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Ia-Associated Invariant Chain Is Fatty Acylated before Addition of Sialic Acid†

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ABSTRACT: The murine invariant chain (Ii) was found to incorporate radioactive palmitic acid. This binding of fatty acid inhibits the formation of interchain S–S bonds, probably because the cysteine residue in the transmembrane region of the Ii chain is palmitylated. The inhibition of fatty acylation by cerulenin blocks further posttranslational maturation of the invariant chain as shown by two-dimensional gel electrophoresis of Ii immunoprecipitates. In particular, the addition of sialic acid residues is blocked. Thus, it appears that fatty acylation is essential for carbohydrate processing of the Ii chain.

The immune response to soluble antigens is regulated by Ia antigens encoded by the I region of the major histocompatibility complex on chromosome 17 of the mouse. Ample evidence suggests that T lymphocytes can recognize antigens only in association with Ia antigens on the surface of antigen-presenting cells [for a review, see Unanue (1981)]. However, little is known about the molecular mechanism of antigen presentation.

Ia antigens consist of polymorphic integral membrane glycoproteins which form two heterodimers, $A_\alpha A_\beta$ and $E_\alpha E_\beta$. These dimers are expressed on the surface of macrophages, blymphocytes, and several other cell types (Hämmerling, 1976). Early after their synthesis Ia antigens are linked to a nonpolymorphic polypeptide of M_r 31 000, designated the invariant (Ii) chain (Jones et al., 1978). This Ia–Ii complex is transient and is dissociated during transport of Ia antigens to the plasma membrane. The cytoplasmic site of this separation of Ia and Ii is not known.

Previously we and others have observed that there is more than one species of invariant chains associated with murine Ia antigens. Of this family of invariant polypeptides the Ii chain is the most abundant (Koch & Hämmerling, 1982; Zecher et al., 1984). At least two of the polypeptides, the Ii chain and a polypeptide of M_r at 41 000, are structurally closely related and are encoded by the same gene (Yamamoto et al., 1985).

Analogues of the mouse invariant chain have been observed in human (Charron & McDevitt, 1979), in rat (Frelinger et al., 1979), in hamster (Sung et al., 1982) and in guinea pig (Quill & Schwartz, 1983) cells. The function of the Ii chain is not known. However, the fact that in all species investigated so far invariant chains are associated with Ia antigens suggests that invariant chains are involved in expression or assembly of Ia, or in its biological function.

Human (Strubin et al., 1984; Claesson et al., 1983) and mouse (Singer et al., 1984) invariant chain cDNA sequences are known. The deduced amino acid sequences exhibit a homology of 73% [reviewed in Long (1985)]. This highly conserved primary structure explains many features common

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to both the mouse and human invariant chains. Both proteins are very basic with a p*I* value of about 8. They bear two N-linked glycans, and they are strongly labeled with [^{35}S]-methionine (McMillan et al., 1981) because they contain 14 methionines. The processed form of the human invariant chain was found to contain also O-linked oligosaccharides and sialic acids (Claesson & Peterson, 1983; Machamer & Cresswell, 1982). In contrast, no O-linked glycosylation and sialylation have been reported for the mouse Ii chain.

In this report we investigate further the maturation of the Ii chain. We demonstrate that Ii polypeptides bind palmitic acid and that sialic acids are added to Asp-linked and presumably Ser/Thr-linked glycans. The fatty acylation of the Ii chain is a prerequisite for sialylation.

Materials and Methods

Cell Lines and Monoclonal Antibodies. WEHI 267 is a plasmacytoma of BALB/c origin, kindly provided by Dr. A. W. Harris, Melbourne. The in vitro growing B-lymphoma CH1.1 derived from BlO.-H-2^a,H-4^b congenic mice was described by S. Koch et al. (1984). The monoclonal antibody (mAb)[1] In1 was obtained by fusion of rat spleen immunized with CH1.1 cells (Koch et al., 1982). MAb In1 (IgG2b) is specific for the mouse invariant chain (Ii) and a polypeptide with M_r 41 000, which is another member of the Ii family (Yamamoto et al., 1985). MAb MAR 18.5 (IgG2a) is directed against rat κ chain (Lanier et al., 1982). Cell lines and hybridomas were cultured in RPMI 164o containing 10% FCS.

Biosynthetic Labeling of Cells and Immunoprecipitation. These procedures were performed as described by (Koch & Hämmerling, 1982). After being washed with medium lacking methionine, cells were labeled for 1 h with medium containing 20 μCi of [^{35}S]methionine/mL (600 Ci/mmol; Amersham) and 10% dialyzed FCS. Labeling with [^{3}H]palmitic acid (New England Nuclear; 30 Ci/mmol) was performed with medium containing 10% dialyzed FCS, supplemented with 0.5 mCi of [^{3}H]palmitic acid/mL (added as 80 mCi/mL) and 10^6 cells/mL for 1 h.

After being labeled, 10^7 cells were resuspended in 500 μL of Tris-buffered saline, pH 7.4, containing protease inhibitors 1:1000 Trasylol (Sigma) and 10 mM PMSF (Serva) and lysed by addition of 50 μL of 10% Nonidet P-40 (Fluka). Cell debris was removed by centrifugation in an Eppendorf centrifuge (10000*g*). The cell lysate was precleared with protein A–Sepharose (Pharmacia) (50 μL of packed beads) for 2 h at 4 °C. For immunoprecipitation the cell lysate and 50 μL of In1 hybridoma culture supernatant (20 × concentrated), 50 μL of MAR 18.5 (mouse anti-rat κ chains) concentrated supernatant as a protein A binding sandwich, and 5 μL of packed beads of protein A–sepharose were gently rotated over night. The protein A–Sepharose was washed 3 times with Tris-buffered saline, pH 7.4, containing 0.25% NP40, PMSF, and Trasylol.

Inhibition of N-Glycosylation with Tunicamycin. WEHI 267 cells (5 × 10^5/mL) were cultured in the presence of 4 μg/mL tunicamycin (Sigma) for 150 min as described previously (Yamamoto et al., 1985). Lower concentrations of tunicamycin and shorter incubation times did not completely inhibit N-glycosylation of the Ii chain (data not shown).

Inhibition of Fatty Acylation by Cerulenin. Cells were cultured for 4 h in the presence of cerulenin (Calbiochem) (5–100 μg/mL), which is an inhibitor of fatty acylation (Schlesinger & Malfer, 1982). With 10–20 μg/mL cerulenin cell growth was not affected and the synthesis of the invariant chain was not decreased.

Neuraminidase Treatment. The invariant chain was immunoprecipitated from 1-h labeled material as described above. For neuraminidase treatment 20 milliunits (20 μL) of neuraminidase (Boehringer) were added to the immunoprecipitate after 0, 30, 60, and 120 min and incubated for 4 h at 37 °C. The precipitates were washed 3 times with 0.25% NP40.

Two-Dimensional Gel Electrophoresis. Nonequilibrium pH gradient gel electrophoresis (NEPHGE) separation and nonreduced SDS–PAGE were performed as described (O'Farrel et al., 1977; Koch & Hämmerling, 1982). The second dimension (SDS–PAGE) was as described by Laemmli (1970). Briefly, for the first dimension NEPHGE, immunoprecipitates were treated with lysis buffer containing 50 mM dithiothreitol, 2% Triton X-100, 9.5 M urea, and 2% Pharmalytes pH 3–10. NEPHGE gels were prepared from polyacrylamide (Bio-Rad), Triton X-100 (Merck), urea (Schwarz/Mann), and Pharmalytes pH 3–10 (Pharmacia). Nonequilibrated pH gradient electrophoresis was performed for 5 h at constant voltage (550 V). The first dimension (nonreduced SDS–PAGE) was carried out with immunoprecipitates treated for 5 min at 95 °C with sample buffer containing 1% SDS in 0.06 M Tris, pH 6.8, and electrophoresed in 3-mm rod gels with 1 mA per gel. For the second dimension gels were treated for 1 h with sample buffer containing 1% SDS and 50 mM dithiothreitol in 0.06 M Tris, pH 6.8. The first-dimensional rod gels were fixed on top of the second-dimensional gel (13% acrylamide) with hot agarose (1%) melted in sample buffer. The second dimension was performed with constant current (200 mA).

Results

Murine Ii Chain Contains Sialic Acid Bound to N- and O-Linked Glycans. The invariant chain family is expressed as a group of proteins of approximate molecular weight of 41 000 (p41), 31 000 (Ii), and 25 000 (p25). Both the p41 and the Ii chain bear the determinant recognized by the monoclonal antibody In1, as recently shown by immunoblotting studies (Yamamoto et al., 1985). Probably p25 is also a member of the Ii family as suggested by the observation that transfection of the Ii gene into Ii negative cells leads to expression of p41, Ii, and p25 (unpublished data). For the present investigation we have chosen a cell line that expresses large amounts of the Ii chain, small amounts of p41 and p25 polypeptides, and no Ia antigens (Koch & Harris, 1984). Therefore, the complexity of the two-dimensional gel pattern is reduced when this line is used for analysis of Ii.

WEHI 267 plasmacytoma cells were labeled for 1 h with [^{35}S]methionine. The invariant chain was immunoprecipitated from cell lysates with mAb In1. The immunoprecipitates of Ii were separated in two dimensions, in the first by nonequilibrium pH gradient electrophoresis (NEPHGE) and in the second dimension by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The invariant chain and three spots with approximate molecular weight of 25 000 (p25) were obtained (Figure 1A). The Ii chain itself resolves into six to eight additional acidic spots with increasing molecular weight (bracket in Figure 1A). Pulse and pulse–chase experiments suggest that the acidic spots were indeed derived from the Ii chain (data not shown). In contrast, the p25 chain, which is characterized by two to three distinct spots, does not exhibit additional charge heterogeneity after chase. When the

[1] Abbreviations: mAb, monoclonal antibodies; FCS, fetal calf serum; Hepes, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid; CAS, concanavalin A conditioned medium; PMSF, phenylmethanesulfonyl fluoride; NP40, Nonidet P-40; NEPHGE, nonequilibrium pH gradient gel electrophoresis; Tris, tris(hydroxymethyl)aminomethane; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

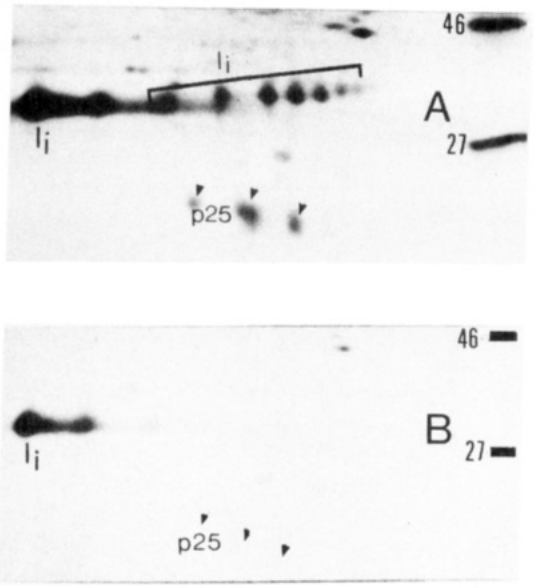

FIGURE 1: Neuraminidase treatment of Ii immunoprecipitates. Mouse plasmacytoma cells WEHI 267 were labeled for 1 h with [^{35}S]-methionine and immunoprecipitated with mAb In1. (A) Nontreated or (B) neuraminidase-treated immunoprecipitates were electrophoresed in the first dimension by NEPHGE and in the second dimension by SDS–PAGE.

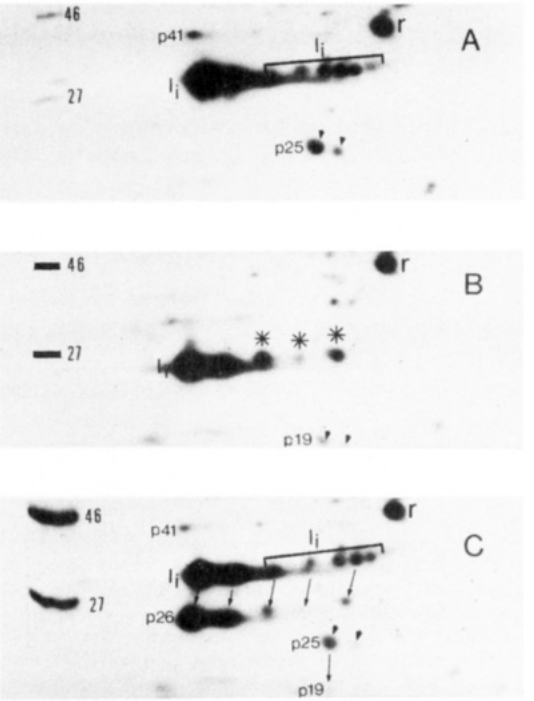

FIGURE 2: Ii chain maturation after inhibition of N-glycosylation. WEHI 267 cells were cultured in the presence or absence of tunicamycin and subsequently labeled for 1 h with [^{35}S]methionine. Immunoprecipitates obtained with mAb In1 were separated as in Figure 1. (A) Without tunicamycin; (B) tunicamycin treated; (C) immunoprecipitates mixed from (A) and (B). r represents a contaminant not sensitive to tunicamycin which can be used as a reference. Spots marked with an asterisk (B) represent sialylated Ii forms on O-linked carbohydrates. Arrows in (C) relate the invariant chains containing N-linked carbohydrates to their respective precursors.

cells were precultured with conditioned medium (CAS) which is known to increase expression of the Ii chain (Koch, N., et al., 1984), also the p41 polypeptide is present as a series of acidic spots (data not shown).

Next we investigated whether sialic acid residues were involved in the formation of the acidic Ii spots. Immunoprecipitates of Ii were treated with neuraminidase and separated in two-dimensional gels. The neuraminidase treated material (Figure 1B) lacks the acidic Ii spots when compared to the nontreated material (Figure 1A). The invariant chain treated with neuraminidase (Figure 1B) is indistinguishable in charge and size to the invariant chain observed when 10-min pulse-labeled material is used (not shown). These findings indicate that the acidic Ii spots are derived from Ii by addition of sialic acid residues.

The sequence of the human and the murine Ii chains contain two Asp-X-Thr residues which may serve as N-glycosylation sites (Claesson et al., 1983; Strubin et al., 1984; Singer et al., 1984). In accordance with these data treatment of cells with tunicamycin showed that the Ii chain contains two Asp-linked carbohydrate side chains (Sung & Jones, 1981), whereas the p41 polypeptide may have three to four N-linked carbohydrate side chains (Yamamoto et al., 1985). In contrast to the human invariant chain so far no O-glycosylation has been found in the mouse Ii chain (Swiedler et al., 1983; Sung & Jones, 1981).

WEHI 267 cells were cultured in the presence or absence of tunicamycin and subsequently labeled for 1 h with [^{35}S]-methionine (Figure 2). As expected the high mannose form of Ii (Figure 2A) is about 5000 larger in molecular weight than its precursor, which was obtained after tunicamycin treatment (Figure 2B). Mixing of material from untreated and tunicamycin-treated cells confirms this shift in molecular weight (Figure 2C). The N-glycosylated form of Ii appears to be slightly more acidic than its nonglycosylated precursor. It can also be seen in Figure 2 that the molecular weight of the associated p25 component is decreased by about 6000, suggesting the presence of two N-linked carbohydrate side chains. After inhibition of N-glycosylation with tunicamycin, several of the acidic Ii spots found in Figure 2A are not present (part B or C of Figure 2), which indicats that these sialic acids are located on the N-linked carbohydrate chains. However, three of the acidic spots are still present. They are reduced in size due to lack of N-linked carbohydrates and are only slightly altered in charge (Figure 2C and marked with asterisks in Figure 2B). The resistance of the acidic components to tunicamycin treatment suggests the presence of sialic acids on Ser/Thr (O-linked) carbohydrates. This is supported by data of Claesson & Peterson (1983) and Machamer & Cresswell (1982), who found O-linked glycans on the human invariant chain.

Immature Ii Chains Can Form Covalent Interchain S–S Bonds. Nonreduced/reduced SDS–PAGE separates S–S-linked Ii dimers from the respective monomeric forms (Koch & Hämmerling, 1982; Koch et al., 1982). The Ii chain contains only one cysteine residue which must be responsible for the formation of S–S bonds. According to the amino acid sequence this cysteine is located either within the membrane or close to it (one or two amino acid positions apart) on the cytoplasmic portion of the Ii chain (Long, 1985). Because of the strong reducing conditions inside the cell (Kosower & Kosower, 1978) it is possible that these intermolecular S–S bonds are formed during lysis of cells and therefore are artificial. However, the existence of these S–S bonds suggests a previous noncovalent association of Ii chains.

Separation of 1 h labeled immunoprecipitates of Ii by nonreduced/reduced electrophoresis demonstrates that only immature high mannose forms of Ii and p41 chains exist as S–S-linked dimers (e.g., Ii–Ii and I–p41; see Figure 3A). In contrast, the mature forms of the Ii chain bearing complex carbohydrates and having a higher approximate molecular weight appear as monomers on the diagonal (bracket in Figure 3A). Presumably on these monomeric Ii chains the cysteines

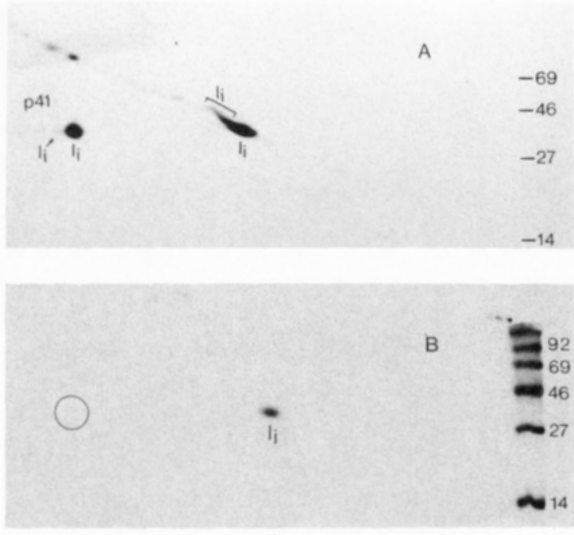

FIGURE 3: Separation of S–S-linked dimers from monomeric invariant chains. Immunoprecipitates obtained with mAb In1 were separated in the first dimension nonreduced SDS–PAGE, reduced, and then separated in the second dimension, SDS–PAGE. Previous S–S linked subunits were situated below the diagonal. (A) WEHI 267 cells were labeled for 1 h with [^{35}S]methionine. Subunits of S–S-linked dimers as derived from Ii–p41 and Ii–Ii were situated below the diagonal. Monomeric molecules are situated on the diagonal. The position of the processed forms of Ii are marked with a bracket. (B) WEHI 267 cells were labeled for 1 h with [^{3}H]palmitic acid. The Ii-marked spot represents the monomeric Ii form whereas in the dimeric region (circle) no labeled Ii is present.

are not available for the formation of intermolecular S–S bonds. This raises the question whether during maturation of the Ii chain the cysteine is blocked.

Ii Chain Is Metabolically Labeled with [^{3}H]Palmitic Acid. Recently it has been reported that some HLA antigens are fatty acylated at their transmembrane cysteines (Kaufman et al., 1984). As mentioned above the Ii chain also contains one transmembrane cysteine at position 28. We addressed the question whether this cysteine can be palmitylated and therefore may not be available for formation of interchain S–S bonds. WEHI 267 plasmacytoma cells were labeled for 1 h with [^{3}H]palmitic acid, immunoprecipitated with mAb In1, and separated in a nonreduced/reduced two-dimensional gel. Figure 3B shows that [^{3}H]palmitic acid is incorporated into the Ii chain of WEHI 267 cells. Since the fatty acid was not removable by boiling in SDS sample buffer, this suggests a covalent linkage of palmitic acid to the invariant chain. However, the label is only present in the monomeric Ii form. No label was observed on the gel at the position of the S–S-linked dimer (marked with a circle in Figure 3B and compare with Figure 3A). Similar observations were made with a B lymphoma, CH1.1, and murine spleen cells (data not shown).

Since the palmitic acid label is only incorporated into the monomeric Ii, it is likely that fatty acylation modifies the cysteine by thio ester bonds as described by Kaufmann et al. (1984) and thus previous formation of S–S bonds. This assumption is supported by the finding that the palmitic acid label is sensitive to hydroxylamine treatment (data not shown).

Fatty Acylation Is Necessary for Addition Of Sialic Acid. Next we studied whether maturation and processing of the carbohydrate side chain are dependent upon fatty acylation. Cells were cultured in the presence of cerulenin, an inhibitor of fatty acylation (Schlesinger & Malfer, 1982) and subsequently labeled for 1 h with [^{35}S]methionine. Cerulenin was used in concentrations of 5–100 μg/mL. Concentrations above 50 μg/mL are toxic and inhibit the synthesis of the Ii chain.

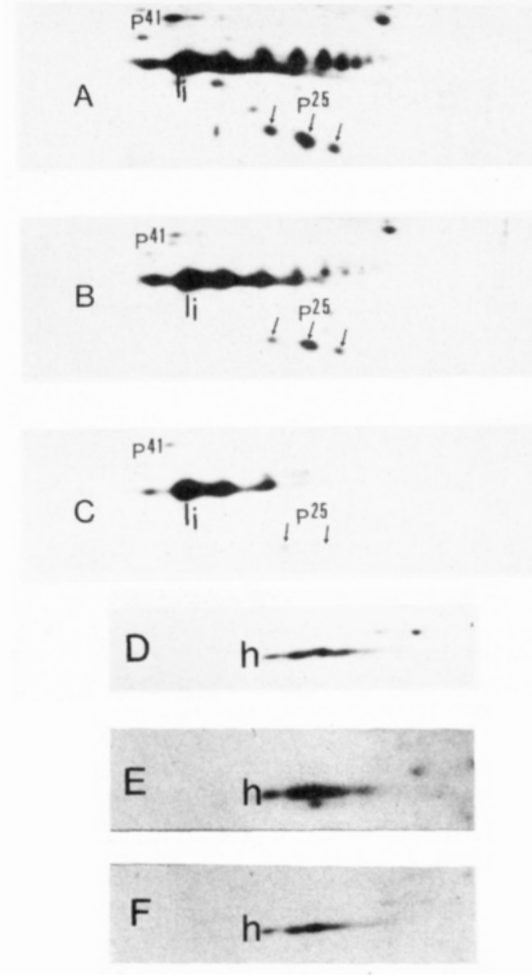

FIGURE 4: Inhibition of fatty acid acylation by cerulenin. WEHI 267 cells were cultured in the absence (A and D) or the presence of (B and E) 5 and (C and F) 10 μg/mL cerulenin. Subsequently cells were labeled for 1 h with [^{35}S]methionine and immunoprecipitated with mAb In1 (A–C). The immunoglobulin heavy chain (h), which is not fatty acylated, was used as a standard obtained in the same experiment and controls the treatment with cerulenin (D–F). Two-dimensional separation was performed as described in Figure 1.

With 5 μg/mL the appearance of sialylated Ii was partially (Figure 4B) and with 10 μg/mL (Figure 4C) almost completely inhibited. However, the glycosylation of the heavy chain of immunoglobulin which is not fatty acylated (h in Figure 4D–F) is not altered by treatment with cerulenin. Since only the immature but no mature forms of Ii chain were obtained after inhibition of fatty acylation with 10 μg/mL cerulenin (Figure 4C), it appears that palmitylation is essential for further processing of the invariant chain.

Discussion

This report describes the covalent linkage of palmitic acid to the murine Ia-associated invariant chain. Palmitylation of the Ii chain was only observed in monomeric but not in dimeric forms of the Ii chain. Thus, the formation of interchain S–S bonds seems to be blocked by fatty acylation. A likely explanation is that the cysteine residue in the transmembrane region of the Ii chain is the target structure for fatty acylation and therefore not available for formation of S–S bonds.

Although covalent linkage of fatty acids was frequently observed for viral and bacterial membrane proteins, so far only few fatty acylated membrane proteins of eukariotic cells have been described [reviewed in Schmidt (1983) and Magee & Schlesinger (1982)]. Notable examples include HLA-B and HLA-DR heavy chains, which are modified by fatty acids via

thio ester bonds (Kaufman et al., 1984), and the transferrin receptor (Omary & Trowbridge, 1981; Schneider et al., 1984). The function of fatty acids on membrane proteins is not yet clear. It has been suggested that fatty acids are important for interaction with membranes in the various cell compartments. There is some evidence that fatty acylation is essential for assembly of the acetylcholine receptor and/or surface expression (Olson et al., 1984). However, fatty acylation of membrane proteins does not seem to be a general feature of membrane proteins. For example, HLA-A1 and -A2 heavy chains lack a transmembrane cysteine and are not modified by fatty acids. In contrast HLA-B7, -B8, and -B27 heavy chains are functionally and structurally very similar to HLA-A but are fatty acylated (Kaufman et al., 1984). Moreover, in some variant forms of vesicular stomatitis virus the G proteins are palmitylated whereas in other strains they are not modified (Kotwal & Gosh, 1984). These authors concluded that fatty acylation is not a general requirement for maturation and budding of envelope viruses.

For the Ii chain it remains to be determined whether or not fatty acid modification is functionally meaningful. Perhaps fatty acids are necessary for transport of Ii through intracellular compartments. Since only monomeric but not dimeric forms of the Ii chain can mature by addition of sialic acid, it is possible that a function of the fatty acid modification consists in prevention of dimerization. In this context it is of interest to note that not only murine but also human Ii chains have a cysteine in their transmembrane region (Long, 1985) which is fatty acylated (own unpublished observation).

It is also interesting to observe that both the transferrin receptor and the invariant chain are inserted with their N-terminus into the membrane. Both proteins lack a cleavable leader sequence and both occur as dimeric structures. During its way to the plasma membrane the transferrin receptor has to cross several membrane compartments (Octave et al., 1981). This transit occurs by fusion of intracellular membranes with transport vesicles. Because of the asymmetric orientation of the transferrin receptor, its acylation site is in the cytoplasm during transport. At this position the fatty acid could serve as an acceptor site for intracellular membrane fusions. Although the similar properties of the Ii chain suggest that its transport mechanism is related to that of the transferrin receptor, more experimental evidence is necessary.

The processing of proteins in various cell compartments has been intensively investigated. Goldberg & Kornfeld (1983) were able to demonstrate the subcellular organization of processing of Asp-linked oligosaccharides. They also defined the stages in which enzymes modify the carbohydrate side chains. In the Golgi apparatus further glycans are added to Ser/Thr. Fatty acylation appears to take place in an early subcompartment of the Golgi complex (Dunphy et al., 1981) shortly before oligosaccharides become resistant to endoglycosidase H (Schmidt & Schlesinger, 1980) or possibly in some compartments of the rough endoplasmic reticulum with acylation activities (M. F. G. Schmidt, personal communication). From these findings and from this and previous work on the Ii chain, the following scheme for the biosynthesis of Ii can be drawn: During insertion of the Ii chain into the membrane of the endoplasmic reticulum two oligosaccharides are attached to asparagines in positions 114 and 120. The transmembrane cysteine residue (position 28) is now available for formation of interchain S–S bonds or for fatty acylation. The N-glycosylated protein will pass through various subcellular compartments where its oligosaccharides are processed. Since we have observed that not all carbohydrate side chains are N-linked, it is likely that the mouse invariant chain contains also Ser/Thr-linked glycans. This would be in agreement with the human invariant chain that is known to contain O-linked carbohydrates (Claesson & Peterson, 1983). Addition of O-linked carbohydrates occurs probably in the Golgi apparatus. Fifteen to thirty minutes posttranslation, and only after fatty acylation, sialic acid residues can be added to the glycans of the invariant chain (data not shown). Modification by sialic acid has also been observed for the human invariant chain (Machamer & Cresswell, 1982; Claesson & Peterson, 1983).

A pool of invariant chains remains in the endoplasmic reticulum (Kvist et al., 1982; Machamer & Cresswell, 1984; Sung & Jones, 1981) and is not processed to its mature form. Some of the invariant chain is still endoglycosidase H sensitive also after 300 min of chase (Claesson & Peterson, 1983). As the invariant chain is usually synthesized in excess compared to Ia antigens, it has been suggested that the invariant chain can only be transported and processed if associated with polymorphic Ia antigens (Claesson & Peterson, 1983). Our observation that the Ii chain can be processed in Ia negative cells as shown in this report does not agree with this view.

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Monoclonal Antibodies Defining Blood Group A Variants with Difucosyl Type 1 Chain (ALe^b) and Difucosyl Type 2 Chain (ALe^y)†

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ABSTRACT: Three hybridomas secreting monoclonal antibodies, HH1, HH2, and HH3, defining different difucosyl A structures (ALe^b or ALe^y), have been established. Antibody HH1 (IgG2a) reacts specifically with the difucosyl A structure irrespective of a type 1 or type 2 chain, while antibody HH2 (IgG3) reacts exclusively with the difucosyl type 2 chain A (ALe^y) and does not react with the difucosyl type 1 chain or monofucosyl type 2 chain. Antibody HH3 (IgG2a) reacts exclusively with the difucosyl type 1 chain A (ALe^b) and does not react with the monofucosyl type 1 chain A or mono- and difucosyl type 2 chain A. These hybridoma antibodies were obtained by immunization of mice with purified glycolipid antigens and were selected by their reactivity with the specific glycolipid structures. These antibodies, together with previously established monoclonal antibody AH-21, specific for monofucosyl type 1 chain A, and monoclonal antibody TH-1, specific for type 3 chain A, are extremely useful to define blood group A variants present in cells and tissues.

The blood group A determinant is a well-established trisaccharide, GalNAcα1→3[Fucα1→2]Galβ1→R; however, the determinant is carried by a large number of core structures as listed in Table I [reviewed by Watkins (1980) and Hakomori (1981)]. With the recent development of the monoclonal antibody approach, the complexity and variation in blood group A determinants have been clearly established by specific monoclonal antibodies that distinguish among these A variants (Abe et al., 1984; Clausen et al., 1984, 1985a). It has become apparent that variation in core structure, but not in the A determinant itself, provides the basis for the presence of structurally and immunologically distinctive variants of A antigens, such as A_1 and A_2 (Moreno et al., 1971; Kisailus & Kabat, 1979; Clausen et al., 1985). Previously, we have established monoclonal antibodies AH-21 defining type 1 chain A (Abe et al., 1984) and TH-1 defining type 3 chain A (Clausen et al., 1985a). Among type 1 and type 2 chain A variants, two types of structures, monofucosyl and difucosyl, have been distinguished, as shown in Table I (1 and 2). Of particular interest is a wide distribution of difucosyl A determinants found in glycolipids of animal and human gas-

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