Review

JMH blood group system: a review

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Dedication

This review is dedicated to John J. Moulds, MT(ASCP)SBB, as he was instrumental in bringing a group of antibodies with interesting serologic characteristics to the blood group it is today. In the classic "Moulds" way throughout his life and career, he always challenged young SBBs and laboratory scientists to question unusual serologic results. He would say, "They are telling you something." The JMH blood group system is a testament to his beliefs. He is coauthor on many papers in this review. John called me to provide additional information he found important to the JMH story. I am honored and privileged to have known John, to have been challenged by John, and to write this review as a tribute to his work.

The JMH blood group system consists of six high-prevalence antigens. These antigens are located on the Sema7A protein. The molecular basis of the JMH1– phenotype is not known; however, single nucleotide changes in the *SEMA7A* gene on chromosome 15 account for the other JMH antigens. JMH1, commonly known as JMH, is most notable because transient depression of the antigen occurs and anti-JMH may develop. These antibodies are most commonly observed and are not significant in transfusion. Antibodies developed in the rare JMH variant types may cause reduced red cell survival. This review provides a general overview of the JMH blood group system, including the serologic and molecular characteristics as well as proposed functions of the Sema7A protein. *Immunohematology* 2014;30:18–23.

Key Words: John Milton Hagen group, semaphorin 7A (CDw108 glycoprotein), *SEMA7A* gene, high-prevalence red cell blood group antigen

Introduction

The JMH blood group system has the distinction of being the blood group with the most "nicknames." The "over 60 group," "John Milton Hagen group," "The Boys," "The Cat," and the "Old Boys' Club" have all been used in reference to the antibody and antibody makers.[1] The earliest accounts in the 1970s were of antibody reactivity, followed by description of the protein carrying the antigens (semaphorin 7A or CD108), and finally with identification of the gene *SEMA7A,* JMH earned its rightful status of blood group system 026 in 2001.

Antibodies to JMH antigens are encountered infrequently but have unique characteristics landing them their nicknames. The "over 60 group," "The Boys," and the "Old Boys' Club" all are derived from early observations that many of the antibodies were found in older gentleman. Older has been defined as >50 or 60 years of age. Like Issitt,[2] I concur that old is recognized as mature, wiser, and experienced patients. Not to leave the ladies out of describing these antibodies, the nickname "The Cat" came from one of the first women who produced anti-JMH. She is said to have claimed the anti-JMH occurred when her cat died (Marilyn Moulds, March 2012, personal communication).

The significance of antibodies to JMH antigens in transfusion and pregnancy are minimal and will be discussed.

History

In March 1973, a male patient in his 60s was to have elective orthopedic surgery. He had no history of transfusions. A weakly reactive antibody detected by the indirect antiglobulin test (IAT) was not reactive with papain- or ficin-pretreated cells. No compatible blood was available, and units collected for autologous transfusions were never given. It was reported as an "antibody to high-incidence unknown factor," and samples were sent to other laboratories for investigation. This result led to a group of antibodies being collected in the 1970s that were compatible with this individual's red blood cells (RBCs) and had similar reactivity. They were first mentioned in print by Issitt in 1975 as the John Milton Hagen group of antibodies, as he termed them "belonging to a group of high-incidence antigens of which little is known about." His remarks were based on personal communication with John J. Moulds.[3] The first antibody was reported to be recognized in 1970 per Sabo et al.,[4] who further characterized 49 sera with similar reactivity and proposed giving this antibody the symbol JMH, naming it after one of the original antibody makers, and adding it to the list of high-titer, low-avidity antibodies. These antibodies were of high titer and weakly reactive in saline IAT with all RBCs tested, except autologous cells and other JMH– RBCs.

In the 1980s, work was done to further define the serologic characteristics of anti-JMH and its reactivity with chemically modified RBCs, namely those treated with proteases, sulfhydryl-reducing agents, and neuraminidase. In addition, attempts were made to predict the clinical significance of anti-JMH in transfusion using chromium-51 RBC survival studies and subclassing.[5] In 1982, a monoclonal antibody named H8 was described with JMH specificity.[6,7] This antibody was important to further work in characterizing the JMH protein. In addition, J.J. Moulds reported evidence that there was heterogeneity in reactivity of different anti-JMH.[8]

Telen et al.[9] reported in 1990 that several high-incidence antigens including the JMH antigen were absent on RBCs of individuals with paroxysmal nocturnal hemoglobinuria (PNH). PNHIII RBCs were previously shown to lack glycosylphosphatidylinositol (GPI)-linked proteins, and when these RBCs were tested with human anti-JMH and monoclonal H8, no reactivity was seen. This result suggested that JMH must reside on a GPI-linked membrane protein. Immunoprecipitation and immunoblotting experiments on human anti-JMH and H8 showed JMH resides on a 76-kD phosphatidylinositol-linked protein.[10] Finally, the location of JMH was determined to be on the CDw108 glycoprotein, now known as semaphorin 7A.[11]

Having knowledge of the location of JMH, the cDNA clone containing the *CDw108* gene was identified in 1999.[12] The gene resides on the middle of the long arm of chromosome 15. This *CDw108* gene is now known as *SEMA7A* and is located on 15q23–24. The genetic information earned JMH its own blood group system in 2001 named John Milton Hagen, 026, and its symbol JMH.[13] JMH1 is the antigen detected by most individuals making anti-JMH. Four additional variant JMH genotypes were added in 2007.[14] JMHQ was proposed in 2011, reported in four Native Americans whose RBCs were JMH1–, with most examples of anti-JMH, and officially recognized later that year.[15]

Today, there are six recognized antigens in the system summarized in Table 1.[16,17] Each antigen is defined by antibodies nonreactive with JMH1– RBCs.

Table 1. The John Milton Hagen blood group system

Number	Name	Prevalence	Molecular basis of antigen-negative phenotype
JMH1	JMH	High	Not known
JMH2	JMHK	High	619C>T R207W
JMH3	JMHL	High	620G>A R207Q
JMH4	JMHG	High	1379G>A R460H
JMH5	JMHM	High	1381C>T R461C
JMH6	JMHQ	High	1040G>T R347L

Nomenclature

Nomenclature resulting from serologic observations and biologic and molecular studies has remained very simple for the blood group with so many nicknames. The antigen name commonly known by serologists remains JMH and is officially recognized by the International Society for Blood Transfusion (ISBT) as JMH1. Confirmed JMH variants are sequentially numbered for example, JMH2 and named with the first letter from the antibody maker's first name (JMHK) following JMH.

JMH Glycoprotein

Antigens in the JMH blood group system are carried on the protein semaphorin 7A, also known as Sema7A and CD108. Mature Sema7A consists of 525 amino acids. Changes in amino acids 207 and 460/461 were noted by Seltsam in JMH-variant individuals. A three-dimensional model of the crystal structure of Sema7A was proposed (Fig. 1A and B).[14] Position 207 is located at the top face of the sema domain, whereas positions 460 and 461 are on the bottom.

As mentioned previously, Sema7A protein binds to the cell membrane by a GPI linkage. Not only is it present on RBCs, it is present on lymphoid and myeloid cells as well as bone cells, neurons of the brain and spinal cord, thymus, spleen, gut, kidney, heart, and placenta. Sema7A is found primarily in activated T cells and thymocytes.[18]

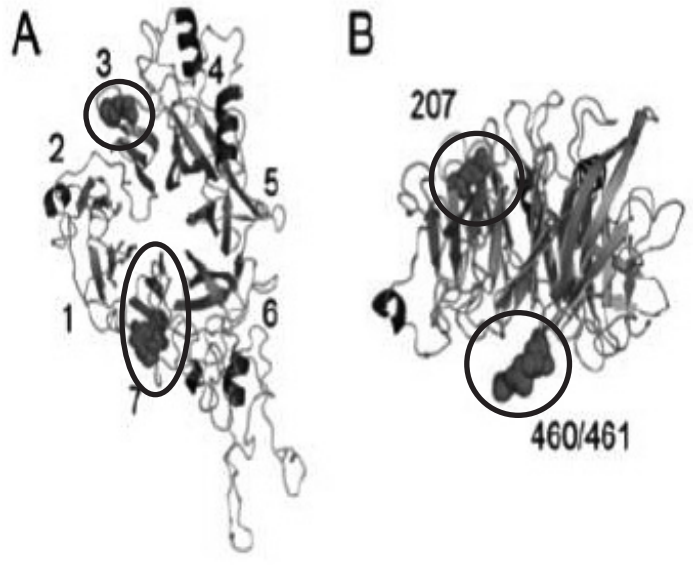

Fig. 1 Three-dimensional model of Sema7A (reprinted with permission from AABB). Image courtesy of A. Setsam. **(A)** A cartoon of the sema domain when looking down on the protein. The numbers represent propellers. **(B)** A side view of the sema domain. The polymorphic amino acid positions 207, 460, and 461 are circled.

Numerous roles for Sema7A have been proposed. It is clear it is involved in cytokine expression, chemotaxis, and axon guidance signaling.[19] In effector T cells, it likely promotes macrophage recruitment to sites of inflammation.[18,20,21] A recent report shows the Sema7A_R461C variant (JMH5) causes different T-cell responses than the wild-type Sema7A.[22] It is able to strongly activate CD4+ T cells and change their phenotype to a more cytotoxic one, and evidence is provided that Sema7A_R461C delivers costimulatory signals to T cells, causing them to increase their release of cytokines such as interleukin (IL)-1β, IL-6, and IL-8.

Sema7A has also been reported to be an important regulator of tissue remodeling. In genetic studies in Korean women with decreased bone mineral density, mutations in *SEMA7A* were found.[23] Sema7A also contributes to regulation of tissue fibrosis and remodeling.[24]

The exact function of semaphorin 7A on RBCs is not known, but it likely plays a role in cell migration as an adhesion molecule.[25] It may also play a role as a receptor for *Plasmodium falciparum*. It was identified as a receptor for the *P. falciparum* merozoite-specific thrombospondin-related anonymous protein (TRAP) homolog in laboratory experiments using recombinant protein.[26]

JMH Antigen Characteristics

JMH1 is the primary antigen in the system and is present in greater than 99 percent of all individuals. The JMH1-negative phenotype occurs as either inherited or more commonly acquired depression of the antigen. The JMH antigen is most commonly depressed in a transient manner. In these individuals, the antigen is often below the level of detection by standard serologic methods and they make anti-JMH. This result likely explains the serologic observation of a positive direct antiglobulin test (DAT) seen in many individuals with anti-JMH. There has been no genetic change identified in the *SEMA7A* gene of these individuals.[14] The transient phenomenon may be triggered by an autoimmune process like that seen in other blood group systems.

There is only one family described with the inherited negative phenotype identified when randomly screening donors. In this family, it was shown that three generations of individuals possessed JMH– RBCs, consistent with autosomal dominant inheritance.[27] None had made anti-JMH, and all had a negative DAT.

Rare JMH variants have been described with reduced or variable expression of JMH antigen (Table 1).[14] All possess an antibody that is not reactive with JMH1– RBCs. However, variable reactivity is seen when the specificity of each antibody is cross-tested with other JMH variants (Table 2).[28] JMH antigens are destroyed by various chemicals, as shown in Table 3, characteristic of other blood group antigens on GPI-linked proteins. PNHIII cells can be used as a source of JMH– RBCs as well.[29]

Table 2. Anti-JMH and JMH-related antibodies tested with JMH– and variant JMH red blood cells

	Anti-					
Phenotype	JMH1	JMH2	JMH3	JMH4	JMH5	JMH6
JMH:-1	0	0	0	0	0	0
JMH:-2	+	0	0	+	+	NA
JMH:-3	+	0	0	+	+	NA
JMH:-4	+	+	+	0	+	NA
JMH:-5	+	+	+	0	0	NA
JMH:-6	+	NA	NA	NA	NA	NA

NA = not available.
Adapted from Daniels.[27]

Table 3. Characteristics of antigens in the John Milton Hagen blood group system

Sensitive to ficin or papain treatment
Sensitive to trypsin and alpha chymotrypsin treatment
Sensitive to 2-aminoethyl-isothiouronium bromide and 200 mmol/L/ 50 mmol/L dithiothreitol treatment
Sensitive to sialidase treatment
Resistant to chloroquine treatment
Absent from paroxysmal nocturnal hemoglobinuria-III red blood cells
Weakly expressed on cord cells

The *SEMA7A* Gene: Genetics and Inheritance

SEMA7A is located on 15q22.3-q23, the middle of the long arm of chromosome 15 (GenBank accession number BC101647), and is organized in 14 exons. To date, no nucleotide changes have been identified in individuals with JMH– RBCs. JMH variants result from changes in nucleotides in exons 6 and 11 (Table 1).

Rare individuals who lack Sema7A on their RBCs possess Sema7A on other cells. Given the information provided from molecular analysis performed to date, this is likely because of a posttranscriptional mechanism.[14]

JMH Antibodies

Anti-JMH is found most often as the acquired form and as such can be found in individuals with no prior transfusions

or pregnancies. JMH antibodies are weakly reactive in a saline IAT. All RBCs tested will show positive reactivity. Using a scale of 0 to 4+, weak macroscopic to 2+ reactivity is common. More consistent, stronger positive reactivity may be seen when testing donor RBCs using column agglutination or solid-phase methods. All anti-JMH, whether found in JMH– or JMH-variant individuals, are negative with JMH:-1 RBCs.

Some JMH antibodies made by JMH-variant individuals are nonreactive with other JMH variants (Table 2). These individuals will not have a positive DAT or positive autocontrol, as seen in the majority of individuals with depressed JMH antigen.

As mentioned previously, PNHIII cells may also be used as a source of JMH– selected cells, as they lack all GPI-linked proteins.[29] The characteristic finding is the autocontrol being weakly positive and weaker than the antibody reactivity with panel cells. The DAT is also weakly positive with polyspecific anti-human globulin (AHG) and anti-IgG. Interestingly, the eluate is usually negative, but there are rare reports of anti-JMH in the eluate.[30]

JMH antibodies are predominantly IgG4 subclass.[31–33] A rare example of a presumed significant IgG3 anti-JMH has also been described.[34] The IgG subclass is important to keep in mind when identifying anti-JMH. Some AHG reagents on the market lack anti-IgG4. If using this AHG reagent, no reactivity will be observed in any IAT regardless of methodology. Additional characteristics of JMH antibodies are summarized in Table 4.

Soluble recombinant JMH proteins have been produced that will inhibit anti-JMH.[14] These soluble proteins are not available commercially at this time. However, they could be useful in confirming anti-JMH specificity as well as in ruling out underlying alloantibodies.

A novel approach to detecting anti-JMH is through the use of particles coated with purified, recombinant Sema7A protein in a gel card format.[35] If recombinant proteins could be manufactured for all common blood group antigens, they could replace the need for donor RBCs. This is an ongoing area of investigation.

Table 4. Characteristics of antibodies to John Milton Hagen antigens

Immunoglobulin G (IgG)
Usually IgG4-negative with anti-human globulin lacking IgG4 anti-IgG
Usually weakly reactive (<2+) in test tube methods
Nonreactive with papain or ficin-treated RBCs
Nonreactive with trypsin or alpha chymotrypsin
Reactive with chloroquine-treated RBCs
Nonreactive with 200 mmol/L/50 mmol/L dithiothreitol treatment or 2-aminoethyl-isothiouronium bromide-treated RBCs
Not inhibited with pooled normal serum
Do not bind complement
Antibodies may be transient (detectable when individuals RBCs have reduced expression of antigen)
Do not cause hemolytic disease of the fetus and newborn

RBC = red blood cell.

Clinical Importance

The acquired type JMH-negative individuals producing anti-JMH have not been associated with adverse transfusion episodes.[36] It is routine transfusion practice today to give crossmatch-incompatible blood to these patients.

Rare, inherited-variant JMH-negative individuals who have made anti-JMH have been associated with decreased RBC survival.[37] One additional patient, later confirmed to be a JMH variant by molecular analysis, was also reported to experience an acute hemolytic transfusion reaction.[14,38]

Another report of an IgG3 antibody and a positive monocyte monolayer assay suggested the antibody was significant, but the patient did not require transfusion.[34] Molecular studies were not performed to determine whether this was a JMH-variant individual.

Very little is known about anti-JMH in pregnancy because most individuals with anti-JMH are older women or men. In addition, JMH is very weakly expressed on cord cells. One example of a 32-year-old pregnant woman with a JMH-weak phenotype and anti-JMH gave birth to a baby with no evidence of hemolytic disease of the fetus and newborn.[14]

Conclusions

The John Milton Hagen blood group system is fairly straightforward, but it took more than 30 years to organize serologic observations of the original group of antibody makers to determine its biochemistry and molecular genetics, and to it finally being named its own blood group system, 026. Investigation of this system, led by the work of John J. Moulds, taught us the value of sharing rare RBCs and fluids to further identify unusual antibodies and to fully evaluate antibody identification results that do not make sense. To build on his legacy, readers are encouraged to continue to question unusual serologic results and use the molecular tools available to find new, interesting, and exciting findings to add to this system and other blood group systems, or perhaps to discover a new system.

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