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Analysis of *H-2*-Linked Immune Responses Involved in Resistance to AKR Tumor Growth

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Abstract. *H-2*-associated immune response gene(s) govern resistance to growth of a spontaneous AKR lymphoma, BW5147. The antigenic specificities recognized by the anti-BW5147 humoral response have been characterized and include: Thy-1, a T-cell differentiation antigen; gp70, a retroviral envelope protein; and several previously uncharacterized proteins, including a 78 000 molecular mass protein, p78, which is restricted to expression on BW5147 cells and five phosphoproteins with molecular masses of 33 000, 29 000, 23 000, 17 000, and 16 000. Only mice which are able to respond to Thy-1, p78, and the phosphoproteins can survive an inoculation of BW5147. Thus, resistance to BW5147 is complex and involves multiple antigens with possible roles in tumor rejection.

Introduction

H-2-associated genes have been shown to govern resistance to growth of transplantable tumors (Sato et al. 1973, Kiessling et al. 1975, Williams et al. 1975, Clark et al. 1977) and to retroviral-induced leukemias (Aoki et al. 1966, Lilly 1966, Tennant and Snell 1968, Lilly 1970, Mühlock and Dux 1971, Chesebro et al. 1974, Lonai and Haran-Ghera 1977, Meruelo et al. 1977, Chesebro et al. 1978, Vlug et al. 1981, Dux 1983). Resistance to growth of a spontaneous AKR leukemia, BW5147, is controlled by an *H-2*-linked *Ir* gene which maps to the *H-2I* locus (Meruelo et al. 1980). Strains of mice which can resist tumor development can mount an effective humoral response against the tumor cells, while mice which succumb to the tumor cannot (Meruelo et al. 1980). The present study further explores the role of the antibody response in tumor resistance and examines biochemically the antigens against which the antibody response is elicited. The genetic control of responsiveness to the antigens and the relationship of responsiveness to survival is further defined.

Abbreviations used in this paper: DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; Ir, immune response; MuLV, murine leukemia virus; NMS, normal mouse serum; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TCA, trichloroacetic acid

Materials and Methods

Mice. The F_1 hybrid and most parental strains of mice used in this study were bred at The New York University Medical Center, New York. The parental strains B10.RQB1, B10.RSF2, and B10.RKD2 were obtained from Dr. C. David. The Mayo Clinic, Rochester, Minnesota. AKR/J mice were purchased from The Jackson Laboratories, Bar Harbor, Maine. The F_1 hybrids used in this study are listed in Table 1. The haplotypes of the non-AKR parents are noted in Table 1. AKR mice have an $H\text{-}2^k$ haplotype.

Antisera. Primary antisera were prepared from the various F_1 hybrids 10 days after an intraperitoneal injection of 5×10^6 BW5147 cells. Hyperimmune anti-BW5147 sera were prepared by the hyperimmunization of the F_1 hybrids $(B10.RQB1 \times AKR/C)F_1$, $(B10.Q \times AKR/C)F_1$, and $(C3H.Q \times AKR/C)F_1$, which are resistant to tumor development, with BW5147 cells. Anti-K^k was prepared by the hyperimmunization of $(A.TL \times C3H.OL)F_1$ hybrids with C3H spleen and lymph node cells. Goat antiviral sera including anti-Tween-ether-disrupted AKR MuLV (4S-97), anti-Rauscher virus p30 (18S-221), and anti-Rauscher virus gp70 (5S-167) were provided by Dr. J. Cole, The National Cancer Institute, Bethesda, Maryland. The monoclonal antibody G8, which is reactive against the Thy-1.1 antigen, was prepared by the fusion of $P3 \times 63$ AgU1 (P3U1) myeloma cells with spleen cells from a $(C3H.Q \times AKR/C)F_1$ hybrid which had been hyperimmunized with BW5147 cells.

Cell lines. BW5147 is a tissue culture-adapted T-cell lymphoma derived from a spontaneous AKR/J thymoma (Hyman and Stallings 1974). BW5147 cells were obtained from The Salk Institute, San Diego, California. The BW5147 Thy-1-negative variants BW5147 G.1 and BW5147.3 (Hyman and Stallings 1974) were obtained from Dr. R. Hyman, The Salk Institute, San Diego, California. Other cell lines used in this study include YAC-1, a Moloney virus-induced A-strain T-cell lymphoma (Cikes et al. 1973); 18-81, an Abelson virus-induced BALB/c B-cell lymphoma (Siden et al. 1979). AQR-RADLV, a RadLV-induced B10.AQR T-cell lymphoma;

Table 1. Mapping of the *H-2*-linked *Ir* genes governing survival and the ability of F_1 hybrids to respond to Thy-1, p78, and phosphoproteins pp33, pp29, pp23, pp17, and pp16

	H-2 haplotype of non-AKR parent					**A** Percent survival*	**B** Response to 10^7 AKR/J thymocytes†	**C** Response to p78 and the phosphoproteins‡
		I						
	K	*A*	*E*	*S*	*D*	(⇦)	(◀)	(←)
(B10.Q × AKR/C)F_1	*q*	*q*	*q*	*q*	*q*	63 (17/27)	+	+
(B10 × AKR/C)F_1	*b*	*b*	*b*	*b*	*b*	0 (0/13)	+	−
(B10.RQB1 × AKR/C)F_1	*q*	*q*	*q*	*q* ⇦ ←	*b*	67 (20/30)	+	+
(B10.BR × AKR/C)F_1	*k*	*k*	*k*	*k*	*k*	0 (0/24)	−	+
(B10.D2 × AKR/C)F_1	*d*	*d*	*d*	*d*	*d*	0 (0/13)	+	−
(B10.RKD2 × AKR/C)F_1	*k*	*k*	*k*	⇦ *q*	*d*	0 (0/11)	−	+
(B10.AQR × AKR/C)F_1	*q* ⇨	*k*	*k*	*d*	*d*	0 (0/20)	−	+
[B10.A(5R) × AKR/C]F_1	*b*	*b*	*k*	*d*	*d*	0 (0/25)	+	−
[B10.A(3R) × AKR/C]F_1	*b*	*b*	*k*	*d*	*d*	0 (0/14)	+	−
[B10/A(4R) × AKR/C]F_1	*k* ◀	*k*	*b*	*b*	*b*	0 (0/9)	−	+
(B10.S × AKR/C)F_1	*s*	*s*	*s*	*s*	*s*	0 (0/15)	+	−
(B10.RSF2 × AKR/C)F_1	*s*	➔ *k* →←	*b*	*b*	*f*	0 (0/17)	−	+
(C3H.Q × AKR/C)F_1	*q*	*q*	*q*	*q*	*q*	61 (48/79)	+	+
(C3H × AKR/C)F_1	*k*	*k*	*k*	*k*	*k*	0 (0/20)	−	+

* Five million BW5147 cells were injected intraperitoneally into the various F_1 hybrids. Strains are considered resistant if > 60% of individual mice survived tumor inoculation for longer than 120 days

† Response to AKR/J thymocytes was determined by radioimmunoassay using serum from individual mice obtained 10 days after injection of 10×10^6 AKR/J thymocytes, as shown in Figure 4

‡ Response to p78 and to the phosphoproteins pp33, pp29, pp23, pp17, and pp16 was determined by immunoprecipitation of BW5147 cell extracts with serum obtained 10 days after injection of BW5147 cells, as shown as Figures 6 and 7

B10.G-X1, a B10.G radiation-induced T-cell lymphoma; AKR/C-1 and AKR/C-2, spontaneous AKR/C thymomas; and AKR/J M4, a spontaneous AKR/J thymoma, were established in our laboratory.

Cell lines were routinely maintained at 37°C with 5% CO_2 in DMEM or in RPMI 1640 (Gibco Laboratories, Grand Island, New York) containing 10% FCS, 1% penicillin/streptomycin.

Radioimmunoassay. Fifty microliters of diluted antiserum was added to 2×10^6 cells and incubated for 30 min at room temperature. The cells were then washed once in PBS, 1% bovine serum albumin, 0.1% sodium azide. Fifty microliters of ^{125}[I]-protein A (70-100 μCi/mg; Amersham Corp., Arlington Heights, Illinois) diluted 1:10 was added to the cells and incubated for 30 min at room temperature. The cells were then washed three times and transferred to a new tube; cpm were determined by a Beckman Biogamma counter (Beckman Instruments, Inc., Fullerton, California).

Cellular absorption of antisera. Fifty microliters of diluted serum was added to various concentrations of cells and incubated for 1 h at 4°C. The absorbed antisera were spun at $1300 \times g$ for 20 min to sediment the cells. The sera were then tested for reactivity by radioimmunoassay. Thymocytes from 1–2 month old AKR/J and AKR/C animals were used. For passive antibody treatment, hyperimmune anti-BW5147 serum was absorbed at a concentration of 20×10^6 AKR/J thymocytes per 25 μl of serum. Previous titration experiments have shown that the anti-Thy-1.1 activity was absorbed out from hyperimmune anti-BW5147 serum using $1–2 \times 10^6$ thymocytes per 25 μl of antisera. Removal of the anti-Thy-1 activity was confirmed by a radioimmunoassay testing the serum against AKR/J thymocytes.

Passive antibody treatment. Three tenths of a milliliter of normal mouse serum or hyperimmune anti-BW5147 sera, unabsorbed or absorbed with AKR/J thymocytes, was administered intravenously to mice of the susceptible strains (C3H × AKR/C)F_1 and (B10 × AKR/C)F_1 3–4 h prior to inoculation with 1×10^3 or 1×10^4 BW5147 cells. Titration experiments showed that an inoculation of as little as 1×10^2 BW5147 cells caused mortality due to tumor growth in susceptible strains of mice.

Cell-surface iodination. Cell-surface proteins were iodinated by incubating 10^8 cells with 2.5 mCi Na^{125}-[I] (350–600 mCi/ml; Amersham) and 200 μg lactoperoxidase (Sigma Chemical Co., St. Louis, Missouri). Twenty-five microliters of 0.03% H_2O_2 was added three times over a 10 min labeling period at room temperature. After labeling, the cells were washed three times in PBS and resuspended in lysis buffer [0.5% Nonidet P40 (NP-40), 50 m*M* Tris-HCl (pH 7.2), 150 m*M* NaCl, 3 m*M* phenylmethylsulfonyl fluoride (Sigma), 1% aprotinin (Sigma)] at 4°C for 30 min. Lysates were clarified by centrifugation at $150\,000 \times g$ for 60 min.

TCA-precipitable counts were determined by adding TCA to 10% a 1:200–1:500 dilution of extract in PBS containing 5% bovine serum albumin, as a protein carrier.

Biosynthetic radiolabeling. Tumor cells were biosynthetically radiolabeled at a radiochemical concentration of 25 μCi/ml in DMEM lacking the

amino acid or phosphates to be used for labeling plus 10% FCS for 18 h at 37°C with 5% CO_2. Radiochemicals used included ^{3}[H]-leucine (> 140 Ci/mmol; New England Nuclear, Boston, Massachusetts), ^{35}[S]-methionine (> 600 Ci/mmol; Amersham), and ^{32} [P]-orthophosphoric acid carrier-free, 50–1000 mCi/mmol; New England Nuclear). After labeling, the cells were washed three times in PBS and lysed as described.

Immunoprecipitation. Aliquots of extracts containing equal TCA-precipitable counts, ranging from 1–20 × 10^6 cpm, were precleared by incubation with 25 µl of normal mouse serum for 30 min at room temperature. Two hundred microliters of a 10% solution of IgGsorb (*Staphylococcus aureus A*, Cowan Strain; New England Enzyme Center, Inc., Malden, Massachusetts) was added and incubated for 30 min at room temperature. The extract was then spun at 4500 × g for 10 min. The supernatant was transferred to a new tube. Appropriate volumes of antisera were added to the precleared supernatant to allow for antibody excess and incubated overnight at 4°C. In general, 25 µl of hyperimmune allogeneic sera or 100 µl of primary antisera was used. Three hundred microliters of IgGsorb was added and incubated for 60 min. The pellet was washed three times with 0.5% NP-40, 50 m*M* Tris-HCl (pH 7.2), 150 m*M* NaCl. The pellet was resuspended in electrophoresis sample buffer [2% SDS, 80 m*M* Tris-HCl (pH 6.8), 10% glycerol, 2 m*M* ethylene diaminetetraacetate, 5% 2-mercaptoethanol, 0.001% bromphenol blue] and boiled for 5 min. The sample was pelleted at 5000 × g for 10 min and run on SDS-PAGE.

SDS-PAGE. SDS-PAGE was run using a discontinuous buffer system as described by Laemmli (1970). 7.5%–15% gradient gels were made from solutions of 30% acrylamide, 0.8% bisacrylamide (Bio-Rad Laboratories, Richmond, California) and contained 0.375 *M* Tris-HCl (pH 8.8) and 0.1% SDS. The stacking gel contained 4.75% acrylamide, 0.125 *M* Tris-HCl (pH 6.8), 0.1% SDS. Gels, 24.5 cm in length, were run at 140 V for 18 h.

The gels were fixed and stained in 0.1% Coomassie Brilliant Blue R (Bio-Rad), 50% TCA for 20 min and destained in 7% glacial acetic acid. ^{3}[H]-containing gels were prepared for fluorography by soaking gels in three times the gel volume of 0.5 *M* sodium salicylate for 60 min. The gels were dried under vacuum and exposed to Kodak XAR-5 film at −70°C.

Samples containing standard molecular mass markers were run with each gel. Molecular mass markers comprised phosphorylase B (M_r 92 500), bovine serum albumin (M_r 66 200), ovalbumin (M_r 45 000), carbonic anhydrase (M_r 31 000), soybean trypsin inhibitor (M_r 21 500), and lysozyme (M_r 14 400) (Bio-Rad) or phosphorylase B (M_r 92 500), bovine serum albumin (M_r 68 000), ovalbumin (M_r 45 000), α-chymotrypsinogen (M_r 25 700), β-lactoglobulin (M_r 18 400), and cytochrome C (M_r 12 300) (Bethesda Research Laboratories, Inc., Gaithersburg, Maryland).

Tunicamycin treatment. One million cells/ml were preincubated at 37°C for 2 h. The cells were then transferred to leucine-free media and incubated with tunicamycin (Calbiochem-Behring Corp., San Diego, California) at a concentration of 2 µg/ml for 1 h. The radiochemical was then added for 18 h as described. Cells were washed and lysed.

Results

Antibody involvement in resistance to growth of an AKR spontaneous T-cell leukemia. H-2 congenic F_1 hybrids

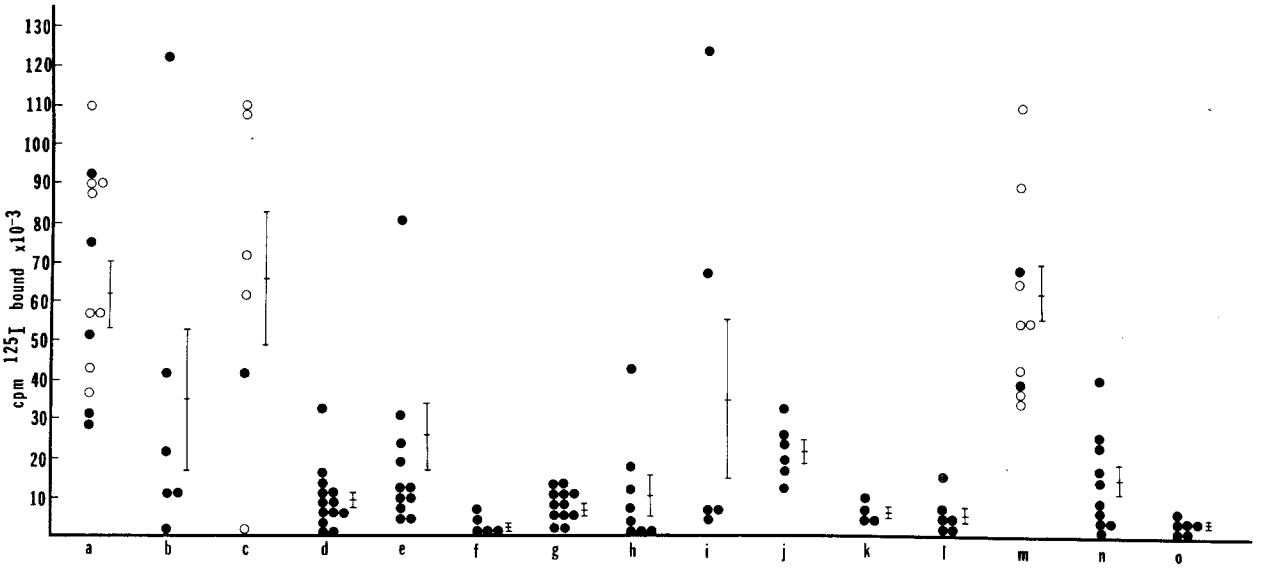

Fig. 1. Magnitude of the antibody response of individual mice of various F_1 hybrids 10 days after an intraperitoneal injection of 5 × 10^6 BW5147 cells. Fifty microliters of serum diluted 1:10 was tested for reactivity against BW5147 cells by a direct radioimmunoassay using ^{125}[I]-protein A. *Open circles* (○) indicate mice which have survived tumor inoculation. *Closed circles* (●) indicate mice which have succumbed to tumor growth. The *center line* indicates the mean antibody response; the *upper* and *lower lines* indicate ± standard error (SE). Lane a represents the antibody response of individuals of (B10.Q × AKR/C)F_1 hybrids, mean antibody ± SE: 62 000 ± 8428; lane b, (B10 × AKR/C)F_1 hybrids, 35 167 ± 18 442; lane c, (B10.RQB1 × AKR/C)F_1 hybrids, 66 167 ± 16 841; lane d, (B10.BR × AKR/C)F_1 hybrids, 9666 ± 1388; lane e, (B10.D2 × AKR/C)F_1 hybrids, 25 833 ± 8453; lane f, (B10.RKD2 × AKR/C)F_1 hybrids, 2333 ± 421; lane g, (B10.AQR × AKR/C)F_1 hybrids, 7000 ± 5329; lane h, [B10.A(5R) × AKR/C]F_1 hybrids, 10 750 ± 5095; lane i, [B10.A(3R) × AKR/C]F_1 hybrids, 35 666 ± 20 617; lane j, [B10.A(4R) × AKR/C]F_1 hybrids, 22 617 ± 2892; lane k, (B10.S × AKR/C)F_1 hybrids, 6750 ± 1182; lane l, (B10.RSF2 × AKR/C)F_1 hybrids, 6333 ± 1783; lane m, (C3H.Q × AKR/C)F_1 hybrids, 62 700 ± 6883; lane n, (C3H × AKR/C)F_1 hybrids, 14 900 ± 3749; lane o, (C3H.Q × AKR/J)F_1 hybrids, 3833 ± 654. Statistical analysis (T-test analysis) demonstrates a highly significant difference ($P < 0.001$) between the mean antibody response elicited by the strains of mice which are resistant to tumor growth and those which are susceptible to the tumor. The SE of the mean antibody response elicited by the susceptible strains (B10 × AKR/C)F_1 and [B10.A(3R) × AKR/C]F_1 (lanes b and i) overlap with the SE of the mean antibody response elicited by the resistant strains (B10.Q × AKR/C)F_1 and (B10.RQB1 × AKR/C)F_1 (lanes a and c). However, a significant difference ($P < 0.01$) exists between the levels of the antibody response elicited by these susceptible and resistant strains

from crosses of AKR/C (*H-2*^{k}) and C3H or C57BL/10 backgrounds (Table 1), were tested for their ability to mount an antibody response after an intraperitoneal injection of 5×10^6 BW5147 cells. BW5147 is a spontaneous AKR/J (*H-2*^{k}) T-cell leukemia. Sera from individual mice were tested by radioimmunoassay for reactivity against BW5147 cells (Fig. 1). The F_1 hybrids (B10.Q × AKR/C)F_1, (B10.RQB1 × AKR/C)F_1 and (C3H.Q × AKR/C)F_1 could mount a strong antibody response against BW5147 cells and survive tumor inoculation (Fig. 1, lanes a, c, and m, respectively). The F_1 hybrids (B10 × AKR/C)F_1, (B10.BR × AKR/C)F_1, (B10.D2 × AKR/C)F_1, (B10.RKD2 × AKR/C)F_1, (B10.AQR × AKR/C)F_1, [B10.A(5R) × AKR/C]F_1, [B10.A(3R) × AKR/C]F_1, [B10.A(4R) × AKR/C]F_1, (B10.S × AKR/C)F_1, (B10.RSF2 × AKR/C)F_1, (C3H × AKR/C)F_1, and (C3H.Q × AKR/J)F_1 do not generate a strong antibody response and succumb to tumor growth (Fig. 1, lanes b, d, e, f, g, h, i, j, k, l, n, and o, respectively).

The antibodies produced in response to BW5147 cells are cytotoxic to the tumor cells in vitro when extraneous rabbit complement is added. The only strains of mice whose individuals consistently show a strong response, as assayed by cytotoxicity at a serum dilution of 1:10 and 1:40, are (B10.RQB1 × AKR/C)F_1 and (B10.Q × AKR/C)F_1 hybrids, the strains which can survive tumor inoculation (Fig. 2). Individuals of the F_1 hybrids, (B10 × AKR/C)F_1, (B10.BR × AKR/C)F_1, (B10.D2 × AKR/C)F_1, (B10.RKD2 + AKR/C)F_1, (B10.AQR × AKR/C)F_1, [B10.A(5R) × AKR/C]F_1, [B10.A(3R) × AKR/C]F_1, [B10.A(4R) × AKR/C]F_1, (B10.S × AKR/C)F_1, (B10.RSF2 × AKR/C)F_1, which did not mount a strong antibody response as detected by radioimmunoassay, had low cytotoxic activity (Fig. 2).

Mapping of the Ir gene involved in resistance to tumor growth. Comparison of the *H-2* haplotypes of the F_1 hybrids (Table 1, column A, ⟨⊢), maps the gene governing resistance to tumor growth to the I region of the *H-2* complex. The F_1 hybrids (B10.Q × AKR/C)F_1 and (B10.RQB1 × AKR/C)F_1 survive tumor inoculation, while (B10 × AKR/C)F_1 hybrids succumb to tumor growth. These results map the gene governing resistance to the left of the *H-2D* region. (B10.BR × AKR/C)F_1, (B10.D2 × AKR/C)F_1, (B10.RKD2 × AKR/C)F_1, and (B10.AQR × AKR/C)F_1 hybrids also succumb to tumor growth. These observations map the gene governing resistance to growth of BW5147 cells to the left of the *H-2S* region and to the right of the *H-2K* region, or to the *I* region of the *H-2* complex. Mice with at least one copy of the *q* haplotype in the *I* region could mount a strong antibody response to BW5147 and survive tumor growth.

Passive administration of anti-BW5147 confers resistance to normally susceptible mice. When mice of the susceptible strain (C3H × AKR/C)F_1 were inoculated with 1×10^3–1×10^4 BW5147 cells, none (0 out of 11) of the mice survived tumor inoculation (Table 2, experiments 1, 2, and 3). When the mice were treated intravenously with 0.3 ml of hyperimmune anti-BW5147 serum produced in the resistant strain (C3H.Q × AKR/C)F_1 3–4 h prior to tumor inoculation, 92% (12 out of 13) of the mice survived the tumor inoculation. If normal mouse serum was administered intravenously instead of anti-BW5147 serum, none of the mice (0 out of 9) survived tumor inoculation. Passively administered hyperimmune anti-

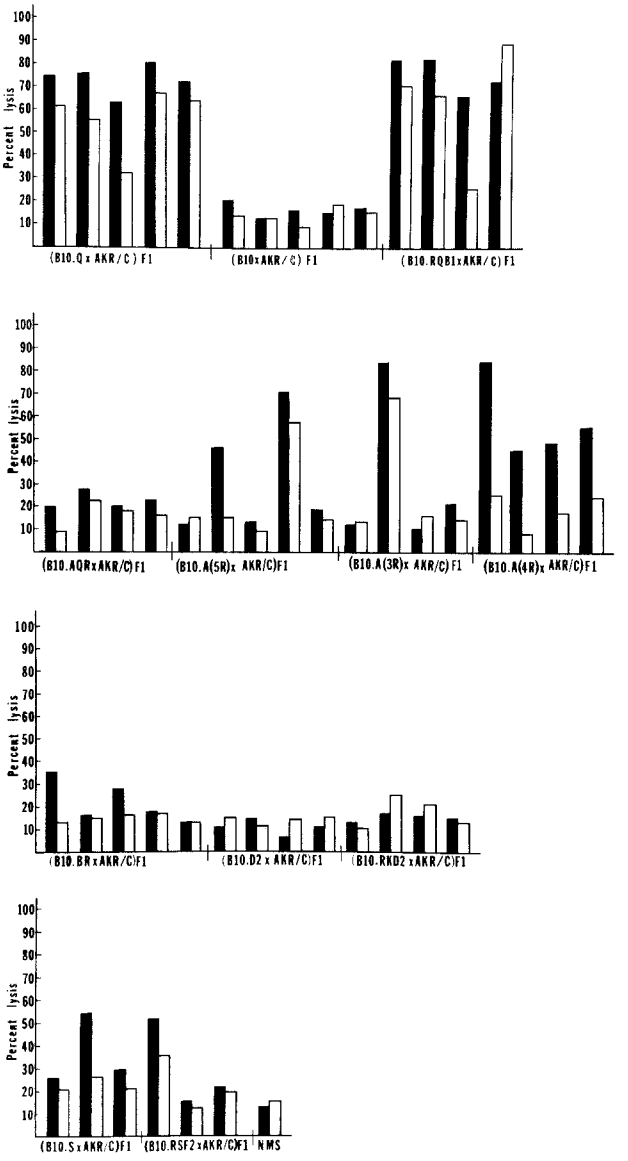

Fig. 2. The antibody produced in response to BW5147 cells is cytotoxic in the presence of complement to the tumor cell. Fifty microliters of serum diluted 1:10 (*solid bars*) or 1:40 (*open bars*) from individual mice of the various F_1 hybrids was tested for complement-dependent cytotoxic activity against BW5147 cells. A cytotoxic response is considered positive if the percent lysis of cells incubated with antiserum plus complement is greater than the percent lysis of cells incubated with NMS and complement

Table 2. Passive antibody treatment of susceptible F_1 hybrids with hyperimmune anti-BW5147 serum produced in resistant mice

Experiment	Strain	Treatment*	Percent survival (> 70 days)	Mean survival time of mice succumbing to tumor (days) ± SD	Number of BW5147 cells injected
1	(C3H × AKR/C)F_1	–	0 (0/2)	35 ± 19	10^3
		NMS	0 (0/3)	33 ± 8	
		Anti-BW5147	75 (3/4)	49	
2	(C3H × AKR/C)F_1	–	0 (0/4)	28 ± 7	10^4
		NMS	0 (0/3)	26 ± 4	
		Anti-BW5147	100 (5/5)	–	
3	(C3H × AKR/C)F_1	–	0 (0/5)	26 ± 6	10^4
		NMS	0 (0/3)	39 ± 13	
		Anti-BW5147	100 (4/4)	–	
		Anti-BW5147 absorbed with AKR/J thymocytes†	75 (3/4)	62	
4	(B10 × AKR/C)F_1	–	13 (1/8)‡	32 ± 5	10^3
		NMS	0 (0/4)	25 ± 3	
		Anti-BW5147	100 (6/6)	–	
		Anti-BW5147 absorbed with AKR/J thymocytes†	50 (3/6)	34 ± 1	

* Mice were either inoculated with BW5147 cells or treated with an intravenous injection of 0.3 ml of NMS or hyperimmune anti-BW5147 serum, unabsorbed or absorbed with AKR/J thymocytes, 3-4 h prior to intraperitoneal tumor injection

† Anti-BW5147 serum was extensively absorbed with AKR/J thymocytes as described in *Materials and Methods* to remove anti-Thy-1 activity. The removal of the anti-Thy-1 activity was shown by radioimmunoassay

‡ In virtually all other experiments, (B10 × AKR/C)F_1 mice showed 0% survival to inoculation with BW5147 cells. This low survival rate is thus not considered significant

BW5147 serum also inhibited tumor development in the (B10 × AKR/C)F_1 strain. Thirteen percent (1 out of 8) of the mice survived an inoculation of 1×10^3 BW5147 cells (Table 2, experiment 4). When hyperimmune anti-BW5147 serum produced in the resistant strain (B10.Q × AKR/C)F_1 was administered prior to tumor inoculation, 100% of the mice (6 out of 6) survived tumor inoculation. None of the mice (0 out of 4) survived when normal mouse serum was administered prior to tumor inoculation.

Analysis of the antigenic activities present in anti-BW5147 serum: the role of Thy-1; absorption analysis. It was thought that the anti-BW5147 serum would contain activity against the T-cell differentiation antigen Thy-1, because allelic differences in Thy-1 exist between the tumor cell (Thy-1.1) and the recipient mice (Thy-1.2). Hyperimmune anti-BW5147 serum was absorbed with various concentrations of AKR/C thymocytes (Thy-1.2), AKR/J thymocytes (Thy-1.1), and BW5147 cells (Thy-1.1). AKR/J and AKR/C are sublines of the AKR inbred line of mice which differ at the *Thy-1* locus, but they contain few other genetic differences (Acton et al. 1973). Absorption of anti-BW5147 with AKR/C thymocytes did not remove any substantial activity of anti-BW5147 serum to AKR/J thymocytes (Fig. 3, panel A) or to BW5147 cells (Fig. 3, panel B). Absorption of anti-BW5147 serum with AKR/J thymocytes or BW5147 cells removed all of the activity to AKR/J thymocytes (panel A), indicating the presence of anti-Thy-1.1 activity. However, absorption of the serum with AKR/J thymocytes removed only a portion (~ 55%) of the activity to BW5147 cells (panel B). Absorption of the serum with BW5147 cells removed the majority of the activity to BW5147 cells (panel B). All of the reactivity to the BW5147 cells could be removed by absorption of the serum with greater numbers of BW5147

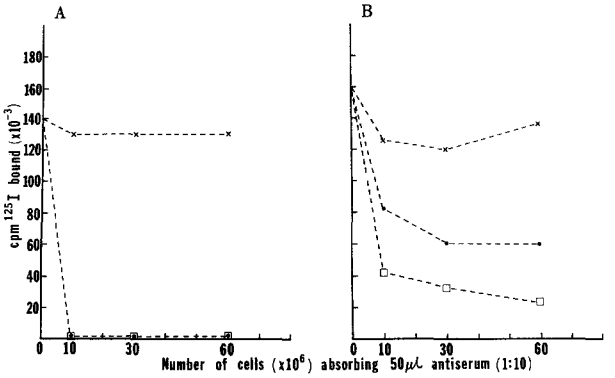

Fig. 3. Analysis of the antigenic activity present in anti-BW5147 serum. Anti-BW5147 serum was absorbed with increasing concentrations of AKR/C thymocytes (x), AKR/J thymocytes (●), and BW5147 cells (□) and was then tested for reactivity against AKR/J thymocytes (panel A) and BW5147 cells (panel B) by a direct radioimmunoassay using ^{125}[I]-protein A

Table 3. Reactivity of various sera against BW5147 and Thy-1-negative variants*

	Anti-BW5147†	Anti-Thy-1.1†	Anti-H-2K†	Anti-gp70‡
BW5147	98925 ± 36706	182246 ± 25761	26223 ± 921	69354 ± 5600
BW5147 G.1	26361 ± 7601	1263 ± 294	1177 ± 116	33487 ± 5597
BW5147.3	29236 ± 1385	10366 ± 467	9015 ± 452	60184 ± 213

* Reactivity of sera was measured by radioimmunoassay using ^{125}[I]-protein A, as described in *Materials and Methods*. Figures indicate mean cpm of duplicate samples minus mean cpm of cells incubated with NMS as a control plus or minus the SD

† Reactivity of sera was measured by a direct radioimmunoassay

‡ Reactivity of sera was measured by an indirect radioimmunoassay incubating cells which had previously been incubated with goat gp70-specific antibody and rabbit goat-specific antibody (as the second antibody) prior to incubation with ^{125}[I]-protein A

cells (120×10^6 BW5147 cells per 50 μl of serum; data not shown). The results indicate that additional activities besides the anti-Thy-1 activity are present in the anti-BW5147 serum. Clearly, however, an antibody response against Thy-1 appears to be important for survival against tumor growth, as (C3H.Q × AKR/J)F_1 hybrids which express the same *Thy-1* allele as the tumor succumb to the tumor, while the (C3H.Q × AKR/C)F_1 hybrids, which recognize the allelic differences in Thy-1, survive (Fig. 1).

BW5147 Thy-1-negative variants can grow in mice resistant to BW5147 cells. Thy-1-negative variants were utilized to examine the role of the anti-Thy-1.1 response in resistance to growth of BW5147 cells. The BW5147 Thy-1-negative variants BW5147 G.1 and BW5147.3 were derived by immunoselection with anti-Thy-1.1 plus complement (Hyman et al. 1974). The Thy-1 content of the variants was examined by radioimmunoassay using the Thy-1.1-specific monoclonal antibody G8 plus iodinated protein A, as described in *Materials and Methods* (Table 3). BW5147 G.1 cells express less than 1% of the Thy-1 present on the surface of the wild-type BW5147 cells. BW5147.3 cells express approximately 7% of the Thy-1 present on the surface of the wild-type BW5147 cells. The Thy-1-negative variants also showed reduced activity with anti-BW5147 serum and with antibodies to H-2K and the retroviral envelope protein, gp70 (Table 3).

When the Thy-1-negative variants were inoculated into the H-2 congenic F_1 hybrids of strains which are resistant to growth of wild-type BW5147 cells, only 13% (5 out of 38) of the mice could survive growth of the Thy-1-negative variant BW5147 G.1 (Table 4). Only 17% (2 out of 12) of the mice could resist growth of the Thy-1-

Table 4. Percent survival and survival rates of H-2 congenic F_1 hybrids after the inoculation of the Thy-1-negative variants BW5147 G.1 and BW5147.3

BW5147 Thy-1-negative variant injected	Recipient F_1 hybrids which are resistant to BW5147*	Preimmunization†	Percent survival (> 120 days)	Mean survival time (days) ± SD
BW5147 G.1	(C3H.Q × AKR/C)F_1	–	13 (5/38)	37 ± 10
BW5147 G.1	(C3H.Q × AKR/C)F_1	Immunized once with BW5147	29 (2/7)	34 ± 12
BW5147 G.1	(C3H.Q × AKR/C)F_1	Hyperimmunized with BW5147	100 (5/5)	–
	(B10.RQB1 × AKR/C)F_1		100 (6/6)	–
BW5147 G.1	(C3H.Q × AKR/C)F_1	Immunized twice with AKR/J thymocytes	0 (0/4)	44 ± 17
BW5147.3	(C3H.Q × AKR/C)F_1	–	20 (1/5)	58 ± 20
	(B10.RQB1 × AKR/C)F_1	–	14 (1/7)	41 ± 6
BW5147.3	(C3H.Q × AKR/C)F_1	Immunized once with BW5147	100 (5/5)	–
	(B10.RQB1 × AKR/C)F_1		100 (3/3)	
BW5147.3	(B10.RQB1 × AKR/C)F_1	Hyperimmunized with BW5147	100 (2/2)	–
BW5147.3	(B10.RQB1 × AKR/C)F_1	Immunized with AKR/J thymocytes	100 (10/10)	–

* *H-2* congenic F_1 hybrids from crosses of AKR/C and C3H or C57BL/10 backgrounds are considered to be resistant to an inoculation of BW5147 cells if > 60% of the individuals can resist tumor growth for longer than 120 days

† Mice were injected with 5×10^6 BW5147 Thy-1-negative variant cells either as a primary injection or 40–60 days after primary immunization with 5×10^6 BW5147 cells, AKR/J thymocytes, or after hyperimmunization with BW5147 cells

negative variant BW5147.3 (Table 4). Thus, the reduction in survival of the F_1 hybrids inoculated with Thy-1-negative BW5147 variants compared to Thy-1-positive BW5147 cells supports the evidence that a Thy-1 response is necessary for survival. However, other factors may also contribute to the increased tumorigenicity of the variant cells, since they show multiple differences in antigenic properties compared with BW5147 cells (Table 3).

Mapping of the anti-Thy-1.1 response to the A subregion. In order to see if survival was correlated with the ability of mice to respond to the Thy-1 antigen, the F_1 hybrids were immunized with normal AKR/J thymocytes. Absorption studies were performed using a monoclonal antibody against Thy-1, showing that BW5147 cells contained approximately two times the amount of Thy-1 as 2 month AKR/J thymocytes (data not shown). Similar findings have been reported by Carlsson and Stigbrand (1983) using a competitive binding assay. The various H-2 congenic F_1 hybrids (Table 1) were injected with 10×10^6 AKR/J thymocytes, and their sera were tested for reactivity against Thy-1.1 on AKR/J thymocytes (Fig. 4). The F_1 hybrids (B10.Q × AKR/C)F_1, (B10 × AKR/C)F_1, (B10.RQB1 × AKR/C)F_1, (B10.D2 × AKR/C)F_1, [B10.A(5R) × AKR/C]F_1, [B10.A(3R) × AKR/C]F_1, (B10.S × AKR/C)F_1, and (C3H.Q × AKR/C)F_1 can mount a strong antibody response against Thy-1.1 (Fig. 4, lanes a, b, c, e, h, i, k, and m, respectively). The F_1 hybrids, (B10.BR × AKR/C)F_1, (B10.RKD2 × AKR/C)F_1, (B10.AQR × AKR/C)F_1, [B10.A(4R) × AKR/C]F_1, (B10.RSF2 × AKR/C)F_1, and (C3H × AKR/C)F_1 mount a weaker response to Thy-1.1 (Fig. 4, lanes d, f, g, j, l, and n, respectively). As seen in Figure 4, the response of (C3H × AKR/C)F_1 mice (*H-2*^{k} haplotype) to Thy-1.1 (lane n) is higher than the response of (B10.BR × AKR/C)F_1 mice (also of the *H-2*^{k} haplotype; lane d). Mice of the C3H background have been reported to elicit higher antibody responses to a number of antigens, including the Thy-1.1 antigen, compared to mice of the C57BL/10 background (Freed et al. 1976, Kolsh and Falkenberg 1978, Zaleski and Klein 1978).

Comparison of the *H-2* haplotypes of the F_1 hybrids (Table 1, column B, ←) maps the gene governing high responsiveness to Thy-1.1 on normal AKR/J thymocytes to the *A* subregion of *H-2I*. (B10 × AKR/C)F_1 hybrids are high responders to Thy-1.1, while the F_1 hybrids (B10.BR × AKR/C)F_1 and [B10.A(4R) × AKR/C]F_1 are low responders. This maps the gene governing the responsiveness to the left of the *E* subregion of the *H-2I* region. (B10.S × AKR/C)F_1 hybrids can mount a strong anti-

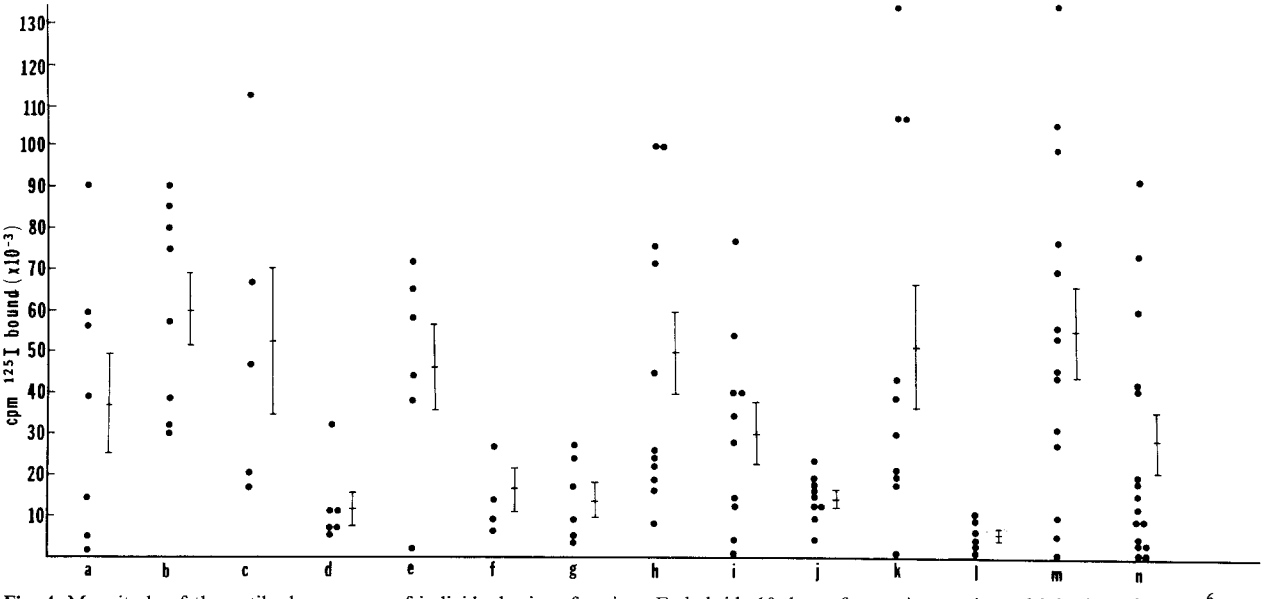

Fig. 4. Magnitude of the antibody response of individual mice of various F_1 hybrids 10 days after an intraperitoneal injection of 10×10^6 AKR/J thymocytes. Fifty microliters of serum diluted 1:10 was tested for reactivity against AKR/J thymocytes by a direct radioimmunoassay using ^{125}[I]-protein A. The *center line* indicates the mean antibody response; *upper* and *lower lines* indicate ± SE. Lane a represents the antibody response of individuals of (B10.Q × AKR/C)F_1 hybrids, mean antibody response ± SE: 37 571 ± 12 507; lane b, (B10 × AKR/C)F_1 hybrids, 60 750 ± 8703; lane c, (B10.RQB1 × AKR/C)F_1 hybrids, 52 800 ± 17 631; lane d, (B10.BR × AKR/C)F_1 hybrids, 12 333 ± 4031; lane e, (B10.D2 × AKR/C)F_1 hybrids, 46 500 ± 10 031; lane f, (B10.RKD2 × AKR/C)F_1 hybrids, 16 500 ± 5268; lane g, (B10.AQR × AKR/C)F_1 hybrids, 14 333 ± 4014; lane h, [B10.A(5R) × AKR/C]F_1 hybrids, 49 900 ± 10 247; lane i, [B10.A(3R) × AKR/C]F_1 hybrids, 30 500 ± 7502; lane j, [B10.A(4R) × AKR/C]F_1 hybrids, 14 222 ± 1839; lane k, (B10.S × AKR/C)F_1 hybrids, 52 200 ± 14 795; lane l, (B10.RSF2 × AKR/C)F_1 hybrids, 5667 ± 1542; lane m, (C3H.Q × AKR/C)F_1 hybrids, 55 286 ± 10 738; lane n, (C3H × AKR/C)F_1 hybrids, 29 118 ± 7690. Statistical analysis (T-test analysis) demonstrates a highly significant difference ($P > 0.001$) between the mean antibody response of the strains of the (AKR/C × C57BL/10)F_1 *H-2* congenics which are high responders to Thy-1.1 on AKR/J thymocytes and those strains which are low responders

body response to Thy-1.1, while (B10.RSF2 × AKR/C)F_1 hybrids mount a low response. This maps the gene governing responsiveness to Thy-1.1 to the *A* subregion. F_1 hybrids with at least one copy of the *q*, *d*, *b*, or *s* allele in the *A* subregion can mount a strong response to the Thy-1.1 antigen on AKR/J thymocytes.

Resistance to growth of BW5147 cells does not merely result from the ability of the F_1 hybrids to respond to the Thy-1 antigen. As shown in Table 1, F_1 hybrids with the *d*, *b*, or *s* allele in the *A* subregion can mount a strong response to the Thy-1.1 antigen on AKR/J thymocytes, yet they succumb to tumor growth.

Activity to other proteins besides Thy-1 are involved in resistance to BW5147. Hyperimmune anti-BW5147 serum produced in the resistant strains (C3H.Q × AKR/C)F_1 and (B10.Q × AKR/C)F_1 was extensively absorbed with AKR/J thymocytes to remove Thy-1.1 activity, as described in *Materials and Methods*. The absorbed antiserum did not contain detectable activity to Thy-1.1, but contained activity to other proteins present on BW5147 cells and not on normal thymocytes (Fig. 3). Passive antibody treatment using absorbed antiserum still provided protection against growth of BW5147 cells in susceptible mice. (C3H × AKR/C)F_1 mice treated with absorbed anti-BW5147 serum had an increased survival rate [75% survival (3 out of 4)], compared to untreated mice, which succumbed to tumor growth (0 out of 5; Table 2, experiment 3). (B10 × AKR/C)F_1 mice also showed an increased survival rate when mice were treated with anti-BW5147 serum absorbed with AKR/J thymocytes [50% survival (3 out of 6)], compared with 13% (1 out of 8) survival of mice which did not receive passive antibody treatment (Table 2, experiment 4).

The involvement of a strong antibody response to other antigens present on BW5147 cells besides Thy-1 in tumor rejection is also apparent from the increased survival rate [100% survival (11 out of 11)] of mice which have been inoculated with the Thy-1-negative variant BW5147 G.1 if the mice were previously hyperimmunized with BW5147 cells (Table 4). Only 13% (5 out of 38) of the mice of strains which are resistant to growth of BW5147 cells could survive a primary inoculation of Thy-1-negative BW5147 G.1 cells (Table 4). Mice which have been preimmunized once with BW5147 cells prior to inoculation of the variant show a slight increased survival rate [29% survival (2 out of 7)]. Mice which have been immunized twice with AKR/J thymocytes do not survive an inoculation of BW5147 G.1 (0 out of 4; Table 4).

Similar studies were repeated using the Thy-1-negative variant BW5147.3. Seventeen percent (2 out of 12) of the mice of strains which are resistant to BW5147 cells could survive an inoculation of BW5147.3 cells. However, in this case the ability of the mice to resist growth of BW5147.3 cells after priming appears to be due to a secondary response elicited to the Thy-1.1 antigen. One hundred percent of the mice could resist growth of BW5147.3 if the mice were hyperimmunized with BW5147 cells, immunized once with BW5147 cells, or immunized with 5×10^6 AKR/J thymocytes (Table 4). As previously discussed, BW5147.3 contains low levels of Thy-1.1, approximately 7% of the level found on the original BW5147 cells.

Identification of other antigens detected by anti-BW5147 sera. In order to examine which surface proteins are recognized by anti-BW5147 serum and their possible involvement in tumor rejection, iodinated cell-surface proteins from BW5147 cells were immunoprecipitated with anti-BW5147 hyperimmune serum. Proteins with molecular masses of 70 000, 33 000, a doublet at 29 000, 26 000, and 23 000 were immunoprecipitated with anti-BW5147 serum (Fig. 5, lane 2).

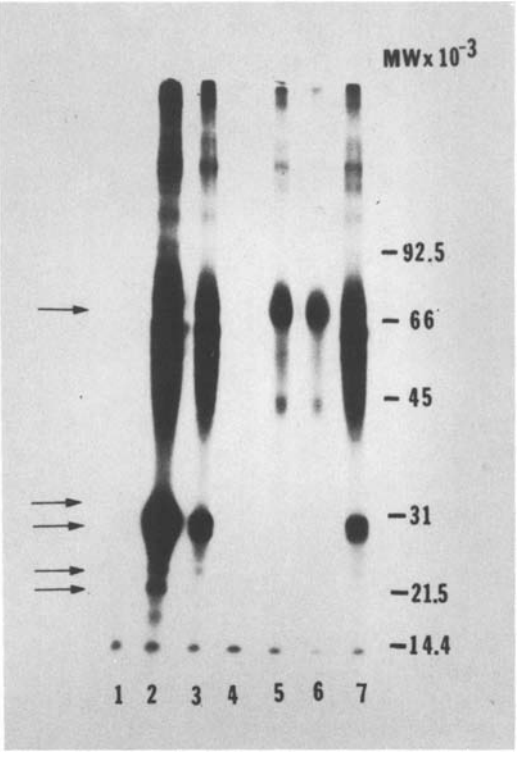

Fig. 5. Iodinated cell-surface proteins which are immunoprecipitated with anti-BW5147 serum. Equal TCA-precipitable counts of ^{125}[I]-labeled cell extracts from BW5147 cells (lanes 1 and 2) and the Thy-1-negative variant, BW5147 G.1 (lanes 3–7) were immunoprecipitated with various sera and analyzed by SDS-PAGE and autoradiography as described in *Materials and Methods*. Lanes 2 and 3 represent extracts immunoprecipitated with hyperimmune anti-BW5147 serum; lanes 1 and 4, NMS; lane 5, primary anti-BW5147 G.1 serum; lane 6, serum from (C3H.Q × AKR/C)F_1 hybrids that were hyperimmunized with BW5147 cells 40 days after the last immunization; lane 7, serum from mice hyperimmunized with BW5147 cells, then injected with BW5147 G.1 40 days after the last immunization

The Thy-1-negative variants express surface p70, p33, p29, p26, and p23, although at reduced levels compared to wild-type BW5147 cells (Fig. 5, BW5147, lane 2; BW5147 G.1, lane 3). The F_1 hybrids generally do not mount a strong antibody response to surface p33, p29, p26, and p23 after a primary injection of the Thy-1-negative variants (Fig. 5, lane 5). Serum from mice which were hyperimmunized with BW5147 cells and then inoculated with the Thy-1-negative variants contained activity to surface p33, p29, p26, and p23 (Fig. 5, lane 7). Serum from hyperimmunized mice 40 days after the last immunization which were not inoculated with the variants did not contain antibody activity to surface p33, p29, p26, or p23 (Fig. 5, lane 6). These observations are consistent with the fact that preimmunization of the mice with BW5147 cells renders the mice resistant to growth of the Thy-1-negative variants.

Biosynthetic labeling of BW5147 cells with various radiochemicals allowed the identification of several other proteins which immunoprecipitated with anti-BW5147 serum. A 78 000 molecular mass protein, p78, was identified with ^{3}[H]-leucine-labeled cell extracts (see Fig. 6, lane 1). BW5147 cells were also radiolabeled with ^{32}[P]-orthophosphoric acid. Five phosphoproteins with molecular masses of 33 000, 29 000, 23 000, 17 000, and 16 000 were immunoprecipitated with anti-BW5147 serum from

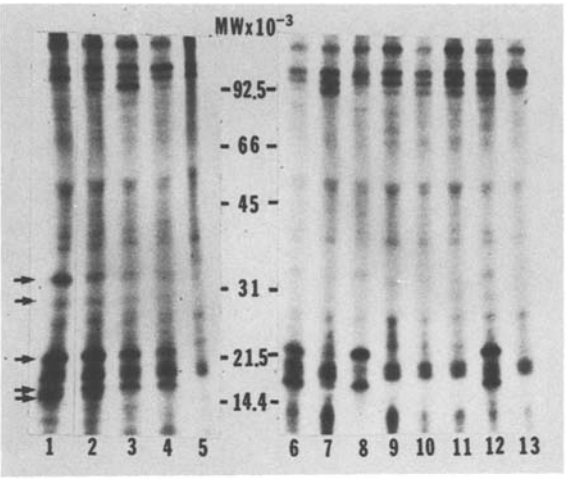

Fig. 7. The ability of various H-2 congenic F_1 hybrids to mount an antibody response to pp33, pp29, pp23, pp17, and pp16 10 days after an intraperitoneal injection of BW5147 cells. ^{32}[P]-orthophosphoric acid-labeled BW5147 cell extracts were immunoprecipitated with sera from the H-2 congenic F_1 hybrids (B10.RQB1 × AKR/C)F_1 (lane 1), (B10.Q × AKR/C)F_1 (lane 2), (B10.RKD2 × AKR/C)F_1 (lane 3), (B10.AQR × AKR/C)F_1 (lane 4), (B10.D2 × AKR/C)F_1 (lane 5), [B10.A(4R) × AKR/C]F_1 (lane 6), [B10.A(5R) × AKR/C]F_1 (lane 7), (B10.RSF2 × AKR/C)F_1 (lane 8), [B10.A(3R) × AKR/C]F_1 (lane 10), (B10 × AKR/C)F_1 (lane 11), (B10.BR × AKR/C)F_1 (lane 12), (B10.S × AKR/C)F_1 (lane 13) and analyzed by SDS-PAGE and autoradiography. Lane 9 is an immunoprecipitate using normal serum as a control

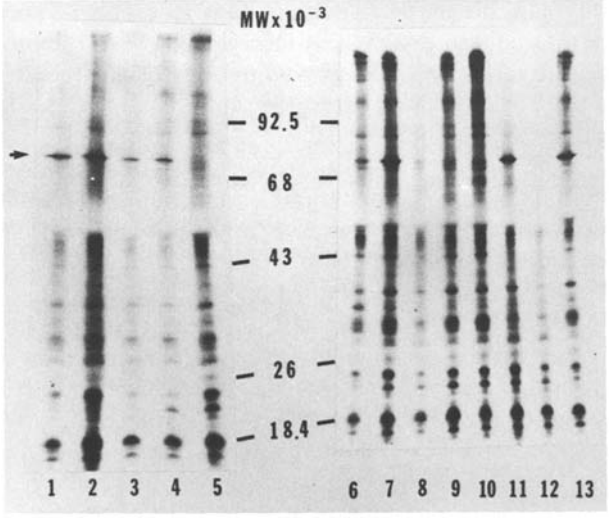

Fig. 6. The ability of various H-2 congenic F_1 hybrids to mount an antibody response to p78 10 days after an intraperitoneal injection of BW5147 cells. ^{3}[H]-leucine-labeled BW5147 cell extracts were immunoprecipitated with sera from the H-2 congenic F_1 hybrids (B10.Q × AKR/C)F_1 (lane 1), (B10.RQB1 × AKR/C)F_1 (lane 2), (B10.RKD2 × AKR/C)F_1 (lane 3), (B10.AQR × AKR/C)F_1 (lane 4), (B10.D2 × AKR/C)F_1 (lane 6), (B10.BR × AKR/C)F_1 (lane 7), (B10 × AKR/C)F_1 (lane 8), [B10.A(5R) × AKR/C]F_1 (lane 9), [B10.A(3R) × AKR/C]F_1 (lane 10), [B10.A(4R) × AKR/C]F_1 (lane 11), (B10.S × AKR/C)F_1 (lane 12), (B10.RSF2 × AKR/C)F_1 (lane 13) and analyzed by SDS-PAGE and fluorography. Lane 5 is an immunoprecipitate using NMS as a control

^{32}[P]-orthophosphoric acid-labeled BW5147 cell extracts (see Fig. 7, lane 1).

Characterization of the proteins recognized by anti-BW5147 serum. The iodinated 70 000 molecular mass protein immunoprecipitated by anti-BW5147 serum appears to be similar to the retroviral envelope protein, gp70. The 70 000 molecular mass protein immunoprecipitated with anti-BW5147 serum from iodinated BW5147 cell extracts generated identical partial protease peptide maps (Cleaveland et al. 1977), compared to gp70 immunoprecipitated from BW5147 cells with anti-gp70 serum (data not shown).

Although the molecular mass of p78 is similar to the retroviral envelope protein gp70, which immunoprecipitates with goat anti-gp70 as a heterogeneous group of proteins, the proteins are not identical. p78 is not accessible to surface iodination, while gp70 is readily iodinated. p78 does not contain carbohydrates attached through N-linked asparagine residues. The mobility of the 78 000 molecular mass protein on SDS-PAGE after treatment of cells with tunicamycin was identical to those on untreated cells (Fig. 8, lanes 9 and 4, respectively). Retroviral envelope protein gp70 (Fig. 8, lane 3) showed increased mobility after tunicamycin treatment to a molecular mass of 64 000 (Fig. 8, lane 8). pr65 and p30, nonglycosylated

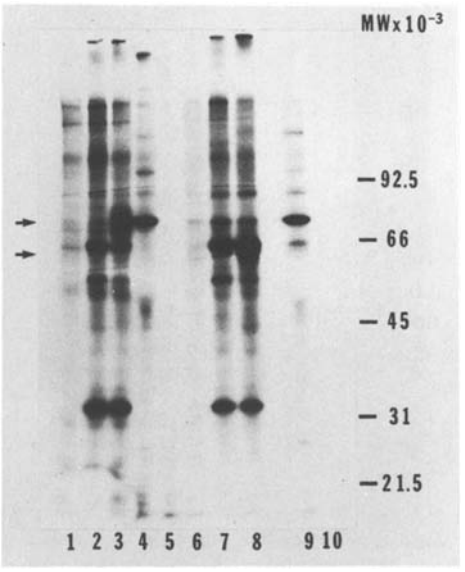

Fig. 8. Tunicamycin-treatment of BW5147 cells. Cell extracts from ^{3}[H]-leucine-labeled BW5147 cells that had been previously incubated with (lanes 6–10) or without (lanes 1–5) tunicamycin were immunoprecipitated with normal goat serum (lanes 1 and 6), anti-p30 (lanes 2 and 7), anti-AKR MuLV (lanes 3 and 8), anti-BW5147 (lanes 4 and 9), and NMS (lanes 5 and 10) and run on SDS-PAGE and examined by fluorography

viral core proteins, do not have an altered molecular mass after tunicamycin treatment (Fig. 8, untreated, lane 2; tunicamycin-treated, lane 7). There was no detectable incorporation of titiated sugars (^{3}[H]-mannose, ^{3}[H]-glucosamine, ^{3}[H]-fucose) into p78, while the sugars were readily incorporated into gp70 (data not shown).

The surface proteins p33, p29, and p23 have similar molecular masses as three of the phosphorylated proteins immunoprecipitated by anti-BW5147 serum. It was thought that p26 might be the Thy-1 molecule, which has a reported molecular mass of 25 000 (Letarte-Muirhead et al. 1975). However, the 26 000 molecular mass protein is immunoprecipitated from the Thy-1-negative variant BW5147 G.1 (Fig. 5, lane 3), which was shown to express less than 1% of the Thy-1 content compared to wild-type BW5147 cells.

Mapping and characterization of Irs to p78, pp33, pp29, pp23, pp17, and pp16. The various H-2 congenic F_1 hybrids were tested for their ability to respond to p78 (Fig. 6) and to the phosphoproteins (Fig. 7) after an intraperitoneal injection of BW5147 cells. The F_1 hybrids (B10. RQB1 × AKR/C)F_1, (B10.Q × AKR/C)F_1, (B10. RKD2 × AKR/C)F_1, (B10.AQR × AKR/C)F_1, (B10. BR × AKR/C)F_1, [B10.A(4R) × AKR/C]F_1, and (B10. RSF2 × AKR/C)F_1 are able to mount an antibody response to the 78 000 protein (Fig. 6, lanes 2, 1, 3, 4, 7, 11, 13, respectively) and to the phosphoproteins (Fig. 7, lanes 1, 2, 3, 4, 12, 6, 8, respectively). Immunoprecipitation of pp33, pp29, and pp16 is often weakly detectable when primary serum is used for immunoprecipitation.

The F_1 hybrids (B10.RQB1 × AKR/C)F_1 and (B10.Q × AKR/C)F_1 (Fig. 7, lanes 1 and 2, respectively) which are resistant to growth of BW5147 cells and tumor development, mount a stronger antibody response to the phosphoproteins than the F_1 hybrids (B10.RKD2 × AKR/C)F_1, (B10.AQR × AKR/C)F_1, [B10.A(4R) × AKR/C]F_1, (B10.RSF2 × AKR/C)F_1, and (B10.BR × AKR/C)F_1 (Fig. 6, lanes 3, 4, 6, 8, 12, respectively), which respond to the phosphoproteins yet are susceptible to tumor development.

The F_1 hybrids (C3H × AKR/C)F_1 (data not shown), (B10.D2 × AKR/C)F_1, (B10 × AKR/C)F_1, [B10.A(5R) × AKR/C]F_1, [B10.A(3R) × AKR/C]F_1, and (B10.S × AKR/C)F_1 cannot mount a response to p78 and the phosphoproteins (Fig. 6, lanes 6, 8, 9, 10, 12, respectively; Fig. 7, lanes 5, 11, 7, 10, 13, respectively). Comparison of the *H-2* haplotype of the F_1 hybrids (Table 1, column C, ←) allows location of the gene governing responsiveness to p78 and to the phosphoproteins, to the *A* subregion of the *H-2* complex. The F_1 hybrids (B10.Q × AKR/C)F_1 and (B10.RQB1 × AKR/C)F_1 respond to p78 and the phosphoproteins, while (B10 × AKR/C)F_1 hybrids do not respond. This maps the gene governing responsiveness left of the *H-2D* region. The F_1 hybrids (B10.BR × AKR/C)F_1 and (B10.RSF2 × AKR/C)F_1 respond to the proteins, while the F_1 hybrids (B10 × AKR/C)F_1 and (B10.S × AKR/C)F_1 do not respond. This maps the gene governing responsiveness to p78 and the phosphoproteins to the *A* subregion of the *H-2* complex. F_1 hybrids with at least one copy of the *q* or two copies of the *k* allele in the *A* subregion can respond to the p78 and to the phosphoproteins.

Comparing the ability of the F_1 hybrids to respond to Thy-1.1, p78, pp33, pp29, pp23, pp17, and pp16 with survival (Table 1, columns A, B, and C), it appears that only mice which are capable of responding to Thy-1.1 and to the other proteins identified by immunoprecipitation with anti-BW5147 serum can resist tumor development. As seen in the response to iodinated proteins, the F_1 hybrids generally do not mount a strong antibody response to p78 or the phosphoproteins after a primary injection of the Thy-1-negative variants (data not shown). However, serum from mice which were hyperimmunized with BW5147 cells and then inoculated with the Thy-1-negative variants also contained activity to p78 and the phosphoproteins (data not shown).

Discussion

H-2 congenic F_1 hybrids from crosses of AKR/C and C3H or C57BL/10 backgrounds which could resist tumor growth could mount a strong antibody response to an

intraperitoneal injection of BW5147 cells. The gene(s) governing resistance maps to the *I* region of the *H-2* complex. Mice with at least one copy of the *q* haplotype in the *I* region could survive tumor inoculation and mount a strong antibody response. The antibody produced in response to BW5147 is cytotoxic to the tumor in vitro in the presence of extraneous rabbit complement. Resistance to tumor growth in genetically susceptible strains could be obtained by the passive transfer of hyperimmune anti-BW5147 serum produced in resistant hosts.

The antigenic activities present in anti-BW5147 serum were analyzed. The antibody response is complex and contains activity against many antigens including Thy-1.1. The anti-BW5147 serum also contains activity to other previously uncharacterized antigens including a 78 000 molecular mass protein, five phosphoproteins with molecular masses of 33 000, 29 000, 23 000, 17 000, and 16 000, and a surface protein of 26 000. Serum from nonimmunized F_1 hybrids (Vlug et al. 1981), as well as from the immunized F_1 hybrids used in this study, contains antibodies reactive against purified MuLV, as seen in this study by the reactivity to iodinated retroviral gp70 molecules. Detection of a number of the identified proteins such as the retroviral gp70 molecule, p26 and the phosphoproteins requires labeling with a high energy radioactive compound such as ^{125}[I]-iodine or ^{32}[P]-orthophosphoric acid.

The Thy-1 response appears to be important for rejection of BW5147 cells, as the F_1 hybrid strain (C3H.Q × AKR/J)F_1, which is genetically similar to the resistant strain (C3H.Q × AKR/C)F_1 but contains the same *Thy-1* allele as the tumor, succumbs to tumor growth. The importance of the anti-Thy-1 response for survival is also shown by decreased survival rates when mice are inoculated with Thy-1-negative variants as compared to wild-type BW5147 cells. However, resistance to growth of BW5147 cells does not correlate with the ability of the F_1 hybrids to respond to Thy-1. F_1 hybrids with at least one copy of the *q*, *d*, *b*, or *s* allele of the *A* gene respond to Thy-1, yet only F_1 hybrids bearing the *q* haplotype can survive inoculation of BW5147 cells.

A strong response to other antigens besides Thy-1 present on BW5147 cells appears to be involved in tumor rejection. Anti-BW5147 serum which has been extensively absorbed with normal AKR/J thymocytes to remove anti-Thy-1 activity still provides protection against tumor development when passively administered to susceptible mice. There was a 60% survival rate when susceptible strains of mice were passively immunized with anti-BW5147 serum which was absorbed with AKR/J thymocytes, compared to 8% survival of untreated mice. However, survival was 95% when mice were passively immunized with unabsorbed antiserum. The decreased survival rate observed when mice were treated with absorbed serum compared to unabsorbed serum could indicate a need for a strong antibody response for tumor rejection. The anti-Thy-1 activity could add to the magnitude of the response.

Mice which have been hyperimmunized with BW5147 cells can resist an inoculation of the Thy-1-negative variant BW5147 G.1 which contains less than 1% of the quantity of surface Thy-1 that is expressed on wild-type BW5147 cells. It does not seem likely that the Thy-1 response is involved in the destruction of BW5147 G.1 cells, as mice that have been inoculated twice with AKR/J thymocytes do not survive inoculation of BW5147 G.1 cells. Hyperimmunization of mice to other antigens present on BW5147 cells besides Thy-1, including p78, the phosphoproteins, and surface p26, allows protection of the mice against tumor development. The Thy-1-negative variants express surface p33, p29, p26, and p23, although at reduced levels compared to wild-type BW5147 cells. Mice do not mount a strong response to p33, p29, p26, p23, and p78 after a primary inoculation of the Thy-1-negative variant. The reduced antibody response may be due to the reduced levels of surface proteins present on the variants compared to wild-type BW5147 cells. The majority of the mice succumb to growth of the variants. However, if mice are hyperimmunized with BW5147 and then injected with the variants, the mice respond to p78, p33, p29, p26, and p23 and can survive tumor inoculation.

The Thy-1 variant BW5147.3 contains low levels of Thy-1, approximately 7% of that found on wild-type BW5147 cells. Mice could resist growth of BW5147.3 if hyperimmunized with BW5147 cells, immunized once with BW5147 cells, or immunized with AKR/J thymocytes. The increased survival rate observed after priming appears to be due to a secondary response elicited to the Thy-1.1 antigen.

These experiments show that multiple antigens are recognized in response to the inoculation of BW5147 cells and that only F_1 hybrid mice which can resist growth of BW5147 are those capable of responding to Thy-1, p78 and pp33, pp29, pp23, pp17, and pp16. One can speculate on the possible roles of the identified antigens. p78, which was shown to differ from the retroviral envelope protein gp70, appears to be restricted to expression on BW5147 cells. Other lymphoma cell lines were examined for the presence of p78 by biosynthetic labeling and immunoprecipitation with anti-BW5147 serum. p78 is detectable only in BW5147 cells and in the Thy-1-negative variants BW5147 G.1 and BW5147.3 (data not shown). p78 was detectable neither in other tissue culture-adapted spontaneous lymphomas tested, including the AKR/C spontaneous lymphomas AKR/C-1 and AKR/C-2, the AKR/J spontaneous lymphoma AKR/J M4, the Abelson MuLV-induced B-cell lymphoma 18-81, the Moloney MuLV-induced T-cell lymphoma YAC-1, the RadLV-induced T-cell leukemia AQR-RadLV, the B10.G radiation-induced lymphoma, B10.G-X1, nor in normal spleen cells

or thymocytes. A response to an antigen with restricted expression may be involved in tumor rejection.

Proteins with molecular masses of 33 000, 29 000, and 23 000 are present on the surface of BW5147 cells. pp33, pp29, pp23, pp17, and pp16 have been detected in all rapidly dividing cells examined, including both lymphoid and nonlymphoid, normal and transformed cells from various species including murine and human (M.-A. Zalman and D. Meruelo, manuscript submitted). The phosphoproteins are associated with the proliferative state of the cell, as studied in many systems, including growth stimulation of normal cells with mitogens, interleukin-2 dependency for growth of a cloned T-cell line, cessation of proliferation by serum starvation of Swiss 3T3 fibroblasts and the retention of the proliferative capacity of SV40-transformed 3T3 fibroblasts, differentiation and inhibition of proliferation of human promyelocytic leukemic cells (M.-A. Zalman and D. Meruelo, manuscript submitted). Thus, the antibody response against the phosphoproteins may be important in inhibiting tumor growth, as these proteins appear to play a role in cell proliferation.

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