The transmembrane protein-tyrosine phosphatase CD45 is required for both thymocyte development and T cell activation (1, 2). CD45 exerts its effects, at least in part, by regulating the phosphorylation state of Src family kinases through the dephosphorylation of a negative regulatory carboxyl-terminal tyrosine residue (3, 4). In addition to the cytoplasmic phosphatase domains, CD45 contains a large extracellular region. Three alternatively spliced exons reside within the external domain of CD45 and contain numerous sites for potential O-linked carbohydrate additions. These exons are developmentally regulated with respect to usage in T cells; and therefore, cells at different developmental stages have the potential to express vastly different forms of CD45. In addition to the O-linked carbohydrate found in the alternatively spliced sequences, there are numerous potential N-linked carbohydrate attachment sites (5). These N-linked carbohydrate additions have been demonstrated to be important for both cell-surface expression and stability of CD45 (6). In addition, we have recently shown that the composition of the CD45 N-linked carbohydrate is developmentally regulated, possibly through the action of the endoplasmic reticulum (ER) \(^1\) enzyme glucosidase II (GII) (7). Intriguingly, no typical cell-surface ligand for the extracellular domain of CD45 has been identified; however, several lectins have been demonstrated to bind CD45 carbohydrate (7–12).

The intracellular transport of proteins from the ER to the cell surface is a tightly controlled process involving the coordinated action of many enzymes and proteins (13). For the most part, as a protein moves through the secretory pathway, a level of control is exerted at each stage of the transport process, from protein folding and vesicle budding at the ER to movement through the Golgi stacks and finally sorting at the trans-Golgi network (TGN) en route to its final destination (14). In many cases, this regulation is necessary for proper function of the protein. For example, transport of Class I and II major histocompatibility complex (MHC) antigen-presenting proteins as well as CD1 follows different routes to the cell surface, and those specific routes are necessary to ensure that the appropriate antigens are loaded into their peptide- or glycolipid-binding grooves (15–17). For CD45, it is clear that cell-surface expression is required for proper function and that the N-linked carbohydrate on CD45 plays a role in this process (6). Recently, mannose-binding lectin has been shown to bind cell-surface CD45, which indicates that CD45 is able to escape complete carbohydrate processing, leaving immature, high mannose carbohydrate (7, 12). Alternative trafficking routes are one possible mechanism CD45 could employ to reach the plasma membrane without complete carbohydrate processing. With the recent examples of carbohydrate influencing biological function such as dendritic cell-specific ICAM-grabbing non-integrin interactions (18, 19), CD8-MHC interactions (20, 21), and T cell receptor clustering (22), as well as the limited information on CD45 trafficking in general, we set out to determine the overall trafficking patterns of CD45. By examining the transport of CD45 to the plasma membrane, we hoped to gain insight into the mechanism of expression of CD45 bearing mannose-binding lectin ligands.

Our results indicate that there is a pool of CD45 that is very rapidly expressed on the cell surface after synthesis. It appears that two different mechanisms exist that allow CD45 to reach

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\(\S\) Alberta Heritage Foundation for Medical Research Senior Scientist.

To whom correspondence should be addressed: Dept. of Medical Microbiology and Immunology, 6-70 HMRC, University of Alberta, Edmonton, Alberta T6G 2S2, Canada. Tel.: 780-492-7710; Fax: 780-492-9828; E-mail: hanne.ostergaard@ualberta.ca.

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\(^1\) The abbreviations used are: ER, endoplasmic reticulum; GII, glucosidase II; TGN, trans-Golgi network; MHC, major histocompatibility complex; Endo H, endoglycosidase H; PBS, phosphate-buffered saline; BFA, brefeldin A; FACS, fluorescence-activated cell sorter; ERGIC, endoplasmic reticulum-Golgi intermediate compartment; CFP, coat protein complex; CFTR, cystic fibrosis transmembrane conductance regulator.
the cell surface: one involving the Golgi complex, resulting in endoglycosidase H (Endo H)-resistant carbohydrate modification; and the other independent of the Golgi, resulting in the maintenance of exclusively Endo H-sensitive carbohydrate on the cell surface. These data support the existence of a transport pathway where cargo can reach the cell surface extremely rapidly, without the requirement of the Golgi complex.

MATERIALS AND METHODS

Cell Lines and Antibody Reagents—The T lymphoma cell line BW5147 (referred to below as BW) was maintained as previously described (23). The CD45RO-expressing fibroblast cell line 82 50.5 was provided by Dr. Pauline Johnson (University of British Columbia, Vancouver, British Columbia, Canada) (24). The anti-pan CD45 extracellular domain monoclonal antibody I3/2 was described previously (23). The anti-Class I MHC D β2 antiserum H137 (25) was kindly provided by Dr. Kevin Kane (University of Alberta). Rabbit antisera H2 and J37, specific for GILβ and the intracellular region of CD45, respectively, were previously described (26). Rabbit anti-Pyk-2 antiserum was previously described (27). Anti-GIα antisera was purchased from Stressgen Biotechnologies Corp. (Vancouver).

Cell-surface Biotinylation, Cell Lysis, Immunoprecipitation, Streptavidin Pull-down Assay, and Endoglycosidase Treatment—Cell-surface biotinylation was performed as previously described (7). Briefly, cells were biotinylated with 50 μl of 10 mm sulfo-NHS-SS-biotin (Pierce/5 × 10^7 cells/ml in phosphate-buffered saline (PBS)) for 20 min on ice. Reactions were quenched by washing cells twice with PBS containing 5 mm glycine. All cells were lysed at a density of 5 × 10^7/ml in 5% Nonidet P-40 (Pierce)/Tris-buffered saline buffer (lysis buffer) and incubated for 20 min on ice. Post-nuclear supernatants were incubated with 13/2-coupled Sepharose 4B for 1–2 h or with 10 μl/ml rabbit antiserum, followed by capture of the immune complexes with protein A-Sepharose 4B. Biotinylated proteins were isolated by incubation with streptavidin-agarose for 1–2 h at 4 °C. Immunoprecipitates and streptavidin pull-downs were washed three times with radiolabeled precipitation assay buffer. After washing, the immunoprecipitates were boiled in reducing sample buffer. Immunoprecipitates were treated with Endo H (Calbiochem) in PBS containing 1% Nonidet P-40, 0.1% SDS, and 1% 2-mercaptoethanol for 16 h at 33 °C.

Pulse-Chase Analysis, Isolation of Cell-surface Proteins, and Brefeldin A Treatment—Cells were washed twice with PBS prior to depletion of intracellular methionine by incubation for 30 min at 37 °C with methionine-free RPMI 1640 medium (Invitrogen). Cells were then pulsed for 10 min at 15 °C or for 5 min at 37 °C (pulse conditions are indicated for each figure) with 0.5 μCi/ml Tran35S-label (ICN Biomedicals) at 5 × 10^6/ml. Cells were washed twice with ice-cold unlabeled cold-containing methionine) prior to initiation of the chase. Cells were chased at 37 °C (or as indicated) in complete medium, followed by washing with PBS prior to lysis. Where indicated, the chase medium was supplemented with 10 mm methionine. All cells were kept on ice after the chase prior to isolation of the cell-surface protein as described below to prevent antibody or biotin internalization. For specific isolation of cell-surface proteins, either an antibody or biotinylation method was employed. For isolation of cell-surface protein by the antibody method, cells were incubated for 20 min with 20 μg/ml I3/2 or 10 μg/ml rabbit antiserum at 4 °C, followed by washing three times with PBS. Cells (5 × 10^6) were then lysed with 500 μl of 2.5 × 107/ml unlabeled lysate. Immune complexes were recovered with secondary antibody-coated protein A-Sepharose 4B. For isolation of cell-surface CD45 by biotinylation, cells were surface-biotinylated as described above and lysed at 2.5 × 107/ml in lysis buffer, followed by total CD45 immunoprecipitation. Captured CD45 was released by boiling for 2 × 5 min in 50 μl of 2% SDS-containing Tris-buffered saline. Eluent was diluted to 1 ml with lysis buffer. Biotinylated CD45 was isolated with immobilized streptavidin as described above. Cell-surface proteins were selectively isolated by the antibody method unless otherwise stated. All immunoprecipitates and pull-downs were washed three times with radiolabeled precipitation assay buffer prior to boiling with reducing sample buffer. In the cases where brefeldin A (BFA) was used to block protein trafficking, cells were preincubated with 2 μg/ml BFA for 30 min at 37 °C. BFA was also present during the pulse-chase at 2 μg/ml. Polyacrylamide Gel Electrophoresis, Autoradiography, and Immunoblotting—Proteins were resolved on polyacrylamide gels and transferred to Immobilon (polyvinylidene difluoride; Millipore Corp., Bedford, MA) as described previously (23). For separation under nonreducing conditions, 2-mercaptoethanol was omitted from the sample buffer. Immunoblotting was performed with the BioMax Tran-Scan intensified system (Eastman Kodak Co.). Western blot analysis was conducted with the indicated antiserum followed by horseradish peroxidase-conjugated protein A (Pierce) and visualized by ECL (PerkinElmer Life Sciences). Densitometry was performed using NIH Image Version 1.62 software.

FACS Analysis—Cells (1 × 10^6) were incubated with 10 μg/ml antiserum H2 for 20 min on ice, followed by two washes with PBS containing 0.1% serum. For detection of bound antibody, cells were further incubated with fluorescein isothiocyanate-conjugated donkey anti-rabbit antibody for an additional 20 min on ice. Cells were then fixed in 1% paraformaldehyde before analysis.

RESULTS

CD45 Acquires Endo H Resistance Quickly after Synthesis—Because CD45 is capable of reaching the cell surface with incompletely processed carbohydrate (7, 12), we wished to follow the processing of CD45 carbohydrate through the secretory pathway using the acquisition of Endo H resistance as a marker of protein location. Treatment of newly synthesized CD45 with Endo H after various chase times indicated that CD45 began to acquire Endo H resistance ~15 min after synthesis and that 80% of newly synthesized CD45 was Endo H-resistant after 60 min (Fig. 1). Class I MHC showed a lag period before acquiring Endo H resistance (Fig. 1). This lag phase in glycoprotein trafficking seen with Class I MHC may reflect the requirement for peptide loading, β2-microglobulin association, and transport to the Golgi before Class I begins to achieve Endo H resistance. It should be noted at this point that CD45 never achieved full Endo H resistance, with ~50% of its carbohydrate remaining Endo H-sensitive (Fig. 1).

Two Pools of CD45 Appear on the Cell Surface, Each with Different Kinetics—in addition to determining the rate of acquisition of Endo H resistance, another key parameter in the trafficking of CD45 is the time required to reach the cell sur-
face. By ascertaining the time required to reach the plasma membrane, we can observe whether or not CD45 traffics directly from the Golgi to the cell surface, or if a route through other compartments occurs. Initial experiments performed to address the time required for newly synthesized CD45 to reach the plasma membrane indicated that a population of newly synthesized CD45 was able to traffic to the surface during a 5-min pulse at 37 °C (data not shown). This time to cell-surface expression seems to be extremely rapid compared with the kinetics reported for the majority of other cell-surface proteins. However, Nori and Stallcup (28) used CD45 as a control in a pulse-chase experiment and reported similar results with respect to rapid CD45 cell-surface expression. To more accurately determine the time required for newly synthesized CD45 to reach the cell surface, a modification to the previous pulse-chase protocol was necessary. A pulse condition was needed that prevented or substantially slowed bulk protein transport, but still allowed for sufficient incorporation of metabolic label. Incubation of cells at 15 °C has previously been demonstrated to block protein transport at the ER-Golgi intermediate compartment (ERGIC) (29); therefore, pulsing cells with 35Smethionine at 15 °C instead of 37 °C should slow protein transport enough to reveal a true zero chase time for cell-surface expression. Using this new protocol, no radiolabeled CD45 was detected on the cell surface at time 0 (Fig. 2A). By 5 min, a lower molecular weight population of newly synthesized CD45 appeared at the plasma membrane, whereas a higher molecular weight population reached the cell surface at ~15 min. The amount of newly synthesized, higher molecular weight CD45 isolated from the cell surface increased through 60 min, whereas the amount of the lower molecular weight population remained fairly constant after 15 min. As determined by densitometric analysis, at 60 min, the lower molecular weight population of newly synthesized cell-surface CD45 composed ~20% of the total newly synthesized cell-surface material; and in examining the steady-state levels of surface CD45, the lower form was ~20% of the total (Fig. 2A). We also observed that the total amount of newly synthesized CD45 increased over the chase time, which raises the possibility that labeled CD45 continues to be synthesized over the chase or that, because the antibody used to isolate CD45 is conformation-dependent, this increase may reflect an increase in the amount of folded CD45. Because the inclusion of 10 mM methionine during the chase did not prevent the increase in labeled CD45 isolated over the chase (Fig. 2A, exp. 2), we suspect that the increase in labeled CD45 recovered is due to an increased ability of the antibody to recognize CD45 over the chase time.

It still remains possible that, during the cell-surface isolation of newly synthesized CD45, we were recovering internal pools of labeled CD45 nonspecifically. This may be particularly problematic at the early time points. For a number of reasons, we feel that this possibility is unlikely. Perhaps the most compelling argument against this possibility is that, using the same isolation protocol with a fibroblast cell line transfected with CD45, we were unable to recover newly synthesized cell-surface CD45 until 30 min, even though a significant amount of newly synthesized CD45 could be recovered over the chase time (Fig. 2B). In fact, with this cell line, cells were pulsed for 5 min at 37 °C before initiating the chase; and even with this pulse procedure, a rapidly expressed population of CD45 was not detected (Fig. 2B). Additionally, there appears to be only one glycoform of CD45 isolated from these cells, in contrast to the two forms isolated from the BW cell line. Furthermore, using Class I MHC as a control for the protocol, the surface expression of newly synthesized Class I MHC followed trafficking kinetics similar to those previously reported (30, 31), with no newly synthesized Class I MHC being isolated from the cell surface until 30 min of chase time (Fig. 2C). Finally, control mixing experiments in which unlabeled surface protein was isolated in the presence of labeled lysates showed that barely detectable levels of labeled material (significantly lower than the amount we detected at the early time points in Fig. 2A) were isolated with the unlabeled cell-surface complexes (data not shown).

Another published method to isolate cell-surface proteins involves surface biotinylation and isolation of the protein of interest, followed by streptavidin affinity enrichment (15, 32). Using the surface biotinylation approach to isolate cell-surface CD45, we obtained results similar to those attained with the surface antibody method described above (Fig. 3A). Newly synthesized surface-biotinylated CD45 began to appear on the cell surface at 5 min and increased through to 60 min of chase time, with the existence of two different forms (Fig. 3A). Between experiments and surface CD45 isolation protocols, the only
CD45 was determined by Western blot resolved by SDS-PAGE. The presence of the CD45 immunoprecipitate (Fig. 3) was performed on the eluted material. Material bound to streptavidin-coated beads was resolved by SDS-PAGE and subjected to autoradiography and Western blot (WB) analysis. B, Pyk-2 immunoprecipitates (ip.) from a surface-biotinylated cell lysate (lane 1) or a lysate biotinylated after lysis (lane 2) were resolved by SDS-PAGE. Western blotting with streptavidin (SA; upper panel) and Pyk-2 (lower panel) was performed. C, I3/2 immunoprecipitation from non-biotinylated BW lysates was performed. The CD45 immunoprecipitate (lane 1) or a streptavidin pull-down from the elution of the CD45 immunoprecipitate (lane 2) was resolved by SDS-PAGE. The presence of CD45 was determined by Western blot analysis.

variability in results occurred in the amount of the higher molecular weight form of CD45 isolated at 15 min. To control for possible nonspecific isolation of cell-surface CD45 by the biotinylation method, two controls were performed. First, a cytosolic protein-tyrosine kinase (Pyk-2) was immunoprecipitated from a surface-biotinylated lysate or a lysate biotinylated after detergent solubilization. Pyk-2 was found to be biotinylated only in the case where biotin was added post-lysis, not during the surface biotinylation procedure (Fig. 3B). Second, non-biotinylated CD45 eluted from a CD45 immunoprecipitate was not captured by streptavidin-coated beads (Fig. 3C). Therefore, it appears that there is a rapidly expressed population of CD45 in BW cells and that the mechanism used to express this form of CD45 does not exist in all cell types.

The Rapidly Expressed, Lower Molecular Weight Cell-surface CD45 Is Endo H-sensitive, whereas the Higher Molecular Weight Form Is Endo H-resistant—Because bulk CD45 acquired Endo H resistance rapidly after synthesis and there were two different forms of CD45 expressed on the cell surface with different kinetics, we wished to determine whether both forms were in fact Endo H-resistant or if one was possibly Endo H-sensitive. To determine the carbohydrate status of the two different forms of newly synthesized cell-surface CD45, surface CD45 was isolated after pulse-chase and subjected to Endo H treatment. The rapidly expressed population of CD45 was entirely Endo H-sensitive, whereas the higher molecular weight form achieved its full Endo H resistance (Fig. 4). These data suggest that the higher molecular weight form is a mature glycoform of CD45 with fully processed carbohydrate, whereas the lower molecular weight form is an immature glycoform with unprocessed carbohydrate. Note again that the fully processed glycoform of CD45 still contains a significant fraction of Endo H-sensitive carbohydrate, but mature cell-surface CD45 always appears to contain this level of Endo H-sensitive carbohydrate in these cells (7).

The Rapidly Expressed Pool of CD45 Reaches the Cell Surface by a BFA-insensitive Mechanism—The finding that CD45 was capable of reaching the cell surface without complete processing of its carbohydrate raises the question of how this material traffics from the ER to the cell surface. The prevailing model of glycoprotein transport states that once a glycoprotein leaves the ER, it is transported through the ERGIC to the Golgi, where the carbohydrate is processed to a complex form, and finally to the cell surface. Our finding that the rapidly expressed pool of CD45 reached the cell surface with exclusively unprocessed N-linked carbohydrate suggests that this pool may by-pass the Golgi entirely en route to the cell surface. One of the most well characterized and commonly used methods to inhibit protein transport through the Golgi is treatment of cells with the fungal metabolite BFA. BFA interferes with the recruitment of the ADP-ribo-sylation factor-1 GTPase to COPI-coated membranes, effectively blocking protein transport through the prevention of ER export and subsequent Golgi redistribution (33, 34). Treatment of cells with BFA effectively inhibited Class I MHC cell-surface expression and the trafficking of the higher molecular weight mature glycoform of CD45, indicating that the BFA-induced blockade was successful; however, it did not inhibit the transport of the rapidly expressed, lower molecular weight glycoform of CD45 to the cell surface (Fig. 5). Examination of the carbohydrate on newly synthesized CD45 after BFA treatment revealed that it contained entirely Endo H-sensitive carbohydrate, as expected (Fig. 5). These data suggest that the rapidly expressed population of CD45 reaches the cell surface independent of the Golgi complex and does not rely on a BFA-sensitive transport mechanism. As we did not observe increasing amounts of CD45 reaching the cell surface after BFA treatment, and the amount of cell-surface CD45 appeared fixed at ~20% in the presence of BFA, the data suggest that there is an early commitment of a significant portion of CD45 to the conventional transport pathway. Appar-
Recently, once it enters the conventional pathway, CD45 cannot be expressed on the cell surface in the presence of BFA by the rapid alternative pathway.

Both 15 and 20°C Blockades Result in Delayed Trafficking Kinetics of the Rapidly Expressed Pool of Cell-surface CD45—To further dissect the pathway used by the rapidly expressed pool of CD45, we took advantage of blocks at the ERGIC and TGN imposed by chasing proteins at 15 and 20°C, respectively (29, 35, 36). Performing the chase at 15°C resulted in delayed and reduced expression of the lower molecular weight form of CD45, whereas this treatment completely prevented the surface expression of the higher molecular weight form of CD45 and Class I MHC, indicating that the blockade was successful (Fig. 6A). Chasing cells at 20°C after pulsing at 15°C and isolation of cell-surface CD45 resulted in the appearance of the lower molecular weight form of CD45 beginning at 15 min and increasing through the entire chase time (Fig. 6B).

By chasing at 20°C, both the higher molecular weight form of CD45 and Class I MHC were prevented from reaching the plasma membrane, indicating that the kinetic block was functional (Fig. 6B). Examination of the remaining CD45 after the chase at 15 or 20°C revealed that it still contained predominantly Endo H-sensitive carbohydrate (Fig. 6, A and B). The observation of reduced kinetics of acquisition of Endo H resistance is in accordance with previously published data (35). Therefore, it appears that a block of glycoprotein traffic through the cis-Golgi or at the TGN does not prevent the expression of the lower molecular weight form of CD45, but it does appear to delay its trafficking kinetics.

GII, a Putative Resident ER Protein, Traffics Rapidly to the Cell Surface and Possesses Immature Carbohydrate—Because CD45 and GII associate stably in the BW cells used for this study and a number of other putative resident ER proteins have been found on the cell surface of numerous cell types (37–40), we wished to determine whether GII is expressed on the cell surface. To first determine whether GII is present on the cell surface, FACS analysis was performed with an antiserum specific for GIIβ. The data in Fig. 7A clearly show that GIIβ was expressed on the cell surface. Given the finding of GII on the surface by FACS analysis, we wanted to determine the kinetics of expression of GII at the plasma membrane. Isolation of surface GII by the antibody method using anti-GIIβ antisera after pulse-chase revealed rapid surface expression of GIIα (Fig. 7B). Newly synthesized GIIα was detected at the plasma membrane 5 min after synthesis, with a further increase at 15 min, after which time the levels remained relatively constant. There did appear to be more newly synthesized GIIα on the cell surface at 30 and 60 min, but there also appeared to be more GIIβ recovered at those time points (Fig. 7B). Because GIIα is glycosylated, we could assess the status of the carbohydrate expressed by surface GIIα to determine whether surface GII expresses N-linked carbohydrate pro-
The mechanism by which CD45 can reach the cell surface with immature carbohydrate.

Unexpectedly, in examining the trafficking of CD45, we observed a striking pattern of CD45 cell-surface expression. There appears to be two different glycoforms of CD45 that reach the cell surface, each with different kinetics. The lower molecular weight glycoform arrived within 5 min and achieved maximum expression at 15 min, whereas the higher molecular weight form appeared after 15 min and increased through 60 min. The rapidity with which both forms reached the cell surface compared with other cell-surface glycoproteins is quite surprising. This rapid trafficking of CD45 was confirmed by examining the rate of acquisition of Endo H resistance. As illustrated in Fig. 1, there was a short lag period in the time required for CD45 to begin to acquire Endo H resistance. This suggests that CD45 does not remain in the ER long after synthesis; rather, it traffics quickly to the Golgi, where its carbohydrate can be processed.

Interestingly, the rapidly expressed, lower molecular weight pool of CD45 contained exclusively Endo H-sensitive carbohydrate, whereas the higher molecular weight CD45 possessed mature carbohydrate (Fig. 4). This finding suggests that the rapidly expressed pool bypasses the Golgi complex, where the enzymes required to convert N-linked carbohydrate from Endo H-sensitive to Endo H-resistant reside. It has been suggested that the minimal time required for transit through the Golgi is 10 min (30), and the finding that the rapidly expressed pool appeared after 5 min supports the hypothesis that this pool of CD45 may by-pass the Golgi. In addition, treatment of cells with BFA, which disrupts the Golgi, still permitted the expression of the lower molecular weight CD45 on the cell surface, whereas the higher molecular weight CD45 and Class I MHC were completely prevented from trafficking to the plasma membrane. Finally, a block at the TGN resulted in abrogation of the higher molecular weight CD45 and Class I MHC from the cell surface, but the lower molecular weight CD45 still appeared. Collectively, these data support the by-pass of the Golgi complex by the rapidly expressed, lower molecular weight cell-surface CD45.

Given the above data, we suggest there are two possible means by which the rapidly expressed population of CD45 could reach the plasma membrane. The first involves a direct fusion event between peripheral components of the ER and the plasma membrane. The second involves a vesicle-mediated transport mechanism. Recently, Gagnon et al. (41) demonstrated a direct ER-to-plasma membrane fusion event during phagosome formation. They found putative resident ER proteins including calnexin in the early phagosome. From this work, they concluded that the plasma membrane is not the only contributor to phagosome formation, but that through direct fusion, the ER provides a source of membrane (41). It has also been found by examination of the internal architecture of the cell that elements of the ER can be found closely juxtaposed to the plasma membrane (42). If newly synthesized CD45 in the ER were found at the site of direct ER-to-plasma membrane fusion, it would reach the cell surface rapidly without transport through the Golgi. In addition, disruption of the Golgi would likely not have any effect on the ability of CD45 to reach the cell surface by this mechanism, nor would any of the kinetic transport blocks have any effect. This direct fusion event could also explain the finding of newly synthesized GII on the cell surface (containing Endo H-sensitive carbohydrate) as well as the mechanism by which the numerous resident ER proteins are found at the plasma membrane (37–40). Finally, the direct fusion could also explain why we detected rapid cell-surface expression of CD45 in BW cells, but not fibroblast cells. One

**DISCUSSION**

We have previously demonstrated that CD45 reaches the plasma membrane with incompletely processed carbohydrate and that mannose-binding lectin can recognize this cell-surface CD45 (7). Because of recent reports implicating carbohydrate in the regulation of cellular processes and the importance of CD45 in T cell function, we have further investigated the transport pathway utilized by CD45 in hopes of determining the mechanism by which CD45 can reach the cell surface with immature carbohydrate.

**Fig. 6.** Expression of the lower molecular weight form of CD45 is delayed by a 15 or 20°C chase. A, cells were pulsed at 15°C for 10 min, followed by chasing at either 37 or 15°C. Cell-surface CD45 (first and second panels) or Class I MHC (third and fourth panels) was isolated by the antibody method and separated by SDS-PAGE. Autoradiography was performed, followed by Western blot (WB) analysis. B, BW cells were pulsed for 10 min at 15°C and chased at 20°C. Cell-surface CD45 (first and second panels) or Class I MHC (third and fourth panels) was isolated by the antibody method and resolved by SDS-PAGE. Autoradiography and Western blot analysis were performed. For both A and B, the remaining CD45 was immunoprecipitated and either mock-treated (M) or subjected to Endo H digestion (H) (fifth panels). The resultant proteins were separated by SDS-PAGE, and autoradiography was performed.
would expect that, because of the more compact architecture of lymphoid cells, ER components would more frequently contact cell-surface membranes in BW cells, whereas in the much larger, less densely packed fibroblast cells, the frequency of the ER membrane contacting the plasma membrane would be predicted to be much lower. Thus, a direct fusion of the ER with the plasma membrane could account for our observations.

Alternatively, a bona fide vesicle-mediated transport mechanism could also be reconciled with our data. There are a few examples of transport pathways that by-pass the Golgi, including rotaviral production and cystic fibrosis transmembrane conductance regulator (CFTR) transport. Examination of CFTR trafficking demonstrated that there is a 2-fold decrease of CFTR in the Golgi compared with the ER, with limited amounts in both the cis-Golgi and TGN (43). Subsequently, it was also observed that CFTR export requires the machinery of the early secretory pathway, namely COPII, but that after leaving the ER in COPII vesicles, the remaining transport utilizes a non-conventional pathway (44). In examining rotaviral particle release, it was observed that viral particle production is dependent on the ER; however, disruption of the Golgi complex by treatment with monensin has no impact on viral particle release (45). Interestingly, cholesterol transport has recently been demonstrated to occur in a BFA-insensitive manner. Cholesterol reached the cell surface with a half-time of ~10 min, but its expression was reduced by a 15 °C chase (46). These characteristics are reminiscent of the transport kinetics and BFA sensitivity shown by the rapidly expressed pool of CD45.

In examining the effect of BFA on the disruption of Golgi structure, it has been observed that different Golgi constituents re-localize to different areas of the cell post-BFA treatment (47). BFA prevents transport by inhibiting a guanine nucleotide exchange factor for the ADP-ribosylation factor-1 GTPase, which is thought to be required for the recruitment of COPI to the vesicle membrane. Interestingly, examination of the ER has revealed that BFA has little to no impact on the structure of ER exit sites (48). ER exit sites are believed to be the place where vesicles bearing cargo leave the ER for trafficking to the intermediate compartment. Because BFA has little impact on the structure of ER exit sites, it is conceivable that vesicles with no requirement for BFA-sensitive COPI trafficking and containing cargo destined for the cell surface independent of the Golgi would be able to effectively reach the cell surface in the presence of BFA. In support of a COPI-independent transport route from the ER to the ERGIC, work by Scales et al. (49) demonstrated the sequential action of COPII and COPI for ER-to-ERGIC trafficking and ERGIC-to-Golgi trafficking, respectively. In the event that COPI is required for ER-to-ERGIC transport, a BFA-resistant guanine nucleotide exchange factor for ADP-ribosylation factor-1 was recently cloned (50). In support of either a COPI-independent or BFA-resistant COPI-dependent mode of transport, a member of the connexin family of gap junction proteins was also shown to traffic to the cell surface in a BFA-insensitive manner (51). In addition, with respect to CFTR trafficking, a dominant-negative ADP-ribosylation factor-1 construct had no effect on the transport of CFTR (44). Therefore, a BFA-insensitive guanine nucleotide exchange factor or a COPI-independent mechanism may be responsible for the trafficking of the rapidly expressed pool of CD45 to the cell surface.

The expression of cell-surface CD45 containing exclusively unprocessed carbohydrate is somewhat unexpected; however, this pool of CD45 may have important biological implications.
The lower molecular weight, fully Endo H-sensitive form of CD45 was detected on the cell surface at steady-state levels (Fig. 2B), suggesting this is a constitutive pathway resulting in a stable pool of protein. Consistent with the stable expression of the lower molecular weight form on the cell surface, we have found that both glycoforms of CD45 expressed on the cell surface of BW cells are recognized by the mannoside-binding lectin (7). Lectin recognition of CD45 could have many implications in T cell biology, including the modulation of adhesion, cell migration, mobility of CD45 within the plasma membrane, and signaling thresholds.

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The Protein-tyrosine Phosphatase CD45 Reaches the Cell Surface via Golgi-dependent and -independent Pathways

Troy A. Baldwin and Hanne L. Ostergaard

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