INDEPENDENT REGULATION OF H-2K AND H-2D GENE
EXPRESSION IN MURINE
TERATOCARCINOMA SOMATIC CELL HYBRIDS*

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Products of the H-2K and H-2D genes of the mouse major histocompatibility complex are associated with β2-microglobulin and are presented on the cell surface. Allelic products of each of these loci are codominantly expressed on somatic cells of heterozygotes and on somatic cell hybrids between cells of differing H-2 haplotypes (1). Embryonal carcinoma cells (ECC), the stem cells of teratocarcinoma, and cleavage stage embryos do not express the H-2K and -2D gene products although they do express these products at a later stage of development or differentiation (2). Two previous investigations of H-2 gene expression on teratocarcinoma somatic cell hybrids have been reported. Miller and Ruddle (3), with H-2 typing sera, found that the H-2Kk products were strongly, and the H-2Kb only weakly, expressed on the cell surface of somatic cell hybrids between the teratocarcinoma cell line PCC4 azaguanine (aza) 1 and C3H thymocytes. Andrews and Goodfellow (4), with an identical fusion system, found that their hybrid cells also expressed H-2Kk antigens, but not the H-2Dk allelic products. These hybrids resembled ECC phenotypically, but unlike ECC, they expressed the H-2 antigens of the differentiated parent. They hypothesized, therefore, that H-2 gene expression in these hybrids was under cis control and that no diffusible regulatory molecules influenced the predetermined expression of parental genetic information (4). These results could also be explained by control of H-2 gene expression that is haplotype specific or by the loss of the chromosomes that contain the H-2 genes from the teratocarcinoma parent. We now report the analysis of H-2 expression on somatic cell hybrid clones derived from fusions of two different teratocarcinoma cell lines with differentiated mouse cells. We present evidence for two levels of control of H-2 expression. In hybrid clones that resemble ECC, the H-2 expression is conditional upon differentiation. In those that do not resemble ECC and are morphologically differentiated, H-2 gene products are found. Expression of the H-2K- and H-2D-coded molecules in these hybrids appears to be independently

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† Abbreviations used in this paper: aza, azaguanine; BrdU, 5-bromodeoxyuridine; DMEM, Dulbecco's modified Eagle's medium; ECC, embryonal carcinoma cells; FBS, fetal bovine serum; MEM, Eagle's minimal essential medium; PBS, Dulbecco's modified phosphate-buffered saline; RIA, indirect antibody-binding radioimmunoassay; SSEA-1, stage-specific embryonic antigen-1.
controlled. In addition, when H-2 is present on these hybrid cell surfaces, the H-2D gene products are always found and the H-2K antigenic specificities may or may not be.

Materials and Methods

Cell Lines and Culture Conditions. The pluripotent ECC line PCC4 aza 1 was derived from embryoid bodies of tumor OTT6050, a retransplantable teratocarcinoma originally produced by grafting a 6-d-old 129/Sv embryo into the testis of an F1 hybrid (A/He × 129/Sv) mouse (5). The cell line is 8-aza resistant (6, 7). We selected the clone F9 5-bromodeoxyuridine (BrdU) 7C12, derived from the nullipotent ECC line F9 (8), for its resistance to BrdU. PCC4 aza 1 and F9 BrdU 7C12 are grown in Falcon tissue culture flasks (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) with Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 15% fetal bovine serum (FBS) (Reheis Chemical Co., Chicago, Ill.) and 15 μg/ml of 8-aza or 30 μg/ml of BrdU, respectively. Both cell lines are derived from 129/Sv mice that are of the H-2^a haplotype, which is serologically indistinguishable from the H-2^b haplotype.

The three cell lines, K129SV (H-2^b), KC3HSV (H-2^k), and KD2SV (H-2^d) are SV40-transformed kidney-derived fibroblasts, maintained as described previously (9). The BW 5147 (H-2^k) cell line is derived from an AKR/J T cell lymphoma adapted to tissue culture (10) and is resistant to 8-aza. BW 5147 cells were maintained in RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) that contained 10% FBS and 15 μg/ml of 8-aza. The EL-4 cell line, H-2^a positive, Ia^b negative (11), was grown in DMEM with 10% FBS. All cell cultures were incubated at 37°C in a humidified atmosphere with a constant flow of 5% CO₂.

Cell Hybridization. Splenocytes were prepared by gentle teasing of freshly dissected C3H/HeJ spleens. The erythrocytes were lysed by incubation in 4 ml of 0.83% ammonium chloride (4 min at 4°C), and the cells were collected after centrifugation through a 2-ml FBS underlay (400 g for 10 min). PCC4 aza 1 and F9 BrdU 7C12 cells were harvested with 0.25% trypsin in Ca++- and Mg++-free Dulbecco’s modified phosphate-buffered saline (PBS) that contained 0.9% EDTA. Single-cell suspensions of BW 5147 cells were prepared by vigorous pipetting.

Spleen cells and PCC4 aza 1 cells were washed once in serum-free DMEM and mixed at a ratio of 10:1. F9 BrdU 7C12 (9 × 10⁶) and BW 5147 (11 × 10⁶) cells were harvested, washed once with serum-free DMEM, and mixed. The pelleted mixture was resuspended in 0.3 ml of 45% polyethylene glycol 1,000 (J. T. Baker Chemical Co., Phillipsburg, N. J.), incubated for 75 s in a water bath at 37°C, diluted by the dropwise addition of 5 ml of serum-free DMEM, and centrifuged (400 g for 5 min) at room temperature (12). The cells were seeded in Linbro FB16-24TC tissue culture plates (Linbro Chemical Co., Hamden, Conn.) in selection medium that consisted of DMEM supplemented with 15% FBS, 13.6 μg/ml hypoxanthine, 0.025 μg/ml methotrexate, 3.9 μg/ml thymidine, and 0.225 μg/ml glycine (13).

Clones were isolated from independent wells that contained a single colony, and subclones were obtained by the limiting-dilution technique. These cloned cell lines were determined to be hybrid by their growth in selection medium, chromosomal analysis, and observation of isozyme heteropolymers.

Antibodies. Anti-H-2 sera were supplied by the Transplantation and Immunology Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md. Anti-H-2^K^b (D-33), derived from (B10.D2 × A)F1; anti-B10.A(5R), detects private specificity 33 (H-2^K^b) along with public specificities 53 and 54, and contains some anti-Ia^a activity. Anti-H-2-D^d (D-2), from [B10.A(5R) × LP.RIII]F1; anti-B10, detects private specificity 2 (H-2^D^b) and public specificity 56. Anti-H-2K^b (D-23), from (B10 × LP.RIII)F1; anti-B10.A(2R), detects private specificity 23 (H-2^K^b), and may contain anti-Ia^b activity. Anti-H-2-D^d (D-32), from (B10.A(2R) × C3H.SW)F1; anti-C3H, detects private specificity 32 (H-2^D^b). Anti-H-2-D^d (D-4), from (B10.AKM × 129)F1; anti-B10.A detects only the private specificity 4 (H-2^D^b).

Ascites fluid (kindly supplied by Dr. K. Rajewsky, University of Cologne, Cologne, Federal Republic of Germany) from mice injected with the B22-249 R1 hybridoma cells (14) was the source of the monoclonal antibody recognizing the H-2.2 private specificity (H-2^D^b). This antibody is of the IgG2a class and was used at a 1:4,000 dilution in indirect antibody-binding radioimmunoassay (RIA).
Ascites fluid from a BALB/c mouse, injected with 11-4.1 hybridoma cells (15) (obtained from Dr. Inga Meichers, Stanford University, Palo Alto, Calif.) was the source of monoclonal antibody recognizing a public specificity common to H-2K\(^k\), H-2K\(^p\), H-2K\(^q\), and H-2K\(^r\). This antibody is also of the IgG2a class and was used at a 1:1,000 dilution.

The monoclonal antibody that detects a stage-specific embryonic surface antigen-1 (SSEA-1) expressed on early embryo and ECC has been described in detail (16). The anti-SSEA-1 reagent used was serum from an ascites-bearing BALB/c mouse that had been injected intraperitoneally with 10\(^6\) anti-SSEA-1 antibody-producing hybrid cells mixed with Freund's complete adjuvant. The anti-SSEA-1 reagent was used at a dilution of 1:32,000 (~75% of the maximal binding on F9 BrdU 7C12 cells).

**Preparation of Cells for Immunoassays.** Tissue culture cells were harvested, counted, and suspended in 0.01 M Hepes-buffered (Sigma Chemical Co., St. Louis, Mo.) Eagle's minimal essential medium (MEM) that contained 10% FBS. Splenic lymphocytes were obtained from freshly dissected and teased spleens after lysis of erythrocytes and centrifugation through a layer of Ficoll-Hypaque (1.077 g/ml) (Nyegaard and Co., Oslo, Norway). Linbro round-bottom microtiter plates (IS-RB-96, Linbro Chemical Co.) were used for all incubations of target cells with antisera.

**Immunoassays.** The RIA and the preparation of \(^{125}\)I-rabbit anti-mouse IgM and IgG are described elsewhere (17). Results are reported as the average counts per minute from triplicate samples minus the average counts per minute bound to cells incubated with a corresponding dilution of control myeloma-induced ascites fluid.

Absorption of conventional H\(^2\) antisera was performed by incubating increasing numbers of hybrid or control cells (0.5 \(\times\) \(10^6\), 2 \(\times\) \(10^6\), and 8 \(\times\) \(10^6\)) in 60 \(\mu\)l of antiserum at a dilution sufficient to lyse 60–85% of target splenic lymphocytes. After incubation at 4\(^\circ\)C for 60 min on a shaker, the cell suspensions were centrifuged and the supernates assayed. For cytotoxicity tests, 9 \(\mu\)l of target cell suspension that contained 3 \(\times\) \(10^5\) cells was added to the 50 \(\mu\)l of absorbed serum, incubated at 36\(^\circ\)C for 30 min on a shaker, and washed once with PBS that contained 2.5% FBS. The target cell pellets were then resuspended in 50 \(\mu\)l of rabbit complement (diluted 1:10 with Hepes-MEM) and incubated for 30 min at 36\(^\circ\)C on a shaker. The percentage of dead cells was determined by erythrosin B dye exclusion. C57BL/6J and 129/J splenic lymphocytes or EL-4 cells were used as targets for anti-H-2K\(^b\) or -2D\(^b\) sera; AKR/J and C3H/He] splenic lymphocytes were used as targets for anti-H-2K\(^k\) and -2D\(^k\) sera; and BALB/c ICR (Institute for Cancer Research, Fox Chase, Pa.) splenic lymphocytes for anti-H-2D\(^a\) serum. Absorption of antisera was always performed with all three cell concentrations; however, for ease of comparison, data are sometimes presented as the percent reduction of cytotoxicity on target lymphocytes after absorption with 2 \(\times\) \(10^6\) cells only.

**Results**

Two series of hybrid clones were investigated: PCC4 aza 1 fused with C3H spleen cells (the 477 series) and F9 BrdU 7C12 fused with BW 5147 (the 978 series). The 477 hybrids resemble ECC morphologically, and both ECC and five independent 477 hybrids bind antibody to the stem cell-specific surface marker SSEA-1 (Table I). In addition, clones of the 477 hybrids do not express the H-2 antigens of the H-2\(^b\) or H-2\(^k\) haplotype. Fig. 1a gives an example in which anti-H-2D\(^a\) serum failed to be absorbed with increasing numbers of cells from 477-1 clones I, DD, and GG. Identical data were obtained with antisera specific for the H-2D\(^b\), -2K\(^b\), or -2K\(^k\) molecules (Table II).

If these clones were maintained without subcultivation, but with medium change every 2nd d for 2–3 wk (aged cultures), differentiated cells appeared and became the dominant subpopulation in the cultures. Data from two representative experiments concerning expression of H-2 antigens on aged cultures are presented in Fig. 1b and Table II. The D4 antiserum is included for control purposes. Cells from the aged 477-1 GG clone absorb the cytotoxic activity from the four H-2 sera specific for the H-
TABLE I

Expression of SSEA-1 on Hybrid and Control Cells

| PCC4 aza 1 × C3H spleen cells | F9 BrdU 7C12 × BW 5147 |
|-------------------------------|------------------------|
| cpm *                         | cpm *                  |
| 477-1 B                       | 10,100                 | 978-1-5                 |
| 477-1 P                       | 7,900                  | 978-1-6                 |
| 477-1 I                       | 11,400                 | 978-2-1                 |
| 477-1 DD                      | 12,800                 | F9 BrdU 7C12           |
| 477-1 GG                      | 12,800                 | BW 5147                |
| PCC4 aza 1                    | 10,800                 | K129SV                 |

*Counts per minute bound to 5 × 10^5 cells after incubation with 50 μl α-SSEA-1 (1:32,000) followed by 50 μl 125I-rabbit anti-mouse IgM (heavy-chain specific). The values represent the average counts per minute bound to triplicate cell samples minus the counts per minute bound to triplicate samples of each cell (200-800 cpm) when incubated with 1:32,000 ascites fluid from mice bearing the P3-X-63-Ag8 myeloma (12).

Fig. 1. Quantitative absorptions of anti-H-2D^k (D-32) with 477 hybrid cells (a) and with aged 477 hybrid (b) cells. After absorption with KC3HSV (O), K129/SV (Δ), PCC4 aza 1 (□), 477-1 I or 477-1 I aged (■), 477-1 DD or 477-1 DD aged (▲), or 477-1 GG or 477-1 GG aged (■), the residual cytotoxic activity of anti-H-2D^k (D-32) was tested by complement-mediated cytotoxicity on spleen lymphocytes from AKR/J mice. u, unabsorbed antiserum; c', complement control.

It was possible that the presence of antibodies against Ia determinants in the two antisera directed against the H-2K specificities could alter the absorption results obtained. To exclude this possibility, we tested the anti-H-2K^b serum after absorption both on H-2K^b- and Ia-expressing C57BL/6J spleen lymphocytes, and on Ia-negative, H-2K^b-positive EL-4 cells. Activity of the absorbed antiserum was reduced to the same extent on both target cells. A monoclonal antibody to a public specificity present on the product of the H-2K^b allele, and not of the H-2K^b allele, was also used to...
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Table II
H-2 Expression on PCC4 × Spleen Hybrid and Control Cells Detected by Absorption Analysis

| Cells              | Antisera* |
|--------------------|-----------|
|                    | H-2K\textsuperscript{a} | H-2D\textsuperscript{b} | H-2K\textsuperscript{k} | H-2D\textsuperscript{k} |
|                    | (D-33)    | (D-2)   | (D-23)    | (D-32)    |
| 477-1 B            | 22.3      | 6.2     | 6.8       | 17.1      |
| 477-1 P            | 5.3       | 20.3    | 3.5       | 14.9      |
| 477-1 I            | 19.5      | 16.6    | 17.2      | 15.2      |
| 477-1 aged         | 12.3      | 38.3    | 24.3      | 20.5      |
| 477-1 DD           | 11.3      | 17.2    | 10.3      | 18.6      |
| 477-1 DD aged      | 30.0      | 69.9    | 26.2      | 54.6      |
| 477-1 GG           | 23.1      | 37.5    | 12.0      | 19.5      |
| 477-1 GG aged      | 83.4      | 78.9    | 83.9      | 66.8      |
| PCC4 aza 1         | 10.0      | 9.4     | 7.1       | 6.5       |
| C3H splenocytes    | 7.0       | 0       | 94.8      | 91.8      |
| 129 splenocytes    | 96.1      | 93.7    | 3.5       | 4.5       |
| KC3HSV             | 3.9       | 1.7     | 70.9      | 59.1      |
| K129SV             | 80.6      | 79.1    | 7.1       | 4.5       |
| KD05V              | ND        | ND      | ND        | ND        |

* Genotypes and private specificities that are recognized by National Institutes of Health typing sera.
† Percent reduction of cytotoxicity of a given anti-H-2 serum after absorption with 2 × 10\textsuperscript{6} cells. Percent reduction = [ci(u) - ci(t)]/[ci(u)] × 100, where ci(u) is the cytotoxic index of unabsorbed serum and ci(t) is the cytotoxic index of the serum absorbed with test cells. Cytotoxic index (ci) = (T - C)/(100 - C) × 100, where T is percentage of cells killed by the serum tested, and C is the percentage killed by complement only. Target cells are C57BL/6J (H-2K\textsuperscript{b} and H-2D\textsuperscript{b}), AKR/J (H-2K\textsuperscript{k} and H-2D\textsuperscript{k}), or BALB/c ICR (H-2K\textsuperscript{d} and H-2D\textsuperscript{d}) splenic lymphocytes. ND, not determined.

detect the presence of H-2K\textsuperscript{k} molecules. This antibody recognizes a different specificity from that detected with the conventional serum and excludes the possibility of contaminant antibodies to Ia\textsuperscript{k} determinants. Analysis of the reactivity of this monoclonal antibody on the hybrid clones (Table III) confirms the results obtained by absorption. The 477-1 GG aged clone reacts with this monoclonal reagent, whereas the two other aged clones do not.

Hybrids of the 978 series (F9 BrdU × BW 5147) are morphologically different from each of the parental cells. These hybrids are large, substrate-attached fibroblastic cells and do not express either the SSEA-1 surface marker of the teratocarcinoma parent (Table I) or the Thy-1.1 marker present on BW 5147 cells (results not shown). In contrast to the 477-1 hybrid clones, H-2 antigens are expressed on each of the three hybrid clones tested. To control the H-2 assays, two SV40-transformed, substrate-attached cell lines (K129SV and KC3HSV) derived from mice of the same H-2 haplotype as the parental cells of the hybrid were used, as well as AKR/J and 129/J lymphocytes and both parental cell lines (F9 BrdU 7C12 and BW 5147). Splenic lymphocytes absorb slightly more of the specific reactivity from specific test sera than the SV40-transformed control cell lines, whereas BW 5147 absorbs considerably less. Results from the F9 BrdU 7C12 cell line parallel the negative control cells. Cells of the 978-1-6 hybrid clone express H-2K\textsuperscript{b}, -2D\textsuperscript{b}, -2K\textsuperscript{k}, and -2D\textsuperscript{k} antigenic specificities;
### Table III

**H-2 Expression on Hybrid and Control Cells Detected by Monoclonal Antibodies**

| Cells                        | Reagent | Cells                        | Reagent |
|------------------------------|---------|------------------------------|---------|
| PCC4 aza 1 × C3H spleen cells| 11-4.1  | F9 BrdU 7Cl2 × BW 5147       | 11-4.1  |
| (anti-H-2K^k)                |         | (anti-H-2K^k)                |         |
| 477-1 B                      | 0       | 978-1-5 D8                   | 1,400   |
| 477-1 P                      | 0       | 978-1-5 D11                  | 420     |
| 477-1 I                      | 0       | 978-1-5 H9                   | 620     |
| 477-1 I aged                 | 0       | 978-1-5 H12                  | 1,400   |
| 477-1 DD                     | 0       | 978-1-6 2B12                 | 155     |
| 477-1 DD aged                | 150     | 978-2-1 2B6                  | 150     |
| 477-1 GG                     | 0       | F9 BrdU 7Cl2                 | 240     |
| 477-1 GG aged                | 1,200   | BW 5147                      | 2,000   |
| PCC4 aza 1                   | 0       | KC3HSV                       | 4,500   |
| K129HSV                      | 4,400   | K129SV                       | 230     |
| *Counts per minute bound to 5 × 10^5 cells after incubation with 50 μl 11-4.1 (1:1,000) or B22-249R1 (1:4,000) followed by 50 μl 125I-rabbit anti-mouse IgG (heavy- and light-chain specific). The values represent the average counts per minute bound to triplicate cell samples minus the counts per minute bound to replicate samples of each cell (200-1,000 cpm) when incubated with 1:1,000 ascites fluid from mice bearing the P3-X-63-Ag8 myeloma (12). |

Each of the original hybrid clones were subcloned, and the subclones were also repeatedly obtained with different passages of these hybrid clones.

Data from the absorption of the D-23 sera (anti-H-2K^k) were correlated with the reactivity of the monoclonal antibody to a public specificity of the H-2K^k allele. Subclones of the 978 hybrids, which were clearly H-2K^k positive in absorption experiments (978-2-1 2B6 and 978-2-1 2F8), do not bind this monoclonal antibody.
Fig. 2. Quantitative absorption of (a) anti-H-2D<sup>b</sup> (D-2), (b) anti-H-2D<sup>a</sup> (D-32), (c) anti-H-2K<sup>b</sup> (D-33), and (d) anti-H-2K<sup>k</sup> (D-23) with 978 hybrid clones and control cells. After absorption with KC3HSV (△), K129SV (O), 978-1-5 (●), 978-1-6 (▲), or 978-2-1 (■), the residual cytotoxic activity of the antisera was tested by complement-mediated cytotoxicity on splenic lymphocytes from C57BL/6J (D-2 and D-33) or AKR (D-32 and D-23) mice. u, unabsorbed antiserum; c', complement control.

(Table III). Subclones that absorbed the reactivity from the D-23 serum bind the monoclonal reagent. Subclones 978-1-5 D11 and H9, which were judged negative in absorption analysis, do bind a small amount of this monoclonal reagent, and, thus, the possibility exists that H-2K<sup>k</sup> is weakly expressed on all or some of these hybrid cells. The reactivity in RIA of the subclones with monoclonal antibody, recognizing H-2.2 (private specificity of H-2D<sup>b</sup>) is in complete agreement with the data from the absorption experiments.

Discussion

The 477 hybrids (PCC4 X C3H spleen) reported here resemble ECC both morphologically and in their expression of cell surface antigens. When teratocarcinoma stem cells differentiate in vitro, they no longer express SSEA-1 (18), and the differentiated cells express H-2 molecules (19). Although the 477 hybrids are SSEA-1 positive and H-2 negative, they differentiate and express H-2 antigens after aging in culture. Extinction of the expression of the H-2 antigens of the nonteratocarcinoma parental cell is at variance with previous reports (3, 4); our results indicate that H-2 expression
### Table IV

**H-2 Expression on F9 X BW 5147 Hybrid Clones, Subclones, and Control Cells Detected by Absorption Analysis**

| Cells     | Antisera* |
|-----------|-----------|
|           | H-2K<sup>b</sup> | H-2D<sup>b</sup> | H-2K<sup>k</sup> | H-2D<sup>k</sup> |
|           | (D-33)     | (D-2)     | (D-23)     | (D-32)     |
| 978-1-5‡ | 22.9       | 87.2      | 27.7       | 82.9       |
| 978-1-5  | 89.9       | 90.5      | 38.9       | 41.3       |
| 978-1-5  | 10.1       | 91.7      | 24.4       | 43.0       |
| 978-1-5  | 10.1       | 84.5      | 25.0       | 37.6       |
| 978-1-5  | 31.4       | 67.9      | 30.1       | 40.5       |
| 978-1-6‡ | 42.3       | 98.4      | 80.6       | 79.3       |
| 978-1-6  | 44.6       | 44.0      | 20.7       | 15.3       |
| 978-2-1‡ | 20.2       | 94.1      | 66.3       | 88.4       |
| 978-2-1  | 7.1        | 0         | 0          | 0          |
| 978-2-1  | 0.8        | 63.1      | 0          | 26.0       |
| F9 BrdU 7C12 | 3.5   | 0         | 11.0       | 4.1        |
| BW 5147  | 0          | 2.3       | 28.4       | 25.5       |
| K129SV‡ | 92.3       | 86.6      | 0          | 4.1        |
| KC3HSV‡ | 0          | 0         | 68.8       | 48.9       |
| EL-4     | 100        | 95.2      | 13.4       | 0          |

* Genotypes and private specificities that are recognized by NIH typing sera.
† Results from Fig. 2.
§ Percent reduction of cytotoxicity of anti-H-2 serum after absorption with 2 × 10⁶ cells is calculated as described in Table II. Target cells are C57BL/6J (H-2K<sup>b</sup> and H-2D<sup>b</sup>) or AKR/J (H-2 K<sup>k</sup> and H-2D<sup>k</sup>) splenic lymphocytes.

The 978 hybrids (F9 X BW 5147) are morphologically different from ECC and rule out cis control of H-2 expression. Moreover, because H-2 antigenic specificities of both parents appear after aging in culture, loss of the chromosomes that bear the structural genes for H-2 is impossible. In previous reports (3, 4), the authors examined the expression of only one of the H-2 gene products from each parental cell (H-2K<sup>k</sup> and -2K<sup>b</sup> or H-2K<sup>b</sup> and -2D<sup>b</sup>, respectively) and did not mention whether the cultures contained mixed populations of differentiated and stem cells. Because we show that H-2K and -2D gene expression are independently controlled and dependent upon in vitro differentiation, our data and those previously reported are not necessarily at variance, though our conclusions differ.

The 978 hybrids (F9 X BW 5147) are morphologically different from ECC and the 477 hybrids. The 978 hybrid clones do not express the teratocarcinoma-specific antigenic determinant SSEA-1 nor the thymoma characteristic Thy-1.1 and they do express H-2. Both in vitro characteristics of the 978 hybrids and the fact that tumors derived from injection of 978 hybrid cells are not teratocarcinoma (data not shown) contrast these hybrid clones with the 477 hybrids and ECC.

Analysis of H-2 expression in these two sets of hybrids suggests two levels of control. If the hybrid cells are indistinguishable from ECC, the H-2 molecules of both parents are not expressed. We feel that positive suppression by regulatory gene products of the teratocarcinoma parent is the most probable mechanism. A second level of control is also suggested for us to find that the H-2K and -2D gene products of both parental
cells can be independently expressed. Furthermore, if H-2 antigenic determinants are present on the hybrid cell surface, the H-2D gene products are always found and the H-2K genes may or may not be expressed. The absence of the H-2K gene product, consequently, can not be explained by the loss of the chromosomes that contain the structural genes for H-2 or β2-microglobulin.

Any attempts to postulate a mechanism for the control of H-2 expression must include the concept that there is no simple on/off control of all of the H-2 genes. The parental cell of the 978 hybrids, BW 5147, is derived from an AKR thymoma. Both the H-2Kk and -2Dk gene products are only weakly expressed on BW 5147 and it may be that the differentiated expression of H-2K in the hybrid cells is the consequence of aberrant gene regulation in this parental cell line. However, the 477 hybrids show independent expression of the H-2D gene products, and some clones of the 978 hybrids do strongly express the H-2K antigenic determinants. Preferential extinction of the H-2K gene product may simply be peculiar to the H-2K haplotype because differential H-2K and -2D gene expression has been primarily observed in this haplotype (20). We believe that this regulatory control is H-2K gene specific and not allele specific because the data from these hybrid cells imply joint control of the H-2Kk and -2Kb gene products. We can postulate mutations that affect the H-2K and not the H-2D genes. The H-2K locus appears to be hypermutable in vivo because most of the mutations that affect H-2 genes were found in the H-2K region (21). Additionally, cell lines derived from AKR thymomas are reported to express products of the H-2Dk gene, but not those of the H-2Kk gene (20). However, attempts to immunoselect for the absence of H-2 expression in cell cultures has resulted in isolation of cells that have lost either H-2K or H-2D expression (22). We feel that mutation of the H-2K structural genes is highly unlikely because three of six independently derived clones from two different parental cell lines would have been affected by such a mutation. In two of these clones the H-2K products from both parental cells are affected. Moreover, two subclones of the 978-1-5 clone express the H-2Kk gene product that is not present on the original clone. Another possible explanation for this differential expression is the loss of some of the chromosomes that contain the β2-microglobulin structural genes; gene dosage would thus result in a limiting supply of β2-microglobulin molecules. This mechanism would imply a preferential affinity of the H-2D gene products for β2-microglobulin. The expression of the H-2Kk specificities in the 978-1-5 subclone D8, once again could argue against this point.

A more likely explanation of our results is that regulatory genes exist with products specific for either the H-2K or the H-2D loci. These regulatory products would allow independent expression of the H-2K and -2D genes. We believe that the H-2D gene is expressed first during differentiation with subsequent activation of the H-2K gene. The H-2D-positive, H-2K-negative hybrids reported here would then represent intermediary steps in the differentiation process. This hypothesis is supported by the recent observation that populations of cells in the thymus are H-2K negative and H-2D positive, whereas peripheral T cells express products of both H-2K and -2D. The hybrid clones and subclones described here, with their differing expression of the H-

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2 gene products, will prove useful in the investigation of the mechanisms that control the regulation of H-2 expression.

Summary

Cells of two teratocarcinoma stem cell lines (PCC4 azaguanine [aza] 1 and F9 5-bromodeoxyuridine [BrdU]) were fused with normal mouse spleen cells and mouse thymoma-derived cells (BW 5147), respectively. Hybrid clones were tested for the expression of molecules coded by the H-2K and -2D genes both by absorption analysis of conventional H-2 sera and by indirect antibody-binding radioimmunoassay with monoclonal antibodies. Somatic cell hybrids between PCC4 aza 1 and spleen cells morphologically resemble teratocarcinoma stem cells and do not express H-2 antigens. However, after differentiation in vitro, one of these hybrid clones expresses the H-2K and -2D gene products of both parental cell lines, one clone expresses H-2D- but not H-2K-coded antigenic determinants, and one clone remains H-2 negative. Somatic cell hybrids between F9 BrdU and BW 5147 resemble fibroblasts. Analysis of a series of hybrid clones revealed some clones that express both the H-2K- and H-2D-coded antigenic specificities of both parental alleles, some that express H-2D gene products strongly and the H-2K gene products very weakly, and some that express H-2D- but not H-2K-coded molecules. These results imply independent regulation of expression of the H-2K and -2D genes. The H-2D gene products appear to be preferentially expressed if the hybrid cells are capable of expressing H-2. The results suggest complex regulatory mechanisms that are H-2K and H-2D specific.

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