Intracellular Transport and Localization of Major Histocompatibility Complex Class II Molecules and Associated Invariant Chain

Jean Pieters, Heinz Horstmann, Oddmund Bakke, Gareth Griffiths, and Joachim Lipp
European Molecular Biology Laboratory, D-6900, Heidelberg, Germany

Abstract. The intracellular transport and location of major histocompatibility complex (MHC) class II molecules and associated invariant chain (Ii) were investigated in a human melanoma cell line. In contrast to the class II molecules, which remain stable for >4 h after synthesis, the associated Ii is proteolytically processed within 2 h. During or shortly after synthesis the N112-terminal cytoplasmic and membrane-spanning segment is in some of the Ii molecules cleaved off; during intracellular transport, class II associated and membrane integrated Ii is processed from its COOH terminus in distinct steps in endocytic compartments.

Intracellularly both Ii and class II molecules were localized in three morphologically and kinetically distinct compartments, early endosomes, multivesicular bodies, and prelysosomes. This localization in several distinct endosomal compartments contrasts with the localization of class II molecules in mainly one endocytic compartment in B lymphoblastoid cell lines. As in these lymphoblastoid cell lines Ii is known to be rapidly degraded it is conceivable that the rate of proteolysis of the class II associated Ii and its dissociation from class II molecules modulates the retention of the oligomeric complex in endocytic compartments, and as a consequence the steady-state distribution of these molecules within the endosomal system.

Maj or histocompatibility complex (MHC) class II molecules consist of two nonidentical glycoproteins, the α-chain and β-chain (for review, see Cresswell et al., 1987). They function in antigen presentation at the surface of a number of cells, including macrophages, B lymphocytes, and some tumor cells (Unanue, 1984). Intracellularly, they are associated with the invariant chain (Ii), and this association occurs directly after insertion into the endoplasmic reticulum (Jones et al., 1978; Kvist et al., 1982). Ii is a transmembrane protein, exposing 30 NH2-terminal amino acids on the cytoplasmic side and ≈160 amino acids on the luminal side of the membrane (Claesson et al., 1983).

The oligomeric complex of class II molecules and Ii is thought to be transported to an endocytic compartment where Ii dissociates from the complex and class II molecules are then transported further to the plasma membrane (Koch et al., 1989; Long, 1989; Neefjes et al., 1990). Ii remains largely intracellularly and is eventually degraded (Owen et al., 1981). Degradation of Ii can partially be inhibited by the addition of the lysosomotropic agent chloroquine or the protease inhibitor leupeptin (Nowell and Quaranta, 1985; Blum and Cresswell, 1988; Nguyen et al., 1989).

The class II associated Ii has been implicated in the regulation of peptide association to class II molecules (Koch et al., 1989; Long et al., 1989). Two distinct functions of Ii in this process have been proposed. First, Ii could prevent MHC class II molecules from binding peptide prematurely in the endoplasmic reticulum or Golgi complex (Elliott et al., 1987; Roche and Cresswell, 1990). This was suggested from the finding that peptide binding to class II molecules was reduced in the presence of Ii or its luminal segment (Roche and Cresswell, 1990; Teyton et al., 1990). Second, the association of Ii with class II molecules may regulate the localization of these molecules in endocytic compartments. This was supported by the finding that the cytoplasmic tail of Ii contains a sorting signal for endosomes (Bakke and Dobberstein, 1990; Lotteau et al., 1990).

It has recently been shown, that in the B lymphoblastoid cell line JY class II molecules were localized to a distinct late endosomal, lysosome related compartment (Peters et al., 1991). Ii could only be found in the endoplasmic reticulum, Golgi, and trans-Golgi network. The failure to detect Ii in endocytic compartment may reflect the rapid degradation of Ii in B lymphoblastoid cell lines (Blum and Cresswell, 1988; Nguyen et al., 1989).

MHC class II molecules expressed in transfected murine L cells were also shown to be located in a late endocytic com-
Materials and Methods

Materials

Materials were obtained from the following sources: [35S]methionine (sp act 1,300 Ci/mM) and [14C]-methylated protein standards were obtained from Amersham International, Amersham, England; protein A-Sepharose was from Pharmacia LKB, Uppsala, Sweden; leupeptin, chymostatin, pepstatin A, aprotinin, and PMSF were from Sigma Chemical Co., St. Louis, MO; endoglycosidase H was from Seikagaku Kogyo Co., LTD, Tokyo, Japan; Neuraminidase (type V) was from Sigma Chemical Co.

Cells and Cell Culture

The human melanoma cell line Mel JuSo was a gift from Dr. Johnson (Institut fur Immunologie Munich, Germany), and described before (Johnson et al., 1981). The cells were cultured in RPMI 1640 medium supplemented with 5% fetal calf serum (RPMI-FCS).

Antibodies

Hybridoma cells L-243 secreting anti-HLA-DR antibodies (Lampson and Levy, 1980) were obtained from the American Type Culture Collection (Rockville, MD). Asites fluid was obtained by culturing the cells intraperitoneally in BALB/c mice. Antiserum 311, a polyclonal antiserum against the α-chain of class II molecules (Sege et al., 1981) was a kind gift of Dr. T. A. Peterson. The polyclonal antiserum against fusion proteins of β-galactosidase and part of IIi expressed in NFl bacteria were described before (Lipp and Dobberstein, 1986; Wraight et al., 1990). Antiserum recognizing an II NH₂-terminal portion was raised against a fusion protein containing the NH₂-terminal 73 amino acids (II73-216) of II and β-galactosidase (anti-IIIN); antiserum recognizing an II COOH-terminal portion was raised against a fusion protein containing amino acids 73-216 of II (Ií73-216) and β-galactosidase (anti-IIIC); VIC YI (Quaranta et al., 1984) is a mouse monoclonal antibody that recognizes an epitope within the NH₂-terminal 30, cytosolic, amino acids of II (Wright et al., 1990), and was a kind gift from Dr. W. Knapp. Clonab LN2 (Biotest AG, Dreieich, Germany) is a mouse monoclonal antibody recognizing an epitope at the outer COOH-terminal portion of II (within amino acids 157-216 of II; Wright et al., 1990), AB4, a mouse monoclonal anti-HLA-DR antibody (Kvalheim et al., 1988) was a kind gift from Dr. Funderud. Rabbit polyclonal antiserum against the (cation-independent) mannose-6-phosphate receptor (MRP) (Griffiths et al., 1988) was a gift from Dr. Hofack. Rabbit polyclonal antiserum against the Golgi enzyme galactosyl transferase (anti-Gal TF) (Berger et al., 1987) was a gift from Dr. E. Berger.

Metabolic Labeling

Cells were grown on tissue culture dishes, and before labeling the medium was replaced by methionine-free medium. After 1 h this medium was replaced by methionine-free medium containing 0.075 mCi/ml [35S]methionine. After 20 min, the radioactive medium was removed, the cells were washed twice in RPMI-FCS containing 2 mM methionine and incubated at 37°C in the same medium.

At the times indicated, the dishes were placed on ice, washed three times with ice-cold PBS and the cells were lysed in 20 mM Hepes pH 7.5 containing 100 mM NaCl, 5 mM MgCl₂, 1% Triton X-100, and 20 μM PMSF. After 10-min incubation on ice, the cell lysates were collected and centrifuged at 13,000 g for 15 min to remove cell debris.

Immunoprecipitation and Electrophoresis

Cell lysates from 1 x 10⁶ cells were incubated with antibody at 4°C for 12 h, followed by the addition of 40 μl protein A-Sepharose (1:1 slurry) and further incubation for 2 h. The beads were washed twice with 1 ml of low salt buffer (10 mM Tris-Cl pH 7.5, 150 mM NaCl, 0.2% NP-40, and 150 mM EDTA), twice with 1 ml of high salt buffer (10 mM Tris-Cl pH 7.5, 500 mM NaCl 0.2% NP-40 and 2 mM EDTA), and twice with 1 ml of 10 mM Tris-Cl pH 7.5. For sequential immunoprecipitation of antibodies, antibody L-243 was used for the immunoprecipitation of the class II molecules and associated proteins. This complex was then denatured by boiling the protein A-Sepharose beads by the addition of sample buffer, denatured, and subjected to SDS-PAGE (10-15%) according to Laemmli (1970), fluorography, and autoradiography.

Immunofluorescence Microscopy

Immunofluorescence on live cells was essentially performed as described by Bakke and Dobberstein (1990). Briefly, viable cells grown on coverslips were incubated on ice with the appropriate antibody to label antigens on the plasma membrane. Then, the cells were fixed with 3% paraformaldehyde, washed, and the cell surface molecules were visualized using FITC or Texas Red-conjugated second antibody. To also label intracellular molecules, cells were fixed with methanol for 4 min at -20°C, washed, and labeled using antibodies and fluorescent or Texas Red conjugates as described in the figure legends. After labeling, coverslips were mounted in Mowiol, and examined using a Leitz Orthoplan fluorescence photo-microscope equipped with a 63× objective and filters for fluorescein or Texas Red.

Electron Microscopy

For the localization of class II molecules and Ii at the EM level, cells were prepared for cryosectioning, and immunolabeled with antibodies against class II molecules followed by 9-nm gold-conjugated protein A (Geuze et al., 1983). To identify the various compartments of the endocytic pathway, cells were allowed to internalize different markers, essentially as described by Parson et al. (1989). For the identification of early endosomes, HRP (10 mg/ml) was allowed to be internalized for 5 or 10 min followed by chase periods up to 30 min to label endosome transport vesicles and the prevacuoles. The HRP was subsequently visualized using anti-HRP antibodies on cryosections, followed by 5 nm protein A-gold complex.

For the identification of lysosomes, a 16-nm BSA-gold complex was internalized for 4 h at 37°C followed by an overnight chase in medium free of BSA-gold. Under this condition the marker distributes between the mannose-6-phosphate receptor (MPR)-enriched prevacuolar compartment (PLC) and the MPR-negative lysosomes, (Griffiths et al., 1988, 1990). These cells were prepared for cryosectioning and immunolabeled with antibodies followed by 5 and 9 nm protein A-gold (Griffiths et al., 1984), as specified in the figure legends.

For quantitation, cells labeled for endocytic compartments were prepared for cryosectioning, and immunolabeled for Ii using anti-IIN followed by 9 nm protein A-gold. Quantitation of the amount of 9 nm gold particles on these cryosections was essentially carried out as described by Griffiths and Hoppeler (1986).

Results

Cleavage of II during Intracellular Transport

II has previously been shown to dissociate from class II mol-
Figure 1. II associated with class II molecules is proteolytically processed during intracellular transport. Mel JuSo cells were incubated with [35S]methionine for 20 min, then washed and chased for the times indicated. Proteins were immunoprecipitated with anti-class II antibody (L-243; A). The immunoadsorbed protein complexes were denatured and reprecipitated with anti-IIIC antiserum (B), or anti-IIIN antiserum (C). In C, P22, P18, and P12 represent 22-, 18-, and 12-kD II-related proteins, respectively. In C, an asterisk indicates forms of II which probably have acquired complex type carbohydrates. Shown are autoradiographs after SDS-PAGE and fluorography.

Figure 2. Proteolytic processing of class II-associated II to P18 and P12, but not to P22 is inhibited by leupeptin. Mel JuSo cells were incubated with 0.3 mM leupeptin 4 h before labeling. Cells were pulse-labeled and chased for the times indicated in the presence of 0.3 mM leupeptin. Proteins were immunoprecipitated using anti-class II antibody L-243 (A). The immunoadsorbed protein complexes were denatured and reprecipitated with anti-IIIC antiserum (B), or anti-IIIN antiserum (C). In C, asterisks indicate forms of II that have acquired complex type carbohydrates. Shown are autoradiographs after SDS-PAGE and fluorography.
chains. A subsequent immunoprecipitation, using antisera recognizing either the luminal Ii COOH-terminal portion (anti-IIIC) or the cytoplasmic Ii NH2-terminal part (anti-IiN), allows thus to identify Ii-related polypeptides associated with class II molecules.

P25 results from an early cleavage in the endoplasmic reticulum. It is not recognized by anti-IiN but by anti-IIIC antiserum (Fig. 1). Therefore we conclude that P25 lacks the NH2-terminal region of Ii, most likely including the membrane-spanning region. The amount of cell-associated P25 decreased during the chase and a portion of P25 can be detected in the culture medium after 2-h chase (Fig. 1).

During the chase period, several small molecular mass proteins appeared after 2 h in the MHC class II immunocomplexes (Fig. 1 A). As Ii associated with class II molecules is degraded during intracellular transport (Blum and Cresswell, 1988; Nguyen and Humphreys, 1989), we suspected that these small molecular weight proteins might be derived from Ii.

Fig. 1, B and C shows that after denaturation of the class II immunocomplexes and reprecipitation with Ii specific antisera, the small molecular mass proteins are immunoprecipitated by the antiserum against the NH2-terminal part of Ii (anti-IiN), but not by the antiserum against the COOH-terminal part (anti-IIIC) (Fig. 1 C). These small molecular weight proteins have molecular masses of 22 (P22), 18 (P18) and 12 kD (P12). As all these molecules react with anti-IiN antiserum, which recognizes the extreme NH2-terminal portion of Ii, these molecules must lack increasing portions from the COOH-terminal side. The amount of P22 decreases between 2 and 4 h of chase, whereas the amount of P12 increases during the same time. This suggests that P22 is processed sequentially to P18 and to P12.

**Effect of Leupeptin on Ii Processing**

The accumulation of P22, P18 and P12 after 2 h of chase and the presence of complex type carbohydrates suggest that cleavage of Ii occurs in a trans-Golgi or post-Golgi compartment. It is known that at least some endocytic compartments possess proteolytic activity (Kornfeld and Mellman, 1989; Diment et al., 1989). Furthermore, it is known that in B lymphoblastoid cells degradation of Ii can be inhibited by the addition of the protease inhibitor leupeptin (Blum and Cresswell, 1988; Nguyen et al., 1989). To test whether proteolytic processing of Ii in Mel JuSo cells occurs in an endocytic compartment, we added leupeptin to the culture medium. Cells were incubated for 4 h in the presence of 0.3 mM leupeptin, labeled for 20 min and chased for the times indicated in Fig. 2. Proteins were immunoprecipitated using anti-class II antibody (L-243), followed by denaturation and immunoprecipitation using anti-IIIC and anti-IiN antisera. Fig. 2 shows that the incubation of the cells with leupeptin resulted in accumulation of complexes of class II molecules and P22 during the chase period. No processing to P18 and P12 is seen (Fig. 2, A and C). Leupeptin had, as expected, no effect on the appearance of P25 (Fig. 2 B).

Incubation of the cells in the presence of a cocktail of the protease inhibitors leupeptin, chymostatin, and pepstatin A did not prevent cleavage of Ii to P22 (data not shown).

**Localization of Class II Molecules and Ii by Light Microscopy**

The biochemical analysis of Ii chains assembled with class II molecules strongly suggested their transport to an endocytic, proteolytic compartment. To localize class II molecules and Ii in Mel JuSo cells, we used immunofluorescence microscopy.

Class II molecules are found at the plasma membrane in Mel JuSo cells, and this is shown in Fig. 3 A. In contrast, no Ii could be detected at the plasma membrane (Fig. B and C), using the monoclonal antibody LN2, recognizing an epitope located at the COOH-terminal portion of Ii (Wraight et al., 1990). After permeabilization of the cells this antibody strongly labeled intracellularly located Ii molecules (results not shown).

As our biochemical data indicate that during intracellular transport of the oligomeric complex of class II molecules and Ii, the luminal, COOH-terminal part of Ii is degraded, we used antibodies against the NH2-terminal part of Ii in the following studies. To analyze the localization of Ii in relation to different markers of intracellular compartments, cells were permeabilized and Ii was visualized using VIC Y1, an antibody recognizing an NH2-terminal, cytoplasmic determinant (Wraight et al., 1990). Ii could be localized in the perinuclear region and in a punctated pattern throughout the cytoplasm (Fig. 4 A). As reference, we used a marker for the Golgi region. Cells were double labeled for Ii and galactosyl transferase, a marker of the trans-Golgi (Berger et al., 1987) (Fig. 4, A and B). As can be seen in Fig. 4, A and B,
Ii is localized in the Golgi area, but also in other distinct structures in the cell periphery.

Inhibition of Ii processing by leupeptin suggested transport of the complex of class II molecules and Ii to an endocytic compartment with proteolytic activity. As the (cation-independent) mannose-6-phosphate receptor (MPR) is known to reside largely in a late endosomal compartment containing proteolytic enzymes (i.e., prelysosomes/late endosomes; Kornfeld and Mellman, 1989; Griffiths et al., 1988; Griffiths et al., 1990), we compared its location with that of Ii. Mel JuSo cells were permeabilized and labeled for both Ii, using VIC Y1, and MPR, using rabbit anti-MPR. As is depicted in Fig. 4, C and D, some Ii seems to colocalize with the MPR, but the bulk of the vesicular structures in which Ii is localized, is MPR-negative.

**Localization of Class II Molecules and Ii at the Electron Microscopy Level**

To further characterize the compartments in which class II molecules and Ii are present, we used electron microscopy. Endocytic compartments were identified by the internalization of HRP for different times (Parton et al., 1989). After 5 min of internalization, HRP is localized to the early endosomes. After 10-15 min of internalization significant amounts of HRP is also found in typical membrane rich, spherical structures (Gruenberg et al., 1989). These structures are especially prominent in Mel JuSo cells, and show a multivesicular body like morphology, therefore, we name them multivesicular bodies (MVB). When HRP is pulsed for 10 min followed by a 30 min chase, HRP is then found distributed between the endosome transport vesicles and the

| Compartment          | Anti-IiN | Anti-IiC |
|----------------------|----------|----------|
| Early endosome       | 3.4 ± 0.9| 10.6 ± 1.4| gold/μm² |
| MVBs                 | 5.5 ± 1.2| 4.0 ± 0.5  | gold/μm² |
| Prelysosome          | 3.3 ± 0.7| 13.3 ± 1.8| gold/μm² |
| Lysosome             | <0.5     | <0.5      | gold/μm² |
| Golgi complex        | 4.7 ± 1.7| 15.5 ± 2.7| gold/μm² |

The labeling of the plasma membrane (0.08 ± 0.01 gold per linear micrometer for anti-IiN and 0.06 ± 0.01 for anti-IiC) while low was over five times higher than background (labeling with an irrelevant antibody gave 0.016 ± 0.004 gold/μm plasma membrane).

Table I. Amount of Ii Present in Double-labeled Cryosections of Mel JuSo Cells as Determined by Immunogold Labeling Using Anti-IiN Antiserum Followed by 9 nm Protein A-Gold

---

Pieters et al. Localization of Class II Molecules and Ii
preparation for cryosectioning and antibody labeling. The compartment against the α-chain followed by 9 nm Protein A–Gold*

Determined by Immunogold Labeling Using Antiserum to MHC Class II Molecules Present in Double-labeled Cryosections of Mel JuSo Cells as

| Compartment       | Anti-α-chain |
|-------------------|--------------|
| Plasma membrane   | 9.8 ± 1.3 gold/μm |
| Early endosome    | 5.9 ± 1.4 gold/μm |
| MVBS†             | 5.6 ± 2.1 gold/μm |
| Prelysosome‡      | 4.8 ± 0.9 gold/μm |
| Lysosome          | 1.7 ± 0.4 gold/μm |
| Golgi complex     | 0.2 ± 0.05 gold/μm |

* For experimental details see legend to Table I.
† Gold particles present on the outer membranes only were quantitated.
‡ For experimentale details see legend to Table I.

Figure 5. Localization of Ii in endocytic compartments (A and B). Mel JuSo cells were allowed to internalize HRP for 10 min to label endocytic compartments. Cryosections were prepared and double labeled with anti–IiN and anti–IiC antiserum. The results are shown in Table I. Ii was found to be present in early endosomes, late endosomes or prelysosomes and MVBS. Very little Ii was found on the plasma membrane.

The quantitative analysis of the labeling of Ii supports the qualitative data showing similar amounts in early endosomes, MVBS and prelysosomes, with negligible amounts in lysosomes (cf. below). To directly compare the amounts of labeling between the different compartments it is necessary to assume that the labeling efficiencies are the same, that is that each antibody has equal access to antigen in the different structures. This may not always be the case (Griffiths and Hoppeler, 1986) and the relatively lower labeling with anti–IiC in the MVBS, for example, may reflect less access of this antibody to the antigen. Alternatively, part of the MVBS population may already contain proteolytic activity which leads to degradation of Ii COOH-terminal fragments (see Figs. 1 and 2). Nevertheless, taken together the data for both antibodies suggest that the amount of Ii in the early endosome, MVBS and prelysosome is similar.

Examples of intracellular compartments which are Ii positive are shown in Fig. 5. Fig. 5 A shows a typical early endosome (Griffiths et al., 1990) in which the lumen is clearly labeled with Ii and HRP, taken up by the cells for 10 min. Also present in this figure is a multivesicular body, in which both the HRP and Ii can be visualized. Fig. 5 B shows two of these MVBS, labeled with both Ii and internalized HRP.

Immunofluorescence microscopy indicated some colocalization of Ii and MPR. This finding was also supported by electron microscopy. Fig. 5 C shows colocalization of Ii with MPR in structures which most probably represent a prelysosome. Also MVBS are shown containing both Ii and MPR. In addition, a MVBS is indicated devoid of both Ii and MPR. The plasma membrane, as expected, is unlabeled.

As class II molecules and Ii remain associated in Mel JuSo cells for >4 h after synthesis and Ii does not appear on the plasma membrane, we were also interested to localize class II molecules in intracellular compartments. Intracellular compartments were identified in a similar manner as described above for Ii.

The amount of class II labeling on cryosections present in the various subcellular compartments was quantitated using the anti–α-chain antiserum. The results are presented in Table II. To be able to compare directly the amount of label on the plasma membrane with that over internal structures we expressed the class II labeling on a per linear micrometer scale. The plasma membrane, as expected, abundantly contained class II molecules; furthermore, the class II molecules were found to be present in equal amounts in the early endosomes, MVBS, and prelysosomes. Lysosomes contained a low but significant amount of class II molecules, whereas the Golgi complex was poorly labeled. The significance of this low Golgi labeling is not known.

Micrographs showing examples of class II molecules localized in the various compartments are shown in Figs. 6 and 7. Fig. 6 A shows colocalization of class II molecules with HRP endocytosed for 5 min in an early endosome. Fig. 6 B shows localization of class II molecules on the plasma membrane (P) and in MPR-rich compartments presumed to be prelysosomes. Some of these vesicular structures were also labeled with a 16 nm gold–BSA complex given to the cells for 4 h and then chased overnight. Not all MVBS which were found positive for class II molecules were also found positive for MPR. This is particular evident in Fig. 7 B, which shows two MVBS devoid of MPR-specific labeling and one which contains relatively large amounts of MPR. Note also that MPR is mostly found at the internal membranes of the MVBS whereas class II molecules are nearly exclusively localized on the peripheral membrane.

The relative distribution of the class II molecules in the endocytic compartments and MVBS is very similar to the distribution of Ii as determined using anti–IiN and anti–IiC antiseras.

As apparently there are distinct populations of MVBS, as judged by the absence or presence of MPR, we were interested to see whether both Ii and class II molecules colocalized to the same MVBS. Fig. 6 B shows that indeed Ii and class II molecules can be identified within the same MVBS.

Discussion

In this study we analyze the intracellular transport and loca-
Figure 6. (A) Localization of class II molecules in early endosomes. Mel JuSo cells were incubated with HRP for 5 min. Cryosections were prepared and labeled with antibody against the α-chain of class II (Ab 311) and 9-nm gold (small arrows), and an antibody against HRP and 5 nm gold (small arrows). Large arrowheads indicate the limiting membranes. (B) Colocalization of class II molecules and Ii in a typical multivesicular body. Cryosections of Mel JuSo cells were labeled with an antibody against the α chain of class II molecules (Ab. 311) and 5-nm gold (arrows), and anti-IiN antiserum and 9 nm gold (arrowheads). Bar, 100 nm.

The Journal of Cell Biology, Volume 115, 1991

1220
The association of Ii or its NH2-terminal fragments with class II molecules might also modulate the distribution of these molecules in the endosomal system. This would explain the finding that class II molecules expressed in cells in which Ii is rapidly degraded (e.g., lymphoblastoid cell lines) are localized in late endosomes/prelysosomes only (Peters et al., 1991). Our results, using a melanoma cell line, suggest that prolonged association of Ii or its NH2-terminal fragments may be responsible for the localization of class II molecules in several distinct endosomal compartments. Differences in Ii proteolysis in different cell lines may therefore explain the apparent discrepancy in class II localization described before (Guagliardi et al., 1990; Peters et al., 1991).

We conclude that knowledge of the type of II molecules at various stages of transport in a given cell line may be essential for understanding transport and localization of MHC class II molecules.

We thank Dr. B. Dobberstein for invaluable advice and discussion throughout this work. We thank Dr. P. A. Peterson (Scripps Clinic and Research Foundation, La Jolla, CA), Dr. W. Knapp (Institut für Immunologie der Universität Wien, Vienna, Austria), Dr. S. Funderud (Laboratory for Immunology, Institute for Cancer Research, Oslo, Norway), Dr. B. Hofack (European Molecular Biology Laboratory, Heidelberg, Germany), and Dr. E. Berger (University of Zurich, Zurich, Switzerland) for kindly providing antibodies.

J. Pieters was supported by a European Molecular Biology Organization long term fellowship.

Received for publication 7 December 1990 and in revised form 19 July 1991.

References

Bakke, O., and B. Dobberstein. 1990. MHC class II associated invariant chain contains a sorting signal for endosomal compartments. Cell. 63:707–716.

Bakke, O., and B. Dobberstein. 1990. MHC class II associated invariant chain contains a sorting signal for endosomal compartments. Cell. 63:707–716.

Berger, E. G., M. Thumker, and U. Müller. 1987. Galactosyltransferase and sialyltransferase are located in different subcellular compartments in HeLa cells. Exp. Cell Res. 173:267–273.

Blum, J. S., and P. Cresswell. 1988. Role for intracellular proteases in the processing and transport of class II HLA antigens. Proc. Natl. Acad. Sci. USA. 85:3975–3979.

Claesson, L., D. Larhammer, L. Rask, and P. Peterson. 1983. cDNA clone for the human invariant γ chain of class II histocompatibility antigens and its implications for the protein structure. Proc. Natl. Acad. Sci. USA. 80:7395–7399.

Cresswell, P., I. S. Blum, D. N. Kelner, and M. S. Marks. 1987. Biosynthesis and processing of class II histocompatibility antigens. CRC Crit. Rev. Immunol. 7:31–53.

Diment, S., K. J. Martin, and P. D. Stahl. 1989. Cleavage of parathyroid hormone in macrophage endosomes illustrates a novel pathway for intracellular processing of proteins. J. Biol. Chem. 264:13403–13406.

Elliott, W. L., C. J. Stille, L. J. Thomas, and R. E. Humphreys. 1987. An hypothesis on the binding of an amphiathic α helical sequence in Ii to the desetope of class II antigens. J. Immunol. 138:2949–2959.

Felder, S., K. Miller, G. Moehren, A. Ullrich, J. Schlessinger, and C. R. Hopkins. 1990. Kinase activity controls the sorting of the epidermal growth factor receptor within the multivesicular body. Cell. 61:633–643.

Griffiths, G., and H. Hoppeler. 1986. Quantiation in immunocytochemistry: correlation of immunogold labeling to absolute number of membrane antigens. J. Histochem. Cytochem. 34:1389–1398.
Griffiths, G. A. McDowell, R. Back, and J. Dubochet. 1984. On the preparation of cryosections for immunosections for immunocytochemistry. J. Ultrastruct. Res. 89: 65–78.

Griffiths, G., B. Hofack, K. Simons, I. Mellmann, and S. Kornfeld. 1988. The mannose 6-phosphate receptor and the biogenesis of lysosomes. Cell. 52: 329–341.

Griffiths, G., R. Matteo, R. Back, and B. Hofack. 1990. Characterization of the cation-independent mannose 6-phosphate receptor-enriched prelysosomal compartment in NRK cells. J. Cell Sci. 95: 441–461.

Gruenberg, J., G. Griffiths, and K. E. Howell. 1989. Characterization of the early endosome and putative endocytic carrier vesicles in vivo and with an assay of vesicle fusion in vitro. J. Cell Biol. 108: 1301–1316.

Guagliardi, L. E., B. Koppelmann, J. S. Blum, M. S. Marks, P. Cresswell, and F. M. Brodski. 1990. Co-localization of molecules involved in antigen processing and presentation in an early endocytic compartment. Nature (Lond.). 343: 133–139.

Johnson, J. P., M. Demmer-Dieckmann, T. Meo, M. R. Hadam, and G. Rietmuller. 1981. Surface antigens of human melanoma cells defined by monoclonal antibodies. I. Biochemical characterization of two antigens found on cell lines and fresh tumors of diverse tissue origin. Eur. J. Immunol. 11: 825–831.

Jones, P. P., D. B. Murphy, D. Hewgill, and H. O. McDevitt. 1978. Detection of a common polypeptide chain in I-A and I-E subregion immunoprecipitates. Immunology. 49: 51–60.

Koch, N., J. Lipp, U. Pessara, K. Schenck, C. Wright, and B. Dobberstein. 1989. MHC class II invariant chains in antigen processing and presentation. Trends Biochem. Sci. 14: 383–385.

Kornfeld, S., and I. Mellman. 1989. The biogenesis of lysosomes. Annu. Rev. Cell Biol. 5: 483–525.

Kvaheim, G., S. Funderud, S. Kvaloy, G. Gaudernack, K. Beiske, E. Jakobsen, and O. Pedstad. 1988. Successful clinical use of an anti-HLA-DR monoclonal antibody for autologous bone marrow transplantation. J. Natl. Cancer Inst. 80: 1322–1325.

Koistinen, S., L. Wiman, L. Claesson, P. A. S. Peterson, and B. Dobberstein. 1982. Membrane insertion and oligomeric assembly of HLA-DR histocompatibility antigens. Cell. 29: 61–69.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.). 227: 680–685.

Lampson, L. A., and R. Levy. 1980. Two populations of la-like molecules on a human B cell line. J. Immunol. 125: 293–299.

Lipp, J., and B. Dobberstein. 1986. The membrane-spanning segment of invariant chain (Aβ) contains a potentially cleavable signal sequence. Cell. 46: 1103–1112.

Long, E. 1989. Intracellular traffic and antigen processing. Immunol. Today. 10: 232–234.

Lotteau, V., L. Teyton, A. Piaud, T. Nilsson, L. Karlsson, S. L. Smid, V. Quaranta, and P. A. Peterson. 1990. Intracellular transport of class II MHC molecules directed by invariant chain. Nature (Lond.). 348: 600–605.

Machamer, C. E., and P. Cresswell. 1982. Biosynthesis and glycosylation of the invariant chain associated with HLA-DR antigens. J. Immunol. 129: 2564–2569.

Neefjes, J. J., V. Stollerz, P. J. Peters, H. J. Geuze, and H. L. Ploegh. 1990. The biosynthetic pathway of MHC class II but not class I molecules intersects the endocytic route. Cell. 61: 171–183.

Nguyen, Q. V., and R. E. Humphreys. 1989. Time course of intracellular association, processing, and cleavage of II forms and class II major histocompatibility complex molecules. J. Biol. Chem. 264: 1631–1637.

Nowell, J., and V. Quaranta. 1985. Chloroquine affects biosynthesis of la molecule by inhibiting dissociation of invariant (γ) chains from α-β dimers in B cells. J. Exp. Med. 162: 1371–1376.

Owen, M. J., S. M. Kissinger, H. F. Lodish, and M. J. Crompton. 1981. Biosynthesis and maturation of HLA-DR antigens in vivo. J. Biol. Chem. 256: 8987–8993.

Parron, R. G., K. Prydz, M. Bomsel, K. Simons, and G. Griffiths. 1989. Meeting of the apical and basolateral endocytic pathways of the MDCK cell in late endosomes. J. Cell Biol. 109: 3259–3272.

Peters, P. J., J. J. Neefjes, V. Oorschot, H. L. Ploegh, and H. J. Geuze. 1991. Segregation of MHC class II molecules from MHC class I molecules in the Golgi complex for transport to lysosomal compartments. Nature (Lond.). 349: 669–676.

Quaranta, V., O. Majdic, G. Stingl, K. Litska, H. Honigsmann, and W. Knapp. 1984. A human la cytoplasmic determinant located on multiple forms of invariant chain (γ1, γ2, γ3). J. Immunol. 132: 1900–1905.

Reid, P. A., and C. Watts. 1990. Cycling of cell-surface MHC glycoproteins through primosome-sensitive intracellular compartments. Nature (Lond.). 346: 655–657.

Roche, P. A., and P. Cresswell. 1990. Invariant chain association with HLA-DR molecules inhibits immunogenic peptide binding. Nature (Lond.). 345: 615–618.

Salamero, J., M. Humbert, P. Cosson, and J. Davoust. 1990. Mouse B lymphocyte specific endocytosis and recycling of MHC class II molecules. EMBO (Eur. Mol. Biol. Organ.) J. 9: 3489–3496.

Sege, K., K. Lask, and P. A. Peterson. 1981. Role of β2-microglobulin in the intracellular processing of HLA antigens. Biochemistry. 20: 4523–4530.

Strubin, M., E. O. Long, and B. Mach. 1986a. Two forms of the la antigen-associated invariant chain results from alternative initiations at two in-phase AUGs. Cell. 47: 619-625.

Strubin, M., C. Berte, and B. Mach. 1986b. Alternative splicing and alternative initiation of translation explain the four forms of the la antigen-associated invariant chain. EMBO (Eur. Mol. Biol. Organ.) J. 5: 3483–3488.

Teyton, L., D. O'Sullivan, P. W. Dickson, V. Lotteau, A. Sette, P. Fink, and P. A. Peterson. 1990. Invariant chain distinguishes between the exogenous and endogenous antigen presentation pathways. Nature (Lond.). 348: 39–44.

Thomas, L. J., Q. V. Nguyen, L. W. Elliott, and R. E. Humphreys. 1988. Proteolytic cleavage of II to p25. J. Immunol. 140: 2670–2674.

Unanue, E. R. 1984. Antigen-presenting function of the macrophage. Annu. Rev. Immunol. 2: 395–428.

Wright, C. J., P. van Endert, P. Moller, J. Lipp, N. R. Ling, I. C. M. MacLennan, N. Koch, and G. Molenhauer. 1990. Human major histocompatibility complex class II invariant chain is expressed on the cell surface. J. Biol. Chem. 165: 5787–5792.