia.4 (Tla) = (A.TL × B10.M)F_1 anti-A.TH; anti-Ia.4, 5 (Tla) = A.TL anti-A.TH; anti-Ia.4 = (A.TL = A.TFR3)F_1 anti-A.TH.

‡ Δ cpm = (cpm of mixed cell culture) − (cpm of responder cells alone + cpm of stimulator cells alone).

TABLE VI
*Pretreatment of Responder SJL/J LN Cells with Anti-Ia Sera and Rabbit C Does not Alter Their Proliferative Response to RCS**

Pretreatment of responder SJL/J LN cells‡ (2×10^5)	Δ cpm§		% of control response
	Exp. 1	Exp. 2	
Normal mouse sera + RC	11,073	7,866	100
Anti-Ia.4 (Tla) + RC	13,586		123
Anti-Ia.4, 5 (Tla) + RC	12,433		112
Anti-Ia. 4 + RC	10,771		97
Anti-Thy 1.2	–	588	8

* 2×10^5 responder SJL/J LN cells were cultured with 5×10^4 γ-irradiated (6,000 R) RCS cells for 96 h, the last 24 of which were in the presence of 1 μCi ^{3}H-thymidine.

‡ Cells (6.6×10^6/ml) were incubated at 37°C for 45 min in the presence of anti-Ia (1:100) or normal SJL (NMS; 1:100) sera and rabbit C (RC) (1:18). Specific antisera as in Table V.

§ Δ cpm as in Table V.

in the absence of C before culture did not diminish their subsequent ability to stimulate or to respond, respectively (data not shown).

Inclusion of anti-Ia sera without C in the culture media inhibits unidirectional MLC (24, 25). In these experiments, it was demonstrated that the inhibition is due to specific reactivity of anti-Ia sera with the stimulator and not with the responder cell. Data presented in Table VII indicate a similar inhibition of the proliferative response of SJL/J LN cells to RCS tumor cells when anti-Ia serum is included in the media. Addition of 1% anti-Ia sera gave from 42 to 72%

TABLE VII

Inhibition by Various Anti-Ia Sera of the Proliferative Response of SJL/J LN Cells to RCS*

Exp. no.	Stimulus	% Mouse serum added	cpm in control culture with NMS (100%)‡	% of control* response in presence of antisera§					
				A.TL × B10.M anti-A.TH	A.TL anti-A.TH	A.TL × $A.TFR_3$ anti-A.TH	A.TL × 5R anti-A.TH	A.AL × B10 anti-A.TL	A.TH × B10 anti-A.TL
1	X-RCS	1.0	23,855	28	46	58			
	X-RCS	2.5	19,312	4	29	44			
	X-RCS	5.0	17,296	0	5	15			
2	X-RCS	3.0	7,177	14	60				
	X-BALB/c Spleen	3.0	6,548	71	70				
	Con A	3.0	14,400	52	55				
	PHA	3.0	1,538	120	123				
3	X-RCS	3.0	11,959		40	52	43	109	114
	X-BALB/c Spleen	3.0	6,653		94	56	112	125	115
	Con A	3.0	6,003		291		176		
	PHA	3.0	4,030		151		135		
	LPS	3.0	4,025		119		49		

* 2×10^5 SJL/J LN cells were cultured in microplates with Con A, PHA, LPS, 5×10^4 RCS cells (6,000 R), or 2×10^5 BALB/c spleen cells (1,500 R) in the presence of the indicated percentage of anti-Ia serum in 0.2 ml RPMI 1640 with 10% fetal calf serum for 72 or 96 h (RCS and BALB/c spleen cells; exp. 1 and 2). 1 μCi of ^{3}H-thymidine was added for the last 24 h of culture.

‡ (A.TL × B10.M)F_1 anti-A.TH – specificity, anti-Ia.4 anti-Tla. A.TL anti-A.TH – specificity, anti-Ia, 4, 5 anti-Tla. (A.TL × $A.TFR_3$)F_1 anti-A.TH – specificity, anti-Ia.4. (A.TL × 5R)F_1 anti-A.TH – specificity, anti-Ia.4. (A.AL × B10)F_1 anti-A.TL – specificity, anti-H-2.19. (A.TH × B10)F_1 anti-A.TL – specificity, anti-Ia.1, 2.

§ cpm of stimulated cultures containing 1% NMS were equal to those without NMS (exp. 1). Background cpm were reduced from 982 to 150, 63, and 47 by 1.0, 2.5, and 5.0% NMS, respectively (exp. 1), from 72 to 5 with all anti-Ia sera at 72 h and from 259 to 20 at 96 h (exp. 2), and from 1,178 to 200 with control and anti-Ia sera at 96 h (exp. 3).

inhibition with three different antisera and almost total inhibition at 5%, when compared to cultures containing similar percentages of normal mouse serum (exp. 1). As with allogeneic MLC, the block appeared to occur on the RCS stimulator cell since the response to nonspecific T-cell stimulators such as PHA remained unaffected. The partial inhibition of concanavalin A (ConA) responses (exp. 2) and of lipopolysaccharide (LPS) responses (exp. 3) are in keeping with reports of others (26, 27). Inhibition of LPS responses have also been noted with 5% normal mouse sera (28) but at the 3% concentration studied here such an inhibition was not seen with normal control sera.

It would appear that inhibition of the response to RCS by anti-Ia^s is specific for that response since the one to allogeneic BALB/c spleen cells showed much less of a reduction in both exps. 2 and 3. In exp. 3, additional control sera were included, such as normal A.TL, which showed no inhibition of any responses, and antiserum to *H-2K*^{s}, as well as antiserum to Ia^k. Neither of these latter antisera inhibited the response to RCS cells or to BALB/c cells (Table VII).

Discussion

Stimulation of normal SJL/J T cells is apparently induced by all syngeneic RCS cells whether derived from primary, as shown here, or from transplanted (1) tumors or even from in vitro cell lines developed from RCS (29). The most actively stimulating cells appeared to be those of low density, but all density

fractions contained stimulating cells; evidence for a soluble stimulating factor produced or liberated from these tumor cells was not seen.

The height of the response for LN cells was several-fold that of allogeneic cells (Table III), suggesting the notion of presensitization of the SJL/J T cells to some antigen on the tumor cells. However, the fact that adult thymus cells (1) and even thymus cells of neonatal SJL/J mice already give proliferative responses to RCS[2] does not support this idea. The response is also much higher than that of thymus or spleen to syngeneic normal LN cells in the SJL/J strain (1). However, responses to LPS-induced normal SJL/J blast cells are seen with syngeneic LN cells,[2] and a similarity between this type of proliferation and that to RCS cells warrants consideration. Another strikingly similar phenomenon is found in the responsiveness of human peripheral T cells to lymphoid tissue culture cell lines (9), mitogen-induced blast cells (30), and even normal B cells (31, 32) from autologous individuals.

The possibility that an ecotropic or xenotropic virus on RCS cells induced the stimulation of T cells was investigated, but no positive results were obtained. Although it is known that a wide variety of inbred strains naturally produce antibody against endogenous ecotropic leukemia virus (MuLV) (33, 34), there is no evidence that MuLV was present on RCS cells since such tumor cells did not absorb activity from anti-G_{IX} sera while normal SJL thymus cells did (E. Stockert and N. M. Ponzio, unpublished observations). While xenotropic virus is found on SJL/J spleen cells (H. C. Morse, III, T. Chused, B. Mathieson, T. Sharp, M. Boehm-Truipp, and J. Hartley, unpublished results), preliminary results do not indicate its presence on RCS cells since these were not killed by rabbit antiserum to xenotropic virus plus C (F. Weinbaum, H. C. Morse, and N. M. Ponzio, unpublished observations) and there was no blocking in vitro of the proliferative response to RCS in the presence of antisera to xenotropic or ecotropic virus.

The ability of Ia antigens to induce MLR (24, 35) and their importance in the presentation of other antigens to helper T cells, particularly in the proliferative response (36–38), prompted us to look for the presence of Ia antigens on RCS cells. Evidence that these lymphoma cells do exhibit Ia surface antigens was readily obtained. In the first place, RCS cells were found to induce good MLR in cells from allogeneic (BALB/c) mice. Secondly, RCS cells were killed to approximately 60% by anti-Ia plus C and the stimulating cells were particularly sensitive to this cytotoxic effect since removal of anti-Ia killed cells greatly reduced the ability of RCS to induce proliferation. Finally, the ability of the anti-Ia4 antisera to block, consistently, in vitro responsiveness to RCS, while leaving other T-cell responses intact, provided strongly suggestive evidence that the major determinant inducing T-cell proliferation to RCS is either the Ia antigen itself or something else closely associated with Ia. Modification of Ia surface antigen on RCS cells could, for instance, be the result of a virus infection of unknown nature, or of continued proliferation of the tumor cells which might also lead to changes in the surface Ia expression. There might be something peculiar about the way Ia antigen is exposed on the surface of these cells that happens to be particularly stimulating to T cells.

The other possibility is that Ia antigen on RCS cells is extremely effective in

stimulating autologous T cells because the response may represent an exaggerated form of a normal autostimulation process. Kuntz et al. (32) have shown that a subpopulation of nylon wool adherent, Fc-positive cells stimulates nylon wool nonadherent autologous T cells of human peripheral blood. The present findings may reflect a similar phenomenon in the mouse in the sense that RCS cells might represent a tumor of such a stimulating B-cell subpopulation. An additional argument in favor of these considerations is that both the stimulation of neonatal thymus by syngeneic adult spleen cells (39) and of human T by autochthonous "non-T" peripheral blood cells (M. E. Weksler, personal communication) are inhibited by antibodies to Ia antigens.

Since macrophage populations also express Ia antigens (40), it was possible that the anti-Ia sera utilized in these experiments were blocking responses through reactivity with Ia determinants on macrophages presenting a stimulating moiety. However, in separate experiments,[2] in which macrophages were depleted by removal of nylon wool adherent cells from both the responder LN population and from the RCS stimulator cell suspension, no diminution of the proliferative response was observed. These data indicate that the proliferation induced by RCS in syngeneic T cells is independent of the presence of macrophages, and further support the contention that anti-Ia sera blocks at the RCS stimulator cell level.

The distribution of T-cell responsiveness to RCS cells over the different *H-2*^{s} strains was also of interest. The fact that SJL/J, A.SW, and B10.S, which all have a complete *H-2*^{s} but differ in background genotype, all responded to RCS although to varying degrees may, of course, relate to the presence of some unknown virus in these strains. However, it does indicate a peculiar reactivity to the RCS-Ia antigen in all *H-2*^{s} strains, since responses to normal SJL/J cells were virtually absent. A.TL and A.TH differ only in the middle portion of the *H-2* region, including the *I, S,* and *G* regions. While A.TL cells failed to respond to RCS, A.TH cells were almost as reactive as SJL/J cells themselves. This indicates that identity between stimulator and responding cells in the *K* region of *H-2* is not sufficient, but that identity in both *K* and *I* regions confers responsiveness to the T cells. The response may thus be a unique function of the *I* region alone or of the *K* and *I* region combined.

These findings support an interesting parallel with that of Zinkernagel and Doherty (41), Gardner *et al.* (42), and Shearer (43) who demonstrate a requirement for identity in the *H-2 K* or *D* regions in conjunctions with exogenous antigenic determinants for the recognition of target cells by cytotoxic effector T cells. It is now apparent that there may be two distinct lineages of T-cell recognition: one stimulated by the *H-2K*- and *H-2D*-associated antigens [Ly 1.2, 2 and 2,3 (44)] and the other stimulated to proliferate in response to Ia antigen-associated products [Ly 1.2, 1 (45)]. In addition, recent evidence has been reported (46) which suggests that the phenotype of cells cytotoxic for syngeneic tumor cells is Ly 1+ and Ly 2,3+. In the case of RCS stimulation of syngeneic T cells, some sort of tumor-associated antigen may be recognized in combination with the Ia cell surface component, but further studies are needed to identify the responsive T-cell subpopulation. The requirement for identity in the *I* region between stimulator and responder cells is also reminiscent of the restrictions

placed by *Ir* gene products on T-B cell interaction in the immune response (47), particularly since RCS lines may represent early B-cell tumors. Previous studies on localization of radiolabeled RCS cells in SJL/J mice (11) have shown a tendency of these cells to localize in follicles of spleen and LN, suggesting a B-cell nature of this tumor, but to date all other B-cell markers, such as surface Ig and Fc receptors, have been found to be absent from RCS cells.

It should be noted that anti-Ia4 is the only major cytotoxic antibody present in some of the antisera which killed a large percentage of the RCS cells. This antigen is known to be present predominantly on B cells, but also on macrophages (40, 48) and on a subpopulation of T cells (27). These antisera could also contain antibody to the recently described *I-J* region antigens expressed on suppressor T cells (49, 50). Further studies will be carried out using specific anti-I-J^s sera to determine whether RCS cells or the responding T cells bear these antigens.

Summary

The results of studies on the reticulum cell sarcoma (RCS) tumors of SJL/J mice presented here, indicate that spontaneous tumors, which arise in older mice, also possess the capacity to induce the vigorous proliferative response in syngeneic T lymphocytes that are characteristic of the transplantable RCS lines. Analysis of cell surface antigens revealed the presence of Ia determinants on gradient-purified transplantable RCS tumor cells; however, these cells did not express Thy 1.2, Ig, or any of the viral proteins that were tested for by specific antisera. Pretreatment of RCS cells with anti-Ia sera and complement-deleted cells that were stimulatory for syngeneic T lymphocytes, and addition of anti-Ia sera directly to cultures blocked the proliferative response at the stimulator (RCS) cell level.

Lymph node cells from *H-2^s* strains other than SJL/J, including A.SW and B10.S also gave proliferative responses to RCS cells, although lower in magnitude. A requirement on the part of responding cells for identity with RCS cells at the *Ir* region was indicated by the finding that A.TH but not A.TL lymph node cells responded to RCS. It is concluded that RCS cells stimulate *Ir*-region identical T cells (without evidence of presensitization) through a modification in the expression of Ia antigens on the surface of the tumor cells.

The advice of Dr. D. Roos of the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, has contributed much to our ability to prepare linear Ficoll gradients. We are very grateful to him for his help. In addition, we are greatly indebted to Dr. E. Stockert of the Sloan Kettering Institute for Cancer Research, New York, for generous gifts of antiserum (W/Fu × BN)F_1 anti-W/Fu (anti-G_{IX}) and for determining whether RCS cells absorbed anti-G_{IX} activity from such antisera (51). The valuable advice combined with gifts of antisera and of xenotropic virus-producing cultured rabbit cornea cells provided by Doctors H. Morse and F. Weinbaum of the National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Md. is also deeply appreciated. Their generosity in allowing us the liberty of quoting their unpublished data has been of help in the preparation of this manuscript. We are also grateful to Dr. R. A. Lerner of the Scripps Clinic and Research Foundation, La Jolla, Calif. to Dr. E. Fleissner of the Sloan Kettering Institute for Cancer Research, and to Dr. B. Del Villano of the Cleveland Clinic Research Foundation, who generously provided goat anti-MuLV gp 69/70 and rabbit anti-MuLV gs30. We are indebted to Dr. S. Karpatkin, Department of Medicine, New York University School of Medicine, for the use of his Coulter counter. Excellent technical assistance was provided by Ms. Judy Chapman.

Received for publication 28 December 1976.

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