IMBALANCED MHC CLASS II MOLECULE EXPRESSION AT SURFACE OF MURINE B CELL LYMPHOMAS

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The MHC of the mouse (H-2 complex) exerts a marked influence on resistance against murine viral lymphomagenesis (1–7). Resistance genes to various murine leukemia virus (MuLV)[1] isolates have been mapped (1–6) to several regions within the H-2 complex, i.e. the K, I-A, I-E, S, and/or D regions. In one study (8), complementation with respect to resistance between the I-A and I-E regions was observed.

The H-2 influence on susceptibility to MuLV-induced lymphomas is usually compatible with H-2-controlled immunological resistance mechanism(s) directed against MuLV or MuLV cell surface antigens (3, 9–16). H-2-controlled immune mechanisms were shown to operate at the level of antiviral antibodies (11, 14–16), virus-specific proliferating T cells (12), virus-specific cytotoxic T cells (10), or I region–linked inhibition of the outgrowth of preleukemic cells (13).

A second mechanism by which the MHC influences lymphomagenesis in the mouse, besides immune response regulation, is variation in the level of expression of MHC molecules on tumor cells. So far this issue has only been investigated for class I MHC molecules. H-2-linked resistance to radiation leukemia virus infection has been associated (9, 10) with an increased and selective expression of certain class I MHC antigens. Transfection of the AKR K36 cell line, which lacks K^k cell surface expression (17, 18), with a transcriptionally active K^k gene, makes this cell line much less transplantable in syngeneic recipient mice (18), possibly due to enhanced susceptibility to K^k-restricted MuLV-specific cytotoxic T cells (17, 18). Some Moloney MuLV–induced T cell tumors and SJL/J B cell tumors have lost expression of certain class I MHC molecules (19, 20).

Other experimental models also indicate a role for aberrant class I MHC expression in tumorigenesis. Imbalance in the expression of class I H-2 molecules influences the metastatic properties of lung carcinoma (21), sarcoma (22–25) and B16 melanoma cells (26) in mice. The oncogenic potential of adenovirus-

[1] Abbreviation used in this paper: MuLV, murine leukemia virus.

transformed rat cells is correlated with the adenovirus E1A product(s), which influence(s) class I MHC expression (27, 28). Introduction and expression of a transfected class I MHC gene was sufficient to abrogate the oncogenicity of highly tumorigenic adenovirus 12–transformed mouse cells (29).

Previously, we described the induction and phenotypic characteristics of a large number of lymphomas of H-2-congenic C57BL and BALB/c mice that arose spontaneously (6), were induced by milk-transmitted ecotropic MuLV (6), or by neonatal inoculation of cloned MuLV (7, 30). Analysis of class II MHC antigen expression on a series of B10.A(H-2^a) lymphomas indicates that (a) most primary B cell lymphomas express high levels of both I-A and I-E antigens at the cell surface, (b) some B cell lymphomas have an I-A$^-$, I-E$^+$ phenotype, (c) the loss of cell surface I-A expression can be the result of either a diminished Aα or Aβ mRNA expression, and (d) one of the I-A$^-$, I-E$^+$ B cell tumors contains a (11; 17) translocation with a breakpoint on chromosome 17 within or very close to the H-2 complex. To our knowledge this is the first detailed description of imbalanced class II MHC expression in an animal tumor model.

Materials and Methods

Tumor Induction. The development of the C57BL/10ScSn [B10(H-2^b)] and B10.ASgSn [B10.A(H-2^a)] sublines, virus-negative or with milk-transmitted B-ecotropic MuLV, was described in detail before (6). The induction of lymphomas by neonatal inoculation with cloned MuLV isolates has been described (7, 30). B10.A mice were purchased from Olac Ltd., Bicester, United Kingdom. For evaluation of tumor incidence, animals were observed three times a week for signs of tumor development or general illness. Moribund mice were killed for autopsy and viable cell suspensions from lymphomas were frozen with a programmed cooling device and cryopreserved in liquid nitrogen in 10% DMSO as described (6). In addition, tumor tissues were directly frozen in liquid nitrogen for DNA and RNA analyses.

Tumor Cell Characterization. Haematologic neoplasias were classified morphologically according to the Pattengale-Taylor classification (31). The characterization of the cell surface markers expressed by the lymphoma cells was performed by flow cytofluorometry on a Coulter Epics-C. Data were collected from 5,000 viable cells by using logarithmic amplification of signals from the photomultiplier tubes and plotted on a linear fluorescence scale.

Antibodies. The rat mAb directed against Thy-1 (59AD2.2) (32), B220 (RA3-2C2) (33), Lyt-1 (53-7.3) (32), Lyt-2 (53-6.7) (32), T200 (30G12) (32), and mac-1 (M1/70.15) (34) were used at 1:1 dilutions of tissue culture supernatant containing mAb. The characteristics of the mAb directed against the I-A, I-E, and K^k antigens used in this study are summarized in Table I. The haplotype specificity of anti-MHC mAb was confirmed by their reaction with spleen cells of a panel of recombinant C57BL mice. Anti-MHC mAb were used at 1:100 dilutions of ascites containing mAb. As second antibodies in the flow cytofluorometry experiments, the following fluorescein-labeled antisera were used: goat anti–mouse Ig (1:20; Central Laboratory of the Netherlands Red Cross Blood Transfusion Service) and rabbit anti–rat Ig (1:50; Cappel Laboratories, Cochranville, PA).

DNA Analysis. Isolation of cellular DNA was performed as described (40). DNA samples (15 μg) were digested under conditions recommended by the suppliers (Boehringer-Mannheim Diagnostics, Inc., Mannheim, Federal Republic of Germany). Gel electrophoresis, transfer to nitrocellulose filter and hybridization to ^{32}P-labeled probes was carried out as described (41). As molecular weight markers, Hind III–digested phage λ DNA was used. Finally, filters were washed in 0.1× SSC plus 0.1% SDS for 15 min at 60°C (class II MHC–specific probes) or 65°C (AKV [AKR leukemia virus]-LTR probe), dried, and autoradiographed with Kodak X-Omat R film and intensifying screens.

RNA Analysis. For RNA isolation, frozen tissues were homogenized with a polytron

TABLE I

Characteristics of Mouse Anti-MHC mAb

Hybrid cell line	Ig class	Molecule detected	Haplotype reactivity	Reference
B17-263R1	IgM	I-A	b, d, j, p, g	35
B17-123R2	IgM	I-A	b, d, j, p, g, r, (s)*	35
17/227R7	IgG2a	I-A	b, d, j, k̲, (u)	35
25-9-17S	IgG2a	I-A	b, d, (p), (q)	36
34-5-3S	IgG2a	I-A	b, d, (p), (q)	37
28-16-8S	IgG2a	I-A	b, d	36
K25-8-7	IgG2a	I-A	b, f, k̲, r, s, q	G. Hämmerling, unpublished data
11-5.2‡	IgG2b	I-A	k̲	38
11-3.25‡	IgG2b	I-A	f, k̲, r, s	38
13/18	IgG2a	I-E	d, j, k̲, p, r, u, v	35
14-4-4S	IgG2a	I-E	d, k̲, p, r	39
11-4.1‡	IgG2a	H-2K	k̲, r, p, q	38

* Weak crossreactivity.

‡ Obtained from Becton Dickinson and Co. (Mechelen, Belgium).

(30 s, full speed) at 0°C in 3 M LiCl, 6 M urea, and maintained overnight at 4°C. After centrifugation at 15,000 g for 1 h, the pellet was dissolved in 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA. Subsequently the RNA was deproteinized by phenol and subsequent chloroform/isoamyl alcohol (24:1) extraction, and precipitated with ethanol. For dot-blot analysis, RNA was dissolved in 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, and applied to nitrocellulose filters as described (42). Northern blot analysis of RNA with formaldehyde-treated 1.0% agarose gels was performed as described (27). Hybridization to ^{32}P-labeled probes was carried out as described (41). Finally, filters were washed in 0.1× SSC plus 0.1% SDS at 60°C for 15 min, dried, and autoradiographed with Kodak X-Omat R film and intensifying screens. To remove radioactive label, filters were washed at 68°C for 2 h in H$_2$O/formaldehyde 1:1 (vol/vol) and preexposed to x-ray film to ensure that no residual label was left.

Hybridization Probes. The hybridization probe specific for the U3-LTR of AKV-MuLV (AKV-LTR) has been described in detail previously (43). The following class II MHC–specific hybridization probes were used: Aα, an Eco RI fragment, containing an almost full length Aα^d cDNA inserted into the Eco RI site of pBR322 (44); Aβ, pclAβ-1, a 464 bp 3' cDNA probe encompassing the transmembrane, intracytoplasmic, and 3' untranslated region of Aβ^d, inserted in the Pst 1 site of pBR322 (45); Eα, an ~700 bp cDNA clone of Eα^d inserted into the Pst 1 site of pBR322 (R. N. Germain, unpublished data); Eβ, an ~500 bp cDNA clone containing the β2, transmembrane, and cytoplasmic region of Eβ^d subcloned into pUC8 (R. N. Germain, unpublished data).

Cytogenetic Studies. Tumor-bearing mice were injected 20 min before section with 10 μg demecolcine (Sigma Chemical Co., St. Louis, MO) dissolved in HBSS. Tumor-affected organs were removed, mechanically dissociated through a fine metal gauze, and the resulting cells were centrifuged for 10 min at 1,200 rpm. The cell pellet was resuspended in 0.075 M KCl at 37°C and incubated at 37°C for 25 min. Before centrifugation (10 min at 1,200 rpm) a few drops of freshly prepared fixative (methanol/glacial acetic acid, 3:1) was added and mixed thoroughly. After centrifugation, the cell pellet was gently resuspended in fresh fixative, and any remaining pieces of tissue or cell clumps were discarded. The cell suspension was allowed to stand at room temperature for 25 min, centrifuged, and resuspended in fresh fixative. This last step was repeated until a clear cell suspension was obtained. Air-dried preparations were made on alcohol-cleaned glass slides.

Chromosome preparations were stored for ~14 d at room temperature before being stained by a G-banding technique based on that of Herbst et al. (46). Basically, slides were

TABLE II

Characteristics of Two Primary B10.A B Cell Lymphomas with Strongly Reduced I-A Cell Surface
Expression

Primary tumor	Strain	Sex	Age (mo)	Inducing agent	Mouse lymphocyte differentiation markers					Class II MHC expression			Histology*
					T200	Thy	Mac-1	B220	sIg	Control	I-A	I-E	
1189	B10.A	♀	19	Milk-transmitted MuLV	+†	−	−	+	+	70§ 52∥	81 60	90 188	Immunoblastic
1464	B10.A	♀	29	Spontaneous	+	−	−	+	±	20 40	50 48	86 192	Lymphoblastic
1242¶	B10.A	♂	13.5	Milk-transmitted MuLV	+	−	−	+	±	39 60	91 188	86 187	Follicle center cell, large cell type

* Classification according to Pattengale and Taylor (31).
‡ +, strong expression; ±, weak expression; −, no expression.
§ Percentage of cells above channel 30.
∥ Mean intensity of fluorescence (channel number) of cells above channel 30.
¶ Representative primary B10.A B cell with a strong I-A/E expression.

incubated in 2× SSC at 60°C for 60–120 min, rinsed in distilled water, and then in buffer, pH 6.8 (Gurr). A short trypsin treatment of ~10 s followed (0.17% Difco trypsin 1:250 in buffer, pH 6.8 [Gurr]) at 4°C, with rinsing in distilled water and buffer, pH 6.8 (Gurr). Slides were then stained in a 5% Giemsa (BDH Chemicals, Ltd., Poole, United Kingdom) solution in buffer, pH 6.8 (Gurr), rinsed in distilled water, dried, and mounted in fluoromount (BDH, Chemicals, Ltd.). Suitable metaphases were photographed using Kodak technical Pan film 2415, and karyotyped according to the standard nomenclature (47).

Results

Cell-surface I-A/E Expression of Primary B10.A B cell Lymphomas. The cell surface I-A/E expression of 24 primary B10.A (I-A^k, I-E^k) B cell tumors was determined by flow cytofluorometry with a panel of nine I-A- and two I-E-reactive mAb (Table I). 3 spontaneous and 19 MuLV-induced B10.A B cell lymphomas expressed both I-A^k and I-E^k antigens. In addition, in these 22 tumors, no serological aberrations were observed in their reaction with any of the 11 I-A/E–specific mAb (data not shown).

However, two primary B10.A B cell lymphomas were observed that displayed strong I-E^k but only minimal I-A^k expression at the tumor cell surface (Table II). Tumor 1464 arose spontaneously and tumor 1189 was induced by a milk-transmitted B-ecotropic MuLV (6) (Table II). Both tumors had a T200$^+$, B220$^+$, sIg$^{±/+}$, Thy-1$^-$, mac-1$^-$ phenotype (Table II). Strikingly, both primary I-A$^-$, I-E$^+$ tumors were localized exclusively in the greatly enlarged mesenteric lymph node.

Stability of I-A$^-$, I-E$^+$ Tumor Phenotype upon Transplantation. Both B10.A I-A$^-$, I-E$^+$ tumors were transplanted into several syngeneic recipient mice, which developed lymphomas in 4–8 wk (Table III). The transplanted I-A$^-$, I-E$^+$ tumors were much more aggressive than the primary I-A$^-$, I-E$^+$ tumors, as indicated by extensive tumor cell infiltrations into spleen, mesenteric and other lymph nodes, kidney, and liver. Tumor cells from different organs of individual transplants strongly expressed I-E^k, as determined by their reaction with the mAb 13-18

TABLE III

Relationship Between Transplantability of Primary B10.A B Cell Lymphomas and I-A/E Expression of Primary and Transplanted Tumors

Tumor	I-A/E phenotype of primary tumor	Animals with tumor after transplantation*	Latency (d)	I-A/E phenotype of transplants	Transplants tested (n)
Spontaneous tumors					
1252	I-A$^{(+)}$E$^{(+)}$‡	0/3	>100		
1265	NT§	0/3	>100		
1285	NT	4/5	50.2 ± 10.2∥	I-A$^+$E$^+$	2
1359	I-A$^{(+)}$E$^{(+)}$	0/6	>100		
1464	I-A$^-$E$^+$	4/6	55.2 ± 5.7	I-A$^-$E$^+$	4
Milk-transmitted MuLV-induced tumors					
1096	NT	1/9	61	I-A$^+$E$^+$	1
1099	I-A$^{(+)}$E$^{(+)}$	9/9	19.6 ± 1.4	I-A$^{(+)}$E$^{(+)}$	2
1224	I-A$^+$E$^+$	1/10	96	I-A$^+$E$^+$	1
1189	I-A$^-$E$^+$	2/2	36, 40	I-A$^-$E$^+$	2

* Transplantation was performed by i.p. inoculation of 5–20 × 10^6 live primary tumor cells in syngeneic recipients.
‡ +, strong expression (positive cells >50 channels above background fluorescence with FITC-labelled goat anti–mouse Ig); (+), weak expression (positive cells 10–50 channels above background fluorescence); –, no expression (positive cells <10 channels above background fluorescence).
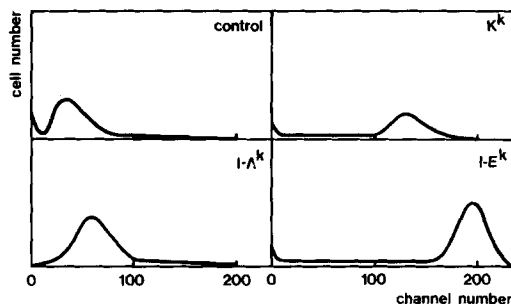§ NT, not tested because no more live cells were available.
∥ Mean ± SD.

FIGURE 1. Expression of H-2 antigens by transplant 2 of B10.A tumor 1464 as determined by flow cytofluorometry with mAb directed against I-A^k (17/227R7), I-E^k (13-18), and K^k (11-4.1) antigens. The primary I-A$^-$, I-E$^+$ B10.A tumors 1464 and 1189, and individual transplants of these tumors showed similar fluorescence patterns (data not shown).

(Fig. 1) and 14-4-4S (data not shown). With mAb 17/227R7 (Fig. 1) and three other I-A^k-reactive mAb (data not shown), only minimal fluorescence above background was seen. With seven mAb that do not react with I-A^k but detect I-A molecules from a wide spectrum of H-2 alleles (Table I), no reaction was observed. Comparable results were obtained with the primary tumor 1464 and the primary and individual transplants of tumor 1189 (Tables II and III). Both the primary (data not shown) and transplanted (Fig. 1) I-A$^-$, I-E$^+$ tumors expressed the K^k molecule on the cell surface (Fig. 1). These findings make it unlikely that the minimal cell surface detection of I-A^k antigens is caused by either (a) a mix-up of tumor cells, or (b) a mutation in the I-A^k molecule.

Relationship between Transplantability of Primary B10.A B Cell Lymphomas and I-A/E Expression of Primary and Transplanted Tumors. To evaluate the biological significance of the I-A$^-$, I-E$^+$ phenotype, we determined the I-A/E phenotype and transplantability of a total of nine B10.A primary B cell tumors, namely seven random B10.A B cell tumors and the two I-A$^-$, I-E$^+$ tumors (Table III). Four of these primary tumors expressed both I-A and I-E antigens, while the other three were not characterized because no viable primary cells were available. Two out of three of these primary tumors most likely had an I-A$^+$, I-E$^+$ phenotype, however, because (*a*) they were transplantable and the transplants had an I-A$^+$, I-E$^+$ phenotype (Table III); and (*b*) all other transplantable tumors also retained the I-A$^+$, I-E$^+$ phenotype of the primary tumor (Table III). Of the seven random B10.A B cell tumors, two were readily transplantable, two transplantable in only a small minority of recipient mice, and three not transplantable in the number of animals tested (Table III). This frequency of takes is comparable to that of I-A$^+$ B10 B cell tumors (three out of eight transplantable; data not shown), but differs significantly from the uniformly excellent transplantability of the T cell tumors from the same experimental model (6, 7), i.e. all 13 B10.A, and all 4 B10 T cell tumors were readily transplantable (data not shown).

Expression of Class II MHC–specific mRNA in I-A$^-$, I-E$^+$ Tumors. To further document the minimal cell surface I-A expression of the two I-A$^-$, I-E$^+$ tumors, and to explore its mechanism(s), we examined the expression of class II MHC–specific mRNA. The results of Northern blot analysis with total cellular RNA are shown in Fig. 2. Two individual transplants of tumor 1189 (lanes 2 and 3) possess a greatly reduced expression of the 1.4 kb Aβ mRNA (Fig. 2), but a strong expression of both the Aα (Fig. 2, lanes 2 and 3), Eβ (Fig. 2, lanes 2 and 3), and Eα mRNA (data not shown). Three individual transplants of tumor 1464 (Fig. 2, lanes 4–6) express a low level of the 1.4 kb Aα mRNA (Fig. 2, lanes 4–6) but high levels of Aβ (Fig. 2, lanes 4–6), Eβ (Fig. 2, lanes 4–6) and Eα mRNA (data not shown). 3 primary B10.A B cell lymphomas (Fig. 2, lanes 7–9) and 11 other primary or transplanted B cell lymphomas (data not shown) with a normal I-A$^+$, I-E$^+$ cell surface expression show a comparably high level of expression of the Aα, Aβ, and Eβ mRNAs. Comparable results were obtained in dot-blot analyses (data not shown).

DNA Analysis of I-A$^-$, I-E$^+$ Tumors. In Southern blot analyses with Eco R1–, Kpn 1–, Eco RV–, and Hind III–digested DNA, no additional restriction enzyme fragments hybridizing with the Aα and Aβ probes are observed in the DNA of the two I-A$^-$, I-E$^+$ tumors, in comparison with control tissues. In addition, no hybridizing fragments are lost by any of the I-A$^-$, I-E$^+$ B10.A B cell tumors (data not shown). Southern blot analyses with the MuLV-specific AKV-LTR probe reveal that the DNA of both the MuLV-induced tumor 1189 and the spontaneous tumor 1464 contains a considerable number of integrated MuLV proviruses. DNA of individual transplants of these tumors contains an almost identical pattern of MuLV integrations in comparison with the primary tumor (data not shown).

Chromosome Analyses. To address the question whether the I-A$^-$, I-E$^+$ phenotype could be correlated with specific chromosomal aberration(s), chromosome
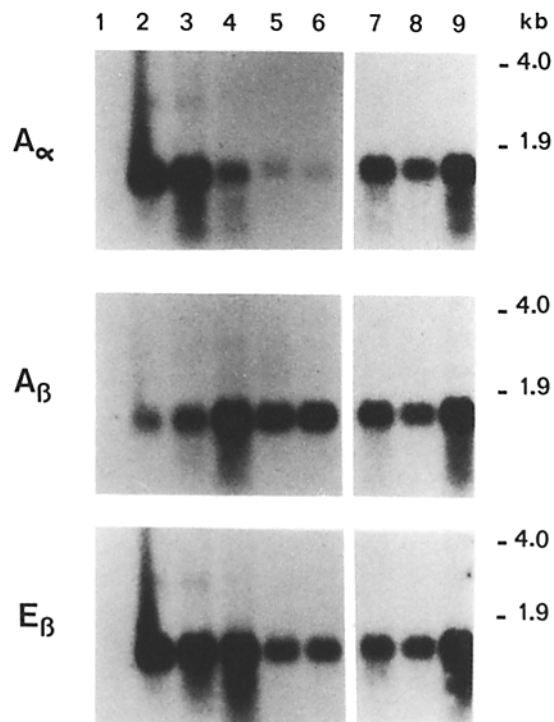
FIGURE 2. Expression of class II MHC–specific mRNA by B10.A B cell lymphomas. Northern blot analysis of 20 μg total cellular RNA on formaldehyde-treated 1.0% agarose gels was performed as described (27). Nitrocellulose filters were successively incubated with the Aβ, Aα, and Eβ specific hybridization probes, washed, and exposed to x-ray film. RNA was isolated from: lane *1*, YAC, T cell line; lanes *2* and *3*, two individual transplants of the I-A⁻, I-E⁺ B10.A tumor 1189; lanes *4–6*, three individual transplants of the I-A⁻, I-E⁺ B10.A tumor 1464; lanes *7–9*, three individual primary B10.A B cell tumors with I-A⁺, I-E⁺ phenotype.

spreads of I-A⁻, I-E⁺ transplants of both tumors were examined by the Giemsa banding technique.

Transplant of Tumor 1189. A total of 48 metaphases were analyzed, 37 from the spleen and 11 from the liver. All 27 cells with an XY constitution had an apparently normal male karyotype and were therefore considered as being derived from dividing host cells. Cells with an XX constitution represented the tumor cell population. The stem line consisted of the following karyotype: 39,XX,t(4;19), t(11;17), der(18)t(15;18), −12, +mar 15 (Fig. 3). The breakpoints for the translocations were as follows: t(4;19)(E2;B), t(11;17)(E2;D), and der(18)t(15;18)(A2;E3). The marker chromosome 15 had an insertion of unknown chromosome material in band A2.

Transplant of Tumor 1464. A total of 63 metaphases were examined, 35 from the spleen and 28 from a lymph node, all of which had an XX constitution and were therefore of tumor cell origin. The numerical range varied between 48 to 49 chromosomes per cell. 31 cells were karyotyped; all had +2, +3, +16, +17, +18, +19, and a translocation t(6;11)(D;A5). The majority of cells also had an extra chromosome 15 as a simple trisomy, with four instances of a tandem
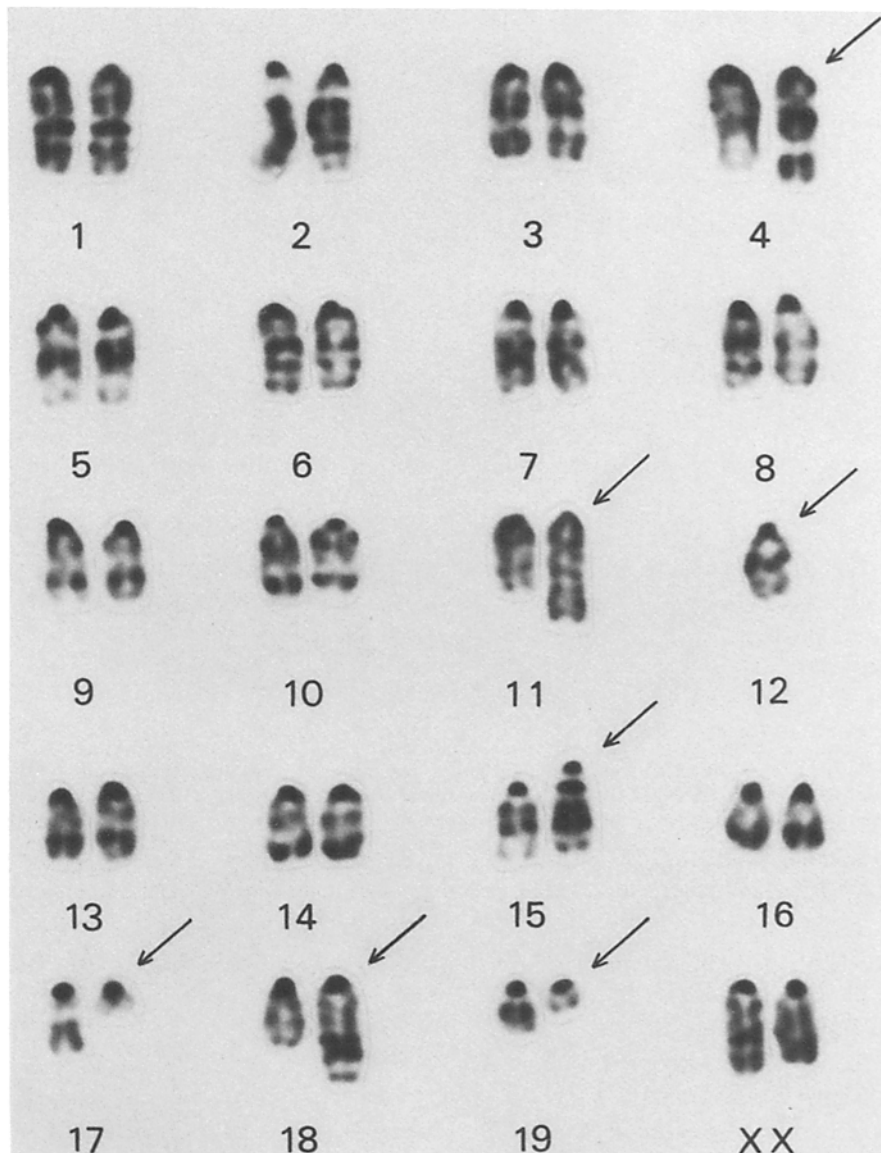
FIGURE 3.   Representative Giemsa-banded karyotype of a transplant of the I-A⁻, I-E⁺ tumor 1189 showing 39,XX,t(4;19)(E2;B), t(11;17)(E2;D), der(18)t(15;18)(A2;E3), −12, +mar 15.

duplication. An additional number 18 was also present in 10 cells, and nearly all metaphases had one or two small unidentifiable marker chromosomes.

Discussion

The minimal I-A^k expression on the cell surface of two B10.A B cell lymphomas is most likely caused by a strongly reduced level of Aα- (tumor 1464) or Aβ- (tumor 1189) specific mRNA. We cannot exclude that some of the residual signal of Aα or Aβ mRNA is caused by cross hybridization of the hybridization probes

used. Several mechanisms could account for these imbalances in Aα and Aβ mRNA expression.

First, soluble mediators (e.g., interferons, growth factors) may induce mRNA expression and cell surface expression of the I-A or I-E molecules selectively. This hypothesis is supported by the observation that, on human endothelial cells and dermal fibroblasts, recombinant human IFN-γ enhances the expression of HLA-DR more strongly than of HLA-DC antigens (48). In addition, in the human myelocytic leukemia cell line HL-60, IFN-γ induces the cell surface expression of DR but not of DC antigens (49). The induction by IFN-γ of DR antigens on HL-60 cells is associated with the induction of high level DRα and DRβ mRNA, without a simultaneous induction of DCα and DCβ mRNA expression (49). In organs of moribund mice, which almost exclusively contain malignant B cells, the relative scarcity of (T cell–dependent) mediators could account for the absence of I-A expression. In pilot experiments, however, we were unable to induce I-A^k expression in an in vitro cell line of the I-A$^-$, I-E$^+$ tumor 1464 by in vitro administration of recombinant mouse IFN-γ (data not shown).

Second, the I-A$^-$ tumors may contain altered promoter and/or enhancer regions of the Aα (tumor 1464) or Aβ gene (tumor 1189). Subtle nucleotide changes or small insertions/deletions in the Aα or Aβ genes may not be detected by the Southern blot analyses performed in this study. The existence of separate enhancer elements for class II MHC genes may be indicated by the differential tissue distribution and ontogeny of DC-1 and HLA-DR antigens in man (50). Moreover, recently, a cell type–specific enhancer element associated with the mouse Eβ gene has been identified (51). Alteration of (a) transcriptional control site(s) may be related to the chromosomal aberrations involving chromosome 17 (trisomy or translocations) observed in both I-A$^-$ tumors, and may thereby influence the relative mRNA expression of the *I-A* and *I-E* genes. In this respect it is striking that one of the I-A$^-$, I-E$^+$ tumors contains a (11; 17) translocation with a breakpoint on chromosome 17 that is localized within or very close to the H-2 complex, which has been assigned to the 17C-E1 region of chromosome 17 (52). Although we have found no confirmation that this breakpoint maps within the I-A region itself, a region close to *I-A* could be involved. Otherwise, this I-A$^-$, I-E$^+$ tumor still possesses a cytogenetically normal chromosome 17. Hence, the (11; 17) translocation may represent an event independent from the loss of I-A^k expression, but associated with the induction of the malignant state of mouse B cell lymphomas with a less differentiated phenotype than murine plasmacytomas, which often contain a (12; 15) or (6; 15) translocation (53, 54). So far we have examined the karyotype of two additional B10.A B cell tumors (which possess an I-A$^+$, I-E$^+$ phenotype). In the karyotype of a transplant of the I-A$^{(+)}$, I-E$^{(+)}$ tumor 1099 (Table III), trisomy of the chromosomes 11 and 15 was observed (data not shown). In a transplant of the I-A$^+$, I-E$^+$ B10.A tumor 1285 (Table III), a large number of chromosomal aberrations was present, including a t(3;9)(D;D) and two chromosomes 11 containing insertion of unknown chromosomal material (data not shown). Hence, in conclusion, the chromosomal aberrations present in the B10.A B cell lymphomas investigated thus far are rather heterogeneous. However, in all four B10.A B cell tumors examined, abnormalities in chromosomes 11 and 15 were observed, resembling the chro-

mosomal aberrations reported for plasmacytomas induced by mineral oil in combination with the Abelson-Moloney virus (54). This raises the possibility that in tumor 1189, as a result of the (11; 17) translocation, one of the cellular oncogenes on chromosome 11 (i.e. p53, erbA, erbB, or others) is brought under control of promotor and/or enhancer regions present within the H-2 complex.

Third, the B10.A B cell lymphomas may contain unaltered $A\alpha$ and $A\beta$ genes but express unknown tumor cell–associated cellular proteins that modify the level of $A\alpha$ or $A\beta$ mRNA. These unknown proteins could influence transcription of the $A\alpha$ or $A\beta$ gene through direct regulation of the activity of the promoter or enhancer regions of the genes involved. Alternatively, these proteins could influence the stability of $A\alpha$ or $A\beta$ mRNA. A direct role of a MuLV protein, in analogy with the adenovirus 12 E1A product (27, 28), seems unlikely, because a large number of MuLV-expressing murine B cell lymphomas show normal expression of I-A/E mRNA and antigens. However, the generation of a mutant/recombinant MuLV that encodes such a protein cannot be excluded.

Two murine B cell tumors were observed with strongly reduced cell surface I-A expression. Noteworthy, in another study, 3 out of 20 B cell non-Hodgkin's lymphomas in man were also shown to lack expression of DC antigens (the human analog of the mouse I-A antigen [55]), in spite of a high expression of DR and SB gene products (56). Perhaps an I-A$^-$, I-E$^+$ phenotype may be a normal phenomenon at some stage in the differentiation of mouse B cells, although to our knowledge such a population of B cells has not yet been reported. However, the observation that in one tumor a reduced $A\alpha$ mRNA but in the other a reduced $A\beta$ mRNA level was observed argues against this hypothesis. A more likely explanation for the minimal expression or lack of I-A antigen is immunoselection to escape from I-A-restricted immune surveillance in vivo. The frequency of aberrant I-A expression in primary B10.A B cell lymphomas (2 out of 24) is low. Although both I-A$^-$, I-E$^+$ tumors were transplantable in syngeneic mice, the overall transplantability frequency of B10.A B cell lymphomas does not allow any conclusion concerning a difference in transplantability of I-A$^-$, I-E$^+$ vs. I-A$^+$, I-E$^+$ tumors, if only because the frequency of testable I-A$^-$, I-E$^+$ tumors is too low. In addition, no selection towards an I-A$^-$, I-E$^+$ phenotype is apparent upon transplantation of I-A$^+$, I-E$^+$ tumors. However, this does not exclude the possibility that immunoselection against a class II MHC–restricted MuLV-specific T cell response may occur in some of the B cell lymphomas. In other individual tumors, immunoselection may involve loss of the viral target antigen rather than MHC molecules from the cell surface. Immunoselection in vivo by class I MHC–restricted Moloney MuLV–specific CTL leading to the loss of the viral target antigen was recently documented by van der Hoorn et al. (57). The ultimate answer of whether the I-A$^-$, I-E$^+$ phenotype of tumors 1189 and 1464 has biological significance has to come from transfection and expression of cloned $A\alpha$ and $A\beta$ genes in these tumors, followed by study of the comparative transplantability of transfected vs. untransfected tumor lines.

Summary

To study the role of class II MHC expression in mouse lymphomagenesis, we examined the cell surface expression of I-A/E antigens on 24 spontaneous or

murine leukemia virus (MuLV)-induced mouse B10.A (I-A^k, I-E^k) B cell lymphomas. Two primary B10.A B cell lymphomas were observed with strong I-E^k expression but with only minimal cell surface I-A^k expression. Both tumors are readily transplantable in syngeneic mice, with maintenance of their I-A$^-$, I-E$^+$ phenotype. Strikingly, one I-A$^-$, I-E$^+$ B cell lymphoma contains a (11; 17) translocation with a breakpoint on chromosome 17 that is localized within or very close to the H-2 complex. DNA of both tumors contains normal restriction enzyme fragments of the Aα and Aβ genes. Northern blot analyses indicated that one I-A$^-$, I-E$^+$ tumor strongly expressed Aα, Eα, and Eβ mRNAs but possessed only a weak expression of Aβ mRNA. The other B cell lymphoma showed Aβ, Eα, and Eβ mRNA expression but only minimal Aα mRNA expression. In 11 primary B10.A B cell lymphomas with a normal I-A$^+$, I-E$^+$ phenotype, no imbalances in Aα/Aβ mRNA levels were observed. The implications of these findings for the role of class II MHC expression in mouse B cell lymphomagenesis are discussed.

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