Trimeric Interactions of the Invariant Chain and Its Association with Major Histocompatibility Complex Class II αβ Dimers*

(Received for publication, April 29, 1996, and in revised form, June 25, 1996)

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The invariant chain (I chain) associates with major histocompatibility complex class II αβ heterodimers upon synthesis, preventing them from binding peptides and unfolded proteins in the endoplasmic reticulum and directing class II transport to post-Golgi endosomal compartments. To assess which regions of the I chain are involved in binding class II molecules, we have studied proteolytic fragments of the I chain generated both by natural proteolytic degradation of αβ dimer-invariant chain complexes (αβI) within human B cells and by in vitro digestion of purified αβI complexes with proteinase K. The 18-kDa luminal I chain fragment generated by proteinase K, called K3, remains associated with αβ dimers and retains the complex (αβ-K3) in a high molecular mass nonameric configuration. The N terminus of the K3 fragment was identified as glycine 110. This indicates that the K3 fragment lies outside of the class II-associated invariant chain peptide region (amino acids 81–104) of the I chain, shown to be important for initial αβI assembly. An N-terminal 12-kDa I chain fragment called p12, generated intracellularly, was also analyzed and was found to remain associated with αβ dimers in a high molecular mass form analogous to the nonameric αβI complex. These results demonstrate that at least two class II contact points exist along the length of the I chain and that different regions of the I chain can stabilize the αβI nonamer. Additional evidence suggests that the O-linked glycan(s) characteristic of the I chain is added to the short C-terminal region absent from the K3 fragment.

Molecules encoded by the major histocompatibility complex (MHC) genes serve to display peptides processed from endogenous or exogenous protein antigens to T cells. This process leads to T cell activation and amplification of both cellular and humoral immune responses to foreign antigen. MHC class I molecules bind peptides in the endoplasmic reticulum (ER) that are delivered from the cytoplasm by the transporters associated with antigen processing (reviewed in Ref. 1). In contrast, MHC class II molecules associate with the invariant chain (I chain) upon synthesis in the ER (2–4). This association prevents the class II αβ heterodimer from binding peptides and unfolded proteins during transport of αβ dimer-invariant chain complexes (αβI) from the ER through the Golgi apparatus (5–10). Inhibition of binding appears to result from the interaction of the class II-associated I chain peptide (CLIP) region of the I chain with the peptide-binding groove of the MHC class II molecule (11–13).

The I chain associates with class II molecules in the ER to form a nine-chain (nonameric) complex containing three I chain molecules and three αβ dimers (14). After assembly with the assistance of the chaperone calnexin (15, 16), the nonameric complex exits the ER, is transported through the Golgi apparatus, and localizes to a compartment in the endocytic pathway. A percentage of αβI complexes may transport to the cell surface and rapidly internalize before localizing in this compartment (17). There is an endosomal localization signal encoded within the cytoplasmic tail of the I chain (18). The exact nature of the class II molecule-containing endocytic compartments is not yet clear, but they are biochemically distinct from classical endosomes or lysosomes (19–21). Class II αβ dimers, the I chain, and endocyotoxed antigen colocalize in these compartments (22–25). It is possible that the I chain encodes additional localization signals elsewhere within the molecule. The four different forms of the human I chain (p33, p35, p41, and p43) may regulate the localization of class II molecules in distinct endocytic compartments (26–28).

Once αβI complexes reach late endocytic compartments, the I chain is degraded through a series of proteolytic steps in which cathepsins B and D have been implicated (29–32). After removal of the I chain, αβ dimers are capable of binding processed peptides and of being transported to the cell surface. A nested set of I chain peptides, from amino acid residues 81 to 104 in the human system, can be found associated with both human and mouse class II molecules (33–36). This is the CLIP region of the I chain, which seems to be important for initial assembly of αβI complexes (37, 38) and the removal of which is mediated by HLA-DM molecules (39–41).

We initiated these experiments to determine how the I chain interacts with αβ dimers and what regions are important in forming the nonameric αβI structure. We have previously demonstrated that αβI complexes remain nonameric until and even during endosomal proteolytic degradation of the I chain (42). To further characterize sites within the I chain that are important for contact with the class II αβ dimer, we have analyzed additional I chain fragments formed in vivo and in vitro.
Materials and Methods

Cells—The human B-lymphoblastoid cell line Pala (HLA-DR3) was grown in Iseove’s modified Dulbecco’s medium supplemented with 25% bovine calf serum (Life Technologies, Inc.). For generation of αβ I complexes, Pala cells (5 x 10^6) were radiolabeled with 0.25 mCi of [35S]methionine (Tran35S-label, ICN Radiochemicals, Irvine, CA) in 1 ml of methionine-free Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 3% dialyzed fetal calf serum and 20 µg/ml gentamicin-Sulfate (Life Technologies, Inc.) for 6 h at 37°C in the presence of 10 µM monensin (Boehringer Mannheim). In some experiments, cells were labeled in the presence of 1 mM leupeptin (Sigma). For generation of αβp12 complexes, Pala cells were first starved in methionine-free medium, pulse-labeled as described above for 30 min in methionine-free medium, and then chased in medium containing excess unlabeled methionine for 3 h.

Antibodies—The hybridoma cell line L243 (anti-DR mAb) (43) was obtained from American Type Culture Collection (Rockville, MD). DIV6.1 (anti-DR α chain mAb) (44) was a generous gift of Dr. V. van Heyningen (Medical Research Council Clinical and Population Genetics Unit, Edinburgh, Scotland). B145 (anti-invariant chain C-terminal mAb) was obtained from Binding Site, Inc. (San Diego, CA). The anti-peptide mAb PIN.1 (amino acids 12-28 of the I chain) (45) represents free K3 fragment, no longer associated with I chain. PIN.1 complexes were purified from the HLA-DR3 homozygous B-lymphoblastoid cell line Pala and digested with proteinase K (Fig. 2). To 10 µg/ml of the material eluting at 18 kDa referred to as K3 (Fig. 1). The K3 fragment of the I chain remained associated with MHC class II αβ dimers. This fragment contains the N-linked glycans at positions 114 and 120, but not O-linked glycan. The data showed that a short segment of the C terminus was also removed (46). These results suggested that the N terminus of the K3 fragment was near or within the CLIP region (amino acids 81-104) of the I chain. To precisely define the N terminus of K3, metabolically labeled αβ I complexes were purified from the HLA-DR3 homozygous B-lymphoblastoid cell line Pala and digested with proteinase K (Fig. 2). At 10 µg/ml proteinase K digestion yields a single I chain fragment of ~18 kDa that remains associated with αβ dimers. The α and β chains are unaffected by proteinase K at this concentration. N-terminal sequencing of the K3 fragment, after transfer to polyvinylidene difluoride membrane, gave the results shown in Fig. 3. [35S]Methionine peaks were detected at cycles 3, 12, and 18 that aligned with only one region in the I chain sequence and mapped the N terminus of the K3 fragment to the glycine residue at position 110. From the known N terminus of K3 and from the fact that it has lost a C-terminal segment of the I chain, at least 8 of the 14 methionine residues present in the intact I chain are absent from K3. This accounts for the reduced intensity of K3 compared with the I chain in Fig. 2.

Size Exclusion Analysis of αβ K3 Complexes—Purified αβ I exists as a nonameric complex (14). To determine whether αβ K3 complexes retain the nonameric structure, purified αβ I was digested with proteinase K and injected over a HPSEC column in the detergent CHAPS. Fractions were then immunoprecipitated with the monoclonal antibody BU45, specific for a luminal epitope within the I chain. The results shown in Fig. 4 show that αβ K3 complexes elute in a broad peak at ~17.5-ml elution volume (V_e). This V_e is only slightly greater than that determined for αβ I complexes in CHAPS (17.25 ml) and suggests that the K3 fragment of the I chain can retain the nonameric association with αβ dimers. The peak at 19.75 ml represents free K3 fragment, no longer associated with αβ dimers. Purified αβ K3 further demonstrated that the material eluting at 17.5 ml represents a nonameric complex of three αβ dimers and three K3 fragments, proteinase K-digested material was separated by SDSPAGE and treated with the chemical cross-linker.
DTSP prior to immunoprecipitation (Fig. 5). Following cross-linking, the material eluting at 17.5 ml has an apparent molecular mass of $\sim 200$ kDa by SDS-PAGE. This is consistent with $\alpha_b K_3$ existing as a nonameric complex. It should be noted that $\alpha_b K_3$ cross-links extremely efficiently, as does $\alpha_b I$ (14). K3 trimers, however, cross-link poorly compared with I chain trimers (47). Thus, the regions of the I chain lost from K3 are important for efficient cross-linking to itself, but not to $\alpha$ and $\beta$ chains.

**Appearance of the p12 Fragment during Intracellular I Chain Proteolysis—** Evidence suggests that once $\alpha_b I$ complexes reach the appropriate endosomal/lysosomal compartment, the I chain is proteolytically digested in a stepwise fashion (29, 48). Human B-lymphoblastoid cells grown in the presence of the sulfhydryl protease inhibitor leupeptin accumulate a partial proteolytic fragment of the I chain called LIP (29). This fragment is $\sim 21$ kDa and includes the N-terminal cytoplasmic region of the I chain as well as a portion of the luminal region including the N-linked glycans at positions 114 and 120 (29–32). Pulse-chase analysis of untreated melanoma cells suggests that the class II-associated LIP fragment is subsequently cleaved by a leupeptin-sensitive protease to a 12-kDa fragment called p12 (49). To confirm this result in the human B-lymphoblastoid cell line, the HLA-DR3 homozygous Pala cells were pulse-labeled for 30 min with $[^{35}S]$methionine and then chased for 3 h. Immunoprecipitation of detergent-solubilized cell lysate (Fig. 6) demonstrates that anti-I chain antibodies (PIN.1.1 and R1.EQLP) precipitate $\alpha_b$ dimers in association with the intact I chain. In addition, these antibodies both recognize two smaller fragments of the I chain at $\sim 12$ and 10 kDa. Immunoprecipitation with the anti-DR monoclonal antibody L243, which recognizes a combinatorial epitope of the $\alpha$ and $\beta$ chains, demonstrates that only the 12-kDa (p12) fragment of the I chain is associated with MHC class II $\alpha_b$ dimers. L243 does not recognize $\alpha_b$ dimers in association with the intact I chain.

**Size Exclusion of $\alpha_b p12$ Complexes—** HPSEC was performed to determine whether $\alpha_b p12$ complexes, like $\alpha_b$LIP and $\alpha_b K_3$, remain associated with $\alpha_b$ dimers in a nonameric complex. Pala cells were pulse-labeled for 30 min with $[^{35}S]$methionine and then chased for 3 h. Immunoprecipitation of detergent-solubilized cell lysate (Fig. 6) demonstrates that anti-I chain antibodies (PIN.1.1 and R1.EQLP) precipitate $\alpha_b$ dimers in association with the intact I chain. In addition, these antibodies both recognize two smaller fragments of the I chain at $\sim 12$ and 10 kDa. Immunoprecipitation with the anti-DR monoclonal antibody L243, which recognizes a combinatorial epitope of the $\alpha$ and $\beta$ chains, demonstrates that only the 12-kDa (p12) fragment of the I chain is associated with MHC class II $\alpha_b$ dimers. L243 does not recognize $\alpha_b$ dimers in association with the intact I chain.
HPSEC columns in CHAPS. Fractions were collected and immunoprecipitated with L243. The results in Fig. 7 show a large peak eluting at 18.75 ml that contains α and β chains and represents mature αβ dimers. In addition, L243 immunoprecipitated a smaller peak of αβ dimers associated with the p12 I chain fragment, which eluted at 17.5 ml. This Ve is only slightly larger than that reported for αβ/I in CHAPS, suggesting that the p12 fragment retains higher order nonameric complexes with αβ dimers. To examine this further, the same experiment was performed using the detergent n-octyl glucoside for HPSEC. In octyl glucoside, αβ/LIP complexes have been shown to dissociate from nonamers into trimeric complexes containing one α, one β, and one LIP molecule (42). HPSEC of αβ/p12 complexes in octyl glucoside (Fig. 8) showed that these complexes also dissociate from higher order nonamers (Ve = 18 ml) into complexes eluting at 19.5 ml, which probably represent αβ/p12 trimers.

**Fig. 4.** Size exclusion analysis of αβ/K3 complexes. αβ/K3 complexes, generated as described for Fig. 2, were equilibrated in 0.6% CHAPS and injected over HPSEC columns as described under “Materials and Methods.” Fractions were collected according to Ve and immunoprecipitated with the BU45 antibody. The positions of MHC class II α and β chains and the K3 fragment are indicated to the left of the gel. The positions of the molecular mass standards are indicated in kilodaltons to the right of the gel. Radioactivity in the protein bands was quantitated by PhosphorImager analysis in arbitrary units to generate the traces shown beneath the gel.

**Fig. 5.** Cross-linking of HPSEC-purified αβ/K3 complexes. αβ/K3 complexes were fractionated by HPSEC as described for Fig. 4. Each fraction was chemically cross-linked with DTSP prior to immunoprecipitation with BU45. The position of cross-linked αβ/K3 is indicated to the left of the gel. The molecular mass standards are indicated in kilodaltons to the right of the gel.

**Fig. 6.** Invariant Chain Interaction with MHC Class II αβ Dimers
enrich for αβp12 complexes, were immunoprecipitated with PIN.1.1, and the precipitated material was treated with neuraminidase and then with O-glycanase to remove O-linked glycans. The results in Fig. 9A demonstrate that although the intact I chain drops slightly in molecular mass after O-glycanase treatment, the p12 fragment does not. The two left lanes are a longer exposure of the two right lanes in order to visualize the p12 fragment. The two right lanes are a shorter exposure to visualize the drop in molecular mass of the intact I chain. These data indicate that the p12 fragment does not contain O-linked glycans.

The LIP fragment of the I chain was analyzed in the same fashion as p12. The C terminus of the LIP fragment is C-terminal to the N-linked glycosylation site at position 120 (see Fig. 1). Pala cells labeled in the presence of leupeptin were lysed and immunoprecipitated with PIN.1.1. Immunoprecipitated proteins were treated with neuraminidase followed by O-glycanase. The results in Fig. 9B show a slight drop in molecular mass of the LIP fragment after removal of sialic acid, but no further reduction after treatment with O-glycanase. The reduction in molecular mass after neuraminidase treatment is attributable to removal of sialic acid residues from the N-linked glycans present on the LIP fragment at positions 114 and 120. As a control, CD45 was immunoprecipitated from the same cell lysate and treated with neuraminidase and O-glycosidase. This heavily O-glycosylated protein showed a large drop in molecular mass.

FIG. 6. Appearance of the class II-associated p12 fragment of the I chain during intracellular proteolytic degradation. Pala cells were pulse-labeled with [35S]methionine for 30 min and chased for 2 h in medium containing excess unlabeled methionine. Cells were then lysed in TS buffer containing 2% C12E9 and immunoprecipitated as described under “Materials and Methods” with PIN.1.1 (anti-I chain N-terminal mAb), L243 (anti-DR αβ chain mAb), or R.I.QLP (rabbit antiserum against the I chain N terminus). The immunoprecipitates were analyzed by 13% SDS-PAGE and fluorography. The positions of the α chain, β chain, I chains, and p12 fragment are indicated to the left of the gel. The positions of the molecular mass markers are indicated in kilodaltons to the right of the gel.

FIG. 7. HPSEC of αβp12 complexes in CHAPS. Pala cells were labeled to enrich for αβp12 as described for Fig. 6 and then lysed and equilibrated in 0.6% CHAPS. Lysates were separated by HPSEC in 0.6% CHAPS, and fractions were collected (Ve; indicated along the top of the gel). Each fraction was then immunoprecipitated with L243 ascites. The immunoprecipitated material was analyzed by 13% SDS-PAGE. Shown beneath the gel are traces of α chain, β chain, and p12 fragment signals in each lane of the gel generated by PhosphorImager analysis and reported in arbitrary units. The positions of the α chain, β chain, and p12 I chain fragment are indicated to the left of the gel. The positions of the molecular mass standards are indicated in kilodaltons to the right of the gel.
lar mass after treatment (data not shown). These data demonstrate that, at least in human B cells, little or no I chain is modified by O-glycosylation in proximity to the plasma membrane. Combined with the earlier data, this argues that the O-linked glycan(s) of the I chain are close to the C terminus, within the region cleaved from the K3 fragment.

**DISCUSSION**

The data presented here demonstrate that at least two sites along the length of the I chain interact with the MHC class II αβ dimer. Analysis of natural and in vitro generated fragments of the I chain also reveals that these fragments remain associated with class II molecules in higher order multimers analogous to nonameric αβ-I complexes. In vitro digestion of purified αβI with proteinase K yields an 18-kDa I chain fragment (K3) containing most of the C-terminal luminal portion of the I chain (46). The N terminus of this fragment was mapped to amino acid 110. Size exclusion (Fig. 4) and cross-linking (Fig. 5) analyses suggest that the K3 fragment remains associated with DR molecules in a nonameric complex. With its N terminus at position 110, the K3 fragment does not include the CLIP region of the I chain extending from positions 81 to 104. Nested sets of I chain peptides from this region have been isolated from mature class II molecules in mouse and human cells (33–36). Evidence suggesting that CLIP is bound in the class II peptide-binding groove (11, 12) has been confirmed by x-ray crystallographic analysis (13), and the CLIP region has recently been shown by deletion analysis to be critical for initial assembly of αβ and I chains (37, 38). However, it is clear from the data presented here that once the αβI complex is formed, a site distal to the CLIP region can maintain the interaction with class II molecules. It should be noted that αβ-K3 complexes are isolated from monensin-treated cells and therefore have immature N-linked glycans. It is conceivable that this may contribute to the stability of the nonameric αβ-K3 complex.

A second site of I chain interaction with class II molecules is present in the p12 fragment. This is a 12-kDa fragment that is transiently generated within a class II-expressing cell during proteolytic degradation of the I chain (49). It includes the N-terminal cytoplasmic domain and, based on its apparent molecular mass by SDS-PAGE, the transmembrane region. The precise C terminus of p12 is not known, but its apparent size on SDS-PAGE leads to a predicted C terminus around amino acids 100–110, consistent with its lack of N-linked glycans. If this prediction is correct, then the p12 fragment should include at least a part of the CLIP region (amino acids 81–104) of the I chain. It is possible, however, that because this fragment is relatively small and includes the transmembrane segment, it might bind an aberrant number of SDS molecules,
disrupting the charge/mass ratio. An anti-CLIP antiserum failed to react detectably with p12 (data not shown), but the same serum reacted only weakly with the intact I chain, and the presence or absence of all or part of the CLIP sequence in p12 remains unknown. If the CLIP region is associated with the peptide-binding groove of class II molecules, it is likely to be protected from proteolysis. It is therefore tempting to speculate that p12 contains CLIP, while p10, which fails to bind class II molecules (Fig. 6), does not.

This report, in combination with data on the LIP fragment of the I chain (42), has detailed the analysis of fragments that cover nearly the entire length of the I chain. p12 represents a small N-terminal fragment. LIP is also a N-terminal fragment, but includes a portion of the luminal domain including the CLIP region. Finally, K3 is a C-terminal fragment that overlaps LIP, but does not include the CLIP region. None of these fragments contain the O-linked glycan(s) characteristic of the intact I chain. Based on the proposed N and C termini of all the fragments (Fig. 1), it seems likely that the O-linked glycan is close to the C terminus of the intact I chain, a portion of which is lacking in K3 (46). All three of the I chain fragments remain associated with MHC class II molecules in a nonameric structure similar to that reported for αβ1 complexes (14). Recombinant I chain constructs that include the C-terminal region have previously been shown to be trimeric (50). The data presented here and elsewhere (19) argue that the N-terminal regions of the I chain also interact to form a trimeric structure. This interaction appears to be variably stable in solution depending upon the detergent used. Thus, both αβLIP and αβp12 retain a nonameric structure in CHAPS, but appear to dissociate into simple trimers in n-octyl glucoside (Figs. 6 and 8) (6). This suggests a possible interaction of the I chain transmembrane regions. The requirement for C-terminal interactions to maintain a stable I chain trimer in conventional detergents may explain the observations of a number of groups that C-terminally truncated I chain constructs fail to trimerize, as detected by chemical cross-linking, when expressed in vivo (38, 51). Alternatively, the C-terminal region may be a primary site for efficient cross-linking. Data from Wiley and co-workers (50, 52) indicate, based on NMR analysis, that the region of the I chain between the membrane and position 118 is extended and that this region is highly susceptible to proteolysis. Thus, a picture is emerging where the I chain interacts at both the N and C termini to generate a trimeric structure, and a class II αβ dimer interacts with each component of the trimer at the CLIP region via the peptide-binding groove and in an unknown fashion with the C-terminal region (53). Whether additional interactions between the class II molecules and the I chain exist, perhaps via the transmembrane region or the cytoplasmic domains, remains unknown.

Acknowledgments—We thank Sebastian Amigorena and Ira Mellman for assistance with the PhosphoImager analysis and also for helpful discussions. We thank Kathy Stone and Ken Williams for N-terminal Edman degradation analysis, B. Arunachalam for help with illustrations, and Nancy Demetios for assistance in preparation of this manuscript.

REFERENCES
1. Germain, R. N., and Margulies, D. M. (1993) Annu. Rev. Immunol. 11, 403–450
2. Strubin, M., Berte, C., and Mach, B. (1986) EMBO J. 5, 3483–3488
3. Machamer, C. E., and Cresswell, P. (1982) J. Immunol. 129, 2564–2569
4. Kvist, S., Wiman, K., Claesson, L., Peterson, P. A., and Dobberstein, B. (1982) Cell 29, 61–69
5. Roche, P. A., and Cresswell, P. (1990) Nature 345, 615–618
6. Newcomb, J. R., and Cresswell, P. (1993) J. Immunol. 150, 499–507
7. Teyton, L., O’Sullivan, D., Dickson, P. W., Lotteau, V., Sette, A., Fink, P., and Peterson, P. A. (1990) Nature 348, 39–44
8. Roche, P. A., Teletski, C. L., Karp, D. R., Pinet, V., Bakke, O., and Long, E. O. (1992) EMBO J. 11, 2841–2847
9. Demetz, S. (1993) Eur. J. Immunol. 23, 2100–2108
10. Busch, R., Cloutier, I., Sékaly, R.-P., and Hammerling, G. J. (1996) EMBO J. 15, 418–428
11. Avva, R., and Cresswell, P. (1994) Immunity 1, 763–774
12. Bangia, N., and Watts, T. H. (1995) Int. Immunol. 7, 1585–1591
13. Ghosh, P., Amaya, M., Mellins, E., and Wiley, D. C. (1995) Nature 378, 457–462
14. Roche, P. A., Marks, M. S., and Cresswell, P. (1991) Nature 354, 392–394
15. Anderson, K. S., and Cresswell, P. (1994) EMBO J. 13, 675–682
16. Schreiber, K. L., Bell, M. P., Huntson, C. J., Bajajopalan, S., Brenner, M. B., and McKean, D. J. (1994) Int. Immunol. 6, 101–111
17. Roche, P. A., Teletski, C. L., Stang, E., Bakke, O., and Long, E. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6581–6585
18. Bakke, O., and Dobberstein, B. (1990) Cell 63, 707–716
19. Amigorena, S., Drake, J. R., Webster, P., and Mellman, I. (1994) Nature 369, 113–120
20. Tulp, A., Verwoerd, D., Dobberstein, B., Ploegh, H. L., and Pieters, J. (1994) Immunity 1, 63–70
21. Roche, P. A., and Cresswell, P. (1990) Nature 345, 615–618
22. Newcomb, J. R., and Cresswell, P. (1993) J. Immunol. 150, 499–507
23. Teyton, L., O’Sullivan, D., Dickson, P. W., Lotteau, V., Sette, A., Fink, P., and Peterson, P. A. (1990) Nature 348, 39–44
24. Roche, P. A., Teletski, C. L., Karp, D. R., Pinet, V., Bakke, O., and Long, E. O. (1992) EMBO J. 11, 2841–2847
25. Demetz, S. (1993) Eur. J. Immunol. 23, 2100–2108
26. Busch, R., Cloutier, I., Sékaly, R.-P., and Hammerling, G. J. (1996) EMBO J. 15, 418–428
27. Avva, R., and Cresswell, P. (1994) Immunity 1, 763–774
28. Bangia, N., and Watts, T. H. (1995) Int. Immunol. 7, 1585–1591
29. Ghosh, P., Amaya, M., Mellins, E., and Wiley, D. C. (1995) Nature 378, 457–462
30. Roche, P. A., Marks, M. S., and Cresswell, P. (1991) Nature 354, 392–394
31. Anderson, K. S., and Cresswell, P. (1994) EMBO J. 13, 675–682
32. Schreiber, K. L., Bell, M. P., Huntson, C. J., Bajajopalan, S., Brenner, M. B., and McKean, D. J. (1994) Int. Immunol. 6, 101–111
33. Roche, P. A., Teletski, C. L., Stang, E., Bakke, O., and Long, E. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6581–6585
34. Bakke, O., and Dobberstein, B. (1990) Cell 63, 707–716
35. Amigorena, S., Drake, J. R., Webster, P., and Mellman, I. (1994) Nature 369, 113–120
36. Tulp, A., Verwoerd, D., Dobberstein, B., Ploegh, H. L., and Pieters, J. (1994) Immunity 1, 63–70

Fig. 9. The p12 and LIP fragments of the I chain do not contain O-linked glycans. A, Pala cells were pulsed with [35S]methionine and then chased for 6 h in the presence of 1 mM leupeptin to accumulate αβp12 complexes. These cells were then lysed and immunoprecipitated with PIN.1.1 ascites. The immunoprecipitated material was treated with 25 milliliters of neuraminidase and then with (+ O-GLY) or without (-O-GLY) 2 milliliters of O-glycosidase. Proteins were analyzed by 13% SDS-PAGE. The two left lanes are a long exposure to visualize the p12 fragment. The two right lanes are the identical lanes are a long exposure to visualize the p12 fragment. The two right lanes are the identical lanes.
Nature 369, 120–126
21. West, M. A., Lucocq, J. M., and Watts, C. (1994) Nature 369, 147–151
22. Cresswell, P. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 8188–8192
23. Lamb, C. A., Yewdell, J. W., Bennink, J. R., and Cresswell, P. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5998–6002
24. Pletscher, M., and Permis, B. (1983) Eur. J. Immunol. 13, 581–584
25. Guagliardi, L. E., Koppelman, B., Blum, J. S., Marks, M. S., Cresswell, P., and Brodsky, F. M. (1990) Nature 343, 133–139
26. O’ Sullivan, D. M., Noonan, D., and Quaranta, V. (1987) J. Exp. Med. 166, 444–469
27. Peterson, M., and Miller, J. (1992) Nature 357, 596–598
28. Arunachalam, B., Lamb, C. A., and Cresswell, P. (1993) Int. Immunol. 6, 439–451
29. Blum, J. S., and Cresswell, P. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 3975–3979
30. Neefjes, J. J., and Ploegh, H. L. (1992) EMBO. J. 11, 411–416
31. Roche, P. A., and Cresswell, P. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3150–3154
32. Reyes, V. E., La S., and Humphreys, R. E. (1991) J. Immunol. 146, 3877–3880
33. Elberdy, J. M., Newcomb, J. B., Surman, M. J., Barbosa, J. A., and Cresswell, P. (1992) Nature 360, 474–477
34. Sette, A., Ceman, S., Kubo, R. T., Sakaguchi, K., Appella, E., Hunt, D. F., Davis, T. A., Michel, H., Shabanowitz, J., Rudersdord, R., Grey, H. M., and DeMars, R. (1992) Science 258, 1801–1804
35. Rudensky, A. Y., Prento-Hurleth, P., Hong, S.-C., Barlow, A., and Janeway, C. A., Jr. (1991) Nature 353, 622–627
36. Chicz, R. M., Urban, R. G., Lane, W. S., Gorga, J. C., Stern, L. J., Vignali, D. A. A., and Strominger, J. L. (1992) Nature 358, 764–768
37. Freisewinkel, I. M., Schenck, K., and Koch, N. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9703–9706
38. Bijmakers, M. J. E., Benaroch, P., and Ploegh, H. L. (1994) J. Exp. Med. 180, 623–629
39. Denzin, L. K., and Cresswell, P. (1995) Cell 82, 155–165
40. Sloan, V. S., Cameron, P., Porter, G., Gammon, M., Amaya, M., Mellins, E., and Zaller, D. M. (1995) Nature 373, 802–806
41. Sherman, M. A., Weber, D. A., and Jensen, P. E. (1995) Immunity 3, 197–205
42. Newcomb, J. R., and Cresswell, P. (1993) J. Immunol. 151, 4153–4163
43. Lampson, L. A., and Levy, R. (1988) J. Immunol. 125, 293–299
44. Guy, K., Van Heyningen, V., Cohen, B. B., Dean, D. L., and Steel, C. M. (1982) Eur. J. Immunol. 12, 942–948
45. Forath, J., Aspergerrg, K., Drevis, H., and Axen, R. (1973) J. Chromatogr. 86, 53–58
46. Marks, M. S., and Cresswell, P. (1985) J. Immunol. 136, 2519–2525
47. Marks, M. S., Blum, J. S., and Cresswell, P. (1990) J. Cell Biol. 111, 839–855
48. Maric, M. A., Taylor, M. D., and Blum, J. S. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2171–2175
49. Pieters, J., Horstmann, H., Bakke, O., Griffiths, G., and Lipp, J. (1991) J. Cell Biol. 115, 1213–1223
50. Park, S. J., Sudegh-Nasser, S., and Wiley, D. C. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 11289–11293
51. Bertolino, P., Staschewski, M., Trescol-Biémont, M.-C., Freisewinkel, I. M., Schenck, K., Christien, I., Forquet, F., Gerlier, D., Rabourdin-Combe, C., and Koch, N. (1995) J. Immunol. 154, 5620–5629
52. Jasanoff, A., Park, S.-J., and Wiley, D. C. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9900–9904
53. Cresswell, P. (1996) Cell 84, 505–507
54. Laemmli, U. K. (1970) Nature 227, 680–685
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*J. Biol. Chem.* 1996, 271:24249-24256.
doi: 10.1074/jbc.271.39.24249

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