Major Histocompatibility Complex Class II Compartments in Human B Lymphoblastoid Cells Are Distinct from Early Endosomes

By Peter J. Peters,* Graça Raposo,* Jacques J. Neefjes,‡ Viola Oorschot,* Richtje L. Leijendekker,* Hans J. Geuze,* and Hidde L. Ploegh§

*From the *Laboratory of Cell Biology, Medical School, University of Utrecht, 3584 CX Utrecht, The Netherlands; ‡Department of Cellular Biochemistry, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands; and §Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

Summary

In human B lymphoblastoid cell lines, the majority of major histocompatibility complex (MHC) class II heterodimers are located on the cell surface and in endocytic compartments, while invariant chain (Ii)-associated class II molecules represent biosynthetic intermediates which are present mostly in the endoplasmic reticulum and Golgi complex. To investigate the origin of the MHC class II–positive compartments and their relation to early endosomes, the intracellular distribution of MHC class II molecules and Ii in relation to endocytic tracers was studied in human lymphoblastoid B cells by immunoelectronmicroscopy on ultrathin cryosections. Cross-linking of surface immunoglobulins, followed by a brief period of internalization of the immune complexes, did not alter the intracellular distribution of MHC class II molecules. While early endosomes were abundantly labeled for the cross-linked immunoglobulins, <1% of total MHC class II molecules were detectable in early endosomes. MHC class II– and Ii-positive structures associated with the *trans*-Golgi network can be reached by endocytosed bovine serum albumin (BSA)-gold conjugates after 30 min of internalization. Prolonged exposure to BSA-gold allowed visualization of later endocytic compartments, in which a progressive loss of Ii was observed: first the lumenal portion, and then the cytoplasmic portion of Ii escaped detection, culminating in the formation of MHC class II–positive compartments (MIIC) devoid of Ii. The loss of Ii also correlated with a transition from a multivesicular to a multilaminar, electron-dense MIIC. The intracellular compartments in which class II molecules reside (MIIC) are therefore a heterogeneous set of structures, part of the later aspects of the endocytic pathway.

In human B lymphoblastoid cells, newly synthesized MHC class II molecules enter the endocytic pathway during biosynthesis, before their arrival at the cell surface (1–3). In the endocytic pathway, antigenic peptides are generated by proteolysis and are made available for binding to MHC class II molecules. However, the exact location of class II molecules in the endosomal/lysosomal route in this cell type is under debate (4–6). Here we report that the distribution of MHC class II molecules in the human B lymphoblastoid cell line IM9 (4) is indistinguishable from that previously reported (5) for the JY cell line, and that we cannot detect MHC class II molecules in early endosomes at levels >1% of total class II content. Colocalization of MHC class II molecules with endocytosed Ig in early endosomes was reported by Guagliardi et al. (4) for the human B cell line IM9, using immunogold labeling of osmium-fixed, plastic-embedded cells. Peters et al. (5), using ultrathin cryosections, reported the presence of class II molecules in lysosomal structures (referred to as MHC class II compartments [MIIC][1]) in the human B cell line JY (5). The discrepancy between these studies might be explained by cell type–specific differences (6, 7), or by exposure of the B cells to anti-Ig reagents to induce internalization of surface Ig in the former (4) but not in the latter (5) study (6, 7). The use of such reagents entails the risk

P. J. Peters and G. Raposo contributed equally to this report.

[1] *Abbreviations used in this paper:* CIIV, MHC class II–enriched fraction; ICC, invariant chain carboxy terminus; Ii, invariant chain; ICN, invariant chain amino terminus; Lamp-1, lysosomal-associated membrane protein; MIIC, MHC class II compartment; MPR, mannose-6-phosphate receptor; Tf/HRP, transferrin–horseradish peroxidase; TGN, *trans*-Golgi network.

of false positive labeling. We therefore examined the distribution of MHC class II molecules in ultrathin frozen sections from the IM9 cell line and found no evidence for the presence of class II molecules in early endosomes. The point of entry of class II molecules in the endocytic route and the relative position of MIIC in the antigen processing pathway have not been adequately resolved. This issue is of relevance in light of the proposed function of the class II–associated invariant chain (Ii). The Ii chain targets class II molecules to endocytic structures and dissociates from class II molecules soon after the $\alpha\beta$Ii complex has left the Golgi. Release of Ii from the $\alpha\beta$ heterodimer is a prerequisite for peptide binding. To explore in more detail the site of encounter of endocytosed materials with class II molecules in relationship to removal of Ii, we followed in parallel the fate of both endocytic tracers and Ii. We provide evidence that endocytic tracers contact class II molecules in a compartment that is still positive for Ii, while the MIIC in its mature form, the most abundant source of intracellular class II molecules, is devoid of Ii. We conclude that antigen and class II molecules may first meet in *trans*-Golgi network (TGN)-associated, Ii-positive structures, from which MIIC are developed, a process accompanied by destruction of the Ii chain.

Materials and Methods

Cells. The human B cell line IM9 was obtained from the American Type Culture Collection (Rockville, MD) and maintained in RPMI 1640 medium supplemented with 10% (vol/vol) FCS, 2 mM glutamine, and penicillin/streptomycin at 100 IU and 100 μg/ml, respectively. The human B cell line JY has been described (2, 5).

Immunostaining. Before incubation with a transferrin–horseradish peroxidase (Tf/HRP) conjugate (8), to deplete the cells of transferrin, the cells were maintained in serum-free RPMI 1640 medium for two consecutive incubations of 20 min each. The cells were then incubated with 25 μg/ml of Tf/HRP (8) for 5 min or 10 min at 37°C, followed by fixation in 0.5% glutaraldehyde in 0.1 M phosphate buffer at 37°C, and processed for electron microscopy as described (5). MHC class II molecules were labeled with rabbit anti–class II α serum (2, 5) at a dilution of 1:600. HRP was immunolabeled with anti-HRP (Sigma Immunochemicals, St. Louis, MO) at a dilution of 1:300. Antisera directed against the invariant chain carboxy terminus (ICC) and invariant chain amino terminus (ICN) were used at a dilution of (1:200). ICC and ICN have been generated by Phil Morton (Monsanto Chemical Co., St. Louis, MO). For ICC and ICN, a 0.2% glutaraldehyde fixative for 30 min was used. Cathepsin D antibodies have been described (5). Immunoglobulins were visualized using the protein A–gold technique (9). Surface immunoglobulins were labeled by a sandwich procedure essentially as described by Guagliardi et al. (4). However, to eliminate the risk of false positive labeling, endocytosed Ig was visualized after fixation, rather than by using prelabeled anti-Ig reagents during endocytosis. Specifically, IM9 cells were washed twice with PBS and were incubated with rabbit anti–human κ light chain immunoglobulin (Central Laboratories of the Red Cross Blood Transfusion Service Amsterdam, The Netherlands) diluted 1:40, for 30 min at 0°C. Unbound Ig were removed by washing with PBS at 0°C. Cells were then transferred to 37°C for 2 or 10 min and fixed with glutaraldehyde as described above. Ultrathin sections were immunolabeled with swine anti–rabbit immunoglobulins (dilution 1:3,000; Nordic Immunological Laboratories, Capistrano Beach, CA) and protein A–gold. BSA-gold was applied to the cells for 10 min and chased for 20 min as described. Sections were processed for and examined by electron microscopy as described (5).

Results

To examine whether class II molecules localize to compartments in which cross-linked surface Ig is found after a brief period of internalization, we examined the B lymphoblastoid cell line IM9 and looked for the presence of class II molecules in early endosomes. After immunolabeling of ultrathin cryosections prepared from IM9, we observed a subcellular distribution (including endoplasmic reticulum [ER] and Golgi region) for MHC class II molecules that was very similar to that observed in JY cells (2, 5). We labeled early endosomes in IM9 cells (4) by internalization of a Tf/HRP (10) for 5 min (Fig. 1, *A–C*). The cell surface was labeled for MHC class II molecules (Figs. 1 *A* and 2, *A* and *B*) and also Tf/HRP (Fig. 1 *B*). Tf/HRP, but no MHC class II, was present in coated pits (Fig. 1 *B*) and in early endosomes (Fig. 1 *A–C*) (8). The level of class II staining (measured as gold particles per square micrometer) observed in early endosomes of IM9 cells and JY cells (5) did not exceed 1% of the total. No Tf/HRP could be detected in MIIC. We next labeled early endosomes by an alternative method (4). Surface immunoglobulins were cross-linked with heterologous antibodies in a three-step sandwich procedure (at 0°C) and allowed to internalize for 2 min at 37°C (4) (Fig. 2). Early endosomal structures contained heterologous Ig, as reported (4), but no class II molecules were observed in the immunoglobulin-positive endosomes.

In contrast to the earlier report (4), early endosomes, as defined by internalization of Tf/HRP (8), were negative for cathepsin D, whereas other membrane-delimited structures did stain for it (Fig. 3). Early endosomes, therefore, are compartments distinct from MIIC.

Compartments labeled for class II, but not for class I molecules and mannose-6-phosphate receptors (MPR), were also detected in IM9 cells (data not shown). These compartments contained the lysosomal markers lysosomal-associated membrane protein (Lamp-1) and cathepsin D (14) (data not shown) and have been defined as MIIC (5), which are late endocytic structures closely related to lysosomes. When the appearance of BSA-gold in class II–positive structures was examined, the endocytic tracer reached MIIC, which are Lamp-1 positive, after 30 min. We examined the distribution of MHC class II molecules and of Lamp-1 in compartments that were labeled by endocytosis of BSA-gold. As shown in Table 1, there is a progressive increase in endocytic delivery of BSA-gold to MIIC that express Lamp-1. Of the class II–positive structures that do not stain for Lamp-1, we find only modest numbers that are labeled by the endocytic tracer. Lysosomes not labeled for MHC class II molecules were present (5). Structures colabeling for Lamp-1, cathepsin D, and MHC

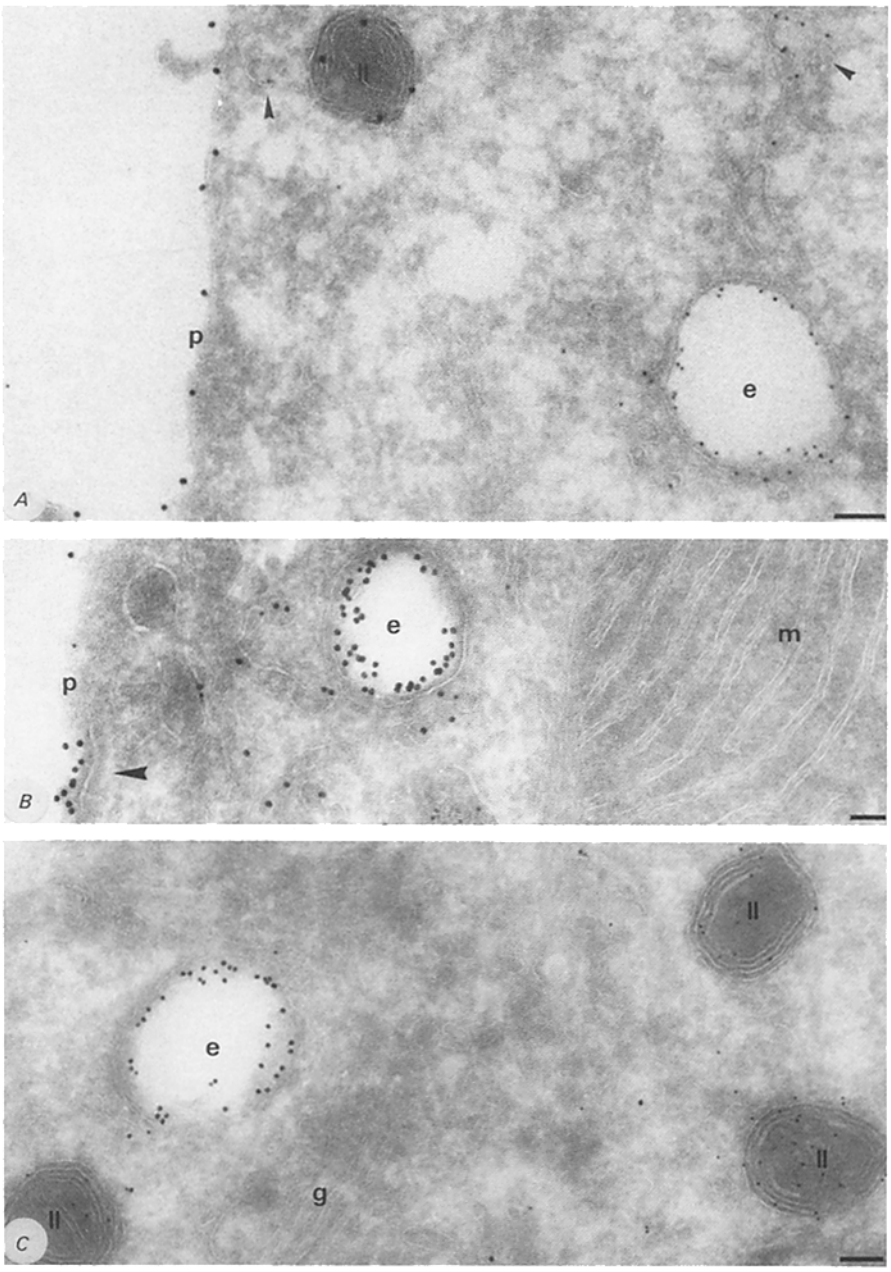

Figure 1. Early endosomes and MHC class II compartments are distinct. Early endosomes were identified in IM9 cells by allowing internalization of Tf/HRP for 5 min. After fixation, ultrathin sections were prepared and labeled with anti-class II and anti-HRP antibodies. No colocalization of Tf/HRP and MHC class II molecules is observed in these early endocytic vesicles. Bar, 100 nm. (*A*) Class II molecules were labeled with 15 nm gold and Tf/HRP with 10 nm gold. (*B* and *C*) Class II molecules were labeled with 10 nm gold, and Tf/HRP with 15 nm gold. MHC class II molecules are located at the plasma membranes (*p*; *A*) and in MIIC (*II*; *A* and *C*). Tf/HRP is located in small vesicles, in vacuolar and tubular (*A*, *arrowhead*) structures. The Golgi apparatus (*g*) is not labeled for Tf/HRP (*C*). Note the absence of class II molecules in Tf/HRP-containing endosomes (*e*) Mitochondria (*m*) are devoid of label.

class II molecules have likewise been observed in mouse macrophages (13).

Having established that the site of encounter between class II molecules and endocytosed material is not the early endosome, we sought to determine more precisely at which stage of the maturation pathway of class II molecules the intersection with the endocytic pathway occurs. For that purpose, we exploited antibodies directed against the lumenal and cytoplasmic portions of Ii, since it is known that during the course of class II molecule maturation, Ii is lost by proteolysis. We examined the distribution of Ii in relation to endocytosed (10-min pulse, 20-min chase) BSA-gold conjugates. The invariant chain was followed using rabbit antibodies directed against the NH_2-terminal, cytoplasmic portion (ICN) and against the COOH-terminal, luminal part (ICC). Immunoreactivity with both antibodies was observed in the ER, indicative of the presence of intact Ii at that location (Fig. 4 *A*). Whereas the lumenal Ii epitope was detectable in the Golgi and in some MIIC (Fig. 4 *B*), it was not usually observed in MIIC devoid of BSA-gold under these conditions (Fig. 4 *B*). The MIIC strongly labeled for class II molecules in the same section are negative for endocytosed BSA-gold (Fig. 4 *B*). We conclude that MIIC, devoid of the lumenal portion of Ii, are located late in the endocytic pathway. However, on occasion we did observe ICC-positive structures that were multivesicular and reachable by BSA-gold after a 10-

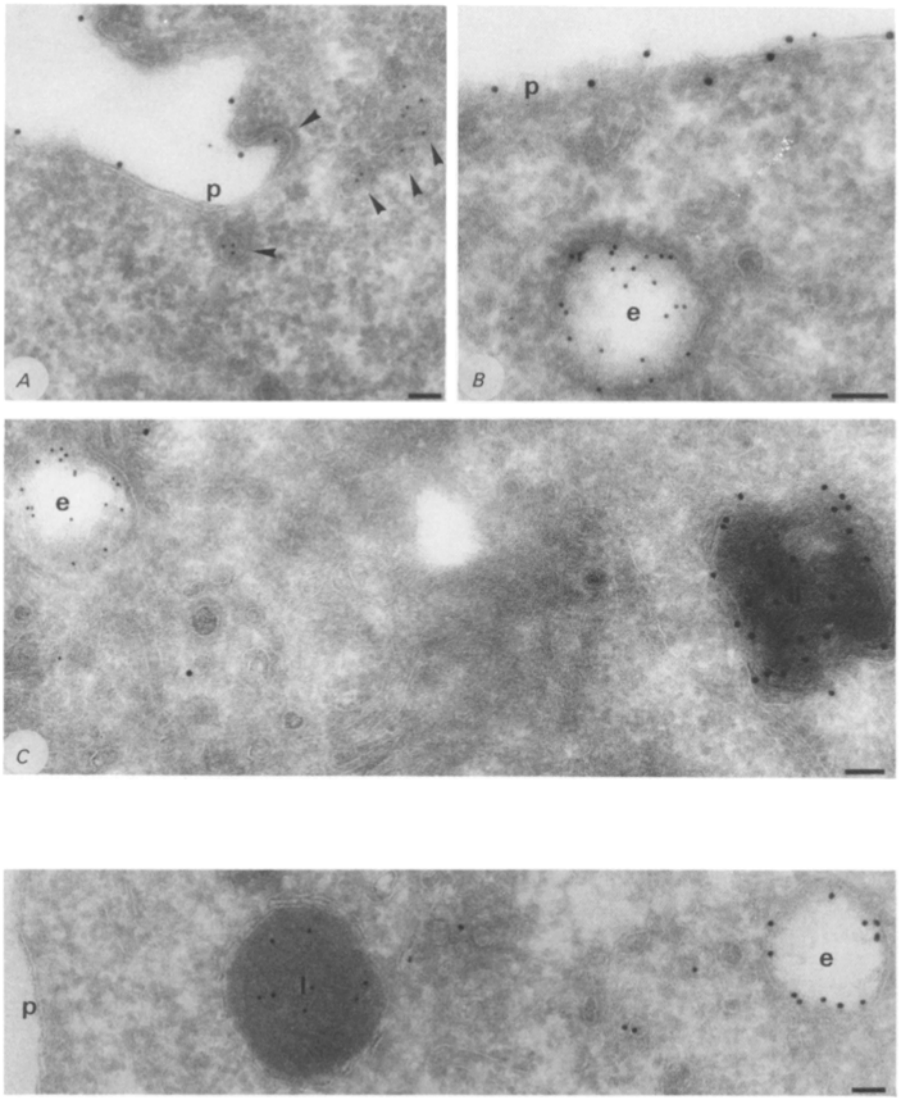

Figure 2. MHC class II molecules do not colocalize with internalized immunoglobulin in early endosomes. Surface immunoglobulin was labeled by a sandwich procedure essentially as described by Guagliardi et al. (4). However, endocytosed Ig was visualized after fixation rather than by using prelabeled anti-Ig reagents during endocytosis (4). (*A*) Class II molecules (gold 15 nm) are located on the cell surface and heterologous Ig (gold 10 nm) is located at the plasma membrane (*p*), in a coated pit and endocytic vesicles (*arrows*). These vesicles are of a size (~100 nm) that is characteristic for early endocytic structures. (11, 12). Bar, 100 nm. (*B*) MHC class II molecules (gold 15 nm) are located at the plasma membrane (*p*), and heterologous Ig is located at the plasma membrane and in endocytic vacuoles (*e*) with a characteristic size of ~500 nm (11, 12). Note that no class II molecules are detected in these endocytic structures. Bar, 100 nm. (*C*) Class II molecules (gold 15 nm) are located in characteristic, electron dense MIIC (13). Heterologous Ig (small gold, 10 nm) is located in endosomal structures (*e*). Note the complete segregation of class II molecules and endocytosed heterologous Ig. Bar, 100 nm.

Figure 3. Endocytic structures containing internalized Ig do not label for cathepsin D. Immunoglobulins on IM9 cells were allowed to internalize for 2 min, as described in Fig. 2. Ultrathin sections were double labeled for immunoglobulin (15 nm gold) and cathepsin D (10 nm gold) The early endosomes (*e*) labeled for endocytosed immunoglobulin were not labeled for cathepsin D. Lysosomes (*I*) labeled for cathepsin D were not labeled for internalized heterologous immunoglobulin. The plasma membrane (*p*) is not labeled for cathepsin D. Bar, 100 nm.

Table 1. *Appearance of BSA-Gold in MHC Class II–positive Intracellular Structures*

Time*	MIIC (Lamp-1$^+$)	Other (Lamp-1$^-$)
min		
10	–	–
30	37‡	2
0	58	8

* Time of exposure to BSA-gold (5 nm)
‡ Number of BSA-gold positive structures.
MIIC were identified based on their class II content and morphological appearance. Most MIIC are Lamp-1 positive (5). We detect small numbers of class II positive structures that do not stain for Lamp-1, referred to as "other."

min pulse and a 20-min chase (Fig. 5). These compartments, positioned distal from the Golgi because of their endocytic nature, showed weak staining for MHC class II products, possibly because of steric hindrance caused by the anti-ICC protein A–gold complex used in the first incubation step. We conclude that endocytosed material can first contact class II molecules in a compartment containing intact Ii. Compartments can be classified as MIIC on morphological criteria: they are either multivesicular (Fig. 4 *B*), multilaminar (Fig. 1), or both (Fig. 5). MIIC occur in three manifestations based on Ii content: those that contain intact Ii because they are positive for both lumenal (ICC) and cytoplasmic (ICN) epitopes of Ii; those that contain the proteolysed NH_2-terminal fragment of Ii, positive for the cytoplasmic (ICN) Ii epitope only; and MIIC that are altogether negative for Ii. Fig. 6 shows MIIC that are positive for both Ii epitopes (*upper left quadrant*), MIIC that are positive for the cytoplasmic (ICN) Ii epitope only (*lower left quadrant*), and an Ii-negative MIIC in the central portion of the micrograph. At a lower mag-

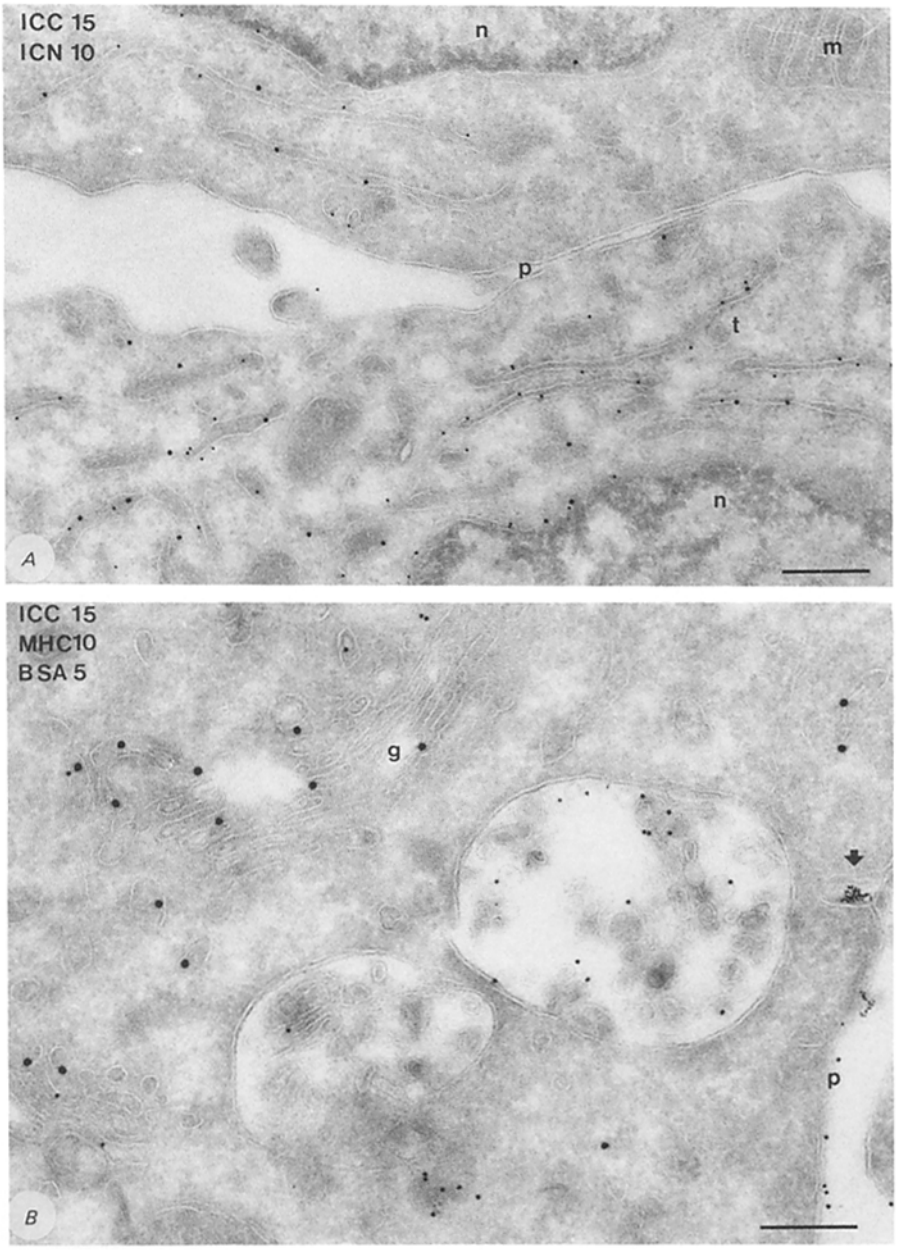

Figure 4. Intact invariant chain is found in the ER and in the Golgi. (*A*) JY cells were sectioned and stained with antibodies against the COOH- and NH_2-terminal portion of invariant chain, ICC, and ICN, respectively, and decorated with 15 (ICC) and 10 (ICN) nm gold. *n*, nucleus; *m*, mitochondrion; *p*, plasma membrane. Bar, 100 nm. (*B*) Same as *A*, but on cells exposed to BSA gold for a 10-min pulse and a 20-min chase. Note the presence of the BSA-gold (5 nm) conjugate at the cell surface and in an early endosome. In this field, all immunoreactivity with ICC (Ii lumenal portion) is confined to the ER. MIIC, identified by the 10-nm gold particles, are negative for Ii and endocytosed BSA-gold.

nification that shows MIIC visible in greater numbers, it is clear that MIIC that are negative for the lumenal epitope of Ii far outnumber those that contain it (Fig. 7, *arrowheads*, Ii, lumenal fragment; *asterisks*, Ii-negative MIIC). Quantitative analysis (50 cells analyzed) reveals that 46% of all MIIC lack any detectable Ii. The remaining 54% have retained the cytoplasmic portion of Ii, and of these, 31% are positive for the lumenal domain. Importantly, all of the ICC-positive MIIC also stain for ICN. Similar values were obtained for two other EBV-transformed B lymphoblastoid cell lines (data not shown). We therefore suggest that the loss of the lumenal fragment precedes that of the cytoplasmic portion, in agreement with the results from biochemical experiments. Inhibition of Ii breakdown, as brought about by blocking the activity of the vacuolar H^+ ATPase, results in the generation of ~11-kD fragments that contain the cytoplasmic portion of Ii (16). These fragments are of a size that could still contain the CLIP region that is inhibitory for peptide binding (19). Results from subcellular fractionation experiments on murine B cells pulsed in the presence of the protease inhibitor leupeptin reveal the existence, during the pulse, of a similar 11-kD fragment in compartments in which peptide loading of class II molecules is inhibited in the presence of leupeptin; during a chase, in the absence of leupeptin, the disappearance of the 11-kD fragment is accompanied by a conversion of class II molecules to the peptide-occupied form (20).

Discussion

The data shown here are consistent with proposals for the site of generation of peptides for presentation by class II mol-

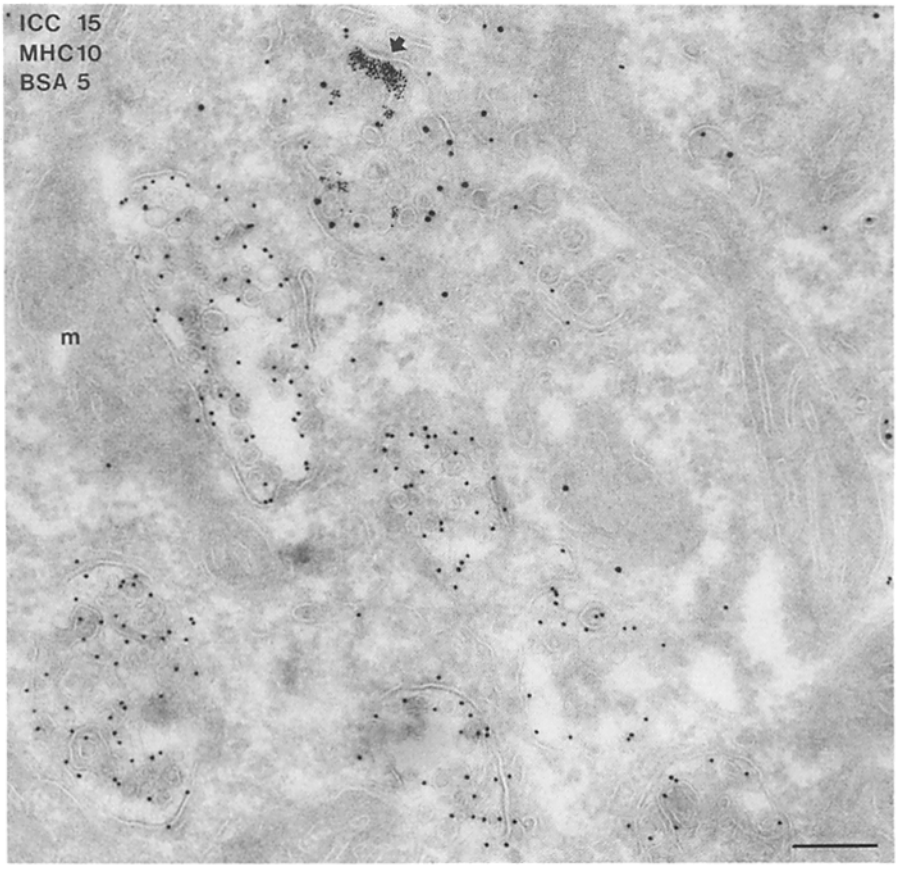

Figure 5. Encounter of endocytosed BSA-gold in MIIC that contain the lumenal portion of Ii. JY cells were exposed to BSA-gold (5 nm) and prepared for immunoelectron microscopy, as described in Fig. 4. Most of the MIIC visible in this field, while positive for class II (10 nm gold), are negative for endocytosed BSA-gold and negative for the lumenal epitope of Ii (15 nm gold). The single MIIC that is positive for BSA-gold (*arrow*) is positive also for the lumenal epitope (ICC) of Ii. Staining intensity for class II is reduced in this compartment for reasons discussed in the text. *m*, mitochondrion. Bar, 100 nm.

ecules in mouse cells (21, 22) and human cells (17), in which emphasis is placed on late aspects of the endocytic route (15, 17, 18). It has been suggested that a sizable fraction of MHC class II molecules may reach endocytic compartments by insertion at and rapid internalization from the cell surface (24, 25). In class II–positive mouse B cell lines, a significant fraction of class II molecules have been reported to recycle, and they have been recovered from early endosomal fractions (26). In mouse macrophages, class II molecules do not recycle and are found predominantly in late endocytic structures. For

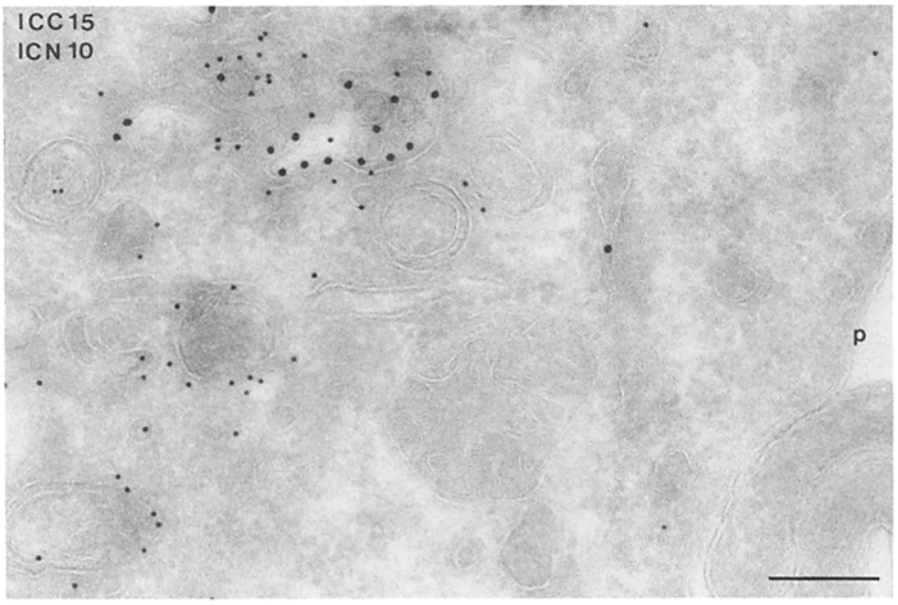

Figure 6. Two distinct types of invariant chain–positive MIIC. JY cells were prepared for immunoelectron microscopy as described in the legend to Fig. 4. Staining was performed with antibodies against the cytoplasmic NH_2-terminal portion of Ii (ICN; 10 nm gold) and with antibodies directed against the lumenal COOH-terminal portion of Ii (ICC; 15 nm gold). The MIIC that is positive for ICC is also positive for ICN (*upper left quadrant*). The two MIIC in the lower left quadrant are positive for ICN, but negative for the lumenal epitope. The MIIC in the central portion of this field is positive for neither. *p*, plasma membrane. Bar, 100 nm.

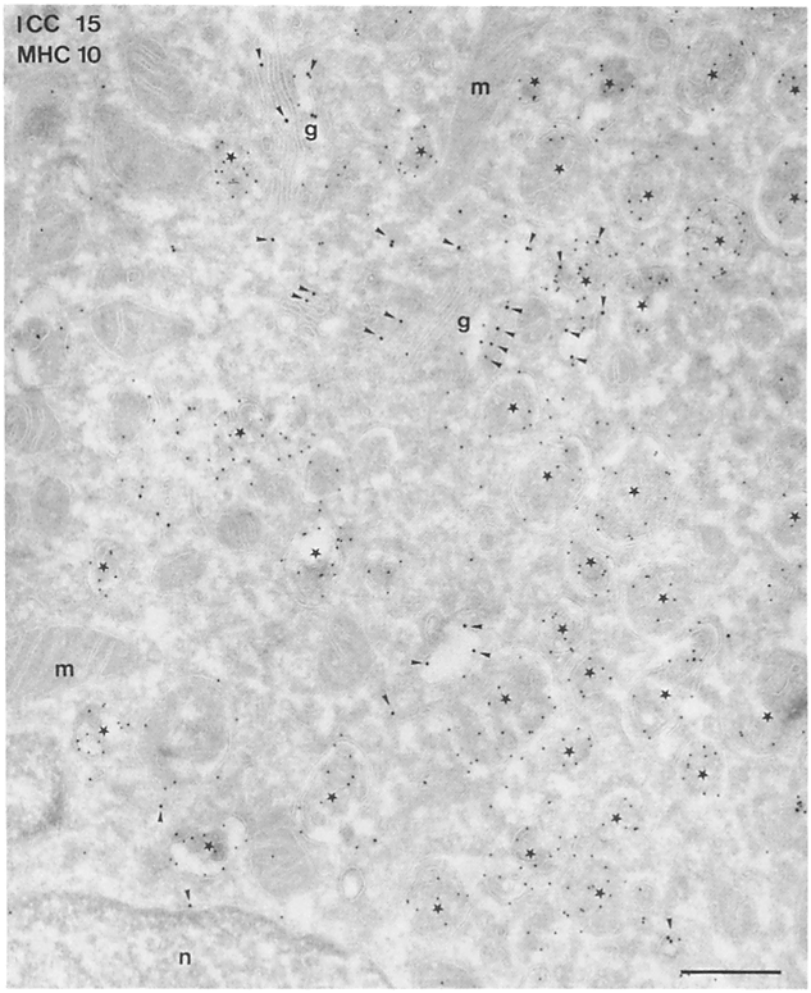

Figure 7. Most MIIC are negative for the lumenal portion of invariant chain. Most of the MIIC present in this field are positive for class II (10 nm gold) and negative for the lumenal epitope of Ii (*asterisks*). A far smaller number of ICC-positive structures (15 nm gold) is observed. *m*, mitochondrion; *n*, nucleus; *g*, Golgi apparatus. Bar, 500 nm.

human B lymphoblastoid cell lines, spontaneous internalization of surface-disposed class II molecules is a minor process (2, 27), but internalization and recycling have been observed (28). Intracellular trafficking of MHC class II molecules may be modified considerably in cells in which the intracellular acidic compartments have been neutralized (16). Our recent results obtained in human B cells exposed to the vacuolar H^+ pump inhibitor concanamycin B, an agent that blocks early to late endosomal trafficking, show the direct route of delivery of class II molecules from the TGN to the endocytic pathway to be the most prevalent (16). Under normal conditions, a fraction of class II molecules is delivered to the cell surface and indeed reaches the endocytic pathway by internalization, but this pathway appears to be rather minor (16), and it can account for our inability to detect any class II molecules in early endosomes. The results shown here allow the conclusion that at steady state, no more than 1% of all class II molecules are found in early endosomes. However, immunocytochemistry is unlikely to reveal compartments that are used in transit and are occupied by the molecules of interest for a brief period only: their steady state levels would be too low to allow detection by immunoelectron microscopy, a method quantitative only as far as equilibrium levels are concerned.

An earlier report (1) was the first to describe the susceptibility of biosynthetically labeled class II molecules to enzymatic attack by an endocytosed transferrin–neuraminidase conjugate, suggestive of involvement of early endosomes as the site of encounter. However, not all of this conjugate was found to recycle, nor could all of the neuraminidase in the conjugate be immunoprecipitated with antitransferrin antibodies (1), leaving open the possibility of the conjugate or free enzyme penetrating deep into the endocytic pathway, and reaching late endosomes or even lysosomes in an active form. While establishing the important concept of intersecting intracellular routes of class II molecules and endocytosed materials, these experiments did not address the issue of whether early, as opposed to late endosomes, are the intracellular site of encounter of antigen and class II molecules.

The present results also establish that in human B lymphoblastoid cells, the most likely site of encounter of MHC class II molecules and antigen, is not the early endosome, but a late endocytic structure. We show that structures that are Ii-positive and emanate from the TGN (29) can be ad-

dressed by endocytosed BSA-gold, but only after prolonged incubation (30 min). The majority of characteristic MIIC is devoid of Ii, as detected by antibodies directed against the luminal epitope (Fig. 7). We observed MIIC that stained for the cytoplasmic portion of Ii, and less frequently did we observe MIIC, as identified by class II content and morphology-positive for the lumenal portion of Ii; these MIIC invariably stained for the cytoplasmic portion of Ii as well. We therefore suggest that the MIIC are derived from TGN-associated, Ii-positive structures, a process accompanied by progressive destruction of Ii. The lumenal epitope is destroyed first, followed by the removal of the NH_2-terminal cytoplasmic fragment.

Removal of Ii from the class II $\alpha\beta$Ii complex is required for peptide binding to occur. Class II molecules, located in the TGN-associated, Ii-positive compartments, are unlikely to be fully occupied by peptide and the peptide loading process may not be complete until the class II molecules have reached MIIC. The shortest fragment of Ii capable of stable interaction with class II molecules, as studied by in vitro translation (19), is segment 1–104, and prevents peptide binding. If its removal is a necessary condition for peptide loading to occur, our data suggest that in human B lymphoblastoid cells, this process indeed takes place mostly in the MIIC. This scheme suggests that endocytosed materials may be found in Ii-positive compartments, and that the pathway traveled by endocytosed antigens may be parallel to that of $\alpha\beta$Ii complexes for the later part of the endocytic pathway. Breakdown of Ii and breakdown of antigen into suitably sized peptides would thus take place synchronously, and a class II–binding site, liberated by removal of Ii, could immediately be occupied by newly generated peptide. Our earlier electron microscopy observations (5) were made using predominantly anti–class II antibodies. The availability of antibodies directed against the luminal and cytosolic domain of Ii allowed us to explore the generation of MIIC and the steps that precede it. Our earlier model proposed a direct transfer of class II molecules to MIIC, a site at which the encounter with antigen was proposed to take place (Fig. 8 *B*). The earlier data together with the present results indicate that MIIC clearly represent a heterogeneous set of structures, both by morphology and by access to endocytic tracers. MIIC addressed relatively early by endocytic tracers are mostly multivesicular, whereas those that are accessible later are often multilaminar. The present data are consistent with a model in which the intersection of the pathway travelled by endocytosed material, as well as that of newly synthesized class II molecules takes place at a site in close proximity of, if not contiguous with, the TGN (Fig. 8 *A*). The recent reports (15, 17, 18) of the isolation of compartments positioned in the endocytic pathway and positive for class II may now be interpreted in the light of the scheme proposed in Fig. 8 *A*. The occurrence of a heterogeneous set of endocytic structures, encompassing elements of the TGN (Geuze, H. J., personal communication) as well as MIIC, would be the expected result. Amigorena et al. (15) identified a class II–enriched fraction called CIIV that is Lamp-negative, and by this criteria, is a likely candidate for the TGN-associated endocytic structures identified in the present study. The morphological data and the subcellular fractionation experiments would be consistent with the identity of CIIV with the TGN-associated endocytic structures, which may be considered early MIIC. Note that the majority of MIIC are Lamp-1 positive (15), unlike CIIV (Table 1). Structures resembling MIIC were found infrequently (1–2 per cell cross-section) in the murine A20 cells used for the subcellular fractionation experiments (15). In the human B lymphoblastoid cells analyzed in the present study, the average number of MIIC is in excess of 20 per cell (see Fig. 7 for a typical example). CIIV are connected to the endocytic pathway, although not all fractions addressed by endocytic tracers were class II positive (15). The presence of transferrin receptors in the CIIV fraction is a further argument in favor of the notion that CIIV are

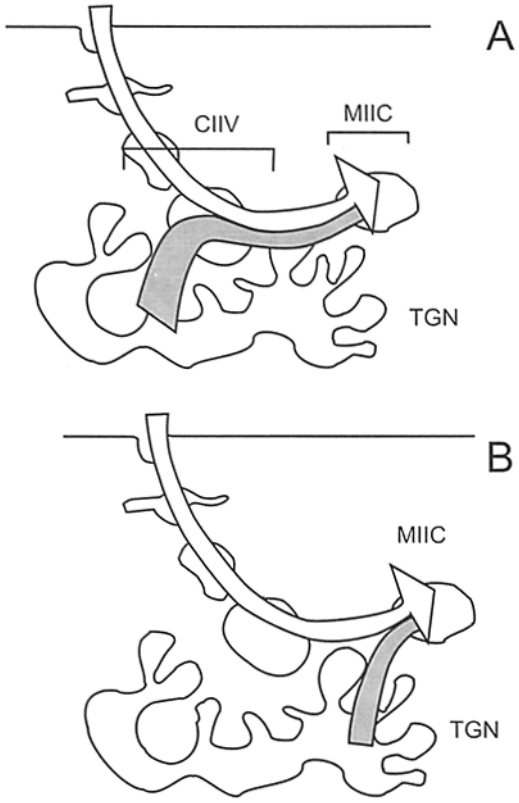

Figure 8. Trafficking of MHC class II molecules and the endocytic pathway. (*A*) Scheme based on the data presented in (15, 16) and in the present study; the endocytic pathway is indicated by a large open arrow, and includes early endosomes, late endosomes, and MIIC in linear progression. The pathway traveled by MHC class II molecules is indicated by the shaded portion of the arrow. We propose that the MIIC identified by Amigorena and colleagues (15) may be positioned in the area of the TGN, immediately upstream of MIIC. As CIIV and MIIC are similar yet distinct, they may represent overlapping populations of a heterogeneous MHC class II–positive, endocytic compartment. (*B*) Scheme based on earlier studies (5) in which a direct transfer of class II molecules from the TGN to MIIC was proposed. Note that in this scheme, there is little room for maturation of MIIC through progressive loss of Ii or for heterogeneity in class II–positive structures that would account for the observed differences between CIIV (15) and MIIC (5, 17, 18).

distinct from MIIC, and that CIIV are positioned closer to the TGN, both spatially and temporally. This argument makes the assumption that the CIIV fraction is not contaminated with a Tfr-positive compartment that displays electrophoretic properties identical to CIIV. Note that the scheme proposed in Fig. 8 *B* is not easily reconciled with the existence of CIIV, whereas the alternate scheme (Fig. 8 *A*) requires it. The relative abundance of CIIV and MIIC may well relate to the differentiation state of the APC under consideration. Our present data do not address the mechanism of class II molecule transfer between CIIV and MIIC, but we suspect this may be a rapid process, in agreement with the observations of Amigorena et al. (15), who conclude that loss of Ii must take place around the time class II molecules have reached CIIV. Our observations indicate that a sizable number of MIIC stain for the cytoplasmic portion of Ii, and it is this fraction that could perhaps correspond to the CIIV identified by Amigorena et al. (15).

The route travelled by peptide-occupied class II molecules to the cell surface has not yet been visualized. Lysosomal constituents can be released to the extracellular milieu by regulated secretory events (29, 30). Whether a similar situation applies to delivery of peptide-loaded class II molecules from MIIC to the cell surface of the APC is a possibility that seems likely in light of recent results obtained for human dendritic cells (31). Exposure of dendritic cells to GM-CSF and IL-4 causes redistribution of intracellularly disposed class II molecules to the cell surface. This observation raises the exciting possibility that cytokines, produced early during an immune response, contribute to more efficient antigen presentation by controlling trafficking and display of peptide-loaded class II molecules. The slow transfer of peptide-occupied class II molecules to the cell surface from the MIIC in B lymphoblastoid cells may represent a rate-controlling default mechanism that would otherwise be a target for cytokine-imposed control.

We thank Dr. J. W. Slot for advice during the course of these experiments, and Dr. P. Morton for his generous gift of the ICC and ICN antisera.

This research was supported by the Netherlands Organization for Scientific Research and by the National Institutes of Health (AI34893 to H. L. Ploegh). Data not shown are available upon request.

Address correspondence to Hidde Ploegh, Center for Cancer Research, Massachusetts Institute of Technology, 40 Ames Street, E17-322, Cambridge, MA 02139-4307.

Received for publication 24 January 1995 and in revised form 14 March 1995.

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