TECHNICAL NOTE

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Gm(11) Grouping of Dried Bloodstains

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ABSTRACT: An absorption inhibition method for the detection of gamma marker Gm(11) in dried bloodstains is described. Particular reference is made to the association of Gm(11) with Gm(−1,−2). When a dried bloodstain fails to inhibit anti-Gm(1) and anti-Gm(2), this may represent a true Gm(−1,−2) result or there may be insufficient material to inhibit either antibody. The detection of Gm(11) in a bloodstain extract provides an objective means of confirming the apparent absence of Gm(1) and Gm(2) as representing a true Gm(−1,−2) result. This antigen compares very well with other blood group systems with regard to the amount of bloodstain required for analysis and its stability. No evidence is available for preferential loss of Gm(1) and Gm(2) relative to Gm(11) in dried bloodstains.

KEYWORDS: criminalistics, genetic typing, blood, Gm blood grouping, bloodstain, Gm(11), anti-Gm(11), absorption inhibition, gamma marker

Immunoglobulin G (IgG) molecules are composed of two heavy chains (γ) (molecular weight [MW] ~ 55 000) and two light chains (MW ~ 20 000). The γ chains occur in four immunological isotypes or subclasses (γG1, γG2, γG3, γG4) and the light chains in two subclasses (Kλ). A heavy chain consists of approximately 446 amino acid residues and a light chain of 214 residues. In any one individual, the frequency of heavy chain subclasses is approximately 71% G1, 18% G2, 8% G3, and 3% G4.

The gamma marker (Gm) allotypes or blood groups are a system of inherited antigenic determinants present on constant domains of heavy chains, the γ-polypeptide chains of immunoglobin G(IgG) molecules, and are specific for one IgG subclass. There are now about 27 different, clearly recognized antigens in the Gm system. Gm(11) is present in IgG Subclass 3. Grubb and Laurell were the first to describe Gm genetic marker system [*1*]. The applicability of Gm typing in dried blood and physiological fluids has been established [*2,3*]. Attempts have been made in the past to group in the Gm system, but with limited success [*4,5*]. The Gm system is valuable for its stability, discrimination, potential, and usefulness to determine the racial origin of a stain [*6,7*]. Bloodstains have been grouped satisfactorily for Gm(1), Gm(2), and Gm(10) for the past three years in our laboratory system and their importance has been significant. Khalap et al described an absorption inhibition method for

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the detection of Gm(1) and Gm(2) in dried bloodstains [8]. A problem with this technique arises when interpreting an absence of inhibition of both anti-Gm(1) and anti-Gm(2) in dried bloodstains. Does this represent a true Gm(−1,−2) result or was there insufficient IgG to inhibit either antibody? Subjective criteria that help differentiate between these two interpretations (such as apparent amount of stain) must be used with great caution. Grubb stated that some Gm factors, such as Gm(5) and Gm(10), are always present in the serum of Gm(−1,−2) individuals and they can be used as positive markers for Gm(−1,−2) [9]. Khalap and Divall described a procedure for the detection of Gm(5) as means to confirm Gm(−1, −2) results [10]. Khalap and Divall also described a procedure for Gm(10) detection in seminal stains as a positive marker with very successful results [11]. Gm(11) is part of the Gm^b complex. It is commonly associated with Gm(5) in all races. Gm(5) and Gm(10) both are part of the Gm^b complex. Therefore, Gm(11) can also be used as a positive marker to confirm the true negatives Gm(−1,−2). The purpose of this study is to describe a method for the detection of Gm(11) in dried bloodstains which could be used as a positive marker for identifying (Gm−1,−2).

Material and Methods

Blood samples used in this study were obtained from Interserum Exchange, Chicago. Liquid blood samples received in this laboratory for routine case work were used in this study as sources of whole blood.

Antisera

Anti-Gm(11) and anti-D-Gm(11) (incomplete anti-D) were obtained from Interserum Exchange, Chicago.

Indicator Cells

Group O, homozygous D-positive (R_1R_2) red blood cells were used. Ortho Diagnostics cells were washed three times in saline prior to sensitization.

Sensitization of Indicator Cells

One drop of packed (R_1R_2) indicator cells, one drop of saline, and one drop of undiluted anti-D-Gm(11) were incubated at 37°C for 90 min. The cells were washed three times to remove excess anti-D and used to prepare a 5% suspension in saline. One drop of the suspension was mixed with one drop of anti-human globulin (Ortho Diagnostics) on a glass slide. Strong agglutination indicated the cells had been sensitized. The negative control consisted of one drop of saline and one drop of 5% cell suspension on a glass microscope slide. The control was negative. The sensitized cells were made to a 1% suspension in bovine saline albumin (Ortho Diagnostics) (0.3% bovine serum albumin in isotonic saline) and used in titrations and inhibitions.

Titration of Anti-Gm(11) Sera

Linear dilutions of anti-Gm(11) were made in saline in glass test tubes. One drop of 1% sensitized cells, two drops of 100 m*M* Tris-hydrochloric acid buffered saline (pH 7.5) were added to one drop of diluted antisera. The tubes were shaken, left at room temperature for 75 min, then centrifuged at (3400 rpm/1000 g) for 1 min. The cell buttons were carefully transferred to small drops of saline on a glass plate with the help of a Pasteur pipette and examined under a microscope for agglutination. The highest dilution of antiserum giving

complete agglutination was used as the working dilution of antiserum in subsequent inhibition tests.

Sample Preparation

Blood samples were centrifuged, serum was separated and prepared as a 1-in-15 dilution in saline. Control bloodstains were made from whole blood samples on a cotton bedsheet. The stains were dried at room temperature at least 24 h and then extracted. Pieces of bloodstained material, approximately 5 mm^2 were extracted in eight drops of Tris-hydrochloric acid saline buffer (pH 7.5) at 37°C for at least 3.5 h. The extract was enough to test Gm(1), Gm(2), and Gm(11). Control pieces of material taken adjacent to the bloodstains were also extracted in the same manner as the bloodstains. The extraction was carried out in conical 1.9-mL polypropylene microcentrifuge tubes.

After the extraction, a small hole was made in the attached cap of the microcentrifuge tube with a hot probe. Then the piece of material being extracted was removed with forceps and placed in the top portion of the attached cap and centrifuged at 11 600 rpm for 5 min. This method is used for two reasons. One purpose is to remove as much extract from the piece of material as possible. The other purpose is to remove any insoluble debris. Following centrifugation, the supernatants were transferred to clean tubes by means of double-drawn Pasteur pipettes.

Inhibitions

One drop of diluted test serum (1-in-15) or two drops of bloodstain extract were added to one drop of suitable diluted anti-Gm(11) and the tubes were shaken to mix the contents. Inhibitions using serum samples were left at room temperature for a minimum of 45 min and bloodstain extracts were left overnight (16 h) at 4°C.

After the inhibition period, one drop of indicator cells (1% suspension in saline albumin) was added to each tube. The tubes were shaken and left at room temperature for 75 min and then centrifuged at (3400 rpm/1000 g) for 1 min. The cell buttons were removed and examined for agglutination as described for the titration.

Positive and negative control sera and stain extracts were included in each set of inhibition tests. A control that tested for the presence of antiglobulin antibodies in each serum sample or stain extract was also included. This consisted of one drop of diluted serum or two drops of bloodstain extract, one drop of saline albumin, and one drop of 1% indicator cells. Extracts of stains were diluted 1/100, 1/200, 1/400, 1/600, 1/800, 1/1000, 1/1200, and 1/1400 to check the preferential loss of Gm(1) and Gm(2) relative to Gm(11).

Gm(1) and Gm(2) Typing

Tests for Gm(1) and Gm(2) were made in the same manner as described for Gm(11), using the appropriate antiserum and corresponding anti-D for red cell sensitization.

Results

One-hundred serum samples were used in this study. All the serum samples and their corresponding dried bloodstains were tested for the presence and the absence of Gm(11) antigen. All the phenotypes in serum match with the types obtained from the stains. No false positives and negatives were observed.

All the 43 serum samples and their corresponding stain extracts grouped as Gm(−1,−2) were positive for Gm(11). Antiglobulin antibodies were detected in a few serum samples, but not in the corresponding stain extracts. In this study Gm(11) was detected in up to seven-

month-old stains. Twenty-seven samples and their corresponding stain extracts were typed as Gm(+1,−2,+11) and four were Gm(+1,+2,+11). In diluted extracts Gm(1) and Gm(2) were detected up to 1/800 dilution where as Gm(11) was detected in 1/200 dilution. None of the samples was observed where Gm(1,2) was lost because of dilution, but Gm(11) could be detected. After extraction, the pieces of material were dried and typed for the ABO blood group system by the Howard and Martin [*12*] technique. All the samples were grouped correctly in the ABO system.

Discussion

Gm(11), like Gm(1), Gm(2), Gm(5), and Gm(10) compares very well with other blood group systems with regard to the amount of bloodstain required for analysis, persistence, and stability. Gm(11) can be used in combination with other genetic markers to discriminate between two bloodstains. The greatest value of Gm(11) is in the combination Gm(−1, −2,11). The presence of the positive marker Gm(11) provides an objective means to interpret the absence of inhibition of anti-Gm(1) and anti-Gm(2) as Gm(−1,−2). Gm(1) and Gm(2) are present on IgG Subclass 1 which constitutes 71% of the IgG. In comparison Gm(11) is on IgG Subclass 3 which is only 8% of the IgG. Since Gm blood groups are found on IgG, the greater the percentage of the IgG, the greater the amount of Gm blood group substance present. If Gm(11), which is on 8% of the IgG, causes inhibition and Gm(1), Gm(2), which is on 71% of the IgG, does not cause inhibition, then this is an objective basis to confirm (Gm−1,−2) results. More bloodstain material is required for Gm(5) as compared to Gm(1) and Gm(2) [*11*]. The same is true for Gm(11); that is, more bloodstain material is required as compared to Gm(1) and Gm(2). For this reason, an apparent Gm(−11) must be recorded as inconclusive even though complete inhibition of anti-Gm(1) and anti-Gm(2) may be obtained with the same sample extract.

The stability of Gm(11) was studied and was detected in stains up to seven months old. Since in diluted samples extracts, none of the samples were observed where Gm(1) and Gm(2) were lost because of dilution, but Gm(11) was still present. When the sample extract was diluted, Gm(11) was lost first in relation to Gm(1) and Gm(2). This eliminates the possibility of a Gm(1,2,11) stain degrading to Gm(−1,−2,+11) which would otherwise lead to an erroneous interpretation. Gm(11) is a very useful marker especially in its use as a positive marker for Gm(−1,−2). No evidence is available for preferential loss of Gm(1) and Gm(2) relative to Gm(11) in dried bloodstains.

All 43 serum samples in this study typed as Gm(−1,−2) were Gm(11) positive. ABO blood groups were detected from the same pieces of material after extraction for Gm, which is an added advantage to Gm blood grouping.

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