



Table I: Panel of Hybridomas<sup>a</sup>

hybridoma	parental cells		isotype	IgG		references
	splenocyte	myeloma		antigenic specificity		
CSAT	BALB/c	SP2/0	2b	anti-integrin	Neff et al., 1982	
34-5-8S	C3H	SP2/0	2a	anti-H-2D <sup>d</sup>	Ozato et al., 1982	
H36.15.3	BALB/c	SP2/0	2a	anti-hemagglutinin	Staudt et al., 1983	
H36.4.5	BALB/c	SP2/0	2a	anti-hemagglutinin	Staudt et al., 1983	
H36.2.6	BALB/c	SP2/0	2a	anti-hemagglutinin	Staudt et al., 1983	
H36.15.6	BALB/c	SP2/0	2a	anti-hemagglutinin	Staudt et al., 1983	
S-S.1	BALB/c	SP2/0	2a	anti-sheep red blood cell		
B137.17.1	BALB/c	.653	2a	?	Perussia et al., 1987	
L5.1	BALB/c	.653	2a	anti-transferrin receptor	Lebman et al., 1982	
D4IE4	BALB/c	.653	1	anti-P <sub>0</sub>	Miller et al., 1984	

<sup>a</sup> The parentage and serologically defined polypeptide structure of the IgG secreted are indicated for each member of the panel of hybridomas employed in the clonal analysis.

for the host-dependent and tissue-dependent variability in glycosylation (Hsieh et al., 1983; Sheares & Robbins, 1986; Parekh et al., 1987).

In the present study, we describe the clonal repertoire for the glycosylation of IgG secreted by a panel of murine hybridomas, formed by the fusion of immune splenocytes with myelomas. The effect upon glycosylation of the hybridoma's parentage and the serologically defined polypeptide structure of the monoclonal IgG which it secretes are assessed. The contribution of generational differences, resulting from continuous culturing, and differences in culturing conditions are evaluated as further sources of variability in glycosylation.

#### MATERIALS AND METHODS

**Materials.** D-[U-<sup>14</sup>C]Glucosamine hydrochloride (284 Ci/mol), D-[6(N)-<sup>3</sup>H]glucosamine hydrochloride (23.4 Ci/mmol), and En<sup>3</sup>Hance were purchased from New England Nuclear (Boston, MA). Sephadex G-50 (Superfine), Sephadex G-10, Con A-Sepharose,<sup>1</sup> DEAE-Sephadex A-25, and protein A-Sepharose were obtained from Pharmacia (Piscataway, NJ). LcH (E-Y Laboratories, San Mateo, CA) was conjugated to Affi-Gel 10 (Bio-Rad, Richmond, CA) (7 mg/mL of resin) according to an established procedure (Knudsen et al., 1981). Bio-Gel P-2 (200–400 mesh) was also from Bio-Rad. Pronase (grade B) was purchased from Calbiochem (San Diego, CA).

**Culturing and Metabolic Labeling of Hybridomas.** Hybridomas (Table I) were obtained either from American Type Culture Center (Rockville, MD) or from various laboratories at the Wistar Institute (Philadelphia, PA). The fusion partners were BALB/c-derived, nonsecreting myelomas. For contrast, one hybridoma (34-5-8S) was chosen for its allogeneic C3H background, and the allotypically different IgG which it secretes. The H36 clones were a cluster of related hybridomas derived from a single fusion of one myeloma variant with immune splenocytes from one animal. All H36 clones secreted an IgG2a directed against the same antigen.

Cells were seeded at  $5 \times 10^5$ /mL and cultured in Dulbecco's modified Eagle's medium with low glucose (Gibco, Grand Island, NY), supplemented with 10% heat-inactivated fetal bovine serum, 10 mM sodium bicarbonate, and 25 mM Hepes (pH 7.2). After a 20-h preincubation, cultures were metabolically labeled with [<sup>3</sup>H]glucosamine (2  $\mu$ Ci/mL) for an

additional 48 h. Parallel cultures of CSAT hybridomas were labeled alternatively with [<sup>14</sup>C]glucosamine (0.5  $\mu$ Ci/mL).

**Isolation of IgG and Preparation of Glycopeptides.** IgG in the spent culture medium was affinity purified chromatographically on protein A-Sepharose essentially by the procedure of Ey et al. (1978). The purified IgG was incubated with predigested Pronase for 3 days, according to an established procedure (Blithe et al., 1980). For <sup>1</sup>H NMR analysis, IgG also was obtained from the ascites fluid of CSAT hybridomas passaged in pristane-primed BALB/c mice. The IgG was affinity purified and digested with Pronase similar to IgG obtained from tissue culture.

**Structural Characterization Studies.** To ensure consistency of elution profiles, a common <sup>14</sup>C-labeled CSAT IgG Pronase digest was added as an internal standard to all <sup>3</sup>H-labeled samples. The resulting doubly labeled Pronase digests were analyzed chromatographically by various techniques. The radioactive distribution in each fraction, determined by liquid scintillation counting, was plotted by computer-aided graphics, which also provided peak area summations.

(1) **Gel Filtration Chromatography.** Blue dextran and phenol red, added to the sample, served as exclusion and inclusion volume markers, respectively. Samples were analyzed on columns (1 cm  $\times$  135 cm) of Sephadex G-50 developed with an alkaline borate buffer composed of 45.5 mM boric acid/4.5 mM sodium tetraborate/2 mM Na<sub>2</sub>EDTA/0.02% sodium azide (pH 8.2) (Rothman & Warren, 1988).

For preparative purposes, selected fractions of the <sup>3</sup>H-labeled Pronase digests were pooled. Borate was removed by evaporation under a stream of nitrogen after repeated treatment with 1% acetic acid in methanol. The residue in a minimal volume of water was desalted further on a Bio-Gel P-2 column (1 cm  $\times$  15 cm) with a 5-cm overlayer of Sephadex G-10 that was developed with water (Blithe et al., 1980).

(2) **Anion-Exchange Chromatography.** The <sup>3</sup>H-labeled IgG Pronase digest was fractionated batchwise on a column of DEAE-Sephadex (0.7 cm  $\times$  4 cm). After the column was washed with 10 mM pyridine/17 mM acetic acid (pH 4.5) to remove the neutral glycopeptides, the acidic glycopeptides were eluted with 2 M ammonium acetate/3.5 M acetic acid (pH 4.5). Volatile buffers were removed by lyophilization.

(3) **Lectin Affinity Chromatography.** After being boiled for 5 min, IgG glycopeptides were analyzed on columns of Con A-Sepharose or LcH-agarose (0.7 cm  $\times$  4 cm) according to the procedure of Cummings and Kornfeld (1982). After the glycopeptides lacking lectin affinity were removed by washing with TBS buffer, the column was eluted stepwise. First, glycopeptides with low lectin affinity were eluted with 10 mM methyl  $\alpha$ -glucoside in TBS; then, those with high affinity were eluted with 100 mM methyl  $\alpha$ -mannoside in TBS, prewarmed to 60  $^{\circ}$ C.

<sup>1</sup> Abbreviations: Con A, concanavalin A; LcH, *Lens culinaris* agglutinin; GFC, gel filtration chromatography; DEAE, diethylaminoethyl; mAb, monoclonal antibody; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Pipes, 1,4-piperazinediethanesulfonic acid; Tes, 2-[[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino]ethanesulfonic acid; Hepps, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid; Tris, 2-amino-2-(hydroxymethyl)propane-1,3-diol; TBS, 150 mM NaCl/10 mM Tris/1 mM CaCl<sub>2</sub>/1 mM MgCl<sub>2</sub>/0.02% NaN<sub>3</sub> (pH 8).

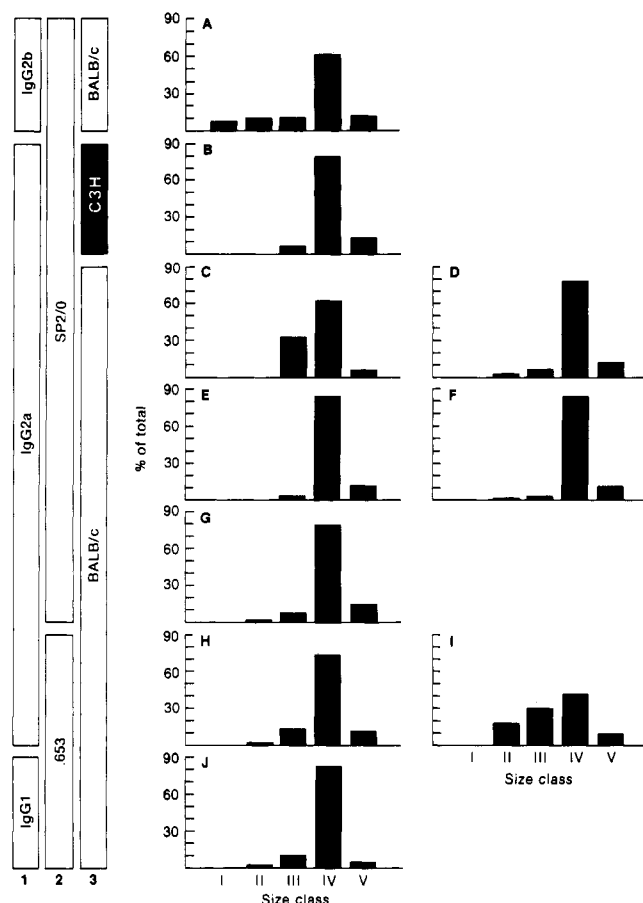


FIGURE 1: Clonal comparison of IgG glycopeptides by alkaline borate GFC. The  $^3\text{H}$ -labeled IgG Pronase digests were analyzed by alkaline borate GFC. The size class distributions are shown for the IgG glycopeptides of CSAT (A), 34-5-8S (B), H36.15.3 (C), H36.4.5 (D), H36.2.6 (E), H36.15.6 (F), S-S.1 (G), B137.17.1 (H), L5.1 (I), and D4IE4 (J). The panel to the left schematically represents how the hybridomas are related to each other by the isotype (1) of IgG secreted, as well as by their parentage of myeloma (2) variant and splenocyte (3) strain.

For  $^1\text{H}$  NMR analysis, glycopeptides of IgG isolated from ascites fluid were resolved as described above on a column (1 cm  $\times$  8 cm) of Con A-Sepharose.

(4) *500-MHz  $^1\text{H}$  NMR Spectroscopy.* Glycopeptides were repeatedly exchanged in  $^2\text{H}_2\text{O}$  with intermediate lyophilization and finally dissolved in  $^2\text{H}_2\text{O}$  which was 99.96 atom %  $^2\text{H}$  (Aldrich).  $^1\text{H}$  NMR spectra were recorded on a Bruker WM-500 spectrometer (SON hf facility, Department of Biophysical Chemistry, University of Nijmegen, The Netherlands) operating at 500 MHz and a probe temperature of 27  $^\circ\text{C}$ . Resolution enhancement of the spectra was achieved by Lorentzian-to-Gaussian transformation. Chemical shifts are given relative to sodium 4,4-dimethyl-4-silapentane-1-sulfonate but were actually measured indirectly to acetone in  $^2\text{H}_2\text{O}$  ( $\delta = 2.225$ ) (Vliegthart et al., 1983).

## RESULTS

### *Characterization of IgG Glycopeptides by Apparent Size.*

In an attempt to observe what influence differences in polypeptide structure and parental background might have upon glycosylation, the Pronase digests of IgG secreted by the panel of hybridomas were fractionated according to apparent size by alkaline borate GFC. Clonal analysis revealed that as few as three and as many as five size classes of glycopeptides could be distinguished (Figure 1). Although there is some apparent variability between clones in the relative distribution of these

various classes, the predominant size present was always class IV. The molecular weight of this glycopeptide size class is approximately 2000 (Rothman & Warren, 1988).

A second feature common among all the clones was the presence of class III, IV, and V glycopeptides. Of the 10 monoclonal IgGs surveyed, 2 (CSAT and L5.1) were found to have a pattern different by the presence of additional glycopeptides of size classes I and/or II. Although CSAT mAb differs, isotypically, L5.1 mAb is the same as the majority of the panel. Thus, a priori, the absolute number of size classes does not appear to correlate with the isotype.

The clonal differences in the elution profile of CSAT IgG glycopeptides characterized by class I and II glycopeptides have been studied more fully (Rothman & Warren, 1988). Glycopeptide classes I and II were found to differ from the other classes chiefly by sialylation. Their presence reflects the expression of an additional glycosylation site, presumably located within the Fab region, whereas, typically, IgG is glycosylated solely at a single conserved site, located within the Fc portion (Beale & Feinstein, 1976). Furthermore, extensive sialylation appears to be a feature more characteristic of the Fab (and the related Bence Jones proteins) than of the Fc (Taniguchi et al., 1985; Abel et al., 1968; Burton, 1985; Chandrasekaran et al., 1981). Typically, the site of all additional carbohydrate is located within the idiotypically defined variable region of IgG (Spiegelberg et al., 1970; Sox et al., 1970). However, this additional carbohydrate probably does not correlate with the idiotype or the related antigenic specificity. MAbs with either identical specificities (characterized by the H36 series of anti-hemagglutinin mAbs) or different specificities (characterized by the remainder of the panel) generally have similar GFC elution profiles of their glycopeptides, although they differ from that of CSAT mAb (Figure 1). Rather, the expression of an additional site of glycosylation would appear to have been acquired adventitiously in the process of generating antibody diversity.

*Characterization of IgG Glycopeptides by Charge.* Despite differences in structure and parental background, all hybridomas of the panel yielded IgG glycopeptides which were found to be predominantly neutral by chromatography upon DEAE-Sepharose (Figure 2). Subsequent analysis of the glycopeptides by alkaline borate GFC indicated that the neutral glycopeptides were predominantly of size classes IV and V, whereas classes I and II were composed entirely of acidic glycopeptides. A variable but significant fraction of class III glycopeptides also was acidic. This distribution of acidic glycopeptides by size is an agreement with the sensitivity of CSAT IgG glycopeptides to neuraminidase (Rothman & Warren, 1988).

*Characterization of IgG Glycopeptides by Affinity for Con A.* Among the clonally common glycopeptide size classes III, IV, and V, the predominant IgG glycosylation phenotype was characterized by glycopeptides of low affinity for Con A-Sepharose (Figure 3A-F). Class IV glycopeptides of low affinity for Con A-Sepharose accounted for 42–62% of the total radioactivity incorporated into IgG. One of the most prominent differences between clones occurred in the relative distribution of class IV glycopeptides of high affinity and those of low affinity for Con A-Sepharose. The ratio between these two phenotypes varied between 1:10 and 2:3. Together, these two phenotypes comprised 50–90% of the total radioactivity incorporated into IgG.

*Characterization of IgG Glycopeptides by Affinity for LcH.* When analyzed by LcH-agarose, glycopeptides of high affinity for the lectin were characteristic of the predominant phenotype

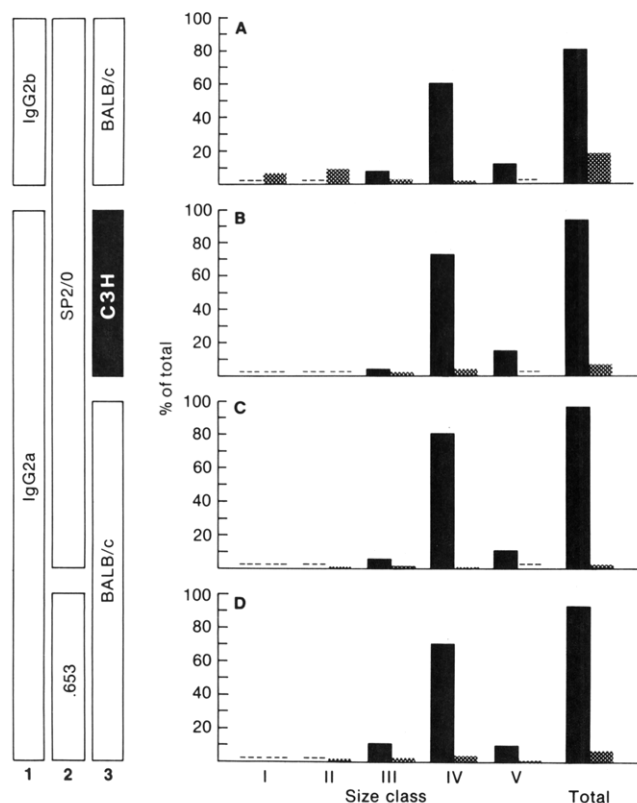


FIGURE 2: Clonal comparison of IgG glycopeptides by DEAE-Sephadex chromatography. The  $^3\text{H}$ -labeled IgG Pronase digests of CSAT (A), 34-5-8S (B), S-S.1 (C), and B137.17.1 (D) were fractionated batchwise on a column of DEAE-Sephadex into neutral (black bars) and acidic (stippled) glycopeptides. An aliquot of each charge-resolved glycopeptide pool was counted to determine the distribution of acidic and neutral glycopeptides within the total Pronase digest. The balance of each pool was analyzed by alkaline borate GFC to determine the size class distribution. This was weighted for the relative distribution of acidic and neutral glycopeptides within the total digest. The panel to the left is described in Figure 1.

(Figure 3G–L). By themselves, class IV glycopeptides of high affinity for LcH-agarose accounted for 50–75% of the total radioactivity incorporated into IgG.

Compared with classes IV and V, however, glycopeptides of size class III were found to be relatively enriched in glycopeptides of either low or no affinity for LcH-agarose. Furthermore, qualitative differences were apparent in the LcH elution profiles of glycopeptides of high affinity. Those derived from glycopeptides of size class III were eluted somewhat more quickly than the analogous ones derived from classes IV and V (data not shown). Similar subtle alterations in the Con A-Sepharose elution profiles of IgG glycopeptides have been reported to result from small differences in oligosaccharide structure (Narasimhan et al., 1979).

Another interesting common feature was that greater than 90% of the IgG glycopeptides of classes III, IV, and V, which are assumed to be derived from the Fc portion, had affinity for both LcH and Con A. Although both lectins share many carbohydrate specificities, core fucosylation is required for binding to LcH, but not to Con A (Kornfeld et al., 1981). Thus, the lectin binding data in the present study would suggest that virtually all murine monoclonal IgG expresses core fucosylation of the Fc with very little, if any, variability in its expression.

**Characterization by  $^1\text{H}$  NMR Spectroscopy of the Carbohydrate Structure Derived from the Predominant Phenotype.** To elucidate the carbohydrate structure expressed in the predominant phenotype, the CSAT IgG glycopeptide of size

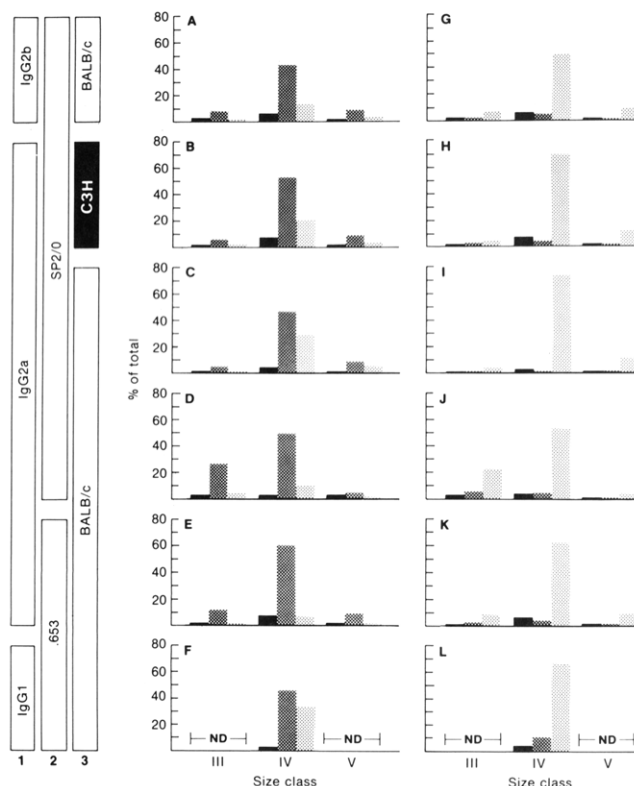


FIGURE 3: Clonal comparison of IgG glycopeptides by lectin affinity chromatography. The  $^3\text{H}$ -labeled IgG Pronase digests were fractionated by alkaline borate GFC. Next, each of the isolated and desalted glycopeptides was resolved either by Con A (A–F) or LcH (G–L) affinity chromatography into glycopeptides with either no (black bars), low (dark stipple), or high (light stipple) affinity for the particular lectin. The distributions by lectin affinity were then weighted for the relative distribution of the alkaline borate GFC size classes within the total digest (as determined from Figure 2). The lectin affinity distributions are shown for the IgG glycopeptides of CSAT (A and G), 34-5-8S (B and H), S-S.1 (C and I), H36.15.3 (D and J), B137.17.1 (E and K), and D4IE4 (F and L). ND, distribution by lectin affinity not determined. The panel to the left is described in Figure 2.

Table II:  $^1\text{H}$  Chemical Shifts of Structural Reporter Groups of the Monosaccharides Present in the Predominant Glycopeptide of CSAT IgG, Together with Those of a Reference Compound

reporter group	residue <sup>a</sup>	chemical shift (ppm)	
		CSAT	GP-II-3 (G Gn)
H-1	1	5.069	5.071
	2	4.681	4.682
	3	n.d.	4.766
	4	5.117	5.115
	4'	4.922	4.926
	5	4.554	4.553
	5'	4.580	4.578
H-2	6'	4.472	4.471
	3	4.250	4.253
NAc	4	4.186	4.188
	4'	4.108	4.114
	1	2.012	2.010
H-1	2	2.093	2.091
	5	2.053	2.051
	5'	2.046	2.045
CH <sub>3</sub>	Fuc	4.872	4.873
	Fuc	1.201	1.210

<sup>a</sup> Residue positions refer to Figure 4.

class IV and low affinity for Con A-Sepharose was analyzed by 500-MHz  $^1\text{H}$  NMR spectroscopy. Its deduced structure is depicted in Figure 4. Summarized in Table II are the relevant  $^1\text{H}$  NMR parameters, which are compared with those



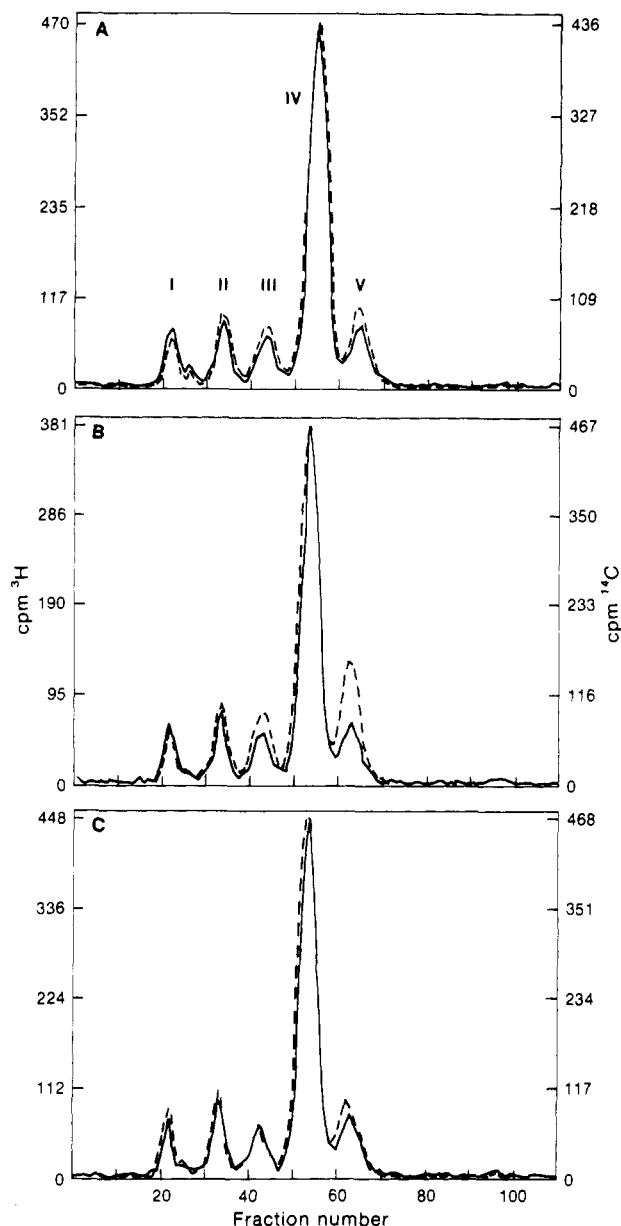


FIGURE 7: Effect of growth inhibitors upon IgG glycosylation. Parallel cultures of CSAT were metabolically labeled with [ $^3\text{H}$ ]glucosamine in the absence (A) or presence of either 0.1  $\mu\text{g/mL}$  vinblastine sulfate (B) or 10 mM thymidine (C) by the protocol described in under Materials and Methods. An additional culture of CSAT was metabolically labeled with [ $^{14}\text{C}$ ]glucosamine in the absence of inhibitors. The secreted IgG was affinity purified and digested with Pronase. Aliquots of the [ $^{14}\text{C}$ ]labeled digest (—), added as an internal standard, were cochromatographed by alkaline borate GFC with the [ $^3\text{H}$ ]labeled digest (---). Treatment with either inhibitor resulted in a 60% reduction in cell number.

chosen to synchronize the growth of the hybridomas at various stages of the cell cycle. Whereas thymidine (inhibition at  $G_1/S$  interphases) had no apparent influence upon glycosylation, vinblastine (inhibition in M phase) had a modest effect. This effect was particularly noticeable in the enhanced percentage of class V glycopeptides (Figure 7).

## DISCUSSION

Previously, we have shown that the CSAT hybridoma repertoire for glycosylation of IgG comprises a minimum of eight distinct phenotypes (Rothman & Warren, 1988). In the present study, six of these eight phenotypes were found to be commonly expressed by all ten clones analyzed and presumably reflect glycosylation of the same site located within the Fc

portion of IgG. These clonally common phenotypes were characterized by glycopeptides of either low affinity, high affinity, or no affinity for Con A-Sepharose and were distributed differently by size between classes III and IV (plus V). Class IV and V glycopeptides differ from each other only by the size of the peptide remaining after exhaustive Pronase digestion (Rothman & Warren, 1988). The inability of Pronase to completely remove all amino acid residues surrounding the glycosylation site is characteristic of most glycoproteins and appears to be dependent upon the nature of the oligosaccharide (Kobata, 1984).

Despite the phenotypic heterogeneity in glycosylation of IgG, there is considerable similarity between different clones. In particular, virtually all Fc-derived oligosaccharides are believed to express a core fucose residue, as, typically, greater than 90% of IgG glycopeptides have affinity for LCH-agarose. Moreover, the majority of the IgG glycosylation by all hybridomas is of only two phenotypes. Of these, the predominantly expressed phenotype is the same for all clones. Upon digestion of IgG by Pronase, this phenotype is characterized chromatographically by neutral glycopeptides of size class IV ( $M_r \sim 2000$ ) which are of high affinity for LCH-agarose but of low affinity for Con A-Sepharose. The second most prevalent phenotype is characterized by neutral glycopeptides also of size class IV, but which are of high affinity for Con A-Sepharose.

For CSAT IgG, the carbohydrate structure derived from the predominant phenotype has been identified by  $^1\text{H}$  NMR. Similar structures are also the predominant ones expressed on rabbit (Taniguchi et al., 1985) and bovine (Tai et al., 1975) serum IgG, as well as on human myeloma IgG (Kornfeld & Kornfeld, 1980; Kornfeld et al., 1971). Chromatographic similarity of glycopeptides by four criteria (apparent size, charge, and affinity for two lectins) suggests that a similar oligosaccharide also is the predominant one expressed on all IgG secreted by our panel of murine hybridomas. Due to its high affinity for Con A-Sepharose, the second most prevalent phenotype is consistent with the expression of an oligosaccharide similar to the predominant one, but lacking the single galactosyl residue, such that both branches terminate with *N*-acetylglucosamine (Narasimhan et al., 1979).

Very recently, Mizuochi et al. (1987) have elucidated the structure of the oligosaccharides expressed on IgG from pooled murine serum. The major structure is identical with the predominant one identified for CSAT IgG in the present study. Furthermore, they have reported that 94% of the oligosaccharides are core fucosylated and that 80% bear either a single galactose residue or none, which is consistent with our analysis by lectin affinity.

Interestingly, though, we find that a lesser percentage of sialylated structures are expressed on IgG secreted by murine hybridomas than Mizuochi et al. (1987) reported for murine serum IgG. Whereas a comparable percentage of murine, rabbit, and human serum IgG is sialylated, there is considerable variability in the (Fc) sialylation of human myeloma IgG (Mizuochi et al., 1987, 1982; Taniguchi et al., 1985). Thus, the reduction in sialylation we observe may be a feature of murine hybridomas, perhaps as a consequence of the somatic hybridization of two cell types.

*Variability in Expression of Glycosylation Phenotypes.* As anticipated, there is some variability in the relative expression of the various phenotypes. In part, some of this variability is clonal in origin. For example, significant variability between different hybridomas exists in the relative expression of the two major phenotypes characterized by glycopeptides which

bind to Con A-Sepharose but which differ in their affinity. In some instances, other differences between clones may reflect the expression of an additional site which is glycosylated differently. This is exemplified by class I and II IgG glycopeptides derived from CSAT, which are sialylated and lack affinity for Con A (Rothman & Warren, 1988). However, there is no phenotype which appears to be uniquely characteristic of either the hybridoma's parentage or the serologically defined polypeptide structure of the IgG which it secretes.

Instead, there appears to be a continuum of shared phenotypes. Although the predominantly expressed one is always the same, these phenotypes otherwise are variably expressed in an apparently random fashion by the different clones. To some extent, this may be a consequence of the variability in glycosylation evident between different generations of the same hybridoma upon continuous culturing. This generational variability does not influence the number of phenotypes expressed. Rather, it results in a random drift in the relative expression of these phenotypes, particularly the one characterized by class III glycopeptides.

Anderson et al. (1985) also have reported what appears to be a generational variability in glycosylation of mouse myeloma IgM. By contrast, Sweidler et al. (1985) have reported stability in glycosylation of major histocompatibility complex (MHC) antigens by lymphomas. Whether this discrepancy reflects intrinsic differences between cell surface and secreted glycoproteins is uncertain. Alternatively, generational variability may be an intrinsic feature of immunoglobulins.

Lastly, some variability in phenotypic expression of glycosylation may be a consequence of differences in culturing conditions of the hybridomas. However, typical culturing differences which would be anticipated, such as culture medium pH and proliferation status, have only a modest influence upon the IgG glycopeptide elution profile. In fact, a survey of environmental challenges, including a comparison of the effect of growth of hybridomas in vitro with that in vivo, revealed only occasional differences in the relative expression of the various phenotypes, whereas the nature of the predominant phenotype remained conserved (unpublished data).

The relative clonal similarity in IgG glycosylation poses an intriguing question. If a single, predominant phenotype is sufficiently important enough to have been conserved, why is there phenotypic heterogeneity? One possibility might be that glycosylation defines functional subsets of mAb. Thus, heterogeneity in effector function might be a consequence of structural heterogeneity in glycosylation. Studies to determine the effect of metabolically induced structural alterations in glycosylation upon IgG effector function should help to answer this question.

#### ACKNOWLEDGMENTS

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Registry No. IgG carbohydrate, 70308-40-6.

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## Effect of Homo Poly(L-amino acids) on Fibrin Assembly: Role of Charge and Molecular Weight

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**ABSTRACT:** Positively charged molecules such as protamine, leukocyte cationic protein, and the carboxyl terminus of platelet factor 4 have been shown to increase fibrin fiber thickness. Synthetic homo poly(L-amino acids) were used to explore the role of charge and molecular weight of cationic molecules on fibrin assembly. The effects of poly(L-lysine) (PLL), poly(L-glutamic acid) (PLG), poly(L-aspartic acid) (PLA), poly(L-histidine) (PLH), and poly(L-arginine) (PLArg) on the assembly and structure of fibrin gels were studied by using light-scattering techniques. At a PLG ( $M_r$  60 000) concentration of 80  $\mu\text{g/mL}$  and a PLA ( $M_r$  20 000) concentration of 64  $\mu\text{g/mL}$ , neither of these negatively charged polymers produced a detectable change in either fibrin assembly kinetics or final structure. Positively charged PLArg (16  $\mu\text{g/mL}$ ) caused a 30% increase in fibrin fiber mass/length ratio without calcium. In contrast, PLH (16  $\mu\text{g/mL}$ ), also positively charged, had no effect in the absence of  $\text{CaCl}_2$  but produced a 40% increase in fiber mass/length ratio with 5 mM  $\text{CaCl}_2$ . At concentrations as low as 1  $\mu\text{g/mL}$ , positively charged PLL increased the initial fibrin assembly kinetics and led to larger fiber mass/length ratio. The impact on fibrin mass/length ratio was equivalent for three different molecular weight preparations of PLL ( $M_r$  25 000, 90 000, and 240 000). The lack of a molecular weight effect on fiber thickness and the low polymer concentrations required to produce the perturbation argue against an excluded volume effect as the mechanism by which lateral fiber growth is augmented. Mechanisms by which poly(L-amino acids) may perturb fibrin assembly are discussed.

**F**ibrinogen normally circulates as a soluble asymmetric protein,  $M_r$  340 000, until the amino-terminal peptide, fibrinopeptide A, is enzymatically removed by thrombin. The subsequent fibrin polymerization kinetics and resulting fibrin structure are exquisitely sensitive to and modified by the microenvironment in which fibrin assembly occurs (Jones & Gabriel, 1988). Small shifts in pH, ionic strength, or calcium concentration during the assembly process result in dramatic changes in the material properties of fibrin (Ferry, 1947;

Shulman et al., 1953a,b; Shen et al., 1975; Latallo et al., 1962; Carr et al., 1986a,b). Agents with known positive charge, including protamine (Stewart et al., 1969), dextran (Carr & Gabriel, 1980), and hydroxyethyl starch (Carr, 1986), have been shown to modify the final clot structure. Recently, native plasma proteins such as immunoglobulins (Gabriel et al., 1983), histidine-rich protein (Leung, 1986), leukocyte cationic protein (Carr et al., 1986a,b), thrombospondin (Bale et al., 1986), platelet factor 4 (Carr et al., 1987), and actin (Janmey et al., 1985) have been reported to alter fibrin assembly kinetics and fibrin material properties. The roles of molecular charge and size of nonpolymerizing perturbing molecules in determining fibrin assembly kinetics and structure remain incompletely examined. This study reports the effects of charged synthetic homo poly(L-amino acids) of varying size and charge

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