Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 757–761, February 1986
Immunology

Intracellular transport blockade caused by disruption of the disulfide bridge in the third external domain of major histocompatibility complex class I antigen

(H-2L^d antigen/site-directed mutagenesis/cell surface expression)

JUN-ICHI MIYAZAKI*, ETTORE APPELLA†, AND KEIKO OZATO*

*Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Human Development, and †Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Communicated by Igor B. Dawid, September 17, 1985

ABSTRACT **The third external domain of major histocompatibility class I antigens has a highly conserved disulfide bridge between cysteine-203 and cysteine-259. To elucidate the functional significance of this disulfide bridge, we have produced a mutant *H-2L^d* gene by site-directed mutagenesis in which the codon for cysteine-203 is changed to a codon for serine, which is unable to form a disulfide bridge. The mutant *H-2L^d* gene was introduced into mouse L cells and its expression has been studied. No measurable expression of the H-2L^d antigen was detected on the cell surface of the transformants by antibody-binding assays. However, a large quantity of the mutant H-2L^d antigen was found in the cytoplasm of the transformants as observed by immunoprecipitation of metabolically labeled lysate and by immunocytochemistry of membrane-permeabilized cells, using an antibody specific for the first external domain of the H-2L^d antigen. The mutant antigen was glycosylated and associated, at least in part, with β_2-microglobulin. Subcellular fractionation experiments indicated that the transport of the antigen was blocked between the endoplasmic reticulum and the plasma membrane. It is concluded that structural integrity of the third external domain is a prerequisite for intracellular transport of class I antigens. On the basis of these findings we suggest that the domain structure containing the disulfide bridge serves as a signal structure necessary for receptor-mediated intracellular transport and that this requirement is the evolutionary basis for high conservation of similar structures present throughout the immunoglobulin supergene family.**

Major histocompatibility complex (MHC) class I antigens are a set of integral cell surface glycoproteins involved in many aspects of the immune response. These antigens consist of two noncovalently associated chains (1): a heavy chain ($M_r \approx$ 45,000) encoded by the *H-2K*, *-D*, or *-L* gene in mice and β_2-microglobulin (β_2m; $M_r \approx$ 12,000). One of the most striking features of the MHC class I products is their high degree of polymorphism. About 50 different alleles are known at the *H-2K* and *-D* loci, respectively (2).

Class I antigens consist of three regions, an extracellular region, a transmembrane region, and a cytoplasmic region at the carboxyl terminus; the extracellular region is subdivided into three domains designated N, C1, and C2, each comprising about 90 amino acid residues (3, 4). The latter two domains (C1 and C2) each have an intradomain disulfide bridge, between Cys-101 and Cys-164 and between Cys-203 and Cys-259, respectively. The domain structure containing a dilsulfide bridge is found in common among all the class I antigens so far examined (5–10), suggesting an important role of this structure in the expression and/or functions of class I antigens. Previously we have shown that site-directed mutagenesis can be employed to study structure–function relationships of class I antigens (11, 12). In this report, we describe a mutant H-2L^d antigen lacking the highly conserved disulfide bridge in the C2 domain. The C2 domain is less polymorphic than the N and C1 domains and plays a minor role in T-cell recognition (13–15), and there is an implication that it is associated with β_2m (16) and that this association may be necessary for the intracellular transport of class I antigens (17, 18).

We demonstrate here that the disulfide bridge of the C2 domain is essential for intracellular transport of class I antigens from the endoplasmic reticulum to the plasma membrane, and we discuss implications for receptor-mediated transport of the class I antigen.

MATERIALS AND METHODS

Site-Directed Mutagenesis. The basic strategy for mutagenesis is the same as described previously (11, 12). Single-stranded M13 phage DNA (m9-33.6), which includes an expressible *H-2L^d* gene of about 4 kilobases, was used as the template DNA for mutagenesis. The mutagenic oligonucleotide 5′-GGGCCCAGCTCCTCAGGGT-3′ was synthesized by the solid-phase phosphotriester method (19) on an automated synthesizer (Vega Biochemicals, Tucson, AZ), purified by high-pressure liquid chromatography, and then subjected to electrophoresis through a 20% polyacrylamide gel after 5′-phosphorylation with T4 polynucleotide kinase. Ten picomoles each of the mutagenic oligonucleotide, M13 hybridization primer (New England Biolabs), and M13 universal primer (Bethesda Research Laboratories) were annealed with 2 pmol of the template and then the product was allowed to elongate in the presence of DNA polymerase (Klenow fragment) and T4 DNA ligase in a total volume of 30 μl at 14°C for 24 hr. A portion of the reaction mixture was used to transfect competent *Escherichia coli* JM103 cells. The resulting plaques were pooled and replated with JM103 cells. Twenty-four plaques were picked, grown in 2-ml cultures, and screened by dot-blot hybridization on a nitrocellulose filter, using the 5′-end-labeled mutagenic oligonucleotide as the probe (20). Three positive clones were identified after washing the filter at 71°C and the mutation was confirmed by DNA sequence determination using the chain termination method (21).

Reconstruction of the Mutant DNA. To remove possible secondary mutations in the mutants obtained, we transferred the *Bgl* II 630-base-pair (bp) fragment harboring the desired mutation into the background of the wild-type *H-2L^d* gene.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: β_2m, β_2-microglobulin; TK, thymidine kinase; MHC, major histocompatibility complex; mAb, monoclonal antibody; bp, base pair(s).

Briefly, we first removed a 630-bp *Bgl* II fragment of the *H-2L*^{d} gene from m9-33.6 and the corresponding *Bgl* II fragment from one of the mutants was inserted into this wild-type *H-2L*^{d} gene. The *Bgl* II fragment contains a sequence starting 26 bp upstream of the mutation site and ending within the fifth intron. The presence of a *Bgl* II site in the essential gene *2* of the M13 vector did not interfere with this construction, because phage growth is inhibited by insertion into this site. Reconstruction of the complete *H-2L*^{d} gene was confirmed by digestions with several restriction enzymes, including *Bgl* II, *Pst* I, *Bam*HI, and *Xba* I; one such clone, designated SH38B, was used for subsequent studies. Further, the 630-bp *Bgl* II fragments both from m9-33.6 and from SH38B were recloned in the *Bam*HI site of M13mp11 in both orientations and their sequences were compared with each other by the chain termination method (21). There were no differences between the two sequences apart from the desired point mutation in SH38B.

DNA-Mediated Gene Transfer. DNA-mediated gene transfer of the mutant *H-2L*^{d} gene to thymidine kinase-deficient (TK$^-$) mouse L cell line DAP-3 was carried out as previously described (22, 23). Twenty micrograms of double-stranded replicative form DNA prepared from *E. coli* JM103 cells infected with SH38B mutant phage and 500 ng of pBR-TK DNA containing the herpes virus *TK* gene (24) were coprecipitated with calcium phosphate and then added to a 100-mm plate seeded with 1×10^6 DAP-3 cells. Selection with hypoxanthine/aminopterin/thymidine (HAT) medium (25) yielded TK$^+$ transformed colonies, which were subsequently isolated and analyzed for expression of the mutant *H-2L*^{d} gene.

Immunocytochemical Analysis. Detection of intracellular H-2 antigens was carried out with membrane-permeabilized cells. Briefly, cells were fixed with 0.25% glutaraldehyde in phosphate-buffered saline for 5 min at room temperature, followed by treatment with 0.3% Triton X-100 in phosphate-buffered saline for 15 min at room temperature. After removal of the detergent by rinsing, the cells were incubated with monoclonal antibody (mAb) in culture supernatant. As described elsewhere (26), bound antibodies were visualized by the subsequent reaction with peroxidase-coupled goat anti-mouse IgG F(ab')$_2$ (Cappel Laboratories, Cochranville, PA), using diaminobenzidine as a substrate.

Metabolic and Surface Labeling of Cells. Cells were washed twice with methionine-free medium and incubated in the same medium supplemented with 10% fetal bovine serum for 1 hr. Then they were trypsinized, washed, and resuspended at 7×10^6 cells per ml in the above medium plus [^{35}S]methionine (Amersham, 1000 Ci/mmol; 1 Ci = 37 GBq) at 450 μCi/ml. After incubation at 37°C for 3 hr, the cells were washed in regular medium and lysed in 0.2 ml of lysis buffer (10 mM Tris·HCl, pH 7.5/150 mM NaCl/1% Nonidet P-40/0.1 mM phenylmethylsulfonyl fluoride) on ice for 15 min. Nuclei were removed by centrifugation for 10 min in an Eppendorf centrifuge. The supernatants were stored at −70°C prior to immunoprecipitation.

For preparation of the microsomal and cytosolic fractions, labeled cells were suspended in 0.3 ml of hypotonic buffer (10 mM Tris·HCl, pH 7.5/0.1 mM phenylmethylsulfonyl fluoride) and sonicated for 15 sec three times. The samples were centrifuged for 1 min at $2000 \times g$ to remove a nuclear fraction. A 0.2-ml fraction of the supernatants was centrifuged for 5 min at $137,000 \times g$ to obtain a microsomal pellet and a cytosolic fraction. The pellet was washed once and lysed in 0.2 ml of lysis buffer. Insoluble materials were removed by centrifugation for 10 min in an Eppendorf centrifuge.

Cells were surface labeled with ^{125}I by the lactoperoxidase method (27) with minor modifications. Briefly, cells were scraped from Petri dishes, washed, and incubated in HAT medium at 1×10^6 cells per ml for 2 hr. After washing twice, cells were surface labeled with Na^{125}I (Amersham, 14.5 mCi/μg) at 0.4 mCi/ml in the presence of β-D(+)-glucose, lactoperoxidase, and glucose oxidase. Labeled cells were then washed five times and lysed as described above.

Immunoprecipitation. Labeled lysates were cleared once with fixed *Staphylococcus aureus* Cowan I strain (28) pretreated with ascites fluid of an unrelated mAb and once with *S. aureus* pretreated with unlabeled DAP-3 lysate. Then aliquots of the lysates were incubated with an excess of the indicated mAb (used as undiluted ascites fluid) at 4°C overnight. The immune complexes were bound to *S. aureus* and then extensively washed in high-salt buffer. Bound proteins were solubilized by boiling in elution buffer containing 2% (wt/vol) $NaDodSO_4$ and 2% (vol/vol) 2-mercaptoethanol and then were analyzed by electrophoresis in $NaDodSO_4$/10% or 11% polyacrylamide gels (29) and fluorography. Molecular weights were estimated by reference to the mobilities of commercially available protein standards.

RESULTS

Mutagenesis of the *H-2L*^{d} Gene. Site-directed mutagenesis was employed to convert the cysteine residue (codon UGC) to a serine residue (codon AGC) at amino acid position 203 of the H-2L^d antigen. To minimize the effects caused by amino acid substitution other than the breakage of the disulfide bridge, we chose serine as the amino acid residue that replaced cysteine, since the two amino acids differ only at a single atom: a sulfur atom in cysteine is substituted with an oxygen atom in serine. We synthesized a mutagenic oligonucleotide (19 bases long) that is complementary to the sequence corresponding to amino acid positions 200 to 206 in the *H-2L*^{d} gene cloned in M13 single-stranded phage vector but has a single base substitution (A → T) to induce the mutation. The mutagenesis was performed as previously described (11, 12). Of 24 phages subjected to dot hybridization screening, 3 remained positive under increasing stringency and were subsequently shown by sequencing to possess the expected mutation. Double-stranded DNA was prepared from *E. coli* infected with one of these mutant phages and was introduced into TK$^-$ mouse L cells (DAP-3) together with pBR-TK DNA. Ten HAT-resistant transformants were tested for the expression of the H-2L^d antigen on the cell surface by antibody-binding assay using 16 H-2L^d-specific mAbs (30, 31). None of the transformants showed positive binding to any antibody tested (data not shown). Considering that usually more than 90% of transformants are positive for the H-2L^d antigen expression after transfer of wild-type genes by these methods, it seemed likely that most of the transformants were producing the H-2L^d antigen but were deficient in surface expression or missing antigenic sites detectable by the antibodies used here.

Before proceeding to examine these possibilities, we wished to confirm that the failure in detection of the H-2L^d antigen in these transformants was attributable to the designed mutation and not to any other mutations that might have been concomitantly or spontaneously introduced in the process of the mutagenesis. For this reason, we made a new construct named SH38B in which a 630-bp *Bgl* II fragment containing the mutation site was inserted into the remaining part of the wild-type *H-2L*^{d} gene, m9-33.6 (see *Materials and Methods*). The sequence of the 630-bp *Bgl* II fragment from SH38B was analyzed along with that of the wild-type gene. Results showed that a codon of serine (AGC) had replaced the original codon of cysteine (TGC) at amino acid position 203 (Fig. 1) with no other alterations in this fragment.

Detection of the Mutant H-2L^d Antigen by Intracellular Staining. Double-stranded DNA of the mutant SH38B was introduced into DAP-3 cells, and 10 HAT-resistant transformants were isolated and subjected to antibody-binding assay. Again, we did not detect any binding of mAbs to the

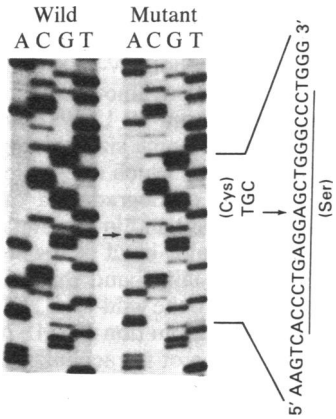

FIG. 1. Comparison of the mutant (right) and the wild-type (left) DNA sequences. The 630-bp *Bgl* II fragments were recloned in the *Bam*HI site of M13mp11 and the sequences were determined by the chain-termination method (21), using the universal primer (Bethesda Research Laboratories). An autoradiograph of a 6% polyacrylamide gel is shown. The mutagenic oligonucleotide is complementary to the underlined region; arrows indicate the mutation site.

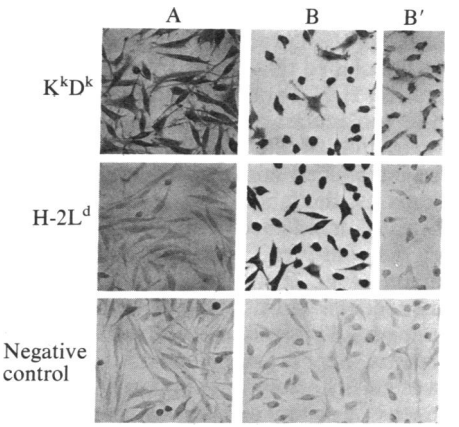

FIG. 2. Intracellular staining of the mutant H-2L^d antigen. Untransformed cells DAP-3 (*A*) and mutant H-2L^d transformant SMB2-9 (*B*, *B'*) cultured in chamber slides were stained by the immunoperoxidase method (26), using anti-H-2K^{k}D^k mAb 16.3.1 (*Top*), anti-H-2L^d mAb 64.3.7 (*Middle*), and an unrelated mAb, PS67 (*Bottom*), after treatment with Triton X-100 (*A*, *B*) or without treatment with Triton X-100 (*B'*).

cell surface of the transformants. Since it was unlikely that the mutagenesis affected the efficiency of transformation, we tested the alternative possibility that the mutant antigens were present within the cells but not on the cell surface. Immunoperoxidase staining was employed for transformed cells after brief treatment with Triton X-100, which allowed passage of antibodies through the plasma membrane. Antibodies specific for the N and C1 domains were examined, because the conformation of these domains would be less altered by the mutagenesis than the C2 domain (11). As can be seen in Fig. 2*B*, specific diaminobenzidine staining was demonstrated in SMB2-9 transformant cells with the N-domain-specific anti-H-2L^d mAb 64.3.7 (11). Of 10 transformants tested 2, including SMB2-9, were heavily stained. An additional 6 transformants were also stained with a lower intensity, while the 2 remaining cells were negative. In contrast, no detectable staining was observed in untransformed DAP-3 cells, indicating the specificity of the staining (Fig. 2*A*). Similar results were obtained with the C1-specific mAb 30.5.7, though the staining was not as strong as with mAb 64.3.7 (data not shown). No detectable staining was seen with the C2-domain-specific mAb 28.14.8 (see following section, on immunoprecipitation). In control experiments, mAb 16.3.1 specific for the H-2K^{k}D^k antigens, which are endogenously expressed in L cells, stained all the cells tested, including untransformed DAP-3 cells; in contrast, unrelated mAb PS67 did not stain any of the cells tested (Fig. 2). It should be noted that the staining by anti-H-2L^d mAb 64.3.7 represented detection of the intracellular H-2L^d antigen, since no staining was observed without Triton X-induced membrane permeation (Fig. 2*B'*). As expected, anti-H-2K^{k}D^k mAb was positive for cells not treated with Triton X-100. These observations demonstrate that the H-2L^d antigen is present in most of the mutant transformants but is absent from the cell surface. The failure of surface expression of the mutant antigen is due to the mutation in the *H-2L^d* gene disrupting the disulfide bridge in the C2 domain rather than to a defect in the general transport system caused fortuitously by the transformation, because the surface expression of the endogenous H-2K^{k}D^k antigens is normal in all the transformants.

Analysis of the Mutant *H-2L^d* Gene Product by Immunoprecipitation. To investigate certain properties of the mutant H-2L^d antigen further, immunoprecipitation and NaDodSO$_4$/PAGE were carried out. The mutant transformant SMB2-9, which stained most densely in the above experiments, was used. Cells were labeled with [^{35}S]methionine, immunoprecipitated with mAbs, and analyzed by NaDodSO$_4$/PAGE (Fig. 3). Anti-H-2L^d mAb 64.3.7 efficiently precipitated the wild-type H-2L^d antigen, the migration of which is consistent with the previous report (32). The same antibody precipitated a large amount of materials with the same molecular weight from the mutant SMB2-9 but not from untransformed DAP-3 cells (Fig. 3*B*). Specificity of the precipitation was confirmed by the absence of the corresponding band when the lysates of DAP-3, W-12, and SMB2-9 were precipitated by the unrelated mAb 34.2.12 (H-2D^d) or anti-H-2K^{k}D^k mAbs 11.4.1 and 3.83 (Fig. 3 *A* and *D*). The latter antibodies revealed a band

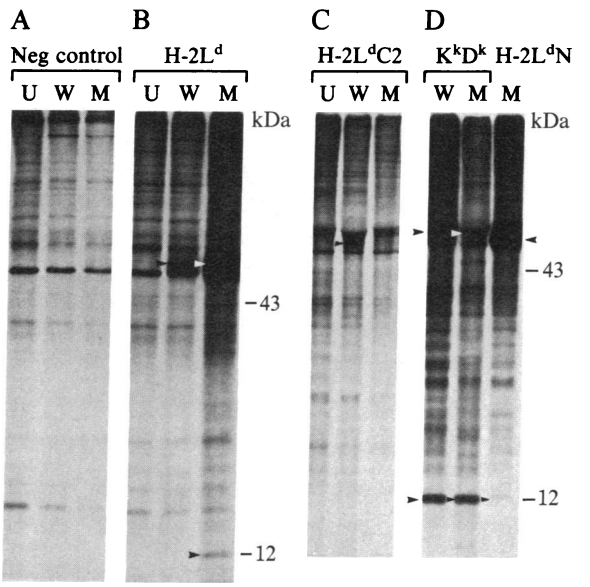

FIG. 3. Immunoprecipitation of ^{35}S-labeled transformants. DAP-3 [untransformed L cells (U)], W-12 [wild-type H-2L^d transformant (W)], and SMB2-9 [mutant H-2L^d transformant (M)] were labeled with [^{35}S]methionine and lysed, and the lysate was immunoprecipitated with unrelated mAb 34.2.12 (Neg control), H-2L^d N-domain-specific mAb 64.3.7 (H-2L^{d}N), H-2L^d C2-domain-specific mAb 28.14.8 (H-2L^{d}C2), or H-2K^{k}D^k-specific mAbs 3.83 and 11.4.1 (K^{k}D^k). Precipitates were analyzed by electrophoresis in NaDodSO$_4$/10% (*A*, *B*) or 11% (*C*, *D*) polyacrylamide gels. Upper and lower arrowheads indicate the positions of the H-2 antigen and of β_2m, respectively.

corresponding to the H-2K^{k}D^k antigens that migrated slightly slower than the protein precipitated by the anti-H-2L^d mAb. Thus, it was concluded that the mutant cells synthesized and accumulated the H-2L^d antigen precipitable by mAb 64.3.7 and that the molecular weight of the mutant antigen is comparable to that of the wild-type antigen. To examine whether the introduced mutation affected the determinants on other domains, the labeled lysates were immunoprecipitated by the C2-domain-specific mAb 28.14.8. This antibody precipitated the wild-type H-2L^d antigen but not the mutant H-2L^d antigen (Fig. 3*C*), indicating the absence of the determinant recognized by this antibody.

We examined if β_2m is associated with the mutant H-2L^d antigen, because there is an implication that β_2m binds to the C2 domain of class I antigens and plays a role in transport of class I antigens. As shown in Fig. 3*B*, there was a weak but distinct band of $M_r \approx 12{,}000$, corresponding to β_2m, coprecipitated from the mutant antigen preparation by mAb 64.3.7. The anti-H-2K^{k}D^k mAbs precipitated the K^{k}D^k products and β_2m, the latter of which showed the same migration pattern as that coprecipitated by the anti-H-2L^d mAb (Fig. 3*D*). The corresponding band was not detected from the wild-type preparation (Fig. 3*B*), presumably because of the well-known weak association of the H-2L^d antigen with β_2m (32) and because of a lower amount of the antigen present in the wild-type than in the mutant transformant. However a faint band corresponding to β_2m was observed in the wild-type H-2L^d immunoprecipitates after prolonged exposure (data not shown). These data suggest that the mutant H-2L^d antigen retains the ability to associate with β_2m and that affinity to β_2m is not considerably different between the mutant and wild-type H-2L^d antigens.

The failure of the mutant transformants to express the H-2L^d antigen on the cell surface was further established by immunoprecipitation from ^{125}I-surface-labeled lysates. mAb 64.3.7, which precipitated the mutant H-2L^d antigen from

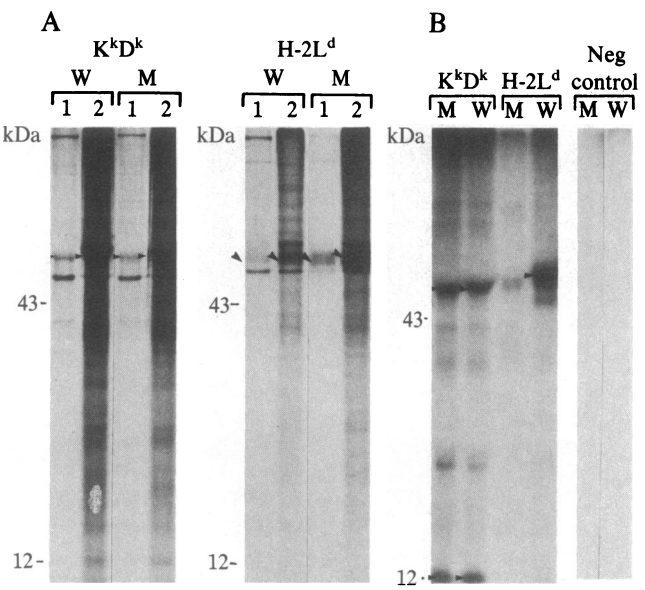

FIG. 4. Subcellular localization of the H-2L^d antigen in wild-type H-2L^d transformant W-12 (W) and mutant H-2L^d transformant SMB2-9 (M). (*A*) Cells labeled with [^{35}S]methionine were fractionated into microsomes (lanes 1) and cytosol (lanes 2). Fractionated materials were immunoprecipitated by anti-H-2K^{k}D^k mAb 11.4.1 (left four lanes) or by anti-H-2L^d mAb 64.3.7 (right four lanes). (*B*) Cells were surface labeled with ^{125}I and immunoprecipitated with anti-H-2K^{k}D^k mAbs 3.83 and 11.4.1, anti-H-2L^d mAb 64.3.7, or unrelated mAb 34.2.12 (Neg control). Precipitates were analyzed by 11% (*A*) or 10% (*B*) NaDodSO$_4$/PAGE. Arrowheads indicate the position of the H-2 antigen.

^{35}S-labeled lysates, was employed. As seen in Fig. 4*B*, anti-H-2K^{k}D^k mAbs 3.83 and 11.4.1 precipitated the cell surface K^{k}D^k antigens from both W-12 and SMB2-9 lysates in similar amounts. A significant amount of the surface H-2L^d antigen was precipitated from W-12 lysates but not from SMB2-9 lysates. There was some size heterogeneity seen in the wild-type H-2L^d antigen, which may reflect a variability of terminal glycosylation or degradation of carbohydrate moieties (33). There was no band in the mutant preparation that corresponds to the major band of the wild-type H-2L^d antigen (Fig. 4*B*). A very faint band that migrated slightly faster than the major band of the wild-type H-2L^d antigen was observed in the immunoprecipitation of SMB2-9. This may be explained either by labeling of intracellular antigens caused by cell damage during iodination or by the presence of an extremely low level of the mutant antigen expressed in the plasma membrane. In either case, the surface expression of the mutant H-2L^d antigen is negligible, consistent with the results of the antibody binding assay and flow cytofluorometric analysis (data not shown). Thus the mutant antigen appears to carry a specific defect that prevents transport of the antigen to the plasma membrane. An alternative possibility is that the mutant H-2L^d antigen is exported to the plasma membrane and is rapidly shed. We regard this as unlikely because of the large amount of the antigen accumulated in the cells. Moreover, concentrated culture supernatants prepared from the mutant transformants did not contain a detectable amount of the H-2L^d antigen as tested by inhibition of antibody binding to the H-2L^d antigen expressed on W-12 cells (data not shown).

Subcellular Localization of the Mutant H-2L^d Antigen. Most membrane proteins, including major histocompatibility antigens, are thought to be inserted into the endoplasmic reticulum simultaneously with their synthesis on ribosomes (34). If this insertion process were affected by the introduced mutation, it would result in the accumulation of the antigen in the cytosol. To examine this possibility, cells labeled with [^{35}S]methionine were fractionated into microsomes and cytosol and then subjected to immunoprecipitation. As shown in Fig. 4*A*, both H-2K^{k}D^k and H-2L^d antigens from W-12 and SMB2-9 were localized exclusively in the microsomes. Precipitation of smaller amounts of the H-2 antigens from the cytosol was not due to low recovery of cytosol proteins, since the total incorporated radioactivity was similarly distributed between the cytosolic and microsomal fractions for either cells tested. Thus, the mutant H-2L^d antigen is evidently integrated into microsomal membrane, as are the wild-type H-2L^d and K^{k}D^k antigens.

DISCUSSION

The present study demonstrates that a single nucleotide substitution resulting in the breakage of a disulfide bridge renders an MHC class I antigen incapable of being expressed on the plasma membrane. The defect cannot be attributed to a deficiency of general transport machinery, as the endogenous H-2K^{k}D^k antigens are transported normally. Nor can it be explained by a secondary defect due to possible glycosylation deficiency (see below). Rather, the defect is a direct consequence of the structural alteration caused by the disruption of the disulfide bridge in the third external domain. The disruption of the disulfide bridge that occurred in the second domain, in contrast, does not prevent cell-surface expression of the antigen, as has been shown before (11). Because of the strict structural constraints imposed on the class I antigens for intracellular transport, one may postulate the presence of a receptor-mediated mechanism for class I antigen transport that guides the movement of newly synthesized antigens from endoplasmic reticulum to the plasma membrane. Receptor-mediated transport has been indicated

for other glycoproteins (see below). In this model, nascent antigens do not passively follow the flow of intracellular membrane movement, but rather they are specifically sorted and directed by a carrier-mediated mechanism involving ligand–receptor-like interactions. On the basis of this concept we propose that the domain structure containing the disulfide bridge serves as a signal structure that is crucial for the interaction with the receptor.

Receptor-mediated transport has been proposed for other membrane or secretory glycoproteins. Evidence is compelling for various hepatocyte glycoproteins (35) and for murine leukemia viral glycoproteins (36), in which each protein is shown to travel through intracellular membrane to the plasma membrane with unique kinetics. An intracellular receptor has been identified for transport of lysosomal hydrolases, which, through binding to the signal structure, mannose 6-phosphate, achieves specific delivery of the enzymes to lysosomal membrane (37).

It has been indicated that β_2m, which noncovalently associates with class I antigens, plays an important role in the transport of the antigen. Cells lacking β_2m are unable to express the antigens on the cell surface, an inability that can be corrected by providing β_2m by cell fusion with normal cells (38, 39). Transport of class I antigen is reported to be facilitated by co-injection of β_2m mRNA with class I mRNA into frog oocytes (40). Further, β_2m appears to associate with the C2 domain of the class I antigen (16). Thus one may assume that β_2m functions at least as a part of the postulated receptor. However, there are arguments against the participation of β_2m in the transport of class I antigens; it was suggested (41) that β_2m is not required for surface expression of the H-2D^b antigen, the C2 domain of which is identical to that of the H-2L^d antigen in the amino acid sequence (42). Furthermore, association of β_2m was not abolished in the mutant described in this paper (Fig. 3). Recently, the protein E3/19K encoded by adenovirus type 2 was shown to bind to human MHC class I antigens and block their intracellular transport without abrogating the association of β_2m (43). These data suggest that the binding of β_2m is not sufficient for the transport of class I antigens. Therefore, it is more likely that another receptor, independent of β_2m, plays a predominant role. Our results, however, do not exclude the possibility of participation of β_2m in the transport.

It is noteworthy that members of the immunoglobulin supergene family, derived presumably from a common ancestral gene, have, without exception, an intrachain disulfide bridge in the domain most proximal to the plasma membrane (44). Its high evolutionary conservation would be best explained by the strict requirement of the disulfide bridge for the receptor-mediated intracellular transport that would allow efficient cell-surface expression of the gene products. It may then follow that all members of the immunoglobulin supergene family share a common receptor for their intracellular transport, through which they reach the plasma membrane.

Interaction of class I antigens with the postulated receptor would be complex. As will be presented elsewhere, carbohydrate moieties of class I antigens also affect intracellular transport, although they are not an absolute requirement, suggesting that interaction with the receptor(s) is mediated by carbohydrate as well. There may even be multiple receptors in various steps of transport, and they may require different parts of the class I molecule for efficient interaction.

Our present work did not clarify the exact location at which the transport is blocked. Because the mutant H-2L^d showed the same apparent molecular weight with the wild-type antigen (Fig. 3*B*), the mutant antigen must be almost fully, if not completely, glycosylated [carbohydrate moieties can be removed from the H-2L^d antigen by site-directed mutagenesis, which results in a significant reduction of molecular weight (unpublished results)]. Thus the mutant antigen may be trapped in Golgi cisternae, where the N-linked glycosylation is completed. Further analysis of the defect in this mutant and others generated by similar methods may help elucidate pathways of intracellular transport of membrane glycoproteins, which are at present not fully understood.

We are grateful to Dr. T. Shiroishi for help in the initial stage of this work, Dr. J. T. Russell for help with subcellular fractionation, and Drs. R. Lieberman and T. Hama for helpful advice. We thank Dr. D. H. Sachs for his generous gift of a monoclonal antibody. Technical assistance provided by Mr. M. Walker and Ms. B. Orrison is gratefully acknowledged.

1. Rask, L., Lindblom, J. B. & Peterson, P. A. (1974) *Nature (London)* **249**, 833–834.
2. Klein, J. (1979) *Science* **203**, 516–521.
3. Coligan, J. E., Kindt, T. J., Uehara, H., Martinko, J. & Nathenson, S. G. (1981) *Nature (London)* **291**, 35–39.
4. Nathenson, S. G., Uehara, H., Ewenstein, B. M., Kindt, T. J. & Coligan, J. E. (1981) *Annu. Rev. Biochem.* **50**, 1025–1052.
5. Orr, H. T., Lopez de Castro, J. A., Parham, P., Ploegh, H. L. & Strominger, J. L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4395–4399.
6. Tragardh, L., Rask, L., Wiman, K., Fohlman, J. & Peterson, P. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1129–1133.
7. Moore, K. W., Sher, B. T., Sun, Y. H., Eakle, K. A. & Hood, L. (1982) *Science* **215**, 679–682.
8. Evans, G. A., Margulies, D. H., Ozato, K., Camerini-Otero, R. D. & Seidman, J. G. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1994–1998.
9. Reyes, A. A., Schold, M., Itakura, K. & Wallace, R. B. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3270–3274.
10. Kvist, S., Roberts, L. & Dobberstein, B. (1983) *EMBO J.* **2**, 245–253.
11. Shiroishi, T., Evans, G. A., Appella, E. & Ozato, K. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7544–7548.
12. Shiroishi, T., Evans, G. A., Appella, E. & Ozato, K. (1985) *J. Immunol.* **134**, 623–629.
13. Ozato, K., Evans, G. A., Shykind, B., Margulies, D. H. & Seidman, J. G. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2040–2043.
14. Reiss, C. S., Evans, G. A., Margulies, D. H., Seidman, J. G. & Burakoff, S. J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2709–2712.
15. Arnold, B., Burgert, H.-G., Hamann, U., Hammerling, G., Kees, U. & Kvist, S. (1984) *Cell* **38**, 79–87.
16. Yokoyama, K. & Nathenson, S. G. (1983) *J. Immunol.* **130**, 1419–1425.
17. Owen, M. J., Kissonerghis, A.-M. & Lodish, H. F. (1980) *J. Biol. Chem.* **255**, 9678–9684.
18. Sege, K., Rask, L. & Peterson, P. A. (1981) *Biochemistry* **20**, 4523–4530.
19. Ito, H., Ike, Y., Ikuta, S. & Itakura, K. (1982) *Nucleic Acids Res.* **10**, 1755–1769.
20. Zoller, M. J. & Smith, M. (1983) *Methods Enzymol.* **100**, 468–500.
21. Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. & Roe, D. A. (1980) *J. Mol. Biol.* **143**, 161–178.
22. Wigler, M., Pellicer, S., Silverstein, R., Axel, G., Urlaub, G. & Chasin, L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1373–1376.
23. Camerini-Otero, R. D. & Zasloff, M. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5079–5083.
24. Enquist, L. W., Vande Woude, G. F., Wagner, M., Smiley, J. R. & Summers, W. C. (1979) *Gene* **7**, 335–342.
25. Szybalski, W., Szybalski, E. H. & Ragni, G. (1962) *Natl. Cancer Inst. Monogr.* **7**, 75–88.
26. Ben-Barak, Y., Russell, J. T., Whitnall, M. H., Ozato, K. & Gainer, H. (1985) *J. Neurosci.* **5**, 81–97.
27. Hubbard, A. L. & Cohn, Z. A. (1975) *J. Cell Biol.* **64**, 438–460.
28. Kessler, S. (1975) *J. Immunol.* **115**, 1617–1624.
29. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
30. Murre, C., Choi, E., Weis, J., Seidman, J. G., Ozato, K., Liu, L., Burakoff, S. J. & Reiss, C. S. (1984) *J. Exp. Med.* **160**, 167–178.
31. Hansen, T., Ozato, K. & Sachs, D. H. (1983) *Adv. Immunol.* **34**, 39–69.
32. Margulies, D. H., Evans, G. A., Ozato, K., Camerini-Otero, R. D., Tanaka, K., Appella, E. & Seidman, J. G. (1983) *J. Immunol.* **130**, 463–470.
33. Jones, P. P. (1977) *J. Exp. Med.* **146**, 1261–1272.
34. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67**, 835–851.
35. Fitting, T. & Kabat, D. (1982) *J. Biol. Chem.* **257**, 14011–14017.
36. Lodish, H. F., Kong, N., Snider, M. & Strous, G. J. A. M. (1983) *Nature (London)* **304**, 80–83.
37. Sly, W. S. & Fischer, H. D. (1982) *J. Cell. Biochem.* **18**, 67–85.
38. Hyman, R. & Stallings, V. (1977) *Immunogenetics (NY)* **4**, 171–181.
39. Fellous, M., Kamoun, M., Wiels, J., Dausset, J., Clements, G., Zeuthen, J. & Klein, G. (1977) *Immunogenetics (NY)* **5**, 423–436.
40. Severinsson, L. & Peterson, P. A. (1984) *J. Cell Biol.* **99**, 226–232.
41. Potter, T. A., Boyer, C., Verhulst, A.-M. S., Golstein, P. & Rajan, T. V. (1984) *J. Exp. Med.* **160**, 317–322.
42. Maloy, W. L. & Coligan, J. E. (1982) *Immunogenetics (NY)* **16**, 11–22.
43. Burgert, H.-G. & Kvist, S. (1985) *Cell* **41**, 987–997.
44. Hood, L., Kronenberg, M. & Hunkapiller, T. (1985) *Cell* **40**, 225–229.