cultured for 5 days. The results showed that the initial transcriptional ability is lost at this time in all the incomplete systems. This differs, as shown in Fig. 2, from the situation after only 2 days in the incomplete systems; under these conditions some of the initial transcriptional ability was still retained in the incomplete systems. After 5 days of culture the only nuclei capable of transcribing the 25K casein gene were those isolated from tissue that had been exposed to all three hormones (Table 2). These nuclei had a high level of this transcriptional activity; α-amanitin (5 μg/ml) eliminated the activity (not shown). Thus, transcription of the 25K casein mRNA in mammary cells requires both insulin and prolactin. In contrast, synthesis of total RNA (derived largely from epithelial nuclei) was hardly affected by the step-down conditions.

Earlier work (*11*) has shown that prolactin has a dual role in the enhancement of rat 25K casein mRNA accumulation. It stimulates its formation and markedly increases the half-life of the 25K transcript. Those experiments were conducted in the presence of insulin, but this study shows that prolactin's effect on the half-life of the mRNA does not require insulin. However, prolactin exerts no effect on the transcription of the 25K casein mRNA in the absence of insulin. Both insulin and prolactin are essential for formation of the transcript. In other words, stimulation of transcription of the 25K casein gene cannot be ascribed to a single hormone.

Insulin has been reported to favor the accumulation of mRNA's for albumin (*2*), amylase (*3*), tyrosine aminotransferase (*4*), pyruvate kinase (*5*), and δ-crystallin (*6*). It has also been reported to inhibit the transcription of the phosphoenolpyruvate carboxykinase gene (*12*). However, the effect of insulin reported here and its role in the insulin-estrogen-ovalbumin system (*13*) suggest that this hormone may be an essential component in a number of transcription systems.

PIOTR CHOMCZYNSKI
Laboratory of Biochemistry and Metabolism, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Bethesda, Maryland 20205

PRADMAN QASBA
Laboratory of Pathophysiology, National Cancer Institute, Bethesda, Maryland 20205

YALE J. TOPPER
Laboratory of Biochemistry and Metabolism, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases

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Carbohydrate Dramatically Influences Immune Reactivity of Antisera to Viral Glycoprotein Antigens

Abstract. *Analysis of the ability of heteroantisera, monoclonal antibodies, and antibodies to synthetic peptides to react with viral glycoproteins deglycosylated with endoglycosidase F revealed that the reactivities of most of the antibodies to these glycoprotein antigens were influenced by the attached carbohydrate moieties. All heteroantisera prepared in rabbits or goats to either fully glycosylated retroviruses or influenza virus were virtually unreactive toward the viral glycoproteins after carbohydrate removal. Analyses with a panel of monoclonal antibodies to purified Rauscher murine leukemia virus gp70 indicated that the reactivity of most of these antibodies improved while the reactivity of others decreased or remain unchanged after carbohydrate removal. Most of the antibodies to synthetic peptide sequences in the influenza virus hemagglutinin also improved in reactivity after carbohydrate removal. These data indicate that carbohydrate side chains on viral glycoproteins influence the immune response to these antigens, and the more native the glycoprotein immunogen, the more dramatic the carbohydrate influence. Thus the immune response to these glycoproteins is not simply a function of the immunogenicity of certain domains over others but rather is a direct measure of carbohydrate influences on the host's perception of the foreign antigen.*

The pathways and mechanisms by which carbohydrate is added to glycoproteins are now relatively well defined (*1*). However, little is known about the role carbohydrates play in the function of this class of proteins. A glycosylated molecule may have increased hydrophilicity in certain of its regions or be protected from proteolytic attack; glycosylation may somehow facilitate the secretion of certain proteins or their mobilization to the cell surface (*2*). One of the primary concerns of our studies was to determine what role or roles carbohydrate may play in the immune response to viruses, in particular to retroviruses and influenza viruses. There has been considerable speculation on this subject, but until recently, reagents were not available that could effectively remove carbohydrate without proteolysis or modification of the primary amino acid backbone. The discovery of an endoglycosidase [Endo F (*3*)] that efficiently cleaves both *N*-linked high mannose and complex glycans from glycoproteins allowed us to test directly certain aspects of the carbohydrate-protein interaction. We now report that the carbohydrate moieties of the viral glycoproteins have a dramatic effect on the antigenicity of these glycoproteins.

Untreated and deglycosylated Rauscher leukemia virus (R-MuLV) were studied by Western blot analysis of heteroantisera prepared to purified R-MuLV glycoprotein (gp70), to intact R-MuLV, and to R-MuLV disrupted with a mixture of Tween and ether (Fig. 1). All three antisera showed diminished reactivity to gp70 after treatment of the virus with Endo F. The reactivity of the antiserum to purified gp70 (Fig. 1A) was only 40 percent of that of control after carbohydrate was removed from the viral glycoprotein (calculated by slicing and counting the isotopically labeled bands from the nitrocellulose strips). The reactivities of the antisera to intact virion (Fig. 1B) and to Tween-ether–disrupted virion (Fig. 1C) with deglycosylated gp70 were 1 to 3 percent of that observed with

untreated virus. Immune reactivity of the antiserum to whole virion with the nonglycosylated core protein p30 (major band at 30,000 molecular weight in Fig. 1, B and C) remained unchanged after the protein was treated with Endo F, underscoring the glycoprotein specificity of the enzyme action. These results indicate that when the glycoprotein immunogen is presented in a more native conformation (that is, as whole virion), the influence of carbohydrate on the immune response is enhanced.

The results of immune precipitations with monoclonal antibodies to R-MuLV gp70 (*4*) against untreated and deglycosylated R-MuLV (Fig. 2) revealed that some antibodies precipitated control and Endo F–treated gp70 poorly (Fig. 2A); others precipitated gp70 only after carbohydrate was removed (Fig. 2B); another group only reacted with untreated R-MuLV gp70 (Fig. 2C). Most of these (35 of 52 precipitating antibodies) improved in reactivity to various degrees after deglycosylation of gp70 (Fig. 2, D and E). Still others reacted equally well with both treated and untreated gp70 (Fig. 2F).

It is clear that this panel of monoclonal antibodies did not accurately reflect the heteroantisera response because most of them improved in reactivity after removal of carbohydrate from the glycoprotein. However, most of this panel was influenced by carbohydrate moieties on R-MuLV gp70. A large part of this influence is not via actual contribution of carbohydrate side chains to the epitope recognized by the antibody because of the improvement in antibody reactivity after carbohydrate removal. A small proportion (8 of 52 precipitating antibodies) did not react with R-MuLV gp70 after carbohydrate side chains were removed and thus may recognize carbohydrate as all or part of an epitope. However, it is also possible that carbohydrate removal causes a shift in the conformation of the polypeptide backbone such that the epitope is no longer recognized.

To study further the effects of glycosylation on the immune response to glycoproteins, we examined a molecule for which the entire primary structure, including glycosylation sites, was known. We chose the X47 influenza virus (H3N2 subtype) hemagglutinin (HA) molecule (*5*) because of the availability of antisera to synthetic peptides [specific for peptides spanning more than 75 percent of the entire HA molecule (*6*)] and subjected it to Western blot analysis (Fig. 3). When we reacted a rabbit heteroantiserum prepared to X47 with glycosylated and deglycosylated virus (Fig. 3A) the

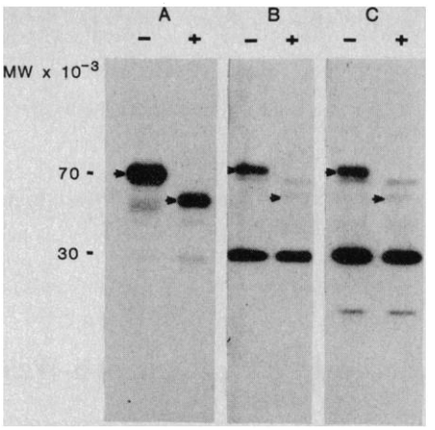

Fig. 1. Reactivity of heteroantisera to R-MuLV gp70 before and after deglycosylation of the glycoprotein with Endo F. Immune reactivity was assessed against R-MuLV either untreated or deglycosylated with Endo F as described (*3*) with subsequent separation on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (*9*) and transfer to nitrocellulose [Western blot analysis modified as described (*10*)]. (A) Reactivity of R-MuLV gp70 to a heteroantiserum prepared to purified goat R-MuLV gp70 as described (*11*) before (−) and after (+) treatment with Endo F. (B) Reactivity of R-MuLV proteins to a goat heteroantiserum to intact virus (*12*) before (−) and after (+) treatment with Endo F. (C) Reactivity of a goat heteroantiserum to Tween-ether–disrupted virus (*12*) before (−) and after (+) treatment with Endo F. Arrows denote bands corresponding to gp70 before and after Endo F treatment. Isotopically labeled bands were sliced and counted to measure changes in reactivity after deglycosylation. Reaction with a monoclonal antibody that recognizes both untreated and deglycosylated gp70 verified that the amounts of gp70 remained unchanged after deglycosylation (not shown).

results were similar to those obtained with heteroantisera to the retroviruses (Fig. 1B); that is, deglycosylation completely abolished the reactivity of the antiserum to X47. Of the 20 antibodies to synthetic peptides, five gave no reaction either with or without deglycosylation; these results are similar to those with normal rabbit serum (Fig. 3B). Of the ten antisera prepared to defined peptide regions of the influenza HA molecule which contain attached carbohydrate moieties (*7*), seven showed improved reactivity (Fig. 3C) with the deglycosylated molecule; one had unchanged reactivity and two were unreactive with ei-

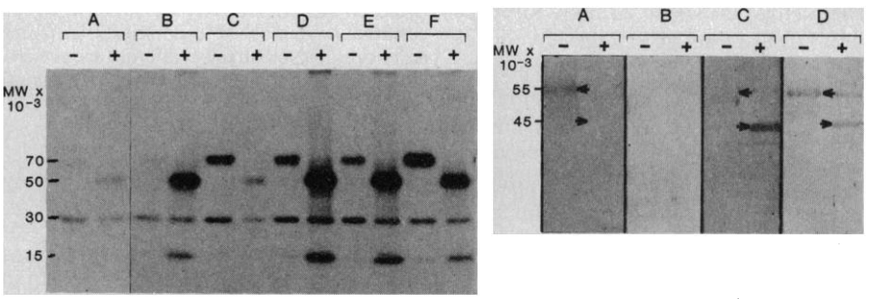

Fig. 2 (left). Effect of deglycosylation of R-MuLV gp70 on reactivity of monoclonal antibodies. Immunoprecipitations were performed with 78 monoclonal antibodies prepared to purified R-MuLV gp70 as described (*4*). R-MuLV disrupted with Nonidet P-40 and isotopically labeled with ^{125}I (Amersham) as described (*11*) was used as antigen. Samples were deglycosylated by treatment with Endo F. The precipitates were then separated by SDS-PAGE (*9*) and visualized by autoradiography. Of these antibodies, 26 gave only background bands in immune precipitation (A); 6 reacted only after carbohydrate removal (B); 8 reacted only with the untreated gp70 (C); 29 improved in reactivity to various degrees after deglycosylation (D and E); and 9 reacted equally well before and after deglycosylation (F). Fig. 3 (right). Reactivity of antibodies specific for a peptide region with the X47 influenza hemagglutinin before and after deglycosylation with Endo F. X47 influenza virus was grown in embryonated eggs. The virus was concentrated from allantoic fluid by centrifugation. Half of the sample was treated with Endo F (*3*), and the other half was used as a buffer control. Samples of the glycosylated and deglycosylated virus representing approximately 10^6 plaque-forming units of virus were run on adjacent lanes by SDS-PAGE (*9*) and subsequently blotted onto nitrocellulose (*10*). Strips containing a lane of each of the glycosylated and deglycosylated samples were incubated with the various rabbit antibodies to peptides analogous to regions of the X47 influenza HA molecule (*6*). After washing, the blots were incubated with peroxidase-coupled goat antibody to rabbit immunoglobulin G. The blots were developed with *O*-diamisidine. Reactivity before (−) and after (+) deglycosylation with Endo F is shown. (A) Reactivity of rabbit heteroantiserum to intact X47 influenza virus was decreased toward the deglycosylated HA molecule. (B) Antiserum 15 (specific for HA peptide sequence positions 140 to 156, which contains no site of glycosylation) reacted with neither glycosylated nor deglycosylated HA. (C) Antiserum 2 (specific for HA peptide sequence positions 1 to 36, which contains two sites of glycosylation at positions 8 and 23) improved in reactivity when the HA was deglycosylated. (D) Antiserum 17 (specific for HA peptide sequence positions 174 to 196 with no site of glycosylation) reacted equally well with either glycosylated or deglycosylated HA. The arrows denote bands corresponding to the influenza HA molecule before and after Endo F treatment.

ther the glycosylated or deglycosylated form of the HA molecule. Of the ten antibodies to influenza HA peptide sequences which do not contain sites of glycosylation, three improved in reactivity after deglycosylation of the HA molecule, four were unchanged in reactivity (Fig. 3D), and three showed no reactivity. Thus, most site-specific antibodies to a peptide sequence of the influenza HA molecule containing a site of glycosylation improved in reactivity against the HA molecule after removal of carbohydrate.

These results suggest that the carbohydrate moieties of glycoproteins play a role in the host's immune recognition and response of these molecules. The experiments with monoclonal antibodies to the R-MuLV gp70 molecule and the antibodies to specific peptide regions of the influenza HA molecule (containing sites of carbohydrate attachment) show that the carbohydrate portion of these glycoproteins can effectively block the interaction of antibodies with the underlying polypeptide regions (*8*). In the influenza system this was true even with regions of the HA molecule that did not have attached carbohydrate, presumably by some distal effect. In contrast, heteroantisera prepared to either R-MuLV or influenza virus lost virtually all reactivity toward the gp70 and HA molecules, respectively, after deglycosylation. This result implies that not only can the carbohydrate moieties block the interaction of antibodies to protein antigenic determinants but that the carbohydrate either becomes the major immunogenic target of the glycosylated protein or directs the immune response to areas under influences of carbohydrate attachment. Thus, the carbohydrate moieties perform a dual role by masking certain polypeptide sites and at the same time directing the immune response, acting as immune decoys.

STEPHEN ALEXANDER
JOHN H. ELDER
Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California 92037

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Pollen Feeding in an Orb-Weaving Spider

Abstract. *Juvenile orb-weaving spiders appear in spring, when insect prey are scarce but when aerial plankton, such as pollen and fungus spores, is abundant. Microscopic organic matter may be the main food of orb-weaving spiderlings, with insects providing only a dietary supplement. Pollen, which is caught on the sticky spirals of* Araneus diadematus *orb webs, doubles the life expectancy of spiderlings and alters their web-spinning behavior, so that they spin more frequently than do fasting controls. Fungus spores do not have the same nutritional value as pollen and may be deleterious to the spiderlings.*

Orb-weaving spiders take down and eat their old webs at fairly regular intervals—a well-documented behavior (*1*) that is usually explained as a mechanism for recovering some of the costs of producing silk (*2*). Speculations about other possible benefits of web eating were raised after we observed young orb-weavers (Araneidae) spin and dismantle several successive webs without apparently capturing any insect prey.

Because the energy costs of web build-

Table 1. Means and confidence intervals for four variables tested on second and third instars of *Araneus diadematus*. Abbreviations: FF, fungus-fed (*9*); PF, pollen-fed (*10*); S, starved controls; *N*, number of spiderlings; and *n*, number of observations. Means followed by the same letter are not statistically different from each other [Student-Newman-Keul's a posteriori test (*28*)]. Probabilities determined by analysis of variance (ANOVA) (*29*).

Instar	Treatment	Mean	Confidence interval (95 percent)	*N*	*n*	*P*
		*Life-span (12-hour intervals)**				
2†	FF	10.96a	8.10 to 14.82	22		0.005
	PF	20.17	13.86 to 29.34	22		
	S	9.90a	7.25 to 13.51	22		
3	FF	17.22b	13.07 to 21.38	9		0.05
	PF	27.11	18.88 to 35.34	9		
	S	17.33b	9.11 to 25.06	9		
		*Frequency of spinning (webs spun per total number of 12-hour intervals alive)**				
2†	FF	0.25c,d	0.19 to 0.30	21		0.018
	PF	0.30c	0.25 to 0.36	21		
	S	0.19d	0.13 to 0.25	21		
3	FF	0.15	0.10 to 0.19	9		0.001
	PF	0.26e	0.22 to 0.30	9		
	S	0.21e	0.17 to 0.25	9		
		Number of 12-hour intervals with each web remaining intact‡				
2	FF	2.94	2.63 to 3.25		66	0.55
	PF	3.10	2.89 to 3.30		204	
	S	3.22	2.58 to 3.85		46	
3	FF	3.57	2.46 to 4.67		23	0.31
	PF	3.13	2.72 to 3.55		67	
	S	3.32	2.86 to 3.78		28	
		Number of 12-hour intervals between webs‡				
2	FF	3.29	2.38 to 4.20		66	0.001
	PF	1.98	1.71 to 2.24		204	
	S	4.11	2.78 to 5.44		46	
3	FF	5.39	3.47 to 7.32		23	0.003
	PF	2.72	2.04 to 3.39		67	
	S	4.57	3.27 to 5.87		28	

*Probabilities based on one-way ANOVA. †These data did not meet the assumption of equal variance of ANOVA and were log- or arcsin-transformed. Means and confidence intervals represent back-transformed values. (*28*). ‡Probabilities based on Kruskal-Wallis one-way ANOVA (*29*).