CORRESPONDENCE

In order to facilitate an exchange of ideas and opinions a correspondence section is included in the Journal. It is hoped that this will create a forum for discussion of matters of general glycoconjugate interest. Comments, both positive and negative, regarding this Journal are also wellcomed, to enable us to cater for the requirements of our readers.

"...A great complexity of the M-antigens and the M antibodies [1]"

The above statement made over 45 years ago [1] is still valid today.

Sinaÿ, Verez Bencomo, and their colleagues took a novel approach: They synthesized glycopeptides (GPs) corresponding to the amino-terminal region of the human blood group M as well as N erythrocyte (RBC) glycoproteins, which carry Thomsen-Friedenreich (T) haptens, Galβ1-3GalNAc, α-*O*-linked to Ser/Thr as vicinal clusters [cf. 2]. They report that both the synthetic T-N and T-M GPs inhibited a single human anti-blood group N antibody [2]. This important avenue must be seen in context of the genetic, biochemical and immunochemical advances during the last 25 years; for summary see refs. in [2-5]. These include the establishment of the dimorphism of the first and fifth amino acids of M and N blood group glycoproteins, and the biosynthesis of T-, N- and M- specific immunodeterminants with human serum glycosyltransferases from homozygous M and N donors as determined with antisera licensed by the Div. of Blood Products Branch of Diagnostic Reagents, U.S. Food and Drug Administration [6]. Because of frequent overlapping and uncertainties of reactions with anti-M and -N sera, it is necessary to use a battery of well characterized sera, see already Friedenreich [1].

The Verez Bencomo-Sinaÿ report is in accord with earlier observations on the minimal requirements for blood group N specificity: "Fully desialized (RBC) M and N antigens also specifically inhibited four of seven human anti-N and one of three rabbit anti-N sera; agglutination of N RBC by numerous human anti-N sera was inhibited by oligosaccharides (OS) and GPs with only non-reducing terminal β-Gal" [cf. 7, 8]. N-activity of fully sialylated N glycoprotein was destroyed by β-galactosidases, with the concomitant release of ~ 3 mol of galactose per subunit of N glycoprotein (mol mass ~ 50 000); no other substance was released. Sialylated M glycoprotein was not inactivated by β-galactosidase and there was no significant galactose release [5]. Other evidence for the decisive role of terminal β-Gal groups in blood group N specificity includes: (a) All anti-*asialo*ganglioside sera (specific for terminal Galβ1-3GalNAc) tested reacted much more strongly with O,N than with O,M RBC, as shown by absorption, agglutination and inhibition; none of the other anti-ganglioside sera did so [9]. (b) Horse anti-pneumococcus Type XIV serum (anti-β-Gal), precipitated with gently isolated, highly active O,N but not O,M glycoprotein. Precipitation was specifically inhibitable by OS with non-reducing terminal β-Gal [5, 9]. (c) Upon immunizing with desialylated NN RBC glycoprotein exhibiting mainly non-reducing β-Gal termini, we obtained, in 1966, an 8 to 16-fold increase in anti-N but not anti-M agglutinin titers in 3 of 6 rabbits [3]. (d) Mild periodate oxidation destroyed >3 times as much galactose in O,N as in O,M glycoprotein [7].

Sinaÿ and Verez Bencomo also report that a single human anti-M serum was inhibited

by their synthetic T-M GP but not by the corresponding T-N GP, indicating that NeuAc may not always be a precondition for blood group M activity. My colleagues and I in 1972 defined two major categories of anti-M sera: Those that were well inhibited only by M antigens that had lost $\leqslant 15\%$ of their NeuAc, and those reasonably well inhibited by $>90\%$ desialylated M antigen [8]. Only ordinary ("normal") M and N antigens were studied. These findings are in partial agreement with those of Judd *et al.*, who used an equal vol of sialidase-treated RBC and completely absorbed anti-M and -N agglutinins of some sera [10]. We found that a serum /RBC ratio of 50/1 removed two thirds of the antibodies from comparable sera [6]. Some of the agglutination results by Judd *et al.* [10], however, cannot be explained this way.

The MN glycoprotein (glycophorin A) is widely believed to contain chains of Winzler's tetrasaccharide structure (W.T.) delineated in [2] as the only *O*-linked OS [cf. 2]. All differences in specificity between M and N would then be based on the dimorphism of the first and fifth amino acids. However, we and Ebert *et al.* [11] demonstrated the presence of OS chains larger than W.T., in sialylated and the corresponding desialylated GPs. We also isolated active M- and N-specific OS fractions [12].

In addition, our studies, using desialylated M and N penta- to hepta-GPs, indicate *O*-glycosylation of the M NH_2-terminus as determined by: (a) β-elimination after *prior* blocking of the free terminal NH_2 group [13] by either acetylation or carbamylation; (b) periodate oxidation, with benzyl-Ser and Ser-Ala as controls [14]; (c) comparison of the products of one-step isothiocyanate degradation with those of one-step subtractive phenylisothiocyanate degradation [15]; and (d) there was unexpected biological support for such substitution *via* Ashwell's hepatic asialoglycoprotein receptor [16], where efficiency of uptake depends on the density of suitable terminal, non-reducing carbohydrate clusters. Certain murine cancer cells which metastasize preferentially to the liver and adhere *in vitro* to hepatocytes possess T and Tn antigens on their cell membrane. These antigens are essential for the adhesion, because minute quantities of T- and Tn-specific GPs competitively inhibited cancer cell adhesion to hepatocytes. The blood group M-derived asialo-GPs were $\sim$ 10 times more active in inhibiting adhesion than the corresponding N-derived ones (coded samples were tested at the German Cancer Research Center) [16]. The additional *O*-glycosidically linked carbohydrate at the NH_2-terminal Ser of the M-derived GP must be reponsible for this large activity increase. Furthermore, we have identified immunogenic M- and N-specific glycolipids that do not stain with fluorescamine in significant quantity in the guinea pig L-10 carcinoma. They have the properties of gangliosides [17].

Because antibody-antigen interactions are limited to a distance that is considerably less than 1 Å [18] most anti-M and -N interact with the outermost structures, i.e., the antigen's peripheral carbohydrate chains. "That biological activities of the important cell-membrane glycoproteins (M,N glycoproteins) depend not only on their terminal carbohydrates but in part on the conformation of their peptide constituents" was made likely earlier [19].

Our interim conclusion would be that polyclonal human anti-M and -N sera define a heterogeneous group of related but not identical specificities, with distribution of specificities following a Gaussian curve. The areas farthest away from the apex of this bell-shaped curve represent mutations that have been largely defined and assigned subspecificities.

Establishing the precise structures responsible for M and N specificities by chemical synthesis will be difficult prior to full immunochemical and structural definition of all that is involved in these specificities. Added complications are the absence of precipitating anti-M and -N sera, and therefore precise quantitation, as well as the generally much lower inhibitory power (which may show up to 40-fold differences for various human antisera against the same intact antigen) of the isolated M and N glycoproteins and proportionally their haptenic structures, as compared to the ABH(O) blood group glycoproteins [cf. 3]. Synthesis of such complex structures may even come to grief when full analytic characterization is presumed to have been achieved. This was shown for neuraminic acid in classic studies by Saul Roseman and Donald S. Comb: Neuraminic acid had been synthesized correctly - in a way accidentally - using incorrect starting material on the basis of an incorrect structural analysis [20].

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