SEROLOGICAL AND BIOCHEMICAL INVESTIGATIONS ON THE N.E. VARIETY OF THE DANTU RED CELL PHENOTYPE

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Studies during the last decade have established that the MNSs blood group locus corresponds to two, presumably adjacent, genes which encode the amino-acid sequences of two (MN and Ss) sialoglycoproteins (SGPs) or glycophorins (A and B) in human red cell (RBC) membranes (reviews: 1-4). The structural difference between the M and N antigens is determined by amino-acid polymorphisms at the 1st (Ser/Leu) and 5th (Gly/Glu) positions of the MN SGP, the complete sequence of which was elucidated (5-8). The sequence of the N-terminal 26 residues (res.) of the Ss SGP is identical with that of the N-specific MN SGP (8,9). This explains the occurrence of an additional N antigen, denoted as 'N', on the Ss SGP. A Met/Thr polymorphism at the 29th position of the Ss SGP represents the structural difference between the S and s antigens (8). Recently (10), the sequence of the intramembraneous domain (res. 36-72) of the Ss SGP was determined and found to be rather similar to the corresponding region (res. 65-101) of the MN SGP (Fig. 1).

At a previous congress of this society (11) we described an individual (N.E.) exhibiting a 'new' phenotype within the MNSs system. As described below, a number of pitfalls have provoked the initial (11) wrong conclusion that N.E. possesses two 'new' alleles at the MNSs locus. Subsequent studies by Contreras et al. (12) have extended our serological observations. RBC from N.E. and other Black individuals were shown to carry the low incidence antigen Dantu which is encoded by the MNSs locus. The Dantu allele gives rise to the following serological features: the Dantu antigen, a protease-resistant N antigen, a weak s antigen, a M receptor and a weak or absent U antigen. Ph RBC, a hybrid SGP variant described by Tanner et al. (13), were also shown to exhibit the Dantu antigen (12). However, the Ph and N.E. RBC types are clearly different, since they contain different amounts of MN and hybrid SGPs (ratios about 1:1.25 and 1:2.5, respectively) (11,13).

We have studied RBC samples from 10 relatives of N.E. (6 Dantu+) and 18 additional unrelated Black Dantu+ individuals by serological methods. Mr. Dantu, Vi., Sh., Ox. II-1 and II-3 are mentioned in ref. (12). The other donors were detected due to the formation of anti-Dantu (14) or anti-U during pregnancy, discrepancies during SsU typings or by screening for the Dantu phenotype. Most of these samples (n = 19) were also studied by sodium-dodecylsulfate polyacrylamide gel electrophoretic (SDS-PAGE) techniques.

Our previous studies (11) had shown that N.E. RBC membranes exhibit a decreased MN SGP content (approx. 50% of normal) and a 'new' SGP, present in about 2.5-fold higher molar quantity than the MN SGP, which was assumed to represent a hybrid of the anti-Lepore type possessing the N-terminal portion of the Ss SGP (res. 1- about 30) and the C-terminal domain of the MN SGP (res. about 60-131). Similar SDS-PAGE data were obtained for the additional Dantu+ samples, indicating that all belong to the N.E. variety, rather than the Ph type (13), of the Dantu phenotype. Further indirect evidence for the assumption that the 'new' SGP exhibits the cytoplasmic domain of the MN SGP was obtained by incu-

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bation of $NaJO_4/NaBH_4^3$ labelled Ox. II-3 membranes with anti-CB2 serum (5) and subsequent SDS-PAGE analysis of the antigen-antibody complexes that bound to protein A-Sepharose, after solubilization with Triton.

The data of Contreras et al. (12) had provided evidence that the Dantu gene complex expresses N (protease-resistant and located on the hybrid SGP) and M activity, although the MN SGP content of membranes from Dantu heterozygotes is only approx. 50% of normal. Further evidence for the assumption that the Dantu allele encodes an MN SGP possessing M activity was obtained from the following results: 1) All Dantu+ RBC samples that we have studied (n = 24) were found to exhibit the phenotype M+N+. 2) Studies on one pedigree (H.P.; 14) in which a mother had made anti-Dantu during pregnancy yielded the phenotypes M-N+S-s+Dantu- (mother) and M+N+S-s+Dantu+ (father and child), respectively. 3) Separation of membranes by SDS-PAGE and subsequent incubation with radio-iodinated anti-N lectin from Vicia graminea, in order to visualize the SGPs carrying N activity (15), revealed that 3 (H.P., Sh., Vi.) of 12 Dantu+ RBC exhibit N activity on the MN SGP. Typical patterns obtained by this method are shown in ref. (15) and Fig. 2. The presence of M activity on the MN SGP in all Dantu+ samples is suggested by the destruction of this receptor by trypsin treatment of RBCas well as amino-acid composition and sequence analysis of a peptide (C1, res. 1-64), isolated from the MN SGP of N.E. In order to account for the data described above, it has to be assumed that the hybrid SGP, produced in large amounts, suppresses the incorporation of the MN SGP, encoded by the Dantu allele (M active) and its normal counterpart, into the RBC membranes of Dantu heterozygotes.

In our previous report (11) we had described that the major membrane protein (coomassie band 3) in N.E. ghosts exhibits a decreased molecular weight. This phenomenon was attributed to a shortening of the carbohydrate chains on band 3, due to sterical hindrance of glycosyl-transferase(s) resulting from the formation of complexes with the MN SGP and the hybrid molecule. Further evidence for this hypothesis was obtained by galactose oxidase/$NaBH_4^3$ labelling of N.E. ghosts which revealed a strong (about 80%) decrease of label in the gel region corresponding to band 3. This alteration of band 3 could be detected for all (n = 18) Dantu+ samples that were investigated by SDS-PAGE and coomassie staining.

In our initial SDS-PAGE experiments (11), weak bands (10-20% of normal) at the positions of the mono- and dimer of the Ss SGP were detected for N.E. and her baby (D.E.) that had suffered from hemolytic disease of the newborn, due to anti-U and anti-D. In subsequent experiments we found that this minor component in N.E. and other Dantu+ membranes exhibits a slightly higher mobility than the monomer of the Ss SGP. Due to some variability of the SDS-PAGE separation, it is not resolved from the Ss SGP in some electrophoretic experiments. The significance of this minor componentwhich was detected for all Dantu+ samples is not yet clear - it might represent a degradation product of the hybrid SGP. It is also detectable in Dantu+ membranes of the Ph variety (13). When the SDS-PAGE analyses on D.E. were repeated about one year after delivery, the Ss SGP content of membranes from D.E. and an additional child (K.E.) of N.E. was found to be approx. 50% of normal, suggesting that both are heterozygous for the allele u. It has to be concluded that coating of the cord RBC sample from D.E. with anti-U or -D had caused a selective degradation of the Ss SGP. The data described above suggest that N.E. is heterozygous for the genes Dantu and u. As judged from our results, 4 other individuals (Sh., Vi., Bl., H.B.) also exhibit the genotype Dantu/u.

Contreras et al. (12) have suggested that N.E., Sh. and Vi. RBC exhibit weak U antigens. According to our previous (11) and further data, this receptor is absent from RBC of all individuals assumed to possess the genotype Dantu/u. SDS-PAGE analyses of membranes from all Dantu+U+ individuals revealed an Ss SGP content of about 50%, consistent with the genotype Dantu/U.

In our initial studies (11) N.E. was found to react weakly with all anti-s sera and with most of our anti-S reagents. Subsequent studies by Contreras et al. (12) revealed that the agglutination of Dantu/u and Dantu/sU RBC by anti-S sera is attributable to a separable anti-Dantu which occurs frequently in sera containing antibodies against low incidence antigens. Our further data confirm this conclusion. Conversely, the weak reactions of anti-s sera with Dantu/u and Dantu/SU RBC can be attributed to a weak s antigen produced by the Dantu allele. We have detected one potent anti-s that fails to react with such RBC, suggesting that this s receptor is altered in a qualitative manner. Just like the N antigen (11,12), the weak s and Dantu receptors are not destroyed by protease treatment of intact Dantu+ RBC. This suggests that they are located on the hybrid SGP which is not degraded by incubation of RBC with various proteinases (trypsin, chymotrypsin, ficin, papain, pronase, V8 proteinase, thermolysin), as revealed by SDS-PAGE. Anti-Dantu could be inhibited by Triton X-100 (0.5%) extracts from Dantu+ membranes which contain the hybrid SGP, but not by SGPs prepared by the phenol and butanol methods. This suggests that the Dantu antigen represents a labile receptor which is located in proximity to the lipid bi-layer of the membrane.

Since the hybrid SGP, in contrast to the MN and Ss SGPs, is not degraded by proteinase treatment of intact RBC, we have used trypsin (and chymotrypsin) treatment of cells, followed by membrane isolation, phenol extraction and gel filtration in the presence of Ammonyx-LO, in order to isolate the hybrid SGP from N.E. and a pool of RBC from Dantu+ individuals who are not related to N.E. The complete structure of the hybrid SGP from N.E. was deduced from manual DABITC/PITC and carboxypeptidase Y sequencing of the intact molecule and various peptides prepared by trypsin, chymotrypsin, V8 proteinase and cyanogen bromide treatment as well as a comparison of the amino-acid composition of peptide C2 (res. 67-86) with the res. 115-118 of the MN SGP (5). We have only sequenced the N-terminal 16 res. of fragment C2 (res. 67-86) and not established an overlap between peptide C2 and the adjacent fragment C3 (res. 87-99). 10 Edman-degradation cycles on the intramembraneous chymotryptic peptide (res. 35/37-66) of the hybrid SGP isolated from the pool of Dantu+ RBC served to establish that the relevant part of the sequence is identical with that of the hybrid molecule from N.E. (Fig. 1).

As shown in Fig. 1, the Dantu-specific hybrid molecule exhibits the res. 1-38 (or 39) of a blood group s-specific Ss SGP and the res. 71 (or 72)-131 of a MN SGP. As judged from its lability, the Dantu antigen appears to be located within the res. approx. 30-40 of the molecule. The proposed structure also explains the absence of the U antigen from u/Dantu RBC, since this receptor is located C-terminal of position 32 or 34 of the Ss SGP (3).

The above-described phenomenon that the Dantu allele expresses M and N antigens of roughly normal strength is of forensic significance. Similarly, the presence of a weak s antigen in Dantu+ RBC and the frequent occurrence of anti-Dantu in anti-S sera represent sources of error in forensic blood typing of Blacks. As judged from the data of Contreras et

al. (12), the Dantu phenotype appears to the rather rare in Caucasians. However, recently (16) we have detected a Dantu+ RBC sample from a Caucasian. The ratio of the MN and hybrid SGPs in the membranes from that donor is similar to that in Ph ghosts (13). However, the Dantu gene complex of this individual appears to produce an Ss SGP, as judged from the amount of this molecule in the membranes, in contrast to the Dantu alleles of the N.E. and Ph varieties. We had initially (11) assumed that the Dantu allele in N.E. had been generated by an unequal crossing over between a u and an En allele. On the basis of the data described above it is likely that the Dantu alleles in N.E. and Ph were generated by a misalignment between a u and a normal gene complex. The reason for the different amounts of MN and hybrid SGPs in N.E. and Ph RBC remains to be elucidated.

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Fig. 1: Amino-acid sequences (one-letter-code) and glycosylation sites (+) of MN SGP, Ss SGP (partial) and hybrid SGP from Dantu+ RBC.

MN SGP:

1 S/L-S-T-T-G/E-V-A-M-H-T-S-T-S-S-S-V-T-K-S-Y-I-S-S-Q-T-N-D-T-H 29

30 K-R-D-T-Y-A-A-T-P-R-A-H-E-V-S-E-I-S-V-R-T-V-Y-P-P-E-E-E-T-G-E-R-V-Q 63

64 L-A-H-H-F-S-E-P-E-I-T-L-I-I-F-G-V-M-A-G-V-I-G-T-I-L-L-I-S-Y-G-I-R 96

97 R-L-I-K-K-S-P-S-D-V-K-P-L-P-S-P-D-T-D-V-P-L-S-S-V-E-I-E-N-P-E-T-S 129

130 D-Q 131

Ss SGP:

1 L-S-T-T-E-V-A-M-H-T-S-T-S-S-S-V-T-K-S-Y-I-S-S-Q-T-N-G-E-M/T 29

30 G-Q-L-V-H-R-F-T-V-P-A-P-V-V-I-I-L-I-I-L-F-V-M-A-G-I-I-G-T-I-L-L-I-S 63

64 Y-X-I-R-R-L-I-K-K 72

Hybrid SGP:

1 L-S-T-T-E-V-A-M-H-T-S-T-S-S-S-V-T-K-S-Y-I-S-S-Q-T-N-G-E-T 29

30 G-Q-L-V-H-R-F-T-V-P-E-I-T-L-I-I-F-G-V-M-A-G-V-I-G-T-I-L-L-I-S-Y-G-I 63

64 R-R-L-I-K-K-S-P-S-D-V-K-P-L-P-S-P-D-T-D-V-P-L-S-S-V-E-I-E-N-P-E-T-S 97

98 D-Q 99

Fig. 2: Densitometric scans of autoradiographs obtained after SDS-PAGE of membranes and incubation of the gels with radio-iodinated Vicia graminea lectin. A.) M-N+ control, B.) M+N+S-s+U+Dantu+ sample exhibiting N activity on the MN, Ss and hybrid (H) SGPs. MN, Ss and H = apparent monomer of the respective SGP; MNx2 = dimer of MN SGP; MN+H = heterodimer involving the monomers of the MN and hybrid SGPs etc.