Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 5252–5256, July 1986
Immunology

Genetic engineering of an H-2D^d/Q10^b chimeric histocompatibility antigen: Purification of soluble protein from transformant cell supernatants

(DNA-mediated gene transfer/immunoaffinity chromatography/protein secretion)

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Communicated by Matthew D. Scharff, March 28, 1986

ABSTRACT **We have constructed a recombinant class I gene in which 5′ sequences of H-2D^d are linked to the 3′ half of a Qa subregion gene, *Q10^b*. This hybrid gene would be expected to direct the synthesis of a protein containing the N and C1 domains of H-2D^d covalently linked to the C2 domain of the secreted, nonpolymorphic, Q10^b antigen. Following DNA-mediated gene transfer into mouse L cells, transformants were analyzed by radiolabeling and immunoprecipitation. These cells secreted a molecule reactive with anti-H-2D^d monoclonal antibodies that identify epitopes on the N and C1 domains as well as with an anti-Q10 carboxyl-terminal peptide antiserum. The H-2D^d-derived antigen is associated with β_2-microglobulin and is readily purified in milligram amounts from culture supernatants by immunoaffinity chromatography.**

The class I molecules encoded by the major histocompatibility complex (MHC) are highly polymorphic integral cell-surface glycoproteins that are expressed on almost all cell types in noncovalent association with a light chain β_2-microglobulin (1–3). These molecules, the products of the *H-2K*, *H-2D*, and *H-2L* loci in the mouse, serve as the major determinants for corecognition of foreign antigen and for allorecognition by cytotoxic T lymphocytes. Nonclassical class I-like molecules, known as the Qa and TL antigens in the mouse, have been identified as the products of the Tla region that maps distal to the MHC (4–6). These differ from the classical class I antigens in that (*i*) their tissue distribution is more restricted; (*ii*) they are less polymorphic; and (*iii*) their function is unclear. One of the Qa-encoded genes, *Q10*, directs the hepatic synthesis of a nonmembrane bound molecule that is secreted in association with β_2-microglobulin and accumulates in the serum (7–12).

Despite the application of improved biochemical methods and recombinant DNA technology to the study of MHC-encoded proteins, our detailed knowledge of the molecular events that are responsible for recognition by and triggering of T lymphocytes is severely limited in that we have only incomplete understanding of the structure of the major critical components—the MHC-encoded class I and class II antigens, the T-cell receptor, and nominal antigen. Detailed analysis of the binding interactions of these molecules, as well as the determination of their secondary and tertiary structure, rests on the ability to generate large amounts of highly purified protein.

Although one approach to this problem is to purify proteolytically or detergent-solubilized material from either tissue or tissue culture cells by classical immunochemical and biochemical methods (13–16), an alternative is to generate *in vitro* genetic mutants that might be expected to lead to expression of secreted analogues after introduction into tissue culture cells. There are at least three different strategies for the generation of mutants resulting in the secretion rather than membrane expression of surface glycoproteins: (*i*) deletion of the hydrophobic transmembrane portion of the protein by introduction of an in-phase termination of translation proximal to the sequences encoding this portion of the protein; (*ii*) deletion of the transmembrane portion of the protein by specific deletion of the transmembrane encoding region of the gene, leaving the carboxyl terminus of the encoded protein intact; or (*iii*) construction of a chimeric protein whose carboxyl-terminal sequences are derived from a homologous gene that is known to encode a secreted protein. A mutation of the first type has been introduced into the mouse histocompatibility antigen H-2L^d (17). This resulted in a transformant cell line that expressed the H-2L^d-derived mutant as secreted protein. However, this cell line was not reported in detail, and it is unclear whether purification of the secreted protein is feasible. A mutation of the second type was introduced into the vesicular stomatis virus G protein, resulting in a molecule that was secreted at a relatively low rate (18). We have taken advantage of the fact that the *Q10^b* gene encodes a secreted class I-related molecule and have constructed a chimeric H-2D^d/Q10^b gene that directs the synthesis of a secreted hybrid protein after introduction into mouse L cells.

MATERIALS AND METHODS

Genomic Clones and Construction of the H-2D^d/Q10^b Chimeric Gene. The genomic clone of H-2D^d and its plasmid subclone pD^d-1 have been described (19, 20). pD^{d}LNC1 was derived from pD^d-1 as detailed in the legend to Fig. 1. The plasmid subclone of Q10^b (pUC3A) was the generous gift of J. Devlin and R. Flavell (Biogen, Cambridge, MA) (9). Fragment isolation, treatment with calf intestinal phosphatase, repair of overhanging ends with T4 polymerase or the Klenow fragment of DNA polymerase I, and ligation with T4 ligase have been described in detail elsewhere (21).

Cell Lines, Culture Conditions, and DNA-Mediated Gene Transfer. The thymidine kinase-deficient L-cell line DAP-3 was used for DNA-mediated gene transfer. Col 15, a Q10-transformant cell line, was the kind gift of A. Lew, J. Devlin, and J. Coligan and has been described in detail elsewhere (11). Cells were maintained as described (19). Col 15 was maintained in hypoxanthine/aminopterin/thymidine (HAT) medium, whereas all other transformant lines were initially isolated by cotransformation with pSV2neo (22), selected for resistance to G418 (GIBCO) at 1 mg/ml, and maintained in

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Abbreviation: MHC, major histocompatibility complex.

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G418 at 0.2 mg/ml. DNA-mediated gene transfer was carried out by calcium phosphate precipitation (19), followed by glycerol shock.

Antibodies. The monoclonal antibodies 34.5.8, 34.4.20, 23.5.21, and 34.1.2 have been shown to recognize H-2D^d determinants dependent on the N and C1 domains of H-2D^d (20). 34.2.12 has been proven to bind to an epitope on the C2 domain of H-2D^d (20, 23). The anti-Q10 carboxyl-terminal peptide antibody was the gift of A. Lew, W. L. Maloy, and J. Coligan and has been described in detail in other publications (10, 24). A rabbit anti-mouse β_2-microglobulin antiserum was the gift of M. Rogers (National Cancer Institute, National Institutes of Health). Rabbit anti-mouse H-2 antiserum was prepared against purified H-2K^k and has broad reactivity with mouse class I molecules (D.H.M., unpublished). For immunoprecipitation all murine monoclonal antibodies were used as the undiluted ascites fluid. For column chromatography, antibodies were purified from hybridoma culture supernatants by affinity chromatography on protein A-Sepharose (25).

Metabolic Labeling, Immunoprecipitation, and Gel Electrophoresis. These were all performed essentially as described (19, 26), with variations noted in the figure legends. Immunoprecipitation from culture supernatants was accomplished without the addition of detergents.

Purification of the H-2D^d/Q10^b Chimeric Protein. An immunoadsorbent column of the anti-H-2D^d monoclonal antibodies 34.5.8 (4 mg), 34.4.20 (2.5 mg), 34.1.2 (6.5 mg), and 23.5.21 (3.0 mg) was made by coupling purified monoclonal antibody protein to CNBr-activated Sepharose CL-4B (10 ml of gel) (Pharmacia). Purification of the chimeric protein was accomplished by passage of filtered culture supernatant over a Sepharose CL-4B column (10 ml) and then passage over the mixed immunoadsorbent, at 4°C at a flow rate of 25 ml/hr. The column was washed with 0.15 M NaCl/0.010 M Tris·HCl, pH 7.6/0.02% NaN_3 and preeluted with 0.45 M NaCl/0.010 M Tris·HCl, pH 7.6/0.02% NaN_3, and the absorbed chimeric protein was eluted with 0.15 M NaCl/0.015 M $NaCO_3$, pH 11.5/0.02% NaN_3. A single homogeneous peak was eluted, pooled, and dialyzed against phosphate-buffered saline (PBS) containing 0.02% NaN_3.

RESULTS

Rationale for and Construction of an H-2D^d/Q10^b Chimeric Gene. Evidence indicates that major structural determinants of polymorphism responsible for serological and T-cell recognition of class I molecules reside in the N and C1 domains (20, 27, 28). Studies of the structure of the *Q10^b* gene and its encoded protein show that this monomorphic class I-like molecule is secreted, rather than membrane bound (7–12), due to a deletion within the transmembrane exon leading to a frameshift mutation and premature termination of translation. This eliminates a contiguous stretch of hydrophobic amino acid residues of the transmembrane domain of the protein. Thus, it seemed likely that a molecule consisting of the polymorphic amino-terminal N and C1 domains of H-2D^d and the carboxyl-terminal C2 domain of Q10^b would both preserve the major H-2D^d polymorphic epitopes and have the secretory phenotype of Q10^b. Thus, we constructed a gene that contained the L, N, and C1 exons of H-2D^d covalently coupled to the C2 exon and 3′ sequences of Q10^b (see Fig. 1).

The chimeric gene described in Fig. 1 was introduced into mouse L cells by DNA-mediated gene transfer and transformants were selected for resistance to the neomycin analogue G418. Nine independently derived pools of uncloned transformants were labeled with [^{35}S]methionine, and the cell culture supernatants harvested after 24 hr of labeling were

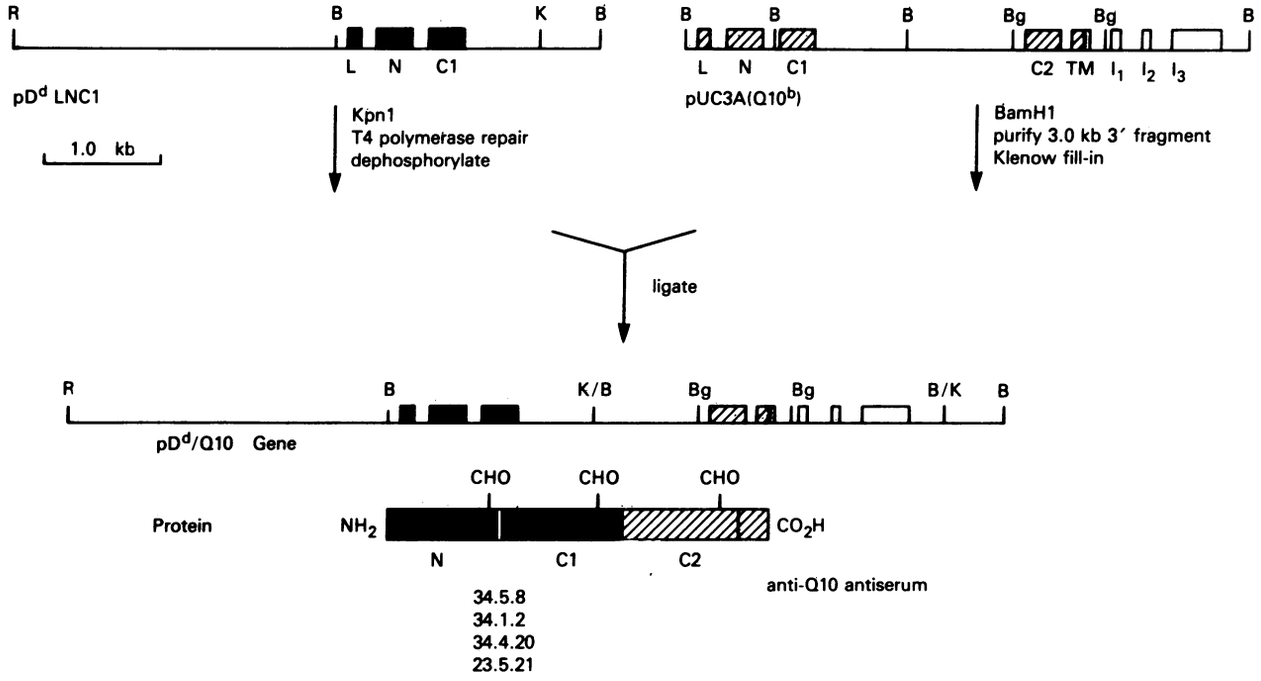

FIG. 1. Construction of the H-2D^d/Q10^b gene, *pD^d/Q10*. Plasmid subclone pD^{d}LNC1 was derived from pD^d-1 (20) by digestion with *Bam*HI, ligation, and screening of resulting subclones by restriction endonuclease mapping. The pBR327 vector is not shown. pUC3A (9) was digested with *Bam*HI, the 3.0-kilobase (kb) 3′ fragment was purified by electrophoresis in a 1% agarose gel, and the ends were repaired by a fill-in reaction using the Klenow fragment of DNA polymerase I. This fragment was cloned into the dephosphorylated, T4-repaired, *Kpn* I-linearized pD^{d}LNC1 by blunt-end ligation and transformation into *Escherichia coli* strain MC1061. Plasmid DNA from resulting colonies was subjected to restriction endonuclease digestion and gel electrophoresis to establish the orientation of the cloned fragment. Schematically, solid boxes indicate the exons encoding the domains of protein derived from H-2D^d, shaded boxes indicate the exons encoding Q10^b protein, and open boxes indicate presumed 3′ untranslated region encoding exons from Q10^b. TM, transmembrane. The expected reactivity of the chimeric gene-derived protein with several antibodies is illustrated.

subjected to immunoprecipitation with a mixture of rabbit anti-H-2 antiserum and the anti-H-2D^d monoclonal antibody 34.5.8. As shown in Fig. 2, in all cases except for the pSV2neo transfection control (lane A), detectable amounts of specifically immunoprecipitable protein were identified. This protein consisted of a 45- to 50-kDa heterogenous chain that coprecipitated with a lower molecular mass protein that migrated with the dye front on this 10% polyacrylamide gel.

Serological Identity of the Chimeric Protein. To establish the serological identity of the molecule secreted by these transformant cells, the pool expressing the greatest amount of radiolabeled antigen was examined in further detail. Transformant pool DMT44.9 (shown in Fig. 2, lane I) was again labeled with [^{35}S]methionine, supernatants were harvested after 24 hr, and this material was subjected to immunoprecipitation with a number of different monoclonal antibodies and antisera. For comparison, the *bona fide* Q10^b transformant line Col 15 was labeled and its supernatant was simultaneously treated with the same panel of antibodies and antisera. Fig. 3 demonstrates that the Q10^b protein secreted by Col 15 was precipitated by the rabbit anti-Q10 antiserum and rabbit anti-β_2-microglobulin and migrated at ≈40–43 kDa. By contrast, the protein secreted by DMT44.9 (the H-2D^d/Q10^b transformant) was precipitable not only with the

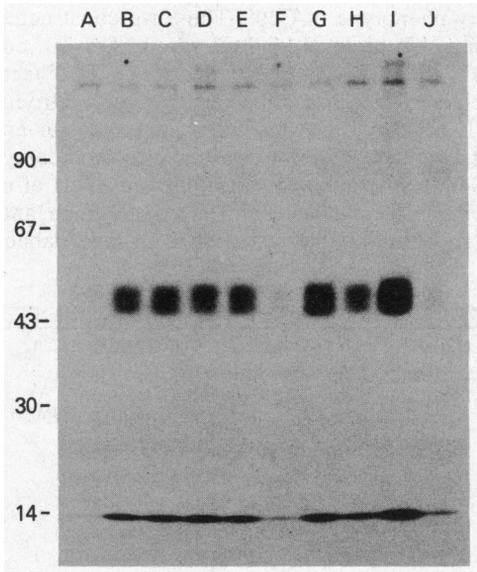

FIG. 2. Immunoprecipitated, secreted, proteins from primary transformant populations. DNA-mediated gene transfer of the *pD^d/Q10* gene was performed in nine independent experiments. Following introduction of DNA into mouse L cells and selection in G418, cells (10^6) were labeled in 1 ml of Dulbecco's modified Eagle's medium (DMEM)/10% fetal calf serum containing 15 μCi of [^{35}S]methionine per ml (1 Ci = 37 GBq), at 37°C in a 10% CO_2 in air atmosphere, for 24 hr. Cell supernatants were harvested, and residual cells were removed by centrifugation in an Eppendorf centrifuge for 2 min and subjected to immunoprecipitation by reaction for 18 hr with 10 μl of a 1:1 mixture of 34.5.8 ascites and rabbit anti-H-2 antiserum. Immune complexes were insolubilized by binding to an excess of protein A-Sepharose, washed, solubilized in $NaDodSO_4$ sample buffer containing 0.15 M 2-mercaptoethanol, and electrophoresed in a 10% polyacrylamide gel containing $NaDodSO_4$. The gel was subjected to fluorography, dried, and autoradiographed. Lanes A–J were all labeled and processed in parallel. Lane A, labeling of the pSV2neo clone transformant control, line DMT44.1; lane B, line DMT44.2; lane C, line DMT44.3; lane D, line DMT44.4; lane E, line DMT44.5; lane F, line DMT44.6; lane G, line DMT44.7; lane H, line DMT44.8; lane I, line DMT44.9; and lane J, line DMT44.10. All lines resulted from transformation utilizing 0.5 μg of pSV2neo and lines DMT44.2 to DMT44.10 also received from 0.5 to 6 μg of two independent preparations of the *pD^d/Q10* gene construct. Molecular masses are given in kDa.

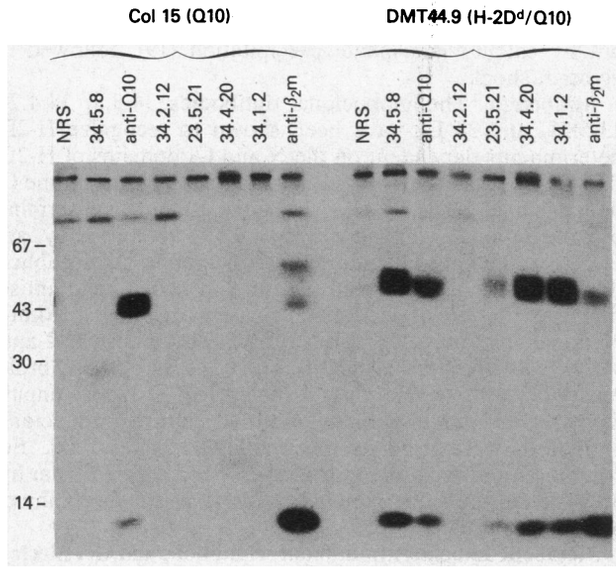

FIG. 3. Antibody reactivity of H-2D^d/Q10^b transformant DMT44.9. T25 flasks of Col 15 (11) and DMT44.9 containing 3 × 10^6 cells were fed with 3 ml of complete medium containing 15 μCi of [^{35}S]methionine per ml, and following incubation for 24 hr, the cell supernatants were harvested, centrifuged, and aliquoted for precipitation with the indicated sera or monoclonal antibody ascites fluid. Following incubation, precipitation, washing, and solubilization as described in the legend to Fig. 2, samples were electrophoresed in a 12.5% polyacrylamide gel, fluorographed, and autoradiographed. β_2m, β_2-microglobulin. Molecular masses are given in kDa.

rabbit anti-Q10 antiserum and rabbit anti-β_2-microglobulin but also with those monoclonal antibodies that recognize epitopes on the N and/or C1 domains of H-2D^d (34.5.8, 34.1.2, 34.4.20). As expected, the protein secreted by DMT44.9 was not precipitated by an antibody that recognizes epitopes specific for the C2 domain of H-2D^d (34.2.12). The H-2D^d/Q10^b molecules migrate as a heterogeneous band of about 45–50 kDa. The hybrid protein contains N-linked carbohydrate addition sites on the N and C1 domains of H-2D^d as well as the N-linked site on the C2 domain derived from Q10. Thus, the H-2D^d/Q10^b protein contains three glycosyl units, whereas the Q10^b protein has only two. As expected, the secreted molecule derived from the chimeric gene is associated with β_2-microglobulin, which is clearly resolved from the dye front on this 12.5% polyacrylamide gel. Quantitative densitometry of these and other autoradiographs, with appropriate correction for the molar representation of methionine in the two protein chains, indicates that the ratio of the heavy chain to β_2-microglobulin in the complex is 1:1.

Time Course of H-2D^d/Q10^b Protein Secretion. To evaluate the time course of the secretion of the H-2D^d/Q10^b chimeric molecule, and to establish the stability of the secreted protein, a pulse–chase experiment was performed. DMT44.9 was incubated with [^{35}S]methionine for 1 hr, and the culture was chased with a 10-fold excess of unlabeled methionine. As shown in Fig. 4, the bulk immunoprecipitable material labeled in the first hour remained in the cell as a 40-kDa molecule for as long as 9 hr (lane C), and minimal amounts remained detectable for as long as 21 hr (lane E). Some secreted fully glycosylated 45- to 50-kDa molecules appeared in the supernatant even after 1 hr, but this peaked at about 9 hr (lane J) and remained essentially constant for as long as 28 hr (lane N). At all time points studied, the H-2D^d/Q10^b heavy chain coprecipitated with the light chain of the complex, β_2-microglobulin. Endoglycosidase F treatment of the mature, secreted molecule resulted in a degradation product of

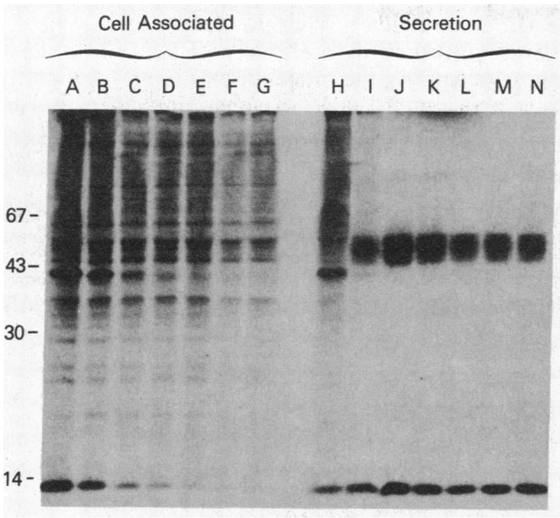

FIG. 4. Pulse–chase labeling of H-2D^d/Q10^b transformant pool DMT44.9. Cells (1.8×10^7) were centrifuged, resuspended in 10 ml of RPMI medium lacking methionine, and starved for 30 min at 37°C. Cells were centrifuged free of the starvation medium and resuspended in 1 ml of prewarmed RPMI medium containing 300 μCi of [^{35}S]methionine (Amersham, >29.6 TBq/mmol), incubated for 1 hr at 37°C, and centrifuged again; the supernatant was removed and cells were resuspended in 10 ml of prewarmed RPMI medium containing 0.15 mg of L-methionine per ml (10× the concentration in RPMI medium), glutamine at 0.15 mg/ml, and nonessential amino acids at 0.15 mg/ml. At various times, 1-ml aliquots were removed onto ice, the cells were centrifuged, and the samples were split into pellet and supernatant. Cell pellets were lysed in 100 μl of lysis buffer (26), nuclei were removed by centrifugation, and the lysates, following preclearance with an anti-H-2K^k, -D^k monoclonal antibody 16.1.2 (29) and protein A-Sepharose, were subjected to immunoprecipitation with 34.5.8 and protein A-Sepharose. Complexes were washed, solubilized in sample buffer containing NaDodSO$_4$ and 2-mercaptoethanol, and electrophoresed on a 12.5% polyacrylamide gel containing NaDodSO$_4$. Supernatants were centrifuged again to ensure the removal of insoluble debris and were subjected to immunoprecipitation with 34.5.8 (18 hr at 4°C). Following addition of protein A-Sepharose, complexes were washed, solubilized, and analyzed on the same polyacrylamide gel as the cell-associated material. The indicated lanes represent 0 (A, H), 4 hr (B, I), 9 hr (C, J), 16 hr (D, K), 21 hr (E, L), 24 hr (F, M), and 28 hr (G, N) of chase. Molecular masses are given in kDa.

40 kDa, indicating that the intracytoplasmic 40-kDa molecule contained only core carbohydrate (data not shown).

Clonal Stability of Transformant Cells. To examine the stability of the H-2D^d/Q10^b transformant cells, as well as to identify cellular clones expressing greater amounts of the hybrid protein, 15 clones derived from two independent transformant pools were labeled with [^{35}S]methionine and their supernatants were allowed to react with monoclonal antibody 34.5.8. As shown in Fig. 5, all 15 clones secreted detectable amounts of an electrophoretically identical heterodimeric protein consisting of a 45- to 50-kDa heterogeneous heavy chain and a 12-kDa light chain. The clones that appeared to be secreting the H-2D^d/Q10^b protein in the greatest amount (clones DMT44.7.4, DMT44.7.7, DMT44.7.8, and DMT44.9.5; lanes E, H, I, and N) were expanded and again analyzed. Based on this experiment, clone DMT44.7.4 was chosen to serve as the basis for further experiments.

Quantitative Purification of H-2D^d/Q10^b Protein from Transformant Cell Culture Supernatants. The L cells transformed with the H-2D^d/Q10^b chimeric gene synthesize and secrete readily detectable amounts of the corresponding protein. Estimates based on measurement of the number of dpm immunoprecipitated from steady-state labeling experiments are that the rate of secretion is 0.5–1.0 μg per 10^6 cells per 24 hr. This represents about 0.5% of the total trichloroacetic acid-precipitable secreted protein (data not shown). To concentrate and purify the secreted antigen, we prepared an anti-H-2D^d immunoadsorbent column. The material purified (as described in the legend to Fig. 6) was subjected to electrophoresis, fixed, and stained. Four different preparations show that the predominant protein purified is the same as the radiolabeled, immunoprecipitable chimeric H-2D^d/Q10^b. These elution conditions preserve the association of the heavy chain with β_2-microglobulin, since both chains can be precipitated by each of the anti-H-2D^d monoclonal antibodies following elution of the purified protein from the column (data not shown). Electrophoresis of the purified protein in the absence of reducing agents leads to the same pattern, indicating that this association is not covalent (data not shown). To a variable degree these preparations are contaminated with a homogeneous 28-kDa protein thought to be a fetal calf serum component, since it is not observed in radiolabeled cell supernatant eluted from the immunoadsorbent.

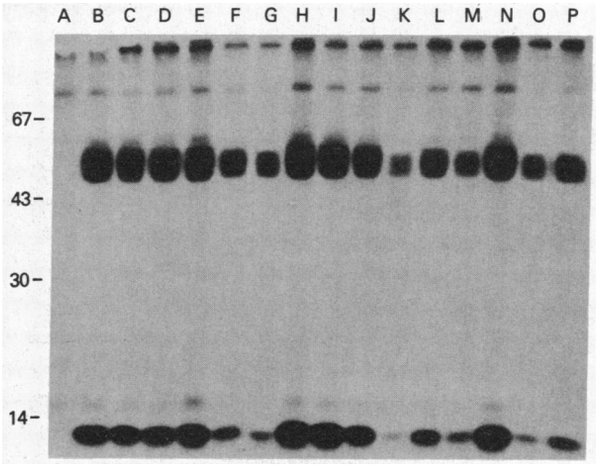

FIG. 5. Clonal analysis of H-2D^d/Q10^b transformants. Clonal cell lines derived from DMT44.1 (pSV2neo transformant), DMT44.7 (see Fig. 2, lane G), or DMT44.9 (see Fig. 2, lane I) were labeled as described in the legend to Fig. 1 and the cell supernatants were subjected to immunoprecipitation with monoclonal antibody 34.5.8, electrophoresis in a 12.5% polyacrylamide gel containing NaDodSO$_4$, fluorography, and autoradiography. Lane A, DMT44.1.1 (negative control); lane B, DMT44.7.1; lane C, DMT44.7.2; lane D, DMT44.7.3; lane E, DMT44.7.4; lane F, DMT44.7.5; lane G, DMT44.7.6; lane H, DMT44.7.7; lane I, DMT44.7.8; lane J, DMT44.7.9; lane K, DMT44.9.1; lane L, DMT44.9.2; lane M, DMT44.9.3; lane N, DMT44.9.5; lane O, DMT44.9.6; and lane P, DMT44.9.7. Molecular masses are given in kDa.

DISCUSSION

This paper describes the construction of a chimeric H-2D^d/Q10^b gene, which, following DNA-mediated gene transfer into mouse L cells, leads to the secretion of a glycosylated 45- to 50-kDa class I protein in equimolar association with its light chain, β_2-microglobulin. The secreted molecule retains the amino-terminal serological identity of H-2D^d, as demonstrated by its interaction with four monoclonal antibodies that identify epitopes previously mapped to the N and/or C1 domains of H-2D^d by exon-shuffling experiments. In addition, the secreted molecule has lost the monoclonal specificity that maps to the C2 domain of H-2D^d (34.2.12) and has gained the serological epitope defined by the anti-Q10 antipeptide antibody. Since this antibody is specific for the carboxyl-terminal peptide (10), its reactivity indicates the integrity of the carboxyl-terminal sequence in this hybrid protein. The precipitation of this molecule from the super-

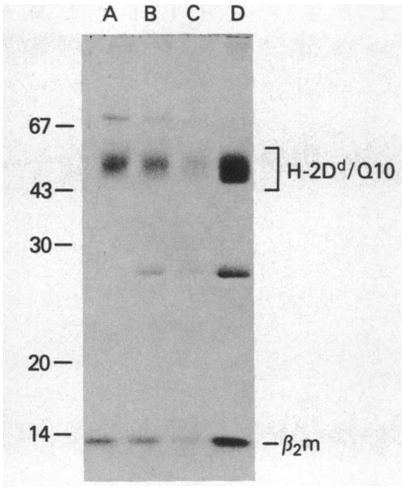

FIG. 6. Electrophoretic analysis of immunoadsorbent-purified H-2D^d/Q10^b protein. DMT44.9 and DMT44.7.4 cells were grown to confluence in 850-cm^2 roller bottles in 200 ml of DMEM containing 5% fetal calf serum. Five independent preparations of cell supernatant were passed over the anti-H-2D^d immunoadsorbent column, washed, and eluted. Two such preparations were pooled and further concentrated by ultrafiltration through Amicon C25A membranes. Twenty-microliter aliquots of the eluted material were boiled in $NaDodSO_4$ sample buffer containing 2-mercaptoethanol and electrophoresed in 12.5% polyacrylamide gels, stained in 0.05% Coomassie brilliant blue (Kodak R-250)/50% CH_3OH/7% CH_3COOH overnight, and destained in 5% CH_3OH/7% CH_3COOH, and photographed. Lane A, pool of ultrafiltration-concentrated preparations 3 and 4; lane B, unconcentrated preparation 5; lane C, preparation 6; lane D, preparation 7. β_2m, β_2-microglobulin. Molecular masses are given in kDa.

natant by anti-β_2-microglobulin antiserum demonstrates the association of the two chains. Polyacrylamide gel electrophoresis of immunoadsorbent-purified H-2D^d/Q10^b under denaturing, but nonreducing, conditions indicates that this association is noncovalent. These transformant cells are stable since transformant pool DMT44.9 and clone DMT44.7.4 have been maintained in culture without selection for several months without apparent variation in the secretory phenotype. The secreted H-2D^d/Q10^b molecule has an apparent molecular mass of 45–50 kDa, consistent with the 301 amino acid residue chain (calculated molecular mass of ≈32 kDa) containing N-linked glycosyl units at positions 86, 176, and 256. As suggested (11), the apparently large contribution to the molecular mass of N-linked carbohydrate may be due to the particular glycosylation pattern of the L cell.

The utility of producing relatively large amounts of an immunologically reactive, yet water-soluble polymorphic class I protein overcomes some of the major obstacles to performing critical experiments related to the structure and function of class I molecules. This may offer a general approach to the production of large amounts of different class I molecules of mouse and other species to provide the substrate for the growth of crystals for x-ray crystallography. [Although classical purification methods have provided a sufficient quantity of the human class I molecules HLA A2 and HLA A28 for crystallization and low-resolution structure determination (30), we expect that chimeric gene products will be technically more amenable to generalized high-quantity production.] Introduction of similar gene constructs into cells more highly differentiated for a secretory phenotype, such as myeloma cells or hepatoma cells, may prove valuable. In addition, the availability of such soluble class I molecules should allow focal questions of T-cell immunology to be addressed directly: What are the binding parameters for T-cell receptors thought to interact with H-2D^d? Do allosteric changes accompany the interaction of H-2D^d with antigen? Finally, if this approach is of general applicability for the engineering of soluble class I molecules, it may be adapted to other integral membrane proteins, including the class II glycoprotein and the T-cell receptor.

We thank S. Starnes for preparation of the manuscript and A. Singer, A. Lew, and J. Coligan for their encouragement and critical comments. A.L.R. was supported by the McKnight Foundation, Minneapolis, MN.

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