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Calreticulin and Calnexin Interact with Different Protein and Glycan Determinants During the Assembly of MHC Class I

Michael R. Harris,* Yik Y. L. Yu,* Cathy S. Kindle,* Ted H. Hansen,2* and Joyce C. Solheim†

Before peptide binding, a variety of endoplasmic reticulum (ER) proteins are associated with class I including calnexin, TAP, calreticulin, and tapasin. Although the selective functions of any of these ER proteins have been difficult to define, individually or in combination they perform two general chaperone functions for class I. They promote assembly of the class I heterotrimERIC molecule (heavy (H) chain, β2m, and peptide) and they retain incompletely assembled complexes in the ER. In this study, we present evidence that calreticulin clearly differs from calnexin in how it associates with class I. Regarding the structural basis of the association, the oligosaccharide moity in the α1 domain and the amino acid residue at position 227 in the α3 domain were both found to be critical for the interaction of class I with calreticulin. Interestingly, calreticulin displayed sensitivity to class I peptide binding even in TAP-deficient human or mouse cells. Thus, calreticulin is clearly more specific than calnexin in the structures and conformation of the class I molecule with which it can interact. The Journal of Immunology, 1998, 160: 5404–5409.

During its assembly, the class I MHC heavy (H)3 chain associates with a variety of proteins in the endoplasmic reticulum (ER). One of these proteins, TAP, enables peptides to enter the ER and interacts with the class I H chain until it binds a peptide (1–5). In studies of the HLA-A2 mutant T134K, the association of class I with TAP has been shown to require for presentation of intracellular Ags to T cells (6–7). A recently discovered molecule, tapasin (gp48), also participates in class I assembly (8–9). Tapasin is a type I transmembrane glycoprotein with a C terminal ER retention signal (10–11). The LCL72.220 cell line, which does not express tapasin (8), has low surface expression of class I and impaired interaction between class I and TAP (9). Thus, tapasin seems to be a physical intermediary between TAP and the class I H chain (8–9).

Calreticulin and calnexin, which are ER chaperones with lectin-like activity, also bind to class I and several other glycoproteins (8, 12–14). The calcium-binding domains of these two chaperones are also markedly similar in sequence (15). Binding of both calnexin and calreticulin has been shown to be regulated by glucose trimming of nascent N-linked oligosaccharides (14, 16–17). Calnexin acts initially to stabilize free class I H chains and protect them from degradation (18). Furthermore, calnexin has been reported to promote subunit assembly of class I with molecules in the ER (19–20). In regard to the role of calnexin in ER retention, it is important to note that these studies were done in cells that lack TAP (19) or in cells where class I does not associate with TAP due to a lack of β2m (20). The importance of this observation is that in wild-type cells class I is associated with the complex of TAP, tapasin, and calreticulin before peptide binding (8). Thus the role of calnexin in ER retention in wild-type cells remains to be determined. In any case, considering the evidence showing calnexin to be a class I chaperone (cf. 18), it was surprising that class I assembly with β2m, peptide loading, and surface expression were found to be normal in calnexin-deficient cells (21–22). Thus calnexin appears not to have a unique role in chaperoning class I. Based on this result and the above noted similarities in their binding specificity, it was suggested that calnexin and calreticulin may have redundant functions in class I assembly and expression (22–23).

Calreticulin has been shown to display steady state association with class I/TAP/tapasin complexes (8, 17, 24). Indeed, the majority of nascent class I molecules associated with calreticulin are simultaneously associated with TAP (17). In contrast, calnexin does not display stoichiometric association with the class I/TAP/ calreticulin complex (11, 17, 24) but may bind a small number of assembling TAP complexes (11, 25). Furthermore, at least in human cells, calnexin associates predominantly with free class I H chains (26–27), whereas calreticulin association requires the H chain be assembled with β2m (8, 24). Similarly, calreticulin was found to be more restrictive than calnexin in its binding to folding intermediates of influenza hemagglutinin (28). These observations suggest that calnexin and calreticulin have different binding specificities in regard to glycoprotein folding, and that their chaperone functions may be successive and distinct. Furthermore, since calreticulin and not calnexin shows predominant association with the TAP complex, calreticulin could facilitate the retention of β2m-assembled H chains awaiting peptide.

In this report, we demonstrate that a mutation in the α3 domain of the H chain or selective removal of the N-linked glycans from the α1 domain of the H chain ablates association of class I with calreticulin but not calnexin. Since the binding of calreticulin to class I is TAP- and tapasin-independent, the simplest explanation of our
findings is that calreticulin, like calnexin, binds directly to the class I H chain but that the two chaperones bind to different sites. We also report here that calreticulin preferentially associates with open forms of both mouse and human class I, and is selective for the class I open form even in the absence of TAP. These data suggest that calreticulin aids the stabilization and/or ER retention of peptide-free H chain/β2-m heterodimers. Furthermore, peptide-induced folding of class I in cell lysates does not alter its association with calnexin (5, 25), whereas calreticulin and tapasin are associated with class I only before peptide-induced folding. These cumulative findings clearly establish that the interactions of calreticulin and calnexin with class I are quite distinct.

Materials and Methods

Cell lines

L-Ld cells were generated by introducing the Ld gene into murine Ltk- DAP-3 (H-2b) fibroblast cells (29), T1 and T2 cells (30) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). T1 (174 × CEM.T1) is a cloned hybrid of 721.174 (an immunoselected variant of LCL 721) and CEM.6.3. T1 has lost only one copy of CEM.6.3-derived chromosome 6; it expresses TAP1, TAP2, and class II DR Ag. T1 is the parental cell line of T2 (174 × CEM.T2). T2 has lost both copies of CEM.6.3-derived chromosome 6 and so does not express class II MHC, TAP1, or TAP2. T2-Ld (an Ld transfectant of T2) was a gift from Peter Cresswell (Yale University, New Haven, CT). RMA-S (31), a C57BL/6-derived thymoma, was a gift from Jeffrey Bluestone (University of Chicago, IL). RMA-S has a mutation that causes premature termination of the TAP2 protein, and therefore it does not have a functional TAP heterodimer. RMA-S-Ld is an Ld transfectant of RMA-S (32). Mutants of Ld that lack α1 domain or α2 domain glycosylation sites were produced by the PCR in the case of mutants LdN86K and LdN176Q, or by the Quik Change method (Stratagene, La Jolla, CA) in the case of mutants LdN86Q and LdS88W. These Ld mutants are named as follows: original amino acid residue, position, and substituted amino acid residue. The mutant cDNAs were cloned into the expression vector pRSV.Sneo (33), transfected into L cells with Lipofectin (Life Technologies, Gaithersburg, MD), and selected in 0.6 mg/ml G418. An Ld mutant that lacks all three glycosyl groups was donated by Keiko Ozato (National Institute of Child Health and Human Development, Bethesda, MD) (34). Ld mutant LdD227K has been previously described (5). Cells were maintained at 37°C, 5% CO2 in RPMI 1640 (Life Technologies) containing 10% FCS (HyClone Laboratories, Logan, UT), glutamine, pyruvate, and penicillin/streptomycin, or were cultured at 37°C, 7% CO2 in DMEM (Life Technologies) containing 10% FCS (HyClone Laboratories), glutamine, pyruvate, and penicillin/streptomycin.

Antiseras and mAb

The mAb 64-3-7 is an IgG2 Ab that recognizes the α1 domain of open Ld (29, 35–38), and mAb 50-5-7 is an IgG2 Ab that recognizes the α2 domain of folded Ld (37–39). Ld forms that possess the 64-3-7 serologic epitope lack the epitopes of several conformation-sensitive mAbs (e.g., 30-5-7) that surround the peptide-binding groove, which suggests that the Ld64-3-7 form has an open/unfolded cleft (35). The interaction of 64-3-7 with peptide causes a conversion to the folded (30-5-7) form, as demonstrated by titration of radiolabeled peptide into cell lysates and immunoprecipitation of the Ld molecules. With increasing concentrations of peptide ligand, the amount of 64-3-7 Ld decreased and the amount of 30-5-7 Ld increased (37). Furthermore, the labeled peptide was coprecipitated in dose-dependent fashion with the 30-5-7 Ld form and could be simultaneously visualized on the autoradiograph (37). In vivo, a precursor-product relationship exists between 64-3-7 Ld and 30-5-7 Ld, as shown by pulse-chase analysis. As the chase time increases, 30-5-7 Ld levels rise and 64-3-7 Ld levels drop (37).

HCl10 is an IgG2a that reacts preferentially with open HLA-B and -C heavy chains (40–41). W6/32 is an IgG2a that reacts with a monomorphic determinant on all folded HLA/β2-m heterodimers (41–42). Experiments by Carreno and Hansen showed a reciprocal relationship between HCl10 HLA forms and W6/32 HLA forms (41). For example, culture of L-B27/hH2-m cells with peptides decreases the number of surface HCl10-reactive molecules and increases the population of W6/32-reactive molecules. Furthermore, in the presence of peptide ligands, the half-life of W6/32-reactive molecules on L-B27/hH2-m is extended by about 3 h and the half-life of HCl10-reactive molecules is reduced by the same amount of time.

The 1478 anti-TAP1 antisera was made against the same TAP1 peptide as the R.RING4C antisera (43). Anti-calreticulin serum (44) was purchased from Stressgen (Victoria, BC, Canada). MAb 11.4.1 is an IgG2 Ab specific for K* (45). Affinity-purified rabbit anti-human tapasin Ab (8) was provided by Dr. Peter Cresswell. The anti-calnexin serum specific for a C-terminal segment from mouse and human calnexin (46) was donated by David McKeen (Mayo Clinic, Rochester, MN). Anti-TAP2 hybrid-oma 435.3 (47) was developed by Dr. Peter van Endert (Institut National de la Santé et de la Recherche Médical (INSERM) Unité, Paris, France) and sent to us by Dr. Peter Cresswell. The 15-5-5 mAb is an IgG2 Ab that recognizes Dq (48).

Immunoprecipitations

Cells were preincubated for 30 to 60 min at 37°C in culture media that lacked methionine. Then [35S]methionine (125–250 μCi/ml) was added, and the cells were radiolabeled for 15 to 30 min. The cells were then washed three times in PBS containing 20 mM iodoacetamide (IAA, Sigma, St. Louis, MO) and lysed in buffer that contained 1% 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonate (CHAPS, Boehringer-Mannheim, Indianapolis, IN) in Tris-buffered saline, pH 7.4, freshly added 0.2 mM iodoacetate (Sigma), and 20 mM iodoacetamide. Alternatively, cells were lysed in Tris-buffered saline that contained 1% digitonin (Wako, Richmond, VA), 0.1 mM 7-amino-1-chloro-3-tosylamido-2-heptone (TLCK), and 0.5 mM freshly added PMSF. The lysis buffer with either CHAPS or digitonin was supplemented with a saturating volume of mAb or rabbit Ab before its addition to pelleted cells. After incubation for 30 min on ice, nuclei were removed by centrifugation, and lysates were incubated with protein A-Sepharose beads (Pharmacia, Piscataway, NJ). The beads were washed four times with 0.1% CHAPS or 0.1% digitonin in Tris-buffered saline, pH 7.4, and the samples were eluted by boiling in 0.125 M Tris pH 6.8/2% SDS/12% glycerol/2% bromophenol blue. All immunoprecipitates were electrophoresed on 4–20% or 8% acrylamide SDS-PAGE gels (Novex, San Diego, CA) with the Laemml buffer system (49). Gels were treated with Amplify (Amersham, Boston, MA), dried, and exposed to BioMax MR film (Eastman Kodak Co., Rochester, NY) at −70°C for varied lengths of time. All immunoprecipitation lanes shown within a figure are from a single experiment.

In the peptide binding experiments (Figs. 4 and 5), cells were radiolabeled as indicated above and divided into four aliquots of 2 × 10⁷ cells. The aliquots were solubilized in lysis buffer supplemented with 0.05 or 0.5 μM of a synthetic Ld peptide ligand derived from murine cytomegalovirus (MCMVpp89, residues 168–176) (50) or with 50 μM of a negative control D-binding peptide derived from influenza nucleoprotein (NP 365–380) (51). Samples were incubated on ice for 1 h and nuclei were removed by centrifugation. Each sample was divided into two aliquots, one which was precipitated with mAb 64-3-7 and one which was precipitated by mAb 30-5-7 by addition of the respective Ab and incubation on ice. After 1 h, the lysate was transferred to protein A-Sepharose, and further steps in the immunoprecipitation protocol were performed as described above.

Western blots

Immunoprecipitates electrophoresed on SDS-PAGE as described above were transferred to Immobilon P membranes (Millipore, Bedford, MA). After overnight blocking, membranes were incubated in a dilution of Ab for 2 h, washed three times with PBS/0.05% Tween-20, and incubated for 1 h with biotin-conjugated goat anti-mouse or anti-rabbit IgG (Caltag Laboratories, San Francisco, CA). Following three washes with PBS/0.05% Tween-20, membranes were incubated for 1 h with streptavidin-conjugated horseradish peroxidase (Zymed, San Francisco, CA), washed 3 times with PBS/0.3% Tween-20, and incubated with Western blot developing reagents (Amersham). Membranes were exposed to BioMax MR film (Eastman Kodak Co., Rochester, NY) at −70°C for varied lengths of time. Western blot lanes shown within a figure are from a single experiment, beginning from the same immunoprecipitation samples displayed in the “Immunoprecipitation” 35S-methionine autoradiographs above the Western blot lanes.

Results and Discussion

Calnexin and calreticulin share sequence similarities and known lectin-like activity (14–16). Despite these similarities, we have found that calreticulin interacts with different structures on class I than calnexin, and unlike calnexin it is responsive to the peptide-binding status of the class I MHC heavy chain.
Calreticulin can associate with the open H chain even in the absence of TAP

To determine whether calreticulin was associated with open vs folded class I molecules, we used mAb that distinguish these alternative class I conformations. For the mouse Ld molecule, open forms were specifically detected with mAb 64-3-7, and folded forms were detected with 30-5-7. Lower panels, coprecipitated TAP and calreticulin were identified by probing blots of the Ld immunoprecipitates with anti-TAP1 or anti-calreticulin serum. Shown are ECL enhanced luminescence images of Ab-probed blots of the immunoprecipitations displayed above. The data shown are representative of two separate experiments that yielded similar results.

Calreticulin can include with the open H chain even in the absence of TAP

The data shown are representative of two separate experiments that yielded similar results.

FIGURE 1. Calreticulin preferentially associates with the open form of the murine class I MHC H chain. Upper panel, autoradiograph of 35S-methionine-labeled, immunoprecipitated open and folded forms of Ld from lysates of L-Ld cells. “Anti-open Ld” is mAb 64-3-7 and “anti-folded Ld” is mAb 30-5-7. Lower panels, coprecipitated TAP and calreticulin were identified by probing blots of the Ld immunoprecipitates with anti-TAP1 or anti-calreticulin serum. Shown are ECL enhanced luminescence images of Ab-probed blots of the immunoprecipitations displayed above. The data shown are representative of two separate experiments that yielded similar results.

Calreticulin and tapasin are not associated with the class I H chain after peptide-induced folding

It has been previously reported that addition of peptide can cause the dissociation of class I from TAP (3–5). To determine whether this dissociation was dependent upon TAP itself, or the TAP-associated proteins (tapasin and calreticulin), we monitored peptide-sensitive molecular associations in TAP-deficient cells. As shown in Figures 4 and 5, peptide-induced folding of class I sharply diminishes the population of open class I molecules and causes a parallel reduction in calreticulin association in mouse RMA-S cells and human T2 cells transfected with the Ld gene. Using an Ab specific for human tapasin (8), we also monitored the tapasin association in T2-Ld cells and found its binding paralleled that of calreticulin (Fig. 5). Thus, even in the absence of TAP, the association of calreticulin and tapasin with class I is specific for open forms before peptide-induced folding. In contrast to these findings, the association of calnexin with class I is not affected by peptide-induced folding (5, 25).

The N-linked glycan in the α1 H chain domain is uniquely required for the interaction of H chain with calreticulin and not calnexin

Earlier reports demonstrated that calreticulin and calnexin, consistent with their lectin-like activity, interact with a glycosyl group on the class I H chain (8, 17). The experiments presented in these reports involved drugs that affect processing of all glycosyl moieties of all proteins. To study the effects of class I glycosylation without altering other cellular proteins and to determine whether certain class I H chain oligosaccharides are of predominant importance, we created a panel of mutants with selected omissions of N-linked glycans from the α1, α2, and/or α3 domains of Ld. We have found that the removal of the Ld α1 domain glycosylation site by site-directed mutagenesis (LdN86K) prevents association of calreticulin and TAP with the class I molecule (Fig. 6). Calnexin, however, still binds strongly to LdN86K (Fig. 6).

To ensure that it was indeed the absence of the α1 domain carbohydrate group and not the amino acid substitution per se that prevented interaction of the H chain with calreticulin and TAP, two other mutants were tested that had different amino acid substitutions but lacked the same glycosyl group (LdN6Q and LdS88W). As shown in Figure 6, these two mutants also were not associated with calreticulin or TAP, but were associated with calnexin. As a further confirmation of this result, an additional Ld mutant that lacks all three carbohydrate groups (LdΔCHO-) was tested and found not to associate with calreticulin and TAP (Fig. 7).

We next determined whether the α1 domain glycosyl group alone was necessary for calreticulin and TAP association with...
class I, or whether absence of a different carbohydrate moiety would have the same effect. An L<sup>d</sup> mutant with an amino acid substitution that prevented glycosylation in the α2 domain (L<sup>d</sup>N176Q) was made, and it was found to have the same level of calreticulin, TAP, and calnexin interaction as wild-type L<sup>d</sup>. This indicates that the α1 domain carbohydrate is uniquely required for calreticulin association with class I, whereas calnexin’s association with class I is clearly not dependent on the α1 domain carbohydrate. Indeed, calnexin may also contact the H chain polypeptide chain (52–54), even though the specificity of complex formation with non-MHC glycoproteins appears to be provided by the carbohydrate moieties (55). In any case the findings reported here provide clear evidence that the two lectins, calnexin and calreticulin, bind glycan moieties at different locations on the H chain. This difference may reflect differential accessibility of glycan moieties for the membrane-associated calnexin vs the lumenally located calreticulin. Our finding that the α1 glycan is necessary for calreticulin association with class I is interesting in light of the fact that all mouse and human H chains have an α1 glycan at this position, even though mouse H chains have a second, and sometimes a third, glycan in the α2 and α3 domains (56–60).

**Mutation in the α3 H chain domain affects class I association with calreticulin and not calnexin**

Studies of both L<sup>d</sup> (5) and D<sup>d</sup> (25) have shown that mutations in the α3 domain of class I can prevent class I association with TAP. For example, an aspartic acid—lysine mutation at position 227 of the α3 domain of L<sup>d</sup> (L<sup>d</sup>D227K) ablates TAP association (Ref. 5, and Fig. 7). Given previous results, which demonstrated that the binding of calreticulin to class I is TAP and tapasin independent (8, 24), calreticulin, like calnexin, seems to bind directly to class I. Thus it was of considerable interest whether the D227K α3 mutation ablated the interaction of class I with calnexin and calreticulin. As shown in Figure 7, this mutation disrupts calreticulin interaction

**FIGURE 3.** Calreticulin associates with the open conformation of the class I MHC H chain even in the absence of TAP. *Upper panel,* autoradiograph of 35S-methionine-labeled immunoprecipitates of open and folded forms of class I H chains made with mAb 64-3-7 and mAb 30-5-7, respectively. The mAb 11-4-1 (anti-K<sup>1</sup>) was used as a negative control. Lysates of RMA-S-L<sup>d</sup> and T2-L<sup>d</sup> cells were tested. *Lower panel,* coprecipitated calreticulin was identified by probing a Western blot of the L<sup>d</sup> immunoprecipitates with anti-calreticulin serum. Shown is an ECL enhanced luminescence image of an Ab-probed blot of the immunoprecipitations displayed above. The data shown are representative of two separate experiments that yielded similar results.

**FIGURE 4.** Calreticulin is responsive to peptide-induced folding of the class I MHC H chain in mouse cells, even in the absence of TAP. Immunoprecipitations with mAb 64-3-7 (specific for open forms) and with mAb 30-5-7 (specific for folded forms) were performed on RMA-S-L<sup>d</sup> cell lysates in the presence of increasing concentrations (0, 0.5, or 50 μM) of an L<sup>d</sup> peptide ligand from murine cytomegalovirus (YPHFMTPTNL) or in the presence of 50 μM negative control D<sup>d</sup>-binding influenza-derived peptide. Immunoprecipitations with mAb 30-5-7 (specific for folded L<sup>d</sup>) were also performed on T2-L<sup>d</sup> cell lysates in the presence of the same peptide concentrations (data not shown). Lanes labeled “Immunoprecipitations” are autoradiographs of 35S-methionine-labeled immunoprecipitates of open and folded forms of class I H chains made with mAb 64-3-7 and mAb 30-5-7, respectively. Coprecipitated calreticulin was identified by probing Western blots of the L<sup>d</sup> immunoprecipitations with anti-calreticulin serum. Lanes labeled “Western Blot” are ECL enhanced luminescence images of Ab-probed blots of the immunoprecipitations displayed above. The data shown are representative of two separate experiments that yielded similar results.

**FIGURE 5.** Calreticulin and tapasin are responsive to peptide-induced folding of the mouse L<sup>d</sup> class I MHC H chain in human cells, even in the absence of TAP. Immunoprecipitations with mAb 64-3-7 (specific for open forms) were performed on T2-L<sup>d</sup> cell lysates in the presence of increasing concentrations (0, 0.5, or 50 μM) of an L<sup>d</sup> peptide ligand from murine cytomegalovirus (YPHFMTPTNL) or in the presence of 50 μM negative control D<sup>d</sup>-binding influenza-derived peptide. Immunoprecipitations with mAb 30-5-7 (specific for folded L<sup>d</sup>) were also performed on T2-L<sup>d</sup> cell lysates in the presence of the same peptide concentrations (data not shown). Lanes labeled “Immunoprecipitations” are autoradiographs of 35S-methionine-labeled immunoprecipitates of open and folded forms of class I H chains made with mAb 64-3-7 and mAb 30-5-7, respectively. Coprecipitated tapasin and calreticulin were identified by probing Western blots of the L<sup>d</sup> immunoprecipitations with anti-tapasin or anti-calreticulin serum. Lanes labeled “Western Blot” are ECL enhanced luminescence images of Ab-probed blots of the immunoprecipitations displayed above. None of the 30-5-7- L<sup>d</sup> immunoprecipitates were found to be calreticulin or tapasin associated (data not shown). The data shown are representative of two separate experiments that yielded similar results.
with class I and does not interfere with the association between calnexin and class I (data not shown). Therefore, the α1 domain glycan and the α3 position 227 are both uniquely required for the association of the class I H chain with calreticulin, but not calnexin.

Overall, our data point to discrete roles for calreticulin and calnexin in class I assembly and expression. Our data extend earlier models (8, 17) of calnexin and calreticulin function by better distinguishing structural features necessary for class I binding. We have observed that calreticulin and calnexin bind to different sites on class I. More specifically, the α1 domain glycan and the α3 residue at position 227 both affect class I association with calreticulin and not calnexin. Importantly, the experiments showing the role of the α1 domain carbohydrate moiety were done by site-directed mutagenesis and not by chemical treatment, which would broadly affect protein glycosyl groups. In addition, we demonstrate that calreticulin is responsive to class I folding since it, unlike calnexin, preferentially binds to open conformations of class I. Furthermore, we show here that calreticulin’s sensitivity to peptide-induced folding is TAP independent.

Our data and those from other laboratories (17, 23) suggest sequential interaction of calnexin and calreticulin with class I. In this model, the open H chain initially associates with calnexin. Before interaction with the TAP/tapasin complex, the class I H chain exchanges calnexin for calreticulin. It should be noted that this switch from calnexin to calreticulin is not strictly governed by β2m assembly. Although β2m assembly is a requisite for H chain association with calreticulin, mouse and human H chains with or without β2m are detectable in association with calnexin (5, 61). It is likely that calnexin functions in the ER retention of free H chains. Indeed, this conclusion is strongly supported by the observation that calnexin retards class I transport in β2m-deficient cells (20). However, the data presented here, that calreticulin and tapasin are sensitive to peptide loading of class I, suggest that these proteins could be involved in ER retention of peptide-empty class I/β2m heterodimers. In total, these data show that the interactions of calreticulin with class I are structurally and functionally distinct from those of calnexin.

Note added in proof.

Consistent with findings reported here showing that calreticulin and calnexin interact with different glycan determinants, Q. Zhang and R. D. Salt (1998. J. Immunol. 160:831) added a second N-glycan at position 176 of the HLA-A*0201 molecule and found that this mutant interacted better with calnexin and less well with calreticulin.

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References


