Blut (1987) 55: 33–43

Blut

The Dantu Erythrocyte Phenotype of the NE Variety

II. Serology, Immunochemistry, Genetics, and Frequency*

P. Unger[1], J. L. Procter[1], J. J. Moulds[2], M. Moulds[2], D. Blanchard[3], M. L. Guizzo[4], L. A. McCall[5], J. P. Cartron[3], and W. Dahr[6]**

[1] Blood Center of the Michael Reese Research Foundation, 530 East 31st Street, Chicago, IL 60616, USA
[2] Gamma Biologicals Incorporated, 3700 Mangum Road, Houston, TX 77092, USA
[3] Centre National de Transfusion Sanguine Institut et INSERM U76, 6 rue Alexandre Cabanel, F-75739 Paris, Cedex 15, France
[4] Community Blood Center, 349 South Main Street, Dayton, OH 45402, USA
[5] Tidewater Regional Center of the American Red Cross, 611 West Brambleton Avenue, Norfolk, VA 23501, USA
[6] Abteilung für Transfusionswesen, Universitätskliniken Köln, Stelzmann Str. 9, D-5000 Köln 41, Federal Republic of Germany

Summary. Red cells (RBC) possessing the low-frequency MNSs antigen Dantu from 36 Black individuals (21 propositi) were found to exhibit the NE variety of this phenotype, as judged from the electrophoretic glycophorin (GP) pattern, described in an accompanying article, and/or from the polybrene test which detects the decreased NeuAc level of these RBC. All known Dantu^{NE} RBC (53) exhibit the phenotype M+N+. This finding as well as family studies and immunochemical investigations demonstrate that the *Dantu^{NE}* allele encodes a blood group M-specific GP A. Thus, the strongly decreased GP A level of RBC from *Dantu^{NE}* heterozygotes represents the product of the *Dantu* allele and its normal counterpart. It is suggested that the formation of a complex with the anion channel protein (band 3) represents the prerequisite for optimum incorporation of GP A into normal RBC membranes. The hybrid GP in Dantu^{NE} RBC, produced in large quantity, might suppress the incorporation of GP A in a *cis* and *trans* manner via the formation of a complex with band 3. The hybrid GP in Dantu^{NE} RBC lacks U activity, but expresses N activity and a qualitatively altered s antigen, thus proving its GP B-GP A hybrid nature in conjunction with data described in the accompanying article. Screening of ficin-treated RBC with *Vicia* lectin revealed that the Dantu phenotype exhibits a frequency of about 0.005 in American Blacks and less than 0.001 in Germans.

* Preliminary results of this work were presented at the 9th and the 11th International Congress of the Society for Forensic Haemogenetics [14, 40], the 26th Annual Meeting of the South Central Association of Blood Banks [25] and the 19th Congress of the International Society of Blood Transfusion [32]

** *Present address:* Institut für Immunologie und Serologie der Universität Heidelberg, Im Neuenheimer Feld 305, D-6900 Heidelberg 1, Federal Republic of Germany

Offprint requests to: W. Dahr, Institut für Immunologie und Serologie der Universität Heidelberg, Im Neuenheimer Feld 305, D-6900 Heidelberg 1, Federal Republic of Germany

Key words: Erythrocyte membrane – Glycoprotein – MNSs blood groups – Dantu antigen – Glycophorin

Introduction

The '*MNSs* blood group locus' encodes the amino acid sequences of two NeuAc-rich, homologous glycoproteins in human erythrocyte (RBC) membranes that are denoted as glycophorin A (GP A; MN sialoglycoprotein, MN SGP or α) or GP B (Ss SGP or δ) (for reviews see [6, 7, 23, 26, 31, 36]). As described in an accompanying article [17], Dantu RBC of the NE variety that were first described by Unger *et al.* [40] are characterized by a decreased quantity of GP A (approx. 43%), a decreased level (about 50%) or the complete absence of GP B, in *Dantu/U* or *Dantu/u* heterozygotes, respectively, and a large quantity (molar ratio to GP A about 2.4) of an apparent GP B-GP A hybrid molecule. Moreover, these RBC exhibit an alteration of the carbohydrate unit on the anion channel protein (band 3).

Contreras *et al.* [5] have demonstrated that RBC of the NE type and a similar RBC variant (Ph), described by Tanner *et al.* [28, 38], exhibit the low-frequency MNSs antigen Dantu. Anti-Dantu was detected in anti-S and -s reagents as well as several sera containing multiple antibodies against low frequency antigens [5]. The NE and Ph varieties of the Dantu phenotype have so far only been detected in Blacks. Recently, however, a further *Dantu* gene complex (MD) has been discovered in a Caucasian [34]. The GP patterns of Ph and MD RBC differ from those of cells of the NE type.

Previous studies [5, 14, 34, 38, 40] revealed that the hybrid molecule in all Dantu+ RBC exhibits N specificity. Moreover, the investigations of Contreras *et al.* [5] disclosed that the *Dantu* allele expresses a weak s antigen. In the Ph family [38] and in one pedigree (Ox), studied by Contreras *et al.* [5], the *Dantu* gene complex was also shown to encode an M antigen. In this communication we describe studies on the serology, immunochemistry, genetics and frequency of the Dantu^{NE} phenotype. Our report will particularly focuss on certain aspects that are not or only partially covered by the contribution of Contreras *et al.* [5]. As described in a preliminary report [14], the hybrid molecule, encoded by the Dantu^{NE} gene complex, appears to comprise the residues (res.) 1–39 of blood group s-specific GP B and the res. 71–131 of GP A. The details of these sequencing studies will be published elsewhere (see Appendix).

Materials and Methods

Ascertainments

NE (II-7), a Rh(D)-negative 32 year old Black gravida VI, para IV, was admitted to hospital in Chicago at 27 weeks of gestation with premature rupture of the placental membranes. Anti-D and -U with a titer of 4096 or 8, respectively, were detected in her serum. The infant (BE, III-6), born by ceasarian section after 37 weeks of gestation, required phototherapy and five exchange transfusions with Rh(D)- and U-negative blood during the first month of life and 14 simple transfusions during hospitalization for 3.5 months. Cord RBC from III-6 yielded a 4+ direct antiglobilin test (DAT). Anti-D and -U could be eluted from the cells. RBC from NE did not react with anti-U. However, positive results were obtained with some anti-S and-s sera. Discontinuous sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (disc SDS-PAGE) of her RBC membranes revealed alterations of the GPs and the presence of a proteinase-resistant

hybrid GP [40]. Studies in collaboration with M. Contreras (North London Blood Transfusion Centre, UK) and P. Tippett (MRC Blood Group Unit, London, UK) revealed that NE exhibits the low-frequency antigen Dantu and that the reactions with anti-S sera are caused by anti-Dantu in these reagents [5].

The Black patient MB, a gravida V, who had made anti-U (titer 16) and delivered an infant with a weakly positive DAT, was detected in Norfolk, because her RBC were typed as S−s+U−, and identified as Dantu+ in Houston.

Blood samples from the Black family HP (I-1, I-2 and the newborn II-2) were referred to Gamma Biologicals by L. K. Wagoner (St. David's Community Hospital, Austin, TX, USA), because I-2 had made an antibody (titer 64) reacting with the RBC from I-1 and II-2 (after chloroquine-diphosphate treatment) that was identified as anti-Dantu. Cord RBC from II-2 yielded a 3+ DAT. The mild hemolytic disease of the newborn of II-2 was successfully treated by phototherapy [25]. A similar family (T.; comprising a Dantu+ father and a Dantu+ newborn as well as a M+N+Dantu− mother who had made anti-Dantu) that was also studied at Gamma Biologicals has been described by Contreras *et al.* [5].

HB and WR were detected by screening of 600 Black donors in Norfolk with anti-U (18 negatives), because their RBC were typed as S−s+U−, and identified as Dantu+ at Gamma Biologicals.

Since studies on NE had revealed that her RBC exhibit a blood group N-active, proteinase-resistant hybrid molecule [40], blood samples from 1,000 Black, 1,000 German or 2,200 predominantly Black donors were screened with *Vicia graminea* lectin, after ficin treatment of RBC, in Chicago, Cologne or Dayton, respectively. Samples studied in Chicago were, in part, obtained from the American Red Cross Blood Services, the Rush Presbyterian St. Luke's Medical Center or the Medical Center of the University of Illinous (Chicago, IL, USA). RBC that reacted with *Vicia* lectin were subsequently tested with anti-Dantu and anti-St^a.

Materials

Blood samples from Mr. Dantu, Sh, Vi, Ox II-1, II-3, and II-5 as well as four members of a large South African pedigree [30] (IV-4, IV-5, IV-13, all Dantu+U+; III-3, Dantu+U−) were generously provided by T. Stout (Vancouver, Canad), K. Skradsky (Minneapolis, MI, USA), the Lewisham Blood Transfusion Service, M. Contreras (London, UK) [15] or Ph. Moores (Durban, SA) [30], respectively. Blood samples from relatives of NE (II-7) (father, I-2, Dantu+; mother, I-3; four paternal half sisters, II-1, Dantu+, II-2, Dantu+, II-3, II-4, Dantu+; four children, III-1, Dantu+, III-2, Dantu+, III-3, III-6) were also studied. RBC membranes from NE I-2, II-1, II-4, II-7, III-1, III-2, III-3, III-6, Mr. Dantu, MB, HB, WR, HP I-1 and I-2, Sh, Vi, Ox II-1, II-3 and II-5 and three additional Dantu+ donors, detected by screening with *Vicia* lectin, had been studied by disc SDS-PAGE, as described in the accompanying article [17]. Disc SDS-PAGE of membranes from the four South African donors [30] followed by periodic acid/Schiff-(PAS-) staining also yielded patterns, typical for $Dantu^{NE}$ heterozygotes. Three Dantu+U+ relatives of one of the donors (And.), detected during the screening in Dayton, were also studied by serological methods.

Purified *Vicia graminea* lectin was generously provided by M. J. Prigent (Paris, France). Anti-Duclos [22] or anti-M^v plus -N were gifts of B. Habibi (Paris, France) or H. Gershowitz (Ann Arbor, MI, USA), respectively. Other antisera were obtained from several commercial sources, donated by colleagues or detected in our laboratories. Other materials have been described in previous publications [1, 2, 9, 10, 12, 13, 15–17].

Methods

Agglutination tests were carried out by standard techniques and scored according to Marsh [27]. Elution of antibodies from RBC was performed with ether [35], chloroquine-diphosphate or the ELU-KIT according to the instructions of the manufacturer (Gamma Biologicals Inc., USA). Hemagglutination inhibition tests and enzyme treatments of intact RBC were carried out as described elsewhere [12, 17]. Some studies were carried out on RBC that had been frozen in liquid nitrogen.

Membranes were isolated from native or trypsin-treated RBC [17, 19] and extracted by the phenol/saline method [13, 21]. The partially purified hybrid GP in extracts from trypsin-treated NE RBC was isolated by gel filtration in the presence of Ammonyx-LO [13, 20]. An N-terminal

peptide (T 1, res. 1–39 [39]) from GP A of two Dantu+U+ donors was purified by phenol/saline partitioning [13, 21] of the supernatant from trypsin-treated RBC at room temperature and subsequent gel and ion exchange chromatography [10, 39]. A chymotryptic peptide (C 1, res. 1–64) of GP A [39] was isolated by gel filtration after enzyme treatment [16] of extracted GPs from RBC of NE.

Amino-acid composition and sequence analyses were carried out as described elsewhere [3, 4, 10, 12]. Disc SDS-PAGE was performed by the technique of Laemmli [1, 24]. Gels were either stained by the PAS-procedure [19] or incubated with radio-iodinated *Vicia graminea* lectin [1, 2]. The gels were dried subsequently and subjected to autoradiography.

Results

Serological Studies

All Dantu+ RBC samples studied by us ($n = 36$) exhibited the phenotype M+N+. The strength of the agglutination by most rabbit anti-M and -N sera was roughly similar to that of M+N+ control cells. However, titrations using four human or mouse monoclonal anti-M sera revealed that some Dantu+ cells ($n = 4$) react more strongly than others ($n = 4$) (Table 1, data not shown). The reactions obtained with anti-N reagents were also variable. For example, anti-M^v plus -N reacted much more strongly with Dantu+ RBC than with M+N+ control cells. Conversely, most ($n = 14$) Dantu+ cell samples were only weakly agglutinated by *Vicia* anti-N, whereas some ($n = 7$) reacted more strongly. Four or five of the samples reacting more weakly or more strongly, respectively, with *Vicia* lectin were not studied by biochemical techniques for investigating the MN blood type of GP A, described below.

Five out of ten different anti-s sera reacted weakly with Dantu+U+S+ ($n = 2$) and Dantu+U−S− RBC ($n = 7$). Those reagents that failed to react in agglutination assays were also non-reactive in absorption/elution studies. Anti-S failed to bind to Dantu+S− RBC ($n = 34$), as judged from agglutination or absorption/elution tests. Cells from patient NE were tested with nine different examples. Positive reactions, obtained with some anti-S reagents, are attributable to a separable anti-Dantu in these sera, as described by Contreras *et al.* [5] and below.

Contreras *et al.* [5] have reported that RBC from NE, Vi and Sh exhibit a weak U antigen. However, we obtained negative results with cells from these and three additional (HB, WR, MB) individuals in agglutination or absorption/elution studies. RBC from patient NE were tested with 11 different examples of anti-U. Anti-Duclos [22] reacted only weakly with cells from three Dantu+U− (NE, Sh, MB) individuals. Confirming and extending previous data [5], the U, Duclos, S or s antigens of Dantu+U+S+s+ weak or Dantu+U+S−s+ RBC, respectively, were similar to those of Dantu−S+s+U+ cells, as judged from titration studies.

Contreras *et al.* [5] have detected a separable anti-Dantu in one anti-s, at least one anti-S and 10 sera containing antibodies against several low-frequency antigens. We have found anti-Dantu in anti-S sera from at least four different donors and eight additional sera containing multiple antibodies against low-frequency antigens. Anti-Dantu could not be detected in any of our anti-s and anti-U sera.

The hybrid GP in Dantu+ RBC is not, or only partially, degraded by various proteinases, in contrast to GP A and GP B [14, 17, 40]. The agglutination of Dantu+ cells by anti-Dantu, -s, and -N was strongly (about 4- to 16-fold) enhanced after proteinase treatment, suggesting that these receptors are associated with the hybrid GP.

Table 1. Agglutination of Dantu^{NE} RBC by various antisera

Agglutinin	RBC samples from M+N+S+s+ control		Dantu+U− (NE II-7)		Dantu+U+ (HP I-1)	
	Titer	Score	Titer	Score	Titer	Score
Anti-M[a]	8	15	16	20	4	11
Vicia lectin	8	33	4	18	8	27
Anti-Mv plus -N	8	25	32	51	32	58
Anti-S[b]	16	32	0	0	0	0
Anti-s[b]	32	29	16	13	32	30
Anti-U[b]	16	24	0	0	16	23
Anti-Duclos	64	41	8	16	64	38

[a] Average of data obtained with two human and two mouse monoclonal antisera
[b] Average of data obtained with four human antisera

Table 2. Inhibitory activity of GPs from donor NE II-7 against various antisera

GP preparation	Inhibitory activity[a] against				
	Anti-M	Anti-N	Anti-S	Anti-s	Anti-Dantu
GP mixture	0.63	0.63	>10.0	1.25	>10.0
Purified hybrid GP	>10.0	0.32	>10.0	0.63	>10.0

[a] Inhibitory activity is expressed as the minimum quantity (mg/ml) of substance inhibiting the agglutinin with a titer of 4. The average values of data obtained with two rabbit (anti-M, anti-N) or two human (anti-S, anti-s, anti-Dantu) antisera are presented

After treatment with ficin or other proteinases (papain, pronase, thermolysin, V8 proteinase) that destroy the MNSs antigens of normal RBC, the abnormal s antigen could also be demonstrated in Dantu+U+S−s+ RBC ($n = 16$). Contreras *et al.* [5] have reported that the M antigen of Dantu+ RBC is only partially destroyed by trypsin treatment. In our experiments, the M receptor of Dantu+ cells ($n = 14$) was completely inactivated, as measured with potent antisera, suggesting that the M antigen is associated with the trypsin-sensitive portion of GP A (res. 1–39 [39], rather than with the proteinase-resistant hybrid GP (data not shown).

Since the NeuAc levels of RBC from *Dantu^{NE}* heterozygotes are decreased [17, 40], they reacted much more weakly (1+) in the polybrene test than control samples (4+). Dantu+ cells from ten individuals that had not been studied by SDS-PAGE, were only weakly aggregated by polybrene, indicating that they also exhibit the NE variety of the Dantu phenotype, rather than the Ph [28, 38] or MD [34] types.

Immunochemical Studies

Inhibition tests with the mixture of extracted GPs and the purified hybrid GP, devoid of component X [17], from patient NE (Dantu+U−) showed that the hybrid molecule exhibits N- as well as s-activity (Table 2). Anti-M was inhibited by the GP mixture containing GP A, but not by the hybrid GP, thus providing further evidence that GP A carries M-activity (Table 2). Anti-Dantu could not be inhibited by extracted GPs.

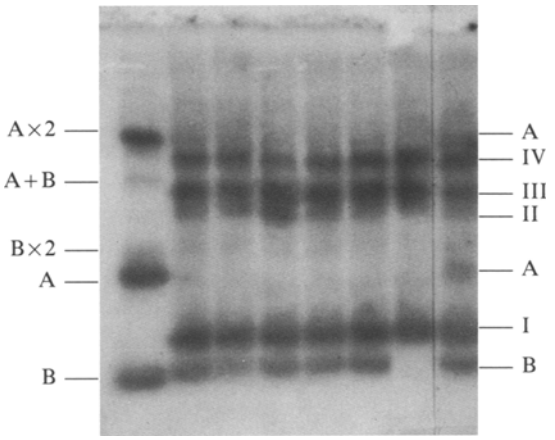

Fig. 1. Visualization of blood group N-specific components in Dantu+ membranes by radio-iodinated anti-N from *Vicia graminea*. RBC membranes (about 150 μg of protein per lane) were separated by disc SDS-PAGE [24]. The gel was incubated with radio-iodinated *Vicia* lectin and subjected to autoradiography [1, 2]. Lanes 1 through 8: M−N+ control, NE III-1, NE III-2, NE I-2, NE II-1, NE II-4, HB (Dantu+U−) and HP I-1. A or B denote the monomer of GP A or GP B, respectively. A×2 = GP A dimer, B×2 = GP B dimer, A+B = dimer of GP A and GP B, I = monomer of hybrid, II, III and IV = dimers involving the hybrid [17]

Amino-acid composition and sequence analyses (six Edman-cycles) on peptides C1 (res. 1−64) or T1 (res. 1−39) of GP A from patient NE or two Dantu+U+ donors, respectively, established that this molecule exhibits only blood group M-specificity in these individuals. The M-specific residues serine or glycine were detected at the first or fifth position, respectively (data not shown).

Binding of labelled *Vicia graminea* anti-N to membrane components, after separation by SDS-AGE, represents a powerful technique for investigating the location and relative quantity of N-active molecules [1, 2, 18]. Analyses by this method confirmed that Dantu+U− ($n = 6$) or Dantu+U+ ($n = 14$) RBC lack, or contain a decreased quantity of, GP B [14, 17] (Fig. 1, data not shown). The monomer (band I) of the hybrid and the dimers (II, III, IV) involving this molecule [17] also bound the labelled lectin. The GP A of most samples ($n = 17$) was devoid of N-activity. However, the labelled lectin bound weakly to the mono- and dimer of GP A from some individuals (HP I-1, Vi, Sh). RBC from these donors were agglutinated more strongly by *Vicia* anti-N or more weakly by anti-M, respectively, than those from other Dantu+ donors. A comparison of densitometric scans of autoradiographs and PAS-stained gels indicated that about half of the GP A molecules in the RBC from HP I-1, Vi and Sh exhibits N-activity. After separation of extracted GPs from Dantu+U−, Dantu+U+, or normal RBC by disc SDS-PAGE, no binding of *Vicia* lectin to the components J or X [17] could be detected (data not shown).

Inheritance and Frequency of Dantu

Studies on the Ox pedigree had shown that Dantu is inherited as a dominant character and provided evidence that the $Dantu^{NE}$ allele encodes an N and an M antigen [5].

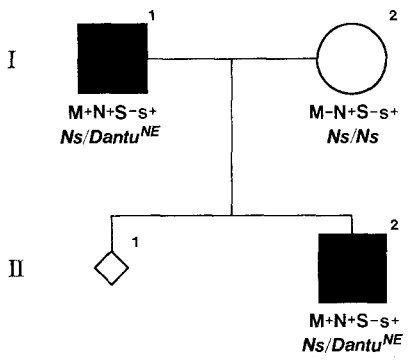

Fig. 2. HP pedigree demonstrating that the *Dantu* gene complex transmits an M antigen. ● or ○ = Dantu+ or Dantu−, respectively, ◇ = stillbirth. The proposed genotypes were deduced from serological and biochemical studies

The HP pedigree proved this possibility unambiguously (Fig. 2). Since the RBC from I-2 do not exhibit M-activity, II-2 must have inherited the N-active hybrid and an M-specific GP A from I-1. Since SDS-PAGE had revealed that two children (III-3, III-6) of NE (II-7) exhibit the genotype *Uu* [17], NE must possess the genotype *Dantu/u.* However, the large NE pedigree (not shown) comprising six Dantu+ U+S−s+ individuals was not informative with respect to the question whether the $Dantu^{NE}$ allele encodes an M antigen. Similarly, the T. and And. pedigrees (not shown) did not solve this question.

Screening of 1,000, 2,200 or 1,000 ficin-treated RBC with *Vicia* lectin revealed six, ten, or no positive samples in Chicago, Dayton, or Cologne, respectively. Five each of the donors detected in Chicago and Dayton were found to be Dantu+U+. The remaining six individuals exhibited the St(a+) phenotype.

Discussion

Data described in this and the accompanying article [17] indicate that 36 Black individuals (21 propositi) exhibit the NE variety of the Dantu phenotype, as judged from SDS-PAGE and/or the polybrene test. Thus, the $Dantu^{Ph}$ [38] and $Dantu^{MD}$ types [34] appear to be rather rare. The quantity of GP A in RBC from $Dantu^{NE}$ heterozygotes is only about 43% of normal [17]. However, three lines of evidence suggest that the GP A in these cells represents the product of the $Dantu^{NE}$ allele and its normal counterpart and that the GP A encoded by the $Dantu^{NE}$ gene complex exhibits M-specificity: 1.) Our experiments involving labelled *Vicia* lectin and trypsin-treatment demonstrate that the RBC from three individuals contain M- and N-specific GP A in a ratio of about 1: 1. Five additional donors appear to exhibit the same phenotype, as judged from titration studies with anti-M sera and *Vicia* anti-N. 2.) The Ox [5] and HP pedigrees as well as that of Moores *et al.* ([30] Ph. Moores, pers. commun.) demonstrate that the $Dantu^{NE}$ gene complex encodes blood group N- as well as M-specificity. 3.) The phenomenon that all known $Dantu^{NE}$ RBC (53) exhibit the phenotype M+N+ provides statistical evidence for this conclusion. Of course, our data do not exclude the possibility that the *Dantu* allele is heterogeneous and encodes a blood group N-specific GP A, or no GP A at all, in a minority of the individuals.

The data discussed above indicate that the *Dantu*^{NE} allele suppresses the expression of GP A in a *cis* and *trans* manner. The possibility that the *Dantu*^{NE} gene exerts its inhibiting effect at the DNA, RNA or protein synthesis level cannot be excluded. However, we consider it most likely that the suppression occurs at the stage of the insertion of GP A into the membrane. Data on RBC from *En, sU/M*^{k} and *sU/u* heterozygotes, in which the quantity of GP A and/or GP B is decreased by about 50%, indicate that glycoprotein synthesis represents one factor limiting the insertion of these molecules into the membrane. The GP B content of cells from *SU* hemizygotes was consistantly decreased to a lesser extent (about 35%), in comparison to that of S+s− RBC, suggesting that the expression of this molecule is slightly impaired in S+s− cells [11]. As judged from data on Rh_{null} RBC [15], the formation of a complex with protein(s) encoded by the *Rh* locus might represent a factor limiting the incorporation of GP B into the membrane. There is good evidence that GP A (about 850,000 copies per cell) forms a complex with the anion channel protein (band 3, about one million copies) in normal RBC [7, 9, 16, 33]. The finding that the carbohydrate unit of band 3 is altered in Dantu^{NE} cells [17, 40] provides provisional evidence that the hybrid also aggregates with this molecule. Therefore, we suggest that the formation of a GP A-band 3 complex facilitates the incorporation of GP A into normal membranes. The hybrid in Dantu^{NE} cells that is produced in large quantity might inhibit the insertion of GP A by competition *via* the formation of a complex with band 3. The hypotheses described above are not unique: The linking protein band 4.1 that forms complexes with GP C (D SGP or β) and GP D (E SGP or γ) appears to facilitate the incorporation of these molecules into the membrane, as judged from data on 4.1-deficient RBC [37].

Our data demonstrating that the purified hybrid from Dantu^{NE} RBC exhibits N- and s-specificity, in conjunction with other results [14, 17], prove the GP B-GP A hybrid nature of this molecule. Dantu^{NE} RBC react much more weakly with certain anti-N reagents than M−N+ cells, although they exhibit a higher number of N-specific molecules. This phenomenon may be caused by the fact that the extracellular domain of the hybrid is much shorter than that of GP A [14, 39].

Since our sequence studies [14] suggest that the hybrid comprises the N-terminal 39 res. of blood group s-specific (Thr at position 29) GP B, it is peculiar that Dantu^{NE} RBC react only weakly or not with anti-s or -U, although the quantity of the hybrid is about 4.5-fold higher than that of GP B in S−s+ cells. Since a GP B-Rh protein(s) complex might be important for s- and U-activity [15], the weak, or lack of, expression of these antigens in Dantu+U− RBC might, in part, be caused by a hybrid-band 3 complex. Since the U antigen is located C-terminal of position 32 of GP B [7, 8], the absence of U-activity from Dantu+U− cells might also be caused by the different sequence C-terminal of position 39 [14] of the hybrid or by a conformational change. The finding that anti-Dantu was not inhibited by extracted GPs does not exclude that the Dantu antigen is located on the hybrid. Our results ([14], unpubl. data), suggest that this receptor represents a labile structure which is denatured during extraction, just like the En^{a}FR, Wr^{b} and U antigens [8, 16] (see Appendix).

Dantu+U− (NE, Sh, MB) RBC reacted only weakly with anti-Duclos, just like S-s-U- cells [22]. Patient NE exhibits the genotype *Dantu/u* [17] and the other Dantu+U− individuals are likely to possess the same genotype. Therefore, our data do not disclose whether the *Dantu*^{NE} allele expresses weak Duclos activity or none at all.

Since the *Dantu*^{NE} allele appears to encode an M-specific GP A and the GP B-GP A hybrid, but no GP B, it might have been generated by a misalignment between a normal *Ms* allele and a *u* gene complex, that is frequent among Blacks, and subsequent unequal crossing over. Since the GP B gene appears to be deleted in the *u* gene complex (O.O. Blumenfeld, pers. commun.), this might have triggered the misalignment. Our data presented here and elsewhere [14, 17] are not compatible with suggestions that the hybrid exhibits M-activity [5, 29] or that the *Dantu*^{NE} allele encodes a GP A-GP B-GP A hybrid and GP B, but no GP A [29].

Contreras *et al.* [5] found only one Dantu+ Black individual among 44,000 donors in London. Vengelen-Tyler and Mogck [41] failed to detect any Dantu- or St^a-positive individual among 100 American Blacks. Our data demonstrate that both these phenotypes exhibit frequencies in the range of 0.005 to 0.001 in Blacks living in the United States.

Acknowledgments. We thank several colleagues for blood and serum samples and R. Anders for typing the manuscript. This work was supported by grants from the 'Deutsche Forschungsgemeinschaft', the 'Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen', the 'Institut National de la Sante et de la Recherche Medicale (INSERM)', and through a Heisenberg research fellowship by the 'Deutsche Forschungsgemeinschaft' to W. Dahr.

References

1. Blanchard D, Asseraf A, Prigent MJ, Cartron JP (1983) Miltenberger Class I and II erythrocytes carry a variant of glycophorin A. Biochem J 213: 399–404
2. Blanchard D, Asseraf A, Prigent MJ, Moulds JJ, Chandanayingyong, Cartron JP (1984) Interaction of *Vicia graminea* anti-N lectin with cell surface glycoproteins from erythrocytes with rare blood group antigens. Hoppe-Seyler's Z Physiol Chem 365: 469–478
3. Chang JY, Creaser EH, Hughes GJ (1977) Separation of 4-*N,N*-dimethylaminoazobenzene-4′-thiohydantoins of amino acids by thin-layer chromatography on silica gel. J Chromatogr 140: 125–128
4. Chang JY, Brauer D, Wittmann-Liebold B (1978) Micro-sequence analysis of peptides and proteins using 4-*NN*-dimethylaminoazobenzene-4′-isothiocyanate/phenylisothiocyanate double coupling method. FEBS Lett 93: 205–214
5. Contreras M, Green C, Humphreys J, Tippett P, Daniels G, Teesdale P, Armitage S, Lubenko A (1984) Serology and genetics of an MNSs-associated antigen Dantu. Vox Sang 46: 377–386
6. Dahr W (1981) Serology, genetics and chemistry of the MNSs blood group system. Rev Franc Transf Immunohematol 24: 85–95
7. Dahr W (1986) Immunochemistry of sialoglycoproteins in human red blood cell membranes. In: Vengelen-Tyler V, Judd WJ (eds) Recent advances in blood group biochemistry. American Association of Blood Banks, Arlington, VA pp 23–65
8. Dahr W, Krüger J (1983) Solubilization of various blood group antigens by Triton X-100. In: Proceedings of the 10th International Congress of the Society for Forensic Haemogenetics, Munich. Schmitt & Meyer, Würzburg, pp 141–146
9. Dahr W, Uhlenbruck G, Leikola J, Wagstaff W, Landfried K (1976) Studies on the membrane glycoprotein defect of En(a-) erythrocytes. I. Biochemical aspects. J Immunogenet 3: 329–346
10. Dahr W, Beyreuther K, Steinbach H, Gielen W, Krüger J (1980) Structure of the Ss blood group antigens. II. A methionine/threonine polymorphism within the N-terminal sequence of the Ss glycoprotein. Hoppe-Seyler's Z Physiol Chem 361: 895–906
11. Dahr W, Weber W, Kordowicz M (1981) The allele S^u in Caucasians – medico-legal, immunochemical and genetic aspects. In: Hummel K, Gerchow J (eds) Biomathematical evidence of paternity. Springer, Berlin, Heidelberg, New York, pp 131–138

12. Dahr W, Newman RA, Contreras M, Kordowicz M, Teesdale P, Beyreuther K, Krüger J (1984) Structures of Miltenberger class I and II specific major human erythrocyte membrane sialoglycoproteins. Eur J Biochem 138: 259–265
13. Dahr W, Moulds J, Baumeister G, Moulds M, Kiedrowski S, Hummel M (1985) Altered membrane sialoglycoproteins in human erythrocytes lacking the Gerbich blood group antigens. Biol Chem Hoppe-Seyler 366: 201–211
14. Dahr W, Moulds J, Unger P, Blanchard D, Cartron JP (1985) Serological and biochemical investigations on the N.E. variety of the Dantu red cell phenotype. In: Brinkmann B, Henningsen K (eds) Advances in forensic haemogenetics 1. 11th Congress of the Society for Forensic Haemogenetics, Copenhagen. Springer, Berlin, Heidelberg, New York, pp 1–7
15. Dahr W, Kordowicz M, Moulds J, Gielen W, Lebeck L, Krüger J (1986) Characterization of the Ss sialoglycoprotein and its antigens in Rh_{null} erythrocytes. Blut 54: 13–24
16. Dahr W, Wilkinson S, Issitt PD, Beyreuther K, Hummel M, Morel P (1986) High frequency antigens of human erythrocyte membrane sialoglycoproteins. III. Studies on the En^aFR, Wr^b and Wr^a antigens. Biol Chem Hoppe-Seyler 367: 1033–1045
17. Dahr W, Moulds J, Unger P, Kordowicz M (1987) The Dantu erythrocyte phenotype of the NE variety. I. Dodecylsulfate polyacrylamide gel electrophoretic studies. Blut 55: 19–31
18. Duk M, Lisowka E, Kordowicz M, Wasniowska K (1982) Studies on the specificity of the binding site of *Vicia graminea* anti-N lectin. Eur J Biochem 123: 105–112
19. Fairbanks G, Steck TL, Wallach DFH (1971) Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10: 2606–2617
20. Furthmayr H, Tomita M, Marchesi VT (1975) Fractionation of the major sialoglycopeptides of the human red membrane. Biochem Biophys Res Commun 65: 113–121
21. Glöckner WM, Newman RA, Dahr W, Uhlenbruck G (1976) Alkali-labile oligosaccharides from glycoproteins of different erythrocyte and milk fat globule membranes. Biochim Biophys Acta 443: 402–413
22. Habibi B, Fouillade MT, Duedari N, Issitt PD, Tippett P, Salmon C (1978) The antigen Duclos. Vox Sang 34: 302–309
23. Issitt PD (1985) Applied blood group serology. 3rd ed. Montgomery Scientific Publications, Miami, FL
24. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685
25. Laird-Fryer B, Moulds JJ, Wagoner LK (1984) Hemolytic disease of the newborn due to anti-Dantu. In: Cooper ES (ed) Journal of the South Central Association of Blood Banks, Austin, TX, 25: 16 (abstract)
26. Lisowska E (1987) Antigenic properties of human erythrocyte glycophorins. In: Wu AM (ed) Molecular immunology of complex carbohydrates. Plenum Press, New York NY (in the press)
27. Marsh WL (1972) Scoring of hemagglutination reactions. Transfusion 12: 352–354
28. Mawby WJ, Anstee DJ, Tanner MJA (1981) Immunochemical evidence for hybrid sialoglycoproteins of human erythrocytes. Nature 291: 161–162
29. Merry AH, Hodson C, Thomson E, Mallinson G, Anstee DJ (1986) The use of monoclonal antibodies to quantify the levels of sialoglycoproteins α and δ and variant sialoglycoproteins in human erythrocyte membranes. Biochem J 233: 93–98
30. Moores P, Dinnematin H, Smart E (1984) Dantu and MNSs blood groups in a large natal family. In: 18th Congress of the International Society of Blood Transfusion, Munich. S. Karger, Basel, p 166 (abstract P 8–14)
31. Moulds J, Dahr W (1987) MNSs and Gerbich blood group systems. In: Litwin SD, Scott D, Flaherty L, Reisfeld RA, Marcus DM (eds) Human immunogenetics: An advanced text. Marcel Dekker Inc., New York NY (in the press)
32. Moulds JJ, Case J, Moulds M, Northam C (1986) Heterogeneity of anti-s disclosed by red blood cells from *Dantu/S^u* individuals. In: Abstract volume of the 19th Congress of the International Society of Blood Transfusion, p 653 (abstract P-F-311-9)
33. Nigg EA, Bron C, Girardet M, Cherry RJ (1980) Band 3-glycophorin A association in erythrocyte membranes demonstrated by combining protein diffusion measurements with antibody-induced cross-linking. Biochemistry 19: 1887–1893
34. Pilkington P, Dahr W, Lacey P, Moulds JJ, Wheeler N (1985) A Dantu-positive Caucasian demonstrating a variant hybrid sialoglycoprotein. Transfusion 25: 464 (abstract)

35. Rubin H (1963) Antibody elution from red cells. J clin Pathol 16: 70–72
36. Salmon C, Cartron JP, Rouger P (1984) The human blood groups. Masson Publishing USA Inc., New York NY
37. Sondag D, Alloisio N, Blanchard D, Ducluzeau MT, Colonna P, Bachir D, Bloy C, Cartron JP, Delauney J (1987) Gerbich reactivity in 4.1 (–) hereditary elliptocytosis and protein 4.1 level in blood group Gerbich deficiency. Br J Haematol 65: 43–50
38. Tanner MJA, Anstee DJ, Mawby WJ (1980) A new human erythrocyte variant (Ph) containing an abnormal membrane sialoglycoprotein. Biochem J 187: 493–500
39. Tomita M, Furthmayr H, Marchesi VT (1978) Primary structure of human erythrocyte glycophorin A. Isolation and characterization of peptides and complete amino acid sequence. Biochemistry 17: 4756–4770
40. Unger P, Orlina A, Dahr W, Kordowicz M, Moulds J, Newman RA (1981) Two new gene complexes in the MNSs blood group system. In: Engelfriet CP (ed) Proceedings of the 9th International Congress of the Society for Forensic Haemogenetics. Schmitt & Meyer, Würzburg, pp 189–193
41. Vengelen-Tyler V, Mogck N (1986) A new test useful in identifying red cells with a ($\alpha-\delta$) hybrid sialoglycoprotein. Transfusion 26: 231–233

Received January 17, 1987/Accepted March 18, 1987