Non-H-2 antigens on fibroblasts and an embryocarcinoma cell line react with xenoantisera against H-2 antigens

(cell surface antigens/morula stage/transplantation antigens/F9 cells)

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ABSTRACT The murine embryocarcinoma cell line F9 lacks the classical transplantation antigens. However, rabbit anti-H-2 antigen sera, recognizing "backbone structures" of H-2 K and D antigens, react with three types of molecules manufactured by F9 cells. A 40,000- and a 25,000-dalton chain are glycoproteins located on the cell surface. The third component, with an apparent molecular weight of 15,000, and the 25,000-dalton chain seem to be unrelated to the 49,000-dalton glycoprotein. The 25,000- and 15,000-dalton components are not manufactured by splenocytes, thymocytes, hepatocytes, or various lymphoma, mastocytoma, and plasmacytoma cell lines. However, fibroblasts derived from both embryos and adult animals synthesize 25,000- and 15,000-dalton molecules reactive with the rabbit anti-H-2 antigen sera. The 49,000-, 25,000-, and 15,000-dalton molecules are not recognized by a syngeneic anti-F9 cell serum.

The murine embryocarcinoma cell line F9, which was established from a teratocarcinoma isolated from 129/Sv mice (1), has been shown by immunizations, absorptions with alloantisera directed against transplantation antigens, and indirect immunoprecipitation analyses of radioactively labeled cell membrane molecules to be devoid of measurable amounts of H-2 antigens (2, 3). Despite the apparent lack of classical transplantation antigens, the F9 cells may prime the development of cytolytic T lymphocytes (4). Cytolytic T lymphocytes are believed to recognize H-2 K and D antigens in addition to foreign antigens (5). We have examined the possibility that F9 cells express analogues of the classical transplantation antigens. Our data suggest that there exist cell surface molecules on F9 cells that may be antigenically related to H-2 antigens.

MATERIALS AND METHODS

Cells. Splenocytes and thymocytes, used as single cell suspensions, were prepared from 129/Sv mice as described (6). Single cell suspensions of hepatocytes were isolated from rats of the Hooded-Lister strain according to a slight modification of the procedure of Seglen (7). The in vitro-grown lymphoma lines EL-4 and YAC, the mastocytoma P815, and the plasmacytoma X-83 were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, kanamycin at 50 µg/ml, and Fungizone at 2.5 µg/ml. Primary rat fibroblast cultures were maintained in the same medium. The fibroblast cultures were established from skin of embryos and of adult animals of the Hooded-Lister strain. Skin from the two sources were trypsinized (0.25% trypsin) in phosphate-buffered saline (P/NaCl), pH 7.4, containing 0.02% EDTA. Single cells were plated in 75-cm² Falcon flasks. F9 cells, obtained from M. McBurney, Oxford, were cultured in gelatin-coated Falcon flasks in Dulbecco's modified Eagle's medium. All cells were incubated at 37°C in a moist 5% CO₂/95% air atmosphere, except the F9 cells that were grown at 12% CO₂.

Antiserum. The rabbit antisera against highly purified H-2 antigens and β₂-microglobulin have been shown to react exclusively with H-2 K and D and related antigens such as TL (8, 9). The anti-H-2 antigen serum contains antibodies against both subunits (8). The rabbit antiserum against murine Ia-antigens was prepared by B. Curman. This antiserum, after absorption on EL-4 cells, is specifically cytotoxic for B cells and macrophages but does not react with T cells. Only the two Ia-antigen subunits were obtained in indirect immunoprecipitation analyses of internally labeled spleen cell glycoproteins. The detailed reactivity pattern of this antiserum will be described elsewhere. D. B. Wilson kindly provided a rat alloantiserum against Ag-B antigens. This serum was raised by injecting Lewis rats with spleen and lymph node cells from the August strain. The H-2b alloantiserum was prepared by injecting (A × BALB/c)F₁ mice with spleen and lymph node cells from C57BL/6 mice. A syngeneic antiserum against F9 cells was prepared by injecting 129/Sv male mice with irradiated F9 cells. The immunization protocol was essentially the same as that described by Artzt et al. (1). This antiserum displayed the same cytotoxicity pattern against F9, PYS-2, PCC-3, and PCC-4 cells as that described by Jacob (10).

Radioactive Labeling and Indirect Immunoprecipitations. Cells were incubated overnight in tyrosine-deficient medium supplemented with [³H]tyrosine at 100 µCi/ml (1 Ci = 3.7 ¥ 10¹⁰ bequereles). When [³H]fucose labeling was performed the normal medium contained 200 µCi/ml. Neuraminidase-digested cells were treated with galactose oxidase (Kabi, Stockholm) and sodium borohydride as described by Gahmberg and Hakomori (11). Labeled molecules were passed over a Lens culinaris hemagglutinin column, bound glycoproteins were desorbed with 10% methyl-α-mannoside, and indirect immunoprecipitations were carried out as detailed elsewhere (12).

Other Methods. Sodium dodecyl sulfate (NaDodSO₄)/polyacrylamide gel electrophoresis was in the discontinuous system of Laemmli (13). Papain-solubilized H₂-antigens were prepared according to a previously described method (14). Separation and isolation of ³H]-labeled (15) H-2 antigen subunits were achieved by methods outlined elsewhere (14).

Peptide mapping experiments of [³H]tyrosine-labeled cell surface antigens, digested with trypsin, were performed as outlined elsewhere (16). Fab fragments were isolated from the IgG fraction of the rabbit anti-H-2 antigen serum (17). Cytotoxicity mediated by antibody and complement was analyzed by the ⁵¹Cr release method as described by Sanderson (18) and Wiggell (19). Absorption of antibodies in the rabbit anti-H-2 antigen serum was performed on a single cell suspension of individual class I antigens. The cells were incubated at 37°C in a moist 5% CO₂/95% air atmosphere, except the F9 cells that were grown at 12% CO₂.

Abbreviations: NaDodSO₄, sodium dodecyl sulfate, P/NaCl, phosphate-buffered saline, H and L, immunoglobulin heavy and light chains.
splenocytes obtained from 129/Sv mice. The cells, 5 x 10^6, were treated with 2% (vol/vol) paraformaldehyde in 50 ml of P/NaCl, pH 7.4, at 0°C for 20 min. The washed cells were then incubated with 0.1 ml of the antiserum diluted 1:100 with P/NaCl for 45 min at 0°C. The cells were subsequently washed three times with Hanks' balanced salt solution. Finally, antibodies bound to the cells were desorbed by treatment with 0.2 M sodium citrate buffer, pH 3.0. Desorbed antibodies were exhaustively dialyzed against several changes of P/NaCl before concentration.

RESULTS

Cytotoxicity of a Rabbit Anti-H-2 Antigen Serum against F9 Cells. A rabbit antiserum against highly purified H-2 antigens was cytotoxic for the F9 cells (Fig. 1). However, the antiserum could not be diluted to the same extent when tested on F9 cells as when tested on syngeneic (129/Sv) lymph node cells. A syngeneic mouse antiserum against F9 cells was cytotoxic for the F9 cells but not for lymph node cells (Fig. 1B and ref. 1) or various lymphoma, mastocytoma, plasmacytoma, and fibroblast cell lines. The relationship between the target molecules for the two antisera was investigated. Fig. 1C and D demonstrates that whereas Fab fragments derived from the rabbit anti-H-2 antigen serum abolished the cytotoxic action against lymph node cells of an anti-H-2 beta alloantiserum, the same Fab fragments, even at high concentrations, had no measurable effect on the anti-F9 serum-induced cytotoxicity on the F9 cells.

Specificity of the Anti-H-2 Antigen Serum. Previous analyses had shown that the rabbit anti-H-2 antigen serum was specific for H-2 K and D antigens and related molecules, such as TL antigens (8). However, it was important to establish that the reactivity against F9 cells was not due to antibodies raised against trace contaminants present in the H-2 antigen preparation used for immunization, which might have escaped detection. To examine this, advantage was taken of the fact that antibodies display two identical antigen-binding sites. If cells are exposed to a large excess of antiserum at least some antibodies should bind to the cells by only one Fab portion, leaving the symmetrical site unoccupied. Consequently, free antigen should be able to react with such a cell-bound antibody. Table 1 confirms this reasoning. The rabbit antibodies against H-2 antigens, which react with both H-2 antigen subunits (8), after having bound to the spleen cells, bound 100-fold more 125I-labeled H-2 antigen heavy chains than did normal rabbit serum or antibodies against beta-2-microglobulin. The cell-bound rabbit antibodies against beta-2-microglobulin and against the H-2 antigens bound 3-fold more 125I-labeled beta-2-microglobulin than did normal rabbit serum. The high background binding in the latter case is due to the occurrence of a beta-2-microglobulin "receptor" on spleen cells.

Whereas similar experiments demonstrated that antibodies against the H-2 antigen heavy chains indeed bound to F9 cells, antibodies against beta-2-microglobulin displayed no significant reactivity with these cells (Table 1). These experiments are consistent with the view that rabbit antibodies against H-2 antigen heavy chains react with molecules expressed on F9 cells. That antibodies raised against the H-2 antigen heavy chain were responsible for the cytotoxic activity against F9 cells was also supported by the observation that

* This study details results obtained with one rabbit anti-H-2 antigen serum (8). Identical results have been obtained with other rabbit antisera raised against other preparations of highly purified pH-2 and Ag-B (rat) transplantation antigens. However, neither of these antisera nor any of the available rabbit anti-HLA-A,-B, or -C antigen sera react with human lung fibroblast molecules similar to those described here.

† K. Sege, L. Ostberg, and P. A. Peterson, unpublished data.

![Fig. 1. Cytotoxicity of a rabbit anti-H-2 antigen serum and a syngeneic anti-F9 cell serum on 129/Sv lymph node cells and F9 cells. (A) Cytotoxicity of the rabbit anti-H-2 antigen serum against equal numbers of 129/Sv lymph node cells (●) and F9 cells (▲). (B) Cytotoxicity of the anti-F9 cell serum on 129/Sv lymph node cells (●) and F9 cells (▲). (C) The F9 cell antiserum was diluted 1:10 more than indicated in B before being subjected to serial dilutions. (D) Inhibition of H-2 beta alloantiserum-induced cytotoxicity against 129/Sv lymph node cells with various concentrations of Fab fragment derived from the IgG fraction of the rabbit anti-H-2 antigen serum (▲) and of normal rabbit serum (●). (E) Inhibition of syngeneic anti F9 cell serum-induced cytotoxicity against F9 cells with Fab fragments. The sources of the Fab fragments and the symbols are the same as in C.

In A and B, 25 µl of the diluted antisera were mixed with 25 µl of 51Cr-labeled cells (4 x 10^6 cells per ml) and 25 µl of 1:10-diluted agarose-absorbed rabbit serum. After 30 min of incubation at 37°C the amount of 51Cr released from the cells was measured. In C and D, 51Cr-labeled cells, 4 x 10^6 in 25 µl, were mixed with 25 µl of serially diluted Fab fragments derived from the rabbit anti-H-2 antigen serum IgG or from normal rabbit IgG. The initial concentration of the Fab fragments was 0.5 mg/ml. After 30 min at 4°C, 25 µl of an alloanti-H-2 serum, diluted 1:800, or the anti-F9 cell serum, diluted 1:30,000, was added together with complement. The incubation mixtures were then treated as described above. The values given in the figure represent the mean of the specific release of 51Cr of analyses performed in triplicate. The background release, measured by substituting normal rabbit or mouse serum for the antisera, never exceeded 12% in any of the experiments.

nanogram amounts of highly purified H-2^a, H-2^d, and H-2^b antigens (14) but no microgram amounts of beta-2-microglobulin abolished the antiserum-induced cytotoxicity.

Characteristics of the F9 Molecules Recognized by the Rabbit Anti-H-2 Antigen Serum. Those molecules present on the F9 cells that reacted with the rabbit anti-H-2 antigen serum were isolated by indirect immunoprecipitation and analyzed by NaDodSO_4/polyacrylamide gel electrophoresis. Fig. 2 summarizes the results. The rabbit anti-H-2 antigen serum recognized only regular H-2 antigens among spleen cell glycoproteins. An antisera against beta-2-microglobulin and an anti-H-2 beta alloantiserum reacted with the same type of spleen cell membrane molecules; i.e., only the H-2 antigen subunits with the apparent molecular weights 45,000 and 12,000 were visualized (see Fig. 2 A, C, and E). Sequential immunoprecipitations corroborated earlier findings that antibodies against beta-2-microglobulin precipitated all molecules reactive with the
Table 1. Binding of 125I-labeled β2-microglobulin and papain-solubilized H-2 K/D antigen heavy chains to antibody-coated 129/Sv mouse spleen cells and F9 cells

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Spleen cells</th>
<th>F9 Cells</th>
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<tr>
<td></td>
<td>β2µg</td>
<td>H-2 K/D</td>
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<tr>
<td>Normal rabbit serum</td>
<td>12,600</td>
<td>566</td>
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<tr>
<td>Anti-β2µ serum</td>
<td>37,400</td>
<td>510</td>
</tr>
<tr>
<td>Anti-H-2 antigen serum</td>
<td>39,100</td>
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Single cell suspensions, 2 x 10^6 129/Sv spleen cells and 1 x 10^6 F9 cells in 100 μl, were separately incubated with 50 μl of the various sera. After 30 min at 4°C the cells were washed four times with ice-cold Hanks’ balanced salt solution and resuspended in 200 μl of the balanced salt solution containing bovine serum albumin at 5 mg/ml, 0.1% NaDodSO_4, and 190,000 cpm of β2 -microglobulin (β2µ) or H-2 K/D antigens. After 45 min of incubation the cells were washed four times and the cell pellets were subjected to radioactivity measurement.

rabbit anti-H-2 antigen serum (see ref. 8). As expected, the anti-β2 -microglobulin and the alloanti-H-2α antigen sera did not measurably react with any glycoproteins derived from the F9 cells (Fig. 2 D and F). However, the rabbit anti-H-2 antigen serum precipitated three to four polypeptide chains from [3H]tyrosine-labeled F9 cells (Fig. 2D). Molecules with the apparent molecular weights 49,000, 25,000, and 15,000 were invariably precipitated. Occasionally a polypeptide chain with the apparent molecular weight 42,000 was also encountered. The syngeneic anti-F9 serum did not precipitate measurable amounts of radioactivity from the F9 cell glycoprotein fraction. Nor did molecules that passed the lectin column unretarded react with this antiserum.

It is clear from these data that the rabbit antisera raised against highly purified papain-solubilized H-2 antigens reacts with proteinaceous molecules present on F9 cells. Because the reactive molecules were isolated on a L. culinaris hemagglutinin column, one or more of the components should contain carbohydrate. This was corroborated because [3H]fucose labeled the 49,000- and 25,000-dalton components (Fig. 3). Moreover, galactose oxidase and sodium borohydride treatment incorporated radioactivity into the same two polypeptide chains. Significant amounts of radioactivity in the 15,000-dalton chain were not observed. Whether this signifies that the smallest chain is devoid of carbohydrate or that carbohydrate is present in amounts too low to be detected remains to be elucidated. However, at least two of the chains are glycosylated and present on the surface of the cells.

Antigenic Relationship between Molecules Derived from F9 Cells and H-2 Antigens. To further establish the antigenic relationship between H-2 antigens and the F9 cell-derived molecules, three types of experiments were performed. First the rabbit anti-H-2 antigen serum was mixed with a single cell suspension of paraformaldehyde-fixed spleen cells from 129/Sv mice. After the cells had been washed repeatedly, antibodies bound to the cells were desorbed by acid treatment. The eluted antibodies were used in indirect immunoprecipitation analysis of [3H]tyrosine-labeled F9 cell glycoproteins. The NaDodSO_4/polyacrylamide gel pattern of the precipitated molecules was indistinguishable from that shown in Fig. 2B. Second, various amounts of unlabelled cell membrane glycoproteins derived from isolated spleen cells were mixed with a constant amount of [3H]tyrosine-labeled F9 cell glycoprotein and a limiting amount of the rabbit anti-H-2 antigen serum. The indirectly immunoprecipitated labeled molecules were analyzed by NaDodSO_4/polyacrylamide gel electrophoresis. When the amount of unlabeled spleen cell glycoproteins in the incubation mixture was increased, less radioactivity was precipitated. However, the relationships among the amounts of precipitated radioactivity present in the 49,000-, 25,000-, and 15,000-dalton components were very similar if not identical regardless of the number of competing spleen cell molecules added. Third, appropriate dilutions of the rabbit antisera against H-2 antigens and a rabbit antisera against murine Ia antigens were mixed with [3H]tyrosine-labeled spleen cell glycoproteins and added to serial dilutions of unlabeled F9-cell glycoproteins. Labeled molecules were isolated by indirect immunoprecipitations and subjected to NaDodSO_4/polyacrylamide gel electrophoresis. When the amounts of F9 cell glycoproteins in the incubation mixtures were increased progressively, fewer of the H-2 antigen subunits were precipitated.

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**Fig. 2.** NaDodSO_4/polyacrylamide gel electrophoreses of [3H]-tyrosine-labeled 129/Sv spleen cell (A, C, E) and F9 cell (B, D, F) glycoproteins reactive with an anti-H-2 antigen serum (A and B) anti-β2 -microglobulin serum (C and D), or an H-2α alloantiserum (E and F). The labeled molecules were isolated by indirect immunoprecipitation. Counting efficiency was X%, ---. Radioactivity profiles of molecules precipitated by the antiserum; - - - molecules nonspecifically precipitated with normal rabbit serum (A-D) and normal mouse serum (E and F). The arrows denote the migration positions of marker IgG heavy (H) and light (L) chains and β2 -microglobulin (β2µ).

**Fig. 3.** NaDodSO_4/polyacrylamide gel electrophoreses of radioactive carbohydrate-containing molecules on the F9 cells reactive with the rabbit anti-H-2 antigen serum. In A the cells were biosynthetically labeled with [3H]fucose and in B the F9 cells were surface-labeled by treatment with galactose oxidase and sodium borohydride. ---, Molecules precipitated with the rabbit anti-H-2 antigen serum; - - - , material precipitated with normal rabbit serum.
(Fig. 4). That this was due to the presence of crossreacting antigens among the F9 cell glycoproteins and not to unspecific inhibition was ascertained by the fact that the rabbit anti-Ia antigen serum precipitated the same amount of Ia-antigen subunits regardless of the amount of F9-cell glycoproteins added. Thus, these data make it highly likely that the 15,000-, 25,000-, and 49,000-dalton chains share antigenic determinants with the classical transplantation antigens.

Structural Relationship among H-2 Antigen Heavy Chains, the 25,000- and the 49,000-Dalton Molecules. The relationship between the 25,000- and the 49,000-dalton chains obtained from F9 cells and H-2 antigen heavy chains isolated from 129/Sv mouse splenocytes was examined by peptide mapping. The [3H]tyrosine-labeled molecules were isolated separately and subjected to trypsin treatment, and the digests were analyzed by high-pressure liquid chromatography. Each one of the three types of molecules gave rise to several well-separated radioactive peptides (Fig. 5). Because the peptide mapping procedure was highly reproducible, it is reasonable to conclude that the three types of chains are distinct entities; i.e., the 49,000-dalton chain is not an antigenically altered H-2 antigen heavy chain and the 25,000-dalton component is not a fragment of either of the other two chains. Whether similarities exist in primary structure among the three types of molecules has to await sequence analyses, because some peptides may fortuitously exhibit identical chromatographic behavior.

Presence of Anti-H-2 Antigen-Reactive Molecules on Various Types of Cells. It is obvious from Fig. 2 that the rabbit anti-H-2 antigen serum did not precipitate any molecules from spleen cells with the apparent molecular weights 25,000 or 15,000. Whether the 49,000-dalton molecule present on F9 cells is also expressed by spleen cells could not be revealed by Na-DodSO4/polyacrylamide gel electrophoresis because the H-2 antigen heavy chain has a similar molecular weight. Thymocytes, labeled with [3H]tyrosine, and galactose oxidase/sodium borohydride-labeled rat hepatocytes apparently did not manufacture the 25,000- and 15,000-dalton chains. Likewise, [3H]tyrosine-labeled glycoproteins derived from the lymphoma line YAC and the plasmacytoma line X-63 did not constitute measurable quantities of the 25,000- and 15,000-dalton molecules. The lymphoma line EL-4 and the mastocytoma cell line P815 displayed barely detectable amounts of a 25,000-dalton component that seemed to be specifically recognized by the rabbit anti-H-2 antigen serum. No 15,000-dalton chain could be visualized in these analyses. However, primary fibroblast cell lines established from rat embryos and from skin of adult animals incorporated significant amounts of [3H]tyrosine into 25,000- and 15,000-dalton polypeptide chains, as can be seen in Fig. 6. Whereas an alloantiserum precipitated only labeled molecules of the expected type—i.e., the 12,000- and 47,000-dalton transplantation antigen subunits—the rabbit anti-H-2 antigen serum reacted with 25,000- and 15,000-dalton molecules in addition to the transplantation antigen chains. Thus, it seems reasonable to conclude that fibroblasts and the F9 cell line synthesize similar types of 25,000- and 15,000-dalton chains that are recognized by a rabbit anti-H-2 antigen serum.

**DISCUSSION**

The embryocarcinoma cell line F9 expresses cell surface glycoproteins crossreacting with several xenonantisera raised against purified H-2 antigens, but it is obvious that the F9 cell membrane molecules are not identical to H-2 antigens. This can be inferred from the observation that the small subunit is not β2-microglobulin (20–22). Moreover, the peptide mapping experiments show that the 49,000- and 25,000-dalton components are distinct from the H-2 antigen heavy chain. Despite

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**Fig. 4.** Na-DodSO4/polyacrylamide gel electrophoreses of [3H]-tyrosine-labeled 129/Sv spleen cell glycoproteins reactive with rabbit antisera specifically directed against H-2 (A) and Ia antigens (B). The two rabbit antisera were individually diluted so as to precipitate about 70% of the maximally precipitable H-2 and Ia antigens from about 3×10⁶ labeled spleen cells. (C, D, E, and F) Amounts of H-2 and Ia antigens precipitated by the antisera mixture from the same amount of labeled spleen cell glycoproteins mixed with unlabeled glycoproteins derived from about 50 mg (D), 200 mg (E), and 1000 mg (F) of F9 cells. —, Radioactivity precipitated by the antisera; - - - , labeled molecules reactive with a normal rabbit serum.

**Fig. 5.** Peptide patterns of [3H]tyrosine-labeled 49,000- and 25,000-dalton molecules isolated from F9 cells and of H-2 K/D antigen heavy chains isolated from 129/Sv mouse splenocytes. All molecules were obtained by indirect immunoprecipitation with a rabbit anti-H-2 antigen serum and Na-DodSO4/polyacrylamide gel electrophoresis. The purified chains were separately extracted from the gel, digested with trypsin, and subjected to high-pressure liquid chromatography on a C-18 column. The details of the procedure are described in ref. 16. For comparison, the peptide pattern of the 49,000-dalton molecule derived from the F9 cells is shown in both A and B (---), whereas --- denotes the radioactive peptide profiles of the 25,000-dalton component (A) and the H-2 K/D heavy chains (B).
considerable efforts to reveal the existence of contaminating antibodies directed against non-H-2 antigens in the antisera employed, we have been unable to obtain such evidence, which would of course provide a trivial explanation for the results described. Therefore, it seems likely that it is the antibodies against the heavy chain that react with the F9 cell molecules. Whether the rabbit antibodies against H-2 antigen heavy chains bind to all three polypeptide chains isolated from F9 cells is unknown. However, the peptide mapping experiments demonstrate that the 49,000-dalton chain is distinct from the 25,000-dalton chain. Moreover, isoelectric focusing, gel chromatography, and sedimentation velocity analyses under non-denaturing conditions allow separation of the 49,000- and 25,000-dalton chains. Such analyses show that the 15,000-dalton molecule is bound to the 25,000-dalton component, 4 which might suggest that the rabbit antibodies react with only one of the two smaller chains. Thus, it seems reasonable to propose that rabbit anti-H-2 antigen sera recognize at least two separate types of cell surface molecules expressed on F9 cells. Vitetta et al. (3) have demonstrated that a syngeneic anti-F9 cell serum precipitates molecules with apparent molecular weights of 45,000, 23,000, and 12,000. Although similar in molecular weight to those described here, the molecules of Vitetta et al. seem to differ from the present ones. Thus, rabbit antisera against F9 cell surface molecules do not react with H-2 antigens, and rabbit sera raised against spleen cells do not precipitate the 45,000-, 23,000-, and 12,000-dalton F9 cell surface antigens (22). Moreover, the expression of the F9 cell surface antigens recognized by the syngeneic antiserum is restricted to the morula and early blastocyst stages (see ref. 10), whereas the components examined here occur on fibroblasts and perhaps on other types of cells. The molecules described here were not antigenically related to molecules recognized by the syngeneic anti F9 cell serum available. A possible explanation for this is that syngeneic anti-F9 cell sera may be multispecific and vary with regard to the titer of the individual antibodies. Alternatively, our failure to confirm the results of Vitetta et al. (3) could be due to the possibility that F9 cells maintained in various laboratories may be phenotypically different. Thus Morello et al. (23) recently reported that a rabbit anti-H-2 antigen serum did not react with F9 cells. However, in our hands the same antiserum precipitated at least the 25,000- and 15,000-dalton chains from the F9 cells. 13 Although the indirect immunoprecipitations were performed differently in the two laboratories, the notion that the expression of cell surface antigens on F9 cells may depend on subtle variations in the culture conditions must be entertained.

It is tempting to suggest that cytotoxic T lymphocytes against F9 cells (4) recognize the molecules described here. If so, this would strengthen the view that the described cell surface antigens are antigenically related to H-2 antigens. However, much further structural work on the isolated F9 cell molecules is required to firmly establish their relationship to H-2 antigens. Such analyses may also give clues as to the biological function of the molecules.

The expert technical assistance of Ms. Elisabeth Harfeldt is greatly appreciated. This research was supported by grants from the Swedish Cancer Society and the Swedish Medical Research Council.

4 This rabbit anti-H-2 antigen serum was kindly supplied by F. Jacob.
