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Gm Typing by Enzyme-linked Immunosorbent Assay (ELISA)*

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Summary. A solid-phase ELISA for Gm typing is described. A mixture of anti-Gm serum (or monoclonal anti-Gm antibody) and test serum was incubated in microtiter wells coated with IgG or its fragments of appropriate Gm type. After washing of the wells, the bound antibody was detected with peroxidase-labeled second antibody. The Glm(3), G3m(16), and G3m(21) antigens could be identified by this technique. Since some of the human anti-Gm sera and anti-Rh_0 sera required for the conventional hemagglutination-inhibition method are hard to obtain, the ELISA system using anti-Gm antibodies and no anti-Rh_0 sera may serve as an alternative to the conventional method.

Key words: Forensic immunology Gm, ELISA – Enzyme-linked immunosorbent assay (ELISA), Gm

Zusammenfassung. Gm-Typisierung wurde mit ELISA durchgeführt. Eine Mischung aus Anti-Gm-Antikörper und zu untersuchendem Serum wurde in mit IgG oder seinen Fragmenten von passendem Gm-Typus überzogenen Mikrotitertüpfel inkubiert. Nach Waschen der Tüpfel wurde der gebundene Antikörper mit einem enzymisch markiertem zweiten Antikörper nachgewiesen. Bei dieser Teknik waren die Antigene Glm(3), G3m(16) und G3m(21) nachweisbar. Das ELISA-System mittels Anti-Gm-Antikörper ohne Anti-Rh_0-Seren soll den Hämagglutinations-Hemmtest ersetzen, da einige humane Anti-Gm-Seren und Anti-Rh_0-Seren nicht leicht erhältlich sind.

Schlüsselwörter: Forensische Immunologie, Gm-Typisierung (ELISA) – Gm-Typisierung, ELISA

Despite its practical utility in paternity testing, personal identification, and population genetics, Gm typing has not come into widespread use in forensic

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science practice. This is mainly because not all of the anti-Gm sera of human origin and not all the incomplete anti-Rh_0 sera of desired Gm specificities are readily available. The conventional hemagglutination-inhibition method involves the use of anti-Gm sera and anti-Rh_0-coated red blood cells. In testing for the Glm(3) antigen, for instance, a drop (25 µl) of anti-Glm(3) serum and a drop of 1/10 to 1/30 dilution of an unknown serum sample are mixed on a microflocculation slide. After shaking for 10 min, a drop of 0.2% suspension of red cells coated with anti-Rh_0 of the Glm(3) specificity is added. The mixture is shaken for 1–2 h and observed for agglutination. The absence of agglutination indicates the presence of the Glm(3) antigen in the sample.

To cope with the shortage of human anti-Gm sera, we prepared anti-Gm sera by immunizing rabbits with normal IgG subclass proteins and their fragments [5, 6]. To enable Gm typing with these rabbit antisera, even when no anti-Rh_0 antibodies of desired Gm specificities are available, we used indicator red cells to which purified normal IgG subclass proteins were coupled by the chromic chloride method [4]. In this way we could detect the Glm(2), Glm(3), and G3m(21) antigens but no G3m(16) antigen. Moreover, the indicator cells were inferior to the anti-Rh_0-coated cells in sensitivity and in length of shelf life. We felt the need for a typing technique requiring no indicator cells.

Enzyme-linked immunosorbent assay (ELISA) has found wide application in immunologic work. The solid-phase micro-ELISA using microtiter plates is particularly suitable for forensic science practice because of its simplicity. Here we report an ELISA system for Gm typing.

Materials and Methods

Coating and Immunizing Antigens

Human plasma obtained from a blood center of the Japanese Red Cross was Gm-typed by the conventional method. Anti-Rh_0 sera and a panel of Gm standard sera were generously provided by Professor H. Matsumoto, Department of Legal Medicine, Osaka Medical School. IgG of appropriate Gm phenotype was isolated by ammonium sulfate precipitation and DEAE-cellulose column chromatography. IgG subclass proteins were isolated by pH gradient elution of the IgG that had been bound to protein A-Sepharose CL-4B (Pharmacia Fine Chemicals) [1]. $F(ab')_2$ fragments were prepared by digestion of IgG with pepsin [3].

Antisera

Rabbit anti-Gm sera were prepared as described previously [5, 6]. Briefly, anti-Glm(3) serum was prepared by immunization of rabbits with $F(ab')_2$ fragments of IgGl(1, 3). Anti-G3m(16) serum was produced by immunization with IgG3(13, 15, 16). Rabbit anti-Gm antibodies were purified by affinity chromatography on Sepharose coupled with IgG positive for the Gm antigen under study, followed by absorption with IgG negative for that antigen. Peroxidase-labelled anti-rabbit IgG and anti-mouse IgG were purchased from Miles Laboratories.

Monoclonal Antibody

Monoclonal anti-G3m(21) antibody was produced by a modification of the hybridoma technique described by Galfre et al. [2], as reported elsewhere [7]. Since the monoclonal antibody had an extremely high titer (1:32,000) against a 0.2% suspension of anti-Rh_0-coated red cells, it was diluted to have a titer of 1:4 before use.

Solid-Phase Micro-ELISA

Twenty samples each of Gm-positive and Gm-negative standard sera were Gm-typed by a solid-phase micro-ELISA derived from the work of Voller et al. [8]. All the steps below were carried out at room temperature. Microtiter wells were coated for 30 min with 100 µl of a dilute solution (20 µg/ml) in 0.05 *M* carbonate buffer, pH 9.6, of IgGl $F(ab')_2$(3), IgG(1, 13, 15, 16), or IgG3(21). The wells were washed three times with 0.05% Tween 20 in 0.01 *M* phosphate-buffered saline (PBS-Tween), pH 7.4. To each well were added 80 µl of a 1:1 mixture of an appropriate dilution of anti-Gm solution and a 1:10 dilution of a test serum in PBS-Tween. After incubation for 30 min, the wells were rinsed with PBS-Tween. Eighty microliters of a 1:400 dilution of enzyme-labeled second antibody in PBS-Tween was added to each well and incubated for 30 min. After washing of the wells, 80 µl of the substrate mixture containing 0.0028*M* *o*-phenylenediamine and 0.006% hydrogen peroxide in 0.05*M* phosphate-0.024*M* citrate buffer, pH 5.0, was added to each well. After incubation in the dark for 15 min, the enzymic reaction was stopped by addition of 80 µl of 12.5% sulfuric acid. The absorbance of the colored product was measured on a Micromini Reader (Dynatech) at 490 mm, and the percentage of inhibition for each sample was calculated as follows:

$$\% \text{ inhibiton} = \frac{AT - AS}{AT}$$

where AS and AT are the absorbances for the wells receiving the serum sample and PBS-Tween instead, respectively.

Results and Discussion

A preliminary study showed that the optimal titers of the anti-Gm reagents for use in our ELISA system were 1:4 against a 0.2% suspension of anti-Rh_0-coated cells.

Figure 1 represents a graph in which % inhibition values in a typical ELISA for Glm(3) typing are indicated. Glm(3)-positive samples gave values of more than 54.5%. In contrast, the % inhibition values for Glm(3)-negative samples were less than 35.7%. Thus, the positive and negative samples could be clearly distinguished from each other. Similar results were obtained with the rabbit anti-G3m(16) serum (Fig. 2). Repeated ELISAs gave similar results.

With the monoclonal anti-G3m(21) antibody, G3m(21)-positive samples showed a more striking contrast with G3m(21)-negative samples than with the

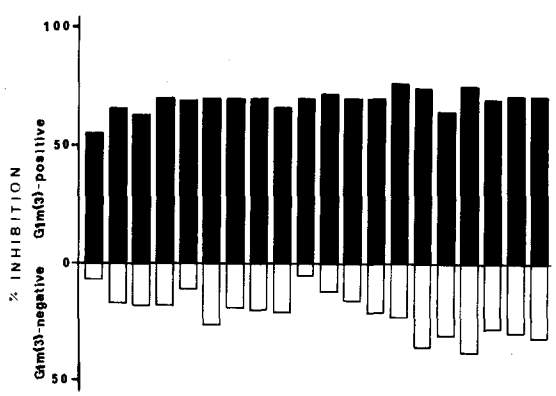

Fig. 1. ELISA for Glm(3) using rabbit anti-Glm(3). Mean value for positive sample = 68.95 ± 4.94, mean value for negative sample = 21.26 ± 9.14

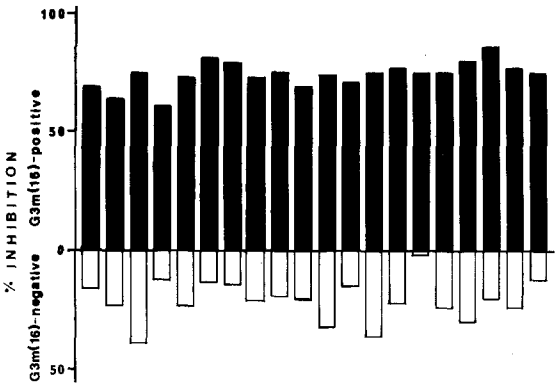

Fig. 2. ELISA for G3m(16) using rabbit anti-G3m(16). Mean value for positive sample = 74.14 ± 5.61, mean value for negative sample = 20.67 ± 8.91

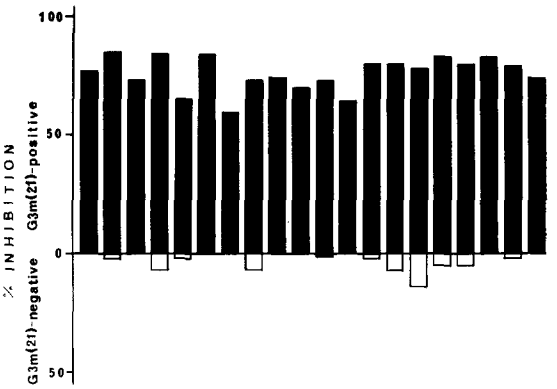

Fig. 3. ELISA for G3m(21) using monoclonal anti-G3m(21). Mean value for positive sample = 76.35 ± 7.25, mean value for negative sample = 2.68 ± 3.68

rabbit anti-Gm sera (Fig. 3). The % inhibition values for G3m(21)-positive samples were more than 58.7% and those for G3m(21)-negative samples were less than 14.1%. This was probably due to a minimal degree of nonspecific adsorption of the antibody. Moreover, the monoclonal antibody gave much more reproducible results than the rabbit antisera.

Since the Japanese have 9 Gm phenotypes resulting from the $Gm^{1,21}$, $Gm^{1,2,21}$, $Gm^{1,3,5,13}$, and $Gm^{1,13,15,16}$ haplotypes, routine Gm typing in this population can be performed, if anti-Glm(2), either anti-Glm(3) or anti-G3m(5), anti-G3m(16), and anti-G3m(21) antibodies are available. The corresponding antigens, except Glm(2), could be identified by the ELISA described here. These results suggest that the ELISA system, which requires no anti-Rh_0 coats, may serve as an alternative to the conventional hemagglutination-inhibition method.

A weak point of this ELISA is the much larger consumption of anti-Gm antibody than the conventional method for the following reasons. Appropriate dilutions of anti-Gm whole serum can be used in the conventional method. In the solid-phase ELISA, however, antiserum should be affinity-purified to minimize nonspecific adsorption of rabbit IgG to the well. The recovery of the antibody is rather low.

In ELISAs for isotypic antigens, very high dilutions of whole serum can be used because of its extremely high antibody titer so that nonspecific adsorption is negligible in the presence of Tween 20. On the other hand, rabbit anti-Gm sera have agglutinin titers of only 256 or less against a 0.2% suspension of anti-Rh_0-coated cells and cannot be used at high dilutions. In addition, a larger volume of antibody solution per test serum is needed than in the conventional method.

Thus, high-titered, monospecific anti-Gm sera must be produced for Gm typing by ELISA. Since monoclonal antibodies promise to become the anti-Gm reagents of choice for ELISA, further attempts are in progress to produce hybridoma antibodies to other Gm antigens.

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References

1. Duhamel RC, Schur PH, Brendel K, Meezen E (1979) pH gradient elution of human IgG1, IgG2 and IgG4 from protein A-Sepharose. J Immunol Methods 31:211–217
2. Galfre G, Howe SC, Milstein C, Butcher GW, Howard JC (1977) Antibodies to major histocompatibility antigens produced by hybrid cell lines. Nature 266:550–552
3. Hudson L, Hay FC (1980) Pepsin digestion. In: Practical immunology. Blackwell, Oxford London Edinburgh Boston Carlton, pp 169–199
4. Kishida T, Fukuda M, Takahashi N, Tamaki Y (1982) Gm typing without anti-D coats. Jpn J Legal Med 36:1000 (in Japanese)
5. Kishida T, Tamaki Y (1983) An improved method for the production of antisera to Glm allotypes. Jpn J Hum Genet 28:269–272
6. Tamaki Y, Kishida T, Shibata K, Takahashi N, Fukuda M (1979) Preparation of anti-Gm sera by immunization of rabbits with protein A-fractionated normal IgG proteins: Further study. J Immunol Methods 45:177–182
7. Tamaki Y, Takahashi N, Kishida T, Ishikawa K (1984) Production of monoclonal anti-G3m(21) antibody. Jpn J Legal Med 38:152–154
8. Voller A, Bidwell DE, Huldt G, Engall E (1974) A microplate method of enzyme-linked immunosorbent assay and its application to malaria. Bull WHO 51:209–211

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