INCREASED SYNTHESIS AND EXPRESSION OF H-2 ANTIGENS ON THYMOCYTES AS A RESULT OF RADIATION LEUKEMIA VIRUS INFECTION: A POSSIBLE MECHANISM FOR *H-2* LINKED CONTROL OF VIRUS-INDUCED NEOPLASIA*

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The murine major histocompatibility complex (MHC),[1] *H-2*, has been shown to have a marked effect on the outcome of infection by murine leukemia viruses, including Gross, Friend, BALB-tennant leukemia virus BT/L, and mammary tumor viruses (1). The mechanism of action of *H-2* linked loci in virus-induced leukemogenesis is unknown.

Some investigators (1) have suggested that *H-2* linked resistance to virus-induced leukemogenesis may result from genetically controlled variation in immune response to virus-induced antigens. This hypothesis arose from several observations. First, several immune response genes were found to map within *H-2*. These Ir genes regulate quantitatively and qualitatively the humoral response to a variety of natural and synthetic antigens (2). In addition, *Rgv-1*, a gene conferring resistance to Gross-virus induced tumorigenesis maps near or within the *I* region of the *H-2* complex (1). Furthermore, *H-2* gene(s) were found to influence a late event in the disease induced by Friend virus. No effect of *H-2* linked genes could be detected on the initial infection by Friend

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[1] *Abbreviations used in this paper*: AFSPBS, sodium azide and fetal calf serum supplemented phosphate-buffered saline; BT/L, BALB-Tennant leukemia virus; BSA, bovine serum albumin; CMC, cell-mediated cytotoxicity; FACS, fluorescence activated cell sorter; FCS, fetal calf serum; FV, Friend virus; *Fv-1*, Gene governing relative spleen focus response to F-S strain of FV; GAMIG, goat anti-mouse IgG; H, Heavy chain of IgG; Ir, Immune response; i.t., intrathymic; L, light chain of IgG; MHC, major histocompatibility complex; MuLV, murine leukemia virus; NMS, normal mouse serum; NP-40, Nonidet-P40 detergent; OVA, ovalbumin; PBS, phosphate-buffered saline; RadLV, radiation-induced leukemia virus; *Rgv-1*, Gene governing resistance to Gross virus; SDS-PAGE, sodium dodecyl-sulfate-polyacrylamide gel electrophoresis; 2-D, two dimensional.

virus (FV) but recovery from the splenomegaly it induced was significantly affected by genes within the MHC (3).

Support for this hypothesis was provided by studies of Aoki et al. (4) demonstrating that serum levels of anti-Gross virus antibodies were higher in mice homozygous or heterozygous for the resistant *H-2* haplotype than in animals homozygous for the susceptible *H-2* type. Similar findings were provided by Sato et al. (5) in studies of resistance to certain leukemias derived from BALB/c mice. Furthermore, Blank et al. (6) have found that animals of the *H-2* resistant haplotype can mount a cell-mediated cytotoxic (CMC) response to FV induced, tissue culture adapted, tumor cells, whereas mice of the susceptible *H-2* type cannot. By contrast, despite the clear association between *H-2* type and resistance or susceptibility to FV-disease, no evidence for *Ir* type mechanisms was uncovered by studies of Chesebro et al. (7–9). Using a different experimental system from that of Blank et al. (6), Chesebro and Wehrly (8, 9) found that *both* susceptible and resistant animals were capable of mounting vigorous humoral and CMC responses to FV-infected cells.

Further difficulties with the hypothesis that *Ir* type mechanisms are involved in resistance to virus-induced neoplasia come from mapping studies. Genes conferring resistance to FV (7), radiation-induced leukemia virus (RadLV) (10, 11) and mammary tumor virus (12) map to the *H-2D* region of the MHC complex. However, almost all *Ir* genes mapped to date map to the left of *H-2D*, in the *I* region of the complex. Thus, at the present moment, it is not clear what role *H-2* linked Ir genes play in conferring resistance to MuLV-induced neoplasia.

Our previous studies (10, 11) have shown that *H-2* associated resistance or susceptibility to RadLV maps to the *D* end of the complex. Resistance to RadLV has been mapped to a separate *H-2* region by other investigators (P. Lonai, personal communication), but the origin of the virus used by the latter investigators, and the clear-cut differences in susceptibility to the two viruses among the same inbred strains of mice indicate that their studies probably involve a different RadLV than the one described in this report. The virus used in our work was discovered by Lieberman and Kaplan (13). The $H\text{-}2D^d$ allele confers resistance to the disease, the $H\text{-}2D^q$ and $H\text{-}2D^s$ haplotypes susceptibility (Table I).

The present report investigates the mechanism by which *H-2D* linked genes confer resistance to RadLV-induced neoplasia. We show that *H-2* linked genetic control apparently does not influence initial virus replication, but instead alters virus proliferation and spread. The effect is evident 5 wk after RadLV inoculation. Furthermore, dramatic and almost immediate changes can be detected after RadLV inoculation on the quantitative expression of H-2 antigens on the cell surface of the great majority of thymocytes. Expression of H-2K determinants is significantly increased on the cell surface of infected mice, whether of susceptible or resistant *H-2* types. An even greater increase in H-2D antigen expression can be detected on thymocytes of resistant *H-2* haplotypes, but *not* on thymocytes of susceptible *H-2* types. This increased expression of H-2K and H-2D molecules is the result of increased synthesis of H-2 glycoproteins, and not the result of exposure of "buried" determinants normally present in the membrane of uninfected thymocytes.

Materials and Methods

Mice. All animals used in the present study were bred and maintained at Stanford. Since RadLV is a B-tropic virus, all mice tested were $Fv\text{-}1^b$.

Virus. RadLV preparations were cell-free extracts obtained from virus-induced lymphoid

TABLE I

H-2 Haplotypes and Relative Susceptibility to RadLV of Mouse Strains used in Present Studies

Strain designation	*H-2* haplotype									Relative susceptibility to RadLV
	K	*A*	*B*	*J*	*E*	*C*	*S*	*G*	*D*	
B10.S	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	Susceptible
B10.S (7R)	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s**	*d*	Resistant
B10.G	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*q*	Susceptible
B10.T (6R)	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*q**	*d*	Resistant

* Vertical bar designates position of crossing over.

tumors of C57BL/Ka mice as previously described (11, 13) C57BL/Ka is a substrain of C57BL ($H\text{-}2^b$) maintained by Dr. Henry S. Kaplan at Stanford University. This substrain is characterized by 90–100% lymphoma incidence after whole body irradiation (reviewed in reference 14).

H-2 Antisera. H-2 alloantisera 048 [(BALB.B × A.AL)F_1 anti-A.TL] (anti-H-$2K^s$); 056 [(B10.BR × A.SW)F_1 anti-B10.S(7R)] (anti-H-$2D^d$); and 020 (A.TH anti-A.SW) (anti-H-$2D^s$) were raised in our laboratory as previously described (15). Antisera D-30 [(B10.A × LP.RIII)F_1 anti-B10.AKM] (anti-H-$2D^q$), and D-11b [(A.SW × B10)F_1 anti-DA/Sn] (anti-H-$2K^qI^q$) were kindly provided by Dr. John G. Ray, Jr., of the Research Resrouces Branch, National Institutes of Health, Bethesda, Md.

Antisera to Virus Determinants. Goat anti-AKR virus, 3T3 cell origin, tween-ether disrupted (Catalogue no. 45-0097) was kindly provided by Dr. Jack Gruber, Chief, Office of Program Resources, National Cancer Institute, Bethesda, Md.

Virus Inoculation. Under ether anesthesia, 0.05 ml of the virus preparation was inoculated into each lobe of the thymus of 3- to 6-wk old mice.

Immunofluorescence Studies. Thymus and spleen cells were obtained from normal animals or mice previously infected with RadLV at the stated intervals. Animals were killed by cervical dislocation and their thymuses and/or spleens were excised and placed in phosphate-buffered saline (PBS). Single cell suspensions were prepared by gentle teasing and pipetting. For all studies documented in this report, cell viabilities were greater than 90%.

For immunofluorescence studies, 1–5 × 10^6 cells were incubated in 50 μl of anti-*H-2* or anti-murine leukemia virus (MuLV) antisera appropriately diluted (dilutions were made in PBS supplemented with 5% fetal calf serum (FCS) and 0.1% sodium azide (AFSPBS) to inhibit antibody-induced capping of surface determinants [16]). After 30 min at room temperature, cells were either underlaid with a 1 ml cushion of FCS or diluted in 1 ml of AFSPBS. The cells were then pelleted at 1,000 rpm for 10 min and resuspended in 50 μl of 1/50 dilution of fluorescein conjugated rabbit anti-mouse IgG or rabbit anti-goat IgG (Microbiological Associates, Bethesda, Md.) also in AFSPBS. After 30 min at room temperature, the cells were diluted in 1 ml AFSPBS and centrifuged or pelleted through FCS. The cell pellet obtained was resuspended in 1 ml AFSPBS and analyzed with the aid of a Fluorescence Activated Cell Sorter, FACS (Becton-Dickinson Electronics Laboratory, Mountain View, Calif.). The operation, design, and capabilities of the FACS have been extensively described (16, 17). All antisera dilutions were carefully calibrated to give optimal fluorescence.

Absorption Studies. The expression of H-2D and H-2K antigens in normal and RadLV infected thymocytes was determined by measuring the ability of these cells to absorb the alloantibody. Thymocytes were incubated in varying numbers with a fixed volume of the appropriate antiserum specific for either H-2D or H-2K for 1 h at 4;dgC. After 1 h, cell samples were centrifuged and the absorbed serum collected and tested against normal spleen cells for remaining cytotoxic activity using the microcytotoxicity assay of Amos et al. (18) as modified by Frelinger et al. (19).

^{125}I Labeling of Intact Lymphocytes and One Dimensional Tube Sodium Dodecyl-Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis. Lymphocytes were obtained from the thymus and spleen as described above, except that mouse erythrocytes were lysed by incubation

in 1 ml of ammonium-chloride-Tris lysing buffer (20) before spleen preparations were labeled and analyzed. The cells were then centrifuged and washed in PBS before being labeled. 5×10^7 cells were incubated in 1 ml of PBS with 200 μg lactoperoxidase (Calbiochem, San Diego, Calif.) (1 mg/ml in PBS), 1–3 mCi Na ^{125}I (NEZ-033L, New England Nuclear, Boston, Mass.) and 25 μl 0.03% H_2O_2. After 5 min at room temperature, a second aliquot of 25 μl 0.03% H_2O_2 was added. After another 5 min, the reaction was stopped with 50 ml of cold PBS. The cells were washed three times with cold PBS and then lysed in 0.5% Nonidet-P40 detergent (NP-40) in PBS for 30 min at 4°C. The lysate was then centrifuged at 100,000 *g* for 1 h. The supernate was aliquoted and stored at −70°C until further use. For immunoprecipitation aliquots from 7.5×10^6 cells were cleared of labeled immunoglobulins with goat anti-mouse Ig (GAMIG) and carrier normal mouse serum (NMS) at equivalence. The resulting precipitate was removed by centrifugation at 8,000 rpm for 10 min. The supernate was then incubated at 37°C for 30 min with specific antiserum or control serum. GAMIG was then added and the samples incubated at 4°C overnight. The precipitate was then collected by centrifugation at 8,000 rpm for 20 min, washed at least four times in 1% Triton-X-100 — 1% deoxycholate in PBS, pH 7.4, dried, and then subjected to SDS-PAGE according to Witte and Weissman (21).

^{35}S-Methionine Labeling of Lymphocytes and Two Dimensional Gel Electrophoresis. Lymphocytes were prepared as above. Labeling with ^{35}S-methionine and extraction with isoelectrofocusing sample buffer or 0.5% NP-40 were done as previously described (22). Immunoprecipitation of H-2 was carried out as detailed elsewhere (22), using heat-killed, formalin-fixed *Staphylococcus aureus*, Cowan I strain, to isolate the antigen antibody complexes formed by addition of alloantisera to the NP-40 extracts.

Two dimensional (2-D) gel electrophoresis of whole cells and immuno-precipitates, both solubilized with isoelectrofocusing sample buffer, were done by the method of O'Farrell (23).

Results

H-2 Effects on Cell Surface Viral Antigen Expression. Genes in the *H-2* complex could presumably affect RadLV-induced leukemogenesis at any of several points: at initial infection by the virus; during its subsequent replication and spread; in the transformation of infected cells; or at a step beyond transformation. As shown in Fig. 1, if susceptible (B10.S) or resistant (B10.S(7R)) mice are infected with RadLV and the course of the disease is followed over a 12 wk period with the aid of an immunofluorescent anti-MuLV serum, no difference is found in total viral cell surface antigen expression 3 wk after virus inoculation. However, a major difference is discernible 5 wk after infection. In susceptible mice, the number of immunofluorescent-positive thymus cells increases markedly during the 3–9 wk interval after virus inoculation, while little increase in virus-positive cells is seen in thymocytes of resistant mice. This finding suggests that initial infection is similar in susceptible and resistant mice and that the *H-2* effect on viral infection is manifested at a later stage, i.e. more than 3 wk after infection.

Changes in Expression of H-2 Antigens after RadLV Infection as Detected by Immunofluorescent Antibody. Using the FACS, a significant change in quantitative expression of H-2 antigens can be detected on the cell surface of thymocytes after intrathymic RadLV inoculation (Fig. 2). Each panel in Fig. 2 compares the intensity of staining between thymocytes from normal and virus infected mice. The upper panels compare the intensity of labeling when the alloantisera are directed against the H-2D determinants on the surface of thymus cells and the lower panels the immunofluorescence intensity when the sera recognize H-2K antigens. Animals were examined at 3, 5, 7, and 9 wk after intrathymic RadLV inoculation. A shift in the curves from left to right

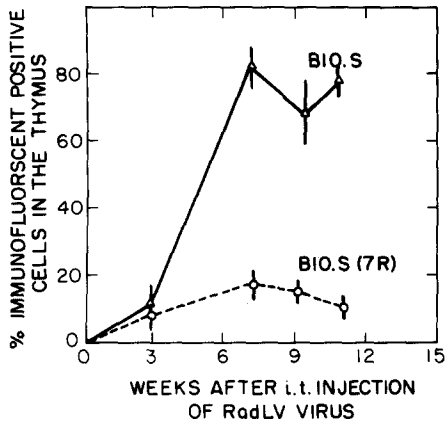

FIG. 1. Virus replication at various intervals after injection. The percentage of immunofluorescent-positive cells in the thymus was determined from the percentage of thymocytes from RadLV infected mice that stain with greater than 3 fluorescein units (a measure of fluorescence intensity defined by a known standard used to calibrate the FACS [16]) minus the percentage of similar thymocytes from control mice. This analysis is similar to that indicated for percentage of *H-2*-positive cells in the legend of Fig. 3. Anti-AKR virus serum (see Materials) was used at 1/250 dilution.

indicates increased fluorescence and therefore a greater number of antigen molecules detected on the cell surface.

To quantitate these data, the mean fluorescence intensity and the number of cells staining with intensity greater than 3 fluorescein units were calculated. The data thus obtained have been plotted in Fig. 3. The upper panel displays the ratio of mean fluorescence intensities of infected versus normal thymocytes at various intervals after virus inoculation. The bottom panel shows the actual number of fluorescing thymus cells from RadLV-infected B10.S(7R) and B10.S mice at various times subsequent to virus inoculation.

The data from Figs. 2 and 3 can be summarized by stating that after intrathymic inoculation of RadLV there is an early increase in cell surface expression of H-2K and H-2D molecules on thymocytes of B10.S(7R) and B10.S mice. The subsequent patterns of expression of H-2K molecules on thymocytes appears similar when these two strains of mice are compared. However, the subsequent effect of viral infection on levels of H-2D molecules differs in the two strains. Expression of H-2D molecules appears to be more markedly increased, for a more prolonged period, in thymocytes of RadLV-infected B10.S(7R) (resistant) mice than in thymocytes of RadLV-infected B10.S (susceptible) animals. Furthermore, the increased expression of H-2D on B10.S(7R) cells is greater and persists longer than the changes observed in expression of H-2K molecules on these same cells.

The difference observed in H-2D expression between B10.S(7R) and B10.S thymus cells is the opposite from the difference in viral antigen expression. B10.S(7R) mice, which are resistant to leukemogenesis, do *not* appear to show a marked increase in the number of virus-positive cells in the thymus during the first 9 wk after infection, and show dramatically increased cell surface expression of H-2D molecules. Exactly the reverse occurs with B10.S mice (susceptible to RadLV).

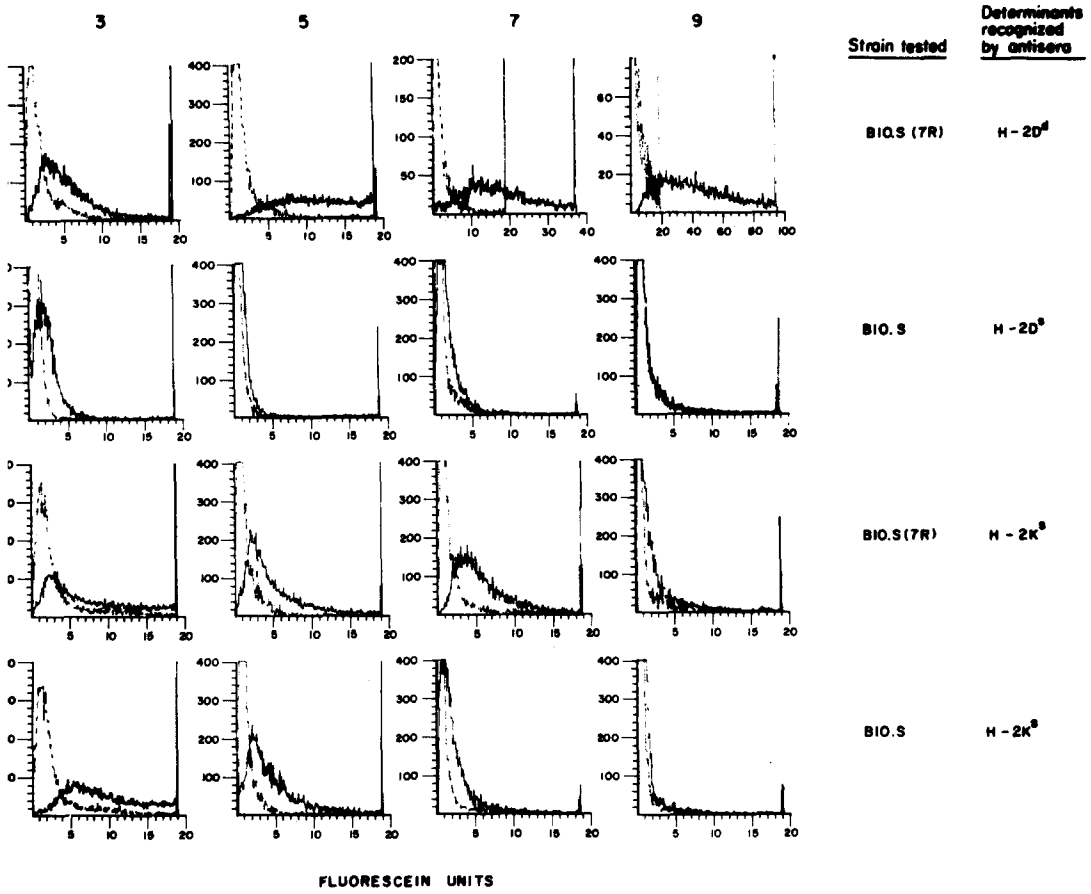

FIG. 2. FACS analysis of H-2 antigen expression on the surface of thymocytes from RadLV infected (solid lines) vs. control mice (dotted lines) at various intervals after intrathymic (i.t.) inoculation of virus. Indirect immunofluorescence with monospecific H-2 alloantisera and a fluorescein conjugated rabbit anti-mouse IgG was carried out to detect H-2 antigen expression. A shift in the curve from left to right corresponds to increased fluorescence (note that the abscissa and ordinate are different in uppermost right panels indicating that the infected thymocyte populations were very bright).

The anti-H-2D^s serum utilized in the present studies was extremely weak compared to other reagents used in the above studies (Table II). Nevertheless, when this reagent was used as a first step reagent, it bound a sufficient number of antibody molecules to the cell surface of B10.S spleen cells so that the fluorescein conjugated rabbit-anti-mouse IgG labeled these cells brightly (data not shown), though not as brightly as 056 stained B10.S(7R) spleen cells. Furthermore, the anti-H-2D^s serum stained B10.S thymocytes infected for 3 wk more than normal thymocytes, but not longer did so for thymocytes infected 7 or 9 wk. These observations argue that this serum would have been able to demonstrate a persistent increase in expression of H-2D^s on B10.U infected thymocytes after 3 wk, had such elevated antigen expression been manifest.

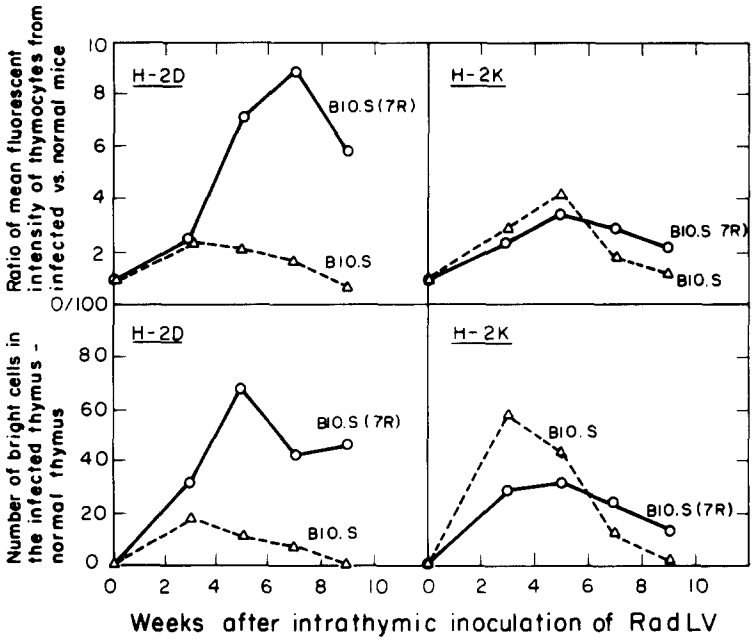

FIG. 3. Numerical presentation of data taken from Fig. 2. The ratio of mean fluorescence of thymocytes from RadLV infected vs. uninfected mice is plotted in the upper panels. The bottom panels give plots of the percentage of thymocytes from RadLV infected mice that stain with greater than 3 fluorescein units minus the percentage of similar thymocytes from control mice. The panels on the left represent effects of infection on H-2D antigen expression, while effects on H-2K determinants are shown in the panels on the right.

A similar FACS analysis of the susceptible B10.G(K^qD^q) mice and resistant B10.T(6R) (K^qD^d) was carried out. As shown in Fig. 4, 7 wk after RadLV infection, B10.T(6R) animals express 13.6 times (ratio of mean fluorescein intensity of infected cells to mean fluorescein intensity of normal cells) the normal level of H-2D^d antigen(s) and 3.1 times the normal level of H-2K^q antigen(s), whereas B10.G animals express only 2.0 times the normal level of H-2D^q determinants and 1.4 times the normal level of H-2K^q determinants. In the latter studies, the anti-H-2D^q reagent had a higher cytotoxic titer than the anti-H-2D^d reagent, was an excellent immunoprecipitating reagent and, as determined by immunofluorescence, it stained spleen cells of H-2D^q mice as strongly as the anti-H-2D^d reagent stained spleen cells of H-2D^d animals.

Changes in Expression of H-2 Antigens after RadLV Infection as Detected by Absorption Analysis. A second independent method to quantitatively measure H-2 antigen expression was sought to support the above findings. Therefore, an absorption analysis was carried out, with thymocytes from infected and normal animals. Small aliquots of sera were absorbed with increasing numbers of thymus cells from either infected or normal mice. All sera were then tested by microcytotoxicity assay (18, 19) against normal spleen cells of the appropriate strains. It is again important to note that the various antisera differed significantly in cytotoxic titer (Table II), but all antisera were used at the cytotoxic inflection point, that is, at the highest dilution at which 100%

TABLE II
Some Properties of Alloantisera Used in the Present Studies

Serum designation	Recipients	Donors	Predicted H-2 reactivity	Tested against target cells	H-2 type of target cells								50% titer
					K	*A*	*B*	*J*	*E*	*C*	*S*	*G*	*D*	
020	A.TH	A.SW	*H-2D*^{s}	B10.S	s	s	s	s	s	s	s	s	s	1:80
				B10.S(7R)	s	s	s	s	s	s	s	s|*	d	0
				A.TH	s	s	s	s	s	s	s	s|	d	0
048	(BALB.B × A.AL)F$_1$	A.TL	*H-2K*^{s}	B10.S(7R)	s	s	s	s	s	s	s	s|	d	>1:1,280
				B10.S	s	s	s	s	s	s	s	s	s	>1:1,280
				B10.A(5R)	b	b	b|	k	k	d	d	d	d	0
				BALB/B	b	b	b	b	b	b	b	b	b	0
				A.AL	k	k	k	k	k	k	k	k|	d	0
				A.TL	s	k	k	k	k	k	k	k|	d	>1:1,280
056	(B10.BR × A.SW)F$_1$	B10.S(7R)	*H-2D*^{s}	B10.S(7R)	s	s	s	s	s	s	s	s|	d	>1:1,280
				B10.S	s	s	s	s	s	s	s	s	s	0
				B10.D2	d	d	d	d	d	d	d	d	d	>1:1,280
				B10.BR	k	k	k	k	k	k	k	k	k	0
				A.SW	s	s	s	s	s	s	s	s	s	0
D-30	(B10.A × LPRIII)F$_1$	B10.AKM	*H-2D*^{q}	B10.G	q	q	q	q	q	q	q	q	q	>1:1,600
				B10.T(6R)	q	q	q	q	q	q	q	q	d	0
D-11b	(A.SW × B10)F$_1$	DA/Sn	*H-2K*^{s}	B10.G	q	q	q	q	q	q	q	q	q	>1:640
			H-2I^q‡	B10.D2	d	d	d	d	d	d	d	d	d	0

* Vertical bar indicates crossover position.

‡ This reagent was produced using a combination of donor and recipient animals that were not congenic pairs and therefore reactivities to other than *H-2* products may be present. An additional known cross-reactivity exists with other *H-2* haplotypes, such as *H-2*^{k}.

cytotoxicity could still be observed. Thus, while some antisera were absorbed at a 1/5 dilution (anti-H-2D^s), others were used at 1/500 dilution (anti-H-2D^d).

The results, shown in Fig. 5, allow several conclusions. In resistant mice, a marked and persistent increase in H-2D antigenic determinants was found in infected thymocytes compared to normal thymocytes. A much smaller and less persistant increase in H-2D antigenic determinants was found in susceptible mice. In all strains, H-2K determinants showed an intermediate increase after infection. These results are similar to those obtained by FACS aided analysis. Furthermore, since the cytotoxicity tests were performed against normal spleen cells, the H-2 determinants detected were presumably those present on normal cells.

Evidence that Changes Observed in Antigen Expressed on the Cell Surface Reflect Changes in Expression of H-2 Coded Determinants. Mouse alloantisera directed against H-2 determinants often contain antibodies to virus determinants (24). Evidence that the antigens detected by the antisera used in the above studies were, in fact, *H-2* associated determinants was provided by immunoprecipitation studies. When anti-H-2D^d or anti-H-2K^s sera are used to immunoprecipitate from NP-40 extracts of normal and infected B10.S(7R) thymocytes, and this material analyzed by SDS-PAGE, the precipitated material showed a major peak of approximately 40,000–45,000 mol wt, and often a second peak of approximately 11,000 mol wt, which presumably represents the mouse analogue of β2-microglobulin (Fig. 6). Other monospecific sera gave the same pattern. This result indicates that these sera were not recognizing any of the usual virus-associated antigens, such as gp69/71, p30, etc. In addition, infected thymocytes expressed greater amounts of precipitable *H-2K* and *H-2D* products, in confirmation of the data from the FACS and from absorption studies.

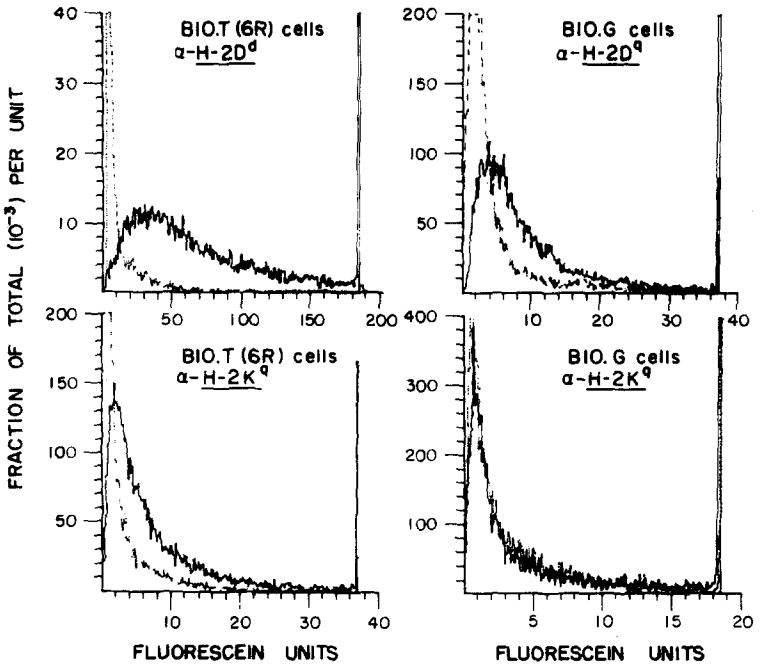

FIG. 4. H-2 antigen expression (as measured with the aid of the FACS) on the surface of thymocytes from RadLV infected (solid lines) vs. control mice (dotted lines) 7 wk after intrathymic virus inoculation. Comparison of effects of virus infection on expression of K and D coded determinants on thymus cells of resistant (B10.T(6R)) and susceptible (B10.G) mice. See legend of Fig. 2 for additional information.

Demonstration of Increased Synthesis of H-2 Coded Molecules after Virus Infection. To determine whether the increased amounts of H-2 on the surface of thymocytes from infected mice reflect increased synthesis of *H-2* coded molecules, thymocytes from normal and infected mice were labeled internally with ^{35}S-methionine. Radiolabeled proteins from these cells were then separated by 2-D electrophoresis and visualized by autoradiography. This procedure has been previously documented (23) and shown to be applicable to analysis of *H-2* gene products (22) with marked reproducibility. In this system certain sets of spots have been shown to define *H-2K* or *H-2D* when *H-2* molecules are immunoprecipitated from NP-40 extracts (22). Using this technique to analyze whole cell extracts, levels of H-2K^s appeared elevated in 7-wk infected thymocytes from both B10.S and B10.S(7R) compared to cells from normal mice. However, the amounts of H-2D are increased in infected B10.S(7R) thymocytes, but not in thymus cells from infected B10.S mice (data not shown). Because this approach does not require the use of antisera for measuring levels of *H-2*, it avoids the complications introduced by using antisera which differ in titer. Thus, the elevated surface levels of H-2K^s and H-2D^d detected by immunofluorescence and absorption, in fact, reflect increased cellular synthesis of these proteins.

To show this more clearly, H-2K^s and H-2D^d molecules were immunoprecipitated from NP-40 extracts of normal and 5 wk infected B10.S(7R) thymocytes

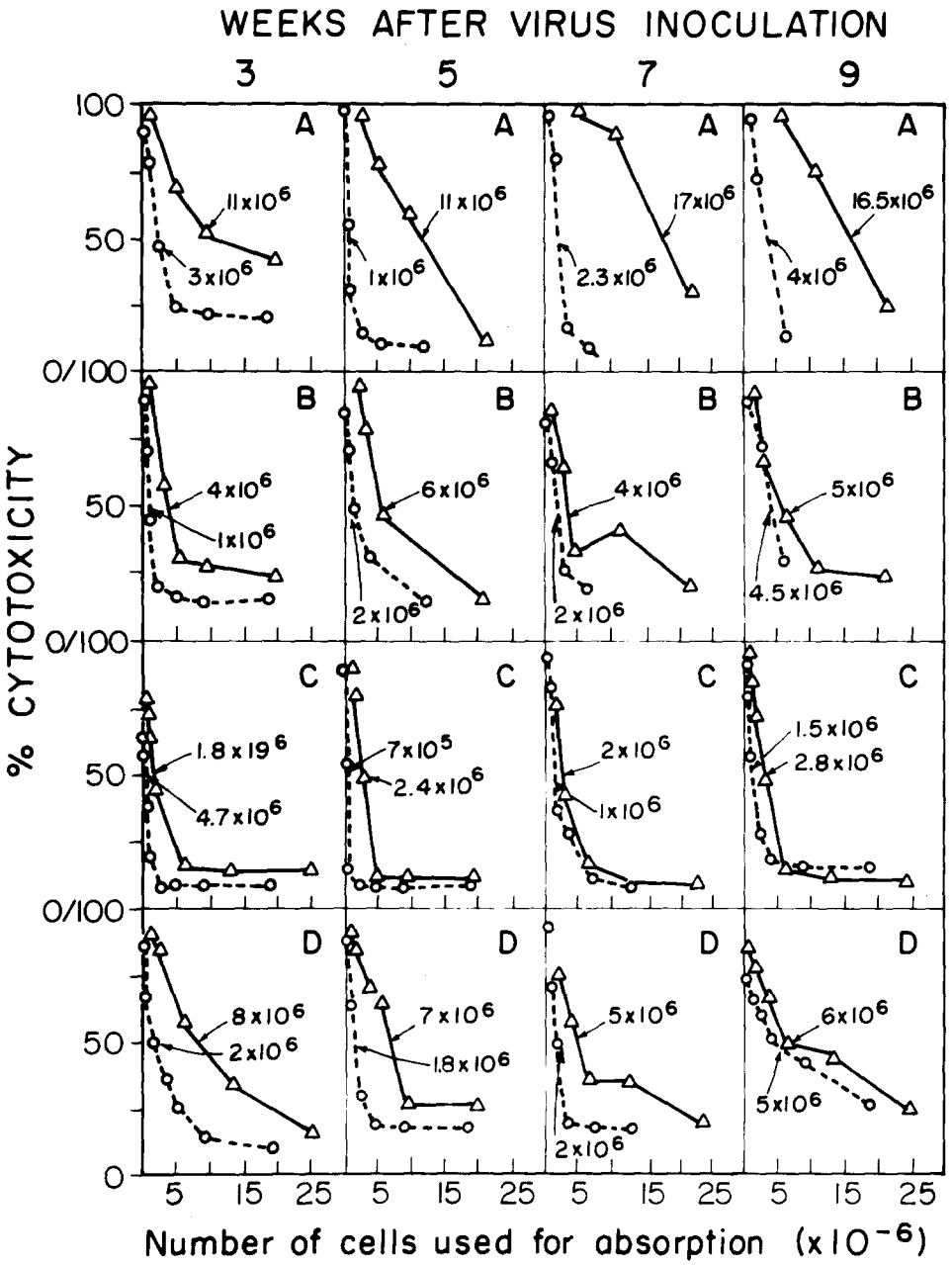

FIG. 5. Expression of H-2D and H-2K antigens in normal and RadLV infected thymocytes determined by measuring alloantiserum absorption capacity. Appropriate antisera specific for either H-2D (panels A and C) or H-2K (panels B and D) determinants were absorbed with increasing numbers of thymocytes from strain B10.S(7R) (panels A and B) or B10.S (panels C and D). Absorptions with infected cells are indicated by dotted lines and with normal thymocytes by solid lines. The number of cells required to absorb 50% of the cytotoxic activity are indicated.

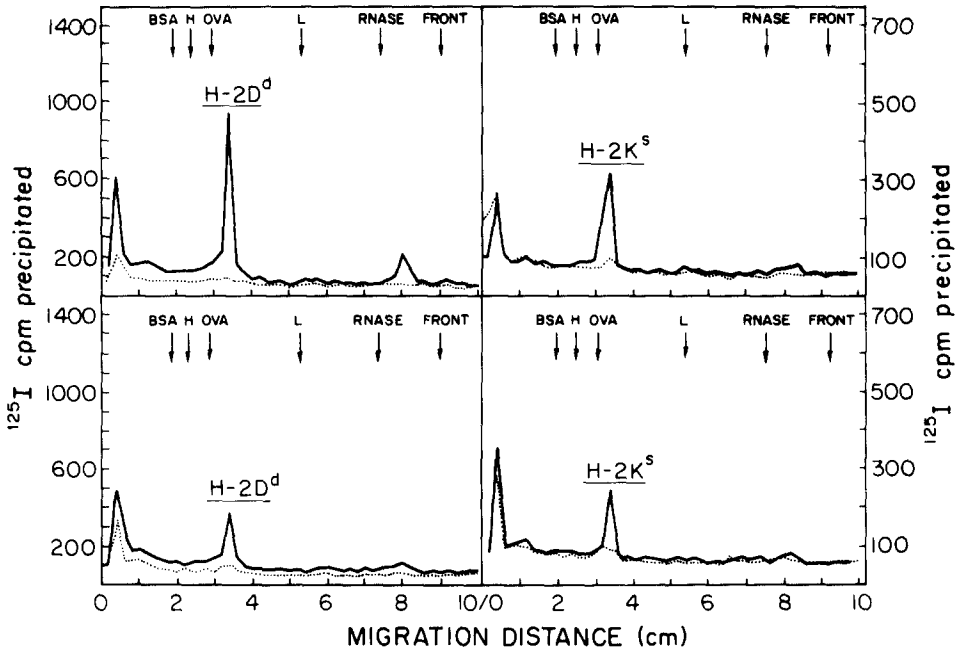

FIG. 6. Representative experiment showing immunoprecipitation and one dimensional gel electrophoretic analysis of H-2 antigen expression on the surface of RadLV infected and control thymocytes. Immunoprecipitation was done with monospecific anti-H-2 sera specific for the expected determinants in the cell lysate (solid lines) or for determinants not present in the extract (dotted line). Electrophoresis was carried out in 10% SDS tube gels as described in the text. Upper panels are the results obtained with thymocytes from virus infected mice. Lower panels give results from uninfected animals. All mice were of strain B10.S(7R). Molecular weight markers given are bovine serum albumin (BSA), 65,000; heavy chain of mouse IgG(H), 50,000; ovalbumin (OVA), 45,000; light chain of mouse IgG(L), 25,000; ribonuclease, 13,000.

which had been labeled for 4 h with ^{35}S-methionine. The immunoprecipitate of H-2K from normal (Fig. 7 b) and infected (Fig. 7 g) cells and H-2D from normal (Fig. 7 c) and infected (Fig. 7 h) cells labeled for 4 h demonstrate the marked increases in amounts of H-2K and H-2D present in infected cells that were seen in the total cell extracts.

To show that these elevated levels of H-2 result from increased rates of synthesis of the proteins as opposed to increased accumulation of those molecules, immunoprecipitates were prepared from cells which had been pulsed with ^{35}S-methionine for only 15 min, a period sufficiently brief that most radiolabeled molecules have not yet reached the cell surface (22). Even after such a short labeling period both H-2K (Fig. 7 i) and H-2D (Fig. 7 j) from infected mice are elevated compared to samples from normal mice (Figs. 7 d and 7 e), suggesting strongly that the rates of synthesis of H-2K and H-2D are higher in cells from RadLV-infected mice. Spot patterns generated by H-2 from cells labeled for 15 min are different from those obtained from cells labeled for 4 h because they represent cytoplasmic precursors which are modified (probably by glycosylation [22]) before they are expressed on the cell surface. Both the

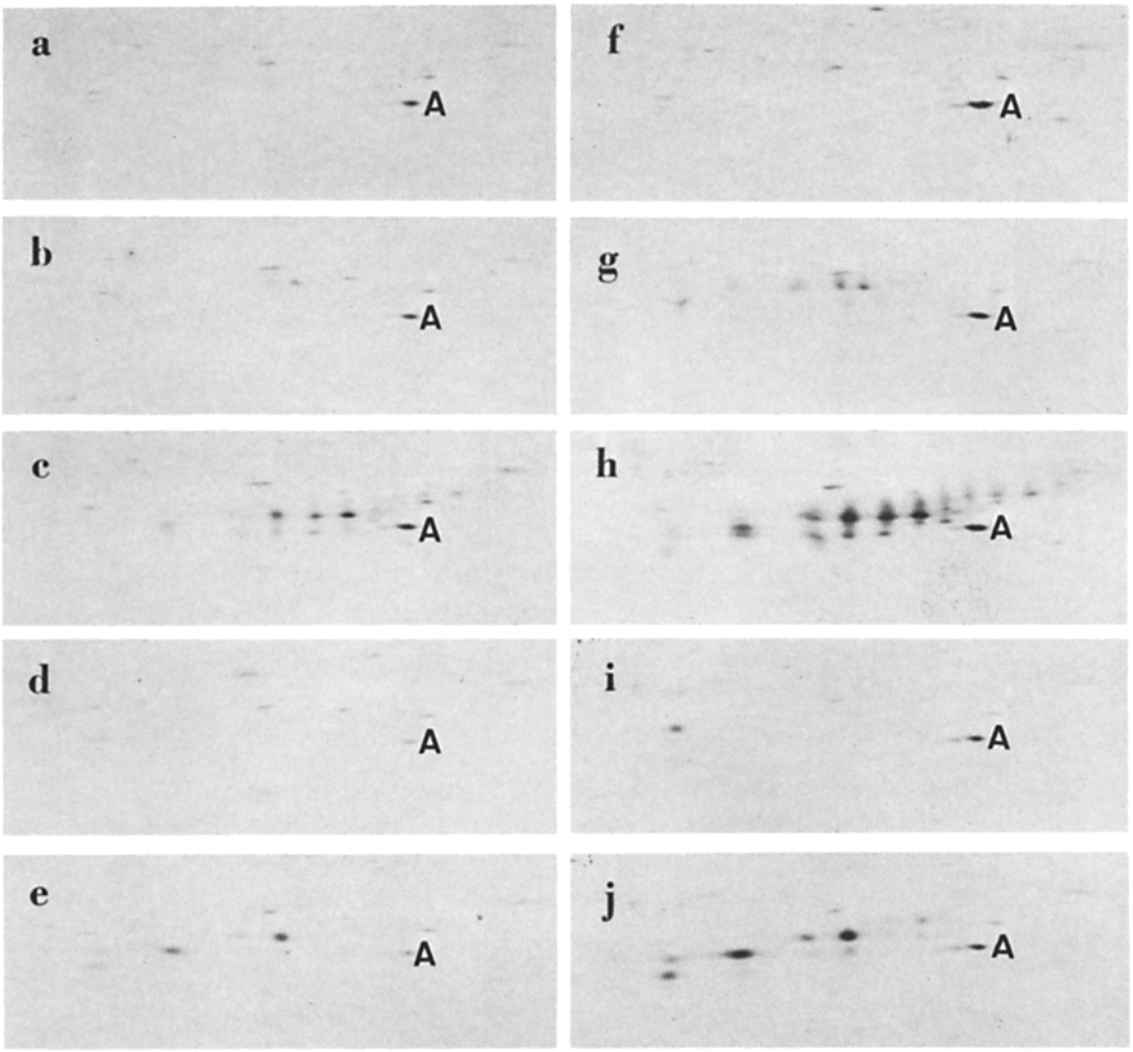

FIG. 7. 2-D gels of H-2K and H-2D proteins immunoprecipitated from NP-40 extracts of control and RadLV-infected B10.S(7R) thymocytes labeled for 4 h or 15 min with ^{35}S-methionine. Amounts of extract containing 2.5 × 10^6 trichloroacetic acid insoluble cpm were immunoprecipitated as described in the text; one-half of each immunoprecipitate was applied per gel. Panels a–e are from control thymocyte extracts; panels f–j are from RadLV-infected thymocyte extracts; a,f) 4-h labeled cells, NMS; b,g) 4-h labeled cells, anti-K^s; c,h) 4-h labeled cells, anti-D^d; d,i) 15-min labeled cells, anti-K^s; e,j) 15-min labeled cells, anti-D^d. The position of actin, mol wt 44,000 daltons, is indicated by the letter A.

precursors and their modified products appear in the gels prepared from 4-h labeled cells.

Increased Antigen Expression Observed Does Not Result from Proliferation of Cells Bearing High Levels of H-2. Chazan and Haran-Ghera (25) have previously reported that cells bearing high levels of H-2 antigens appear in the thymus in greater than usual numbers after RadLV inoculation. The data

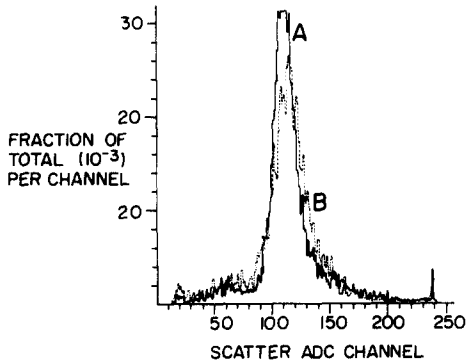

FIG. 8. The light scattering (size) distribution of thymus cells of mice injected intrathymically with either PBS (A) or RadLV (B) 36 h before FACS analysis.

reported below argues that increased synthesis and expression of H-2 molecules after virus infection occurs on all cells of the thymus and does not involve subpopulation expansion.

Fig. 8 shows the light-scatter (size) distribution of thymocytes of PBS and RadLV-inoculated mice 36 h after infection. No significant difference in distribution was detected.

All thymocytes, regardless of size, from RadLV infected animals show increased expression of H-2 antigens on their surface compared to their counterparts from either PBS-inoculated or normal animals (Fig. 9). In this particular experiment, more than 95% of all thymus cells (of any given size) from RadLV infected mice show a fluorescent intensity greater than 15 fluorescein units. By contrast, less than 10% of the thymus cells (of any given size) of PBS injected mice stain with greater than 15 fluorescein units. Thus, at least 85% of the cells show increased H-2D antigenic expression on their cell surfaces in as little as 36 h after virus inoculation. It is unlikely that such dramatic increases in antigen expression result in such a brief time from the rapid proliferation of a few cells bearing high levels of antigen. However, in view of the extremely rapid proliferative rate of a small percentage of thymus cells (26) and the possibility of selective thymolytic effects by the virus, the subpopulation expansion hypothesis cannot presently be ruled out.

The Fate of H-2 Antigens of RadLV Induced Thymomas. RadLV-induced thymomas appear in susceptible animals sometime between 10 and 22 wk after virus inoculation. In resistant animals, thymomas, if they occur, appear after the 20th week after virus infection. These thymomas can be routinely adapted to grow in tissue culture. When examined by absorption analysis, these thymoma cultures lack detectable expression of H-2 antigens (Fig. 10). These results have also been obtained in similar absorption analysis of 10 additional RadLV induced tumor cultures. These findings are in sharp contrast with the increased expression of H-2 antigens during the *early* course of the disease. However, these results are presently incomplete in the sense that it is not yet known whether the absence of detectable H-2 antigens results from masking of H-2 determinants on the cell surface or a true lack of synthesis of these membrane glycoproteins. Furthermore, the phenomenon may result from in

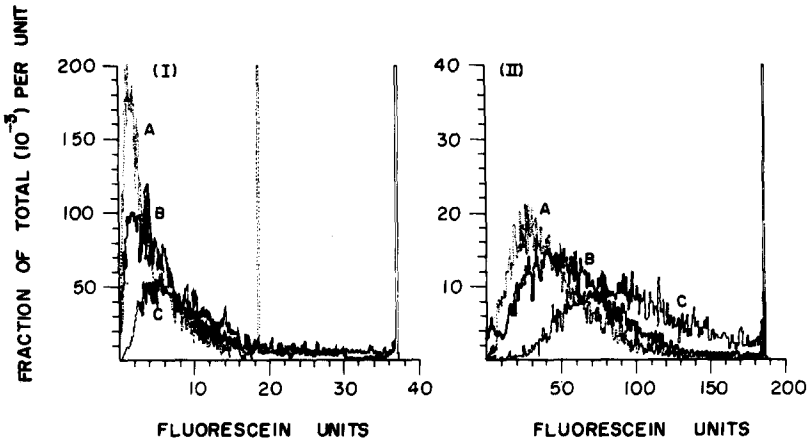

FIG. 9. Comparison of H-2D antigenic expression on the cell surface of B10.S(7R) thymocytes as measured by a FACS analysis of immunofluorescence intensity. Cells were labeled for immunofluorescence as described in the text. Each panel contains three curves, which represent the immunofluorescent distribution of different subpopulations varying in size: (A) small cells (in ADC scatter channels 95–120; see Fig. 8); (B) medium cells (in channels 120–150); (C) large cells (150–250). Panel I represents thymocytes of animals injected with PBS intrathymically 36 h earlier and Panel II thymus cells from mice inoculated simultaneously with RadLV.

vitro adaptation of the cultures although initial findings reveal that at least some RadLV-induced thymomas are H-2 negative immediately after removal from the animals. This phenomenon happens in tissue culture adapted cells of all *H-2* haplotypes, whether susceptible or resistant.

Discussion

The mechanism whereby *H-2* linked genes confer resistance to virus-induced neoplasia is unknown. The MHC antigens have been postulated to play a major role in cell-cell recognition and antigen presentation (27–32). The experiments reported here are the first demonstration that regulation in expression of MHC products occurs rapidly in response to virus infection and that such regulation is *H-2* haplotype dependent. Resistance or susceptibility to RadLV is associated with the *D* region of the *H-2* complex. The $H\text{-}2D^d$ allele confers resistance to the disease, whereas the $H\text{-}2D^q$ and $H\text{-}2D^s$ haplotypes are associated with susceptibility.

H-2 genetic control of virus proliferation and spread is evident 5 wk after RadLV inoculation. In addition, dramatic and *almost immediate* changes can be detected in the quantitative expression of H-2 antigens on the cell surface of thymocytes after RadLV inoculation. Similar studies of Ia, Thy-1, Ly-1, and Ly-2 associated determinants showed that virus infection has no effect on their expression (data not shown). While expression of H-2K determinants is significantly increased on the cell surface of thymocytes of infected mice, whether of susceptible or resistant *H-2* types, an even greater increase in H-2D antigen expression can be detected on thymocytes of resistant *H-2* haplotypes, but not on thymocytes of susceptible *H-2* types.

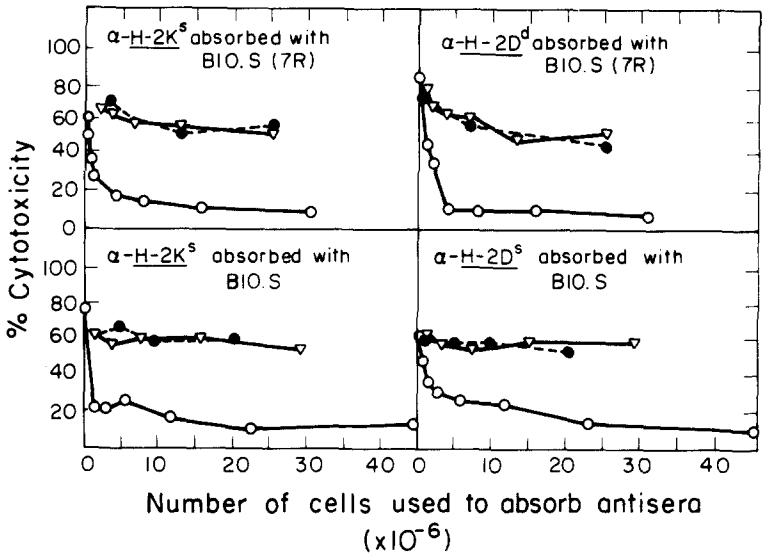

FIG. 10. Failure of RadLV-induced, tissue culture-adapted cells to absorb anti-H-2 activity from monospecific alloantisera. Normal thymocytes (○——○); RadLV-induced, tissue culture-adapted cells (▽——▽); or thymus cells from congenic mice lacking the specificities detected by the antisera used (●——●) (nonspecific absorption controls) were incubated with 40 μl of serum at an appropriate dilution. The activity remaining in the sera after absorption was determined by a microcytotoxicity test.

Increased expression of H-2K and H-2D molecules is the result of increased synthesis of H-2 glycoproteins, and not the result of exposure of "buried" determinants present on the membrane of uninfected thymocytes.

In addition, such changes probably do not represent the selective proliferation of a subpopulation of thymocytes bearing high levels of H-2 antigens (25).

Changes in K and D antigen expression after virus inoculation have been described by other investigators (25, 33). For example, Lilly (33) reported that during the terminal stage of the FV disease (about 21–38 days after virus inoculation) there is an apparent decrease in the level of expression of H-2 antigens in BALB/c mice (susceptible), which is not seen in BALB.B (resistant) animals. However, in none of the reported instances can a clear-cut relation be shown between the changed antigenic expression and the genetic localization of genes conferring resistance to the virus itself. For example, the data of Lilly (33) suggested that both H-2K^d and H-2D^d determinants decreased in the susceptible animal, but the decrease was more pronounced for H-2K^d antigens. However, genetic control of resistance to FV induced neoplasia maps to the *H-2D* region. Studies by Chazan and Haran-Ghera (25), indicating a general increase of H-2 expression in AKR mice (susceptible) with age and onset of leukemogenesis, did not provide comparable data for AKR.*H-2*^{b} (resistant) animals. In addition, no attempt was made to follow independently the fate of H-2K, Ia, or H-2D antigens with monospecific sera.

However, a clearer picture emerges in this respect in the present studies. While the mechanism leading to H-2 increases is as yet unknown, a number of factors suggest that these changes may be important in the host's response to

infection by RadLV. Changes in H-2 expression occur very rapidly. In addition, genes in the *H-2D* region confer resistance to RadLV induced neoplasia, and antigens coded by loci of this region show the most marked and prolonged changes in expression and differential regulation between susceptible and resistant animals. Furthermore, there is an inverse relation between expression of H-2D and viral antigens. Finally, H-2 antigens apparently disappear from the surface of RadLV transformed cultures. Thus, while resistance to the disease is associated with increased H-2 antigen expression, the diseased state is associated with disappearance of these antigens.

Changes in H-2 antigen expression may overtly influence the course of the disease by any of several mechanisms. For example, increased H-2 antigen expression may lead to impairment of virus penetration into or out of the cell membrane. Alternatively, in view of recent data (31, 34) suggesting that serologically detectable H-2 antigens are required for successful killing of target cells by CMC effectors, changes in H-2 expression may affect the role the immune system may play in surveillance for and elimination of virus-infected cells. Further studies are in progress to elucidate the mechanism by which virus infection elicits changes in H-2 expression and how such changes affect the course of the disease.

Summary

Previous studies from this laboratory have mapped resistance and/or susceptibility to radiation-induced leukemia virus (RadLV)-induced neoplasia to the *H-2D* region. *H-2* linked effects on virus replication can be detected subsequent to the initial virus infection, and clear-cut differences in numbers of virus infected thymus cells can be detected as early as 5 wk after RadLV inoculation. Rapid increases in cellular synthesis and cell surface expression of H-2 antigens are detectable immediately after virus inoculation. These changes have been studied by immunofluorescence, absorption, cell surface iodination followed by sodium dodecyl-sulfate-polyacrylamide gel electrophoresis, and two dimensional gel electrophoretic analysis of internally labeled lymphocyte proteins. Expression of H-2K molecules is significantly increased in cells of susceptible and resistant animals. However, significant increases in expression of H-2D antigens occurs only on thymus cells from resistant strains ($H\text{-}2D^d$). Transformed cells of resistant and susceptible *H-2* haplotypes adapted to tissue culture lack detectable H-2 antigens as determined by serological absorption studies. It is argued that altered expression of H-2 antigens plays a very significant role in the mechanism of host defense to virus infection.

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