Characterization of *N*-linked oligosaccharides of an HLA-DR molecule expressed in different cell lines

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In order to determine the factors that influence the glycosylation of an integral membrane protein, we investigated the *N*-glycosylation of a molecule of the human major histocompatibility complex (MHC) class II, the HLA-DR antigen. This glycoprotein was studied in a human Epstein–Barr-virus-transformed B cell line and in a mouse fibroblastic cell line co-transfected with DR α and DR β genes. We observed that the HLA-DR-antigen glycosylation pattern depends on the cell line in which processing takes place and is closely related to the glycosylation pattern of the overall cellular glycoproteins. Furthermore, when comparing the glycosylation of the separated α - and β -chains, differences were noticed within the same molecule, showing the importance of the individual peptide backbone for the glycosylation process.

INTRODUCTION

The main enzymic pathway steps of *N*-linked oligosaccharide processing are well understood (Kornfeld & Kornfeld, 1985), and the elucidation of the factors that determine the structural completion of the carbohydrate moieties is now of great interest. The oligosaccharide processing could be influenced by the structure of the protein itself and/or by the cell where it takes place.

The influence of the cell type on the oligosaccharide processing has been shown by ovalbumin, a secreted glycoprotein (Sheares & Robbins, 1986). Different cell lines were transfected with the same gene encoding this protein, and the glycosylation of the secreted ovalbumin differs according to the transfected cell line. This kind of investigation on an integral membrane glycoprotein has not hitherto been reported.

The influence of protein structure has been documented for mouse major-histocompatibility-complex (MHC) antigens, H-2K and Ia antigens (Swiedler *et al.*, 1985). Differences in the sialic acid content of Ia antigens have been evidenced between B lymphocytes and macrophages (Cowing & Chapdelaine, 1983). This is of importance in view of the implication of these glycoproteins in cellular interactions. For example, major-histocompatibility-complex antigens are involved in the co-operation between B and T cells and in the presentation of antigens to immunocompetent cells (McDevitt *et al.*, 1976; Bodmer & Bodmer, 1984).

The present study deals with the glycosylation of a human major-histocompatibility-complex class II molecule, the HLA-DR antigen. This glycoprotein, constitutively expressed on macrophages, B cells and activated T cells, consists of two non-covalently associated polypeptide chains, the α -chain (M_r 34000) and the β -chain (M_r 28000). The α -chain is structurally mono-

morphic, whereas the β -chain is highly polymorphic (Charron & McDevitt, 1979; Shackelford *et al.*, 1982). Both the α - and β -chains are *N*-glycosylated and contain respectively two and one *N*-linked carbohydrate moieties (Shackelford *et al.*, 1982). A third glycosylated chain, the invariant Ii (M_r 31000), can be found, generally associated with the DR antigens, but this chain is not encoded by the major-histocompatibility-complex genes (Day & Jones, 1983).

In the present work, we compared the oligosaccharide structures of HLA-DR antigens from two different cell lines expressing the same serological DR W6/W6 phenotype: the 430 cell line, an Epstein–Barr-virus-transformed B-cell line, and the TF 22.9 cell line, a mouse fibroblastic cell line co-transfected with DR W6 genes (Rabourdin-Combe & Mach, 1983). The overall cellular glycoprotein extracts were studied as a comparison.

MATERIALS AND METHODS

Cells and monoclonal antibody

Two different cell lines were used: a B cell line of DR W6/W6 phenotype transformed by Epstein–Barr virus (430), and a mouse fibroblastic cell line obtained from an L cell co-transfected with DR α W6 and β W6 genes (TF 22.9) (Rabourdin-Combe & Mach, 1983). Immunoprecipitation was performed with a monoclonal monomorphic anti-DR antibody, D1.12 (Accolla *et al.*, 1982).

Labelling and preparation of the glycopeptides

The cells were labelled with [2-³H]mannose for 24 h, as previously described (Cowan *et al.*, 1982; Néel *et al.*, 1985). Glycoprotein extracts were obtained by suspending the cells (3×10^7 /ml) in 0.15 M-NaCl/0.02% NaN₃/0.01 M-Tris/HCl buffer, pH 7.4, containing 0.5% Lubrol PX (Sigma Chemical Co., St. Louis, MO, U.S.A.)

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Fig. 1. Two-dimensional non-equilibrium pH-gradient polyacrylamide-gel electrophoresis of [$2\text{-}^3\text{H}$]mannose-labelled cell extract immunoprecipitated with D1.12, a monomorphic anti-HLA-DR monoclonal antibody

(a) TF 22.9 cell line; (b) 430 cell line.

for 30 min. The Lubrol was used as detergent to avoid any contamination of the HLA-DR antigens with the invariant chain Ii (Moosic *et al.*, 1982). This detergent probably weakens the electrostatic bonds between the invariant chain and the DR chains: it allows specific immunoprecipitation of the latter without the Ii chains (Cowan *et al.*, 1982). Moreover, no differences in the glycosylation patterns were found between the total extracts obtained with Lubrol or with other frequently used detergents such as Nonidet P-40 (results not shown). When necessary the HLA-DR antigens were immunoprecipitated by using the two-step procedure described by Kessler (1975). The constitutive chains were separated by preparative non-equilibrium pH-gradient gel electrophoresis (O'Farrel *et al.*, 1977). The antigen preparation was loaded on two separate slab gels. After the first run, an SDS/polyacrylamide-gel electrophoresis (Laemmli, 1970) in the second dimension was performed with one of the gels to identify the different fractions (Fig. 1). The α -chains were separated into two fractions, α^1 and α^2 . The α -chains are known to be structurally monomorphic (Charron & McDevitt, 1979), so that the α^1 and α^2 polypeptide structures should be identical and their electrophoresis migration differences could be related to differences in oligosaccharide structures or other post-translational maturation process. The preparative non-equilibrium pH-gradient gel electrophoresis gel was cut into 1 mm slices; to elute the different chains the gel was ground and incubated in 0.15 M-NaCl (1 ml/slice) for 48 h at 4 °C. Glycopeptides were obtained by Pronase (Calbiochem, Los Angeles, CA, U.S.A.) treatment of the glycoprotein extracts as described by Cowan *et al.* (1982) and Quill & Schwartz (1984).

Serial affinity chromatography

Serial affinity chromatography was performed in accordance with Cummings & Kornfeld (1982). Briefly, glycopeptides were sequentially applied to concanavalin A-Sepharose, E-phytohaemagglutinin-agarose, *Lens culinaris* lectin-Sepharose, L-phytohaemagglutinin-agarose and wheat-germ agglutinin-agarose columns. Concanavalin A-Sepharose *Lens culinaris* haemagglutinin-Sepharose and wheat-germ agglutinin-agarose were purchased from Pharmacia, Uppsala, Sweden; E- and L-phytohaemagglutinin-agarose were from E.Y. Laboratories, San Mateo, CA, U.S.A. The minimal oligosaccharide structural requirement for binding to the various insolubilized lectins are indicated in Fig. 2, which refers to the glycopeptide fractions named further below.

Concanavalin A affinity chromatography. This was done in Tris-buffered saline (0.15 M-NaCl/0.001 M-CaCl₂/0.02% NaN₃/0.01 M-Tris/HCl buffer, pH 8). The glycopeptides were separated into three pools: I, unbound tetra- and tri-antennary structures; II, bi-antennary structures eluted with Tris-buffered saline supplemented with 0.01 M-methyl α -glucoside; III, essentially high-mannose-type structures eluted with Tris-buffered saline supplemented with 0.1 M-methyl α -mannoside.

E-Phytohaemagglutinin affinity chromatography. This was done in phosphate-buffered saline (Dulbecco's formula; Flow Laboratories, Irvine, Ayrshire, U.K.); pool I was applied to an E-phytohaemagglutinin-agarose column to separate glycopeptides containing a bisecting *N*-acetylglucosamine residue plus the minimum oligosaccharide structures described by Yamashita *et al.* (1983) (retarded pool I2') from the other tri- and tetra-antennary structures (pool I1') not retarded on this column.

***Lens culinaris* lectin affinity chromatography.** This was done in Tris-buffered saline to separate pool II into two fractions: the non-fucosylated bi-antennary structures (unbound) and the fucosylated ones (eluted with Tris-buffered saline supplemented with 0.5 M-methyl α -mannoside). Furthermore, the tri-antennary structures bearing both the α -linked mannose residue substituted at positions C-2 and C-6 and a fucose residue on the *N*-acetylchitobiose core were separated from pool I1' by the same technique (pool I1'B, eluted with the Tris-buffered saline supplemented with methyl α -mannoside). Pool I1'A contained the unbound remaining tri- and tetra-antennary chains.

L-Phytohaemagglutinin affinity chromatography. This was done in Tris-buffered saline to separate tri- and tetra-antennary structures bearing both outer galactose residues and the α -linked mannose residue substituted at positions C-2 and C-6 (I1'A2 and I1'B2 pools) from the other tri- and tetra-antennary chains (I1'A1 and I1'B1 pools).

Wheat-germ agglutinin affinity chromatography. This was done in 0.01 M-sodium phosphate buffer, pH 7.3, containing 0.15 M-NaCl to separate pool III into two fractions, IIIa and IIIb, the unbound high-mannose-type

Fig. 2. Minimal oligosaccharide structures of the glycopeptides found in the separated fractions from the serial lectin affinity chromatography (from Cummings & Kornfeld, 1982)

The arrows indicate the positions where a substitution can occur without influencing the binding to a particular lectin. The structures of the II'A1-fraction glycopeptides are not presented. They are tri- and tetra-antennary complex-type chains that do not bind to any of the lectins. Glycopeptides on the II fractions are either fucosylated on the *N*-acetylglucosamine residue at the reducing end (IIB) or not (IIA).

and the main part of hybrid structures eluted with the same buffer supplemented with 0.1 M-*N*-acetylglucosamine.

Endo- β -*N*-acetylglucosaminidase (endoglycosidase H) digestion

The pool III glycopeptides obtained by concanavalin A affinity chromatography of the whole-cell extracts were dissolved in 240 μ l of 0.05 M-sodium citrate buffer, pH 5.0, and 5 munits of endoglycosidase from *Streptomyces griseus* (Miles Laboratories, Elkhart, IN, U.S.A.) in 10 μ l diluted in the same buffer were added. The samples were incubated for 24 h at 37 °C in a toluene atmosphere. Digestion was stopped by boiling for 1 min. Endoglycosidase H recognizes simple high-mannose-type oligosaccharides and cleaves the glycosidic bond of the *NN'*-diacetylchitobiose core structure adjacent to the asparagine residue (Tarentino & Maley, 1974).

Gel filtration

Oligosaccharides and/or glycopeptides were discriminated by filtration on either a Bio-Gel P-6 (200–400 mesh; Bio-Rad Laboratories, Richmond, CA, U.S.A.) column (120 cm $\times$ 1 cm; 110 $\times$ 1 ml fractions) or a Bio-Gel P-4 (>400 mesh; Bio-Rad Laboratories) column (180 cm $\times$ 1 cm; 300 $\times$ 0.5 ml fractions) equilibrated in 0.1 M-pyridine/acetate buffer, pH 5.0. Void and bed volumes were determined by bovine serum albumin and [2-³H]mannose elutions. High-mannose-type structures used as standards were kindly provided by Dr. Saunier, Université Paris 7, Paris, France.

Measurement of the mannose–fucose interconversion

Hydrolysis of oligosaccharide chains was performed with 2 M-HCl. The samples were incubated at 100 °C for 4 h and dried in a rotary evaporator. Labelled

Table 1. Lectin-affinity-chromatography analysis of [2-³H]mannose-labelled surface glycoproteins and isolated DR antigens from a B cell line (430) and a mouse fibroblastic cell line (TF 22.9) co-transfected with DR α and DR β genes

The Table shows the distribution of the glycopeptides fractionated by lectin affinity chromatography as described in the Materials and methods section. The results are expressed as percentages of the total radioactivity incorporated in the glycopeptide Pronase digest.

Glycopeptide	Distribution (%)			
	430 cell-surface glycoproteins	430 DR antigen	TF 22.9 cell-surface glycoproteins	TF22.9 DR antigen
Complex-type structures				
Tri- and tetra-antennary				
I1'A1	10	5	31	24
I1'A2	0	0	11	7
I1'B1	8	7	0	0
I1'B2	0	0	0	0
I2'	3	2	0	0
Bi-antennary				
IIA	3	1.5	8	11
IIB	4	3.5	0	0
High-mannose-type structures				
IIIa	72	81	49.5	58
IIIb	0	0	0	0
Total radioactivity (d.p.m.)	8×10^4	6×10^4	8×10^4	6×10^4

monosaccharides were separated and identified on descending paper chromatography (Schleicher and Schull 2043 B) run in butanol/pyridine/0.1 M-HCl (5:3:2, by vol.) for 40 h. Chromatograms were scanned for their ³H content with an automatic t.l.c. linear analyser (Berthold, Wildbad, Germany) connected with an Apple IIc microcomputer. Mannose and fucose, used as standards, were identified by the alkaline-AgNO₃ technique (Trevelyan *et al.*, 1950).

RESULTS

N-Glycosylation of the isolated HLA-DR antigens and of the whole glycoprotein extracts

The *N*-glycosylation of either the isolated DR antigens or the whole glycoprotein extracts was investigated by serial affinity chromatography of the [³H]mannose-labelled glycopeptides obtained from the both 430 and TF 22.9 cell lines. The minimal oligosaccharide structures of the glycopeptides found in the separated fractions are reported in Fig. 2, and the quantitative results are presented in Table 1.

Most of the radioactivity was recovered as mannose. Since cells are able to convert mannose into fucose, this interconversion could contribute to apparent differences between the two cell lines. Thus the presence of [³H]fucose was checked in the whole glycoprotein extracts of the two cell types. No significant amounts of [³H]fucose were found in the TF 22.9 cell line, and less than 4% of the radioactivity was detected as fucose in the 430 cell line. This could lead to a slight overestimation of the proportion of fucosylated fractions in this cell line, but cannot alter significantly the interpretation of the data.

The first affinity chromatography (concanavalin A) was used to separate the glycopeptides into three

Fig. 3. Characterization of the high-mannose type of structure of the pool III glycopeptides obtained by concanavalin A affinity chromatography

The fraction components were filtered on a Bio-Gel P-6 column (120 cm $\times$ 1 cm) equilibrated in 0.1 M-pyridine/acetate buffer, pH 5.0, before (-----) and after (—) treatment with endoglycosidase H.

fractions, known as tri- and tetra-antennary complex structures (pool I), bi-antennary complex structures (pool II) and high-mannose structures (pool III). The high-mannose-type character of the pool III glyco-

Fig. 4. Characterization of the high-mannose-type structures released by endoglycosidase H treatment

Oligosaccharides released by treatment with endoglycosidase from pool III glycopeptides obtained by concanavalin A affinity chromatography were filtered on a Bio-Gel P-4 column (1 cm $\times$ 120 cm) equilibrated in 0.1 M-pyridine/acetate buffer, pH 5.0. (a) 430 cell line; (b) TF 22.9 cell line. The arrows, marked M5 to M9, indicate K_{av} positions of known [3 H]mannose-labelled standards: M5, $\text{Man}_5\text{GlcNAc}$; M6, $\text{Man}_6\text{GlcNAc}$; M7, $\text{Man}_7\text{GlcNAc}$; M8, $\text{Man}_8\text{GlcNAc}$; M9, $\text{Man}_9\text{GlcNAc}$.

peptides was confirmed by their sensitivity to endoglycosidase H. The fraction was filtered on a Bio-Gel P-6 column before and after treatment with endoglycosidase H (Fig. 3). After treatment, the whole radioactivity was shifted towards lower- M_r fractions, confirming the high mannose content of these glycopeptides.

Furthermore, the size of these oligosaccharides was estimated, by filtration on a Bio-Gel P-4 column (Fig. 4). The elution patterns were similar for both cell lines and showed the predominance of the high-mannose-type structures containing eight and nine mannose residues. Consequently, the difference in the radioactivity contents found in the pool III fractions obtained by concanavalin affinity chromatography actually results from various proportions of high-mannose-type structures of the same size.

All the affinity-chromatography results are presented in Table 1 as percentages of the total radioactivity found in the different fractions. As an example, two series of column effluents, corresponding to both the 430 and

TF 22.9 cell line DR antigens, are presented in Figs. 5 and 6 respectively. The whole cellular extract and the HLA-DR antigen isolated from the same cell exhibited similar oligosaccharide patterns. In contrast, major differences were detected in the glycosylation patterns of the two cell lines for both the DR antigens and the whole extracts.

First, as for the complex-type structures, the radioactivity found in all the fractions was much less than half (430 cell line) or nearly half (TF 22.9 cell line) of the total radioactivity incorporated in the glycopeptides. In both cell lines, major amounts of branched (tri- and tetra-antennary) structures were found, i.e. three-quarters and four-fifths of the radioactivity incorporated in all the complex-type structure fractions, which represented respectively 21% and 42% of the total radioactivity in 430 and TF 22.9 cell lines, as reported in Table 1. However, the qualitative distributions of complex-type structures were different, and the two cell lines could be distinguished by their own oligosaccharide structures. Thus the oligosaccharide containing a bisecting *N*-acetylglucosamine residue with the minimal structural unit described by Yamashita *et al.* (1983), pool I2', and the fucosylated either bi-antennary (IIB) or tri- and tetra-antennary (II'B1) structures were present in small amounts in the 430 cell line but not detected in the TF 22.9 cell line. Conversely, galactosylated tri- and tetra-antennary structures (pool II'A2) were not detected in the 430 cell line whereas they represented 11% of the radioactivity incorporated in the TF 22.9 cell line.

Secondly, large amounts of high-mannose-type structures were detected in both cell lines, more in the 430 cell line than in the TF 22.9 cell line (respectively 72% and 49.5% of the total radioactivity).

N-Glycosylation of the isolated DR α - and β -chains

The fine structure of the HLA-DR antigen carbohydrate moiety was further investigated on the α - and β -chains isolated by non-equilibrium pH-gradient gel electrophoresis (O'Farrell *et al.*, 1977). The α -chains were separated, according to their acidic character, into two fractions, α^1 and α^2 , identical in their polypeptide backbone. The lectin-affinity-chromatographic analysis of the different DR chains is shown in Table 2.

In both cell lines, the glycosylation patterns of α^2 - and β -chains seemed similar, whereas several discrepancies were observed on α^1 -chains.

First, the comparison of the distributions of the complex-type structure fractions between bi-antennary and more branched chains, in both cell lines, showed a higher proportion of bi-antennary structures on α^1 -chains than on α^2 - and β -chains. Namely, in the 430 cell line there were respectively 4% and 7% of the total radioactivity incorporated in the bi-antennary and the branched structures on α^1 -chains, whereas there were 5% and 35% on α^2 -chains and 6.5% and 31.5% on β -chains. In the same way, in the TF 22.9 cell line there were 9% and 23% respectively incorporated in the bi-antennary and the branched structures on α^1 -chains, whereas there were 4% and 80% on α^2 -chains and 4.5% and 77% on β -chains. However, as found on the whole cellular extracts and the isolated HLA-DR antigens, the two cell lines could be distinguished by their own specific structures, the same as those described above, but their relative amounts vary with the chain. The distributions on the α^2 - and β -chains were still similar. Among complex-type structures, in the

Fig. 5. Lectin-affinity-chromatography profile of [^3H]mannose-labelled DR antigen glycopeptides from the 430 cell line

Glycopeptides were applied sequentially to columns of concanavalin A-Sepharose (ConA), E-phytohaemagglutinin-agarose (E-PHA), lentil haemagglutinin-Sepharose (Lentil), L-phytohaemagglutinin-agarose (L-PHA) and wheat-germ agglutinin-agarose (WGA) as described in the Materials and methods section. Abbreviations: αMG , methyl α -glucoside; αMM , methyl α -mannoside; GlcNAc, *N*-acetylglucosamine.

430 cell line the I1'A1 tri- and tetra-antennary structures predominated on α^2 - and β -chains, whereas the I1'B1 tri-antennary ones did so on α^1 -chains. In the TF 22.9 cell line the I1'A1 fraction predominated on every chain, but in a larger proportion on α^2 - and β -chains than on α^1 -chains.

Secondly, on both cell lines a higher proportion of high-mannose-type structures was found on the α^1 -chains than on the α^2 - and β -chains, i.e. 89%, 60% and 62% respectively in the 430 cell line and 68%, 16% and 18.5% in the TF 22.9 cell line. It may be noticed that in the 430 cell line the amounts of high-mannose-type structures on

Fig. 6. Lectin-affinity-chromatography profile of [^3H]mannose-labelled DR antigen glycopeptides from the TF 22.9 cell line

Details are as indicated in Fig. 5 legend.

α^2 - and β -chains remained important whereas they were low in the TF 22.9 cell line.

DISCUSSION

Cell influence on the glycosylation pattern

An important change in the HLA-DR antigen glycosylation was observed according to the cell line in

which the molecule was synthesized. Similarly, the overall protein glycosylation patterns varied extensively between different cell types, as if a cell could be characterized by the absence, the presence, or specific amounts of some structures. Since no fucosylated structures (IIB and I1'B1) could be found in the TF 22.9 cell line, this suggests a defect of fucose metabolism in this fibroblastic line. In the same way, each cell line exhibited other specific structures not detected in the

Table 2. Lectin-affinity-chromatographic analysis of [2-³H]mannose-labelled separated α^1 -, α^2 - and β -chains of DR antigens

The α^1 -, α^2 - and β -chains were separated by preparative non-equilibrium pH-gradient gel electrophoresis. Values indicate the distribution of the glycopeptides fractionated by lectin affinity chromatography as described in the Materials and methods section. The results are expressed as percentages of the total radioactivity incorporated in the glycopeptide Pronase digest.

Glycopeptide	Distribution (%)					
	430 α^1 -chain	430 α^2 -chain	430 β -chain	TF22.9 α^1 -chain	TF22.9 α^2 -chain	TF22.9 β -chain
Complex-type structures						
Tri- and tetra-antennary						
I1'A1	1.5	22	17	19	69	61
I1'A2	0	0	0	4	11	16
I1'B1	4	11	13.5	0	0	0
I1'B2	0	0	0	0	0	0
I2'	1.5	2	1	0	0	0
Bi-antennary						
II	4	5	6.5	9	4	4.5
High-mannose-type structures						
IIIa	89	60	62	68	16	18.5
IIIb	0	0	0	0	0	0
Total radioactivity (d.p.m.)	5×10^4	2×10^4	3×10^4	5×10^4	2×10^4	3×10^4

other one, i.e. galactosylated tri- and tetra-antennary structures (I1'A2) in the TF 22.9 cell line and *N*-acetylglucosamine bisecting structures (I2') in the 430 cell line. These data could indicate differences in specific glycosyltransferase activities in each cell line.

Moreover, TF 22.9 and 430 cell lines were different in their relative amounts of tri- and tetra-antennary structures or high-mannose-type structures; the TF 22.9 cell line contained many of the first and few of the second, whereas the opposite was found for the 430 cell line. The tri- and tetra-antennary structures have been proposed as mature chains, with the high-mannose-type structures being intermediate stages in the glycosylation process (Hubbard & Ivatt, 1981). The efficiency of the conversion of high-mannose-type into the complex-type structures could vary with the cell type.

Such an influence of the cell context on the glycosylation pattern of identical molecules is supported by the studies by Hsieh *et al.* (1983) showing that *N*-linked glycosylation of a viral glycoprotein varies with the host in which it is expressed. Fulcher *et al.* (1983) have reported that purified pig endopeptidases from either intestine or kidney were differently glycosylated, but they could not state that the polypeptide sequences were identical.

Likewise, Cowing & Chapdelaine (1983) have shown differences in the Ia antigen sialylation when it is expressed either in monocytes or in B-lymphocytes. Moreover, Alexander *et al.* (1984) have reported that HLA-DR antigens of autologous melanoma and B lymphoblastoid cell lines exhibited different proportions of endoglycosidase H-sensitive oligosaccharide structures.

Importance of the protein structure on the glycosylation pattern

We observed similarities between the glycosylation patterns of the isolated HLA-DR antigens and the overall glycoproteins (Table 1). However, in studying the

separated α^1 -, α^2 - and β -chains some differences can be noticed (Table 2). The presence of specific structures is constant, but their relative amounts vary with the chain, showing that the influence of the cellular context in the glycosylation processing can be modulated. As far as the HLA-DR molecules are concerned, some specific explanations should be considered. The DR-associated Ii chain (Charron *et al.*, 1983), which is bound to the DR chains during the assembly and glycosylation process, might influence the composition of the linked oligosaccharides (Claesson-Welsh & Peterson, 1985). In contrast with the 430 cell line, the DR + TF 22.9 cell line does not synthesize the human Ii chain, but synthesizes the mouse Ii chain, which is associated with the DR α and β products and may have a similar function (results not shown).

More general explanations could arise from the studies on the separated α - and β -chains.

It has been shown by Lee *et al.* (1982) that the α -chains possess two potential *N*-linked glycosylation sites. Shackelford & Strominger (1983) suggested the presence of one complex-type structure and one high-mannose-type structure on α -chains. We found that 60% and 68% of the radioactivity was incorporated in the high-mannose-type fractions of 430-cell-line α^2 -chains and TF 22.9-cell-line α^1 -chains respectively. Because high-mannose-type structures contain five to nine mannose residues and complex-type ones only three, that represented about 50% of the oligosaccharide chains. These results are in good agreement with the presence of one complex-type structure and one high-mannose-type structure on each α -chain.

However, the 430-cell-line α^1 -chain high-mannose-type fraction contained 89% of the radioactivity, corresponding to 75% of the oligosaccharide chains; it suggests the presence of some α -chains with two high-mannose-type structures. Conversely, the TF 22.9-cell-line α^2 -chain high-mannose-type fraction contained 16% of the radioactivity, corresponding to 10% of the oligosaccharide chains. Some α -chains in this cell line could ex-

hibit two complex-type structures; however, we cannot exclude, as suggested by Swiedler *et al.* (1985), the possibility of α -chains glycosylated on only one of the potential sites.

Thus the separation of the α -chains into two populations reveals the glycosylation heterogeneity of this monomorphic chain. The α^1 -chain and α^2 -chain fractions were separated according to their acidic character, the α^1 -chain fraction being the more acid fraction. Sialic acid generally contributes to the acidic character of glycoproteins, it is usually bound to a galactose residue at the non-reducing end of complex-type structures, and high-mannose-type structures are devoid of sialic acid. In both cell lines, even though they may be different, the α^1 -chain fraction contained a higher proportion of high-mannose-type structures than did the α^2 -chain one and the more acidic character of α^1 -chains might therefore seem paradoxical. However, α^1 -chain but not α^2 -chain complex-type structures could bear sialic acid, and the lectin-affinity-chromatographic analysis does not take into account the presence or absence of sialic acid on the complex-type structures. Apart from this, the acidic character could be related to other post-translational events, such as sulphation or phosphorylation of either the oligosaccharide or the polypeptide chains (Kornfeld & Kornfeld, 1985).

As far as the β -chains are concerned, they possess only one potential N-glycosylation site (Lee *et al.*, 1982), and Shackelford & Strominger (1983) postulated a complex-type structure on this site.

In both cell lines a microheterogeneity among the complex-type structures was observed and a high-mannose-type structure fraction was detected (Table 2). As for the latter, the two cell lines were different in their relative amounts, i.e. 62% of the radioactivity corresponding to 50% of the oligosaccharide chains in the 430 cell line and 18.5% of the radioactivity corresponding to 12% of the oligosaccharide chains in the TF 22.9 cell line. The presence of 12% of high-mannose-type structures might have been considered as a mixture of the TF 22.9 cell-surface antigen β -chains, bearing complex-type structures, with chains being processed within the cell. However, in the 430 cell line half the carbohydrate chains were high-mannose-type ones and should probably be considered not as chains being processed within the cell but as part of the glycosylation microheterogeneity of the surface antigen polymorphic β -chains.

The observed differences between the two cell lines seem mainly to be due to the cells themselves. However, although they are closely related, there is a heterogeneity in the polypeptide structure of different DR W6 types (Hazirot *et al.*, 1986). The DR β -chains in 430 cell line are composed of three different DR W6 types, only one of which is present in the TF 22.9 line chains, and different β -chains might affect the pattern of glycosylation of the DR antigens. Although this cannot be ruled out, a previous study has shown that a B cell line from a different DR type exhibits an oligosaccharide profile similar to that of DR W6 430 cell line (Néel *et al.*, 1985).

A site-specific microheterogeneity induced by the polypeptide backbone has been established by Swiedler *et al.* (1985) when comparing two different, although related, class II major-histocompatibility-complex (I-A^k and I-E^k) synthesized simultaneously in a mouse B-cell lymphoma. However, these two mouse antigens, homologous to the human DQ and DR antigens, are more

different from each other than are the different DR antigen haplotypes.

One of the interesting results obtained when studying the isolated α^1 -, α^2 - and β -chains was the strong similarity in both cell lines between α^2 -chain and β -chain glycosylation patterns, whereas those of the α^1 - and α^2 -chains were different. The α^2 -chains that possess two potential N-glycosylation sites exhibited the same microheterogeneity as the β -chains that possess only one glycosylation site. This could be in agreement with the hypothesis that only one of the sites is glycosylated in α^2 -chains, both of them being glycosylated on α^1 -chains.

The glycosylation patterns of α^1 -chains (Table 2) showed that at least one of the two oligosaccharide structures was a high-mannose-type one. The homologous site on α^2 -chains could be non-glycosylated.

As regards the second glycosylation site, the differences in quantitative complex-type structure distributions between α^1 and α^2 -chains involved that the oligosaccharide structures were different on α^1 - and α^2 -chains. It is possible that the presence of a high-mannose-type structure on one of the glycosylation sites could modify the accessibility to the second site for glycosyltransferases on α^1 -chains. The glycosylation of this second site on the α^2 -chains could be identical with that on β -chains.

We demonstrated the cellular influence on the glycosylation pattern of the same HLA-DR antigens synthesized in different cell lines. It remains to be determined whether this cell print is related to species-specificity (mouse versus human) and/or lineage-specificity (fibroblast versus B-cell line). Progress in transfection methods, particularly in human cells, could give new material for such studies. However, the polypeptide backbone influences the microheterogeneity of the glycosylation at one specific site.

It is now of interest to know if cell-type- and polypeptide-backbone-specificities of glycosylation represent two different parts of the contribution to the glycosylation process. One common property could be shared by all the glycoproteins of one cell type, and each glycoprotein could influence the microheterogeneity at its glycosylation sites to specify a role for these glycosylated structures.

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