Review of a terminology proposed to supersede Miltenberger

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The term Miltenberger has been used since 1966 to describe phenotypes for a subsystem of MNS-related blood group antigens. Initially, Miltenberger was used to describe red blood cells (RBCs) with one of four phenotypes that were agglutinated by the serum of Mrs. Miltenberger.[1] Mi.V was added because although these RBCs are not agglutinated by the Miltenberger serum, they expressed the Hil antigen (which is also expressed by Mi.III RBCs).[2] To date, there are ten antigens associated with the Miltenberger subsystem, and the number of classes has now expanded to 11 (Table 1). References for subclasses Mi.I to Mi.IX can be found in recent reviews.[3–5] Subclass Mi.X(HF) was reported recently[6] and, based on reactivity with anti-TSEN[7] and anti-MINY,[8] it has been suggested that JL be regarded as Mi.XI.[4] Only some of these RBCs are agglutinated by Mrs. Miltenberger's serum, and the Mi^a antigen (MNS7) is not included in Table 1 because no separable anti-Mi^a is available.[5] The Miltenberger phenotypes have been numbered chronologically, and it is becoming increasingly difficult to remember which antigens are associated with each phenotype. In addition, since anti-DANE agglutinates M^g-positive RBCs,[9] such cells would become a new Miltenberger class and anti-M^g a further Mi-related antibody. Tippett et al.[5] suggested that the Miltenberger terminology has outlived its usefulness and that, unless it is abolished, it is inevitable that other phenotypes will be added to the subsystem. Now that the biochemical and molecular basis of these phenotypes has been established, a terminology based on these findings might be more useful, practical, and descriptive. The terminology recommended by Tippett et al.[5] and detailed here not only is practical and descriptive, but also can be used for other glycophorin (GP) molecules, as described in this review.

Abolition of Miltenberger Subsystem and Replacement Terminology

All antigens in the Miltenberger subsystem are carried on glycophorin A (GPA), glycophorin B (GPB), or hybrid molecules composed of different proportions of GPA and GPB. For this reason, they belong in the MNS system and there appears to be no further value in separating them into a subsystem.

The phenotype is represented by the abbreviation GP (for glycophorin), followed by a period and an abbreviation of the name of the first propositus or

Table 1. Existing Miltenberger classes and associated antigens

	Antigens									
	Vw	Hut	Mur	MUT	Hil	TSEN	MINY	Hop	Nob	DANE
MNSs:*	9	19	10	35	20	33	34	26	27	32
Mi.I	+	0	0	0	0	0	0	0	0	0
Mi.II	0	+	0	+	0	0	0	0	0	0
Mi.III	0	0	+	+	+	0	+	0	0	0
Mi.IV	0	0	+	+	0	+	+	+	0	0
Mi.V	0	0	0	0	+	0	+	0	0	0
Mi.VI	0	0	+	+	+	0	+	+	0	0
Mi.VII	0	0	0	0	0	0	0	0	+	0
Mi.VIII	0	0	0	0	0	NT	0	+	+	0
Mi.IX	0	0	+	0	0	0	0	0	0	+
Mi.X	0	0	0	+	+	0	+	0	0	0
Mi.XI	0	0	0	NT†	0	+	+	0	0	0

*MNS blood group system number assigned by the ISBT Working Party for the Terminology of Red Cell Surface Antigens[10]
†Not tested

familiar name (Table 2). In most cases, the initial or abbreviation represents one of the significant antigens and is therefore more user-friendly than a subsystem number. A period after GP is used to distinguish the phenotype from the notation for the glycophorin variant. The antigen profiles for GP.Vw and GP.Hut phenotypes can be defined simply as Vw+ and Hut+, respectively, since these antigens are unique to these phenotypes. However, since the specific antigens associated with the other phenotypes are shared by more than one Miltenberger class, our suggestion is to document the minimum number of antigens needed to distinguish the phenotypes. The antigens can be recorded as symbols or as International Society of Blood Transfusion (ISBT) numbers.[10] Documentation of the absence of some antigens as well as the presence of other antigens is required to distinguish some phenotypes. The suggested serological phenotypes shown in Table 2 are those most easily determined in the authors' laboratories. Other phenotypes might be used if other antibodies were readily available. For example, GP.Dane could be precisely defined as DANE+ Mg– if anti-DANE were available.

Using this terminology, it is easy to cluster the phenotypes, based on their constituent GP hybrid molecules (Table 3). GP(A–B) and GP(B–A) hybrid

Table 2. Suggested terminology for Miltenberger phenotypes

First propositus	Shorthand symbol		Serological phenotype	
	Current	Proposed	Symbolic	Numeric
Vw	Mi.I	GP.Vw	Vw+	MNS:9
Hut	Mi.II	GP.Hut	Hut+	MNS:19
Mur	Mi.III	GP.Mur	Mur+Hil+Hop–	MNS:10,20,–26
Hop	Mi.IV	Gp.Hop	Mur+Hil–Hop+	MNS:10,–20,26
Rog	Mi.V	GP.Hil*	Mur–Hil+MUT–	MNS:–10,20,–35
Bun	Mi.VI	GP.Bun	Mur+Hil+Hop+	MNS:10,20,26
Nob	Mi.VII	GP.Nob	Hop–Nob+	MNS:–26,27
Joh	Mi.VIII	GP.Joh	Hop+Nob+	MNS:26,27
Dane	Mi.IX	GP.Dane	Mur+DANE+MUT–	MNS:10,32,–35
HF	Mi.X	GP.HF	Mur–Hil+MUT+	MNS:–10,20,35
JR	Mi.XI	GP.JL†	Mur–TSEN+	MNS:–10,33

*The familiar name instead of the name of the first propositus is used for the Mi.V phenotype.
†The molecular basis of this phenotype was first determined on JL.

Table 3. Phenotypes arranged according to their constituent glycophorins

Gene	Glycophorin	Phenotype symbol	Novel antigens on RBCs
GYP(A–B)	GP(A–B)	GP.MEP (En[a–]UK)	None known
		GP.Hil (Mi.V)	Hil, MINY
		GP.JL (Mi.XI)	TSEN, MINY
		GP.TK	SAT
GYP(B–A)	GP(B–A)	GP.Sch (St[a+]M^{r})	St^{a}
		GP.Dantu	Dantu
GYP(A–ψB–A)	GP(A–B–A)	GP.Vw (Mi.I)*	Vw
		GP.Hut (Mi.II)*	Hut, MUT
		GP.Nob (Mi.VII)	Nob
		GP.Joh (Mi.VIII)*	Nob, Hop
		GP.Dane (Mi.IX)	Mur, DANE
	GP(A–A)	GP.Zan (St[a+]M^{z})	St^{a}
GYP(B–ψB–A–B)	GP(B–A–B)	GP.Mur (Mi.III)	Mur, MUT, Hil, MINY
		GP.Bun (Mi.VI)	Mur, MUT, Hop, Hil, MINY
		GP.HF (Mi.X)	MUT, Hil, MINY
		GP.Hop (Mi.IV)†	Mur, MUT, Hop, TSEN, MINY
GYPA nucleotide substitution‡	GPA	GP.EBH	ERIK, St^{a}

*Previously thought to be the consequence of a nucleotide substitution, but now considered more likely to be due to gene conversion resulting in GP(A–B–A) hybrid molecules.[15] GP.Vw and GP.Hut could also, theoretically, arise from a single nucleotide substitution in the *GYPA* gene.
†Deduced from antigen typings.
‡Gene results in more than 1 transcript.

molecules arise from a single homologous crossover between *GYPA* and *GYPB* genes on misaligned chromosomes. At the protein level (but not necessarily at the DNA level), GP.Hil and GP.JL are reciprocal products to GP.Sch[3] (previously the M^r type of St[a+]), while GP.TK is the reciprocal product to GP.Dantu (C-H Huang and OO Blumenfeld, personal communication). No novel blood group antigens have been associated with GP.MEP, and the expected reciprocal GP(B–A) hybrid molecule has not been found. GP(A–B–A) and GP(B–A–B) hybrid molecules most probably arise through gene conversion.[3]

Further Details of Some Phenotypes

When the biochemical structure of the variant glycophorin has been determined, this information could be used in conjunction with the identifying symbols of the first propositus. For example, the GP.Bun phenotype, which is associated with a GP(B–A–B) hybrid molecule, can be represented as GP.(B–A–B)Bun or, if the Ss status is important, as GP.(B–A–B^S)Bun. A more extended phenotype description is unlikely to be useful but, if the glycophorin or its gene has been sequenced, the contributions of each parent glycophorin can be indicated to describe the glycophorin molecule, e.g., GP(B–A–B^{Thr29})(A58, B27)Bun; GP(B–ψB–A–B^{Thr29})(A58,B27)Bun; or, if the pseudo GPB to GPA junction is known, GP(B–ψB–A–B)(B28,A56;A59,B27). Thr^{29} (or Met^{29}) written as a superscript denotes amino acid 29 of the parent glycophorin B, which would normally express s (or S) antigen. Information in the second set of parentheses represents amino acid involved in junctions (determined or deduced) between the two glycophorins; the junction is denoted by a comma. If detail is not required, this glycophorin could be referred to as GP.Bun or GP(B–A–B)Bun. The terminology described above uses numbers for amino acid residues associated with the parent glycophorin molecules. Each variant glycophorin molecule can also be represented by the amino acid residues within it (Table 4).

Different genetic events can generate the same amino acid sequence and therefore give rise to the

Table 4. Phenotypes arranged according to their constituent glycophorin hybrid molecules and terminology to denote glycophorin alteration

Molecular basis	Glycophorin	Phenotype symbol	Associated novel antigens	Glycophorin alterations‡	Variant glycophorins§
GYP(A–B)	GP(A–B)	GP.MEP(En[a–]UK)	none known	GP(A–B)	GP(A–B)MEP
		GP.Hil(Mi.V)	Hil, MINY	GP(A–B^{Thr29}) (A58,B27)	GP(A^{1-58}–B^{59-104})Hil
		GP.JL(Mi.XI)	TSEN, MINY	GP(A–B^{Met29}) (A58,B27)	GP(A^{1-58}–B^{59-104})JL
		GP.TK	SAT	GP(A–B) (A70,B39)	GP(A^{1-70}–B^{71-104})TK
GYP(B–A)	GP(B–A)	GP.Sch(M^r)	St^a	GP(B–A) (B26,A59)	GP(B^{1-26}–A^{27-99})Sch
		GP.Dantu	Dantu	GP(B–A) (B38,A71)	GP(B^{1-38}–A^{39-99})Dantu
GYP(A–ψ–B–A)	GP(A–B–A)	GP.Dane(Mi.IX)	Mur, DANE	GP(A–B–A) (A34,B;B,A42)	GP(A^{1-34}–B^{35-40}–A^{41-131})DANE
		GP.Nob(Mi.VII)	Nob	GP(A–B–A) (A48,B49;A52,A53)	GP(A^{1-48}–B^{49-52}–A^{53-131})Nob
		GP.Joh(Mi.VIII)	Nob, Hop	GP(A–B–A) (A48,B49,A50)	GP(A^{1-48}–B^{49}–A^{50-131})Joh
		GP.Vw(Mi.I)	Vw	GP(A–B–A) (A27,$B28^{Lys->Met}$,A29)	GP(A^{1-27}–B^{Met28}–A^{29-131})Vw
		GP.Hut(Mi.II)	Hut, MUT	GP(A–B–A) (A27,B28,A29)	GP(A^{1-27}–B^{28}–A^{29-131})Hut
*	GP(A–A)	GP.Zan(M^z)	St^a	GP(A–A) (A26,A59)	GP(A^{1-26}–A^{27-99})Zan.t1.15
			none known	GP(A–A) (A26,A72)	GP(A^{1-26}–A^{27-86})Zan.t2
GYP(B–ψ–B–A–B)	GP(B–A–B)	GP.Mur(Mi.III)	Mur, MUT, Hil, MINY	GP(B–A–B^{Thr29}) (ψB,A49;A58,B27)	GP(B^{1-48}–A^{49-57}–B^{58-103})Mur
		GP.Bun(Mi.VI)	Mur, MUT, Hop, Hil, MINY	GP(B–A–B^{Thr29}) (ψB,A56;A58,B27)	GP(B^{1-50}–A^{51-57}–B^{58-103})Bun
		GP.HF(Mi.X)	MUT, Hil, MINY	GP(B–A–B^{Thr29}) (ψB,A35;A58,B27)	GP(B^{1-34}–A^{35-58}–B^{59-104})HF
		GP.Hop(Mi.IV)†	Mur, MUT, Hop, TSEN, MINY	GP(B–A–B^{Met29}) (A58,B27)	GP(B^{1-50}–A^{51-57}–B^{58-103})Hop
GYPA nucleotide substitution*	GPA GP(A–A)	GP.EH	ERIK St^a	GPA(59Gly–>Arg) GP(A–A) (A26,A59)	GPA^{Arg59}EBH.t1.37 GP(A^{1-26}–A^{27-99})EBH.t2.15

Note: If GP.Nob, GP.Joh, GP.Vw, and GP.Hut also arise from nucleotide substitutions, they would be written as follows:

GP.Nob	GPA(49 Arg–>Thr;52Thr–>Ser)	$GPA^{Thr49,Ser52}$Nob
GP.Joh	GPA(49Arg–>Thr)	GPA^{Thr49}Joh
GP.Vw	GPA(28Thr–>Met)	GPA^{Met28}Vw
GP.Hut	GPA(28Thr–>Lys)	GPA^{Lys28}Hut

*Genes that result in more than 1 transcript.[11,12]
†Deduced from antigen typings.
‡Numbers refer to amino acids derived from the parent glycophorin molecules.
§Superscript numbers represent residue number in variant glycophorin.

same blood group antigen. Thus, different glycophorin molecules have been shown to carry the same novel antigen. For example, the Hil antigen is found on the GP(B–A–B) hybrid molecules associated with GP.Mur (Mi.III), GP.Bun (Mi.VI), and GP.HF (Mi.X), as well as on the GP(A–B) hybrid molecules that bear its name, GP.Hil (Mi.V). In each case, the amino acid sequence that gives rise to the novel antigen is identical.

A Single Genomic DNA Sequence That Can Encode More Than One mRNA Transcript

Some genetic events lead to genes that generate more than one mRNA transcript from a single sequence of DNA; in such cases, the glycophorin product of one transcript can carry one antigen, and the glycophorin product of a second transcript can carry a different antigen. For example, the nucleotide substitution with the *GYPA* gene found in GP.EBH people results in two distinct glycophorin molecules in the red cell membrane: transcript 1 (t1) encodes a GPA with one amino acid substitution that is responsible for the low-incidence antigen ERIK,[12,13] while transcript 2 (t2) encodes a shortened form of GPA that carries the St^a antigen. The same gene is responsible for two other transcripts, but the third transcript (t3) and a fourth transcript (t4) do not encode a membrane-bound protein.[12] The novel antigen characteristic of the protein encoded by a particular transcript can be designated by the ISBT blood group antigen number after the period following the transcript number (Table 4). For example, St^a antigen is expressed by GP(A^{1-26}–A^{27-99}) Zan.t1.15 in GP.Zan (previously called M^Z) and by GP(A^{1-26}–A^{27-99}) EBH.t2.15 in the recently reported GP.ERIK phenotype (Table 4).

Glycophorins From Different Species

As suggested by Huang et al.,[3] when referring to glycophorins from different species, the initial of that species can precede the glycophorin abbreviation. Thus, HGP is used for human glycophorin, CGP for chimpanzee, and OGP for orangutan, etc.

Identical Hybrid Glycophorins Arising From Different Intronic Crossover Sites

Huang and colleagues[14] have reported that GP.Sch (M^r) can arise from recombination events at more than one site in intron 3. These investigators have named the three known ones: type A, type B, and type C. No doubt there will be others, and when they are found we recommend that this terminology precedent be followed.

Conclusion

It is apparent that this proposed new terminology abolishes the concept of a subsystem. However, since all antigens in the Miltenberger subsystem are carried on altered forms of glycophorin A and glycophorin B, they clearly belong in the MNS blood group system and, in fact, all possess ISBT numbers within the MNS blood group system. Furthermore, information from biochemical and molecular biological studies provides explanations for most of the serologic relationships. The proposed terminology is flexible and can be applied to describe other phenotypes. It may be particularly helpful in cases in which one antigen arises from more than one genetic event. For example, GP.Sch, which carries the St^a antigen, is a GP(B–A) hybrid molecule whose alteration can be denoted as GP(B–A)(B26, A59)Sch. A second mechanism that gives rise to a glycophorin molecule carrying the St^a antigen is associated with a nucleotide substitution in the *GYPA* gene.[12] This type, GP.EBH, has been mentioned above. The third mechanism is one of gene conversion, as seen in GP.Zan.[11] The GP.Zan molecule is encoded by a *GYP(A–pseudo B–A)* gene that gives rise to a shorter form of glycophorin A:GPA(A26,A59) Zan that carries the St^a antigen.

The proposed terminology relates to the altered gene, the variant glycophorin, and the novel antigens associated with it. As with the Miltenberger terminology for phenotypes, the new terminology does not address the contribution of blood group antigens encoded by normal glycophorin genes on the same partner chromosomes.

Acknowledgments

We are grateful to O. Blumenfeld, G. Daniels, C. Green, J. Poole, and C-H Huang for their helpful comments and suggestions. We thank B. Thompson for typing the manuscript.

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