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Immunology

Intracellular class II HLA antigens are accessible to transferrin–neuraminidase conjugates internalized by receptor-mediated endocytosis

(invariant chain/exocytosis/antigen processing)

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ABSTRACT **Newly synthesized class II HLA antigens being transported to the surface of human B-lymphoblastoid cell lines (B-LCL) interact with transferrin–neuraminidase conjugates internalized by means of receptor-mediated endocytosis. Class II antigens, isolated from [^{35}S]methionine-labeled B-LCL after incubation with the conjugates at 37°C, showed extensive desialylation of associated invariant chain and detectable loss of β-subunit sialic acid on analysis by two-dimensional gel electrophoresis. An equal amount of unconjugated neuraminidase had no effect, and desialylation of class II antigen components was blocked when access of transferrin–neuraminidase conjugates to the B-LCL transferrin receptors was competitively inhibited by the addition of excess iron-saturated transferrin. The conjugates were shown to cycle through the cells in the same way as unconjugated transferrin, being first internalized and then rapidly secreted in an undegraded form. The data suggest that the exocytic pathway taken by class II antigens intersects the route followed by recycling transferrin receptors and that the interaction occurs prior to the dissociation of the invariant chain from the class II antigen complex. Similar intracellular interactions between class II molecules and foreign proteins internalized by antigen-presenting cells may be important in class II antigen-restricted recognition by helper T lymphocytes.**

Intracellular class II major histocompatibility complex (MHC) antigens of humans and mice are noncovalently associated with a nonpolymorphic glycoprotein, the invariant chain (I chain) (1–5). The interaction between the α- and β-subunits of the class II heterodimer and the I chain is initiated soon after synthesis in the rough endoplasmic reticulum and persists during transport through the Golgi apparatus (2–4, 6). Evidence for this continued association comes from biosynthetic experiments in which human B-lymphoblastoid cell lines (B-LCL) are pulsed for a short period with [^{35}S]methionine and then chased for various lengths of time with unlabeled methionine. Analysis by two-dimensional gel electrophoresis of class II antigens and associated I chain isolated from such cells has shown that sialic acid addition to the N- and O-linked oligosaccharides of the I chain is detectable as early as 30 min after the initiation of the chase (4). Sialic acid addition is a late or *trans* Golgi event (7). After a prolonged chase period, ≈4 hr, the I chain can no longer be found associated with class II molecules (4). This observation, coupled with the inability of most workers to detect I chain on the cell surface (8, 9), has led to the supposition that the I chain dissociates from the class II αβ complex prior to the expression of the latter on the plasma membrane.

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The apparent intracellular dissociation of the I chain from mature class II antigens is at least superficially analogous to the fate of certain ligand–receptor complexes following receptor-mediated endocytosis. Low density lipoproteins (LDL), epidermal growth factor (EGF), and iron-saturated transferrin (FeTf) are internalized by means of clathrin-coated pits into intracellular vesicles called receptosomes (10) or endosomes (11) following binding to specific receptors on the cell surface (12–17). Dissociation of EGF and LDL from their specific receptors and iron from FeTf occurs intracellularly in the endosome or, possibly, in a more specialized compartment known as CURL ("compartment for uncoupling of receptor and ligand;" ref. 18). Dissociation is often attributed to the acidity of these intracellular vesicles, which are maintained at a pH of ≈5 (19). Following dissociation the receptors are recycled to the cell surface, with apotransferrin still associated in the case of the transferrin receptor (14–17). EGF and LDL are deposited in the lysosomes (12, 13).

Strengthening the analogy of ligand–receptor dissociation with class II antigen–I chain dissociation is the finding that the latter is inhibited by the carboxylic ionophore monensin (20). Monensin inhibits exocytosis and endocytosis (21), probably as a result of its ability to neutralize the acidic vesicles involved in intracellular transport. The inhibition of class II antigen–I chain dissociation by monensin may indicate a role for acidification in this process and raises the theoretical possibility that it occurs in the same intracellular compartment as ligand–receptor dissociation. Following dissociation from the I chain, mature class II molecules might then follow the same route to the cell surface as recycling receptors. In fact, all newly synthesized proteins destined for the plasma membrane may follow the same pathway.

This communication describes experiments that demonstrate that neuraminidase conjugated to FeTf and internalized by means of the transferrin receptor can remove sialic acid from intracellular I chain associated with class II HLA antigens. These data strongly suggest that the endocytic pathway followed by transferrin receptors intersects the exocytic route taken by newly synthesized class II antigens prior to the dissociation of the I chain. The implications of this for class II antigen function are discussed.

MATERIALS AND METHODS

Monoclonal Antibodies. MRC Ox20 (Ox20), a rat IgG monoclonal antibody to mouse κ chain, was a generous gift from Neil Barclay (MRC Cellular Immunology Unit, Ox-

Abbreviations: B-LCL, B-lymphoblastoid cell line(s); EGF, epidermal growth factor; FeTf, iron-saturated transferrin; I chain, invariant chain; LDL, low density lipoprotein(s); MHC, major histocompatibility complex; SPDP, *N*-succinimidyl 3-(2-pyridyldithio)propionate; mU, milliunit(s).

ford). DA6.147, an IgG1 monoclonal antibody to the α-subunit of HLA-DR antigens (22), was donated by Veronica van Heyningen (MRC Clinical and Population Genetics Unit, Edinburgh, U.K.). XD5.A11, an IgG monoclonal antibody to the β-subunit of HLA-DR, -DQ, and -DP antigens, was generated in this laboratory (20).

Isolation and Analysis of Radiolabeled Class II Antigens. The B-LCL Swei (23) was grown, radiolabeled with [^{35}S]methionine, and extracted with Triton X-100 in 0.15 M NaCl/0.01 M Tris, pH 7.4 as described (4). Extracts were stored at −70°C until use.

Class II antigens were isolated from the extracts by a solid-phase immunoabsorbent technique. To prepare the immunoabsorbent, Ox20 anti-mouse κ-chain IgG [50 μl, 50 μg/ml in phosphate-buffered saline (P_i/NaCl)] was added to the wells of flexible 96-well microliter plates (Dynatech, Alexandria, VA). After overnight incubation at 4°C, the antibody was removed, the wells were washed four times with water, and DA6.147 or XD5.A11 monoclonal antibody (tissue culture supernatants, 100 μl) was added to the wells. After 60 min at room temperature, the monoclonal antibody supernatants were removed, the wells were washed four times with water, and residual protein binding sites were blocked by the addition of 1% (wt/vol) bovine serum albumin in P_i/NaCl for 10 min.

Extracts of [^{35}S]methionine-labeled Swei cells were incubated with normal rabbit serum (10 μl/100 μl of extract) and formalin-fixed *Staphylococcus aureus* (Cowan I strain) to remove nonspecific contaminating material as described (4). Aliquots (100–200 μl, 10–20 × 10^6 cpm) were then added to immunoabsorbent wells and incubated for 60 min at room temperature. The labeled extract was removed and the wells were washed five times with water. Antibody-bound radiolabeled antigens were eluted in 50 μl of a solution containing 9.5 M urea (Ultra-Pure, Schwartz-Mann), 2% (wt/vol) Triton X-100, 5% (wt/vol) 2-mercaptoethanol, and 2% (vol/vol) Ampholines (Biolyte 3/10, Bio-Rad). They were then analyzed on two-dimensional polyacrylamide gels by using nonequilibrium pH gradient electrophoresis (24) in the first dimension and electrophoresis in $NaDodSO_4$ in the second dimension as described (4).

As a control in some experiments radiolabeled class II antigens were treated with neuraminidase in the immunoabsorbent wells prior to elution. Fifty microliters of 0.1 M NaCl/0.05 M sodium acetate/0.009 M $CaCl_2$, pH 5.5, was added to the well, followed by addition of 10 milliunits (mU) of *Vibrio Cholerae* neuraminidase (Calbiochem). After 60 min at 37°C, the enzyme was removed, the wells were washed with water, and bound antigen was eluted as described above.

Preparation of FeTf–Neuraminidase Conjugates. Conjugates were prepared by using the cross-linking reagent *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP; Sigma; ref. 25). Human transferrin (Sigma) was first saturated with iron by incubation with ferric ammonium citrate as described (26). For derivatization, SPDP (20 μl, 20 mM in anhydrous ethanol) was added to 0.5 ml of a solution of FeTf (10 mg/ml) in 0.15 M NaCl/0.05 M sodium phosphate, pH 7.5. After 30 min at room temperature, excess unreacted SPDP was removed by overnight dialysis againt P_i/NaCl at 4°C. Spectrophotometric analysis (25) indicated an SPDP:FeTf substitution ratio of 6:1.

For derivatization of neuraminidase, supplied in solution at 1 unit/ml at pH 5.5, the pH of 2 ml of enzyme was first adjusted by the addition of 1 M sodium phosphate at pH 7.5 (0.5 ml). SPDP (52 μl, 20 mM in ethanol) was then added and the reaction was allowed to proceed for 30 min at room temperature. This ratio of SPDP to neuraminidase was determined to cause a detectable but <5% reduction in neuraminidase activity in preliminary experiments. The reaction was stopped by the addition of 0.1 M glycine (50 μl). After addition of 40 μl of bovine serum albumin (100 μg/ml) as a carrier, precipitated calcium phosphate was removed by centrifugation and excess SPDP was removed by overnight dialysis against P_i/NaCl. The bovine serum albumin used was alkylated by reaction with a 30-fold molar excess of iodoacetamide to block any free sulfhydryl groups.

For cross-linking, FeTf–SPDP was first reduced to generate free sulfhydryl groups. Dithiothreitol (75 μl, 0.1 M) was added to an equal volume of FeTf–SPDP. After 20 min at room temperature, the reduced FeTf derivative was separated by gel filtration on a column of Sephadex G-50 (5-ml bed) equilibrated in P_i/NaCl. The FeTf derivative (0.5 mg) was added to the neuraminidase–SPDP conjugate (originally derived from 2 units) and cross-linking was allowed to proceed for 24 hr at 4°C. Complexes were separated from unconjugated reactants by gel filtration on Sephacryl S-300 (12 mm × 50 cm), equilibrated in P_i/NaCl containing alkylated bovine serum albumin (0.1 mg/ml) as a carrier. Fractions were assayed for neuraminidase activity by using bovine submaxillary mucin (Sigma) as a substrate and determining released sialic acid by the method of Warren (27), and the major peak, corresponding to an apparent molecular mass of ≈150 kDa by comparison to standard globular proteins, was pooled and stored at −70°C. This molecular mass closely corresponds to the anticipated size of a heterodimer of FeTf (78 kDa) and neuraminidase (apparently 65 kDa by calibration of the Sephacryl S-300 column). The pooled conjugate had a measured activity of 28 mU/ml, and 82% of the neuraminidase activity could be removed from solution by incubation with rabbit anti-human transferrin antibody immobilized on formalin-fixed *S. aureus*, Cowan I strain (data not shown).

For recycling experiments FeTf–neuraminidase conjugates were prepared by using FeTf derivatized with SPDP and subsequently labeled with ^{125}I (^{125}I-FeTf–SPDP) by the chloramine-T technique as described (26). ^{125}I-FeTf–SPDP (100 μg, 3.9 × 10^6 cpm/μg) was reduced and incubated with neuraminidase–SPDP (0.5 unit) to generate cross-linked material as described above. ^{125}I-FeTf–neuraminidase conjugates were again isolated by gel filtration on a column of Sephacryl S-300.

Recycling Experiments with ^{125}I-FeTf–Neuraminidase Conjugates. Swei cells (18 × 10^6) were incubated for 5 min at 37°C in Iscove's/Dulbecco's modified medium (1.8 ml) containing bovine serum albumin (experimental) or FeTf (control) at 2 mg/ml. ^{125}I-FeTf–neuraminidase conjugate (83 × 10^6 cpm) was then added to each tube and the incubation was continued at 37°C for a further 15 min. The cells were then chilled by the addition of ice-cold P_i/NaCl (10 ml), centrifuged (1000 × *g*, 10 min), and washed twice with ice-cold P_i/NaCl. They were then suspended in medium (3.5 ml) that was prewarmed to 37°C and contained FeTf (0.2 mg/ml). Duplicate samples (0.2 ml) were removed at intervals during incubation at 37°C and added to tubes containing 20 mM iodoacetamide in P_i/NaCl (20 μl) to prevent reduction of the conjugate. The cells were pelleted by centrifugation at 2000 × *g* for 30 sec, and supernatant and cells were assayed for radioactivity separately in an LKB model 1272 Clinigamma counter. Then the labeled material in the supernatant was precipitated with acetone and subjected to polyacrylamide gel electrophoresis in $NaDodSO_4$ (28). Gels were dried and analyzed by autoradiography at −70°C using XAR-5 film (Kodak) and an intensifying screen (Lightening Plus, Dupont).

RESULTS

FeTf–Neuraminidase Conjugates Interact with Intracellular Class II Antigens. In addition to two N-linked glycans, which can be processed to the complex form (4), processed human I chain (Ip; ref. 4) possesses O-linked oligosaccharide (4, 20).

On the basis of their differential susceptibility to endo-α-*N*-acetylgalactosaminidase in denaturing versus nondenaturing conditions, at least two O-linked glycans appear to be present (20). The increment in molecular mass caused by the addition of O-linked carbohydrate can be detected by gel electrophoresis in $NaDodSO_4$. The loss of I chain sialic acid is readily observed on two-dimensional gel analysis by a cathodal shift of desialylated I chain, causing the appearance of doublets of I chain spots (I and Ip) with identical mobility in the nonequilibrium pH gradient electrophoresis dimension (4). This method was selected to determine the effects of FeTf–neuraminidase conjugates on intracellular class II HLA antigens.

Swei cells (2×10^6), labeled with [^{35}S]methionine for 4 hr and suspended in RPMI 1640 medium with 10% fetal bovine serum (0.5 ml), were incubated with FeTf–neuraminidase conjugate (5 mU) for 90 min at 37°C. The cells were then washed and extracted with Triton X-100. A two-dimensional gel of class II HLA antigens isolated from the extract with the monoclonal anti-class II antibody DA6.147 is shown in Fig. 1*E*. Class II antigens isolated from control cells incubated identically but in the absence of conjugate are shown in Fig. 1*A*. The removal of I chain sialic acid by the conjugated neuraminidase can clearly be seen, reflected in the cathodal shift of the processed I chain indicated by the vertical arrows.

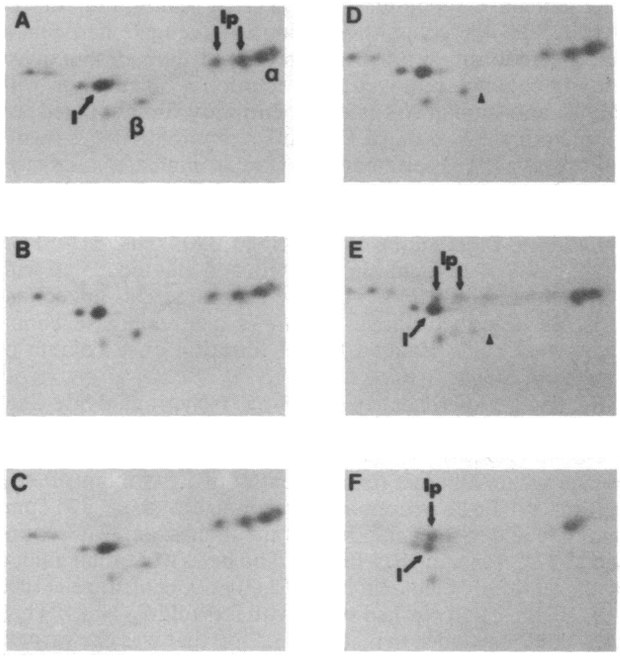

FIG. 1. Effect of transferrin–neuraminidase conjugates on intracellular class II HLA antigens. HLA-DR antigens were isolated from detergent extracts of [^{35}S]methionine-labeled Swei cells by using the monoclonal antibody DA6.147 and were analyzed by two-dimensional gel electrophoresis. Electrophoresis (nonequilibrium pH gradient electrophoresis) was from right to left (acidic to basic) in the first dimension and from top to bottom in 10% acrylamide/$NaDodSO_4$ gels in the second dimension. α, β, and I chains are indicated. Ip is the processed form of the I chain containing O-linked and complex N-linked oligosaccharides. Following labeling and prior to solubilization, the cells were incubated for 90 min at 37°C as follows: in control medium (*A*); in medium containing FeTf (300 μg/ml) (*B*); in medium containing *V. cholerae* neuraminidase (2.5 mU/ml) (*C*); in medium containing FeTf–neuraminidase conjugate (2.5 mU/ml) plus FeTf (300 μg/ml) (*D*); in medium containing FeTf–neuraminidase conjugate (2.5 mU/ml) alone (*E*). (*F*) HLA-DR antigens isolated from control cells treated as in *A* but incubated with *V. cholerae* neuraminidase (1.7 units/ml) after isolation. Arrowheads (*D* and *E*) indicate the position of the most acidic β-subunit, present in panels *A–D* but absent from *E* and *F*.

Although poorly reproduced in the photograph, the most acidic β-subunit spot (indicated by an arrowhead in Fig. 1*D*) is absent in cells exposed to the conjugate (Fig. 1*E*), indicating that β-subunit sialic acid may also be removed by FeTf–neuraminidase conjugates. The product of a limit-digestion with neuraminidase, obtained by treating DA6.147-bound antigen in the immunoabsorbent well prior to elution with sample buffer, is shown in Fig. 1*F*.

Similar results have been obtained by using 247_{HSB}, a rabbit anti-class II HLA antibody (4), and XD5.A11 (4), an antibody to class II β-subunits (data not shown). Neither of these antibodies, nor DA6.147, exhibits any reactivity with free I chain. Consequently, the processed and unprocessed forms of I chain apparent in Fig. 1 are associated with the class II αβ heterodimer and therefore presumably are derived from inside the cell. To further substantiate the intracellular site of the interaction of the I chain with the FeTf-conjugated neuraminidase, two additional control experiments were performed. First, [^{35}S]methionine-labeled Swei cells were incubated with unconjugated neuraminidase. No loss of I chain sialic acid could be detected (Fig. 1*C*). Second, Swei cells were incubated with FeTf–neuraminidase conjugate in the presence of a large excess of unconjugated FeTf (300 μg/ml) to competitively block access of the conjugate to the transferrin receptor. Again no loss of sialic acid was detectable (Fig. 1*D*), indicating that the desialylation of the I chain observed in Fig. 1*E* was receptor dependent. The addition of unconjugated FeTf alone had no effect on the electrophoretic pattern (Fig. 1*B*).

These observations argue that the route taken by FeTf–neuraminidase conjugates and the exocytic pathway followed by class II antigens intersect intracellularly, prior to the dissociation of the I chain.

FeTf–Neuraminidase Conjugates Recycle Normally. Transferrin, after binding to its receptor, is internalized by the cell and, following the loss of associated iron, is recycled to the cell surface and released (14–17). To substantiate the argument that the class II antigen exocytic route intersects the transferrin recycling pathway, experiments were performed to determine whether FeTf–neuraminidase conjugates recycle in the same way as unconjugated FeTf.

In the experiment shown in Fig. 2, Swei cells were preloaded with ^{125}I-FeTf–neuraminidase conjugate at 37°C and, after washing, the release of label was followed during further incubation at 37°C. Release of cell-associated cpm was 85% complete after 60 min, and the half-time of release was ≈7 min. These numbers are comparable to literature values for the release of unconjugated labeled transferrin from a number of cell types (14, 15, 29).

The total label accumulated in the supernatant by 60 min in the experiment shown in Fig. 2 was 93% precipitable by trichloroacetic acid. This indicates that the released material was not substantially degraded. To determine whether any degradation occurred prior to the release of the conjugate, the supernatants were precipitated with acetone and analyzed by polyacrylamide gel electrophoresis in $NaDodSO_4$ followed by autoradiography. The results of this analysis are presented in Fig. 3. The major product released by Swei cells that can be detected on electrophoresis under nonreducing conditions (Fig. 3*A*) is a band with an apparent molecular mass of 140 kDa. A small amount of a larger molecule that barely enters the gel and a lower band with an apparent molecular mass of 68 kDa are also detectable. All three bands are present in the original labeled conjugate at similar relative intensities to those apparent in the recycled material shown in Fig. 3*A* (data not shown). The 140-kDa band closely corresponds to the expected molecular mass of an FeTf–neuraminidase dimer, based on its elution position from Sephacryl S-300 during purification. The higher molecular mass band presumably consists of more extensively cross-linked material. The lower

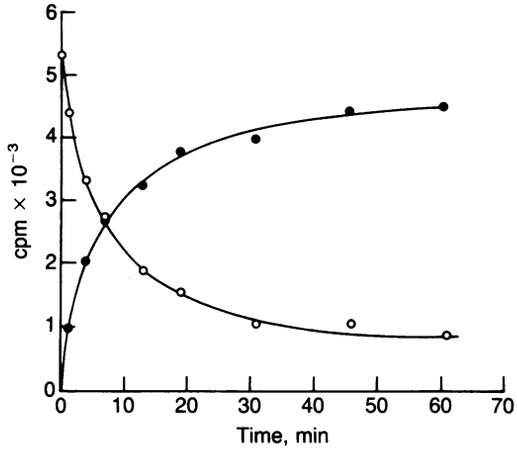

FIG. 2. Transferrin–neuraminidase conjugate cycle through B-LCL. Swei cells were preloaded with ^{125}I-FeTf–neuraminidase conjugates for 15 min at 37°C. After washing at 4°C, they were resuspended at 37°C in medium containing FeTf (0.2 mg/ml). Aliquots of the suspension were removed during subsequent incubation at 37°C and centrifuged. Cell-associated cpm (○) and supernatant cpm (●) are shown.

band comigrates with transferrin run under nonreducing conditions. All three labeled species appear to be released equally well by the cells, indicating that FeTf–neuraminidase conjugates cycle through the cell in an analogous fashion to unconjugated FeTf. Fig. 3*B* shows that the large majority of

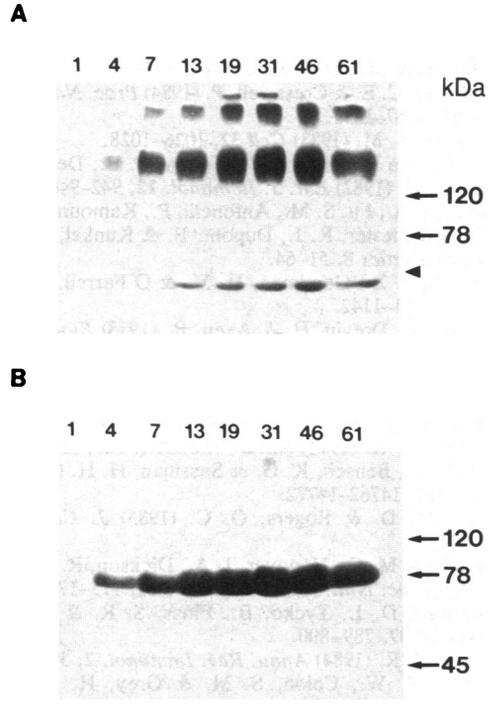

FIG. 3. Transferrin–neuraminidase conjugates are undegraded during cycling through B-LCL. ^{125}I-FeTf–neuraminidase conjugates released into the supernatant after endocytosis by the B-LCL (Fig. 2) were precipitated with acetone and subjected to electrophoresis on 10% acrylamide/NaDodSO$_4$ gels under nonreducing conditions (*A*) or reducing conditions (*B*). The numbers above each lane indicate the time (minutes) of harvest following suspension at 37°C (see legend to Fig. 2). Molecular mass markers β-galactosidase (120 kDa), transferrin (78 kDa), and ovalbumin (45 kDa) were electrophoresed under reducing conditions. The arrowhead in *A* indicates the migration position of unreduced transferrin.

labeled material is present in a single band comigrating with transferrin upon electrophoresis under reducing conditions. Since only the transferrin used in preparing the conjugatewas labeled with ^{125}I, neuraminidase is not visible in this autoradiograph.

DISCUSSION

The results presented in this paper clearly demonstrate an intracellular interaction between transferrin–neuraminidase conjugates, internalized by endocytosis, and newly synthesized class II HLA antigens in the process of exocytosis. The interaction can be blocked by addition of excess FeTf, and the conjugates are cycled through the cell in the same manner as unconjugated transferrin. These observations argue that the interaction of the conjugates and class II molecules occurs as a result of a natural merging of the transferrin recycling pathway and the class II antigen exocytic pathway and is not a result of abnormal handling of the conjugates by the cell.

The precise stage in the exocytosis of class II antigens at which union with the recycling pathway occurs is difficult to determine from the experiments reported here. The primary evidence for the interaction is the observed desialylation of class II antigen-associated I chain. Therefore, the merger must occur prior to the dissociation of the I chain. It could, however, happen either before or after the addition of sialic acid to the class II molecules. If the exocytic and recycling pathways merge prior to the addition of sialic acid, the FeTf–neuraminidase conjugates and class II antigens are presumably cotransported to a compartment containing sialyl transferases. On arrival in this compartment, competition would ensue between transferases adding sialic acid to the complex N-linked glycans and O-linked glycans and the conjugated neuraminidase simultaneously removing them. Favoring this possibility is the reported observation that desialylated transferrin receptors can be resialylated by the K562 human erythroleukemic cell line during the recycling process (30). In a number of experiments, in which the amounts of FeTf–neuraminidase conjugate added to B-LCL and times of incubation were varied, complete desialylation of the I chain, such as that seen in Fig. 1*F*, has never been observed (data not shown). This is also consistent with such a scheme.

Electron microscopic and fluorescence microscopic studies of transferrin receptor recycling have shown that, following endocytosis, transferrin can be detected in the vicinity of the Golgi apparatus, specifically the *trans* region (29, 31, 32). It seems likely that the junction of the recycling pathway and the class II antigen exocytic route is in this region of the cell. Maxfield and co-workers have determined that, in Chinese hamster ovary cells, transferrin can be localized in this region in a compartment with a pH of ≈6.4 compared to the more acidic pH of endosomes (32). Such a compartment is an attractive candidate for the junction of the exocytic and recycling pathways and a plausible candidate for the position of the monensin block in class II antigen transport and also transferrin receptor recycling. It has been shown that class II HLA antigens accumulate in B-LCL cultured in 1–10 μM monensin, failing to arrive at the plasma membrane (20). Sussman and co-workers (29) have shown that monensin blocks recycling by causing transferrin accumulation in dilated multivesicular bodies in the *trans* Golgi region. The acquisition of some sialic acid by the α- and β-subunits of class II antigens in monensin-treated B-LCL (20) suggests that a complete block in transport of class II antigens does not occur before the addition of sialic acid. However, as noted (20), the N-linked oligosaccharides of the class II antigen-associated I chain remain almost totally unprocessed in monensin-treated B-LCL, implying that a pH-dependent

conformational change in the I chain or associated components may be required for the conversion of its N-linked oligosaccharides to the complex form. Unfortunately, the lack of sialic acid addition to the I chain makes it difficult to determine whether an intracellular encounter occurs between FeTf–neuraminidase conjugates and class II antigens in monensin-treated B-LCL.

Class II HLA antigens provide a useful system for examining the interactions of the exocytic and endocytic pathways using the neuraminidase conjugate approach, mainly because of the dramatic effect of sialic acid loss on the electrophoretic properties of the I chain. It is clear, however, that other glycoproteins destined for the plasma membrane or for secretion are likely to follow the same route as class II molecules and to similarly encounter the recycling pathway. However, such intracellular encounters may have greater functional relevance in the case of class II MHC antigens. Class II HLA molecules, and the homologous Ia antigens of the mouse, are critically involved in the recognition by helper T lymphocytes of foreign antigens presented by macrophages, B-lymphoblasts, and other cell types (reviewed in ref. 33). A large body of evidence suggests that protein antigens must be internalized by the antigen-presenting cell, processed in some way, and reexpressed on the cell surface, perhaps in association with class II MHC molecules, before T-cell recognition can occur (33, 34). Antigen presentation to T cells can be inhibited by agents that interfere with lysosomal function and intracellular transport, including monensin (34). Many workers have concluded that proteolysis is a key component in the processing of foreign antigens. However, some recent studies indicate that denaturation, with exposure of hydrophobic regions of the protein, may be a critical factor that is facilitated by proteolysis for the majority of antigens (35).

The intracellular interaction of class II HLA antigens with the recycling pathway of transferrin receptors is clearly not the same as the interaction that occurs between class II molecules and foreign antigens proteolytically cleaved following internalization. The latter are presumably lysosomally derived, whereas the transferrin receptor does not encounter the lysosome during recycling. However, the experiments described here highlight the possibility that critical interactions of class II MHC molecules with foreign antigens may occur inside the antigen-presenting cell, prior to cell surface expression of both components. Conditions inside an intracellular "interaction compartment" could be significantly different from those at the cell surface, in terms of, for example, concentration of components, pH, membrane fluidity, and absence of serum. Thus, interactions between class II molecules and foreign antigens that might occur with only a low probability at the cell surface could be facilitated. Support for this model comes from the observation that surface IgM of tetanus toxoid-specific B-LCL acts as a receptor for the toxoid, promoting its internalization and enhancing presentation of the toxoid to autologous T-helper cells in a class II-restricted fashion (36). In a separate study the intracellular colocalization of class II HLA antigens and internalized surface immunoglobulin in B-LCL has been suggested by immunofluorescence data (37). Thus, antigens may be directed to an intracellular compartment where interactions with newly synthesized class II molecules are enhanced.

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1. Charron, D. J. & McDevitt, H. O. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 6567–6571.
2. Owen, M. J., Kissonerghis, A.-M., Lodish, H. F. & Crumpton, M. J. (1981) *J. Biol. Chem.* **256,** 8987–8993.
3. Kvist, S., Wiman, K., Claesson, L., Peterson, P. A. & Dobberstein, B. (1982) *Cell* **29,** 61–69.
4. Machamer, C. E. & Cresswell, P. (1982) *J. Immunol.* **129,** 2564–2569.
5. Jones, P. P., Murphy, D. B., Hewgill, D. & McDevitt, H. O. (1979) *Mol. Immunol.* **16,** 51–60.
6. Claesson, L. & Peterson, P. A. (1983) *Biochemistry* **22,** 3206–3213.
7. Rothman, J. E. (1981) *Science* **213,** 1212–1219.
8. Quaranta, V., Madjic, O., Stingl, G., Liska, K., Honigsmann, H. & Knapp, W. (1984) *J. Immunol.* **132,** 1900–1905.
9. Accolla, R. S., Carra, G., Buchegger, F., Carrel, S. & Mach, J.-P. (1985) *J. Immunol.* **134,** 3265–3271.
10. Willingham, M. C. & Pastan, I. (1980) *Cell* **21,** 67–77.
11. Helenius, A., Marsh, M. & White, J. (1980) *Trends Biochem. Sci.* **5,** 104–106.
12. Goldstein, J. L., Anderson, R. G. W. & Brown, M. S. (1979) *Nature (London)* **279,** 679–685.
13. Willingham, M. C. & Pastan, I. (1982) *J. Cell Biol.* **94,** 207–212.
14. Karin, M. & Mintz, M. (1981) *J. Biol. Chem.* **256,** 3245–3252.
15. Octave, J.-N., Schneider, Y.-J., Crichter, R. R. & Trouet, A. (1981) *Eur. J. Biochem.* **115,** 611–618.
16. Dautry-Varsat, A., Ciechanover, A. & Lodish, H. F. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2258–2262.
17. Klausner, R. D., Ashwell, G., Van Renswoude, J., Harford, J. B. & Bridges, K. R. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2263–2266.
18. Gueze, H. J., Slot, J. W., Strous, G. J. A. M., Lodish, H. F. & Schwarz, A. L. (1983) *Cell* **32,** 277–287.
19. Tycko, B. & Maxfield, F. (1982) *Cell* **28,** 643–651.
20. Machamer, C. E. & Cresswell, P. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1287–1291.
21. Tartakoff, A. M. (1983) *Cell* **32,** 1026–1028.
22. Guy, K., van Heyningen, V., Cohen, B. B., Deane, D. L. & Steel, C. M. (1982) *Eur. J. Immunol.* **12,** 942–948.
23. Hansen, J. A., Fu, S. M., Antonelli, P., Kamoun, M., Hurley, J. N., Winchester, R. J., Dupont, B. & Kunkel, H. G. (1979) *Immunogenetics* **8,** 51–64.
24. O'Farrell, P. Z., Goodman, H. M. & O'Farrell, P. H. (1977) *Cell* **12,** 1133–1142.
25. Carlsson, J., Drevin, H. & Axen, R. (1978) *Biochem. J.* **173,** 723–737.
26. Larrick, J. W. & Cresswell, P. (1979) *Biochim. Biophys. Acta* **583,** 483–490.
27. Warren, L. (1959) *J. Biol. Chem.* **234,** 1971–1975.
28. Laemmli, A. K. (1970) *Nature (London)* **227,** 680–685.
29. Stein, B. S., Bensch, K. G. & Sussman, H. H. (1984) *J. Biol. Chem.* **259,** 14762–14772.
30. Snider, M. D. & Rogers, O. C. (1985) *J. Cell Biol.* **100,** 826–834.
31. Willingham, M. C., Hanover, J. A., Dickson, R. B. & Pastan, I. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 175–179.
32. Yamashiro, D. J., Tycko, B., Fluss, S. R. & Maxfield, F. (1984) *Cell* **37,** 789–800.
33. Unanue, E. R. (1984) *Annu. Rev. Immunol.* **2,** 395–428.
34. Chesnut, R. W., Colon, S. M. & Grey, H. M. (1982) *J. Immunol.* **129,** 2382–2388.
35. Streicher, H. Z., Berkower, I. A., Burch, M., Gurd, F. R. N. & Berzowsky, J. A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6831–6835.
36. Lanzavecchia, A. (1985) *Nature (London)* **314,** 537–539.
37. Pletscher, M. & Pernis, B. (1983) *Eur. J. Immunol.* **13,** 581–584.