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Immunology

Molecular association between transplantation antigens and cell surface antigen in adenovirus-transformed cell line

(indirect immunoprecipitation/syngeneic cytolysis/AgB antigens/early proteins)

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ABSTRACT **A rat cell line (A2T2C4) transformed with adenovirus type 2 elicited cytotoxic T lymphocytes in syngeneic rats. Cytotoxicity was abolished by a rabbit antiserum directed against the major histocompatibility (AgB) antigens and by a syngeneic rat antiserum raised against the virus-transformed cell line. The syngeneic antiserum immunoprecipitated surface proteins with apparent molecular weights of 45,000, 19,000, 17,000, and 12,000 from the A2T2C4 cells but it displayed no reactivity against primary rat fibroblasts and spleen cells. The rabbit antiserum against AgB antigens precipitated a 19,000-dalton component from the A2T2C4 cells which was not observed in primary rat fibroblasts. Sequential immunoprecipitation revealed identity between the major polypeptides recognized by the two antisera. Because the rabbit anti-AgB antigen serum was specific for the transplantation antigen subunits and because the syngeneic rat antiserum against the A2T2C4 cells failed to react with the AgB antigens in normal cells, it is concluded that the 19,000-dalton component is coprecipitated with the AgB antigens. Antisera directed specifically against β_2-microglobulin and the alloantigenic AgB antigen subunit also coprecipitated the 19,000-dalton component. These results indicate that the AgB antigen subunits form a ternary complex with a virus-coded protein on the surface of the virus-transformed A2T2C4 cells. This molecular complex may be recognized by the cytolytic T lymphocytes.**

During recent years it has become clear that the cellular immune defense against virus infections displays genetic restrictions. Thus, cytolytic T lymphocytes generated during a viral infection can only lyse infected target cells that have the same major histocompatibility antigens as the cells that originally stimulated the development of the cytolytic T cells (for a review, see ref. 1). This genetic restriction which has been mapped to the loci controlling the expression of the classical transplantation antigens suggests a mechanism for how cytolytic T lymphocytes recognize their target antigens. Either the virus product and the histocompatibility antigens are separate entities on the surface of the target cells and the T cells have independent receptors for the two types of molecules (*dual recognition*) or the virus and the transplantation antigens form a molecular complex recognized by one receptor on the T lymphocytes (*altered self*) (2).

So far, evidence in favor of both hypotheses has accumulated. Cocapping experiments (3, 4), antiserum-induced inhibition of T cell-dependent cytolysis (5–9), and selective incorporation of H-2 antigens into enveloped viruses (10, 11) may argue for the altered self concept. However, the development of precursor T cells to mature T lymphocytes with cytolytic activity requires recognition of the major histocompatibility antigens and occurs independently of recognition of non-self antigens (12), a result compatible with the dual recognition concept.

To obtain unambiguous information about the molecular nature of the target recognition site(s) for the cytotoxic T lymphocytes, detailed biochemical studies are needed. We have approached this problem by examining various model systems and have found that adenovirus-transformed cell lines may provide tools for this analysis. The adenoviruses have been extensively studied and their molecular biology is known in considerable detail (for a review, see ref. 13). Adenovirus-transformed cell lines contain adenovirus-specific antigens (14, 15). The gene and the products required for induction of transformation have been identified on the virus genome (16, 17). Less evidence has accumulated concerning the adenovirus tumor transplantation antigens but some, if not all, transformed cells express these antigens (18). In this report we demonstrate that an adenovirus-transformed rat fibroblast cell line (A2T2C4) elicits syngeneic, cytolytic, T lymphocytes. Moreover, a 19,000-dalton glycosylated cell surface antigen, apparently virus-coded, forms a ternary complex with the major histocompatibility antigen subunits.

EXPERIMENTAL PROCEDURE

Animals. Male rats of the hooded Lister strain were used at 3–5 months of age. The rats were kindly supplied by Hans Bennich.

Antisera. A rabbit antiserum was raised against highly purified, papain-solubilized rat AgB antigens. The reactivity of this antiserum was similar to that of rabbit anti-H-2 antigen sera (19). A rabbit antiserum raised against rat β_2-microglobulin was kindly supplied by Ingemar Berggård. The rat anti-AgB alloantiserum, raised by injecting Lewis rats with spleen lymph node cells from August rats, was a gift from Darcy Wilson. Rabbit antisera against rat immunoglobulins were kindly provided by Torbjörn Karlsson. A syngeneic antiserum against the adenovirus-transformed cell line A2T2C4 was raised by injecting male rats of the hooded Lister strain initially with 5×10^6 cells intraperitoneally. After five booster injections with $10–30 \times 10^6$ cells each time, the animals were exsanguinated. All sera were decomplemented before use.

Cell Cultures. The *in vitro*-grown adenovirus-transformed cell lines A2T2C4 and HE4 were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 50 μg of kanamycin per ml, and 2.5 μg of amphotericin B (Fungizone) per ml. The A2T2C4 cells, which contain 60–80% of the adenovirus genome, were originally established from hooded Lister rat embryo fibroblasts (20). About 80% of Ad2DNA is also present in the transformed hamster cells HE4 (21). Both adenovirus 2-transformed cell lines express a virus-coded early glycoprotein confined to the plasma membrane of the cells with a molecular weight of 19,000 (unpublished data). Primary rat fibroblast cultures were maintained in the same medium. The fibroblast cultures were established from skin of embryos of the hooded Lister strain. Skin was trypsinized in pH 7.4 phosphate-buffered saline containing 0.02% EDTA and

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Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.

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0.25% trypsin. Single cells were plated in 75-cm^2 Falcon flasks. The fibroblasts were passaged 10–20 times before use. All cells were incubated at 37°C in a moist atmosphere containing a 5% CO_2/95% air mixture.

Immunization of Rats with Tumor Cells. Rats were immunized by the intraperitoneal injection of 5×10^6 untreated A2T2C4 cells. The rats were used as spleen cell donors 4–8 weeks after the immunization.

Assay of Cytotoxic Activity. Regularly, suspensions of spleen T lymphocytes, isolated by nylon wool filtration (22), were prepared from the immunized rats and 3×10^7 cells were cultured in upright Falcon 3024 flasks together with 3×10^6 A2T2C4 cells previously irradiated with 5000 rads (1 rad = 0.01 Gy). Each culture contained 30 ml of RPMI-1640 (Flow Inc., Irvine, Scotland) supplemented with 10% fetal calf serum, 50 μM 2-mercaptoethanol, and 50 μg of kanamycin and 2.5 μg of Fungizone per ml. Cultures were incubated in humidified air with 5% CO_2 for 6 days.

The cytolytic activity of the effector cells was assessed in quadruplicate in round-bottomed microtiter trays (Cooke M24A, Teddington, Middlesex, England) by mixing serial dilutions of the effector cell suspension (50 μl) with 1×10^3 ^{51}Cr-labeled target cells in 50 μl of the medium. The medium used for these assays had the same composition as that used for the *in vitro* education of the spleen cells.

In experiments to test the effect of various antisera on the cytolysis, 50 μl of the medium or of serial dilutions of the antisera were incubated together with the ^{51}Cr-labeled target cells for 30 min at +4°C prior to the addition of the effector cells. The total counts that could be released from the target cells were determined by incubating the target cells with 1% Triton X-100. The cytolysis assay proceeded for 6 hr at 37°C in an atmosphere of 5% CO_2 in air. After centrifugation, aliquots from the supernatants of each well (usually 0.1 ml) were removed and assayed for radioactivity in a scintillation counter.

Radioactive Labeling and Indirect Immunoprecipitations. For internal labeling, cells were incubated overnight in tyrosine-deficient medium supplemented with 100 μCi of [^{3}H]-tyrosine per ml. Neuraminidase-digested cells were treated with galactose oxidase and sodium [^{3}H]borohydride to label cell surface glycoconjugates. The details were as described by Gahmberg and Hakomori (23). Lactoperoxidase-catalyzed iodination of cell surface proteins was achieved as outlined elsewhere (24). Labeled molecules were subjected to affinity chromatography on columns containing *Lens culinaris* hemagglutinin. Bound glycoproteins were desorbed with 10% α-methylmannoside, and indirect immunoprecipitations were carried out as detailed elsewhere (25).

Other Methods. Sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gel electrophoresis was performed in a discontinuous buffer system (26). Cytotoxicity mediated by antibody and complement was analyzed by the ^{51}Cr-release method (27, 28). Crude plasma membrane fractions were isolated by differential centrifugation, essentially as outlined (29). Glycoproteins were isolated from crude plasma cell membrane fractions of A2T2C4 cells. Membrane proteins were solubilized with 15 mM deoxycholate and the glycoproteins were recovered by affinity chromatography on a *Lens culinaris* hemagglutinin column. For adsorptions of antisera the glycoprotein fraction was covalently coupled to Sepharose-4B (30).

RESULTS

Antiserum Inhibition of T Lymphocyte-Dependent Lysis of A2T2C4 Cells. Experiments were performed to determine if syngeneic rats could elicit cytolytic T lymphocytes directed against the adenovirus-transformed cell line A2T2C4. Spleen cells obtained from hooded Lister rats, primed *in vivo* with the A2T2C4 cells, could lyse the cells, provided the primed spleen cells had been restimulated *in vitro* with the target cells prior to cytolysis. The cytolytic activity of the spleen cells against the A2T2C4 cells was pronounced and lysis was observed at effector-to-target cell ratios as low as 3:1. No cytolytic activity of the educated spleen cells was noted when the target cells were primary fibroblasts from hooded Lister rats or adenovirus-transformed hamster cells (HE4).

After passage of the spleen cells over a nylon wool column to deplete the spleen cells of B lymphocytes, the cytotoxic activity per cell increased about 2-fold. Removal of B cells with anti-rat Ig serum-induced cytotoxicity, which resulted in the lysis of about 40% of the spleen cells, also enhanced the cell-mediated cytolysis of the A2T2C4 cells. Together, these results suggest that T lymphocytes are responsible for the cytolytic activity.

T lymphocyte-dependent lysis of the A2T2C4 cells might involve the recognition of viral genome-coded products in addition to the recognition of the syngeneic AgB antigens. To examine this possibility the cytolytic action of the T lymphocytes was investigated in the presence of various concentrations of antisera. A rabbit anti-AgB antigen serum efficiently blocked lysis of the A2T2C4 cells whereas normal rabbit serum did not affect lysis (Fig. 1). An antiserum raised by immunizing hooded Lister rats with syngeneic A2T2C4 cells also inhibited lytic activity of the T cells although this antiserum was only effective at high serum concentrations. As expected, normal rat serum did not display inhibitory capacity.

Reactivity of the Antisera. We next examined the reactivity of the antisera against molecules on the cell surface of the transformed cells. The syngeneic serum against the A2T2C4 cells was cytotoxic for the A2T2C4 cells when examined in an antibody-dependent, complement-mediated cytolysis assay. The syngeneic antiserum was not cytotoxic against primary fibroblasts or lymph node cells obtained from hooded Lister rats. The rabbit anti-AgB antigen serum was cytotoxic when tested against primary as well as virus-transformed rat fibroblasts.

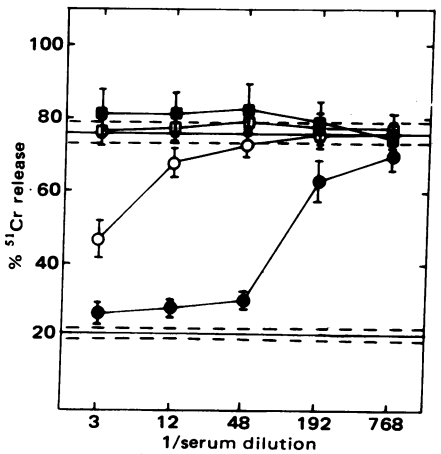

FIG. 1. Inhibition of T cell-dependent cytolysis of A2T2C4 cells. The indicated dilutions of a rabbit antiserum against AgB antigens (●), a syngeneic rat antiserum against the A2T2C4 cells (○), normal rabbit serum (□), and normal rat serum (■) were incubated with 1×10^3 ^{51}Cr-labeled A2T2C4 cells. After 30 min at 4°C, educated rat spleen T lymphocytes were added at an effector-to-target cell ratio of 20:1. The incubations were continued for 6 hr at 37°C, and the ^{51}Cr released was measured after centrifugation. Release of 100% represents the amount of radioactivity recovered in the supernatant after Triton X-100 treatment of the target cells. The solid and the broken horizontal lines denote the mean ± SD of the ^{51}Cr release in the presence (near 80%) and in the absence (at 20%) of the cytolytic T lymphocytes alone. The experimental points and the vertical bars are the means ± SD of analyses performed in quadruplicate.

The cytotoxic activity of the two antisera could be eliminated after absorbtion with a crude plasma membrane fraction from the A2T2C4 cells whereas a membrane fraction from primary rat fibroblasts only abolished the cytotoxic activity of the anti-AgB antigen serum. The glycoprotein fraction from the membranes of the A2T2C4 cells contained most of the antigen recognized by the cytotoxic antibodies in the two sera because absorptions of the antisera on columns containing covalently bound glycoproteins abolished their cytotoxic activity.

The reactivity of the syngeneic anti-A2T2C4 cell serum was then examined by immunoprecipitations. Galactose oxidase and sodium [^{3}H]borohydride-treated A2T2C4 cell surface glycoproteins were isolated and subjected to indirect immunoprecipitation. Only two polypeptide chains, with apparent molecular weights of 45,000 and 19,000, respectively, were immunoprecipitated by this serum (Fig. 2*A*). ^{125}I-Labeling of the surface proteins of the A2T2C4 cells followed by indirect immunoprecipitation with the same serum displayed the same two polypeptide chains. In addition, a small amount of radioactivity migrated with an apparent molecular weight of 12,000 (not shown). When immunoprecipitations were performed with [^{3}H]tyrosine-labeled glycoproteins from the A2T2C4 cells, two additional components with molecular weights of 17,000 and 12,000 were visualized (Fig. 2*B*). That the anti-A2T2C4 cell serum recognized molecules only expressed in the adenovirus-transformed cells was ascertained by the observations that no [^{3}H]tyrosine-labeled glycoproteins from spleen cells or from primary fibroblasts of the hooded Lister rats were precipitated by this antiserum (Fig. 2 *C* and *D*).

The rabbit antiserum against highly purified, papain-solubilized AgB antigens of spleen cells only reacted with the AgB antigen subunits as revealed by indirect immunoprecipitation (Fig. 3). However, this antiserum precipitated a number of [^{3}H]tyrosine-labeled polypeptide chains from the A2T2C4 cells. In addition to the 45,000- and 12,000-dalton chains, distinct peaks were apparent corresponding to molecular weights 25,000, 19,000, and 15,000 (Fig. 3*A*). The 25,000- and 15,000-dalton polypeptide chains were also detected together with the two AgB antigen subunits after immunoprecipitation of [^{3}H]tyrosine-labeled glycoproteins from primary rat fibroblasts (Fig. 3*B*). We have found that the 25,000- and the 15,000-dalton chains, which under physiological conditions form a molecular complex, are antigenically related to the regular transplantation antigens (unpublished data). The precipitation of the 19,000-dalton component by the rabbit anti-AgB antigen serum, however, seemed to be unique for the A2T2C4 cells.

Relationship between Cell Surface Polypeptide Chains Precipitated by Antisera against A2T2C4 Cells and AgB Antigens. The syngeneic rat antiserum and the rabbit anti-AgB antigen serum precipitated molecules that displayed identical molecular weights on NaDodSO$_4$/polyacrylamide gels (cf. Figs. 2 and 3). Sequential immunoprecipitations were performed with the two antisera on the same aliquots of [^{3}H]tyrosine-labeled glycoproteins derived from A2T2C4 cells in order to establish identity between the two sets of polypeptides. Aliquots of labeled molecules were separately immunoprecipitated with normal rat and rabbit sera, the anti-AgB antigen antiserum, and the anti-A2T2C4 antiserum. After the precipitates had been removed by centrifugation, the supernatants were again immunoprecipitated and the precipitates were analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis. Preprecipitation with normal rat serum did not affect the precipitation of the expected 45,000-, 25,000-, 19,000-, 15,000-, and 12,000-dalton chains by the rabbit anti-AgB antigen serum (Fig. 4*A*). However, when the first precipitation with the anti-A2T2C4 cell antiserum was followed by precipitation with the anti-AgB

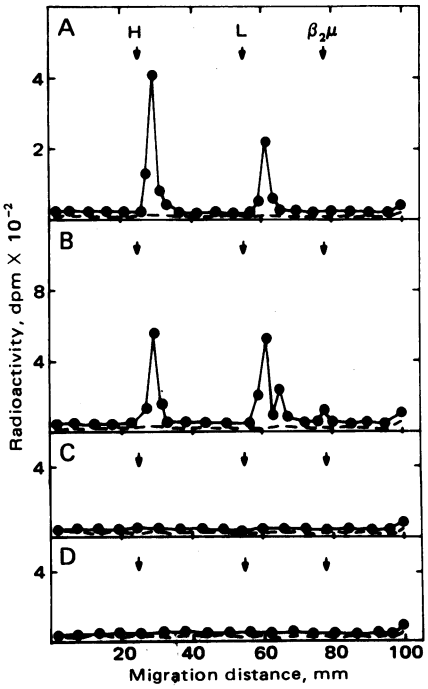

FIG. 2. NaDodSO$_4$/polyacrylamide gel electrophoreses of A2T2C4 cell proteins reactive with a syngeneic antiserum raised against the same cells. Galactose oxidase and Na [^{3}H]borohydride-treated A2T2C4 cell surface molecules (*A*) and [^{3}H]tyrosine-labeled molecules from the A2T2C4 cells (*B*), primary rat fibroblasts (*C*), and rat spleen cells (*D*) were isolated by lectin affinity chromatography and precipitated with the antiserum. Solid lines, radioactive profiles of molecules precipitated by the antiserum; broken lines, molecules nonspecifically precipitated with a normal rat serum; arrows, migration positions of marker IgG heavy (H) and light (L) chains and β_2-microglobulin ($\beta_2\mu$).

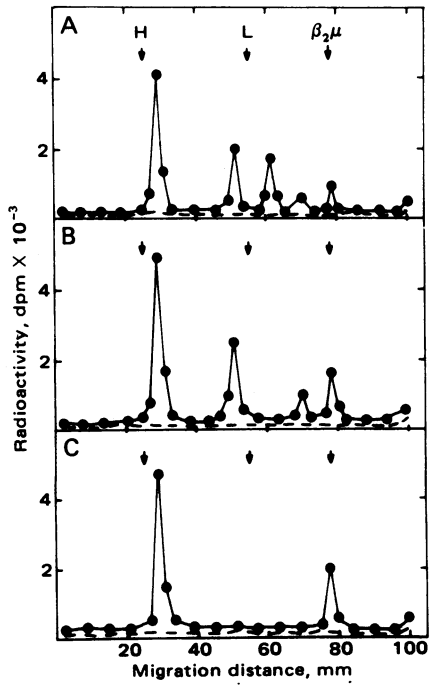

FIG. 3. NaDodSO$_4$/polyacrylamide gel electrophoreses of [^{3}H]-tyrosine-labeled molecules precipitated with a rabbit antiserum against highly purified AgB antigens. Labeled glycoproteins were isolated by lectin affinity chromatography from A2T2C4 cells (*A*), primary rat fibroblasts (*B*), or rat spleen cells (*C*) and indirectly immunoprecipitated with the antiserum (solid lines) or with normal rabbit serum (broken lines). Arrows, migration positions of marker IgG heavy (H) and light (L) chains and β_2-microglobulin ($\beta_2\mu$).

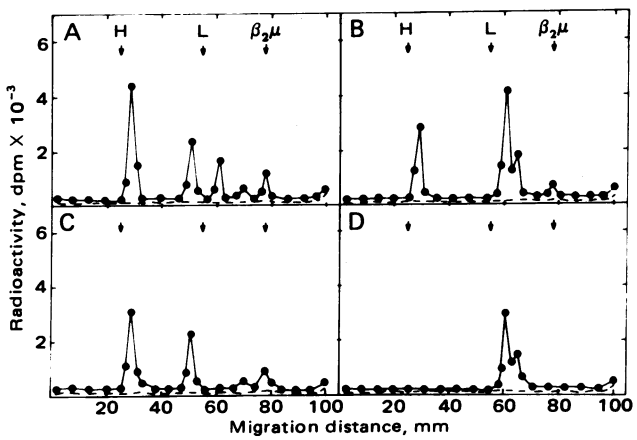

FIG. 4. NaDodSO$_4$/polyacrylamide gel electrophoreses of sequential immunoprecipitates of [^{3}H]tyrosine-labeled A2T2C4 cell glycoproteins. (*A*) Preprecipitation was achieved with a normal rat serum and precipitation with the rabbit anti-AgB antigen antiserum was carried out on molecules remaining in the supernatant after the preprecipitation step. (*B*) Preprecipitation with a normal rabbit serum was followed by precipitation with the syngeneic rat anti-A2T2C4 cell antiserum. (*C*) Syngeneic rat antiserum against the A2T2C4 cells was used for the first precipitation step and the resulting supernatant was treated with the rabbit anti-AgB antigen antiserum. (*D*) Preprecipitation with the anti-AgB antigen antiserum was followed by precipitation with the rat anti-A2T2C4 cell antiserum. Solid lines, radioactivity precipitated by the antisera; broken lines, radioactivity recovered in control precipitates obtained with normal rabbit (*A* and *C*) and rat (*B* and *D*) sera; arrows, migration positions of IgG heavy (H) and light (L) chains and β_2-microglobulin ($\beta_2\mu$).

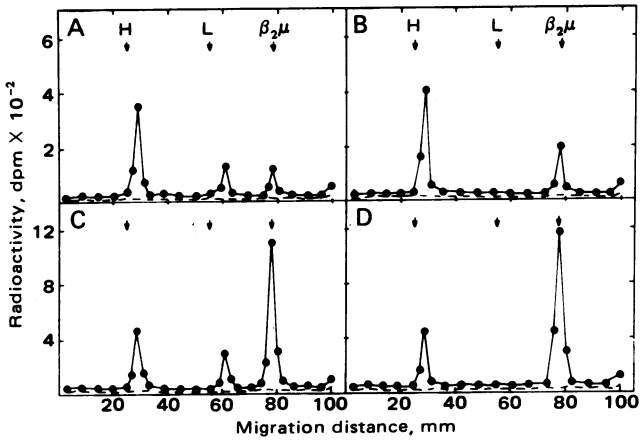

FIG. 5. NaDodSO$_4$/polyacrylamide gel electrophoreses of [^{3}H]-tyrosine-labeled A2T2C4 cell glycoproteins (*A* and *C*) and [^{3}H]tyrosine-labeled hooded Lister primary rat fibroblast glycoproteins (*B* and *D*) isolated by indirect immunoprecipitations with an alloantiserum directed against hooded Lister rat AgB antigens (*A* and *B*) and with a rabbit antiserum against rat β_2-microglobulin (*C* and *D*). Solid lines, molecules specifically precipitated by the antisera; broken lines, radioactivity recovered in indirect immunoprecipitates obtained with normal rat serum (*A* and *B*) and normal rabbit serum (*C* and *D*); arrows, migration positions for the marker IgG heavy (H) and light (L) chains and β_2-microglobulin ($\beta_2\mu$).

antigen antiserum, the 19,000- dalton component was absent (Fig. 4*C*). This suggests that the 19,000-dalton chain precipitated by the anti-AgB antigen antiserum is identical to the component precipitated by the anti-A2T2C4 cell antiserum.

Prior precipitations with normal rabbit serum had no effect on the molecules precipitated by the syngeneic rat antiserum (Fig. 4*B*; cf. Fig. 2*B*) whereas precipitation with the anti-AgB antigen antiserum obviously removed the 45,000- and the 12,000-dalton chains because the supernatant only contained the 19,000- and 17,000-dalton components (Fig. 4*D*). From these analyses we conclude that the anti-A2T2C4 cell antiserum was primarily directed against the 19,000- and 17,000-dalton chains and coprecipitated the AgB antigen subunits.

Evidence for Ternary Complex Consisting of AgB Antigen Subunits and 19,000-Dalton Chain. Because the anti-AgB antiserum reacted with both AgB antigen subunits, it was of importance to investigate whether the 19,000-dalton polypeptide chain was coprecipitated also with antisera against the individual AgB antigen subunits. An AgB antigen alloantiserum precipitated the 19,000-dalton chain together with the AgB antigen subunits (Fig. 5*A*) from the [^{3}H]tyrosine-labeled glycoproteins obtained from the A2T2C4 cells but only the AgB antigen chains were visualized when the labeled molecules were derived from primary rat fibroblasts (Fig. 5*B*). The same results were obtained with the antiserum against β_2-microglobulin (Fig. 5 *C* and *D*). These results are consistent with the view that the two AgB antigen subunits formed a ternary complex with the 19,000-dalton chain.

DISCUSSION

On the adenovirus-transformed cell line A2T2C4, the AgB antigens appear to be bound to a 19,000-dalton polypeptide chain. This polypeptide is encoded in the right-hand end of the viral genome and it is only expressed in some virus-transformed cell lines (unpublished data). After detergent solubilization of the membrane molecules, a substantial amount of the AgB antigens was coprecipitated with an antiserum recognizing the 19,000-dalton component. Likewise, antisera directed against the AgB antigens and their subunits coprecipitated the 19,000-dalton chain. Quantitative aspects of this complex formation are difficult to assess. The antiserum against β_2-microglobulin precipitated small amounts of the complex (Fig. 5), which emphasizes the difficulties in analyzing coprecipitations. It is generally agreed that there is a complex containing equimolar amounts of β_2-microglobulin and the AgB antigen heavy chain on the cell surface (see ref. 31) but antibodies against β_2-microglobulin may dissociate the complex (unpublished data). Antibodies may therefore compete efficiently for binding to the interacting sites of any complex. Other experimental techniques must therefore be used to determine the quantitative aspects of the interaction between AgB antigens on the A2T2C4 cells and the 19,000-dalton polypeptide.

Another potential difficulty in analyzing interacting protein systems by indirect immunoprecipitations is the possibility that, once formed, immune complexes may bind other components nonspecifically. This problem was circumvented in this study by performing preprecipitations, and neither the AgB antigens nor the 19,000-dalton chain appeared to associate with immune precipitates *per se*. Moreover, our unpublished observations demonstrate that the AgB antigens and the 19,000-dalton chain exist as a complex when studied by gel chromatography and by sedimentation velocity analysis.

The antiserum raised against the A2T2C4 cells only precipitated the 19,000-dalton and a 17,000-dalton chain in addition to the AgB antigens when the glycoprotein fraction of the A2T2C4 cells was examined. The 19,000-dalton component was not manufactured by primary rat fibroblasts or spleen cells but was produced by the adenovirus-transformed hamster cell line HE4 and by adenovirus-infected HeLa cells (unpublished data). These results strongly suggest that the 19,000-dalton component is identical to the E19K protein among the early adenovirus-coded proteins (32). Most likely, the 17,000-dalton component, which was not coprecipitated by the anti-AgB antigen antiserum, is a precursor of the 19,000-dalton species. The 17,000- and 19,000-dalton chains exhibit identical peptide maps (unpublished data).

The present analysis indicates that the syngeneic rat antise-

rum only reacts with one cell surface protein and its precursor on the A2T2C4 cells. It is tempting to suggest that this protein may be involved in tumor rejection of adenovirus-transformed cells (18). However, the antiserum used here may only have recognized one of several tumor-specific antigens and it is possible that other immunization procedures may develop reagents against additional antigens. It will be important to determine whether the 19,000-dalton glycoprotein is engaged in the rejection of the A2T2C4 cells. Immunizations with the 19,000-dalton protein should impede tumor growth if the analyzed protein is a tumor-specific transplantation antigen.

The antiserum against the A2T2C4 cells abrogated the lytic effect of the cytotoxic T cells. This result is analogous with similar findings on T lymphocyte-dependent lysis of cells infected with vaccinia virus (6) or vesicular stomatitis virus (9). However, such observations do not necessarily imply that the antibodies react with the actual target antigens for the T cells because steric hindrance by bound antibodies may also cause a blocking effect. This caution is relevant because antibody-mediated capping of a distinct cell surface protein may give rise to a secondary recruitment of transplantation antigens into the cap (33). Moreover, the idea that the antiserum against the A2T2C4 cells contains antibodies directed against cell surface proteins that escaped detection can not be completely refuted at present. However, the findings that the AgB antigens and the 19,000-dalton protein also, after solubilization of the membrane molecules, form a complex provide suggestive evidence that the antibodies may in fact react with the molecule(s) recognized by the cytolytic T lymphocytes.

The antibodies against the AgB antigens also impeded the action of the cytolytic T cells. These antibodies reacted with both the effector and the target cells. However, previous studies have shown that binding of antibodies against the major histocompatibility antigens on effector cells does not influence the activity of these cells (7) whereas, when bound to the target cells, such antibodies abolish cytolysis (7, 8). The rabbit antibodies against the AgB antigens may therefore exert their effect on the surface of the target cells.

It is generally agreed that, whether recognizing allogeneic transplantation antigens (see ref. 34), minor histocompatibility antigens (35), chemically modified cell surface antigens (36), or virus infected cells (1), cytolytic T lymphocytes only react with the neoantigens, provided that they are presented together with the classical transplantation antigens (cf. ref. 37). In view of the structural similarity between the histocompatibility antigens and the immunoglobulins (38, 39), it may be suggested that the transplantation antigens, with weaker interactions and less specificity, may bind foreign substances of various types in a fashion similar to that of antibodies binding specific antigens. Such a molecular interaction, as exemplified by the present results and by recent findings that highly purified transplantation antigens may bind viruses (40) and bacteria (unpublished data), may be a prerequisite for proper presentation of antigens to cytolytic T lymphocytes.

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1. Doherty, P. C., Blanden, R. V. & Zinkernagel, R. M. (1976) *Transplant. Rev.* **29**, 89–123.
2. Zinkernagel, R. M. & Doherty, P. C. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **41**, 505–510.
3. Schrader, J. W., Cunningham, B. A. & Edelman, G. M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 5066–5070.
4. Henning, R., Schrader, J. W. & Edelman, G. M. (1976) *Nature (London)* **263**, 689–691.
5. Koszinowski, U. & Ertl, H. (1975) *Nature (London)* **255**, 552–554.
6. Koszinowski, U. & Ertl, H. (1975) *Eur. J. Immunol.* **5**, 245–251.
7. Germain, R. N., Dorf, M. E. & Benacerraf, B. (1975) *J. Exp. Med.* **142**, 1023–1028.
8. Schrader, J. W. & Edelman, G. M. (1976) *J. Exp. Med.* **143**, 601–614.
9. Hale, A. H., Witte, O. N., Baltimore, D. & Eisen, H. N. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 970–974.
10. Hecht, T. T. & Summers, D. F. (1976) *J. Virol.* **19**, 833–845.
11. Bubbers, J. E., Chen, S. & Lilly, F. (1978) *J. Exp. Med.* **147**, 340–351.
12. Zinkernagel, R. M., Callahan, G. N., Althage, A., Cooper, S., Klein, P. A. & Klein, J. (1978) *J. Exp. Med.* **147**, 882–896.
13. Philipson, L., Pettersson, U. & Lindberg, U. (1975) *Virol. Monogr.* **14**, 1–115.
14. Huebner, R. L., Rowe, W. P., Turner, H. C. & Lane, W. T. (1963) *Proc. Natl. Acad. Sci. USA* **50**, 379–383.
15. Pope, J. H. & Rowe, W. P. (1964) *J. Exp. Med.* **120**, 577–588.
16. Graham, F. L., Abrahams, P. J., Mulder, C., Heyneker, H. L., Warnaar, S. O., de Vries, F. A. J., Fiers, W. & van der Eb, A. J. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 637–650.
17. Lewis, J. B., Atkins, J. F., Solem, R., Gesteland, R. F. & Anderson, C. W. (1976) *Cell* **7**, 141–151.
18. Sjögren, H. O., Minowada, J. & Ankerst, J. (1967) *J. Exp. Med.* **125**, 689–701.
19. Kvist, S., Östberg, L. & Peterson, P. A. (1978) *Scand. J. Immunol.* **7**, 265–276.
20. Gallimore, P. H., Sharp, P. A. & Sambrook, J. (1974) *J. Mol. Biol.* **89**, 49–72.
21. Johansson, K., Persson, H., Lewis, A. M., Pettersson, U., Tibbetts, C. & Philipson, L. (1978) *J. Virol.* **27**, 628–639.
22. Julius, M. H., Simpson, E. & Herzenberg, L. A. (1973) *Eur. J. Immunol.* **3**, 645–649.
23. Gahmberg, C. G. & Hakomori, S. (1973) *J. Biol. Chem.* **248**, 4311–4316.
24. Vitetta, E. S., Bauer, S. & Uhr, J. W. (1971) *J. Exp. Med.* **134**, 242–264.
25. Östberg, L., Sege, K., Rask, L. & Peterson, P. A. (1976) *Folia Biol. (Prague)* **22**, 372–376.
26. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
27. Sanderson, A. R. (1964) *Nature (London)* **204**, 250–252.
28. Wigzell, H. (1965) *Transplantation* **3**, 423–425.
29. Peterson, P. A., Rask, L., Lindblom, J. B. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 35–39.
30. Cuatrecasas, P. (1970) *J. Biol. Chem.* **245**, 3059–3065.
31. Östberg, L., Rask, L., Nilsson, K. & Peterson, P. A. (1975) *Eur. J. Immunol.* **5**, 462–468.
32. Walter, G. & Maizel, J. V., Jr. (1974) *Virology* **57**, 402–408.
33. Bourguignon, L. Y., Hyman, R., Trowbridge, I. & Singer, S. J. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2406–2410.
34. Klein, J. (1974) *Biology of the Mouse Histocompatibility-2 Complex* (Springer, Berlin).
35. Bevan, M. J. (1975) *J. Exp. Med.* **142**, 1349–1364.
36. Shearer, G. M., Rehn, T. G. & Schmitt-Verhults, A. M. (1976) *Transplant. Rev.* **29**, 222–248.
37. Forman, J. & Vitetta, E. S. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3661–3665.
38. Peterson, P. A., Rask, L., Sege, K., Klareskog, L., Anundi, H. & Östberg, L. (1975) *Proc. Natl. Acad. Sci. USA* **74**, 1612–1616.
39. Terhorst, C., Robb, R., Jones, C. & Strominger, J. L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4002–4006.
40. Helenius, A., Morein, B., Fries, E., Robinson, P., Schirrmacher, V., Terhorst, C. & Strominger, J. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3846–3850.