NEURAMINIDASE SENSITIVE ANTIGENIC DETERMINANTS OF PLASMA CELL TUMOR MEMBRANE GLYCOPROTEINS

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1. Introduction

The role played by sialic acids in determining the antigenic specificities of the cell surface is still a matter of discussion. It is known that sialic acids are involved in determining the human blood group M and N specificities [1–3]: neuraminidase treated erythrocytes, other than very briefly, lose both M and N specificities, while a very mild desialylation converts M antigen into N antigen [4–7]. Recently the molecular structure of M and N oligosaccharides has been established, and it has been found that M differs from N substance only in an extra sialic acid residue that covers the terminal galactose of the N determinant [8].

Sialic acids are also involved in erythrocyte antigens such as the F antigen [9] and the Pr_1 and the Pr_2 system [10].

On the other hand, sialic acids seem to exert a peculiar negative role in masking surface antigens expressed on cell membrane other than erythrocytes. On tumor cell surface, tumor specific antigens seem to be masked by neuraminidase-sensitive sialic acid residues [11–13]. Removal of sialic acids increases immunogenicity also of normal cells [14–16]. Moreover, neuraminidase treatment has the same effect on orosomucoid, a soluble sialoglycoprotein antigen [17, 18].

In the present paper the role of sialic acids in the immunological properties of glycoproteins solubilized from the plasma membrane of a mouse plasmacell tumor was investigated.

2. Materials and methods

MOPC-460 plasmacytoma cells were grown in vivo, in syngeneic Balb/C mice, by serial subcutaneous transplantations. For immunofluorescence experiments, cells were cultured in suspension, in Eagle's MEM Spinner, supplemented with 10% horse serum.

Membrane fractions were prepared on discontinuous sucrose gradients, as previously described in detail [19]. Membranes were solubilized in 3 M potassium chloride [20], 10 mM phosphate buffer, pH 7.2, by vigorous stirring overnight at 4°C. The supernatant harvested after centrifugation at 105 000 *g* for 90 min, was dialyzed against phosphate buffered saline (PBS) and recentrifuged. This material was designated as solubilized membrane antigens (SMA).

Protein concentration of SMAs was determined by the Lowry's method; sialic acids were quantitated by the Warren's method using thiobarbituric acid assay [21].

Desialylation of SMAs was performed by Vibrio Cholerae neuraminidase (Koch Light Laboratories) repurified by affinity chromatography on insolubilized *p*-amino-phenyl-*N*-oxamic acid, following the method described by Cuatrecasas [22]. SMAs were incubated with 15 U of neuraminidase/mg SMA protein, in 0.1 M sodium acetate buffer, pH 5.2, 3 mM $CaCl_2$ for 3 hr

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at 37°C. Endogenous proteolytic activity was inhibited by addition of tosyl-phenylalanyl-chloromethyl-ketone (TPCK) and of tosyl-lysyl-chloromethyl-ketone (TLCK) to the final concentration of 30 μM and 200 μM respectively. Control samples were incubated under the same conditions, but neuraminidase was added after incubation. For immunofluorescence experiments, 10^8 tumor cells were washed and incubated at 37°C for 30 min in serum free medium with 100 U of neuraminidase, 3 mM $CaCl_2$, at pH 7.0. This treatment did not affect cell viability that was > 90%, as assessed by Trypan blue exclusion test. Rabbit-anti-SMA antisera were obtained by immunizing New Zealand rabbits with three monthly-spaced subcutaneous injections of 6 mg of SMA protein, emulsified 1:1 in Freund's complete adjuvant.

Precipitating reactions in tubes were performed by the micromethod described by Maurer [23]: all steps were performed at 4°C, in order to prevent the activity of neuraminidase and endogenous proteases.

Immunofluorescence experiments were performed as follows: 10^7 neuraminidase-treated or untreated MOPC-460 cells were incubated for 10 min at 37°C and for 15 min at 0°C with 0.2 ml of the appropriately diluted rabbit antiserum. After repeated washings with complete culture medium, cells were stained for 30 min at 0°C with the immunoglobulin fraction of a goat anti-rabbit L chain antiserum, FITC labelled [24]. Samples were examined with a Leitz Ortholux fluorescence microscope equipped with incident light and immersion objectives.

3. Results and discussion

Neuraminidase released from SMAs about 60% of the total protein-bound sialic acids.

Immune precipitation in tubes of desialylated SMA and of control SMA against rabbit anti native SMA antiserum gave rather regular curves (fig.1). The point of maximal precipitation obtained with control-SMA was reached when about 50 μg of protein were added to the antiserum. When the same amount of desialylated SMA was reacted with the antiserum, the amount of precipitate was reduced by about 10–15%. Moreover, the curve obtained with the desialylated SMA showed a broader plateau and the point of maximal precipitation was reached only at concentrations close

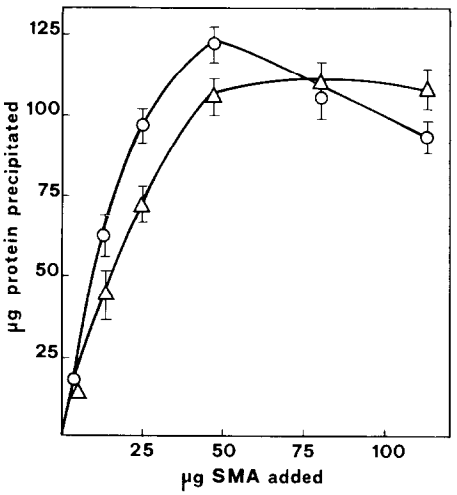

Fig.1. The effect of neuraminidase treatment on the precipitation of solubilized membrane antigens by specific rabbit antibodies. Increasing amounts of antigens were added to constant aliquots of antiserum. (△—△): desialylated membrane antigens; (○—○): control membrane antigens. Vertical bars represent the standard error.

to 80 μg protein. These points were constantly 10% lower than those obtained with the control-SMA.

These findings suggested that neuraminidase treatment of SMA led to the loss of some antigenic determinants expressed by membrane glycoproteins. A set of immunofluorescence experiments was performed to check whether this hypothesis was consistent also for the membrane glycoproteins exposed on the surface of living cells.

The anti-SMA antiserum was tested by indirect immunofluorescence with living MOPC-460 cells. At dilutions of 1:50 100% of the cells were positive. The fluorescence was intense and restricted to the cell surface, that was homogeneously stained. This antiserum, incubated three times, sequentially, with untreated cells (0.25 ml of packed cells/0.45 ml of diluted serum in ice) was completely adsorbed (table 1).

The antiserum was then adsorbed with desialylated cells. Neuraminidase removed 55% of the total sialic acids from living MOPC-460 cells. Since the enzyme does not enter living cells [25], and if the general assumption that the surface sialic acids account for about 70% of the total is valid also for plasmacells, this result means that in our conditions neuraminidase

Table 1
Adsorption of antiserum against solubilized membrane antigens by desialylated MOPC-460 plasmacytoma cells

Cells	Antiserum	Cells showing surface fluorescence**	
		% of the total	S.E.M.
Untreated	Unadsorbed	100	0
Untreated	Adsorbed with untreated cells	0	0
Untreated	Adsorbed with desialylated cells	50.9*	± 3.7
Desialylated	Adsorbed with desialylated cells	5.7*	± 0.9

* $p < 0.001$ (calculated by χ^2 test).
** Cells were incubated with the appropriately diluted (1:50) rabbit antiserum and stained with FITC-labelled goat anti-rabbit L chain antibodies.

removed about 80% of the sialic acids exposed on the cell surface.

At dilutions of 1:50 the antiserum adsorbed with desialylated cells still reacted with 50% of the untreated cells (table 1). In this case the fluorescence was definite but faint and localized in few spots, irregularly distributed over the cell surface. When tested against desialylated cells, the percentage of positive cells was drastically reduced to about 5% (table 1).

This result was consistent with the data obtained from immune precipitation experiments, suggesting the involvement of sialic acids in some antigenic determinants of membrane glycoproteins. Moreover it showed that these sialic acid-dependent antigenic determinants, or at least some of them, are exposed on the surface of intact cells in physiological conditions, when membrane glycoproteins are in their native configuration.

How sialic acid molecules contribute to antigenic determinants of glycoproteins cannot be predicted. Sialic acids may be directly part of the determinants and, thus, directly interact with the active site of the corresponding antibody molecules. Alternatively, sialic acid residues may be close to some determinants, but, being highly hydrophilic, are necessary to pull these determinants to the outer surface of the antigen molecule. Finally, sialic acid residues may only be involved in keeping sialoglycoprotein antigens in a given tertiary structure; desialylation of these antigens would lead to the loss of determinants of conformational nature.

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References

[1] Springer, G. F. and Ansell, N. J. (1958) Proc. Natl. Acad. Sci. USA. 44, 182.
[2] Makela, O. and Cantell, K. (1958) Vox Sang. 5, 492.
[3] Klenk, E. and Uhlenbruck, G. (1960) Z. Physiol. Chem. 319, 151.
[4] Yokoyama, M. and Trams, E. G. (1962) Nature, Lond. 194, 1048.
[5] Baranowski, T. and Lisowska, E. (1963) Arch. Immunol. Terapii Doswiadozalnej 11, 609.
[6] Uhlenbruck, G. (1969) Vox Sang. 16, 200.
[7] Huprikar, S. and Springer, G. F. (1970) in: Blood and Tissue Antigens, (E. Aminoff ed.), p. 170. Acad. Press, New York.
[8] Springer, G. F. and Parimal, R. D. (1974) Ann. Clin. Laboratory Science 4, 294.
[9] Hatheway, C. L., Weseli, D., Ludwick, T. and Hines, H. (1969) Vox Sang. 17, 204.
[10] Ebert, W., Metz, J. and Roelke, D. (1972) Eur. J. Biochem. 27, 470.
[11] Currie, G. A. and Bagshawe, K. D. (1968) Brit. J. Cancer 22, 588.
[12] Currie, G. A. and Bagshawe, K. D. (1968) Brit. J. Cancer 22, 843.
[13] Sanford, B. M. (1967) Transplantation 5, 1273.

[14] Lundgren, G. and Simmons, R. L. (1971) Clin. Exptl. Immunol. 9, 915.
[15] Simmons, R. L., Lipschultz, M. L., Rios, A. and Ray, P. K. (1971) Nature 231, 111.
[16] Simmons, R. L., Rios, A. and Ray, P. K. (1971) Nature 231, 179.
[17] Peters, J. H. (1963) Biochem. J. 2, 137.
[18] Winzler, R. J. (1965) in: The Amino Sugars, (A. Balazs and R. W. Jeanloz eds.) Vol. 2, part B, p. 309. Acad. Press, New York.
[19] Prat, M., Tarone, G. and Comoglio, P. M. (1975) Immunochemistry in press.
[20] Reisfeld, R. A., Pellegrino, M. A. and Kahan, D. B. (1971) Science 172, 1134.
[21] Warren, L. (1959) J. Biol. Chem. 234, 1971.
[22] Cuatrecasas, P. (1972) Methods in Enzymology 28, 897.
[23] Maurer, P. M. (1971) in: Methods in Immunology and Immunochemistry, (C. A. Williams and M. W. Chase eds.) Acad. Press, New York, vol. 3, p. 41. Academic Press, New York.
[24] Clark, M. and Sheppard, C. (1963) Virology 20, 642.
[25] Rosenberg, S. A. and Einstein, A. B. (1972) J. Cell. Biol. 53, 466.