Biochemical Characterization of Class VII and VIII Cells within the Miltenberger System

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INTRODUCTION

The various antigens of the MNSs blood group system are located on two homologous, sialic acid-rich glycoproteins (SGPs) in human red blood cell (RBC) membranes: glycophorin A (GP A; MN SGP; α) and GP B (Ss SGP or δ) (for reviews see Dahr 1986; Lisowska 1987; Moulds & Dahr 1987). The 'MNSs locus', located on chromosome 4, appears to comprise two adjacent genes that encode the amino-acid sequences of these two molecules.

The Miltenberger (Mi-) subsystem of the MNSs system was originally (Cleghorn 1966) defined as a group of four (I-IV) rare RBC classes that share the Mi^a antigen, but differ with respect to other determinants (Vw, Mur, Hut, Hil). Some (V, VII, VIII) of the RBC classes chracterized more recently (Crossland et al. 1970; Giles et al. 1977; Dybkjaer et al. 1981) do not fit with the initial definition of the Mi-system in that they fail to react with anti-Mi^a. Mi-VII and Mi-VIII RBC are rather similar, since both RBC classes exhibit the Anek, Lane and Raddon antigens.

Previous studies (Dahr et al. 1984) revealed that Mi-I and Mi-II RBC exhibit a Thr → Met or a Thr → Lys exchange, respectively, at position 28 of GP A that prevents N-glycosylation of Asn-26. Mi-III, Mi-IV and MI-VI RBC were found to possess a GP B with an increased mol. mass (Dahr et al. 1978; Anstee et al. 1979). The Mi-V gene appears to encode a hybrid molecule of the Lepore-type comprising the residues (res.) 1- approx. 55 of GP A and the res. approx. 30-72 of GP B (Dahr et al. 1978; Anstee et al. 1979; Vengelen-Tyler et al. 1981), but no normal GP A or GP B.

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In a recent study (Dahr et al. 1985) we have shown that the high-frequency, trypsin-resistant, ficin-sensitive antigen En^{a}KT (previously denoted as En^{a}FS), detectable by alloantibodies from En(a-) individuals and autoantibodies from patients with autoimmune hemolytic anemia, is located within the res. approx. 46-56 of GP A. The finding (Laird-Fryer et al. 1986) that one individual (KT) whose RBC were found to contain a normal level of GP A had also made an alloanti-En^{a}KT provoked detailed studies. KT was shown to represent the first example of a Mi-VII homozygote. Her RBC were shown to lack the En^{a}KT antigen and RBC from Mi-VII heterozygotes gave a 'single dose reaction' with anti-En^{a}KT sera suggesting that Mi-VII and En^{a}KT represent alleles. The Anek antigen of KT's RBC was found to be located within the res. approx. 40-57 of GP A.

In more recent experiments we have determined the structural alteration of KT's GP A. Since the Mi-VII and Mi-VIII variants are closely related, we have also performed similar studies on RBC from two Mi-VIII heterozygotes (Dybkjaer et al. 1981; Vengelen-Tyler et al. 1985) (Dahr et al. 1987 and manuscript in preparation).

RESULTS

Just like RBC from Mi-VII heterozygotes, those from the two Mi-VIII heterozygotes yielded a 'single dose reaction' with four anti-En^{a}KT sera, thus suggesting that Mi-VIII and En^{a}KT also represent alleles. Two anti-Anek sera showed a slightly enhanced agglutination of trypsin- or chymotrypsin-treated Mi-VIII RBC and failed to react after ficin, V8 proteinase or sialidase treatment. These data suggest that the Anek antigen of Mi-VIII RBC is also located within the res. approx. 40-57 of GP A (data not shown).

Further evidence for this conclusion was obtained by hemagglutination inhibition assays: Anti-Anek sera were inhibited by the GP mixture, GP A and a tryptic peptide (T3) corresponding to the res. 40-61 of GP A from Mi-VII or Mi-VIII RBC. The mixture of GP B and GP C as well as the mixture of N-terminal tryptic peptides (T1, res. 1-39; T2, res. 1-31) from GP A had no detectable activity (data not shown).

The complete structure of T3 from the GP A of KT was deduced from amino-acid and carbohydrate analyses, manual DABTITC/PITC sequencing of T3 and secondary V8 proteinase fragments, carboxypeptidase Y digestion followed by amino-acid analyses and direct identification of the DABTH-derivatives of glycosylated Ser and Thr residues, as described in detail elsewere (Dahr et al. 1987). The T3 preparations from the two Mi-VIII heterozygotes were also sequenced in an automated gas-liquid solid phase sequenator (res. 40-60). The data demonstrate that the Mi-VII-specific and the MI-VIII-specific GP A molecules exhibit an Arg → Thr (glycosylated) exchange at position 49 (Fig. 1). In addition, Mi-VII-specific GP A was found to possess a Tyr → Ser exchange at position 52. It could not be elucidated whether Ser-52 is carbohydrate-free or glycosylated to a small extent.

Partial structure of normal, EnaKT-specific GP A

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          *     *     *
-A-H-E-V-S-E-I-S-V-R-T-V-Y-P-P-E-E-E-T-G-E-R-
 40                   50                   60
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Proposed partial structure of Mi-VII-specific GP A

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          *     *   * *   ?
-A-H-E-V-S-E-I-S-V-T-T-V-S-P-P-E-E-E-T-G-E-R-
 40                   50                   60
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Proposed partial structure of Mi-VIII-specific GP A

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          *     *   * *
-A-H-E-V-S-E-I-S-V-T-T-V-Y-P-P-E-E-E-T-G-E-R-
 40                   50                   60
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Fig. 1. Proposed partial (res. 40-61) amino-acid sequences (one-letter code) and glycosylation sites (*) of Mi-VII- and MI-VIII-specific GP A molecules.

DISCUSSION

Previous data (Laird-Fryer et al. 1986) and the present results demonstrate that the Anek antigens of Mi-VII and Mi-VIII RBC are located within the res. approx. 40-57 of GP A. Although we have not performed any studies with anti-Lane and -Raddon, it is reasonable to assume that the Lane and Raddon antigens of these RBC are also located in this region of GP A. Laird-Fryer et al. (1986) showed that Mi-VII and EnaKT represent antithetical structures. The weak reactivity of Mi-VIII RBC with anti-En^aKT sera suggests that Mi-VIII and En^aKT are also encoded by alleles. Our finding that Mi-VII-specific GP A and Mi-VIII-specific GP A exhibit structural alterations within the region (res. 46-56) carrying the En^aKT antigen (Dahr et al. 1985) provides an explanation for these data at the molecular level.

Our results demonstrate that Mi-VII-specific and Mi-VIII-specific GP A exhibit an Arg - Thr (glycosylated) exchange at position 49. Mi-VII-specific GP A possesses an additional alteration (Tyr - Ser exchange at position 52). As judged from the genetic code, both sequence alterations can be attributed to two separate exchanges of single DNA bases in the gene for GP A. Therefore, it may be proposed that Mi-VIII represents the evolutionary link between Mi-VII and its normal counterpart.

Since anti-Anek, -Raddon and -Lane react more strongly with RBC from Mi-VIII heterozygotes than with those from Mi-VII heterozygotes (Dybkjaer et al. 1981), it may be concluded that they are predominantly directed against the glycosylated Thr-49. Laird-Fryer et al. (1986) showed that the Anek antigen of Mi-VI RBC is located on their altered GP B. It has been suggested therefore that the abnormal GP B molecules of Mi-III, Mi-IV and Mi-VI RBC were generated by gene conversion involving GP A and GP B as donor and recipient, respectively, during evolution. Structural studies on these RBC are necessary for a detailed understanding of the Mi-system.

LITERATURE

Anstee DJ, Mawby WJ, Tanner MJA (1979) Abnormal blood-group-Ss-active sialoglycoproteins in the membrane of Miltenberger class III, IV and V human erythrocytes. Biochem J 183:193-203

Cleghorn TE (1966) A memorandum on the Miltenberger blood groups. Vox Sang 11:219-222

Crossland JD, Pepper MD, Giles CM, Ikin EW (1970) A British family possessing two variants of the MNSs blood group system, Mv and a new class within the Miltenberger complex. Vox Sang 18: 407-413

Dahr W (1986) Immunochemistry of sialoglycoproteins in human red cell membranes. In: Vengelen-Tyler V, Judd WJ (eds) Recent advances in blood group biochemistry. American Association of Blood Banks, Arlington VA, p 23-65

Dahr W, Longster G, Uhlenbruck G, Schumacher K (1978) Studies on Miltenberger class III, V, Mv and Mk cells. I. Sodium-dodecylsulfate polyacrylamide gel electrophoretic investigations. Blut 37:129-138

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

Dahr W, Müller T, Moulds J, Baumeister G, Issitt PD, Wilkinson S, Garratty G (1985) High frequency antigens of human erythrocyte membrane sialoglycoproteins. I. En^a receptors in the glycosylated domain of the MN sialoglycoprotein. Biol Chem Hoppe-Seyler 366:41-51

Dahr W, Beyreuther K, Moulds JJ (1987 in the press) Structural analysis of the major human erythrocyte membrane sialoglycoprotein from Miltenberger class VII erythrocytes. Eur J Biochem

Dybkjaer E, Poole J, Giles CM (1981) A new Miltenberger class detected by a second example of Anek type serum. Vox Sang 41: 302-305

Giles CM, Chandanayingyong D, Webb AJ (1977) Three antibodies of the MNSs system and their association with the Miltenberger complex of antigens. III. Anek, Raddon and Lane antisera in relation to each other and the Miltenberger complex. Vox Sang 32:277-279

Laird-Fryer B, Moulds J, Dahr W, Min YO, Chandanayingyong D (1986) Anti-En^aFS detected in the serum of a Mi-VII homozygote. Transfusion 26:51-56

Lisowska E (1987 in the press) Antigenic properties of human erythrocyte glycophorins. In: Wu AM (ed) Molecular immunology of complex carbohydrates. Plenum Press, New York

Moulds JJ, Dahr W (1987 in the press) 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

Vengelen-Tyler V, Anstee DJ, Issitt PD, Pavone BG, Ferguson SJ, Mawby WJ, Tanner MJA, Blajchman MA, Lorque P (1981) Studies on the blood of an Mi-V homozygote. Transfusion 21:1-14

Vengelen-Tyler V, Goya K, Green CA, Poole J (1985) The second example of Mi:VIII phenotype. Transfusion 25:464 (abstract)