HOST RESISTANCE DIRECTED SELECTIVELY AGAINST H-2-DEFICIENT LYMPHOMA VARIANTS

Analysis of the Mechanism

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The major, and according to some authors (1), exclusive function of class I molecules of the major histocompatibility complex (MHC)[1] is to guide the T lymphocytes. These molecules are necessary for the triggering, as well as for the effector function of cytotoxic T lymphocytes (CTL) (2). They are composed of a heavy glycoprotein chain with a molecular mass of 45 kilodaltons (kD), encoded by a single gene within or closely linked to the MHC, and the noncovalently associated β_2 microglobulin peptide with a molecular mass of 12 kD (3). The heavy chain, encoded by the H-2K, -D, or -L loci in mice is highly polymorphic and expressed on virtually all cells, including tumor cells.

Tumor cells often show alterations in their expression of H-2 molecules when compared to cells in the corresponding normal tissue. The reported changes are either qualitative or quantitative. The qualitative changes, often referred to as alien H-2 antigens on tumor cells (4), represent a controversial issue which may be resolved by the use of monoclonal antibodies (mAb) and increased insights in the structure and organization of H-2 genes. The quantitative changes are easier to document and interesting also, since, in many cases, they are associated with functional changes of neoplastic cells, such as ability to grow progressively or metastasize in vivo (5–11). These changes have been discussed mainly in relation to immunosurveillance and MHC guidance of T cells. It has been proposed (10) that reduced expression of one or several of the class I allelic products may allow tumor cells to escape detection by CTL. There may thus be a selection for such variants during growth in vivo. There is good evidence for this in the case of some immunogenic tumors (8, 9). However, loss of H-2 expression can also take place during growth in vitro, in the absence of any selective pressure from host factors (11–15). Surprisingly, H-2 expression is often regained if such lines are passaged in vivo (11, 15). Moreover, there are examples where tumor progression or metastasis are closely associated with increased expression of certain or all H-2 antigens (5–7, 16). One explanation of these paradoxical findings is that deletion or reduced expression of H-2 antigens reduces the ability of tumor cells

[1] *Abbreviations used in this paper:* BSS, balanced salt solution; CTL, cytotoxic T lymphocyte; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; mAb, monoclonal antibody; MHC, major histocompatibility complex; NK, natural killer; NRS, normal rabbit serum.

REDUCED TUMORIGENICITY OF H-2-DEFICIENT LYMPHOMAS

to survive and spread in vivo through a mechanism that is independent of the T cell system. Such a mechanism might have evolved to prevent the expansion of H-2-deficient variant clones, which would otherwise represent a danger to the organism by escaping T cell recognition after virus infection or malignant transformation[2] (17). To test this hypothesis, we selected lymphoma cells for loss of H-2 expression and compared these H-2-deficient variants with wild type cells for the ability to grow as tumors in mice. After subcutaneous inoculation into syngeneic mice, the H-2-deficient variants failed to grow out as a solid tumor, whereas the H-2$^+$ control line showed progressive growth at the site of inoculation.[2] In this report, we have analyzed the mechanisms behind this reduced ability to form tumors in vivo. The anti-H-2-resistant and original sublines were compared for in vitro growth parameters, as well as for short-term survival and outgrowth after injection into normal and immunodeficient mice.

Materials and Methods

Animals. Mice of the following inbred strains and F$_1$ hybrids were bred and maintained at the Department of Tumor Biology, Karolinska Institute. C57BL/6, (A.BY × C57BL/6)F$_1$, and (C57BL/6 × C57L)F$_1$, all H-2$^{b/b}$. Athymic *nu/nu* C57BL/6 mice were purchased from Bomholtgaarden, Denmark. All mice were 4–8 wk old at the start of the experiments.

Tumors. RBL-5 is a Rauscher virus–induced T lymphoma, and EL-4 is a benzpyrene-induced T lymphoma. Both are of C57BL/6 (H-2^b) origin. For selection of anti-H-2-resistant lymphoma variants, tumor cells were mutagenized with EMS (ethyl methane sulfonate) (Sigma Chemical Co., St. Louis, MO), 200 μg/ml for 72 h, washed twice, and cultured 1 wk to allow for cell recovery. The cells were then exposed to anti-H-2 alloantisera or mAb, plus rabbit complement (Cedarlane Laboratories, Ltd., Hornby, Ontario, Canada). The surviving cells were expanded and the procedure repeated. Several antibody-complement lytic selections were necessary to obtain stable H-2-low variants. All H-2-low variants used in this study have been selected at least five times. mAb against H-2K^b (28-13-3S) and H-2D^b (28-14-8S) were obtained from the American Type Culture Collection, Rockville, MD (18). All tumors were passaged as ascites lines in H-2-identical C57BL/6 or F$_1$-hybrid mice. For routine passages, the mice were irradiated (400 rad) 24 h before tumor inoculation, to avoid immunoselection.

Analysis of H-2 Expression. Cell surface expression of H-2 was analyzed on a fluorescence-activated cell sorter (FACS IV; Becton Dickinson Immunocytochemistry Systems, Mountain View, CA). For H-2 analysis, 5 × 10^5 cells were incubated with murine anti-H-2 mAb (culture supernatants) for 30 min on ice, washed once, and incubated another 30 min on ice with 200 μl fluorescein isothiocyanate (FITC)-labelled rabbit anti–mouse Ig (DAKO, Copenhagen, Demark) diluted 1:10 in balanced salt solution (BSS) supplemented with 20% fetal calf serum (FCS). After three additional washings with BSS, the cells were analyzed on a FACS IV. The frequency and fluorescence of stained cells were determined using a laser output of 200 mW, photo multiplier adjusted to 600 V, and a constant fluorescence gain for each series of samples.

Treatment with Anti–asialo GM$_1$ Antiserum. Natural killer (NK) cell deficiency in mice was induced by intravenous injections of rabbit anti–asialo GM$_1$ antibody, Wako Chemicals, Düsseldorf, Federal Republic of Germany (19). 30 μl of the antiserum diluted in 0.2 ml BSS was injected in a tail vein every fourth day, starting one day before tumor challenge. A total of 3–5 injections were given.

In Vivo Rejection Assay with ^{125}I-UdR-labelled Cells. Tumor cells were labelled intraperitoneally with ^{125}I-5-iodo-2-deoxyuridine (Radiochemical Centre, Amersham Corp.,

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TABLE I

Nomenclature, Origin, and In Vitro Doubling Time of Lymphoma Cells Used in this Study

Nomenclature used in this paper	Mutageni- zation series	Anti-H-2 selection	In vitro doubling time (h)	Origin
RBL-5	—	−	NT*	RBL-5
RMA	A	−	14.4	RBL-5
RMA H-2 sel	A	+	14.6	RBL-5
RMB H-2 sel	B	+	15.8	RBL-5
EL-4	—	−	NT	EL-4
EMA	A	−	16.5	EL-4
EMA H-2 sel	A	+	16.3	EL-4

* NT, not tested.

Bucks, United Kingdom). 50 μCi were injected intraperitoneally 3 d after intraperitoneal passage of 10^7 viable tumor cells into irradiated (400 rad) syngeneic mice. The cells were harvested and washed after 6 h. Syngeneic and F_1 mice were injected in a lateral tail vein with 10^6 ^{125}I-UdR-labelled tumor cells in 0.2 ml BSS. The mice were killed at different time intervals, and the remaining radioactivity was measured in each organ in a LKB gamma counter, and expressed as percentage of total injected radioactivity.

In Vivo Rejection Test. Doses of 10^3–10^6 tumor cells in a 0.1 ml volume of BSS were inoculated subcutaneously in the flank, and growth was followed by palpations twice weekly. The tumors always appeared at the site of inoculation. In the initial experiments, tumors reaching more than 10 mm in mean diameter grew progressively. In subsequent experiments, the mice were killed when the mean tumor diameter reached 15 mm and no signs of regression were seen. Mice without tumor growth were kept under observation for at least 8 wk after inoculation. All tumor cells had been passaged as ascites lines in irradiated H-2$^{b/b}$ homozygous C57BL/6 or F_1 hybrid mice for several generations before inoculation.

In Vitro Growth of Tumor Cells. Tumor cells were seeded in 50-ml tissue culture flasks (3013; Falcon Labware, Oxnard, CA) in a concentration of 5 × 10^4 cells/ml (total volume, 15 ml) in RPMI 1640 medium supplemented with penicillin-streptomycin and 10% FCS. Viable cells were counted in trypan blue at regular intervals. The doubling time was calculated by exponential regression analysis. The experiments were repeated several times with independently explanted cells.

Cloning Efficiency In Vitro. Tumor cells were seeded in 96-well tissue culture microplates with flat-bottom wells (3596; Costar, Cambridge, MA), starting with 256 cells/well in 0.1 ml RPMI 1640 medium supplemented with penicillin-streptomycin and 10% FCS. Dilutions (1:2) were made, ending with 0.25 cells/replicate well. 10 d after seeding, cloning efficiency was scored by counting wells with viable clones visible in the light microscope.

Results

Selection and Characterization of Anti-H-2-resistant Lymphoma Variants. Anti-H-2–resistant variants have been selected from the C57BL/6-derived RBL-5 and EL-4 lymphomas. The wild type lymphoma cells were mutagenized, then exposed to anti-H-2 serum plus complement in several selection cycles to obtain stable variants. In this study, we used selected sublines from two independent mutagenizations of the RBL-5 wild type line, and one from the EL-4 wild type line. The normal untreated wild type lines, as well as mutagenized but nonselected cell lines, were used as controls (Table I). All variants used in this study showed a
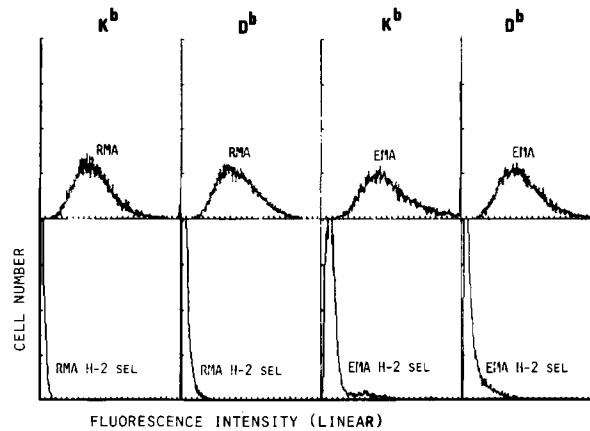
FIGURE 1.   FACS analysis of H-2K^b and H-2D^b expression on control and H-2-variant lymphoma cells after in vivo passage in syngeneic mice.

profound reduction of H-2 cell surface antigens, as determined by serum absorption assays, FACS analysis, and antibody plus complement–mediated lysis with alloantisera or mAb directed against H-2K^b, H-2D^b, or β_2 microglobulin.[2] To avoid changes imposed by prolonged in vitro culture, we used cells that had been passaged in vivo as ascites tumors in irradiated mice in all experiments in this study. It was therefore important to first ascertain that the variants remained stable during such passages in vivo. The expression of H-2D^b and H-2K^b products, analyzed by separate reagents (Fig. 1), was still deficient after 30 in vivo passages. The in vivo– as well as the in vitro–passaged lines showed a marginal, insignificant increase in staining with specific antisera, compared to background staining with fluorescent conjugate alone (not shown). Quantitative absorption experiments performed recently (Ljunggren and Kärre, manuscript submitted for publication) indicate that this is due to a low residual H-2 expression on all or a proportion of the variant cells.

Growth and Cloning Efficiency In Vitro.   There was no significant difference between the control lines and the H-2-deficient variants in growth rate after explantation to tissue culture. Growth curves from one of the observations with the RMA H-2 sel line and the unselected RMA control line are shown (Fig. 2). The lag period before exponential growth, as well as the maximal cell concentration was similar for the two cell lines. Similar results were obtained with the independently selected variants, RMB H-2 sel and EMA H-2 sel; no significant difference with regard to in vitro doubling time, lag periods, nor maximal cell concentration between the wild type control lines and the H-2-selected variants (Table I).

In addition, no significant differences in cloning efficiency were observed between the control lines and the H-2-selected variants of RBL-5 and EL-4. In two independent experiments, with EMA and EMA H-2 sel, it was possible to observe clones of both cell lines in 50% of the wells 10 d after seeding 4 cells/well. A similar pattern was seen in two independent tests with RMA and RMA H-2 sel, although these lines required seeding of 8 cells/well to get 50% plating efficiency.
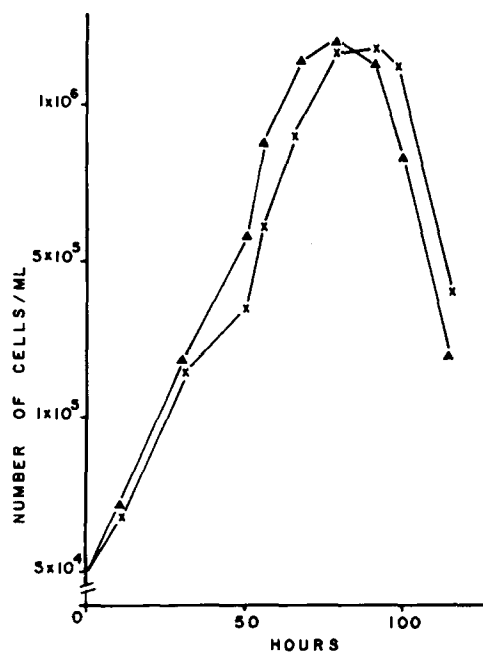
FIGURE 2. In vitro growth rate of RMA (▲) and RMA H-2 sel (x).

Tumor Growth in Normal Mice. Different doses of tumor cells (in this study, 10^3-10^6 cells) were injected subcutaneously in the flanks of mice in groups of 3–6 animals, usually littermates or otherwise age-matched within 2 wk. Rather than using large groups of mice in single experiments, we chose to use relatively small groups of animals in several independent tests throughout the study. This minimized the risk that random fluctuations in the quality of control and variant cell suspensions would be responsible for differences in the ability to form tumors.

After subcutaneous injections of the mutagenized but nonselected control line RMA (as well as the nonmutagenized RBL-5 line) in doses of $\geq 10^3$ cells, these lymphomas always grew out as solid tumors. EMA cells (and the nonmutagenized control, EL-4) were somewhat less malignant, growing in 30–70% of the mice, depending on the injected dose. When tumors appeared, the growth was rapid, and a large solid tumor was palpable within 2–3 wk. The H-2-selected negative variants of RBL-5 and EL-4 (RMA H-2 sel, RMB H-2 sel, and EMA H-2 sel) almost always failed to grow out as solid tumors under the same conditions (Fig. 3). Doses of $\geq 10^6$ cells were needed to get more regular outgrowth of these H-2-deficient cells (not shown). This in vivo difference in tumor growth remained even when the RBL-5 H-2-deficient variant and the control line were injected subcutaneously in the opposite flanks of the same mouse (Table II, Exp. 1 and 2).

Tumor Growth in Irradiated Mice. Irradiation (400 rads) before tumor grafting had a marginal effect on the rejection of 10^3 cells. The same treatment abrogated the rejection potential against 10^4 cells (Table II, Exp. 3 and 4), although the outgrowth of 10^4 H-2-low variant cells in irradiated mice was delayed compared to 10^4 H-2$^+$ cells (RMA median latency period 8 d, range 7–9 d; RMA H-2 sel

10^3 10^4 10^5

PERCENT TUMOR TAKES RBL-5

100   7/7   100   16/16   100   10/10

50   50   50

                1/14   1/12
        0/9 □   0/14 0/16   n.t. □

    H-2⁺   H-2⁻   H-2⁺   H-2⁻   H-2⁺   H-2⁻

PERCENT TUMOR TAKES EL-4

100   100   100

50   50   6/10   50   7/10

        3/10
                    1/14   1/14
            0/16   □   □

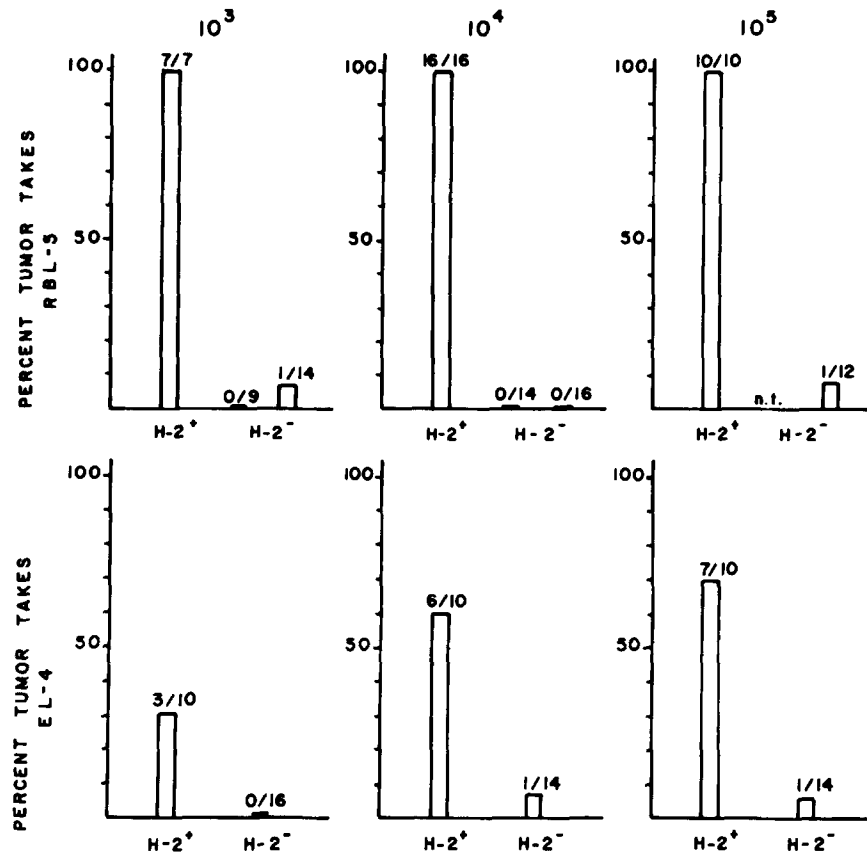   H-2⁺   H-2⁻   H-2⁺   H-2⁻   H-2⁺   H-2⁻

FIGURE 3.   Tumor growth after subcutaneous injections of H-2⁺ RBL-5 and EL-4 lymphoma lines, and H-2-low variants (RMA H-2 sel, RMB H-2 sel, and EMA H-2 sel) in three different doses, in syngeneic C57BL/6 or (C57BL/6 × A.BY)F₁ mice (one lymphoma type inoculated in each mouse). The bars for H-2⁺ control lines of RBL-5 and EL-4 each represent pooled data from tests with the untreated wild type lines, or mutagenized but nonselected lines. There were no differences in number of tumor takes or latency periods between the different types of control lines within each tumor. Only the B series of the RBL-5 H-2⁻ variant (RMB H-2 sel) has been tested with 10⁵ cells. The figures indicate total numbers of mice with tumor growth per total number of mice injected with tumor cells.

median latency period 14 d, range 10–35 d). These results suggested that the reduced tumorigenicity of H-2-variant cells was due to a host mechanism that was weakened, but not completely abrogated by 400-rad irradiation.

Tumor Growth in T Cell–deficient Mice.   To determine whether the inability of H-2-low variant cells to grow out as solid tumors was T cell–dependent, syngeneic nude mice (*nu/nu*) were inoculated with different doses of H-2-low variant and wild type control cells. The tumor cells were injected in separate animals, or in opposite flanks of the same mouse, as described above. Reduced tumorigenicity of the H-2-deficient variant (RMA H-2 sel) was also observed in nude mice. In fact, the nude mice appeared even more resistant, since they remained tumor-free at the RMA H-2 sel inoculation site even after a challenge with 10⁶ cells (Table II, Exp. 5 and 6).

TABLE II

Tumor Growth After Subcutaneous Injection of Wild Type and Variant Lymphoma Cells

Exp.	Mice	Number of cells injected into each flank	RMA tumor takes	RMA H-2-sel tumor takes
1	(C57BL/6 × A.BY)F$_1$	10^4	8/8	0/8
	(C57BL/6 × A.BY)F$_1$	10^3	2/3	0/3
2	(C57BL/6 × A.BY)F$_1$	10^4	5/5	0/5
3*	(C57BL/6 × A.BY)F$_1$	10^4	5/5	—
	(C57BL/6 × A.BY)F$_1$	10^4	—	0/5
	(C57BL/6 × A.BY)F$_1$ irradiated	10^4	5/5	—
	(C57BL/6 × A.BY)F$_1$ irradiated	10^4	—	5/5
	(C57BL/6 × A.BY)F$_1$	10^3	5/5	—
	(C57BL/6 × A.BY)F$_1$	10^3	—	0/5
	(C57BL/6 × A.BY)F$_1$ irradiated	10^3	5/5	—
	(C57BL/6 × A.BY)F$_1$ irradiated	10^3	—	2/5
4	(C57BL/6 × A.BY)F$_1$	10^3	5/5	0/5
	(C57BL/6 × A.BY)F$_1$ irradiated	10^3	5/5	2/5
5*	C57BL/6 *nu/nu*	3×10^5	4/4	—
	C57BL/6 *nu/nu*	3×10^5	—	0/4
6	C57BL/6 *nu/nu*	10^6	4/4	0/4
	C57BL/6 *nu/nu*	10^5	2/4	0/4
	C57BL/6 *nu/nu*	10^4	1/4	0/4
7	C57BL/6 anti–asialo GM$_1$	10^4	4/4	4/4
8	(C57BL/6 × A.BY)F$_1$	10^4	5/5	0/5
	(C57BL/6 × A.BY)F$_1$ anti–asialo GM$_1$	10^4	3/3	3/3

RBL-5 wild type and anti-H-2-resistant variant lymphoma cells were injected into opposite flanks of C57BL/6 or (C57BL/6 × A.BY)F$_1$ mice. Successful tumor growth was compared in normal, irradiated, T cell-deficient, and NK-deficient mice.

* Only one lymphoma type was inoculated in each mouse.

Tumor Growth in NK Cell–deficient Mice. To determine whether the failure of the H-2-deficient cells to grow in vivo was dependent upon host NK cell activity, mice were injected with anti–asialo GM$_1$. This antibody is directed against the glycolipid asialo GM$_1$ expressed on the cell surface of NK cells. Microliter amounts of anti–asialo GM$_1$ depresses NK cell activity in the spleen and other lymphoid organs (19). Mice were injected every fourth day with 25 μl of antibody,
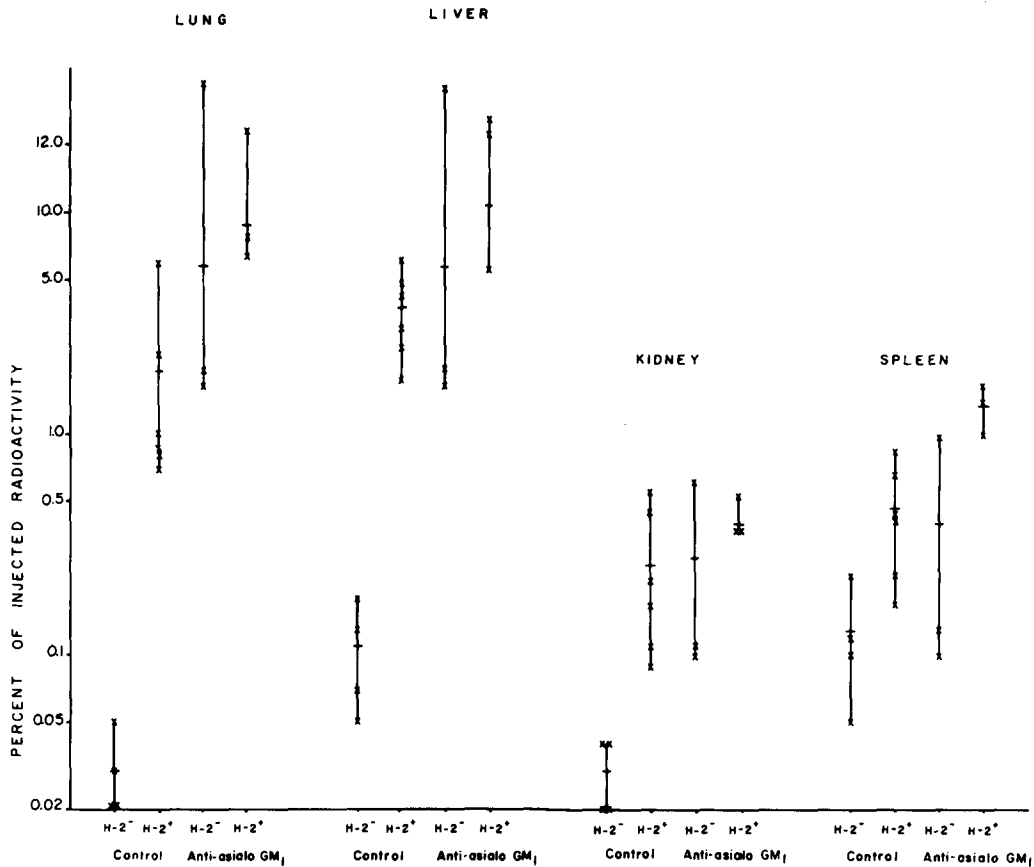
FIGURE 4.   Survival of ^{125}I-UdR-labelled RMA (H-2$^+$) and RMA H-2 sel (H-2$^-$) lymphoma cells in syngeneic C57BL/6 mice 24 h after intravenous injection. The amount of radioactivity remaining in each organ indicated the distribution of surviving cells. Single observations are plotted (x) on a logarithmic scale, and the arithmetic mean is calculated (—).

starting 1 d before tumor challenge, and with a total of 3–5 injections. In mice receiving this treatment, the H-2-low variant grew progressively, like the H-2$^+$ control line (Table II, Exp. 7 and 8).

In Vivo Survival of Isotope-labelled Lymphoma Cells.   Survival and organ distribution of the H-2$^+$ RBL-5 control line (RMA) and the H-2-low variant (RMA H-2 sel) in vivo were studied by monitoring remaining radioactivity in different organs within 12–36 h after intravenous injection of ^{125}I-UdR-prelabelled cells. The ^{125}I-UdR is rapidly released from dead cells, and excreted (20); thus, the amount of radioactivity remaining in each organ indicates the distribution of surviving cells. A 5–100-fold difference was observed in tumor cell survival between the RBL-5 control line and the variant in different experiments. These differences were almost abolished when the mice were pretreated with anti-asialo GM₁ (Fig. 4). This indicated that NK cell activity was necessary for the rapid elimination of the RBL-5 H-2-low variant cells. The effect of anti–asialo GM₁ injections was specific, and not due to injection of serum per se, since the

TABLE III

Survival of ^{125}I-UdR-labelled Tumor Cells in Syngeneic C57BL/6 Mice Pretreated with NRS

Organ	Percent of total injected radioactivity*	
	RMA	RMA H-2 sel
Lung	3.86 ± 3.60	0.37 ± 0.39
Spleen	0.68 ± 0.15	0.11 ± 0.06
Liver	4.04 ± 2.75	0.64 ± 0.64
Kidney	0.29 ± 0.09	0.08 ± 0.06
Hind leg	0.40 ± 0.10	0.18 ± 0.14
Gl tract	1.29 ± 0.40	0.70 ± 0.42
Heart	0.07 ± 0.07	0.02 ± 0.01
Brain	0.04 ± 0.02	0.01 ± 0.00
Thymus	0.02 ± 0.01	0.02 ± 0.01
Thyroidea	0.13 ± 0.00	0.10 ± 0.04

* Mean (±SD) radioactivity remaining 16 h after intravenous injection of RBL-5 wild type control line and anti-H-2–resistant variant, using three mice per group.

differences in survival between the lines were seen also in mice injected with normal rabbit serum (NRS) (Table III). The lower counts in liver, spleen, lung, and kidney in mice injected with RMA H-2 sel was not just due to redistribution, since the amount of detectable radioactivity in other organs was negligible.

Discussion

Three lymphoma variants, selected independently for loss of H-2 expression, showed a significantly reduced ability to form tumors in vivo. In our attempts to analyze the mechanism behind this phenomenon, we failed to find any difference in in vitro growth properties between the variant lymphoma cells and their appropriate controls. The difference observed in vivo must therefore depend on interactions between the tumor cells and host factor(s). One testable interpretation was that the variant and the wild type tumors were in fact equally sensitive to all possible host control mechanisms, and differed only in their ability to evoke a particular systemic host response. The H-2-deficient variant might thus have a unique ability to induce a tumor growth–inhibiting response, or lack the ability to induce a tumor growth–promoting response. These possibilities, however, were excluded in experiments where both tumors were injected simultaneously, in opposite flanks, in the same mouse. Since the difference in tumorigenicity remained under such conditions, it must be due to local tumor-host interactions, or to a difference in sensitivity to the same systemic host factor(s).

Furthermore, the reduced tumorigenicity of the H-2⁻ variant (RMA H-2 sel) did not depend on an intact T cell system. It could be argued that the genetic T cell defect of nude mice is incomplete, since they have CTL precursors detectable under certain conditions in vitro (22). However, it remains clear that nude mice have a strong relative T cell defect in vivo (23). Even if the variants were rejected by occasional T cells in nude mice, we would not expect the nude mice to be even more resistant than the wild type mice and be able to reject a challenge of 10^6 variant cells. Furthermore, while we have been able to protect mice from

RBL-5 challenge by preimmunizing them with irradiated cells from this tumor, we have so far not been able to protect mice from RBL-5, nor high doses of the variant cells by preimmunizing with the variants.[2] Taken together, these findings argue against the possibility that variant cells fail to grow because they evoke a strong adaptive T cell response. They are thus different from the highly immunogenic variants of the tum-negative phenotype derived after mutagenization and cloning without selections, as described by several groups (24, 25).

The strong in vivo resistance of nude mice is reminiscent of earlier findings on their strong rejection potential against certain tumors, attributed to high NK cell activity (26). Our general conclusion in this study is similar. The effects of whole body irradiation are consistent with rejection mediated by NK cells; an effector population with a limited life span, dependent on radiosensitive precursors, but not necessarily on proliferative expansion after tumor grafting (27). This would explain the relatively radioresistant rejection of 10^3 cells, while a dose of 10^4 cells would outnumber the diminishing effector cell population in the absence of continuous influx from the precursor pool. We thus explain the reduced tumorigenicity of the H-2$^-$ variant cells by rapid natural killing in vivo. This was observable within 16–24 h after intravenous injection of radiolabeled cells into the animals. These differences in rapid elimination between the H-2$^-$ and the wild type cells were abrogated by anti–asialo GM$_1$ serum, known to reduce NK cell function in vivo (19). Asialo GM$_1$ is also expressed on a small population of monocytes, and on some immature cells of T cell lineage (19, 28). However, taking into account experiments with radiation, nude mice, and natural killing in vivo, the results strongly support a model wherein cells with the functional and phenotypic characteristics of NK cells are responsible for the rejection. It could also be argued that the rapid elimination would be relevant for intravenous disseminated cells only. However, anti–asialo GM$_1$ treatment also allowed the H-2$^-$ cells to form tumors after subcutaneous inoculation. At this stage, we conclude that the reduced tumorigenicity is largely, if not entirely due to NK-dependent elimination, regardless of the detailed events leading to the final triggering of tumor cell lysis.

The method based on in vivo rejection of radiolabelled tumor cells in this paper was used previously (29–33) in studies of hybrid resistance, and showed that leukemic cells failed to survive in the spleens of mice that are not H-2-identical with the transplant (e.g. H-2^b cells in H-2^{d,d} or H-2^{d,b} hosts). In previous studies (34, 35) of the RBL-5 lymphoma, we found that it was highly malignant in syngeneic H-2^{b,b} hosts, whereas it was rejected in H-2^{d,b} hosts. Thus F$_1$ hybrid resistance (H-2^{d,b} rejecting H-2^b) and the rejection of syngeneic H-2-deficient cells (H-2^b rejecting H-2$^-$) seem to be mediated by the same mechanism, observable as natural killing within 24 h after intravenous injection, and sensitive to anti–asialo GM$_1$ (35). One interpretation, reconciling the two different systems, is that the rejection is dependent upon failure of the grafted cells to present the histocompatibility antigens of the host. NK-mediated rejection of H-2-deficient tumor variants or MHC-disparate bone marrow or lymphoid grafts (36) might then be interpreted to reflect a recognition of incomplete self-H-2 expression[2] (17). A surveillance against cells lacking all or a part of self-MHC would also explain why it is relatively difficult to select complete H-2$^-$ variants in vivo (37).

Preferential outgrowth of cells with reduced or deleted expression of certain H-2 alleles has however been observed for some virally induced, strongly antigenic tumors, for which T cells presumably represent the major surveillance mechanism (8, 9, 38). These experimental systems usually involve transplantation of large cell numbers (i.e. 10^5 cells or more), and long latency periods, both of which may facilitate suppression of the NK-mediated defense or the acquisition of additional changes of the tumor cells, permitting their escape also from this system. One may compare the progressive growth of 10^6 H-2-deficient cells in this study, while 10–1,000-fold lower doses could be efficiently rejected in most mice. The lower doses might better correspond to the size of a variant population newly arising in vivo. Escape from a surveillance against incomplete self could occur through selection for increased H-2 expression during tumor progression, thus explaining the paradoxical increase in the expression of class I MHC alleles associated with in vivo growth or metastasis in several experimental systems (5–7, 15). It is particularly the (re)expression of D-end alleles that has been associated with the metastatic phenotype (5–7). This is interesting if it indeed reflects an escape from a defense system that is also responsible for hybrid resistance and related to the NK cells. Both of the two latter phenomena are strongly influenced by genes mapping to the D end of H-2 (31, 32, 39, 40). In this context, it is interesting that low (or loss of) H-2 expression associated with increased malignancy more frequently involves the K-end locus (7, 8, 38). In some of the latter systems, recent studies with DNA-mediated gene transfer have provided direct evidence for the role of H-2K genes (38, 41, see also 42). Note however that the presence of H-2 would only be expected to delay or prevent growth of relatively antigenic tumors, probably a minority of spontaneous tumors (10). The rejection on the basis of H-2 deficiency suggested in this study would be independent of specific tumor antigens.

We have previously suggested that self-H-2 molecules may constitute (or contribute to the recognition of) an inhibitory signal in the NK-target interaction[2] (17). Lack of inhibitory influence would lead to preferential natural killing of such targets, thus giving the impression of recognition of absence of self. While our results indicate that the H-2-deficient cells are indeed more efficiently rejected by NK cells than their wild type counterparts in vivo, they do not prove the role of H-2 in control of NK sensitivity. At the host level, the NK-mediated elimination could be a nonspecific consequence of earlier events, such as local recruitment and activation of effector cells by H-2-deficient tumor cells, or failure of these cells to home correctly, leading to increased exposure to NK cells. We have recently observed that the H-2-deficient variants are more susceptible to spontaneous spleen-mediated cytotoxicity than the wild type cells in vitro (RMA: mean 8% lysis, range 0–16%; RMA H-2 sel: mean 28% lysis, range 15–45%).[2] This is in line with the possibility that loss of H-2 expression would affect the natural killing directly, although further studies focusing on the NK-target interaction and the role of other cells are required before any conclusions can be drawn. At the tumor cell level, a direct relation between the H-2⁻ and the NK-sensitive/tum-negative phenotype is attractive, but other possibilities must also be considered. The MHC genes could influence the expression or recognition of other cell surface molecules; our results may also merely reflect a common

regulation of MHC genes and genes influencing lymphoma cell interactions with host factors. The role of cell surface H-2 may be directly approached by molecular analysis of the regulatory or structural defects in H-2-variant cells, and by further manipulations of their phenotype using cloned H-2 genes and their derivatives corresponding to truncated (43) or hybrid products (44).

Summary

Three independent variants with a profound reduction of cell surface H-2 have been selected from the C57BL/6 mouse–derived RBL-5 and EL-4 T lymphomas. After subcutaneous inoculation of low cell doses in syngeneic mice, the H-2$^-$ variants failed to grow out, whereas the H-2$^+$ control lines showed progressive growth. No difference in growth rate or cloning efficiency was detectable in tissue culture. The in vivo difference in tumor outgrowth was analyzed in detail for one of the H-2-low lines. The outgrowth difference remained after the H-2-low variant and the control line had been injected subcutaneously in opposite flanks of the same mouse, and it was not dependent upon activity of mature T cells, since the same result was seen in athymic nude mice. The difference was partially sensitive to irradiation of the hosts. When mice were pretreated with anti–asialo GM_1 antiserum, known to depress natural killer (NK) cell activity, the difference in outgrowth was abolished, and both the control line and the H-2$^-$ variant showed progressive growth in vivo. Experiments comparing the distribution and survival of isotope-prelabeled variant and wild type cells indicated that a rapid elimination of the former took place within 24 h after intravenous injection. These differences in tumor elimination were not seen in mice treated with anti–asialo GM_1 antiserum.

We conclude that the reduced tumorigenicity of sublines with impaired H-2 expression is largely, if not exclusively due to rapid elimination by NK cells. These findings may reflect an inverse, indirect relation between factors controlling H-2 expression and NK sensitivity. Another possible explanation is that major histocompatibility complex (MHC)-encoded gene products are directly involved in a regulatory signal in the NK cell system. According to this interpretation, immunological selectivity in the NK cell system would be achieved by the failure to recognize self-MHC, irrespective of the presence of foreign antigens, i.e. by detection of no-self rather than of nonself. This may also explain previous observations on H-2-linked hybrid resistance against lymphoid grafts and changes in H-2 phenotypes associated with tumor progression.

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