EFFECT OF THE H-2 GENE COMPLEX RATES OF FIBROBLAST INTERCELLULAR ADHESION

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ABSTRACT

The rate of collection of embryo fibroblast single cells by an embryo fibroblast monolayer was related to the H-2 haplotype of the fibroblast monolayer. The rate was highest for the H-2^a strains and lowest for the H-2^k strains with all other strains examined being intermediate. As opposed to monolayers prepared from the A and C3H background animals, monolayers from B10 background mice only demonstrated an H-2 haplotype dependent rate differential after treatment with fetal calf serum or neuraminidase. The relationship that was seen between monolayer H-2 haplotype and rate of adhesion with embryonic monolayers was not observed with either congenic 3T3 cell lines or fibroblasts derived from adult tissues.

It was further shown that the rate of single cell pick-up could be substantially reduced by incubating the monolayers with the appropriate polyspecific anti-H-2 antisera. The inhibition observed appeared to be directly related to anti-H-2 antibody binding and was not merely a function of ligand binding to the cell surface, as antisera directed against other fibroblast cell surface antigens did not significantly inhibit the adhesive rate. These results indicate a role for the H-2 gene complex in modulating fibroblast-fibroblast intercellular adhesion.

KEY WORDS H-2 congenics adhesion fibroblast H-2 antisera

The H-2 complex is a small multigenic chromosomal region, which was originally defined because it determined the major transplantation antigens in mice (27). Loci in the H-2 complex have now been shown to affect a variety of immunologic functions. These include the immune responsiveness (IR) of an animal to a wide range of antigens (1, 6), regulation of T-B lymphocyte interaction during immune responses (11), the control of the level of complement components (3), the interaction of sensitized lymphocytes (T cells) with syngeneic target cells that have been infected with virus (33) or modified with hapten (26). Susceptibility to viral leukemogenesis is also linked to the H-2 complex (15). More general physiological traits are also linked to the H-2 complex. Androgen-sensitive organ weights (9), the plasma concentration of, and the ability to bind, testosterone (10), and the steady-state levels of liver cyclic adenosine monophosphate (cAMP) (17) are all modified by H-2 linked genes.

Most loci of the H-2 complex exert their influence primarily at the cell surface. All of the serologically detectable gene products of the H-2 complex, the antigens coded for by the D and K regions (7), the Ia antigens (2), and even the Ss protein (25) appear to be on the cell surface.
Animals

Initially obtained as outgrowth from small pieces (2 mm in diameter) of lung or mesentery that had been placed in Ca++- and Mg++-free Hanks' balanced salt solution designated HH. The cells were suspended in a dissociation solution, CTC, containing 0.2% trypsin (Grand Island Biological Co.), 2.5% (vol/vol) heat-inactivated chicken serum, and 0.002% purified collagenase (Worthington Biochemical Corp.) in a diluent of Ca++- and Mg++-free Hanks' balanced salt solution (Grand Island Biological Co., Grand Island, N.Y.). The fibroblasts were cultured in Eagle's minimal essential medium (Grand Island Biological Co.), supplemented with 15% (vol/vol) fetal calf serum (Dulbecco's modified eagle's medium (Grand Island Biological Co.) with 10% (vol/vol) calf serum (Dulb.10).

A baby hamster kidney cell line (BHK) was kindly provided by Dr. B. Walther. The line was grown in BHK Medium (Grand Island Biological Co.) with 10% (vol/vol) fetal calf serum (FCS).

Preparation of Mouse Fibroblast Strains

Embryo: Fibroblast cell strains were obtained from 15-18-day-old embryos. The embryos were removed aseptically from the mother, eviscerated, decapitated, and the carcasses cut into small pieces. Dissociation was carried out according to a method previously described (20) with 0.25% trypsin (Worthington Biochemical Corp., Freehold, N.J.) in Ca++- and Mg++-free Hanks' balanced salt solution (Grand Island Biological Co., Grand Island, N.Y.). The fibroblasts were cultured in Eagle's minimal essential medium (Grand Island Biological Co.), supplemented with 15% (vol/vol) fetal calf serum (Reheis Co., Inc., Phoenix, Arizona), designated MEM 15. Embryonic fibroblasts were passaged at least twice before assaying, and were then stored in ampoules at −196°C in MEM 15 with 10% (vol/vol) dimethyl sulfoxide (DMSO) until required.

Adult: Fibroblasts from 10-15-week-old mice were initially obtained as outgrowth from small pieces (2 mm in diameter) of lung or mesentery that had been placed in vitro organ culture. This type of preparation was used as it was found that the enzyme treatment of adult tissues produced a low percentage of viable fibroblasts. After the initial isolation, the adult fibroblasts were subsequently grown as monolayers under conditions identical to those of the embryonically derived fibroblasts, and were passaged in vitro at least twice before being assayed.

Cell Lines

3T3 cell lines were established in our laboratory according to the method of Todaro and Green (28), and were passaged at least 20 times before use. The cells were grown in Dulbecco's modified Eagle's medium (Grand Island Biological Co.) with 10% (vol/vol) calf serum (Dulb.10).

A baby hamster kidney cell line (BHK) was kindly provided by Dr. B. Walther. The line was grown in BHK Medium (Grand Island Biological Co.) with 10% (vol/vol) fetal calf serum (FCS).

Adhesion Assay

The assay employed was a modification of that previously described by Walther et al. (29) and measures the rate of binding of suspended single cells to a confluent monolayer.

Fibroblast monolayers, of different H-2 genotypes but of equivalent number of passages, were harvested using a dissociation solution, CTC, containing 0.2% trypsin (Grand Island Biological Co.), 2.5% (vol/vol) heat-inactivated chicken serum, and 0.002% purified collagenase (Worthington Biochemical Corp.) in a diluent of Ca++- and Mg++-free Hanks' balanced salt solution (Grand Island Biological Co.). The cells were washed twice and resuspended in MEM 15 at a concentration of 3 × 10^6 cells/ml, and 0.2 ml of this suspension was placed in each flat bottom well of a microtiter II plate (BioQuest, BBL & Falcon Products, Becton Dickinson & Co., Cockeysville, Md.). This concentration of cells assured a confluent monolayer in the wells within 24 h after plating. In addition, aliquots of the same cell suspension were replated onto petri dishes and labeled for 17-20 h with [3H]leucine (25 Ci/mmol, New England Nuclear, Boston, Mass.). Cell suspensions of the labeled cells were prepared 5 h after the labeled monolayers had been washed and placed in fresh unlabeled medium. CTC was used to harvest the [3H]-labeled cells, which were subsequently washed twice with MEM 15 and twice in N-2-hydroxyethylpiperazine-N′-2-ethane sulfonic acid (HEPES) (0.01 M) buffered Hanks' salt solution designated HH. The cells were suspended in HH at a final concentration of 2 × 10^6/ml. The HH was supplemented with 5% FCS during some experiments as indicated in the text. The test monolayers in the microtiter plates were washed and incubated with HH for 15 min before the experiment. The plates were inverted to remove the HH and then righted and placed in a water bath at 37°C. To each well was added 0.1 ml of [3H]-
labeled cell suspension (2 x 10^4 cells/well). A repeating dispenser (Hamilton Co., Reno, Nev.) allowed the addition of labeled cells to one plate, consisting of 96 wells, within 90 s.

At 5-min intervals after the addition of the labeled cells, supernates from a row of wells were removed by gentle suction, and the monolayers were washed three times with 0.2 ml of HH. After the washing of the last row of cells in a plate was completed, 0.2 ml of 1 M NH_4OH was added per well, and this treatment lysed all cells and solubilized 99% of the radioactivity (29). The combined extracts were counted in a Triton-toluene counting cocktail.

Data obtained from triplicate samples at each time-point were fitted to a straight line by the method of least squares, and the correlation coefficient was determined (r). If r was less than 0.90, the data were discarded. Statistical analysis of the linear regression coefficients was carried out using a variation of Student's t test (13).

Adhesion was quantitated in terms of the rate of pick-up of the single cells by the monolayer, that is, from the slope of the fitted curve.

**Fluorescent Reagents and Antisera**

Fluorescein-conjugated goat anti-mouse IgG (F1-Gam) was prepared as previously described (5). C3H/HeSn anti C3H.SW (anti H-2b) and C3H.SW anti C3H (anti H-2k) were raised in our laboratory and had cytotoxic titers against lymphocytes of 1:5,120 and 1:2,560, respectively; both were heat inactivated before use. AKR anti C3H (anti Thy 1.2) was purchased from Litton Industries.

Sera reacting with the H-2^k K end (D-23) and the H-2^k D end (D-32) of the H-2 complex were obtained from the Transplantation and Immunology Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health. The sera were absorbed with EL4 (C57BL leukemia. H-2^k) cells to remove any anti-MuLV activity (12).

The A.TH anti-A.TL sera detecting Ia antigenic specificities was provided by Dr. H. O. McDevitt.

Antisera raised against a major cell surface protein of chick embryo fibroblasts, CSP (32) in a goat were kindly provided by Dr. K. M. Yamada.

Rabbit anti-rat liver plasma membrane and rabbit anti-mouse spleen cell antisera were prepared in this laboratory.

Rabbit anti-rat brain Thy 1(31) was the generous gift of Dr. A. Williams.

**Indirect Immunofluorescent Staining of Fibroblasts**

Fibroblasts were harvested with CTC then washed twice with HH containing 5% (vol/vol) FCS. Aliquots containing 10^6 cells were resuspended in 50 μl of the antisera and incubated at 4°C for 15–20 min. The cells were then washed twice with HH plus 5% FCS, and 50 μl of fluorescent conjugate was added to each aliquot of cell and further incubated for 15 min on ice. After the final incubation, the cells were washed three times and then examined in a Leitz fluorescence microscope (E. Leitz, Inc., Rockleigh, N.J.) with a 40X n.a. 1.32 objective and a vertical illuminator.

**Neuraminidase Treatment of Cell Monolayers**

Fibroblast monolayers were treated with neuraminidase from Clostridium perfringens (Worthington Biochemical Corp.) for 1 h before the adhesion assay, using 0.01 U/ml diluted in pH 7.35 HH. Although this pH was above that required for optimal release of sialic acid (22), it was the optimal pH for cell survival and monolayer integrity.

**RESULTS**

**Kinetics of Fibroblast-Fibroblast Adhesion**

Fig. 1 is typical of curves obtained using the monolayer collection assay to monitor fibroblast adhesiveness. It can be seen that the rate of cell adhesion was essentially constant between 5 and 30 min. It was also found that the adhesion rate was not dependent on agitation of the plate during this assay (Fig. 1). Variation in adhesion rates of 10–20% was found to occur between experiments; a similar variation has been reported for this assay previously (29), and therefore comparisons in this paper have been made only between
The experiments carried out with the same single cell preparation, performed on the same day, and most often on the same plate. All experiments shown have been performed at least three times, unless otherwise indicated.

The Effect of Passage Number on Cell Adhesion

Table I demonstrates that, for the B10 embryo fibroblast strain in a monolayer, no statistically significant change occurred in the adhesion rate of fibroblasts between 1 and 10 passages. This was true with both BHK and B10 single cells. Later passages of embryo fibroblasts were not studied as these cells did not readily form confluent monolayers. It should be noted that the experiment represented in Table I was carried out on a single plate so as to avoid variations that occur between experiments. This experiment was repeated with C3H fibroblasts, and similarly there was no relationship between passage number and adhesion rate.

The Effect of H-2 Genotype on Rates of Adhesion

Congenic-resistant strains of mice that differ only at the H-2 complex were used to determine the role, if any, of the H-2 gene complex in modulating intercellular adhesion. As shown in Fig. 2 and Table II, with the use of congenic-resistant strains of mice bearing various H-2 haplotypes on the C3H genetic background, significant differences in adhesion rates were observed.

In all experiments, monolayer cells of the C3H/HeJ and the C3H/HeSn strains, which are both H-2<sup>k</sup>, collected single cells at significantly lower adhesion rates than cells of all the other congenic strains on the C3H background. There was no statistically significant difference in adhesion rates between the four other congenic strains on the C3H background. The lower adhesion rate observed for the C3H strain cells was independent of the genotype of the single cell preparation (see Table I). Indeed, Table III demonstrates that, even in noncongenic strains of mice of different genetic backgrounds, the H-2<sup>k</sup> haplotype was associated with a significantly lower rate of adhesion than were other H-2 haplotypes. The H-2 differential effect did appear to be species-specific as baby hamster kidney (BHK) fibroblasts were picked up at the same rate by both C3H and C3H.SW monolayers. The latter observation was of benefit in subsequent assays; a differential uptake of BHK by fibroblast monolayers usually indicated loss of monolayer integrity or subconfluence of one of the monolayers. The fibroblasts from congenic mice of the A background all showed approximately equivalent adhesion rates, with the exception of the A.SW (H-2<sup>*</sup>) which was found to have a significantly higher rate of

\[ \text{Table I} \]

| Passage no. | Adhesion rate | Single cell type |
|------------|---------------|-----------------|
| 1          | 0.98<sup>§</sup> | B10             |
| 1          | 1.40<sup>†</sup> | BHK             |
| 5          | 0.97          | B10             |
| 5          | 1.45          | BHK             |
| 10         | 1.04          | B10             |
| 10         | 1.38          | BHK             |

* Assay performed in HH without FCS.
† Percentage of total cells adhering per minute to monolayer.
‡ No significant difference of adhesion rate with passage using B10 single cells.
§ No significant difference of adhesion rate with passage using BHK single cells.

Figure 2: Adhesion of <sup>3</sup>H-labeled C3H single cells to C3H (▲) and C3H.SW (●) monolayers. The lines have been fitted by the method of least squares. Each point represents the mean and standard deviation of triplicate determinations. The total cpm per single cell aliquot was 23,100. The adhesion rates, calculated as percentage of total cell adhering per minute to the monolayer, were 1.32 and 0.98 for the C3H.SW and C3H monolayers, respectively.

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TABLE II
Effect of H-2 Genotype on Rates of Adhesion
(a) Fibroblasts on the C3H Background

| Single cell type       | Monolayer type | Adhesion rate*± |
|-----------------------|----------------|-----------------|
| C3H(H-2*K)            | C3H(H-2*K)     | 0.85±           |
| C3H.SW(H-2*K)         | C3H.SW(H-2*K)  | 1.25            |
| C3H                   | C3H            | 1.30            |
| C3H.SW                | C3H.SW         | 1.30            |
| C3H.OH(H-2*B)         | C3H.SW         | 1.45            |
| C3H                   | C3H            | 1.10            |
| C3H.OH                | C3H.OH         | 1.35            |
| C3H.JK(H-2')          | C3H.JK         | 1.28            |
| C3H.NB                | C3H.NB         | 1.30            |
| C3H.SW                | C3H.SW         | 1.26            |
| C3H                   | C3H            | 0.98±           |
| C3H.NB(H-2*)          | C3H.NB         | 1.32            |
| C3H.JK                | C3H.JK         | 1.28            |
| C3H.SW                | C3H.SW         | 1.35            |
| C3H                   | C3H            | 0.85±           |
| C3H.NB                | C3H.NB         | 1.35            |
| C3H.JK                | C3H.JK         | 1.30            |
| C3H.SW                | C3H.SW         | 1.29            |
| C3H                   | C3H            | 0.89±           |

* Performed in the absence of serum.
† Percentage of total cells adhering per minute to monolayer.
‡ C3H/HeJ and the C3H/HeSn gave similar results.
§ Adhesion rate significantly different from that of other monolayers in group with identical single cell type. P < 0.05.
* This assay was repeated nine times with four different embryo sources of the fibroblast cell strains, all of which show a significantly higher adhesion rate for the C3H.SW monolayer.

adhesion (Table IV). Unfortunately, no H-2*K was available in strain-A genetic background. Similar experiments, carried out as before with serum-free medium, failed to show any significant differences in adhesion rates between H-2 different fibroblasts from strains with a B10 genetic background (Table V). However, when the single cells were suspended in HH containing 5% (vol/vol) FCS, the differential relationship between H-2 haplotype and adhesion rates was again observed. Single cells adhered to the H-2*K monolayer at a significantly lower rate than to the other H-2 haplotypes. H-2* monolayers were significantly more adhesive than the other congenic-resistant strains on the B10 background (Table V). The addition of FCS to the assay raised the adhesion rate of all monolayers; however, this did not obliterate the differences between haplotypes previously observed with fibroblasts on the C3H background.

Neuraminidase treatment also produced a differential in adhesion rates between B10 background H-2 different fibroblast monolayers, even in the absence of serum. As can be seen in Table VI, neuraminidase treatment resulted in a marked increase in single cell adhesion to B10 (H-2*) monolayers but little if any increase in adhesion to B10.Br (H-2*) monolayers.

The same relationship between monolayer H-2 genotype and rate of adhesion that was observed

TABLE III
Effect of H-2 Genotype on Rates of Adhesion
(b) Fibroblasts of Different Genetic Backgrounds

| Single cell type       | Monolayer type | Adhesion rate*± |
|-----------------------|----------------|-----------------|
| C3H.SW(H-2*B)         | C3H.SW(H-2*B)  | 1.25            |
| A.BY(H-2*)            | A.BY(H-2*)     | 1.21            |
| AKR/J(H-2*)           | C3H.SW         | 1.16            |
| A.BY                  | A.BY           | 1.18            |
| AKR/J                 | AKR/J          | 0.90±           |

* Performed in the absence of serum.
† Percentage of total cells adhering per minute to monolayer.
§ Significantly lower than others in group with same single cell type. P < 0.05.

TABLE IV
Effect of H-2 Genotype on Rates of Adhesion
(c) Fibroblasts on the A Background

| Single cell type       | Monolayer cell type | Adhesion rate*± |
|-----------------------|---------------------|-----------------|
| A.BY(H-2*)            | A.AL(H-2*)          | 1.18            |
| A.SW(H-2*)            | A.SW(H-2*)          | 1.40‡           |
| A(H-2*)               | A(H-2*)             | 1.20            |
| A.BY(H-2*)            | A.BY(H-2*)          | 1.19            |
| A.SW(H-2)             | A.SW                | 1.20            |
| A                    | A                  | 1.15            |
| A.BY                 | A.BY               | 1.18            |

* Performed in the absence of serum.
† Adhesion rate statistically different from others in group with identical single cell type. P < 0.05.
### Table V

**Effect of Fetal Calf Serum on Rates of Adhesion**

| Single cell type | Monolayer cell type | Adhesion rate with FCS | Adhesion rate without FCS |
|-----------------|---------------------|------------------------|--------------------------|
| B10(H-2b)       | B10(H-2b)           | 1.50                   | 0.97                     |
| B10.BR(H-2b)    | 1.15                | 1.05                   |
| B10.A(H-2a)     | 1.44                | 1.04                   |
| B10.BR(H-2a)    | 1.15                | 1.05                   |
| B10.D2(H-2b)    | B10                 | 1.38                   | 1.01                     |
| B10.D2          | 1.10                | 1.05                   |
| B10.S(H-2a)     | B10.S               | 1.70                   | 1.05                     |
| B10.Br          | 1.15                | ND*                    |
| B10.BR          | 1.40                | 1.00                   |
| B10.Br          | 1.40                | ND*                    |

* 5% FCS in HH.
† HH without any serum.
§ Percentage of total cells adhering per minute to monolayer.
|| Adhesion rate significantly different from others in group with identical single cell type. P < 0.05.
* Not done.

### Table VI

**Effect of Neuraminidase on Rates of Adhesion**

| Single cell type | Monolayer cell type | Treatment | Adhesion rate* |
|-----------------|---------------------|-----------|----------------|
| B10(H-2b)       | B10                 | NANASE‡   | 1.34‡          |
| B10.BR(H-2b)    | 1.10                | HH †      | 1.04           |
| B10.BR          | 1.15                | HH        | 1.04           |

* Percentage of total cell adhering per minute to monolayer.
‡ 0.01 U/ml.
§ Adhesion rate significantly different from others in group with identical single cell type. P < 0.05.
† HEPES-Hanks' balanced salt solution.

Though experiments with the adult tissue fibroblasts were somewhat difficult, owing to the great variation in morphology and growth patterns of the cells in vitro, it can be seen (Table VIII) that significant differences in single cell collection were observed. The genotype differential was opposite to that found with the embryo fibroblast strains. Using both lung and mesentery-derived fibroblasts, the B10.Br (H-2b) cell monolayer was more adhesive than the B10 (H-2b) monolayer.

From the results with congenic-resistant strains, it appeared that H-2 haplotype of cells in a monolayer of embryo fibroblasts had some effect on adhesion. Because strains differing at H-2 and many other loci (Table III) showed a similar H-2 effect, we followed segregation of H-2 antigens and the adhesion (collection) rate in fibroblasts prepared from individual (C3H(H-2k) × A(H-2b)) X H-2.

### Table VII

**Effect of H-2 Genotype Upon 3T3 Fibroblasts' Rates of Adhesion**

| Single cell type | Monolayer cell type | Adhesion rate |
|-----------------|---------------------|---------------|
| B10.BR(H-2b)    | 3T3 B10.BR(H-2b)    | 1.56          |
| B10.Br (embryonic) | B10.BR(H-2b)   | 1.01‡         |
| 3T3 B10(H-2b)   | 3T3 B10(Br)        | 1.51          |
| B10 (embryonic) | 3T3 B10          | 1.29§         |
| * Assay performed in the presence of 5% FCS.
‡ Percentage of total cells adhering per minute to monolayer.
§ Adhesion rate significantly higher than others in group with identical single cell type. P < 0.05.

### Table VIII

**Effect of H-2 Genotype on Rates of Adult Fibroblast Adhesion**

| Single cell type | Monolayer cell type | Adhesion rate |
|-----------------|---------------------|---------------|
| B10.Br adult    | B10.Br adult mesentery | 1.52‡         |
| B10 adult mesentery | B10.Br embryonic  | 1.09          |
| B10.Br embryonic | 1.42‡             |
| 1.06             |
| 0.97             |

* Performed in the presence of 5% FCS.
‡ Percentage of total cells adhering per minute to monolayer.
§ Adhesion rate significantly higher than others in group with identical single cell type. P < 0.05.
It may be seen from Table IX that the three strains of k/k fibroblasts collected adherent single cells at a significantly lower rate than did the five a/k fibroblast strains.

Because this second approach again indicated that the H-2 haplotype had some effect on intercellular adhesion, embryo fibroblasts were examined for serologically detectable H-2 products, using polyspecific anti-H-2 antisera and a fluorescein-labeled goat anti-mouse immunoglobulin. It was found that in suspensions of fibroblasts from three different genotypes, 59-65% of the cells were positively stained (Table X). The ring staining observed with polyspecific anti-H-2 sera was not so bright as that observed with anti-Thy-1.2 antibody which detects an unrelated antigenic specificity found predominantly on thymocytes and brain tissue. The anti-Thy-1.2 also stained a far greater proportion of fibroblasts than did the anti-H-2 (Table X).

TABLE IX
Rates of Adhesion to Fibroblasts from (C3H × A)F1 × C3H Backcross*

| Single cell phenotype | Monolayer phenotype | Adhesion rate§ |
|----------------------|---------------------|---------------|
| (C3H × A)F1          |                     |               |
| k/k                  | Exp 1†              | 1.05          |
| k/k                  | Exp 2†              | 0.89          |
| k/k                  |                     | 0.95          |
| a/k                  |                     | 1.01          |
| a/k                  |                     | 1.30          |
| a/k                  |                     | 1.29          |
| a/k                  |                     | 1.25          |
| a/k                  |                     | 1.20          |
| a/k                  |                     | 1.10          |

* Fibroblasts obtained from eight littermates were passaged three times before assay.
† Typed by fluorescence using NIH antisera D4 and D32 which are specific for D^a and D^k private specificities, respectively.
§ Percentage of total cell adhering per minute to monolayer.
\[ Monolayers of k/k phenotype have a significantly lower adhesion rate than the a/k monolayers. P < 0.01. * Not done.

TABLE X
Presence of H-2 Gene Products on Embryo Fibroblasts

| Antisera          | Stained cells (%) |
|-------------------|------------------|
| C3H(H-2^k) nms    | F1-Gam 2         |
| C3H anti-H-2^k    | F1-Gam 2         |
| C3H anti-H-2^k    | F1-Gam 65        |
| C3H anti-Thy-1.2  | F1-Gam 82        |
| C3H anti-H-2.23   | F1-Gam 68        |
| C3H anti-H-2.32   | F1-Gam 48        |
| C3H anti-Ia§      | F1-Gam <2        |
| C3H.SW(H-2^nms)   | F1-Gam 3         |
| C3H.SW anti-H-2^k | F1-Gam 60        |
| C3H.SW anti-H-2^k | F1-Gam 2         |
| C3H.SW anti-Thy-1.2| F1-Gam 86       |
| B10.A(H-2^a) nms  | F1-Gam <1        |
| B10.A anti-H-2^a  | F1-Gam 59        |
| AKR(H-2^nms)      | F1-Gam 2         |
| AKR anti-Thy-1.2  | F1-Gam 1         |

* Dilution of 1:10 used.
† At least 100 cells counted; only cells with complete ring stain were counted. The fibroblast staining with anti-H-2 sera was markedly less intense than that observed with anti-Thy-1.2.
§ Detects Ia specificites 2, 3, and 7 on C3H lymphocytes.
|| DBA/2 anti-B10.A was used at a dilution of 1:6.

(31). Rabbit anti-mouse spleen (antispecies) and rabbit anti-rat liver plasma membrane (a serum reported to contain anti-mouse b-2 microglobulin antibodies [19]) both stained more than 75% of C3H embryo fibroblasts, though fluorescence was not so bright as that of the other two xeno-sera.

Binding of the polyspecific anti-H-2 antisera to the fibroblast monolayer had an effect upon rates of adhesion of single cells. Assays carried out after incubation of the monolayers with antisera or normal mouse serum diluted in HH at 37°C for 30 min showed a marked diminution of the adhesion rates for monolayers incubated with the appropriate anti-H-2 sera. The percentage reduction or inhibition of adhesion rate was between 30 and 40% for the CSW and C3H monolayers incubated with anti-H-2^k and anti-H-2^k, respectively; incubation with anti-Thy-1.2, with antiserum against the wrong H-2 haplotype or with any of the four xeno-antisera resulted in negligible inhibition compared to normal serum controls (Table XI). This experiment has been repeated three times with the inhibition by anti-H-2 sera ranging be-
### Table XI

| Single cell type | Monolayer cell type | Antisera* | Adhesive rate (%) | Inhibition* |
|------------------|---------------------|-----------|------------------|------------|
| (C3H × C3H.SW)F1| C3H.SW(H-2b)        | anti-H-2b | 0.92             | 39.4       |
|                  | C3H.SW              | anti-H-2k | 1.50             | 1.3        |
|                  | C3H.SW              | anti-Thy-1.2 | 1.46         | 3.9        |
|                  | C3H.SW              | nms       | 1.52             | -          |
|                  | C3H(H-2b)           | anti-H-2b | 1.28             | 1.5        |
|                  | C3H                 | anti-H-2k | 0.90             | 30.7       |
|                  | C3H                 | anti-Thy-1.2 | 1.25         | 3.8        |
|                  | C3H                 | nms       | 1.30             | -          |
|                  | B10.Br(H-2a)        | anti-H-2a | 1.05             | 30.0       |
|                  | B10                 | anti-H-2k | 1.41             | 6.0        |
|                  | B10                 | nms       | 1.50             | -          |
|                  | B10.Br(H-2a)        | anti-H-2a | 1.32             | 2.2        |
|                  | B10.Br              | anti-H-2k | 1.08             | 20.0       |
|                  | B10.Br              | nms       | 1.35             | -          |
|                  | C3H(H-2a)           | anti-H-2a | 1.28             | 2.3        |
|                  | C3H                 | anti-H-2k | 0.94             | 28.2       |
|                  | C3H                 | nms       | 1.31             | -          |
|                  | C3H                 | anti-CS$^+$ | 1.21          | 7.6        |
|                  | C3H                 | anti-rat-Thy-1.2$^+$ | 1.28    | 2.3        |
|                  | C3H                 | anti-mouse spleen$^+$ | 1.29    | 1.5        |
|                  | C3H                 | anti-rat liver$^+$ | 1.26     | 3.8        |
|                  | C3H                 | nrs       | 1.29             | 1.5        |

* Antisera used at a dilution of 1:10 in HH solution.
† Calculated as the rate of adhesion with nms minus rate with antisera/rate with nms × 100.
§ See Materials and Methods for details of antisera.

### Table XII

| Single cell type | Monolayer cell type | Antisera* | Adhesive rate (%) | Inhibition* |
|------------------|---------------------|-----------|------------------|------------|
| (C3H × C3H.SW)F1| (C3H × C3H.SW)F1    | anti-H-2b | 1.35             | 15.6       |
| (C3H × C3H.SW)F1| (C3H × C3H.SW)F1    | anti-H-2k | 1.40             | 12.5       |
| (C3H × C3H.SW)F1| (C3H × C3H.SW)F1    | anti-H-2b$^+$ | 1.20       | 22.5       |
| (C3H × C3H.SW)F1| (C3H × C3H.SW)F1    | anti-Thy-1.2 | 1.48          | 4.5        |
| (C3H × C3H.SW)F1| nms                 | 1.55$^+$ | -                | -          |
| C3H             | nms                 | 1.30$^+$ | -                | -          |

* Antisera used at a dilution of 1:10 in HH solution.
† Calculated as the rate of adhesion with nms minus rate with antisera/rate with nms × 100.
§ Monolayer incubated with both anti-H-2b and anti-H-2a simultaneously.
$^+$ (C3H × C3H.SW)F1 significantly higher rate of single cell pick-up compared to C3H. $P < 0.05$.

Between 20 and 60%. Adhesion to a (C3H × C3H.SW)F1 (H-2b × H-2b) monolayer was inhibited between 12 and 15% by either anti-H-2b or H-2b antiserum. Greater inhibition was observed when the monolayer was incubated with both anti-H-2b and anti-H-2k simultaneously (Table XII). It was also shown that the (C3H × C3H.SW)F1, like (C3H × A)F1, gave adhesion rates consonant with those of the "high rate" parent, C3H.SW or A, and significantly above the C3H rate. No effect of H-2 on adhesion rate of B10 background cells was seen with standard monolayers in the absence of fetal calf serum. However, treatment of these monolayers with anti-H-2 sera resulted in significant inhibition of adhesion rates (Table XI).
As mentioned previously, the adhesion rate was determined from the linear portion of the curve, and linearity was usually observed up to 30-40 min. In the antibody experiments, it was observed that in the case of specific inhibition, the linear portion of the curve often was restricted to 20-25 min, whereafter the curve plateaued or even began to fall. Thus, a more striking illustration of the effect of the antibody is illustrated in Fig. 3, which shows the percentage of single cells attaching over a prolonged period of 60 min. The control monolayers continued to pick up an increasing percentage of single cells, whereas the antibody-treated monolayer remained fairly constant in the percentage uptake after 30-40 min. Significant inhibition of single cell pick-up by anti-H-2 antisera was found even at antisera dilutions of 1:160 (Fig. 4).

**DISCUSSION**

Results obtained using three different approaches all demonstrate an association between the H-2 complex and embryo fibroblast adhesion. In all cases, an H-2 linkage is manifest as a differential in rate of collection of single cells by monolayers of different haplotypes, rather than as absolute, all-or-nothing, differences. Comparison of cells from congenic-resistant strains differing only at H-2 and a short stretch of adjacent chromosome, as well as comparison of cells from inbred strains differing at H-2 and many other loci, showed a consistent association between H-2 haplotype and adhesion rate. This association was confirmed in the progeny of a backcross in which H-2 was segregating together with many other loci. A third approach, use of anti-H-2 sera to reduce adhesion rates, implicates serologically detectable products of the complex in adhesion. Indeed, anti-H-2 sera were even effective on cells of mouse strains B10 and B10.Br which otherwise showed no H-2 haplotype differential in adhesion, in a standard assay.

The H-2 differential in collection of single cells appeared to be a function only of monolayer haplotype, and not of single cells' haplotype. It was species specific; BHK single cells did not adhere differentially to any murine cell monolayer. The dominance of monolayer cells in this system was originally shown by Walther (30), who found that even fixation of single cells with 3% glutaraldehyde did not interfere with their uptake, and fixation of the monolayer abolished adhesion.

Single cells are not given time to recover from trypsinization before assay. This may be the reason why the monolayer dominates the process. In any case, the assay may be considered as containing one active cell phase, the monolayer, and one passive cell phase, the single cell suspension. Hence, it is not surprising that, contrary to many

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**Figure 3** The effect of incubating C3H.SW monolayers with C3H anti-C3H.SW antisera at 1:10 dilution (●) or with nms at 1:10 dilution (○) for 30 min before the addition of (C3H.SW × C3H)F1 single cells. In both cases, the monolayers were washed with HH just before addition of single cells. The points represent the mean and standard deviations of triplicate samples.

**Figure 4** The effect of C3H anti-C3H.SW antisera dilution upon the percentage of (C3H.SW × C3H)F1 single cells collected, 30 min after application to C3H monolayers (●). Level of single cell collected with nms (○). The points represent the mean and standard deviations of triplicate samples.
observations on immunocyte interactions (11), no preference for like (syngeneic) H-2 was found in our experiments. Instead, all H-2k monolayers collected cells significantly less well than monolayers of any other haplotype, and H-2k in two different genetic backgrounds collected cells at a rate significantly higher than those of all other haplotypes tested.

Fetal calf serum or neuraminidase treatment was necessary to demonstrate H-2 associated variations in adhesion rate using cells with a B10 genetic background, though in the presence of FCS, or after neuraminidase, the high- and low-rate haplotypes were those seen using cells of other genetic backgrounds. It is known that both FCS and neuraminidase treatment, in general, significantly increase cell aggregation (14, 16). Perhaps, slightly higher surface charge in B10 cells masks any other genetically determined effects on adhesion. Indeed, it has been reported that B10 erythrocytes contain more sialic acid per cell than do erythrocytes of other strains, and that this interferes with assays of hemagglutinating anti-H-2 antibodies (23).

Degree of cellular adhesiveness of embryo fibroblasts is yet another trait associated with the H-2 gene complex that is not involved with the immune system. Several other nonimmunological characteristics have been linked to the H-2 complex (3, 9, 10, 15). One example is the association of basal levels of cAMP in the liver with the major histocompatibility complex (17), and it is of interest to note that in this system, as in the one described in this paper, it was the H-2k haplotype that was associated with the lowest levels of the measured parameter. The low cAMP levels may be associated with hormone-binding deficiencies (17), emphasizing once again the interaction of H-2 gene products with nonimmunological cell surface phenomenon.

The finding of identical rates of cell adhesion for 3T3 lines, whatever their H-2 haplotype, and the results with adult fibroblasts, like those with embryo B10 and B10.Br cells, indicate that H-2 effects on fibroblast adhesion may be masked or altered. In fact, not only are adults' cells more adhesive than embryo fibroblasts, but also there is a reversal in H-2 association with variation in rates of adhesion. For adult fibroblasts, we cannot tell whether the reversal seen is due to the different tissue origins of the fibroblasts or to the age of the cell donors, or to a combination of these and other factors. We emphasize that, in general, H-2-linked differences whether in immune systems or not, are quantitative rather than absolute.

Many of the gene products coded for by the H-2 complex appear to be cell surface glycoproteins, and these include the classical major transplantation antigens coded for by the K and D regions and the I region associated (Ia) antigens. It appears from the fluorescence experiments that the embryonically derived fibroblasts bear products of the D and K regions on their cell surface, but lack detectable amounts of Ia antigens. Hämmerling (8) has also been unable to demonstrate, by adsorption techniques, the presence of Ia antigens on 3T3 fibroblasts, although it should be mentioned that products of the S region of the H-2 complex have been found to be present on the surface of an adult kidney fibroblast cell strain (25).

The reason for the large percentage of H-2 negative fibroblasts in the population may reflect the heterogeneous nature of these cells. The increase in passage number does not appear to result in a more H-2 homogeneous population; in fact, preliminary evidence suggests that H-2 staining becomes even weaker with passage. A similar result, loss of HLA specificities with passage, has been observed with diploid human fibroblasts (24).

The 20-40% inhibition of adhesion rate observed with the appropriate anti-H-2 sera (Fig. 2) appears to be due to the binding of the sera to cell surface moieties coded for by the H-2 gene region. From the immunofluorescence data, it seems most likely that it is the products of the K and D region that are primarily bound. The inhibition observed appears to be directly related to anti-H-2 binding and is not merely a function of antibody binding to the cell surface. Antibodies against other cell surface antigens, which, judging from fluorescence intensity bound at least as extensively as anti-H-2, did not significantly inhibit adhesion. (In fact, it is surprising that one of these, anti-CSP, had no effect, as CSP and similar glycoproteins have been shown to be involved in the aggregation of several cell types (32) and in the adhesion of fibroblasts to collagen (21).) The association of H-2 products with sites for adhesion is also implied by the results with (C3H × C3H.SW)F1 monolayers; it was found that both anti-H-2b and anti-H-2k polyspecific antisera were necessary for maximum inhibition of adhesion. The antiserum effect was, in all cases, titratable, inhibition of adhesion varying inversely with serum dilution.
The inhibitory effect of anti-H-2 sera does not appear to be related to the ability of the sera to cause gross changes in the distribution of cell surface receptors, such as capping. It has been shown that embryo fibroblasts are not capped by prolonged treatment with anti-H-2 sera, even when followed by rabbit anti-mouse Ig (4; Bartlett and Edidin, unpublished observations).

Our results do not distinguish between H-2 products as receptors in adhesion and the possibility that they are closely associated with or otherwise modify the actual receptors. We favor the second possibility, as it is clear that H-2 effects on cell adhesion are conditional upon other aspects of cell structure and physiology.

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