COMMON PRECURSORS OF HUMAN BLOOD GROUP MN SPECIFICITIES*

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Received September 26,1974

SUMMARY: Human blood group MM and NN specific structures have the same precursors. Complete sialic acid removal produced the Thomsen-Friedenreich T antigen which was transformed into Tn antigen by E. coli β-D-galactosidase on red cells as well as on isolated T antigen. MN antigens and their precursors are most clearly defined by isologous human antisera.

The MN antigens are the major antigens of the second human blood group system (1, 2). The first accurate information on the chemical basis of the MN specificities was the finding of their inactivation by neuraminidase (RDE) (3, 4). The antigens are glycoproteins. While there is only one report on the overall chemical composition of an isolated blood group N-active terminal fragment whose carbohydrate part consisted of α-NANA-Gal-(Gal)-GalNAc-R (5), it has recently been shown that the human blood group MM and NN antigens are closely similar and their carbohydrate part differs only by one NANA per immunodominant group, where in the MM antigen it covers that β-D-galactopyranosyl group which together with a non-reducing terminal α-NANA is responsible for N specificity (7-9). M specificity is readily transformed to N by partial desialization with mild acid (6, 8, 9). N appears to be the immediate precursor of M and the allele to the M gene is amorph (7-10). Thus the M and N antigenic specificities are not determined by two allelic genes as believed hitherto (11, 12).

MATERIALS AND METHODS. Isolation and purification of the MM and NN antigens from the ghosts of red cell pools have been described earlier; the preparations employed corresponded to those designated as 'purified' (5).

* This work was supported by National Institutes of Health Grants AI 05681 and CA 14183 and G.F.S. by the Wm. R. Parkes Fund for Cancer Research.

Abbreviations: NANA, N-acetylneuraminic acid; Gal, D-galactose; GalNAc, N-acetyl-D-galactosamine; Glc, D-glucose; GlcNAc, N-acetyl-D-glucosamine; Man, Mannose; OSM, Ovine submaxillary mucin; RDE, receptor destroying enzyme from Vibrio cholerae.

Anti-reagents, Erythrocytes and Suspending Solution. All anti-blood group reagents employed were saline-agglutinating and have been described before (7), except human anti-T and anti-Tn which were both obtained from blood group A_1B persons; the anti-Tn was used after exhaustive absorption with RDE-treated 0 erythrocytes. Phaseolus limensis (13), Dolichos biflorus (14), Arachis hypogaea (15), and Salvia sclarea (16) seed extracts were also used.

Human 0,MM and 0,NN erythrocytes were obtained, stored, washed, and used as described previously (17). The diluent and erythrocyte-suspending solution was aqueous 0.1 M NaCl containing 0.05 M phosphate buffer pH 7.3 (buffered saline). T-activation of 0 cells with RDE was as by Springer and Ansell (3). Tn red cells were donated by Dr. W. D. Bowman (Car. Lip.) and Dr. G. Leonard (Cla. Ric.). We accomplished for the first time artificial Tn activation by a degradative procedure on 0 red cells after their RDE treatment by two E. coli β-D-galactosidase treatments under the conditions described earlier (7), except that 1000 U enzyme in 1.25 ml appropriate buffer made isotonic with NaCl were added to 0.25 ml packed red cells and the total incubation time was 18 hr.

Enzymes. These have been described (5, 7). In addition, washed packed 0,MM and 0,NN red cells were treated with TPCK trypsin (18).

Hemagglutination and Hemagglutination Inhibition Tests. These including positive and negative controls and standards were performed and interpreted as described earlier (5, 7, 17). Red cell agglutination was read microscopically by 3 individuals about 90 min after addition of 0.5 % red cell suspension. Inhibitory activities are given as mg or μmoles/ml before addition of red cells and serum.

Preparation of T Antigen. T antigen was uncovered on 0,MM and 0,NN red cells or prepared from isolated 0,MM and 0,NN antigens by either RDE or acid hydrolysis as described earlier (3, 5, 9).

RESULTS. MN Specificities on Trypsinized Red Cells. Trypsin-treated red cells had lost virtually all their M and N activities with human antisera but not with rabbit anti-M and anti-N sera. The rabbit sera showed a decrease in

specificity, anti-M gave nearly the same titer with trypsinized NN red cells as with homologous cells. Analogous results were obtained with rabbit anti-N sera. Rabbit anti-M and anti-N were extensively absorbed by homologous trypsinized red cells. In addition, rabbit anti-N was removed by trypsinized MM cells while the reverse was not the case. The Vicia reactivity of the trypsinized MM as well as NN cells showed some increase over that of untreated red cells. RDE treatment of the trypsinized cells resulted in their T antigen activation.

T and Tn Specificities. No T activity was demonstrable on the "intact" isolated MM and NN glycoproteins with either human or Arachis anti-T. No enzyme other than RDE uncovered the T antigen. Significant T activation by acid hydrolysis of MM antigen became evident only subsequent to maximal transformation from M to N specificity. Maximal activation of T was obtainable by both weak acid and RDE and to the same extent with both antigens; ca 10 μg antigen completely inhibited human anti-T. Human anti-T was completely inhibited by 15 mg asialoganglioside which has a terminal β-Gal-(1→3)-GalNAc and by 50 μmoles β-Gal-(1→3) or (1→4)-GlcNAc; lactose partially inhibited while melibiose, methyl α- and β-Gal, Gal, Man, GalNAc, GlcNAc and Glc were inactive. These findings indicate that β-Gal is the most important sugar for the human anti-T combining site, but that β-Gal does not possess the entire complementarity for human anti-T as it does with Arachis anti-T as shown by Uhlenbruck et al. (15).

Isolated, fully activated T antigen possessed no Tn activity in inhibition assays with Tn and A_1 cells and properly absorbed human and Dolichos anti-Tn. Beta-D-galactosidase treatment of isolated, fully activated T antigen led to Tn activation as measured with human and Dolichos biflorus anti-Tn but not with the Phaseolus extract. This agrees with earlier findings for the naturally occurring Tn (19). Ordinary O,NN antigen also showed slight Tn activation after β-D-galactosidase treatment.

O red cells, T-transformed and subsequently treated extensively with β-D-galactosidase showed specific Tn agglutination as measured with human anti-Tn, with Salvia sclarea and Dolichos biflorus extracts and also with eluted human anti-A_1.

Salvia sclarea extracts gave a zone phenomenon. Human anti-Tn agglutination was best demonstrable at 12° and with Salvia extract after ca 15 hr incubation.

In accordance with the literature (19, 20), GalNAc was a strong inhibitor of anti-Tn, but we found that on a molar basis OSM had > 4,000 times and Tay-Sachs ganglioside several times the inhibitory activity of GalNAc when measured with human anti-Tn provided they had been desialized. Hog blood group A mucin had some inhibitory activity while human blood group A substances were inactive as was GlcNAc. Gal had slight activity; methyl β-Gal was more active and about twice as much as the α-anomer. Similarly, asialoganglioside showed some activity. Hemagglutination inhibition of Tn cells by Salvia sclarea showed a different inhibition pattern. Only GalNAc was of similar activity with both anti-Tn reagents. With Salvia extract desialized OSM had < 10 % of the activity it exhibited with human anti-Tn and Tay-Sachs ganglioside was inactive whether it had been desialized or not; inactive also were Gal and Gal glycosides. Human blood group A glycoproteins had some activity, but not that from hog.

DISCUSSION. Our results indicate that the human blood group MM and NN antigens are closely similar and that in their carbohydrate structure they differ only by 1 NANA per immunodominant group (Figure 1). The transformation of MM antigen into one which reacted like NN antigen was accompanied chemically solely by partial NANA loss and the uncovering of subterminal β-D-Gal. NN antigen in turn was inactivated specifically by β-D-galactosidase as well as by removal

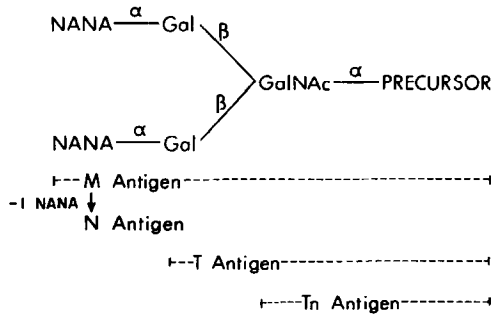

Figure 1: Proposed structural basis of blood group MN and precursor specificities.

of NANA. The NANA required for N specificity is linked to Gal, as shown by transformation to T antigen on NANA removal. We have now demonstrated that the T antigen of Thomsen and Friedenreich (3, 21, 22) is transformed by β-D-galactosidase into one possessing Tn activity.

Figure 1 shows that N has a position in the MN system closely akin to that of H(O) in the ABH(O) system (23). While the scheme is compatible with all our observations, it may be an oversimplification because we found recently (9) that close to 10 % of the total GalNAc of MN specificity carrying glycoproteins (5) was subterminal to NANA. Our findings do not firmly exclude that additional sialic acid may be linked directly to the NANA's depicted in Figure 1, but studies to date do not favor this possibility (24-26). Proof that the two β-Gal are linked to the same sugar is missing, their linkage to adjacent monosaccharides cannot be excluded.

The scheme in Figure 1 covers only the last 4 steps of a longer pathway. The extremely high anti-Tn inhibitory activity of desialized ovine submaxillary mucin which is known to carry solely GalNAc linked α to Ser and Thr (27) permits the speculation that the GalNAc which confers the Tn specificity may also be linked α to Ser and/or Thr. At each step of the scheme a genetic block may occur. The scheme accommodates the deductions of Sturgeon et al. (28, 29) concerning the Tn antigen and of Metaxas et al. on the antigen M^k (30).

Figure 1 is based on findings with human antisera, for even though investigations with animal and plant anti-reagents are most informative, our studies with trypsin have shown that rabbit anti-M and -N sera react with structures substantially different from the M and N determinants detected by human reagents and that Vicia as well as T-active structures may occur without being part of M and N specificities detected by isologous human antisera (5, 21).

The importance of the MN antigens and their precursors extends to the cancer problem, for we have recently found that structures possessing blood group MN specificities are present on the surface of human breast glands and that some precursor substances occur only in malignant human breast tissue (31).

Acknowledgments. We thank Mrs. H. Tegtmeyer, M.T., and Mr. I. Banatwala, M.S., for excellent technical help, and Dr. H. J. Yang for trypsin digests. We are extremely grateful to numerous colleagues who donated rare anti-reagents, red cells, and carbohydrates.

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