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ALTERED MOLECULAR STRUCTURE OF HLA-DR ANTIGENS SYNTHESIZED IN THE PRESENCE OF TUNICAMYCIN

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Summary: Effects of tunicamycin on the synthesis of proteins and glycoproteins, especially the surface antigens of a human lymphoblastoid cell line (Raji), were investigated. Electrophoretic profiles of [^{3}H]leucine labeled membrane components were similar in the tunicamycin treated cells and untreated cells. However, the respective electrophoretic profiles of [^{3}H]glucosamine labeled membrane components were remarkably different. In the presence of tunicamycin, the relative amount of HLA-DR antigens in the newly synthesized membrane was remarkably reduced, and both subunits of the antigens showed reduced apparent molecular weights. Moreover, one of the subunits completely lost its [^{3}H]glucosamine labeled portion(s), and the other subunit still contained [^{3}H]glucosamine labeled portion(s). These results imply that HLA-DR antigens possess either lipid carrier dependent oligosaccharides or other type(s) of oligosaccharide(s).

Introduction: Raji cells possess many well characterized surface markers and antigens on the cell surface(1-5) making them suitable for studies on the biochemical nature and the biosynthesis of components on the cell surface. HLA-DR antigens and HLA-A,B,C antigens are such typical surface antigens, consisting of two dissimilar subunits. Each subunit of HLA-DR antigens is a glycoprotein, whose apparent molecular weight is 34 000 and 29 000, respectively(6). The structure of the carbohydrate moieties in each subunit is not yet elucidated. The gene for the HLA-DR heavy chain is located on the 6th human chromosome(7). Whereas the HLA-A,B,C heavy chain is a glycoprotein, whose apparent molecular

Abbreviations: TM, tunicamycin; FCS, fetal calf serum; KM, kanamycin; PMSF, phenylmethyl sulfonyl fluoride; DOC, sodium deoxycholate; SDS, sodium dodecyl sulfate.

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weight is 44 000(8) having only a single N-linked oligosaccharide(9), the HLA-A,B,C light chain is a simple protein, β2-microglobulin, whose molecular weight is 12 000(10). The gene for the HLA-A,B,C heavy chain is located on the short arm of the 6th human chromosome(11), and the gene for the β2-microglobulin is located on the 15th human chromosome(12). These antigens span the lipid bilayer of the surface membrane, so that the N-terminus and the carbohydrate moieties of these antigens are oriented to the outside, and the C-terminus is exposed on the cytoplasmic face of the surface membrane(7,13,14).

It is unclear whether the carbohydrate moieties of these antigens play an important role in the biosynthesis and the association of each subunit and their integration in the surface membrane. It is known that TM blocks the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine to a polyisoprenyl lipid carrier(15), and consequently inhibits the synthesis of the N-linked oligosaccharide of glycoprotein(16-23).

We report here the effects of TM on the biosynthesis of proteins and glycoproteins of the surface membrane, especially HLA-DR antigens and HLA-A,B,C antigens of Raji cells.

Materials and Methods:

Cell line and cell culture: Raji cells were grown in RPMI 1640 medium supplemented with 10 % FCS and 60 μg/ml of KM.

Bacterium and culture: Staphylococcus aureus strain Cowan 1 was used as an adsorbent of immune complex, and was grown in Kessler's medium(24).

Antisera: Rabbit anti HLA-DR serum was a generous gift from Dr. M. Katagiri, Asahigawa Medical College, Asahigawa, Japan. Rabbit anti human β2-microglobulin serum was purchased from Seikagaku Kogyo Co. Ltd., Tokyo, Japan.

Isotopic labeling: For metabolic labeling studies with [^{3}H]leucine or [^{3}H]glucosamine, the concentration of leucine or glucose in the medium was reduced to 10 % of the normal level. Raji cells at the exponential phase were harvested, washed with the leucine depleted- or glucose depleted-medium supplemented with 10 % FCS and 60 μg/ml of KM, and suspended to 1 x 10^6 cells/ml in this medium. In the case of TM treatment, TM solution(1 mg of TM per ml of methanol) was added to a final concentration of 0.5 μg/ml. Cells were labeled with 5-25 μCi/ml of each radiochemical for 18 hours.

Preparation of membrane fraction: Raji cells were harvested, and washed three times with saline. Membrane fraction was prepared according to the method of Brunette and Till(25), except that 1 mM PMSF was added in all solutions after washing, and both solutions in the aqueous two-phase polymer system were renewed in each centrifugation. The membrane fraction in the interface was collected, diluted with 1 mM PMSF, precipitated by centrifugation at 20 000 rpm for 40 min, and washed with 1 mM PMSF.

Immunoprecipitation: The suspension of isotopically labeled membrane was mixed with the same volume of 1 % DOC-0.3 M NaCl-0.02 M Tris-HCl pH 7.4-0.04 %

NaN_3, and solubilized in ice for 2 hours. The remaining insoluble materials were removed by centrifugation at 100 000 x g for 1 hour. The solubilized membranes were reacted with the specific antiserum in 0.5 % DOC-0.15 M NaCl-0.01 M Tris-HCl pH 7.4-0.02 % NaN_3 for 3 hours in ice. The immune complex was precipitated with the bacterial adsorbent according to the method of Kessler (24). Immunoprecipitates were finally dissolved in the sample buffer(26) with heating at 100°C for 3 min.

Gel electrophoresis and detection of labeled materials: Gel electrophoresis on 8 M urea-0.1 % SDS-8 % polyacrylamide gel was carried out according to the method of Mizushima and Yamada(26). Slab gels were permeated with dimethyl sulfoxide and 2,5-diphenyloxazole, dried on Whatman-3MM paper, and exposed at -70°C to preexposed X-Omat R film XR-1(Kodak) according to the method of Laskey et al(27,28).

Radioisotopes: [^{3}H]leucine(105 Ci/mmole) and [^{3}H]glucosamine(7.3 Ci/mmole) were purchased from the Radiochemical Centre, Amersham, England.

Results and Discussion:

[^{3}H]leucine labeled- and [^{3}H]glucosamine labeled-membrane components from the TM treated and untreated Raji cells. Raji cells at the exponential phase were labeled with [^{3}H]leucine or [^{3}H]glucosamine in the presence or absence of TM, and the membrane fraction was prepared. In the presence of TM, the incorporated radioactivity per mg of membrane protein is reduced to 48.7 % in the case of [^{3}H]leucine labeling, and 14.9 % in the case of [^{3}H]glucosamine labeling as compared with the control. Electrophoretic profiles of [^{3}H]leucine labeled membrane components were almost similar in the TM treated cells and untreated cells[Fig. 1(a) and (b)]. This result shows that the composition of newly synthesized membrane proteins is almost similar in both, although the overall protein synthesis is inhibited to some extent with TM. However, their electrophoretic profiles of [^{3}H]glucosamine labeled membrane components were remarkably different. In the absence of TM, the following bands were dominantly labeled: 215 K, 130 K, 95 K(major band), 46 K, and 37 K[Fig. 1(c)]. In the presence of TM, the following bands were dominantly labeled: 205 K, 165 K, 125 K, 75-56 K(major band), 51 K, 46 K, and 32 K[Fig. 1(d)]. This difference may be due to the fact that, under our conditions, TM inhibits specifically the synthesis of N-linked oligosaccharide of glycoprotein. Therefore glycoproteins that possess other type(s) of oligosaccharide(s) were observed in the TM treated system.

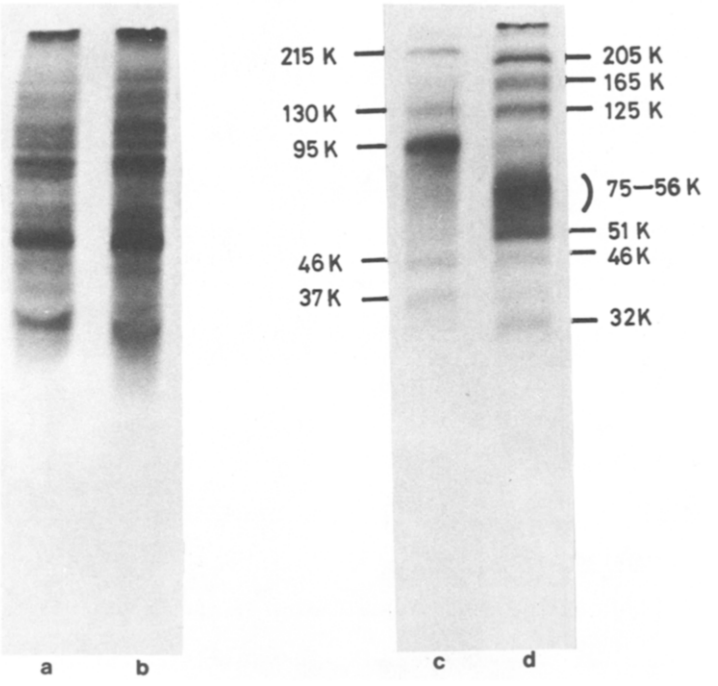

Fig. 1. Effects of TM on the electrophoretic profiles of [^{3}H]leucine labeled- and [^{3}H]glucosamine labeled-membrane components of Raji cells. Approximately 20 000 cpm of samples were applied to each gel well. The membrane fractions used were: (a) labeled with [^{3}H]leucine in the absence of TM; (b) labeled with [^{3}H]leucine in the presence of TM; (c) labeled with [^{3}H]glucosamine in the absence of TM; (d) labeled with [^{3}H]glucosamine in the presence of TM.

Effects of TM on the molecular structure of HLA-DR antigens and HLA-A,B,C antigens in the membrane fraction. When [^{3}H]leucine labeled membrane was analysed by anti HLA-DR serum in our gel system, two components having apparent molecular weights of 41 000(heavy chain) and 34 000(light chain) were detected from the membrane labeled in the absence of TM, but from the membrane labeled in the presence of TM, two components having apparent molecular weights of 37 000 and 30 500 were detected[Fig. 2(a) and (b)]. Other groups that used TM to investigate the biosynthesis of glycoproteins observed a similar reduction of apparent molecular weight, and this reduction is due to a loss of N-linked oligosaccharide(s)(16-21). Judging from these observations, the components having apparent molecular weights of 37 000 and 30 500 seemed to correspond to the HLA-DR heavy chain and light chain, respectively.

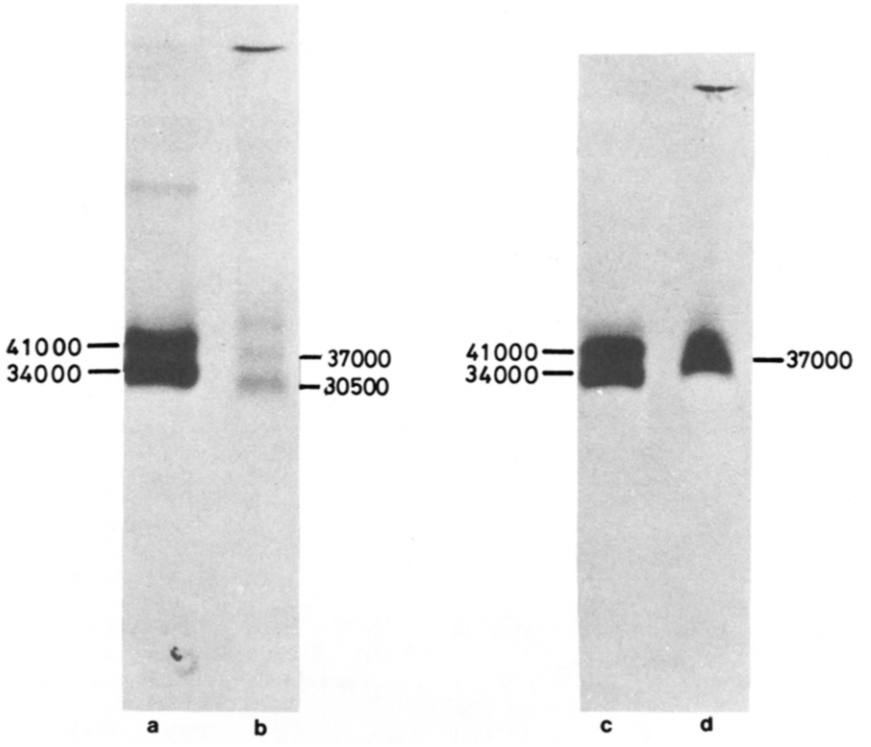

Fig. 2. Effects of TM on the molecular structure of HLA-DR antigens in the membrane fraction. The membrane fraction that was labeled with [^{3}H] leucine or [^{3}H]glucosamine in the presence or absence of TM was solubilized with 0.5 % DOC, and immunoprecipitated with anti HLA-DR serum and bacterial adsorbent. Precipitated materials were analysed by urea-SDS polyacrylamide gel electrophoresis and fluorography. Symbols are the same as described in the legend to Fig. 1.

When [^{3}H]glucosamine labeled membrane was examined by anti HLA-DR serum, two components having apparent molecular weights of 41 000 and 34 000 were detected from the membrane labeled in the absence of TM, but from the membrane labeled in the presence of TM, only a component of apparent molecular weight 37 000 was detected. This result implies that the heavy chain contains either N-linked oligosaccharide(s) or other type(s) of oligosaccharide(s), and that the light chain contains only N-linked oligosaccharide(s). There are mainly two types of oligosaccharide in the glycoproteins, namely N-linked oligosaccharide and O-linked oligosaccharide(29). Some glycoproteins, such as fetuin (30), the human erythrocyte membrane sialoglycoprotein(31,32), human IgA_1(33,

34), and human chorionic gonadotropin(35), contain both N-linked oligosaccharide(s) and O-linked oligosaccharide(s). So one of the other type(s) of oligosaccharide(s) in the heavy chain may be O-linked oligosaccharide(s).

The relative amount of HLA-DR antigens in the membrane synthesized in the presence of TM seemed to be reduced as compared with the control[Fig. 2(c) and (d)]. The proportion of HLA-DR antigens in the membrane proteins synthesized de novo in the presence of TM is reduced to less than 25 % of that of the control.

It is not clear whether the two subunits of HLA-DR antigens in the surface membrane of TM treated cells are associated with each other.

When [^{3}H]leucine labeled membrane was analysed by anti β2-microglobulin serum, two components having apparent molecular weights of 47 000(HLA-A,B,C heavy chain) and 13 000(β2-microglobulin) were detected from the membrane labeled in the absence of TM, but from the membrane labeled in the presence of TM, labeling of β2-microglobulins and HLA-A,B,C heavy chains associated with β2-microglobulin was reduced to the background level[Fig. 3(a) and (b)].

When [^{3}H]glucosamine labeled membrane was examined by anti β2-microglobulin serum, a component of apparent molecular weight 47 000 was detected from the membrane labeled in the absence of TM, but from the membrane labeled in the presence of TM, no specific bands were observed[Fig. 3(c) and (d)].

Further experiments to clarify whether the N-linked oligosaccharide deficient HLA-A,B,C heavy chains that are not associated with β2-microglobulin can exist on the surface membrane are in progress.

The relative amount of HLA-DR antigens and HLA-A,B,C antigens in the membrane synthesized in the presence of TM was reduced as compared with the control. This reduction presumably resulted from the inhibition of the synthesis of the N-linked oligosaccharide(s) of these antigens. This inhibition may cause inefficiency in some or all of the following processes: (a) peptide elongation; (b) integration in the membrane of rough endoplasmic reticulum; (c) association of the two subunits; (d) translocation from rough endoplasmic

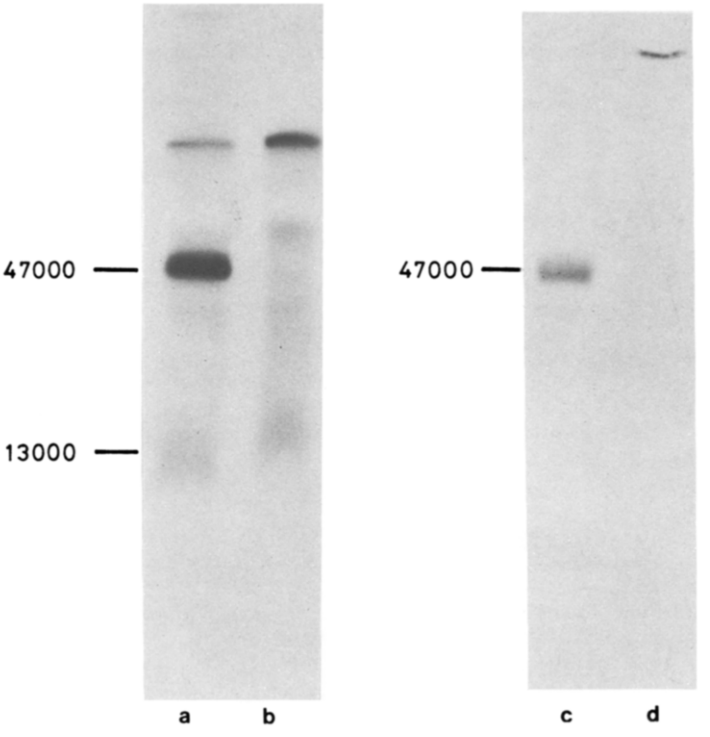

Fig. 3. Effects of TM on the molecular structure of HLA-A,B,C antigens in the membrane fraction. Immunoprecipitations were carried out with anti β2-microglobulin serum and bacterial adsorbent. Other procedures are the same as described in the legend to Fig. 2. Symbols are the same as described in the legend to Fig. 1.

reticulum to the surface membrane; (e) maintenance of these antigens in the surface membrane. Detailed analysis of the effects of TM on these processes is in progress.

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