Proc. Natl. Acad. Sci. USA
Vol. 77, No. 4, pp. 2178–2182, April 1980
Immunology

In vivo or *in vitro* treatments with anti-I-J alloantisera abolish immunity to AKR leukemia

(*Ir* genes/T cells/humoral responsiveness/*H-2*-linked loci/tumor cells)

DANIEL MERUELO*, NANETTE FLIEGER*, DAWN SMITH*, AND HUGH O. MCDEVITT†

*Department of Pathology, Irvington House Institute, New York University Medical Center, 550 First Avenue, New York, New York 10016; and †Department of Medicine, Division of Immunology, Stanford University School of Medicine, Stanford, California 94086

Contributed by Hugh O. McDevitt, November 30, 1979

ABSTRACT **This paper provides evidence for the involvement of immune mechanisms in conferring resistance to a spontaneous AKR leukemia. It is shown that genes in the *B*, *J*, or *E* subregions of the *H-2* complex confer resistance to a spontaneously arisen, tissue culture-adapted AKR thymoma, BW5147. A direct correlation is demonstrated between survival to injected BW5147 cells and humoral responsiveness in various hybrids obtained from crosses of AKR mice and C57BL/10 or C3H/DiSn derived congeneic strains differing at *H-2*. Cellular immunity appears to play no role in resistance to the proliferation of tumor cells. It is further established that development of effective humoral immunity depends on B cells and Ly-1$^+$, 2$^-$, 3$^-$ helper T-cells bearing the I-J^k phenotype. These findings seem directly applicable to the spontaneous disease, and results of studies using transformed cells from an overtly leukemic AKR mouse parallel those obtained using BW1547 cells.**

The involvement of the major histocompatibility complex, *H-2*, in resistance or susceptibility to virus-induced leukemogenesis was first demonstrated experimentally by Lilly and his coworkers (1) and subsequently extended by numerous investigators (2). The mechanisms of action of *H-2*-linked loci in disease associations are unknown. Some investigators (3–7) have suggested that *H-2*-linked resistance to virus-induced leukemogenesis may result from genetically controlled variation in immune responses to virus-induced antigens (8). The present communication provides evidence for the involvement of immune mechanisms in conferring resistance to a spontaneous AKR leukemia and defines the humoral response involved, including the involvement of an I-J$^+$, Ly-1$^+$, 2$^-$, 3$^-$ T cell.

MATERIALS AND METHODS

Mice. All mice used in the present studies were bred at New York University Medical Center from animals derived from H.O.M.'s colony at Stanford University School of Medicine.

Cells. BW5147 cells were originally obtained from the Salk Institute (La Jolla, CA) and have been maintained independently since 1975. Their karyotype and growth conditions have been described (9).

Antisera. Antisera were produced by published protocols (10–12). The following antisera were utilized: α Thy 1.2 (AKR/J anti-AKR/Cum); α J^k ([BALB.B × B10.A(3R)]F$_1$ anti-B10.A(5R)); αA^k ([B10.S(9R) × A.TFR5]F$_1$ anti-A-TL) αA^{k}B^{k}J^{k}E^{k}C^{k}S^{k}G^k (see ref. 13 regarding potential contaminants) (A.TH anti-A.TL); αA^{s}B^{s}J^{s}E^{s}C^{s}S^{s}G^s (A.TL anti-A.TH); αA^{k}B^{k}J^k [(B10.HTT × A.TH)F$_1$ anti-A.TL]; αD^k [(A.TL × B10.A)F$_1$ anti-B10.BR]; αK^k [A.TL × C3H.OL)F$_1$ anti-C3H]; αE^{k}C^{k}S^{k}G^k [B10.S(7R) anti-B10.HTT]. Anti-D^q [B10.A × LP.RIII)F$_1$ anti-B10.AKM] was kindly provided by John G. Ray, Jr. (Research Resources Branch, National Institutes of Health, Bethesda, MD). Rabbit anti-mouse IgG was purchased from Antibodies, Inc. (Davis, CA). Anti-Ly 1.2 [(C3H × B6/Ly 1.1)F$_1$ anti-CE/J or anti-C57BL/6] and anti Ly-2.1 antisera [(BALB.K × C57BL/6)F$_1$ anti CE/J] were prepared and screened as recommended by Shen *et al.* (10).

Purification on Nylon Wood. Purification of T cells was done on nylon wool columns according to the method of Julius *et al.* (14).

Antiserum-Plus-Complement-Mediated Cytotoxicity. Antiserum-plus-complement-mediated cytotoxicity was done as described (15) except that unabsorbed rabbit complement (Dutchland) was used at a 1:8 dilution.

Cell-Binding Radioimmunoassay. Immunoglobulin levels in various antisera were determined either by the binding of antibodies to antigens bound in solid phase (to microtiter plates) as described by Klinman *et al.* (16) or by a modification of cell-binding assays (17). After a 30-min incubation with antisera, cells were washed once and resuspended in 50 μl of a 1:100 dilution of ^{125}I-labeled protein A. After 30 min, the cells were washed thrice and transferred to a counting tube for determination of bound radioactivity in a Beckman Biogamma 4000 counter.

Radioiodination of Protein A. Protein A extracted from *Staphylococcus aureus*, Cowan I strain (Pharmacia), was labeled by a modification of the chloramine-T method (18).

Preparation of Effector Cells, ^{51}Cr-Labeled Target Cells, and Assay for Cell-Mediated Cytotoxicity. Cells were prepared and cell-mediated lympholysis activity was measured as described (9).

Absorption Studies. The expression of H-2 or murine leukemia virus antigens on the surface of lymphocytes was determined as described (17) by measuring the ability of these cells to adsorb the antibody activity(ies) in question.

Irradiation of Mice and Transfers of Cells. Recipient mice were exposed to 600 rads (6 grays) early on the day of transfer (or on the previous day) from a cesium-137 source (model M Gammator, Radiation Machinery, Parsippany, NJ). This machine delivers radiation at a rate of 630 rads/min when the dosage rate is set at 100%. Mice were then reconstituted with immune or nonimmune lymphocytes as described in the text.

Intravenous Injection of Alloantisera. Generally mice received 0.5 ml of the appropriate alloantisera (at 1:50 dilution). Unanesthetized mice were heated for a few minutes under a heat lamp (Fisher Infra-Radiator), inserted into a restraining

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: MCA, methylcholanthene.

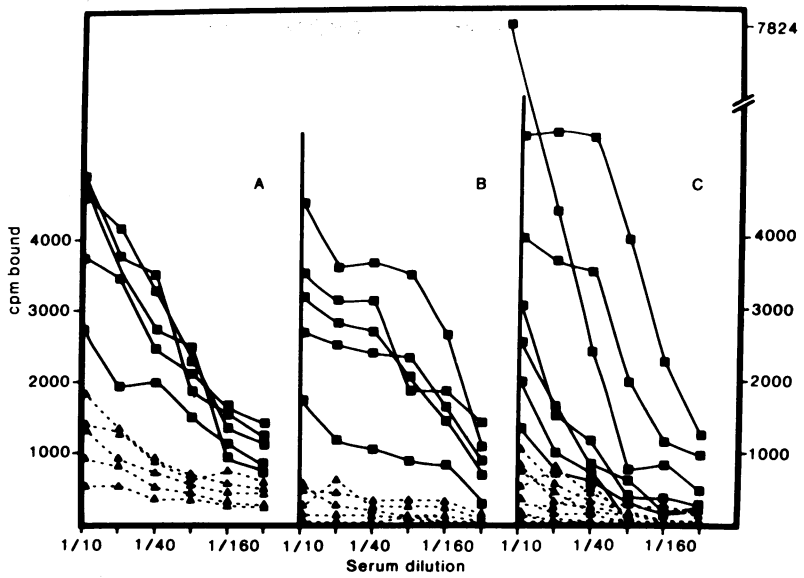

FIG. 1. Humoral immune response of various mice injected 10 days prior with 5×10^6 BW5147 cells, as detected by radioimmunoassay. (*A*) Tested against BW5147 cells; (*B*) tested against supernatants of AKR murine leukemia virus-infected III6A cells (the radioimmunoassay was done by first letting a solution of disrupted AKR murine leukemia virus adsorb onto disposable microcytotoxicity plates so that the virus was attached to a solid substrate) [for *A* and *B*: ■—■, (AKR × B10.6)F_1; ▲---▲, (AKR × B10.BR)F_1]. (*C*) Progeny of an (AKR × B10.G)F_1 × AKR backcross were typed for their *H-2* types [nine $H\text{-}2^{k/k}$ (▲---▲) and seven $H\text{-}2^{k/q}$ (■—■)] and their humoral response was measured against BW5147 cells.

holder, and injected through the tail vein by using a 1-ml disposable syringe fitted with a 27-gauge needle.

RESULTS

Gene(s) in the *B, J,* or *E* Subregions of the *H-2I* Region Confer Resistance to Malignant AKR Cells. In previous studies (9), AKR mice were crossed with animals of various *H-2* congeneic strains on the C57BL/10 or C3H genetic background and the hybrid mice were injected intraperitoneally with BW5147 AKR thymoma cells. Comparison of the cell-mediated immune response of hybrid mice differing in their *H-2* genotype showed a genetically controlled immune response difference. The present study showed that hybrids unable to mount a vigorous CML response to the injected tumor cells [namely, $H\text{-}2^{q/k}$ mice (B10.G × AKR)F_1 and (C3H.Q × AKR)F_1] survive longer than do hybrids capable of responding in a cell-mediated assay [primarily $H\text{-}2^{k/k}$ mice (CKB × AKR)F_1 and (B10.BR × AKR)F_1]. The results of four independent experiments in which various hybrid mice received 5×10^6 BW5147 cells intraperitoneally and were followed for at least 43 days are shown in Table 1. Analysis of the *H-2* haplotypes of the F_1 hybrids demonstrates that gene(s) mapping in the *B, J,* or *E* subregion of the *I* region confer resistance or susceptibility to BW5147 cells. Thus, only hybrids carrying at least one copy of the *q* or *d* allele in these three subregions survived beyond 43 days to any significant degree. These studies were repeated numerous times with the same results each time.

Table 1. Mapping of tumor resistance to the *B, J,* or *E* subregion of *H-2I*

Strain	% survival (day 43)	*H-2* haplotype of non-AKR parent K A B J E C S G D
	Exp. 1	
(B10.D2 × AKR)F_1	67	d d d d d d d d d
(B10.A(5R) × AKR)F_1	0	b b b k k d d d d
(B10.A(3R) × AKR)F_1	0	b b b b k d d d d
B10.BR × AKR	0	k k k k k k k k k
B10 × AKR	0	b b b b b b b b b
	Exp. 2	
(B10.G × AKR)F_1	90	q q q q q q q q q
(B10.AQR × AKR)F_1	0	q k k k k d d d d
(B10.A × AKR)F_1	0	k k k k k d d d d
(B10.BR × AKR)F_1	0	k k k k k k k k k
	Exp. 3	
(C3H.Q × AKR)F_1	80	q q q q q q q q q
(CKB × AKR)F_1	0	k k k k k k k k k
(B10.BR × AKR)F_1	0	k k k k k k k k k
	Exp. 4	
(D2GD × AKR)F_1	0	d d b b b b b b b
(B10.D2 × AKR)F_1	31*	d d d d d d d d d
(B10.G × AKR)F_1	73	q q q q q q q q q
(B10.BR × AKR)F_1	0	k k k k k k k k k

* Repeats of this experiment indicate that (B10.D2 × AKR)F_1 are consistently more resistant than all other F_1 mice except (B10.G × AKR)F_1, and a significant number usually survive tumor inoculation.

Table 2. Effect of intravenous injection of antisera on survival of (AKR × B10.G)F_1 receiving 5×10^6 BW5147 cells

Antiserum*	Mean (± SEM) survival, days	% survival at end of experiment
	Exp. I	
D^k	73.1 ± 12.2	50
$A^kB^kJ^kE^kC^kS^kG^k$	87.1 ± 13.0	67
K^k	87.2 ± 12.1	58
A^k	114.8 ± 5.3	87.5
J^k	51.1 ± 13.2	0
None	105.5 ± 8.9	75
	Exp. II	
$A^kB^kJ^kE^kC^kS^kG^k$	79.9 ± 20.3	57
A^k	97.8 ± 16.5	75
$A^kB^kJ^k$	36.9 ± 12.3	0
E^kC^k	76.3 ± 16.8	63
$A^sB^sJ^sE^sC^s$	81.3 ± 19.7	57
None	89.5 ± 16.3	62

* Defined on basis of antigen potentially recognized.

Table 3. Effect of intravenous injection of various antisera on survival and humoral immunity of (AKR × B10.G)F_1 injected with 5 × 10^6 BW5147 cells

Antiserum*	Mean (± SEM) survival, days	% survival at end of experiment†	% reduction in titer of a BW5147 serum†
D^q	48.2 ± 6.3	67	5
D^k	54.0 ± 6.8	75	7
A^k	51.3 ± 6.5	67	8
J^k	30.9 ± 5.7	22	69
$A^kB^kJ^k$	34.4 ± 6.1	25	50
$A^kB^kJ^k$‡	37.7 ± 7.6	28	53
Ly-1.2	38.8 ± 4.6	36	69
Ly-2.1	52.8 ± 6.4	64	10
θ^{C3H}	37.0 ± 4.9	33	54
None	56.4 ± 6.7	66	0

* Defined on basis of antigen potentially recognized.
† At 90 days. For correlation of % survival with % reduction in titer, coefficient = −0.94; for $n = 10$, $P < 0.01$.
‡ Absorbed with BW5147 cells.

The lack of additional recombinants does not permit more precise assignment of the gene(s) involved in either *B*, *J*, or *E*.

Importance of the Humoral Response and Effects of Passive Antiserum Treatments. Although a reciprocal correlation was found between CML responsiveness and survival, a direct relationship was found between the latter and humoral responsiveness (Fig. 1*A*). (B10.G × AKR)F_1 mice ($H\text{-}2^{q/k}$) showed a stronger response to an intraperitoneal injection of BW5147 cells than did (B10.BR × AKR)F_1 mice ($H\text{-}2^{k/k}$). The humoral response was measurable equally well with BW5147 cells or AKR virus (Fig. 1 *A* and *B*). Linkage of the humoral response to *H-2* was readily demonstrable in a backcross segregation analysis of (B10.G × AKR)F_1 × AKR progeny (Fig. 1*C*).

To study the role of suppression in this system, experiments patterned after those of Greene *et al.* (19) were carried out. Greene *et al.* (19) have shown that daily injections of anti I-J^k antiserum inhibited growth *in vivo* of two different methylcholanthrene (MCA)-induced sarcomas, S1509a and Sa-I, presumably by abolishing tumor-specific suppressor cells. When resistant [(B10.G × AKR)F_1] (Table 2) and susceptible [(B10.BR × AKR)F_1] mice were injected every other day with 10 μl of one of various antisera, no effect of anti-I-J^k antiserum could be detected on survival of susceptible mice (data not shown). In fact, a contrary result was obtained: resistant mice receiving injections of anti-I-J^k showed decreased resistance to the tumor. This effect on resistant mice was examined in a second experiment and similar results were obtained.

A more extensive analysis of the effect of antiserum treatment on resistant mice (B10.G × AKR)F_1 is shown in Table 3. It can be seen from this experiment that: (*i*) treatment with antibodies to $A^kB^kJ^k$ or Ly-1.2 or Thy 1.2 markedly decreased mean survival time and final survival incidence; (*ii*) absorption of anti-$A^kB^kJ^k$ antiserum with BW5147 cells did not affect the serum's activity; and (*iii*) decreased resistance to the injected tumor cells was always associated with a decrease in the antiviral or antitumor humoral response.

Mouse alloantisera directed against H-2 determinants often contain antibodies to virus determinants (20). To ensure against a role of these possible contaminants in the effects observed, the various antisera used in these studies were tested for their reactivity with BW5147 cells. Although significant activity was detected in some antisera, no correlation could be found between this activity and an effect on survival of (B10.G × AKR)F_1 mice (data not shown).

Effect of Various Antisera in Cell Transfer Experiments. To ensure that effects seen with serum injections indeed reflected an effect on lymphocytes involved in the humoral response, a series of cell transfer experiments were carried out. These experiments demonstrated the following. (*i*) Transfer of nonimmune (C3H.Q × AKR)F_1 (resistant) spleen cells to syngeneic, lethally irradiated recipients did not reconstitute the humoral response (Fig. 2 *Right*) although it resulted in a cellular immune response, not normally seen in $H\text{-}2^{k/q}$ mice (Fig. 3), and rendered the mice completely unable to resist tumor growth (Fig. 2 *Left*). (*ii*) On the other hand, when reconstitu-

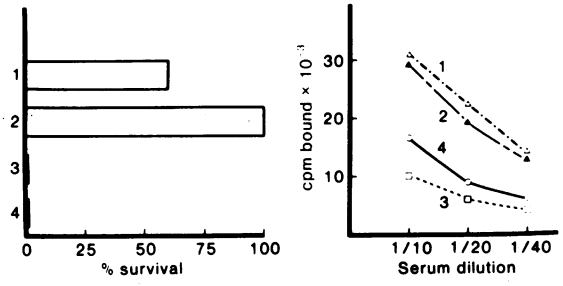

FIG. 2. Transfer of survival of humoral immunity to BW5147 by syngeneic immune lymphocytes. (AKR × C3H.Q)F_1 mice were untreated (bar 1), irradiated and reconstituted with immune cells (bar 2), or irradiated and reconstituted with nonimmune cells (bar 3). Bar 4 shows (AKR × CKB)F_1 mice. (*Left*) Survival after tumor inoculation. (*Right*) Humoral immunity 10 days after challenge with 5 × 10^6 BW5147 cells.

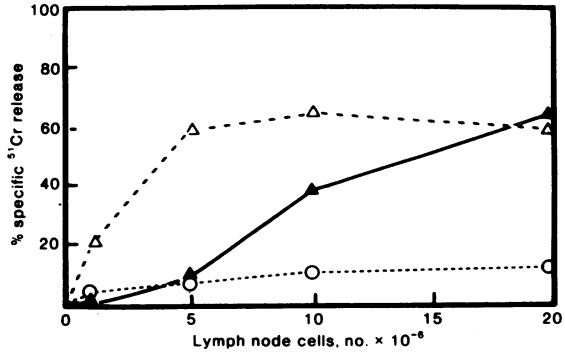

FIG. 3. Cell-mediated immune response of normal responder ($H\text{-}2^{k/k}$; △---△) and nonresponder mice ($H\text{-}2^{q/k}$; ○---○) as well as irradiated nonresponder mice (▲—▲) 10 days after injection of 5 × 10^6 BW5147 cells.

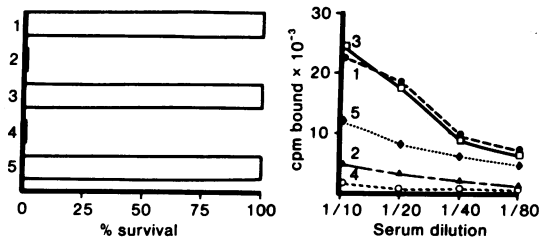

FIG. 4. Role of immune T and B cells in transfer of survival (*Left*) and humoral immunity (*Right*) to an inoculation of 5×10^6 BW5147 cells. Irradiated (AKR × B10.G) mice were reconstituted with: immune whole spleen (bar 1); nonimmune whole spleen (bar 2); nonimmune B cells/immune T cells (bar 3); immune B cells (bar 4); immune B cells/immune T cells (bar 5).

tion of irradiated (AKR × C3H.Q)F_1 mice was carried out with immune, syngeneic spleen cells, the humoral response was equal to that of normal hosts (Fig. 2 *Right*) and rendered the mice completely resistant to tumor growth (Fig. 2 *Left*). (*iii*) The requirement that transferred cells be preimmune applied only to T cells (Fig. 4). When immune or normal T and B cells were transferred, normal B cells functioned to protect the host in the presence of immune but not nonimmune T cells (Fig. 4 *Left*). A similar finding was obtained with respect to the humoral response (Fig. 4 *Right*).

When immune T cells were treated with antiserum to either I-J^k or Ly-1.2 plus complement prior to their transfer into irradiated syngeneic recipients, the capacity of the transferred immune T cells to reconstitute the host's humoral response (Fig. 5 *Right*) or its ability to survive the tumor inoculation (Fig. 5 *Left*) were completely abolished. On the other hand, treatment with anti-Ly-2.1 antiserum plus complement in exactly the same fashion had no effect on the transferred cells.

Only J^k-Positive Helper T Cells Are Required for Effective Humoral Antitumor Immunity. Because anti-$A^kB^kJ^k$ was as effective as anti-I-J^k, one possibility that needed analysis was that two T cells (for example, one A^k-positive and one J^k-positive) were required to help T-dependent B cells make an effective humoral response. Elimination of either one would be sufficient to abolish humoral immunity and resistance to BW5147 cells. Under this assumption, anti-I-J^k would work by abolishing I-J-positive T cells, and anti-$A^kB^kJ^k$ would work by abolishing I-J^k-positive or I-A^k-positive cells. To test this hypothesis, anti-$A^kB^kJ^k$ was absorbed with B10.A(4R) (*kk*bbbbbbb), B10.A(3R) (bbbb*k*dddd), or B10.A(5R) (bbb*kk*dddd) cells. Absorption with B10.A(4R), which removed all anti-A^k activity, rendered the antiserum even more powerful

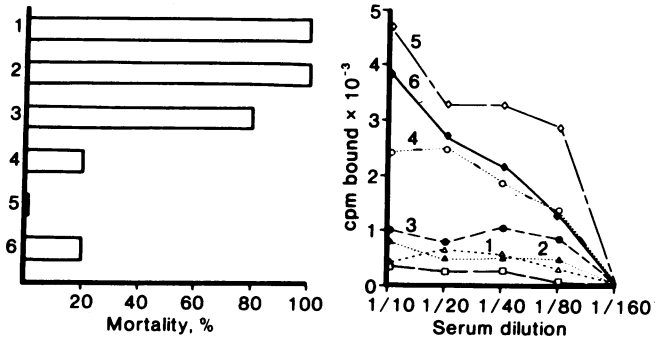

FIG. 5. Effect of various antisera on the capacity of immune T cells to transfer survival (*Left*) and humoral immunity (*Right*) to an inoculum of 5×10^6 BW5147 cells. Antisera: bar 1, $\alpha\,\theta$ + C; bar 2, α Ly-1 + C; bar 3, α I-J^k + C; bar 4, untreated; bar 5, α Ly-2 + C; bar 6, normal mouse serum + C.

Table 4. Result of various absorptions on the effect of anti-$A^kB^kJ^k$ on survival of (AKR × B10.G)F_1 mice after inoculation of 5×10^6 BW5147 cells

Cells used to absorb*	Mean (± SEM) survival, days
None	35 ± 6
B10.A(5R)	75 ± 10
B10.A(3R)	38 ± 3
B10.A(4R)	26 ± 5

* Each 50 μl of antiserum was absorbed with 200×10^6 cells for 1 hr at 4°C.

in abolishing resistance to growth of BW5147 cells (Table 4). In fact, this absorbed antiserum showed the strongest activity of any antiserum tested in the current experiments. Absorption with B10.A(5R), which removed anti-J^k reactivity, rendered the antiserum ineffective in abolishing resistance. Absorption with B10.A(3R) did not alter the antiserum's behavior, as would be expected. Therefore, all the helper T cell(s) involved in resistance to the tumor must display the I-J^k phenotype.

Relevance of Findings to the Spontaneous AKR Leukemia. Malignant AKR cells were extracted from the thymus and spleen of an overtly (spontaneously) leukemic AKR mouse and injected into (B10.G × AKR)F_1 mice being given 10 μl of antiserum every other day. The effect on survival for each antiserum was similar to that obtained in mice receiving BW5147 cells (Table 5).

DISCUSSION

The experiments reported here indicate that a T cell expressing the I-J^k, Ly-1^+, 2^-, 3^- phenotype is crucial for the development of a humoral response capable of rendering mice resistant to the growth of spontaneously developing malignant cells of AKR origin.

The present results differ from several other published reports. For example, Greene *et al.* (19) concluded that injection of anti-I-J^k antiserum inhibits growth of MCA-induced sarcomas in A/J mice by abolishing tumor-specific suppressor T cells and thus permitting a strong immune response in the host. Similarly, Fujimoto *et al.* (21, 22) have found that suppression by splenic T cells of *in vivo* or *in vitro* cell-mediated immunity against a homologous tumor can be eliminated by treatment of the suppressor T cells with anti-I-J antiserum. However, not all of the published data on immunity to autologous tumor cells indicate a key role for Ia-positive suppressor T cells. For example, Frelinger *et al.* (23) have shown that determinants coded in the *A*, *B*, or *J* subregions are expressed on T cells which are necessary for the adoptive transfer of syngeneic tumor immunity. Additional work since the original definition of the *I-J* region (24–26) has shown more functions associated with this subregion than were originally attributable to it. In addition to being found in suppressor cells and suppressor factors, I-J determinants are also found on macrophages (27) and helper T cells (28).

Several obvious differences exist between the present studies and those of Greene *et al.* (19): the size of the tumor inoculum,

Table 5. Survival of (AKR × B10.G)F_1 mice after receiving 10^7 spontaneous AKR leukemia cells intraperitoneally

Antiserum treatment	Mean (± SEM) survival, days	Mortality on day 43, %
αA^k	66 ± 10	17
$\alpha A^kB^kJ^k$	45 ± 7	75
αJ^k	44 ± 3	67

the type of tumor used, the route of administration, and the magnitude of the final effect. For example, when MCA-induced sarcoma cells were injected intramuscularly rather than subcutaneously, the regimen of passive antibody transfer resulted in enhanced growth of the tumor, in a fashion similar to the one reported here for mice receiving BW5147 cells.

Collaborative efforts between our laboratories have indicated that serum effective in eliminating suppressor cells (allowing growth of MCA-induced sarcomas) was also effective in abolishing resistance to BW5147 cells. The simplest conclusion from these experiments is that I-J determinants may be found on helper and suppressor subpopulations of T cells and that their effect will depend on the immunocytes activated by a given tumor challenge.

Several antisera were used in the present studies. Although those having activity only against $I\text{-}J^k$ abolished resistance, others potentially directed against the $I\text{-}J^k$ region (e.g., anti-$A^kB^kJ^kE^kC^kS^kG^k$) did not. This is not surprising because production of alloantibodies does not always lead to activity against all possible antigenic determinants.

Suppression does not seem to play a role in resistance to BW5147 by $H\text{-}2^{q/k}$ mice (treatment with anti-Ly-2.1, which would be expected to eliminate suppressor cells, had no effect), but it does seem to play a role in rendering $H\text{-}2^{k/k}$ mice susceptible to tumor growth. Repeated injections of anti-Ly-2.1 antiserum doubled the mean survival time of (AKR × B10.BR)F_1 mice (data not shown). The role of suppression in $H\text{-}2^{k/k}$ mice needs further study.

In view of the effect of anti-I-J^k antiserum on tumor proliferation in AKR mice, even when a primary tumor is used, it would appear that AKR mice could overcome the spontaneous disease if they could be brought to make the appropriate immune response. [The importance of humoral immunity in resistance to virus-induced leukemogenesis has also been recently demonstrated by Doig and Chesebro (29).] To achieve this experimentally, it would be required that the antigen(s) recognized by the humoral response be clearly identified and that unresponsive $H\text{-}2^{k/k}$ mice be made to respond.

We thank Ms. Anna Paolino for her invaluable help in the breeding of mice, Ms. Diane Teece for the preparation of antisera, and Ms. Beverly Coopersmith for her excellent secretarial assistance. This work was supported by National Institutes of Health Grants CA 22247 and AI 07757, American Cancer Society Grant IM-163, and a grant from the Irma T. Hirschl Foundation. D.M. is a Leukemia Society of America Scholar.

1. Lilly, F., Boyse, E. A. & Old, L. J. (1964) *Lancet* ii, 1207–1209.
2. Meruelo, D. & McDevitt, H. O. (1978) *Semin. Hematol.* **15,** 399–419.
3. McDevitt, H. O. & Bodmer, W. F. (1972) *Am. J. Med.* **52,** 1–8.
4. Lilly, F. & Pincus, T. (1973) *Adv. Cancer Res.* **17,** 231–277.
5. Lilly, F. (1970) *Bibl. Haematol. (Basel)* **36,** 213–220.
6. Aoki, T., Boyse, E. A. & Old, L. F. (1966) *Cancer Res.* **26,** 1415–1419.
7. Benacerraf, B. & McDevitt, H. O. (1972) *Science* **175,** 273–279.
8. Elder, J. H., Gautsch, J. W., Jensen, F. C. & Lerner, R. A. (1978) *J. Natl. Cancer Inst.* **61,** 625–629.
9. Meruelo, D., Deak, B. & McDevitt, H. O. (1977) *J. Exp. Med.* **146,** 1367–1379.
10. Shen, F. W., Boyse, E. A. & Cantor, H. (1975) *Immunogenetics* **2,** 591–595.
11. Reif, A. E. & Allen, J. M. V. (1963) *Nature (London)* **200,** 1332–1333.
12. Murphy, D. B. & Shreffler, D. D. (1975) *J. Exp. Med.* **141,** 374–391.
13. Flaherty, L., Stanton, T. H. & Boyse, E. A. (1977) *Immunogenetics* **4,** 101–103.
14. Julius, M. H., Simpson, E. & Herzenberg, L. A. (1973) *Eur. J. Immunol.* **3,** 645–649.
15. Meruelo, D. (1979 *J. Exp. Med.* **149,** 898–909.
16. Klinman, N. R., Pickard, A. R., Signal, N. H., Geartart, P. J., Metcalf, E. S. & Pierce, S. K. (1976) *Ann. Immunol. (Paris)* **1276,** 489–496.
17. Meruelo, D., Nimelstein, S. H., Jones, P. O., Lieberman, M. & McDevitt, H. O. (1978) *J. Exp. Med.* **147,** 470–487.
18. Hunter, M. W. & Greenwood, F. C. (1963) *Nature (London)* **194,** 495–497.
19. Greene, M. I., Dorf, M. E., Pierres, M. & Benacerraf, B. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5118–5121.
20. Nowinski, R. C. & Klein, P. A. (1975) *J. Immunol.* **155,** 1261–1268.
21. Fujimoto, S., Matanzawa, T., Nakagawa, K. & Tada, T. (1978) *Cell. Immunol.* **38,** 378–387.
22. Fujimoto, S., Yamaguchi, K., Yamada, S. & Tada, T. (1979) in *Symposia of the Immunology Area of the United States–Japan Cooperative Research Program,* in press.
23. Frelinger, J. A., Lukasewycz, O., Hill, S. W. & Hibbler, F. (1978) in *Ir Genes and Ia Antigens,* ed. McDevitt, H. O. (Academic, New York), pp. 417–421.
24. Murphy, D. B., Herzenberg, L. A., Okumura, K., Herzenberg, L. A. & McDevitt, H. O. (1976) *J. Exp. Med.* **144,** 699–712.
25. Tada, T., Taniguchi, M. & David, C. S. (1976) *J. Exp. Med.* **144,** 713–725.
26. Frelinger, J. A., Niederhuber, J. E. & Shreffler, D. C. (1976) *J. Exp. Med.* **144,** 1141–1146.
27. Niederhuber, J. E., Mayo, L. & Shreffler, D. C. (1978) in *Ir Genes and Ia Antigens,* ed. McDevitt, H. O. (Academic, New York), pp. 393–404.
28. Tada, T., Takemori, T., Okumura, K., Nanaka, M. & Tokuhisa, T. (1978) *J. Exp. Med.* **147,** 446–458.
29. Doig, D. & Chesebro, B. (1979) *J. Exp. Med.* **150,** 10–19.