GROWTH OF SJL/J-DERIVED TRANSPLANTABLE RETICULUM CELL SARCOMA AS RELATED TO ITS ABILITY TO INDUCE T-CELL PROLIFERATION IN THE HOST

I. Dominant Negative Genetic Influences of Other Parent Haplotype in F_1 Hybrids of SJL/J Mice*

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In previous studies on the transplantable reticulum cell sarcoma (RCS)[1] of SJL/J mice, it was shown that normal T lymphocytes from these mice gave high proliferative responses to syngeneic γ-irradiated RCS cells (1). Incorporation of antisera to Ia antigens of the SJL/J strain in the medium blocked the ability of the RCS cells to induce a proliferative response (2). The responding cells were found to belong to the Lyt-1 subpopulation of T cells from lymph nodes, spleen, and thymus of normal syngeneic SJL/J mice (3).

Lymph node (LN) cells from recombinant strains A.TH and A.TL were also tested for responses to RCS, and it was noted that A.TL LN cells responded much less than A.TH, whereas A.SW and BIO.S gave good responses (2). This suggested a requirement for a similar I region in responder and stimulator cells. It was further observed that the F_1 hybrids of SJL × A.SW and SJL × C57BL/6 responded with proliferation (1, 4), whereas F_1 hybrids of SJL × BALB/c did not, which perhaps suggests a negative influence from the BALB/c parent (4). Therefore, to evaluate in detail the genetic requirements in responding cells, an extended series of F_1 hybrids of SJL/J mice with a variety of other strains, differing in H-2 subregions and on various backgrounds, were examined for the ability to give a proliferative response in vitro.

It was also noted previously that the readily transplantable RCS tumor failed to grow in syngeneic hosts, if the latter had been irradiated before tumor cell injection (5). Because normal LN cells were shown to enhance growth of subsequently injected tumor cells in irradiated mice, it was suggested that proliferation of normal T cells was needed for growth (5). In these studies, therefore, the ability of F_1 hybrids of SJL/

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; LN, lymph node(s); NK, natural killer; PEG, polyethylene glycol; PHA, phytohemagglutinin; RCS, reticulum cell sarcoma(s).

TABLE I

H-2 Haplotype Subregions of Parental Strains Used to Make F_1 Hybrids with SJL

Strain	K	IA	IB	IJ	IE	IC	S	G	D
SJL/J	s	s	s	s	s	s	s	s	s
A.SW	s	s	s	s	s	s	s	s	s
A.TH	s	s	s	s	s	s	s	s	d
A.TL	s	k	k	k	k	k	k	k	d
A.AL	k	k	k	k	k	k	k	k	d
A/J	k	k	k	k	k	d	d	d	d
A.CA	f	f	f	f	f	f	f	f	f
ATFR.4	f	f	?	?	?	?	s	s	d
ATFR.5	f	f	f	f	k	k	k	k	d
BI0.S	s	s	s	s	s	s	s	s	s
8R	k	k	?	?	s	s	s	s	s
7R	s	s	s	s	s	s	s	s	d
BI0.HTT	s	s	s	s	k	k	k	k	d
9R	s	s	?	k	k	d	d	d	d
BI0.A	k	k	k	k	k	d	d	d	d
BI0.A(5R)	b	b	b	k	k	d	d	d	d
BI0.HTG	d	d	d	d	d	d	d	?	b
BI0.D2	d	d	d	d	d	d	d	d	d
BI0	b	b	b	b	b	b	b	b	b
BALB.K	k	k	k	k	k	k	k	k	k
BALB/c	d	d	d	d	d	d	d	d	d
BALB.B	b	b	b	b	b	b	b	b	b
B6	b	b	b	b	b	b	b	b	b
DBA/2	d	d	d	d	d	d	d	d	d
D2.GD	d	d	b	b	b	b	b	b	b

J mice to support RCS growth was examined in parallel with the proliferative responsiveness of their LN cells to γ-irradiated RCS cells. It will be shown that various subregions of H-2^k and H-2^d haplotypes have strongly negative influences on both of these parameters, whereas H-2^f and H-2^b parental haplotypes have less or no influence on permissiveness for RCS growth and on proliferative responsiveness of T cells to RCS in F_1 hybrids of SJL/J mice.

Materials and Methods

Mice and Tumors. SJL/J mice were purchased from The Jackson Laboratory (Bar Harbor, Maine). All F_1 hybrid mice were bred in our animal facilities. The strains used and their H-2 locus subregions have been outlined in Table I. The RCS-5 and RCS-19 tumors originated from spontaneous RCS in SJL/J mice and were transformed into transplantable lines by Carswell et al. (6, 7); transplantable RCS-X was derived from a primary tumor of an SJL/J mouse in our laboratory. RCS-5 and RCS-X have been maintained by serial intravenous passage of $\sim 10^7$ tumor-infiltrated LN cells into syngeneic young mice, at weekly intervals. RCS-19 has been passaged intraperitoneally at 10^7 cells. For determination of growth in F_1 hybrids RCS-5 or RCS-X LN tumor cells were injected intravenously, 7 d after passage, at 10^7 cells per mouse, into groups of five mice. Because growth of RCS tumor occurs primarily in peripheral lymphoid organs, weights of spleen and mesenteric, brachial, and axillary LN, determined 7 d after injection and expressed as percentage of body weight, were used as indices of tumor growth. γ-Irradiated tumor cells (10,000 γ-RCS) were used to control for apparent growth, i.e., LN and spleen weight increases as a result of the proliferative response to RCS in vivo (4). Therefore, in each experiment an additional group of three mice were injected with 10^7 γ-RCS, and the percentages of LN and spleen weights in terms of body weight in these mice were

subtracted from those obtained in mice injected with unirradiated RCS. Thus, the following formula was used to express growth in F_1 hybrids as percentage of growth in SJL/J mice:

Percent growth

$$= \frac{\text{(index in } F_1 \text{ hybrid with live RCS)} - \text{(index in } F_1 \text{ hybrid with } \gamma\text{-RCS)}}{\text{(index in SJL/J with live RCS)} - \text{(index in SJL/J with } \gamma\text{-RCS)}} \times 100.$$

Overall control values for 14 experiments with viable RCS cells in groups of 5 ♀ SJL/J mice gave indices of 3.604 ± 0.146 for LN and 4.090 ± 0.208 for spleen. The indices in ♀ mice with γ-irradiated RCS cells were 0.758 ± 0.032 for LN and 1.095 ± 0.057 for spleen (eight experiments with three mice each). Control values for viable RCS cells in ♂ mice were 2.347 ± 0.114 for LN and 3.960 ± 0.091 for spleen in six experiments with groups of five mice each, and for γ-irradiated RCS in ♂ mice in three experiments: 0.552 ± 0.053 for LN and 1.009 ± 0.145 for spleen. These results showed better growth in ♀ than in ♂ SJL/J mice. Separate control values for ♀ and ♂ SJL/J mice were, therefore, applied in the determination of growth in F_1 hybrid ♀ and ♂ mice.

Cell Cultures

RESPONSES TO RCS. Lymphocyte cultures from single cell suspensions prepared from mesenteric, brachial, and axillary LN, were set up on flat-bottom Linbro tissue culture plates (No. 76-003-05, Linbro Chemical Co., Hamden, 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 γ-irradiated RCS cells (6,000 R, ^{137}Cs source, Radiation Machinery Corp., Parsippany, N. J.). Responder cells were used at 2×10^5 cells/well, (10^6 cells/ml), whereas irradiated RCS stimulator cells were used at 5×10^4 cells/well. In all experiments nonspecific stimuli were used as a measure of lymphocyte reactivity. These included a 72-h incubation with concanavalin A (Con A 5 μg/ml: Miles-Yeda, Miles Laboratories Inc., Elkhart, Ind.) and phytohemagglutinin (PHA) (PHA-P, 5 μg/ml; Difco Laboratories, Detroit, Mich.). Results of RCS stimulation were considered valid only when Con A and PHA induced appropriate stimulation. Thymidine incorporation (as counts per minute) into cell DNA was measured in five replicate cultures. 1 μCi of [^{3}H]thymidine, (0.36 Ci/mmol, Schwartz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) was added to each well on day 3 of culture. On day 4, the cultures were collected on glass fiber filter disks (H. Reeve Angel & Co., Inc., Clifton, N. J.) with an automated cell harvester. Radioactivity was measured in a Packard tricarb scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.), using Scintiverse (Fisher Scientific Co., Pittsburgh, Pa.) liquid scintillator cocktail. Proliferative responses to RCS were expressed as increases in counts per minute over the counts per minute in control cells cultured in the absence of stimulation. Responses of LN cells from F_1 hybrid mice were expressed as percentages of the response obtained with SJL/J LN cells cultured simultaneously and stimulated with the same γ-RCS preparation:

Percent proliferative response

$$= \frac{(F_1 \text{ hybrid} + \gamma\text{-RCS mixed) cpm} - (F_1 \text{ hybrid cpm} + \gamma\text{-RCS cpm})}{(\text{SJL/J} + \gamma\text{-RCS mixed) cpm} - (\text{SJL/J cpm} + \gamma\text{-RCS cpm})} \times 100.$$

Results were expressed as means ± standard errors (n).

MIXED LYMPHOCYTE CULTURES WITH NORMAL SYNGENEIC STIMULATOR CELLS. Stimulator cells in these cultures were treated with mitomycin C (Drug Synthesis Branch Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.). To enhance the response of LN cells to mitomycin-treated syngeneic spleen cells, 4% (wt/vol) polyethylene glycol (PEG), 6,000 mol wt (Sigma Chemical Co., St. Louis, Mo.), was added to the culture medium (8). 2×10^5 F_1 hybrid nylon wool (9) nonadherent LN cells were used as responder cells and were cultured with mitomycin-treated (25 μg/ml, 45 min at 37°C) syngeneic, parental, or allogeneic spleen cells (2×10^5) or with mitomycin-treated (60 μg/ml, 45 min at 37°C) RCS cells (4×10^4) in medium that contained 4% PEG. Cultures were pulsed with 1 μCi [^{3}H]thymidine 16 h before harvest at 96 h.

TABLE II

Decrease of RCS Growth and of Proliferative Responsiveness to RCS In Vitro under the Influence of H-2 Haplotype Subregions and Non-H-2 Genes in F_1 Hybrids of SJL/J Mice

Strains compared	Back-ground	H-2 haplotype subregions involved	Negative effect on*	
			Growth	Proliferative response (P)
A.SW vs. SJL/J	A	Non H-2: A vs. SJL	+++	+++
ACA vs. A.SW		H-2: f vs. s	+	++
A.TH vs. A.SW		D-end: d vs. s (A)	++	0
A.TFR4 vs. ACA		D-end: d vs. f (A)	++	0
A.TL vs. A.TH		I-G: k vs. s	+++	+++ ($0.001 < P < 0.01$)
A.TFR5 vs. A.TFR4		I-E-G: k vs. f or s	+++	++ ($0.01 < P < 0.02$)‡
A.AL vs. A.TL		K-end: k vs. s	0?§	+?§
BI0.S vs. SJL/J	BI0	Non H-2: BI0 vs. SJL	+++	++
BI0 vs. BI0.S		H-2: b vs. s	0	0
BI0.D2 vs. BI0.S		H-2: d vs. s	++++	++++
BI0.S (7R) vs. BI0.S		D-end: d vs. s	++++	+++ ($0.1 < P < 0.2$)
BI0.D2 vs. BI0.HTG		D-end: d vs. b	++§	0§
BI0.HTT vs. 9R		I-B-I-J: k vs. s	0	0
(BI0.HTT; 9R) vs. 7R		I-E/C: k or d vs. s	+§	++ ($0.02 < P < 0.05$)
[BI0.A(5R); BI0.A] vs. (BI0.HTT; 9R)		K + I-A-I-J: b or k vs. s	?§	++ ($0.001 < P < 0.01$)
BI0.A vs. 9R		K + I-A-I-B: k vs. s	?§	++§
BI0.A(5R) vs. 9R		K + I-A-I-B: b vs. s	?§	++§
BI0.S(8R) vs. BI0.S		K + I-A-I-J: k vs. s	+++	+++ ($0.05 < P < 0.1$)
BI0.HTG vs. BI0		K + I-G: d vs. b	+++	++++ ($P < 0.001$)
BALB.B vs. BI0	BALB	Non H-2:	0	+
BALB.K vs. BALB.B		H-2: k vs. b	++++	++++
BALB/c vs. BALB.B		H-2: d vs. b	++++	++++ ($0.01 < P < 0.02$)
DBA/2 vs. D2.GD	DBA/2	D-end + I-B-G: d vs. b	+++	+++

* A decrease of 1–10% was considered +; of 11–30% ++; of 31–50% +++, and of >50% ++++.

‡ This significance determination was made taking A.TFR4 + A.CA together vs. A.TFR5, because the D end did not appear to affect the proliferative response.

§ Minimal estimate or no value given, because growth and/or proliferative response had reached lowest measurable values.

Results

Growth of RCS in Various F_1 Hybrids. An examination was made of the abilities of various F_1 mice to support growth of 10^7 RCS cells injected intravenously. Fig. 1 shows the results obtained in F_1 hybrids of SJL/J with a number of recombinant mice of A-strain background. It can be seen that SJL × A.SW mice had the highest tumor growth in this series, which was still less than the growth seen in SJL/J mice themselves (49.7 ± 6.1% for LN and 86.5 ± 9.8% for spleen). These results indicate that the A background had a dominant negative effect on tumor growth, particularly in the LN. Growth was further reduced by ~20–30% in the F_1 hybrid with A.TH, which suggests a significant but not very large negative effect of the H-$2D^d$ end. Changing from A.TH to A.TL, as the other F_1 hybrid parent, caused a complete loss of growth, which indicates a strongly negative effect of I^k. In view of the fact that F_1 hybrids with A.TL did not show any significant tumor growth, any further negative effect of H-$2K^k$ could not be analyzed (Table II).

A comparison between the F_1 mice with A.SW and with A.CA showed that H-2^f allowed almost as much tumor growth as did H-2^s, and certainly much more than H-2^k. Comparing A.TFR4 with A.CA again showed a negative effect of the H-$2D^d$ end of ~20–30%. Of particular interest was the comparison between A.TFR4 and A.TFR5 because all growth was lost by a change in regions I-E/C, -S, and -G from H-2^{fs} to H-2^k.

Fig. 2 shows the results obtained with F_1 hybrids of SJL by BIO background mice. Like the A background the BIO background also introduced a highly significant

decrease in growth in the LN (57.8 ± 12.0%) with somewhat less effect on spleen growth (64.5 ± 9.0%). Growth in F_1 hybrids with BIO was at least as good as in F_1 hybrids with BIO.S. Thus, H-2^b was as permissive for tumor growth as was H-2^s itself. The left-hand region of H-2^k had a negative effect of 25–35% (compare BIO.S and BIO.8R), but not as strong as the H-2D^d end, which almost abolished RCS growth [Table II, BIO.S(7R) vs. BIO.S]. When H-2^d was present in every region except in the H-2D end it caused a 40–50% decrease in growth (Table II, BIO.HTG vs. BIO). The effect of the I subregion could not be judged very well on the BIO background because of the strongly negative effect of H-2D^d, but the 5–9% decrease (minimal estimate) between BIO.S(7R) and BIO.HTT or BIO.S(9R) suggested a negative effect of the combined I-E/C, -S, and -G regions.

Fig. 3 shows the results obtained with mice of B6, BALB, and DBA2 backgrounds. The permissiveness of H-2^b as compared with H-2^d or H-2^k was again brought out by examination of F_1 hybrids with B6 and BALB.B mice. Both of these F_1 hybrids were very similar to those of BIO.S and BIO, whereas growth in F_1 hybrids of BALB/c (H-2^d) or BALB.K (H-2^k) was totally lacking. Of all backgrounds examined with H-2^b, the BALB background appeared the most permissive for RCS growth. A strongly negative effect of the presence of H-2^d in regions to the right of I-A was again deduced from the comparison of D2.GD and DBA/2 F_1 hybrids with SJL (Table II).

Various Parameters of RCS Growth in F_1 Hybrids. The effect of time after RCS injection was further examined in some permissive as well as nonpermissive F_1 hybrids. SJL/J mice usually die before the 10th d after injection of 10^7 RCS cells, but most of the F_1 hybrids survived at least until day 14. The results in Table III (expressed as percentage of growth in SJL/J × A.SW) show that growth was progressive during that time both in permissive F_1 hybrids (such as SJL × A.SW and SJL × A.TH) and in relatively nonpermissive F_1 hybrids (such as SJL × 7R), whereas the least-permissive F_1 hybrids (such as SJL × BALB/c and SJL × A.TL) actually showed regression of the tumor with smaller spleen sizes at 14 than at 7 d after RCS injection. Larger tumor cell doses (5×10^7) did not increase tumor growth in the least-permissive strains. 10-fold lower RCS doses (10^6 cells) grew as well in permissive strains (SJL × B6) as did the usual dose of 10^7 (Table IV). Injections of 10^4 RCS cells, although causing at least temporary growth in SJL mice, did not result in detectable growth, even in SJL × B6 (data not shown).

Although by far the majority of experiments were carried out with the tumor line RCS-5, a comparison was made with RCS-X and RCS-19 in a permissive (SJL × B6) and in a nonpermissive strain (SJL × BALB/c). The results in Table IV show that the three RCS lines all grew well in the F_1 of B6, whereas none of the lines grew in the F_1 of BALB/c. Previous studies on induction of T-cell cytotoxicity and on proliferative responses to γ-RCS (1, 4) have also failed to detect differences between these separate transplantable lines.

It should also be noted that SJL/J by other parent invariably gave identical RCS growth, as did the reciprocal F_1 hybrids, (data not shown).

Effect on RCS Growth of Whole Body γ-Irradiation of the Host before RCS Injection. F_1 hybrid mice that were relatively permissive for RCS growth showed a similar inability to grow the tumor when they were irradiated before RCS injection, as was previously described for SJL/J mice themselves (5, 10). In a series of experiments with SJL × B6 mice, for instance, mice that had been irradiated with 650 R 1 d before tumor cell

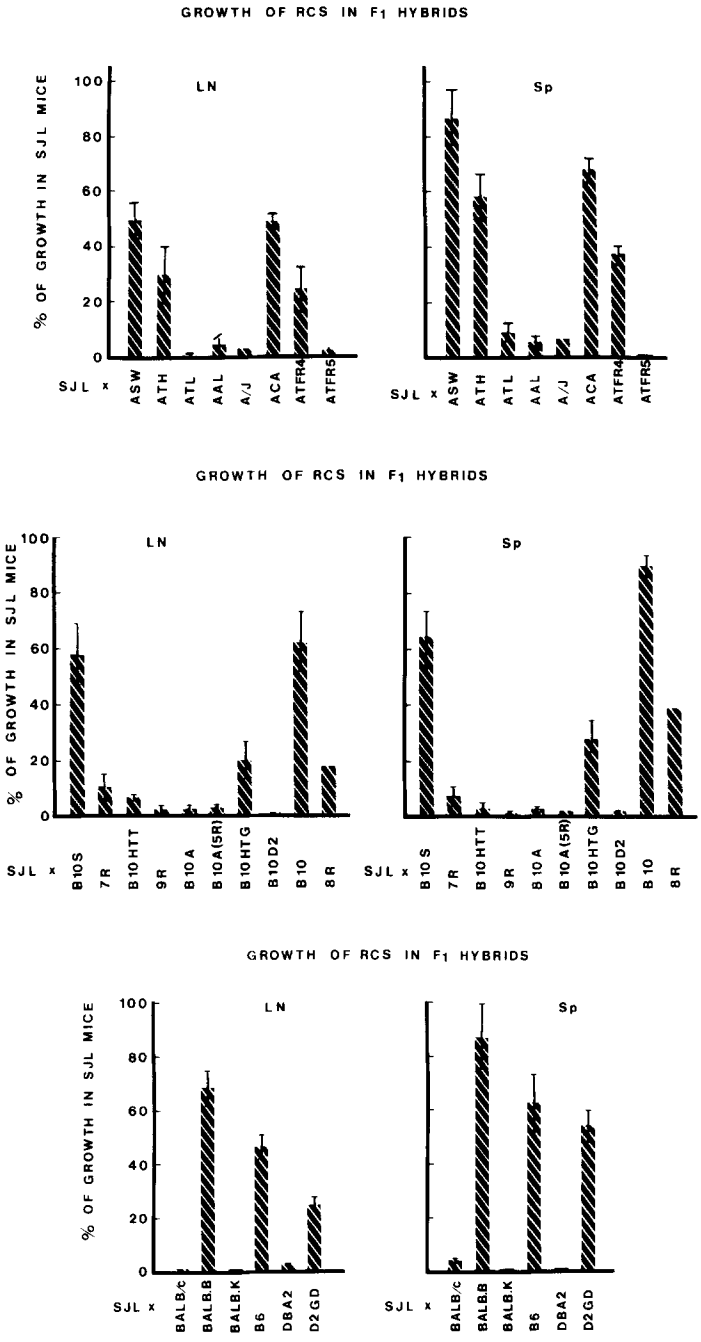

FIG. 1-3. Tumor growth in each experiment was expressed as the percentage of growth in control groups of five SJL/J mice, and corrected for the in vivo proliferative response as indicated in Materials and Methods by use of γ-irradiated RCS cells. Each value represents the mean of two to five experiments. Bars indicate the standard errors for those F_1 hybrids where more than two experiments were performed. Sp, spleen.

TABLE III

Effect of RCS Cell Dose and Time after Injection on Growth in F_1 Hybrids

Strain	Time after injection	Number of RCS cells	(Organ/body weight) × 100		Percent growth in SJL × ASW	
			LN	Spleen	LN	Spleen
SJL × A.SW	1 wk	10^7	1.57*	3.54	100	100
		5×10^7‡	3.08	3.51	100	100
	2 wk	10^7	2.41	5.01	100	100
SJL × SJL	1 wk	10^7	3.56§	4.14	226	117
		5×10^7‡	4.28	3.29	138	93
SJL × A.TH	1 wk	10^7	1.13*	2.54	72	72
		5×10^7	2.54	4.43	82	126
	2 wk	10^7‡	2.80	4.33	116	86
SJL × 7R	1 wk	10^7	0.63‖	1.07	19	30
		5×10^7	0.73	1.30	24	37
	2 wk	10^7	1.61	2.62	67	52
SJL × A.TL	1 wk	10^7	0.53‖	1.07	34	30
		5×10^7	0.70	1.04	23	30
	2 wk	10^7	0.76	0.67	32	13
SJL × BALB/c	1 wk	10^7	0.58	0.97	37	27
		5×10^7	0.75	1.86	24	53
	2 wk	10^7	0.52	0.89	22	18
		5×10^7	0.46	0.70		

* Mean of six experiments.
‡ Grossly enlarged livers at time of death.
§ Mean of 15 experiments; no survivors at 2 wk.
‖ Mean of three experiments; 5 mice/group in each experiment.

TABLE IV

Growth of Three Different Transplantable RCS in SJL/J Mice and F_1 Hybrids*

Time after injection	Tumor dose‡	Strain SJL/J ×	RCS-X		RCS-5		RCS-19	
			LN	Spleen	LN	Spleen	LN	Spleen
1 wk	10^7	SJL	100% (2.89)*	100% (2.66)	100% (2.11)	100% (2.05)	100% (0.49)	100% (2.63)
		B6	41%§	48%	48%	84%	ND‖	83%
		BALB/c	<1%	3%	<1%	4%	ND	3%
1 wk	10^6	SJL	100% (1.31)	100% (1.28)	100% (0.88)	100% (0.82)	ND	ND
		B6	43%	40%	41%	74%	ND	ND
2 wk	10^6	SJL	100% (5.32)	100% (3.99)	100% (4.88)	100% (4.30)	100% (0.63)	100% (3.24)
		B6	65%	98%	69%	123%	ND	103%
	10^7	BALB/c	<1%	<1%	ND	ND	ND	<1%

* Growth was expressed as (organ/body weight) × 100 after injection of live tumor from which was subtracted the value for (organ/body weight) × 100 after injection of X-irradiated (10,000 R) tumor. 5 mice/group in each experiment.
‡ Tumor cells were injected intravenously.
§ Expressed as percentages of values obtained in SJL/J mice with the same RCS cells.
‖ LN values not determined for RCS-19 because growth in LN even in the SJL/J mice was poor with this tumor.

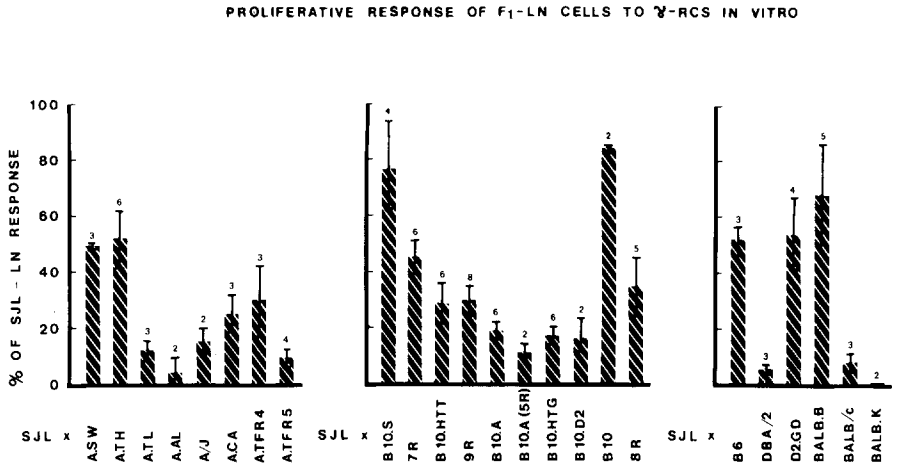

FIG. 4. Proliferative responses in F_1 LN cells are expressed as percentage of the response in SJL/J LN cells to the same preparation of γ-irradiated RCS cells. LN cells pooled from two mice (brachial, axillary, and mesenteric LN) were used in each experiment. Each value represents the mean of the indicated number (*n*) of experiments (given on top of bar). Bars indicate standard errors for more than two experiments, and extreme values where only two experiments are included.

injection showed spleen sizes that were only 33–42% of those in unirradiated mice 1 wk after RCS injection. Comparable experiments in SJL mice showed a somewhat greater reduction in growth, to 26% of the unirradiated SJL mice (data not shown).

Further Examination of the H-2D^d-End Effect. Measures to counteract an effect mediated by the Hh genes of classical F_1 hybrid resistance (11) include treatment with silica (12, 13), carrageenan (14, 15), or cyclophosphamide (16). In a previous study (17) all these M cell-inhibiting agents were shown to inhibit RCS growth in SJL mice (5), which was interpreted as showing a helping effect of cells with the properties of M cells on RCS growth. It was therefore not surprising to find that pretreatment with 3 mg silica i.v. in F_1 hybrids of SJL by A.TFR4, 7R, or A.TH mice did not enhance RCS growth (data not shown).

The only measure, which is also known to overcome the F_1 hybrid resistance linked to the D end and which could be studied here, was that of increasing the tumor dose. The results in Table III with 5×10^7 instead of the usual 10^7 RCS cells showed that the negative effect of the D end in A.TH was significantly reduced. At this high tumor dose, the I-region effect shown by the comparison between F_1 hybrids of SJL with A.TH and A.TL was much greater than it was with the usual dose, whereas the difference between A.SW and A.TH was minimal.

Ability of LN Cells From Various F_1 Hybrids to Give a Proliferative Response to RCS. It was previously found that T cells from certain F_1 hybrids of SJL/J mice did not give as good a proliferative response to γ-irradiated RCS as did T cells from SJL/J mice (1, 4). The results presented in Fig. 4 show relative abilities to give this proliferative responsiveness for all the SJL/J F_1 hybrids examined.

In general, the ability to give this proliferative response varied in a manner similar to the ability of the F_1 hybrids to grow the tumor (Table II). The most obvious exception to this apparent rule was the lack of effect of the H-2D^d end on proliferation in F_1 hybrids with A background, which contrasted with a −20% effect of this locus on growth. There was a negative effect of the H-2D^d end on proliferation in the F_1

hybrids with BIO background, but this effect was more variable and less marked than the H-2D^d-end effect on growth (Table II). Because the change of the H-2D end to H-2^d did not completely abolish the proliferative responsiveness, the effect of the I region on the proliferative responses could be measured, whereas its effect on tumor growth could not. Both the A and the BIO backgrounds caused a decrease in proliferative responsiveness, but the effect of the A background was much greater.

It was evident that the I region, both the I-A and I-E/C loci, had a strong effect on the proliferative responsiveness to RCS regardless of genetic backgrounds. Particularly interesting was the observation that H-2^b and H-2^f caused a loss of proliferation, which appeared as a result of the I-A + K regions (Fig. 4; Table II), whereas H-2^k and H-2^d caused negative effects in both the I-A and I-E/C regions. Moreover the effects of the I-A and I-E/C regions appeared additive in the sense that SJL × A.TH and SJL × A.TL (I^s vs. I^k) differed by 40%, whereas SJL × A.TFR4 and SJL × A.TFR5 (I-E/C^f or -^{s} vs. I-E/C^k) differed by only 20%. Similarly, SJL × BIO.HTT or 9R were 15–16% below SJL × 7R (I-E/C^k or -^{d} vs. I-E/C^s), whereas in turn, they were 11–18% above SJL × BIO.A or BIO.A(5R) (K^k + I-A^k or K^b + I-A^b vs. K^s + I-A^s) in ability to proliferate (Fig. 4; Table II).

Comparison of BIO.S(8R) and BIO.S indicated, however, that the negative effect of K^k + I-A^k in BIO.A was likely to be an underestimation, because the effect of these same regions was much greater (42%) when regions to the right of I were all s. This also indicated that H-2^k in K + I-A had a larger negative effect than did H-2^b or H-2^f, which even as the complete H-2 regions had barely detectable negative effects. Even without the H-2D^d end, H-2^d in K, I, S, and G alone, had a strongly negative effect on both proliferation and growth (Fig. 4; Table II, BIO.HTG vs. BIO). There was no apparent difference between F_1 hybrids with BIO.HTT and BIO.(9R), which indicates that the I-J region was relatively unimportant in this phenomenon and that a change in the I-C region from k to d had no effect.

Comparison Between Responses of F_1 T Cells to Syngeneic Parental and RCS Cells. If the response to γ-RCS were an exaggerated form of the syngeneic MLR, a corresponding variability among F_1 hybrids in their responsiveness to normal SJL cells could be expected. It seemed important, therefore, to determine whether F_1 hybrid T cells could give equally high responses to parental as to syngeneic mitomycin-treated spleen cells.

These responses were all determined in medium that contained PEG because the presence of this agent enhances responses (8). The results in Table V show that the responses to normal non-T cells of syngeneic and parental origin were approximately equally high for each of the F_1 hybrids studied. In contrast, the response to RCS was higher in those strains that also had good responses to RCS without PEG, i.e., SJL × 8R and SJL × A.TH. In the strains that had low to absent responses to RCS without PEG, proliferation to normal SJL spleen and tumor cells was equally high, i.e., SJL × A.TL and SJL × A/J (Table V). Similarly, the responsiveness of thymus cells to RCS as compared with SJL spleen cells was 100- to 200-fold higher in F_1 hybrids with haplotypes that contained I-A-C^s or -^{f}, or even I-E/C^s alone (8R), whereas it was only two- to threefold higher in the ones with I-A-C^k or -^{d}. These findings suggest that those strains that give high responses to RCS recognize something additional on RCS that is not expressed on normal SJL/J cells and that they recognize as foreign.

TABLE V

Proliferation Induced in SJL F_1 Hybrid LN T Cells by Syngeneic and Parental Stimulator Cells

F_1 hybrid responder LN	Responder* cells alone	Δ cpm‡ after stimulation with			
		SJL parental spleen	Syngeneic F_1 spleen	Non-SJL parental spleen	RCS
	cpm				
SJL × A.TH	743	46,736	40,102	38,915	189,348
		(1.0)	(0.9)	(0.8)	(4.1)
SJL × A.TL	4,158	101,655	47,648	84,800	102,644
		(1.0)	(0.5)	(0.8)	(1.0)
SJL × A/J	7,684	47,082	47,373	52,704	36,481
		(1.0)	(1.0)	(1.1)	(0.8)
SJL × 8R	121	47,665	86,024	60,245	227,777
		(1.0)	(1.8)	(1.3)	(4.8)
SJL × 7R	231	71,057	66,794	79,502	99,232
		(1.0)	(0.9)	(1.1)	(1.4)

* Responder cells (2×10^5) were nylon wool nonadherent LN cells; stimulator cells (2×10^5 for spleen and 4×10^4 for RCS) were mitomycin treated (Materials and Methods). The medium for all these cultures contained 4% PEG.

‡ Δ cpm = cpm in cell mixture from which was subtracted the sum of cpm in seperately cultured F_1 hybrid LN and mitomycin-treated spleen or RCS cells. Numbers in parentheses below Δ cpm reflect relative degree of stimulation as compared with responses to normal SJL/J cells.

Discussion

The results indicate a variety of genetic influences in F_1 hybrids of SJL/J, both on the ability to support RCS growth and on the proliferative responsiveness of LN cells to γ-RCS. In the first place there is an effect of background, non-H-2, genes in A, BIO, B6, and less in BALB F_1 hybrids that decreases growth. The negative effect of non-H-2 genes on proliferative responses is most evident with A and B6, and is less marked with BALB and BIO backgrounds. The mechanism of this effect has not been examined further. Examples in the literature of non-H-2 influences on tumor induction in F_1 hybrids have frequently been related to genes controlling permissiveness to murine leukemia virus infections (18–20), but little is known about the possibility that RCS is caused by such a virus in SJL/J mice (21, 22). Previous studies have not suggested the presence of murine leukemia virus antigens in transplantable RCS lines (2). In a recent study, Walker and Phillips-Quagliata (23, 24) have described a strong non-H-2 effect on growth of BALB/c plasmacytomas in F_1 hybrid mice, which suggests that it might be related to the expression of viral antigens on the tumor cells.

Classical F_1 hybrid resistance, as described by Cudkowicz and Bennett (11, 17), relates to the inability of parental H-2^b bone marrow transplants to grow in F_1 hybrids, but also applies to growth of certain lymphomas of H-2^k or H-2^b parental origin (25–28). Previous studies of Warner (29) have shown that such F_1 hybrid resistance to SJL/J bone marrow and myeloid leukemia is also shown by F_1 hybrids of SJL/J with strains carrying H-2^k or H-2^d, but much less in F_1 with H-2^b. Thus, F_1 hybrid resistance directed against homozygous H-2^s cells similar to the one observed here has been described before. Although originally this type of resistance, regulated or controlled by Hh genes, was thought to map to the right of H-2D, in recent studies on the in vitro form of F_1 hybrid resistance (30), which is accompanied by development

of cytotoxicity in vitro, the resistance was mapped to antigens specified by H-2D^b and H-2K^k (31, 32). Although some of the background effect may be a result of similar Hh genes, the clear effect of H-2D^d, and not of H-2D^b, in the F_1 hybrids examined in our studies, suggests an Hh gene-effect that results in resistance to H-2^s, controlled either by H-2D itself or by a locus adjacent to it. Our data do not exclude a similar effect in the H-2K end, particularly in H-2K^k-carrying F_1 hybrids.

Natural killer (NK) cells have recently been suggested as the prime mediators of this form of F_1 hybrid resistance in vivo (33–36). Presumably the effect of H-2D^d is less marked on the A than on the BIO background, as shown in our studies, because the A strain has less NK activity (37). The effect of high tumor cell dosage, partly (on BIO background) or completely (on A background) overriding the H-2D^d-mediated resistance, is in agreement with observations in the literature concerning Hh gene-mediated F_1 hybrid resistance (11). The peculiar behavior of RCS in irradiated SJL/J mice, in which it does not grow, and in which its growth is facilitated by a cell (or more than one cell type), which is sensitive to silica, carrageenan, and trypan blue (5) precludes a detailed analysis of the effect of these agents on the F_1 hybrid resistance. Growth of SJL RCS in the permissive F_1 hybrids is also inhibited by these agents and by irradiation of the host before tumor cell injection, as it is in SJL/J mice themselves (5).

It is of interest that Nakamura and Cudkowicz (38) found a need for "back-stimulation" of F_1 responders by irradiated T cells in the parental stimulator cells for the induction of a cytotoxic response of F_1 anti-parent. It seems likely that the ability of RCS to induce proliferation in F_1 cells would enhance the F_1 hybrid resistance to RCS growth, but, actually a positive correlation between growth and proliferation was usually seen rather than an inverse correlation. For similar reasons, it is of interest to mention here that RCS was recently observed to produce immune type II interferon (39). In view of the activating effect of interferon on NK activity (40) this property would also be expected to enhance any F_1 hybrid resistance that RCS may induce.

The complex effects of the I region on responsiveness of T cells to RCS, and on tumor growth, are of great interest. Previous studies that show I-region effects on tumor growth in F_1 hybrids (41) have suggested the presence of immune response genes in this region that regulate cytotoxic or antibody responses against a tumor-associated viral antigen. Although studies are in progress to examine the possibility that F_1 hybrids give better immune response to RCS than do SJL/J mice, there are other mechanisms that might account for the I-region effects. Previous studies have indicated that either an antigen closely connected with I-A, or I-A itself, induces the proliferation of syngeneic T cells to RCS, because the presence of anti-I-A in the medium specifically inhibits this response (2). The decreasing effect on the proliferative responsiveness in F_1 hybrids of I-A in apparently all haplotypes other than I-A^s, but strongest in I-A^k and weakest in I-A^b, might indicate that a similarity between responder and stimulating cells in the I-A region facilitates this response. Homozygosity in the I-A region could thus increase responsiveness to γ-RCS. However, the most striking effect observed is that of the I-E/C region that appears to correspond entirely to the ability of the haplotype to express a product in this region (42). Thus, a strongly negative effect on proliferation is seen from I-E/C^d and I-E/C^k, but not from I-E/C^b or I-E/C^f. It may be argued that the interaction gene product known to occur between I-A and I-E/C (43), and the resulting influence on I-E/C expression of

the molecular form of I-A (44), are sufficiently changing the responder cell surface of the F_1 hybrid to make responsiveness impossible. In addition, any negative effect of H-2D^d, and possibly also of H-2^k, on the proliferative responsiveness to γ-RCS could perhaps be a result of a regulatory effect of Hh genes on the expression of genes in the I region.

It should be realized that a loss of responsiveness of T cells to γ-RCS could be either on the basis of a progressive loss of similarity of responder to stimulator cell (assuming a syngeneic interaction between T and B cells by a lock- and key-type mechanism) or, in contrast, on the basis of an increase in similarity. The latter interpretation postulates expression of a normally repressed I-E/C^s gene product on RCS cells that is absent from normal SJL/J cells. Thus, the abnormally high response to γ-RCS of some F_1 mice as compared with the normal syngeneic mixed lymphocyte reaction, which T cells of all F_1 mice show to both parental and to F_1 non-T-stimulator cells alike (Table V), would be explained on the basis of an allorecognition by cells from those F_1 mice not expressing an interaction product in the I-E/C region. However, if heterozygosity in I-E/C^s × $^{(k\text{ or }d)}$ would lead to expression of this postulated I-E/C^s product, or else, if the I-E/C^k or I-E/C^d would be very similar (or identical) to this I-E/C^s product, then the T cells of F_1 hybrids of SJL (H-2^s) by H-2^k or H-2^d would no longer recognize RCS as foreign and respond, as they do, to RCS and normal SJL/J non-T cells to the same degree. It should be noted that recent studies by others (45, 46) indicate the expression of foreign H-2 antigens on tumor cells, and that Roman and Bonavida (47) have described the expression of H-2^d-like antigens on in vitro lines of RCS that, however, have some changed properties as compared with the in vivo lines. In our laboratory, no direct evidence for the presence of alien H-2 antigens on in vivo transplantable RCS has been obtained so far.

The correlation between growth and proliferative response still deserves further comment. Analysis of the accessory cell requirements for the RCS growth in SJL/J mice in vivo has led to the suggestion that both T and M cells are needed for its growth (5). Adult thymectomized, irradiated and bone marrow reconstituted as well as nude mice of SJL/J genotype (48) also show less RCS growth than do normal SJL/J mice (~50% reduction). Thus, the proliferative response may itself in some way facilitate RCS growth in vivo, possibly by activation (3) or via production of growth-promoting soluble mediators.

Summary

Growth of three transplantable reticulum cell sarcomas (RCS) was studied in a variety of F_1 hybrids of SJL/J mice by determination of lymph node (LN) and spleen: body weight ratios 7 and 14 d after intravenous injection of RCS cells. Comparison of BIO.S × SJL and A.SW × SJL with SJL/J showed a negative effect of both the A and the BIO non-H-2 genes, particularly on growth in LN. F_1 hybrid resistance was noted with F_1 hybrids that carried H-2D^d and was much more evident with F_1 hybrids from BIO- than from A-background mice. This resistance was less marked at 14 than at 7 d and was partially overcome by injection of higher tumor doses. Changing the I region in the F_1 parent from H-2^s to H-2^b or H-2^f had no effect on growth, but changing to H-2^k or H-2^d virtually abolished the ability to support tumor growth. This effect appeared partially as a result of the I-E/C and partially of the I-A(B) region and was not overcome by higher tumor dose or longer intervals after injection.

There also appeared to be a negative influence on growth of H-2K^k, but this was difficult to differentiate from the I-A^k effect with the available strains.

The known proliferative responsiveness that SJL/J Lyt-1 T cells exhibit to Ia determinants on γ-irradiated RCS cells in vitro was also compared with that of cells from various F_1 hybrids. Responsiveness of F_1 LN cells was expressed as a percentage of the response in SJL/J LN cells to the same RCS cells, measured as [^{3}H]thymidine incorporation. There was a striking degree of correlation between proliferative responsiveness of F_1 LN cells to RCS and the ability of the F_1 mice to support tumor growth. This correlation was especially clear with respect to the negative influences of non-H-2 genes, and of H-2 loci in the I region, particularly of I-A^k or $^{-d}$ and of I-E/C^k or $^{-d}$, but there also appeared to be a (smaller) negative effect of I-A^b or $^{-f}$. Negative influence of H-2D^d on growth, however, was not reflected in a similarly large effect on the proliferative response. Additional findings showed that LN cells from all F_1 hybrids exhibited equivalent syngeneic mixed lymphocyte responses in the presence of polyethylene glycol to mitomycin-treated spleen cells from both the SJL/J and the other parent. The extra high response of F_1 cells to RCS cells, as compared with SJL spleen cells, however, was always absent when I^k or $^{-d}$ was contributed by one of the F_1 parents.

The results suggest a promoting effect of the proliferative response on RCS growth in vivo and, furthermore, an interesting effect of I-A and I-E/C genes, possibly via an interaction product, on the ability of LN cells to be stimulated by Ia determinants on RCS cells.

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