To examine the nature of the factors influencing the galactosylation pattern of the heavy chain of murine immunoglobulin G (IgG), cell fusion was performed between a myeloma (P3X63Ag8) and a hybridoma (Sp2HL/Bu) cell line which secrete different IgGs possessing structurally distinct C\sub{H2}-linked oligosaccharide moieties. The glycosylation patterns of the IgGs of the parental and fused cells were studied. Pronase digestion of the purified heavy chains and subsequent end labeling with fluorescein isothiocyanate produced fluoresceinated glycopeptides which were detected and purified by polyacrylamide gel electrophoresis. Structural information was obtained by enzymatic digestion, lectin affinity chromatography, and methylation analysis. IgGs from both parental lines possessed oligosaccharide units displaying microheterogeneity based upon a common symmetrical biantennary structure terminating in \(\beta\)-GlcNAc. The structures of both IgGs, however, differed in the pattern of the mono- and digalactosylated components. Clones, selected following the fusion of the parental cells, were expanded; and the individual IgGs were purified. All clones produced homodimeric Ig\(\gamma_1\) and Ig\(\gamma_2\) as well as heterodimeric IgG possessing both the \(\gamma_1\) and \(\gamma_2\) heavy chains. Analysis of the carbohydrate moieties of the \(\gamma_1\) chain from the homodimeric and heterodimeric IgGs and of the \(\gamma_2\) chain from the heterodimeric molecule demonstrates that the polypeptide structure of the heavy chain influences the terminal galactosylation of the glycan unit at the conserved site of glycosylation of IgGs.

Plasma membrane and secreted glycoproteins share a common biosynthetic route involving N-glycosylation (1–3). Many of the individual steps involved in the biosynthesis and processing of the oligosaccharide chains of N-linked glycoproteins have been studied in recent years. However, little is known about the mechanisms which determine the fine structures of the completed glycan moieties. The importance of cell type, presumably related to the nature, quantity, and compartmentalization of the individual glycosyltransferases and glycosidases, may contribute to the determination of the final oligosaccharide structure. This has been demonstrated with the expression of viral glycoproteins (4), with the structure of the glycan moiety of ribonuclease B in different in vitro expression systems (5), with the products of transfected foreign genes of naturally occurring glycoproteins (6, 7), and with the structures of the carbohydrate moieties of the same glycoprotein, normally expressed in different tissues (8).

Influence of the acceptor polypeptide on the degree and nature of processing of the glycan unit has been suggested by a number of authors (9–11). However, inferences from these studies which indicate a role for the polypeptide chain in modulating the structure of the glycan must be tempered with the realization that different proteins, synthesized in the same cell, may enter different intracellular compartments and/or exhibit differential rates of synthesis. Presently, there has been no report of the effect of polypeptide primary structure on the fine structure of the glycan moieties of two functionally identical subunits of a single protein in which both subunits possess the same glycosylation site.

Immunoglobulin G (IgG), which is a bilaterally symmetrical molecule composed of two heavy and two light polypeptide chains, is an ideal glycoprotein to examine the influence of a polypeptide backbone on the biosynthesis of N-linked glycans. All human and murine IgG heavy chains possess a conserved site of glycosylation at Asn\(^{292}\) in the C\sub{H2} domain. The N-linked oligosaccharide moieties of all human and murine IgGs are fundamentally similar, based upon a dibranch complex structure (12). Murine IgG rarely contains terminal sialyl units and, unlike the human protein, lacks a bisecting N-acetylgalactosaminyl residue (13, 14). The glycan at heavy chain Asn\(^{292}\) affects a number of important biological properties of the protein. These include the ability to activate complement (15, 16), to induce antibody-dependent cytotoxicity (15), and to bind to the Fc receptor of monocytes (15, 16). The presence and nature of the glycan structure have been suggested to contribute to the maintenance of the spatial relationship between the two heavy chains of the holoprotein (17, 18). Clinical implications of abnormal IgG oligosaccharides have been reported. The presence of agalactosylated IgG, which possesses carbohydrates with abnormal fine structure, correlates with the appearance of symptoms of rheumatoid arthritis in humans (19, 20).

To determine the effect of polypeptide structure on the fine structure of the glycan unit as Asn\(^{292}\) of murine myeloma IgG proteins, we employed cloned hybrid myeloma cell lines. These were constructed by fusion of two cell lines producing IgG of different subclasses. In this study, we demonstrate that both IgGs possess a dibranch glycan moiety, but they differ in the pattern of microheterogeneity associated with the quantity and nature of terminal galactosylation. Structural analyses were performed on the oligosaccharides of the different
IgGs produced by the same hybrid cell. Accordingly, comparisons can be made of the final biosynthetic product of glycosylation of two functionally identical glycoprotein subunits secreted by the same cell, but which differ in polypeptide primary structure. Results of this study demonstrate that the primary structure of the polypeptide influences the addition of terminal galactosyl residues during the biosynthesis of the glycan moiety at the conserved site of glycosylation of murine IgG heavy chains.

**EXPERIMENTAL PROCEDURES AND RESULTS**

**Exoglycosidase Analysis of Pronase Glycopeptides**—The characteristics of the myeloma proteins secreted by the parental and three hybrid clones are described in the Miniprint. When necessary, tryptic glycopeptides from each of the heterodimeric heavy chains were isolated as detailed in the Miniprint. Structural information for the glycans of each tryptic glycopeptide from the heterodimers as well as the whole molecule or isolated heavy chains of the homodimeric IgGs was obtained by Pronase digestion and end labeling of the products with fluorescein isothiocyanate. The resulting FGPs, isolated by Sephadex G-10 chromatography, were detected and purified by PAGE (26). Carbohydrate sequence information was obtained by the use of exo- and endoglycosidases and analysis of the products by PAGE.

**Enzyme Analysis of IgG<sub>ab</sub> Glycopeptides**—Fig. 2 shows the PAGE fluorograms of purified and enzyme-degraded FGPs from the IgG<sub>ab</sub> protein. Pronase digestion and gel filtration of the derivatized products yielded two distinct asparagine-linked glycopeptides (Fig. 2A, lane 4). These were purified by preparative PAGE to yield GP1 (lane 1), GP2 (lane 2), and GP3 (lane 3). As expected for a symmetrical dibranched structure with nonreducing terminal N-acetyl-β-glucosaminyl residues, GP1 is not susceptible to β-galactosidase (Fig. 2B, compare lanes 7 and 9) or α-mannosidase (data not shown). N-Acetyl-β-glucosaminidase, however, is capable of removing two hexose units (compare lanes 7 and 8). Fig. 2C shows that after elimination of the hexosaminyl residues, the glycopeptide is susceptible to α-mannosidase, which removes two hexose moieties. The next most abundant glycopeptide, GP2, is susceptible to β-galactosidase, which removes only 1 residue, yielding a compound of the same mobility as GP1 (Fig. 2B, compare lanes 4 and 6). Treatment of GP2 with N-acetyl-β-glucosaminidase yields two fluorescent bands (lane 5). The predominant component displays an enhanced mobility corresponding to the loss of a single hexose residue from GP2. The minor component has a slower mobility. As is described in the Miniprint, the two fluorescent bands resolved by PAGE are the products of N-acetyl-β-glucosaminidase action on isomeric monogalactosylated dibranch glycopeptides differing in regard to the arm substitution of the galactose. Further exoglycosidase analysis (data not shown) of GP2 confirms that it is related to GP1 except that it contains 2 β-galactosyl residues linked to an N-acetyl-β-galactosaminyi moiety equivalent to the nonreducing terminal of GP1. The least abundant glycopeptide, GP3, has a structure similar to GP1 except that it possesses 2 β-galactosyl residues, each linked to an N-acetyl-β-glucosaminyl residue of GP1. Thus, β-galactosidase treatment of GP3 (lane 1) results in the appearance of a glycopeptide (lane 3) with a mobility similar to GP1. Furthermore, GP3 is resistant to N-acetyl-β-glucosaminidase, indicating the absence of nonreducing terminal N-acetylglucosaminyl residues. These results are consistent with the glycans being related to a dibranch structure exhibiting microheterogeneity with 0, 1, or 2 nonreducing terminal β-galactosyl residues.

**Enzyme Analysis of IgG<sub>ab</sub> Glycopeptides**—Results of similar studies with P3 (IgG<sub>c</sub>) show that the glycan from this protein is virtually identical to that of Sp2 except for the proportion of the two isomeric monogalactosylated glycopeptides present in the microheterogeneous mixture. This difference is most evident in Fig. 5. The action of N-acetyl-β-glucosaminidase on GP2 of the γ<sub>c</sub> chain yields two fluorescent bands which are in approximately equal proportion (lane 2). This contrasts with GP2 from Sp2, which, when treated comparably, produces the two bands in a ratio of 4:1. The two bands are not caused by incomplete enzymatic digestion since prolonged treatment with N-acetyl-β-glucosaminidase of GP2 from either IgG yields products similar to those seen with a 20-min exposure to the enzyme. Such results are suggestive of heterogeneity of the GP2 glycopeptides. Electrophoresis in the presence of borate is capable of resolving FGPs of the same size but of different sugar compositions (28). Borate-PAGE of GP2 from both the γ<sub>c</sub> and γ<sub>ab</sub> heavy chains shows them to be homogeneous (data not shown). Furthermore, sequential degradation of GP2 from P3 by β-galactosidase and N-acetyl-β-glucosaminidase results in: Step 1, the formation of a band corresponding to the loss of a single β-galactosyl unit; and

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1 Portions of this paper (including "Experimental Procedures," part of "Results," part of "Discussion," Figs. 1, 3, 4, and 6, and Tables I and II, and Footnote 2) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

2 The abbreviations used are: FGPs, fluoresceinated glycopeptides; PAGE, polyacrylamide gel electrophoresis.
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then Step 2, the formation of a single band corresponding to the loss of 2 N-acetyl-glucosaminyl residues (data not shown). GP1 from P3 contains 2 terminal N-acetyl-β-glucosaminyl residues (lane 5) and no nonreducing terminal β-galactosyl moieties (lane 6).

Structures of the IgG Glycans from Parental and Hybrid Cell Lines—The ability to separate di-, mono-, and agalactosylated FGP's by PAGE and the resolution of the isomeric monogalactosylated compounds by concanavalin A-Sepharose chromatography allow for determination of the structures of the glycan units isolated from the conserved site of glycosylation. Table III records the percentage of total fluorescence of each FGP corresponding to the variants in the microheterogeneous mixture of glycans from each heavy chain. It is evident that in all cases, regardless of cell type and heavy chain composition of the IgG, the proportion of the variants in the mixture isolated from the IgG, chains is identical. This microheterogeneity is reflected in the di-, mono (isomers A and B)-, and agalactosylated structures in a proportion of approximately 5:20:20:55, respectively. This composition differs from the proportion of the structures associated with the γ1b chains, which is 5:38:9:48 in di-, mono (isomers A and B)-, and agalactosylated glycopeptides, respectively. Most noteworthy, in all the cases studied (regardless of cell type or dimeric form of the IgG), the proportion of each structure within the microheterogeneous mixture isolated from either the γ1 or γ1b chains is virtually identical to the composition determined for the corresponding parental homodimeric γ chains. Interestingly, the proportion of the digalactosylated species is significantly greater in the glycopeptides isolated from the heterodimeric IgG than in structures obtained from the corresponding homodimeric proteins. This increase occurs at the expense of the quantity of agalactosylated glycopeptide in the IgG1 series, but of the monogalactosylated structures of the IgG1b glycopeptides. Nevertheless, the ratio of isomer A to B of the monogalactosylated glycopeptides is not significantly different from that obtained from the appropriate heavy chain in either the homodimeric or heterodimeric protein.

**DISCUSSION**

*Sp2HL/Bu and P3x63Ag8 Produce IgGs Which Exhibit Differences in Glycan Microheterogeneity—Both IgGs from P3 and Sp2 cell lines possess a non-bisected dibranched oligosaccharide, although the proportions of the 1,6- and 1,3-arm monogalactosylated isomers differ in the two proteins. The ratios of the monogalactosylated isomers of IgG from the P3 and Sp2 cells are 1:1 and 4.2:1, respectively. Our results with the myeloma protein from clone P3x63Ag8 are similar to those reported by Rademacher et al. (12) for the protein.*

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**TABLE III**

*Distribution of fluoresceinated glycopeptides*

The numbers indicate percent of structure in the total pool of fluoresceinated glycopeptides recovered from the IgG of each cell line.

| Fluoresceinated glycopeptide | Parent P3x63Ag8 (IgG) | Homodimers (IgG) | Heterodimers (IgG) | Parent Sp2HL/Ba (IgG) | Hybrids, heterodimers (IgG) |
|-----------------------------|-----------------------|------------------|-------------------|-----------------------|----------------------------|
| Gal-GlcNAc-Man              |                       | 27-1             | 15B               | 26A7                  | 27-1                       |
|                             |                       |                  |                   |                       | 15B                         |
|                             |                       |                  |                   |                       | 26A7                        |
|                             |                       | 27-1             | 15B               | 26A7                  | 27-1                       |
|                             |                       |                  |                   |                       | 15B                         |
|                             |                       |                  |                   |                       | 26A7                        |
| Gal-GlcNAc-Man              |                       | 27-1             | 15B               | 26A7                  | 27-1                       |
|                             |                       |                  |                   |                       | 15B                         |
|                             |                       |                  |                   |                       | 26A7                        |
| Gal-GlcNAc-Man              |                       | 27-1             | 15B               | 26A7                  | 27-1                       |
|                             |                       |                  |                   |                       | 15B                         |
|                             |                       |                  |                   |                       | 26A7                        |
| GlcNAc-Man                  |                       | 27-1             | 15B               | 26A7                  | 27-1                       |
|                             |                       |                  |                   |                       | 15B                         |
|                             |                       |                  |                   |                       | 26A7                        |
| Gal-GlcNAc-Man              |                       | 27-1             | 15B               | 26A7                  | 27-1                       |
|                             |                       |                  |                   |                       | 15B                         |
|                             |                       |                  |                   |                       | 26A7                        |
| GlcNAc-Man                  |                       | 27-1             | 15B               | 26A7                  | 27-1                       |
|                             |                       |                  |                   |                       | 15B                         |
|                             |                       |                  |                   |                       | 26A7                        |

*The proportion of isomers of monogalactosylated species is 82:18.*

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secreted by the parent clone, P3K-MOPC-21. The overall microheterogeneous mixture from the P3K protein appears qualitatively similar to P3X63Ag8, although significant amounts of nonfucosylated oligosaccharides were obtained by Rademacher et al. (12). As with the P3 and Sp2 IgGs, however, the glycans from pooled mouse serum IgG (13) and a number of mouse monoclonal antibodies (14) are greater than 94% fucosylated.

**Poly peptide Influences Terminal Galactosylation of IgG Oligosaccharide**—In reports quantifying the relative amounts of the 1,6- and 1,3-arm isomers present in the monogalactosylated species, the 1,6-arm isomer predominates by a factor 1.3–3.5 times the amount of the 1,3-arm isomer (12–14). Never is the 1,3-arm isomer in preponderance. In contrast to what may be expected from the proportions of monogalactosylated isomers observed for IgG, the β-galactosyltransferase isolated from a number of sources (31, 33, 34) preferentially transfers galactose to the 1,3-arm of symmetrically branched acetylated glycopeptide and oligosaccharide acceptors terminating in N-acetyl-ß-glucosaminyl residues. Apparently, either cellular factors or poly peptide structure influences the branch specificity of the reaction catalyzed by the transerase. The ability to obtain fusion constructs of myeloma cells which secrete heterodimeric IgG composed of a heavy chain derived from each parental cell (P3 and Sp2) allows for the determination of the nature of the factors that influence the galactosylation process. Regardless of subclass, IgG is assembled completely with two heavy and two light chains prior to transfer to the Golgi apparatus (36), where glycan processing and galactosylation occur (3). Accordingly, the finding that the glycans moity from each of the heavy chains of the heterodimeric IgG possesses similar levels of microheterogeneity but differs in the proportion of each monogalactosylated species demonstrates that the poly peptide chain is capable of influencing, in vivo, the branch specificity of the β-galactosyltransferase. There is no need to invoke the presence of a β-galactosyltransferase different from that reported previously to explain the nature of the glycan microheterogeneity found in IgG. Furthermore, the structure of the heterodimeric IgG glycan reflects properties representative of the parental cells. The microheterogeneous mixture of the glycopeptides from the γ chain of the heterodimeric protein secreted by the three fused cell lines examined is very similar to that found with the IgG secreted by P3 cells and the homodimeric IG, obtained from the fused constructs. Similarly, the glycopeptide mixture obtained from the IgG of Sp2 cells is virtually identical to the composition of the glycopeptides obtained from the γ chain of the heterodimeric protein produced by the fused cell lines. The possible basis for the observed polypeptide influence on galactosylation is presented in the Mini.

**Acknowledgment**—We would like to thank Dr. Gad Avigard for his critical review of this manuscript.

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Characteristics of IgG from parental and hybrid cell lines - The IgG secreted by each parental and hybrid cell line was isolated from ascites fluid and fractionated into subclasses by Protein A-Sepharose affinity adsorption. The properties of the IgG from the parental cell line, P3X63.Ag8 (21) and Sp2/HyBlue (21) as well as the cloned fusion product 27-I, have been reported elsewhere. Table I shows the characteristics of the polypeptides of the IgG produced by each cell line. Parental cell line, P3, produces a myeloma protein composed of two identical \( \gamma \) heavy chains and \( \kappa \) light chains; Sp2, a hybridoma which was used as a parental cell line, produces a similar homodimeric molecule containing only one \( \gamma \) heavy chain and the heterodimeric IgG (Sp2/HyBlue) which is produced by the hybridoma Sp2HyBlue was used as a parental cell line, produces a hybrid molecular containing only one \( \gamma \) heavy chain and the heterodimeric IgG (Sp2/HyBlue) which is produced by the hybridoma Sp2HyBlue was used as a parental cell line, produces a hybrid IgG containing two identical \( \gamma \) heavy chains and the third is a heterogeneous molecular containing one \( \gamma \) and one \( \kappa \) heavy chain. All of the secreted IgG are associated with two possible light chains derived from the parental cells. SDS-PAGE of the IgG secreted by each hybrid cell line and separated by Protein A-Sepharose affinity chromatography, demonstrates that the grossly contains only the \( \gamma \) heavy chain, while the third IgG contains two identical \( \gamma \) heavy chains and the third is a heterogeneous molecular containing one \( \gamma \) and one \( \kappa \) heavy chain.

Separation of glycopolypeptides from individual glycophosphate sites - Each IgG (10 mg) was reacted with concanavalin A-Sepharose chromatography, using the strength of the carbohydrate link as an indicator of the different heavy chains was analyzed. Trypsin digestion demonstrated that the IgG secreted by each parental cell line possesses only one glycosylation site, presumed to be at the conserved site of glycosylation. The heterogeneous molecules, produced by the fusion cells, contain two tryptic glycopolypeptides. Each is characteristic of the tryptic glycopolypeptide from the heavy chain of the corresponding parental cell line. To accomplish the tryptic peptide analysis, the appropriate cells were grown in vitro in the presence of 2-\( \text{mg} \text{Gp} \) amino acids. Individual cell lines, containing appropriate amounts of gels, were assayed for the presence of the parentally derived heavy chains. Borate-PAGE of the number of neutral hexose units present. The amino acid composition of these proteins was determined by amino acid analysis, employing the instrument by Narasimhan et al. (29) for the separation of analogous oligosaccharides. The fluoresceinated glycopeptides were isolated by gel filtration through Sephadex G-10. Glycosidase treatment of the FGP's and separation of individual FGP's or glycopeptide treated FGP's was performed as described elsewhere (26). Previous laboratories, we demonstrated that the diagnostic properties of two FGP's, which have identical amino acid structures, is proportional to the difference in the number of neutral hexose units present. These glycopeptide was calibrated periodically employing FGP's from known structures. The fact that the FGP's was used to separate FGP's of the same size but which complex with different amounts of bovine \( \kappa \) chain.

Separation of glycophosphate and non-glycosylated glycopolypeptides - Since each GPC from either the \( \gamma \) or \( \kappa \) chain is treated with maltose results in a product consisting of a single, free, and homogeneous glycopolypeptide, two Phase GPC's were isolated from the appropriate ascites fluid and fractionated into subclasses by Protein A-Sepharose affinity adsorption. The properties of the IgG from the parental cell line, P3X63.Ag8 (21) and Sp2/HyBlue (21) as well as the cloned fusion product 27-I have been reported elsewhere. Table I shows the characteristics of the polypeptides of the IgG produced by each cell line. Parental cell line, P3, produces a myeloma protein composed of two identical \( \gamma \) heavy chains and \( \kappa \) light chains; Sp2, a hybridoma which was used as a parental cell line, produces a similar homodimeric molecule containing only one \( \gamma \) heavy chain and the heterodimeric IgG (Sp2/HyBlue) which is produced by the hybridoma Sp2HyBlue was used as a parental cell line, produces a hybrid molecular containing only one \( \gamma \) heavy chain and the heterodimeric IgG (Sp2/HyBlue) which is produced by the hybridoma Sp2HyBlue was used as a parental cell line, produces a hybrid IgG containing two identical \( \gamma \) heavy chains and the third is a heterogeneous molecular containing one \( \gamma \) and one \( \kappa \) heavy chain. All of the secreted IgG are associated with two possible light chains derived from the parental cells. SDS-PAGE of the IgG secreted by each hybrid cell line and separated by Protein A-Sepharose affinity chromatography, demonstrates that the grossly contains only the \( \gamma \) heavy chain, while the third IgG contains two identical \( \gamma \) heavy chains and the third is a heterogeneous molecular containing one \( \gamma \) and one \( \kappa \) heavy chain.
Galactosylation of IgG

Methylation analysis of IgG: fluoroscencence glycopeptides - Methylation analysis of GPI, GP2, and GP3 from the γ chain, as shown in Table 1, is consistent with those glycosyls related to a distinct unit (GP3) of a non-fluorescent di-branching structure terminating in di-α-mannosylotactosyl and possessing a core 1,6 fucose substituent. The 1,6 and 1,3 isomers of GP3 were expected to yield identical methylation derivatives accordingly, the results for this glycopeptide reflect the sum of the two isomers. The analysis were performed on FPs containing less than 5 µg of total glycosylated protein in yields of the core-linked permethylic glucosamine alcohols occurred because of loss of high release of these units.

Aglycosylated and methylated analysis demonstrate the absence of steric acid and blocking of α-galactosyltransferase on any of the glycosylated. Upon the result of these experiments and in analogy to the structure of the glycosyl unit of IgG reported by others (13, 14, 15), the single site of glycosylation of the IgG chain contains a monoheterogenous mixture of glycosyl moieties as shown in Fig. 4.

DISCUSSION

The effect of polypeptide on glycan conformation may influence terminal galactosylation of IgG - Differences in the glycosylation of the glycoprotein disaccharides heavy chains in the heterodimeric IgG may reflect the ability of the β-galactosyltransferase to recognize structural differences in the acceptor, or in the accessibility of the acceptor when it was bound to dipeptide proteins matrix. MOM studies (36, 37) that two relatively stable reverse conformation exist for di-α-mannosyllactosyl terminating in N-acetyl-D-glucosaminyl residues, resulting in the 1,6 am or extending away from the core di-α-mannosyl lactosyl unit. Cryospectroscopic analysis of the Fc portion and whole IgG indicate that the glycosyl unit at the consensus site of glycosylation exhibits limited flexibility and may have positive interactions with the polypeptide chain, primarily involving the 1,6 am extensions (17, 18). Accordingly the presence of the 1,6 am may stabilize conformation of the glycan which are less prevalent in the free oligosaccharide. Such conformers may allow galactosylation of the IgG to be enhanced at the acetylglucosaminyl residue on the 1,6 am, or expressed that resides on the 1,6 am relative to the free oligosaccharide. This would result in an apparent difference in a branch specificity of the transferase. Differences in the ability of the η and γ chains studied to stabilize such conformers would affect the proportion of the 1,3-branched isomer, and not the agalactosylated species of the heterodimeric IgG. Though this process is similar for the enzyme action on glycopeptides (31, 33) and glycosyltransferase (34), the relative efficiency of the different polypeptide matrices in the homodimeric and heterodimeric proteins must reflect the mechanism by which the glycosyltransferase chain influences the terminal galactosylation of IgG.

Stearic factors may affect galactosyltransferase branch specificity - Differences in the nature of peripheral glycosylation may be caused by disparity in the accessibility or the acceptor for the enzyme, when the glycosyl unit is associated with polypeptide chains exhibiting different secondary and/or tertiary structure in the vicinity of the Fc unit. As such, the heavy chain may sterically hinder the approach of the enzyme from the dimannose species the galactosylation of the 1,3 branch, and have no effect on the glycosylation of the 1,6 am as compared to the action of the enzyme on a free oligosaccharide substrate. Accordingly, the steric factor would be expected to be more pronounced in the Fc derived IgG than the Fab product. The presumed differences of the accessibility by processing enzymes for glycosyl of different glycosylation on the same protein may have been involved to explain the biosynthesis of either complex or oligosaccharide structure (39). Alternatively, positive and perhaps specific interactions between the IgG heavy chain and the galactosyltransferase may enhance preferential transfer of the galactosyl unit to the 1,6 am of the glycan. Such a mechanism has been described for the preferential action of UDP-α-D-mannose:glucosamine-1-phosphotransferase on the 1,6 α-D-mannosylactosylglycan in glycoprotein (23, 24, 25, 29). Differences in the IgG, A-D-Section of the enzyme, and the relative efficiency of the different polypeptide matrices in the homodimeric and heterodimeric proteins on terminal galactosylation must be considered.

Table 1. Characteristics of cell lines

| Cell line | Characteristic of secreted IgG | Resistant | Origin |
|-----------|-------------------------------|----------|-------|
| PX13Mg    | S-conjugated | MOPC21   |
| Sc11B2Lu  | α chain | 5-branched | PS1NOd4I |
| 13h       | 2-disaccharides | 31                     |
| 13D       | 2-disaccharides | 31                     |
| 2A27.1    | homodimer | 5a                      |
| 13B       | homodimer | 5a                      |
| 13D       | homodimer | 5a                      |
| 2A27.1    | homodimer | 5a                      |

Fig. 1. HPLC tricic peptide maps of N-mannose labeled immunoglobulins. Tryptic peptides of a mixture of IgG, purified from tissue culture medium of cells labeled with [3H]mannose, and appropriate carrier IgG were resolved by reverse phase chromatography. Panel A, B, and C are the peptide maps of IgG from parental cell lines IgGcB2Lu, PS1NOd4I, and the heterodimeric IgG secreted by fusion construct 31-4, respectively.

Fig. 2. Electrophoretic analysis of IgG, fluoroscencence-detected GP3 expressed by parent cells. Lane 1, homodimer of IgGcB2Lu; lane 2, homodimer of IgGcB2Lu treated with 2-acetylgalactosamine; lane 3, homodimer of IgG secreted by fusion construct 31-4; lane 4, homodimer of IgGcB2Lu.
Table II. Methylation analysis of fluorescent glycopeptides from IgG.

| Methylated Galactosyl Site | Fluorescent Glycopeptide | Glc | Gal | GalNAc | Man | DIGALACTOSE |
|---------------------------|--------------------------|-----|-----|--------|-----|-------------|
| 2,3,4-tri-O-acetyl-1,5-di-O-methyl | 0.64 | 0.49 | 0.63 |
| 2,3,4,6-tetra-O-acetyl-1,5-di-O-methyl | 0.50 | 0.60 | 1.91 |

Proposed structures of the fluorescent glycopeptides isolated from the conserved site of glycosylation on murine IgG:

![](http://www.jbc.org/DownloadedFrom)

1 Molar ratio with 2,4di-O-acetyl-1,5,6tri-O-methyl = 1.00.
The polypeptide of immunoglobulin G influences its galactosylation in vivo.
S O Lee, J M Connolly, D Ramirez-Soto and R D Poretz

J. Biol. Chem. 1990, 265:5833-5839.

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