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Association of Human γ Chain with Class II Transplantation Antigens during Intracellular Transport†

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ABSTRACT: Cell surface expressed human and murine class II transplantation antigens are composed of two polypeptide chains called α and β. During intracellular transport an invariant chain, provisionally called γ, is associated with the class II antigen chains. Since γ chains leave the endoplasmic reticulum only when associated with α and β chains, we have studied the intracellular transport of the γ chain and its possible cell surface expression. Modifications of the carbohydrate moieties of the γ chain during intracellular transport were also examined. The γ chain appears to contain two Asn-linked carbohydrate moieties and maybe also one or more Ser/Thr-linked carbohydrates. At all times during the pulse–chase experiments core glycosylated γ chains resolved into two distinct spots on two-dimensional gel electrophoresis. The occurrence of core-glycosylated γ chains was expected since more γ chains than α and β chains exist in the endoplasmic reticulum. Terminally glycosylated, α, β, and γ chains emerged simultaneously supporting the idea that the three types of chains are brought to the Golgi complex bound to each other. However, terminal glycosylation is temporally related to the dissociation of the γ chain from the α and β chains. Since isolated plasma membranes contained molecules indistinguishable from γ chains, it is concluded that γ chains are transported together with class II antigens from the endoplasmic reticulum to the Golgi complex. After dissociation, class II antigens and some, if not all, γ chains seem to become independently integrated into the plasma membrane.

The major histocompatibility complex (MHC)[1] controls the expression of class II transplantation antigens [for a review, see Benacerraf (1981)]. These molecules are composed of two dissimilar, glycosylated chains, called α and β, which are expressed on the cell surface. In the mouse the I region encompasses two subloci, A and E, which each control the expression of a distinct type of class II antigen (Uhr et al., 1979). Likewise, the human MHC controls at least two types of class II antigens, i.e., HLA-DR antigens and DC antigens (Shackelford et al., 1981; Tosi et al., 1978). In addition, the

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[1] Abbreviations: MHC, major histocompatibility complex; NaDodSO$_4$, sodium dodecyl sulfate; Endo H, endo-β-*N*-acetylglucosaminidase H; Tris, tris(hydroxymethyl)aminomethane; Mops, 3-(*N*-morpholino)-propanesulfonic acid.

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SB locus of the human MHC may also control the expression of a separate type of class II antigens (Shaw et al., 1981). In fact, current efforts to unravel the number of distinct α and β chain genes in the human MHC suggest that at least three types of α and four types of β chain genes may be transcribed in lymphoblastoid cells (Böhme et al., 1983; Wake et al., 1982; unpublished results).

Structural analyses reveal a high degree of homology among α and β chains (Larhammar et al., 1982a). This finding together with the fact that newly synthesized α and β chains of several loci simultaneously occur in the endoplasmic reticulum raises the question why hybrid class II antigens, i.e., molecules consisting of an α chain of one locus combined with a β chain of another locus, have not been reported. One of several possible explanations for the absence of hybrid class II antigens may be that nascent α and β chains become sequestered in such a way that proper association between α and β chains of a given locus is ensured. In this context it is interesting to note that murine and human class II antigens are associated intracellularly with a nonpolymorphic chain, called the "invariant spot" (Jones et al., 1978; Charron & McDevitt, 1979) or the γ chain (Kvist et al., 1982).

In a previous study (Kvist et al., 1982) we demonstrated that this invariant chain, in this paper provisionally called the γ chain, is present in the endoplasmic reticulum in amounts exceeding those of α and β chains. We also showed that the γ chain is transported from the endoplasmic reticulum to the Golgi complex and that this translocation only occurs when the γ chain is associated with class II antigens. Here we have examined the intracellular transport of the γ chain from the endoplasmic reticulum to the cell surface. We demonstrate that the γ chain becomes terminally glycosylated in the Golgi complex, concomitantly with its dissociation from the class II antigens. At least a fraction of the γ-chain pool then seems to be transported to the cell surface.

Materials and Methods

Antisera. A rabbit antiserum (K 311) raised against highly purified, papain-solubilized class II antigens was used. The reactivity of this antiserum has been described (Klareskog et al., 1978; Kvist et al., 1982). Antisera raised against isolated class II antigen α (K 343) and β (K 344) chains were also employed. These antisera preferentially react with nascent, free α and β chains, respectively, although they weakly recognize the complex of α and β chains [see Palacios et al. (1982)]. The rabbit antiserum against class I antigens (K 286) displayed the same reactivity as earlier described (Sege et al., 1981). None of the antisera reacted directly with γ chains (Kvist et al., 1982; Figure 1). Routinely, 1 μL of each antiserum was used for immunoprecipitation.

Cultivation and Radioactive Labeling of Cells. Raji cells, obtained from American Type Culture Collection (Rockville, MD), were cultured in RPMI 1640 supplemented with 10% fetal calf serum (Flow Laboratories, Irvine, U.K.), 100 units/mL penicillin, and 50 μg/mL streptomycin (Sigma Chemical Co., St. Louis, MO). Radioactive labeling of Raji cells with [^{35}S]methionine was carried out as described (Sege et al., 1981). Briefly, the cells, at a concentration of 10×10^6/mL in methionine-free RPMI 1640 supplemented with 10% dialyzed fetal calf serum, were incubated with 1 mCi/mL [^{35}S]methionine (specific activity 800 Ci/mmol, Amersham, U.K.) for 10 min at 37 °C. After this incubation time further incorporation of radioactivity into proteins was terminated by cooling to 0 °C. When chase experiments were carried out, the pulse-labeled cells were incubated for various periods of time in 5 volumes of prewarmed RPMI 1640, containing a 5-fold higher concentration of methionine than the standard medium. Aliquots of cells were withdrawn at appropriate times and were immediately chilled to 0 °C.

When Raji cells were labeled for longer periods of time, the cell concentration was adjusted to 1×10^6 cells/mL and the [^{35}S]- and [^{3}H]methionines (specific activity 70–90 Ci/mmol, Amersham, U.K.) were added to final concentrations of 50–100 μCi/mL. Otherwise the labeling protocol was as described above.

Raji cells were also labeled by lactoperoxidase-catalyzed iodination using Enzymobeads (Bio-Rad, Richmond, CA). Usually 20×10^6 cells in 50 μL of phosphate-buffered saline, pH 7.2, were mixed with 1 mCi of ^{125}I (Amersham, U.K.) and the recommended amount of Enzymobeads. After 20 min of labeling the cells were washed repeatedly with phosphate-buffered saline. Following this procedure 97% of the cells still excluded Trypan Blue.

Indirect Immunoprecipitation. Internally and externally labeled Raji cells were lysed at 0 °C, and glycoproteins were isolated by *Lens culinaris* hemagglutinin affinity chromatography (Cullen & Schwartz, 1976). The details have been outlined elsewhere (Sege et al., 1981). Aliquots of the material desorbed from the lectin column were mixed with 5 μL of normal mouse serum and 15 μL of a rabbit antiserum against mouse immunoglobulins. Immune complexes generated after incubation overnight at +4 °C were removed by using formalin-fixed, heat-killed *Staphylococcus aureus* Cowan 1 strain bacteria. The supernatants were subsequently incubated with 1 μL of the relevant antiserum or normal rabbit serum for 4–8 h. Immune complexes were recovered on protein A containing Sepharose 4B (Pharmacia, Uppsala, Sweden). The Sepharose was washed repeatedly, and bound proteins were then released as depicted elsewhere (Sege et al., 1981). All samples were reduced with 10 mM dithiothreitol and alkylated with 50 mM iodoacetic acid (Sigma Chemical Co. St. Louis, MO) prior to further analyses.

Electrophoreses. One-dimensional $NaDodSO_4$–polyacrylamide gel electrophoresis on 10–15% gradient slab gels was carried out according to Blobel & Dobberstein (1975). All chemicals used were of the highest grade available, and all solutions were filtered through a 0.22-μm Millipore filter (Millipore, Bedford, MA) immediately before use. Preparative $NaDodSO_4$–polyacrylamide gel electrophoresis was accomplished on 10–15% gradient gels cast in 6-mm cylindrical glass tubes. After completed electrophoresis the gel was sectioned into 2 mm wide segments, which were soaked overnight at room temperature in 20 mM Tris-HCl buffer, pH 8.0, containing either 1% Triton X-100 or 0.3% $NaDodSO_4$. Proteins eluted from the gel pieces were monitored by subjecting 15% of the material to radioactivity measurement in a Tricarb dual-channel, liquid scintillation counter (Packard, Downers Grove, IL).

Preparative, nonequilibrium pH gradient gel electrophoresis was performed essentially according to the protocol of O'-Farrell (O'Farrell et al., 1977). The gel, cast in 6 mm wide and 125 mm long glass tubes, contained the following ampholytes (Pharmalyte, Pharmacia): 3% v/v of pH 3–10, 1% pH 2.5–5, 1% pH 8–10.5. Fractionated proteins were isolated as described for preparative $NaDodSO_4$–polyacrylamide gels.

Two-dimensional nonequilibrium pH gradient gel electrophoresis and $NaDodSO_4$–polyacrylamide gel electrophoresis were carried out as described (O'Farrell et al., 1977). The first dimension was accomplished in 3 mm wide cylindrical tubes, and the ampholyte combination was the same as described above. The second dimension was carried out on

10–15% gradient $NaDodSO_4$–polyacrylamide slab gels (Blobel & Dobberstein, 1975). Radioactive components were detected by fluorography (Bonner & Laskey, 1974) with Kodak X-AR 5 films. In all figures of two-dimensional gel patterns the nonequilibrium pH gradient gel electrophoreses are shown in the horizontal position with the anode to the right.

Carbohydrate Modifications. To block glycosylation on asparagine residues, Raji cells were cultured in the presence of tunicamycin (a kind gift of Dr. R. Hamill, E. Lilly Co., Inc.) at a final concentration of the antibiotic of 2 μg/mL (Lehle & Tanner, 1976). The cells were maintained in the tunicamycin-containing medium for 2 h prior to radioactive labeling. To circumvent the possibility of tunicamycin causing an incomplete block of asparagine glycosylation, solubilized material from the labeled cells were routinely passed over a *Lens culinaris* hemagglutinin column to remove glycosylated components. In this fractionation step only a small amount of the radioactivity was bound to the column. Calculations showed that tunicamycin blocked the core glycosylation to greater than 85%.

To remove carbohydrate linked to Ser and Thr of class II antigen chains the [^{35}S]methionine long-term-labeled, immunoprecipitated material was solubilized in 10 μL of 10 mM Tris-HCl buffer, pH 8.4, containing 4% $NaDodSO_4$. After the addition of 250 μL of 0.05 M NaOH containing 1 M sodium borohydride, the samples were incubated at 45 °C for 16 h (Finne, 1975). Following this treatment the class II antigen chains were precipitated by the addition of ice-cold trichloroacetic acid, containing 30 μg of DNA (Sigma Chemical Co., St. Louis, MO). The final concentration of the trichloroacetic acid was 20%.

Asn-linked core sugars were removed from immunoprecipitated class II antigen chains by using Endo H (Seikagaku Kogyo Co., Tokyo, Japan) as described elsewhere (Sege et al., 1981).

Neuraminidase digestions were carried out on membrane glycoproteins prior to immunoprecipitation (Sege et al., 1981). The lysates were diluted to twice their original volume with 0.1 M sodium acetate buffer, pH 5.5, containing 0.3 M NaCl and 14 mM $CaCl_2$. The digestions were allowed to proceed for a total period of 4 h. The enzyme (*Vibrio Cholerae* neuraminidase, 1 IU/mL, Calbiochem, Lucerne, Switzerland) was added in 20-μL portions at 0, 30, 60, and 120 min after initiation of the digestions. To terminate the digestion the pH of the samples were raised to 8.0 by using 1.0 M Tris-HCl buffer. Immunoprecipitation was carried out as described above.

Isolation of Plasma Membrane Components. Plasma membranes of Raji cells, labeled with [^{35}S]methionine for 30 min and chased for 3 h, were isolated by using poly(ethylenimine)-coated polyacrylamide beads (Affi-Gel 731, Bio-Rad, Richmond, CA). The procedure adopted was that of Jacobson (1980). Briefly, 10×10^6 cells with a viability greater than 98% were mixed with an equal volume of beads in 20 mM sodium acetate, pH 4.9–5, containing 0.2 M sucrose for 5 min. Prior to washing with 0.25 M sucrose containing 10 mM Mops, pH 6.0, reactive groups remaining on the beads were coated with bovine serum albumin. Cells attached to the beads were recovered by centrifugation and subjected to sonication. After exhaustive washings the plasma membrane components were released from the beads by heating at 70 °C for 5 min in the presence of 4% $NaDodSO_4$.

As a control labeled cells were mixed with beads previously reacted with bovine serum albumin. The amount of radioactivity recovered from such beads was less than 2% of that recovered from the beads which were mixed with the cells prior to the bovine serum albumin treatment.

Peptide Mapping. γ chains, labeled with [^{35}S]- and [^{3}H]methionine, respectively, were separated by electrophoresis and eluted from the gels. The eluted proteins were mixed, 150 μg of bovine serum albumin was added, and the mixtures were concentrated by precipitation using ice-cold trichloroacetic acid at a final concentration of 20%. The precipitates were washed twice with an ether–ethanol solution (1:1 v/v) and then dried. The proteins were solubilized in 100 μL of 0.2 M NH_4HCO_3, pH 8.1, containing 4 M urea, and when the solutions were clear, 5 μg of trypsin (TPCK; Worthington Biochemicals, Freehold, NJ) in 100 μL of 0.1 M NH_4HCO_3 was added. After 90 min at 37 °C an additional 5 μg of trypsin was added. The enzymatic digestion was terminated by heating at 95 °C for 5 min after a total incubation time of 4 h, and the samples were subjected to lyophilization.

Tryptic peptides soluble in 0.1 M ammonium acetate, pH 7.0, were separated by high-pressure liquid chromatography on a C_{18} μBondapak column (Waters, Milford, MA) equilibrated with 0.1 M ammonium acetate, pH 7.0. Elution of peptides was carried out with a 100-mL linear gradient of ethanol from 0 to 80% in 0.1 M ammonium acetate. Fractions of 0.8 mL were collected at 1-min intervals, and the radioactivity of the effluent was monitored by subjecting aliquots of every fraction to measurement in a dual channel Tri-Carb liquid scintillation counter. Quench correction was accomplished by the external standard channel ratio.

Results

Glycosylation of Class II Antigen α, β, and γ Chains. In a previous study of class II antigens we demonstrated that γ chains were coprecipitated with α and β chains although the antiserum used only recognized α and β chains (Kvist et al., 1982). One-dimensional electrophoresis of pulse–chase labeled class II antigens gave good resolution of core-glycosylated α, β, and γ chains. However, the amount of core-glycosylated γ chains diminished during the chase period while the α-chain band increased in intensity. Whether these quantitative changes were due to terminal glycosylation of the γ chain or to other mechanisms was not elucidated. Thus, in the absence of specific antibodies against the γ chain we decided to investigate the carbohydrate modifications of α, β, and γ chains that take place during intracellular transport as a means to trace the γ chain also at later stages of the intracellular transport. Moreover, two-dimensional electrophoresis was employed to increase the resolution.

It is well established that human class II antigen α and β chains contain Asn-linked carbohydrate moieties (Charron & McDevitt, 1980; Strominger, 1980; Owen et al., 1981). The possible occurrence of Ser/Thr-linked sugars has not been examined. The glycosylation of the γ chain has not been worked out in detail so we had to sort out the electrophoretic behavior of glycosylated and nonglycosylated α and β chains to be able to identify γ chains. To this end we used antisera specific for α and β chains, respectively, and examined the electrophoretic mobilities of core-glycosylated as well as nonglycosylated class II antigen chains. Raji cells were labeled with [^{35}S]methionine for 10 min. After solubilization the class II antigens were indirectly immunoprecipitated with three different antisera. Figure 1 shows that the antiserum (K 311) reacting with both α and β chains precipitated α, β, and γ chains. The antisera against α chains (K 343) and β chains (K 344) exclusively precipitated α and β chains, respectively, but did not coprecipitate γ chains. The latter two antisera also reacted well with α and β chains derived from tunica-

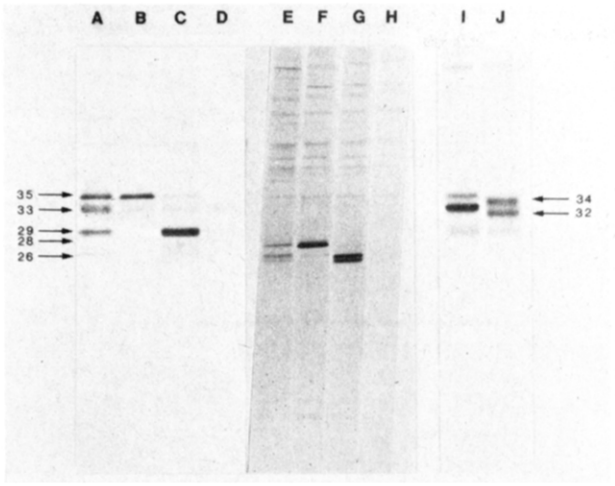

FIGURE 1: Analyses of class II antigen Asn- and Ser/Thr-linked carbohydrate moieties. Raji cells were labeled for 10 min with [^{35}S]methionine in the absence (A–D) and presence (E–H) of tunicamycin. Indirect immunoprecipitations were carried out with antisera against class II antigens (K 311, lanes A and E), against class II antigen α chains (K 343, lanes B and F), and against class II antigen β chains (K 344, lanes C and G). Normal rabbit serum was used as the control (lanes D and H). To examine the presence of Ser/Thr-linked carbohydrate the immunoprecipitated long-term-labeled class II antigens, brought down by antiserum K 311, were incubated for 16 h at 45 °C in the presence (J) and absence (I) of 50 mM NaOH containing 1 M sodium borohydride as described under Materials and Methods. The *figures* denote apparent molecular weights in kilodaltons.

mycin-treated Raji cells (Figure 1). Since tunicamycin inhibits glycosylation on asparagine residues (Tkacz & Lampen, 1975), the decrease in apparent molecular weights of tunicamycin-treated α and β chains is consistent with both chains containing Asn-linked carbohydrate moieties (Owen et al., 1981). This was further verified in experiments where the class II antigens were treated with Endo H. This enzyme removes the entire core sugar moiety, except the Asn-linked glucosamine, provided terminal glycosylation has not occurred (Tarentino & Maley, 1974). After Endo H treatment the α and β chains behaved as the tunicamycin-treated counterparts (not shown).

Figure 1 shows that tunicamycin-treated α chains gave rise to a single electrophoretic band while the β chains resolved into a doublet. Despite the fact that α and β chains are composed of almost identical numbers of amino acids (Larhammar et al., 1982a,b), they differ in electrophoretic mobility also after tunicamycin treatment (see Figure 1, lanes F and G). The possibility that the α chains may contain Ser/Thr-linked carbohydrate in addition to the Asn-linked moieties was examined. To this end [^{35}S]methionine-labeled, terminally glycosylated class II antigens were subjected to mild alkaline hydrolysis (Finne, 1975; Gahmberg et al., 1979) prior to electrophoretic analysis. Figure 1 shows that class II antigen α and γ chains, but not β chains, displayed a decreased apparent molecular weight following alkaline hydrolysis. These and other data[2] indicate that both α and γ chains may contain Ser/Thr-linked carbohydrate moieties.

By use of the two chain specific antisera the experiments of Figure 1 were repeated, and the immunoprecipitates were analyzed by two-dimensional gel electrophoresis (not shown). The positions of the α and β chains, core glycosylated as well as nonglycosylated, were unambiguous and served as references in the experiments described below.

[2] L. Claesson, H. Ploegh, and P. A. Peterson, unpublished observations.

Intracellular Transport of γ Chains. Raji cells were labeled with [^{35}S]methionine for 10 min. Following various periods of chase the class II antigens were isolated by indirect immunoprecipitation. The antiserum (K 311) was mixed with a rabbit antiserum against class I antigens, since it seemed advantageous to include class I antigen heavy chains and β_2-microglobulin as internal markers to allow proper alignment of the two-dimensional gel patterns.

The immunoprecipitates were divided into two parts, one of which was treated with Endo H prior to the electrophoretic analysis. Figure 2 summarizes the results. At the end of the labeling period the core-glycosylated α chains gave rise to one strong (α_1c) and one weak (α_2c) spot. The two α chains differed both in charge and in apparent molecular weight (Figure 2A, 0 min). The β chains (βc in Figure 2) gave rise to two closely spaced, not always resolved, strong spots in addition to one weak spot. The γ chain appeared as a single, apparently homogeneous spot. It is noteworthy that the relative amount of radioactivity associated with the γ chain (Figure 2A, 0 min) is less than that for α and β chains, respectively.

Endo H treatment diminished the apparent molecular weight of the major α chain spot from about 35 000 to about 28 000 (Figure 2B, 0 min). Also the β-chain spots displayed reduced apparent molecular weights as a consequence of the endoglycosidase treatment. The two major β-chain spots coincided with the γ-chain spot after the digestion (Figure 2B, 0 min). Consequently, the Endo H treatment of the γ chain reduced its apparent molecular weight from about 33 000 to 26 000. This observation suggests that two Asn-linked core sugar moieties are associated with the γ chain.

After 40 min of chase the class II antigen chain pattern had become quite complex (Figure 2A,B, 40 min). Several weak, poorly resolved spots (αt) had now emerged. Due to their low p*I* and their high apparent molecular weights, the αt spots most likely represented sialic acid containing and, thus, terminally glycosylated α chains (Figure 2A, 40 min). Since only one of the two Asn-linked carbohydrate moieties of the α chain undergoes terminal glycosylation (Strominger, 1980; Owen et al., 1981), the fact that the αt spots displayed apparent molecular weights reduced by about 3000 after the Endo H digestion (Figure 2B, 40 min) further supported their identification.

A minor fraction of the β chains had undergone changes similar to those of the αt spots (βt in Figure 2A, 40 min). Thus, the βt spots, which exhibited a lower p*I* than the core-glycosylated β chains (βc), were not susceptible to Endo H digestion, while the major portion of the β chains (βc) displayed diminished apparent molecular weights after the enzyme treatment.

The γ-chain spot (γ_1), whose content of radioactivity during 40 min of chase had increased considerably in comparison to that of the α- and β-chain spots (cf. Figure 2A, 0 min), was accompanied by a novel spot, γ_2. The latter displayed a lower p*I* than the γ_1 spot, but its apparent molecular weight was identical with that of γ_1 (Figure 2A, 40 min). Endo H digestion diminished the apparent molecular weights of the γ_1 and γ_2 spots to the same extent, and the two spots were partially superimposed on the βc spots (Figure 2B, 40 min). The finding that the γ_1 and γ_2 spots were equally sensitive to the enzyme treatment suggests that the γ_2 spot is not a γ_1 species whose Asn-linked carbohydrate moieties have become terminally glycosylated.

After 40 min of chase an array of spots with apparent molecular weights similar to those of the α chains was also

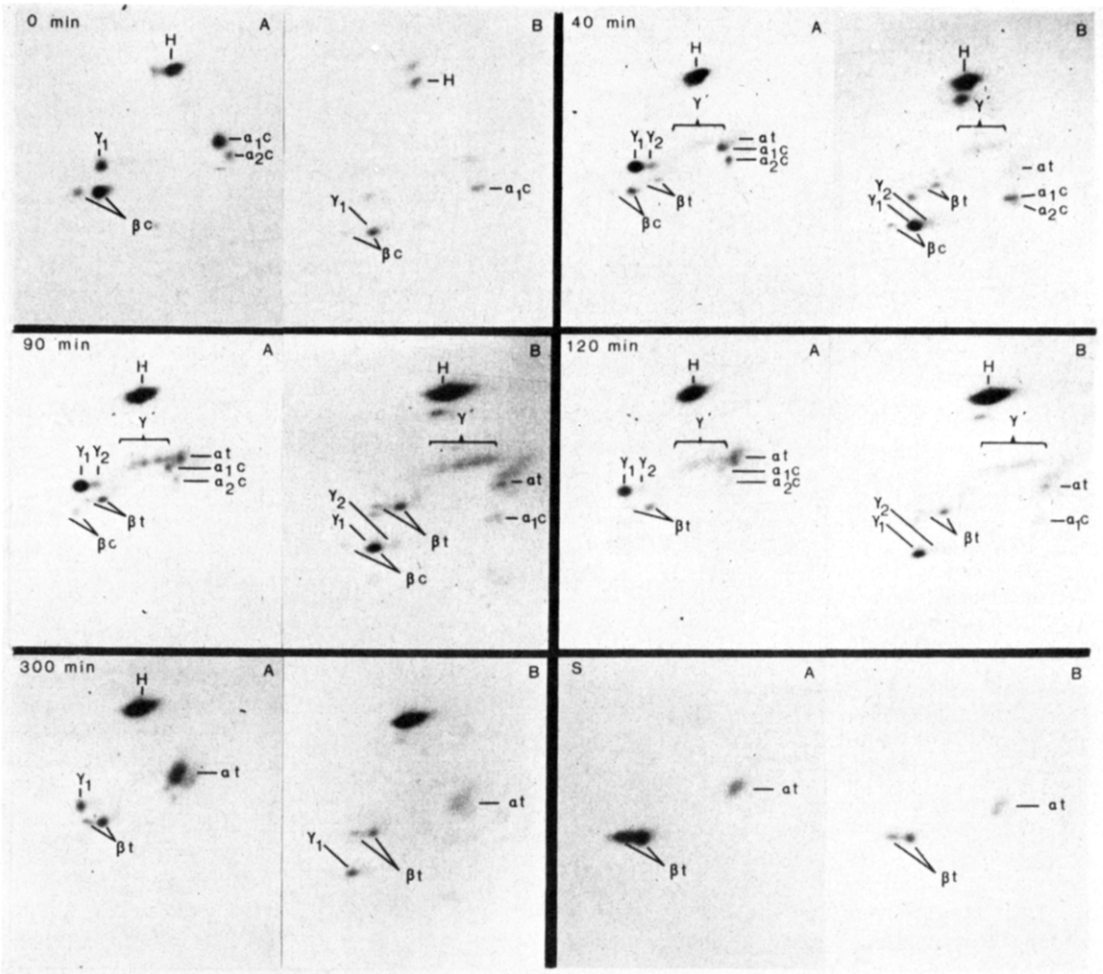

FIGURE 2: Glycosylation of class II antigens during intracellular transport. Raji cells were labeled for 10 min with [^{35}S]methionine and chased for the periods of time indicated. Intact Raji cells were also cell surface labeled by lactoperoxidase-catalyzed iodination (S). Class II antigens were isolated by indirect immunoprecipitation. The antiserum (K 311) had been mixed with a rabbit antiserum against class I antigens prior to use, to ascertain that class I antigens were present in the immunoprecipitates (see below). All immunoprecipitates were divided into two portions. One portion (A) was directly subjected to two-dimensional nonequilibrium pH gradient electrophoresis (horizontal) and NaDodSO$_4$–polyacrylamide gel electrophoresis (vertical) while the other portion (B) was treated with Endo H prior to the electrophoretic separation. The polypeptide patterns of the various gels were aligned by using class I antigen heavy chains (H) and β_2-microglobulin (not shown) as internal markers. Class II antigen α and β chains are denoted as core glycosylated (c) and as terminally glycosylated (t). Note that the Endo H digestion at 0 (B) was incomplete as evidenced by the appearance of unaltered class I heavy chain as well as class II antigen chain spots. For further explanations, see text.

apparent (γ' in Figure 2A, 40 min). The behavior of various forms of the α and β chains ruled out that the γ' spots corresponded to these chains. However, the γ' spots were insensitive to Endo H digestion (Figure 2B, 40 min), which suggests that they contain terminally glycosylated γ_1 and γ_2 chains (see below).

Following a chase period of 90 min the terminally glycosylated α chains (αt) as well as β chains (βt) contained more radioactivity than their core-glycosylated counterparts (Figure 2A, 90 min). The γ_1 and γ_2 spots did not appear to have changed quantitatively, but the γ' spots were more prominent in comparison to the α and β chain spots at 90 min than at 40 min of chase. Endo H digestion of the class II antigen chains after 90 min of chase gave the expected result. The core glycosylated α, β, and γ chain spots as well as the terminally glycosylated α chains displayed diminished apparent molecular weights, while the terminally glycosylated β chains and the set of γ' spots were unaffected by the enzymatic digestion (Figure 2B, 90 min).

After 120 min of chase most of the radioactivity of the class II antigen chains occurred in the positions of the terminally glycosylated α and β chains (Figure 2A, 120 min). While the radioactivity in the γ_1 and γ_2 spots remained unchanged, the radioactivity in the γ' spots diminished. The Endo H digested material displayed the expected behavior (cf. Figure 2B, 90 min and 120 min). It is noteworthy that the γ_1 and γ_2 spots were fully susceptible to Endo H digestion also after 120 min of chase.

The class II antigen chains displayed a simple electrophoretic pattern after 300 min of chase (Figure 2A, 300 min). Terminally glycosylated α and β chains and core-glycosylated γ chains could be visualized. However, core-glycosylated α and β chains, like the γ' material, were virtually absent. As expected, the terminally glycosylated β chains were resistant to Endo H treatment while the terminally glycosylated α chains and the core-glycosylated γ chains were sensitive to the enzyme (Figure 2B, 300 min).

To examine whether the Endo H sensitive γ_1 chain, which remained even after a prolonged chase period, was intracellular or expressed on the cell surface, intact Raji cells were subjected to lactoperoxidase-catalyzed iodination. Labeled, cell surface expressed class II antigens were isolated by indirect immunoprecipitation and analyzed by two-dimensional gel electrophoresis. The labeled class II antigen chains emerged in the positions expected for terminally glycosylated α and β chains (Figure 2A, S), and their electrophoretic behavior after Endo

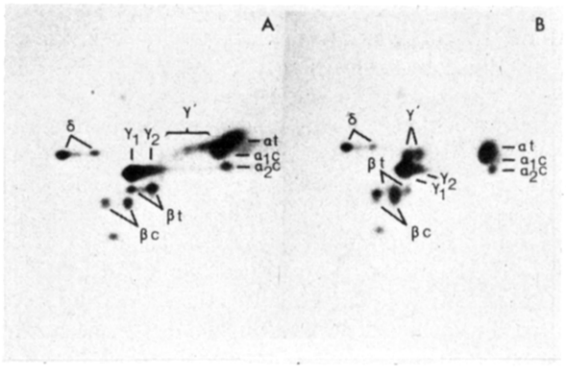

FIGURE 3: Neuraminidase digestion of class II antigens. Raji cells were labeled for 10 min with [^{35}S]methionine and subsequently chased for 90 min. The class II antigens were isolated by indirect immunoprecipitation using antiserum K 311. Prior to the two-dimensional nonequilibrium pH gradient gel electrophoresis (*horizontal*) and NaDodSO$_4$–polyacrylamide gel electrophoresis (*vertical*) the immunoprecipitated class II antigens were divided into two portions, which were incubated at 37 °C for 4 h in the absence (A) and presence (B) of neuraminidase. The various spots visualized after autoradiography were denoted as in Figure 2. However, additional spots (δ), which were not reproducibly found, occur on these two-dimensional gels.

H digestion confirmed this observation (Figure 2B, S). No label occurred in the position of the core-glycosylated γ chain, which may suggest that this form of the γ chain is intracellular.

Terminal Glycosylation of the γ Chain. The results of the pulse chase experiments raised the possibility that the γ′ spots corresponded to terminally glycosylated γ_1 and γ_2 chains. To examine this Raji cells were labeled with [^{35}S]methionine for 10 min and chased for 90 min. Immunoprecipitated class II antigens were divided into two portions, one of which was treated with neuraminidase. Analysis of the two samples by two-dimensional electrophoresis was carried out. Figure 3 shows that the neuraminidase-digested, terminally glycosylated α and β chains (αt and βt, respectively) displayed similar if not identical p*I* values as their core-glycosylated counterparts (α_1c, α_2c, and βc, respectively). However, the apparent molecular weights of the enzyme-treated αt and βt were somewhat greater than for αc and βc (Figure 3B).

The neuraminidase digestion eliminated the array of γ′ spots (Figure 3A) but introduced two new spots (γ′ in Figure 3B) with p*I*s similar to those of the γ_1 and γ_2 spots. The two enzyme-treated γ′ spots displayed slightly greater apparent molecular weights than the γ_1 and γ_2 spots (Figure 3B). This information together with the observation that the γ′ spots were resistant to Endo H digestion (Figure 2) is consistent with the γ′ material corresponding to terminally glycosylated γ_1 and γ_2 chains.

To further explore the relationship between the γ_1 and γ_2 chains with the γ′ material, [^{3}H]methionine-labeled γ_1 and γ_2 chains were mixed with [^{35}S]methionine-labeled γ′ material, digested with trypsin, and subjected to high-pressure liquid chromatography (see Materials and Methods). The peptide maps, shown in Figure 4, were very similar, although a few of the peaks did not coincide exactly. Despite these minor differences, which most likely reflect the differences in glycosylation between the two materials, the peptide maps further support the precursor–product relationship of the core-glycosylated γ chains and the γ′ material.

Cell Surface Expression of γ Chains. The observation that the γ′ material represented terminally glycosylated γ chains raised the question whether γ′ chains are expressed on the cell surface. Since antibodies against the γ chain were unavailable and since α and β chains dissociate from the γ′ chains prior to their cell surface expression (see Figure 2), we had to isolate plasma membranes to analyze their content of γ′ material.

Raji cells, labeled with [^{35}S]methionine for 30 min and subsequently chased for 180 min, were bound to poly(ethylenimine)-coated polyacrylamide beads (Jacobson, 1980). Bound cells were ruptured by sonication, and the beads were washed extensively prior to the desorption of bound plasma membrane components.

The desorbed material was separated by electrophoresis on cylindrical NaDodSO$_4$–polyacrylamide gels. Components with apparent molecular weights of about 25 000–40 000 were recovered and subjected to nonequilibrium pH gradient gel electrophoresis. Material occurring in the region of the gel between the α and β chains was eluted and analyzed by two-dimensional gel electrophoresis. To identify the migration positions of α, β, and γ chains, [^{35}S]methiomine-labeled class II antigens were isolated by indirect immunoprecipitation and run in parallel (see Figure 2A). When the two-dimensional pattern of the isolated plasma membrane components was compared with the gel displaying identified α, β, and γ chains, it became obvious that only terminally glycosylated α, β, and γ chains were apparent in the plasma membrane fraction

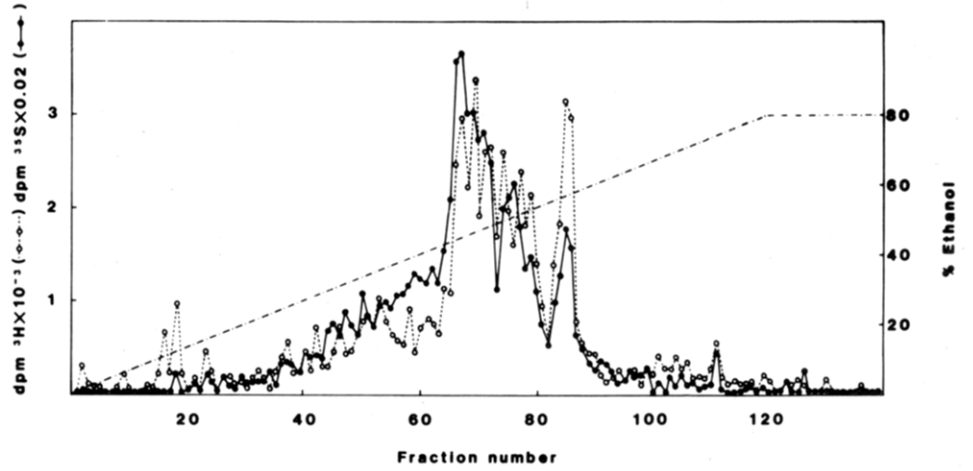

FIGURE 4: Peptide comparison between the γ_1 and γ_2 chains and the γ′ material. A mixture of [^{3}H]methionine-containing γ_1 and γ_2 chains and [^{35}S]methionine-labeled γ′ material was digested with trypsin and separated by reverse-phase high-pressure liquid chromatography. The details are depicted under Materials and Methods. The column was equilibrated with 0.1 M ammonium acetate. A 100-mL linear gradient of ethanol from 0 to 80% in 0.1 M ammonium acetate was used to elute the peptides. Fractions of 0.8 mL were collected at 1-min intervals. Aliquots of each fraction were subjected to radioactivity measurement in a dual-channel liquid scintillation counter.

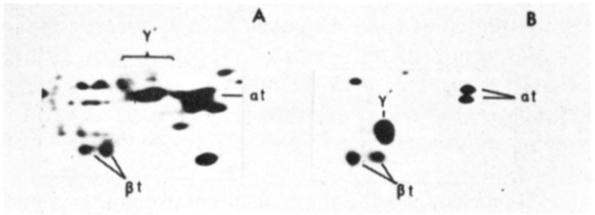

FIGURE 5: Cell surface expression of γ chains. Raji cells were labeled for 30 min with [^{35}S]methionine. After a chase period of 3 h plasma membranes were isolated as described under Materials and Methods. Membrane constituents with apparent molecular weights of 25 000–40 000 were isolated by preparative NaDodSO$_4$–polyacrylamide gel electrophoresis followed by nonequilibrium pH gradient gel electrophoresis. Components with p*I*s intermediate between those of class II antigen α and β chains were recovered from the gel. These plasma membrane proteins were then divided into two fractions, one of which was directly subjected to two-dimensional gel electrophoresis (A), as described in Figure 2. The other fraction was treated with neuraminidase prior to the electrophoreses (B). The spots visualized after autoradiography were denoted according to the nomenclature of Figure 2. Identification of α, β, and γ chains was accomplished by subjecting immunoprecipitated class II antigens, isolated by indirect immunoprecipitation, to the identical treatment (cf. Figure 2).

(Figure 5A). A portion of the isolated plasma membrane material was digested with neuraminidase prior to the two-dimensional electrophoretic analysis (Figure 5B). By comparison with a reference gel displaying neuraminidase-digested class II antigens (Figure 3B) the neuraminidase-digested plasma membrane fraction could be shown to contain two α-chain spots, two β-chain spots, and γ′ material (Figure 5B). Thus, at least a fraction of the γ chains seems to become expressed on the cell surface.

Discussion

The association of γ chains with class II antigen α and β chains has been observed both for murine (Jones et al., 1978; Moosic et al., 1980) and human molecules (Charron & McDevitt, 1979; Shackelford & Strominger, 1980; Owen et al., 1981; Kvist et al., 1982), but data on the characteristics and function of the γ chains are scarce (Shackelford et al., 1982). To ultimately understand the function of γ chains we examined their intracellular transport in relationship to that of α and β chains. This was accomplished by examining the carbohydrate modifications of the α, β, and γ chains at various times during pulse–chase experiments. Two-dimensional electrophoresis was also employed to resolve the various forms of the α, β, and γ chains.

The lymphoblastoid cell line Raji, which was used in this study, displays MHC heterozygosity. Consequently, we had to establish the electrophoretic properties of the Raji α and β chains in their various states of glycosylation to be able to identify the γ chains. By use of antibodies specific for class II antigen α and β chains, respectively, two α-chain and three β-chain containing spots were identified. Whether the various α and β chains only were products of different loci or comprised allelic forms as well was not investigated. All three types of β chains contain a single Asn-linked carbohydrate moiety. The two α chains contain two Asn-linked carbohydrate moieties each. Only the one bound to Asn-118 undergoes terminal glycosylation.[2]

Mild alkaline hydrolysis of class II antigens under conditions known to release Ser/Thr-linked carbohydrate groups (Finne, 1975; Gahmberg et al., 1979) diminished the apparent molecular weights of the α and γ chains provided long-term-labeled material was used. In conjunction with unpublished data[2] this suggests that both α and γ chains may contain Ser/Thr-linked carbohydrate moieties. Endo H digestion of the γ chain reduced its apparent molecular weight by approximately 7000, which is consistent with γ chains containing two Asn-linked carbohydrate moieties.

The major core-glycosylated γ chain species was accompanied by a component with an identical apparent molecular weight but with a lower p*I*. Since the latter component displayed a glycosylation that was very similar to if not identical with that of the major γ species throughout the pulse chase, it is tempting to suggest that two γ chains are expressed in Raji cells. However, the two species may differ only with regard to posttranslational modifications. We have recently succeeded in cloning cDNA corresponding to γ chains[3] so the number of γ chain genes can now begin to be examined by the direct approach.

The pulse–chase experiments and the Endo digestions confirmed our previous observation that γ chains in the endoplasmic reticulum occur in excess over nascent α and β chains (Kvist et al., 1982). This was inferred from the observation that core-glycosylated γ chains existed when all α and β chains had become terminally glycosylated. However, during the course of the pulse–chase experiments terminally glycosylated components termed γ′ emerged. The γ′ components, devoid of sialic acid after neuraminidase treatment, displayed identical p*I*s and similar molecular weights as the core-glycosylated γ chains, in keeping with the idea that the γ′ material represented terminally glycosylated γ chains. This notion was reinforced by the γ chains and the γ′ material exhibiting very similar if not identical peptide maps.

Previous studies from this and other laboratories have shown that γ chains do not usually copurify with cell surface expressed class II antigens [see Klareskog et al. (1979), Wiman et al. (1982), and Walker et al. (1982)]. This observation together with the claim that human γ chains exclusively occur intracellularly (Shackelford & Strominger, 1980; Owen et al., 1982) raised the possibility that the γ chains may circulate between the endoplasmic reticulum and the Golgi complex. However, an array of spots that could not be accounted for by terminally glycosylated α and β chains was evident on two-dimensional electrophoresis at later stages of the intracellular transport (Lloyd et al., 1981; Long et al., 1982). In this study we could demonstrate that they corresponded to terminally glycosylated γ chains (γ′). The appearance of the γ′ material was temporally related to a diminished affinity between γ and α–β chains. Following dissociation of the γ chains from the class II antigens it is difficult to trace the γ chain. However, terminally glycosylated γ chains display a characteristic two-dimensional electrophoretic pattern (γ′), which upon neuraminidase digestion becomes similar to the pattern of the core-glycosylated γ chains. Since the γ′ pattern could be found upon two-dimensional electrophoresis of isolated plasma membranes, this information is consistent with but does not prove that plasma membranes of Raji cells contain γ′ material. The observed γ′ material may be associated with the cytoplasmic side of the membrane or may derive from the interior of a few ruptured cells. When specific antibodies become available, it should be possible to clarify this issue.

The present data show that class II antigen α, β, and γ chains form complexes in the endoplasmic reticulum. The stoichiometry of the three types of chains in the complexes is not yet known. Nonetheless, the three polypeptides seem to be jointly transported to the Golgi complex. Within the trans-Golgi fraction, the terminally glycosylated γ chains seem to dissociate from the α and β chains. Subsequently, the class

[3] L. Claesson, unpublished observations.

II antigens are transported to the plasma membrane and become expressed on the cell surface. Some if not all of the γ chains may also become expressed on the cell surface, but this occurs independently of the class II antigens. This behavior of the γ chains may be of functional significance. Thus, γ chains may quantitatively regulate the cell surface expression of the class II antigens. It has been shown that the cell surface content of the class II antigens varies with the cell cycle (Sarkar et al., 1980). We have found that the synthesis of the γ chain is considerably enhanced when the class II antigen expression on the cell surface is low.[3] Therefore, γ chains may have a role in regulating the exit of the class II antigens from the endoplasmic reticulum. Alternatively, class II antigens may require the presence of cell surface expressed γ chains to accomplish some of their functions.

Added in Proof

Data consistent with those presented in this paper have been published by Machamer & Cresswell (1982).

Acknowledgments

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Registry No. L-Asparagine, 70-47-3; L-serine, 56-45-1; L-threonine, 72-19-5.

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