

A Rapid Biodosimetric Technique at the Human Glycophorin-A Locus

M. Ravi and Solomon F. D. Paul

*Genetic Research Cell, Sri Ramachandra Medical College and Research Institute, Porur,
Chennai 600 116, Tamil Nadu, India*

KEY WORDS Mutations; glycophorin-A; monoclonal antibodies; mutational assay.

ABSTRACT For risk assessment, it is necessary to evaluate the dose of human exposure to a mutagen. Environmental genotoxic factors such as Ionizing Radiations induce dose-dependent somatic mutations. Recent studies support the somatic-mutation theory of carcinogenesis. Mutations at the Glycophorin – A (GPA) loci have been studied for establishment of mutagen exposure particularly at a population level and also to estimate the cumulative dose responses. GPA assay provides lifetime biological dosimetry for exposure to radiation as the mutations are accumulated in the long lived hematopoietic stem cells of the bone marrow. Here we present a novel technique, the RS-1 Assay in quantifying the GPA mutations and its role in biodosimetry and risk assessment.

INTRODUCTION

Dose evaluation of exposed humans to radiation becomes necessary after exposure. Such a dose evaluation can be done by biological dosimetric methods of which increased somatic cell mutant frequencies in exposed individuals can be a reliable index (Hakoda 1988). Biological dosimetry has an important role to play in assessing the cumulative radiation exposure of persons working with radiation and also in estimating the true dose received during accidents involving external and internal exposure. Biodosimetric methods include cytogenetic, immunological and molecular mutational assays. There are currently advancing and new opportunities for biological dosimetry of high-risk groups in the methods for measuring somatic mutations (Mendelshon 1990). Such an evaluation of somatic cell mutations becomes all the more important, as somatic cell mutations can be possible predictors of cancer risk

(Akiyama 1995). Somatic mutations can now be measured in several human genes. The genes currently used include the hemoglobin (Hb) genes on chromosome 11 and 16, the Glycophorin A (GPA) gene on chromosome 4, the hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene on the X-chromosome, the HLA genes on the chromosome 6 and the T-cell receptor (TCR) genes on chromosomes 7 and 14.

GPA is a human blood type antigen that appears on the surface of the erythrocytes. Since the genes for this blood type antigen is inherited in a conventional Mendelian mode, 50% of the population expresses the heterozygous blood type NM. Essentially, all the erythrocytes in a Heterozygous individual contain both the M and N forms of the protein. In cases of Mutagenic insults, one of the two allelic forms of the GPA Gene could be inactivated. As a result, all progeny of such a precursor cell, which are sent into the peripheral circulation, appear as variant erythrocytes. In order to detect the presence of such cells, a fluorescence immunolabelling technique was hitherto used to quantify the variant cells, which in turn gives us an indication of the mutagen dose absorbed. For this, the erythrocytes need to be fixed (Langlois 1985) and immunolabeled with dyes and the cells sorted by a flow cytometer to quantify the variant cell populations.

Heamagglutination assays are sensitive techniques for the quantification of antigens or antibodies present on the erythrocyte surface. As the GPA protein is present on the surface of the erythrocyte membrane, it presents itself as a perfect antigen and thus can be quantified based on the Heamagglutination assays.

Reliable indications as to the GlycophorinA distribution in terms of the variant cells can be obtained by employing suitable antibodies to the two forms of the GPA protein in a heterozygous human subject.

Corresponding Author: M. Ravi
Telephone: +91-44-4765609
Fax: +91-44-4767008
E-mail: maddalravi@hotmail.com

MATERIALS AND METHODS

The RS-1 Assay was performed in 96 well, U bottomed Heamagglutination plates. Antibodies against the two forms of the GPA, the AntiN and AntiM are obtained from the Ortho Clinical Diagnostics, Raritan, New Jersey and were used for the Heamagglutination studies. The dilutions of the anti sera are as given in the Table 1. Fifty micro liters of the various antibody dilution reagents were used in each well.

Table 1: The antibody dilutions required for the assay

Antiserum	40	30	20	10	9	8	7	6
Saline	60	70	80	90	91	92	93	94

One milliliter blood sample from individuals heterozygous for the MN blood type were collected and washed thrice with 15ml of cold saline solution. A 10 % erythrocyte preparation was prepared with the washed erythrocytes in cold saline. 50 micro liters of this suspension was used constantly for the RS-1 Assay.

RESULTS

The results obtained are given in the Table 2. A shift towards the wells containing AntiN antibody was noticed for all the samples, thus indicating the presence of the base-line

Table 2: The results obtained as Heamagglutination patterns

Antiserum		40	30	20	10	9	8	7	6
Saline		60	70	80	90	91	92	93	94
A	M	+	+	+	+	+	+	-	-
	N	+	+	+	+	+	-	-	-
B	M	+	+	+	+	+	+	+	-
	N	+	+	+	+	-	-	-	-
D	M	+	+	+	+	-	-	-	-
	N	+	+	+	-	-	-	-	-
F	M	+	+	+	+	-	-	-	-
	N	+	+	+	-	-	-	-	-
G	M	+	+	+	+	+	+	+	+
	N	+	+	+	+	-	-	-	-
C	M	+	+	+	+	+	-	-	-
	N	+	+	+	-	-	-	-	-
E	M	+	+	+	+	+	+	+	+
	N	+	+	+	+	-	-	-	-

+ Agglutination
- Button formation

mutations with more of Nf variant erythrocytes. The values can be represented as a factor of the antibody dilutions where the agglutination ceases and the button formation starts as given in the Table 3. The figure 1 is a representative of the RS-1 assay.

Table 3: The dilution factors obtained employing the assay

Sample	M	N
A	7/93	8/92
B	6/94	10/90
C	9/91	20/80
D	10/90	20/80
E	6/94	20/80
F	10/90	20/80
G	6/94	10/90

DISCUSSION

Lansteiner and Levine described a blood group system in 1927 called the MN in which two alleles M and N determine the presence of corresponding antigens on red cells. According to this theory, three possible genotypes MM, MN and NN occur. GPA genes code for the determinant molecule for the MN blood group in erythrocytes and are present on the 4q29 chromosome site. The GPA of human erythrocyte is a sialoglycoprotein with a chain of 151 amino acids and a molecular weight of 55,000 Daltons. The M and N forms vary in their amino acid composition in positions (1) and (5) (Furthmayer 1978). The M group has Serine and Glycine in positions (1) and (5) respectively and the N group has Leucine and Glutamic acid in the respective positions.

Increased mutation frequencies were detected hitherto at the GPA locus among high-risk groups and cancer patients (Akiyama 1994). Significant linear response between frequency and radiation exposure were observed for different cell phenotypes (Langlois 1987).

Mutational assays at the GPA sites use monoclonal antibodies to detect the loss of the gene product from either of the two allelic forms (Langlois 1986). Its application is limited to MN heterozygotic individuals who constitute 50% of all human populations. Presumptive mutant cells are of two types: those with the normal signals from one of the alleles and none from the other (hemi zygotes); and those with twice the normal signal from one allele and none from the other (homozygotes), (Mendelshon 1990).

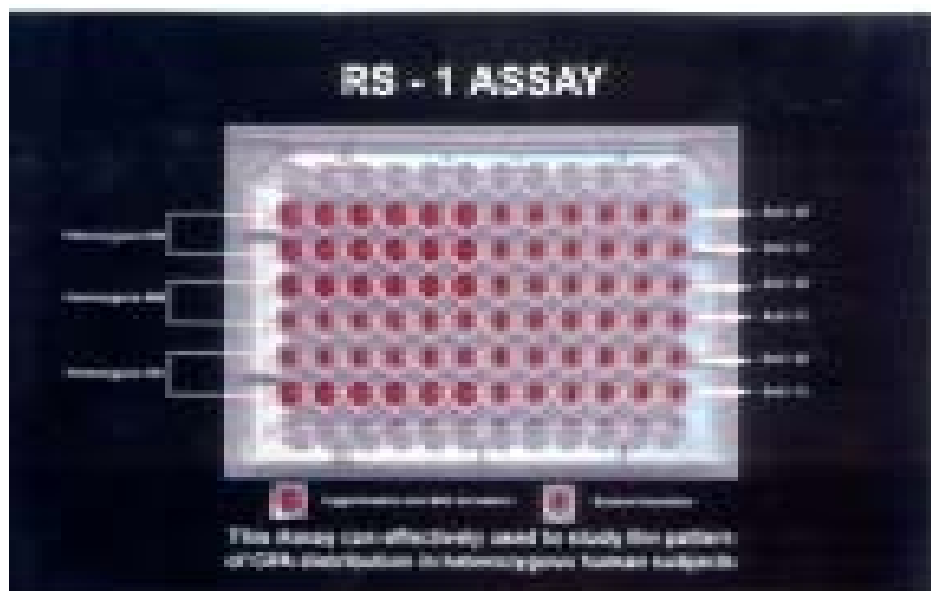


Fig. 1.

GPA known to be the antigenic determinant of the MN blood type can be subjected to a pair of monoclonal antibodies to facilitate detection of mutant erythrocytes lacking either M or N products of GPA alleles among normal erythrocytes from MN heterozygous people. The variant cell types, which can be detected, are those that lack the M form of GPA (hemizygous NØ) and those that lack the N form of GPA (MØ and MM variants) (Akiyama 1994). Typically, for the assay using immunofluorescence, 5 – 30 ml of blood samples are required.

Cell fixation is necessary for these studies to block antibody induced agglutination of the erythrocytes. This is achieved by producing formalin spheres by diluting whole blood in a solution containing Sodium Dodecyl Sulphate, followed by fixation with formalin. Formalin fixation should be avoided for GPA (N) – specific monoclonal antibodies. In yet another technique, the erythrocytes can be fixed with cross-linking agent dimethyl suberimidate (DMS). Such cells are compatible with the binding of all the monoclonal antibodies, but the staining is clear for only one week (Langlois 1986).

The GPA assay was hitherto performed on a single – beam flow cytometer to enumerate variant cells as evidenced by the staining (Jensen 1995). The assay uses two- color

fluorescence labeling of fixed cells with two different monoclonal antibodies, each of which binds specifically one of the two allelic forms of GPA. These erythrocytes can be analyzed by flow-cytometry to enumerate the frequency of the two variant cell types (Jensen 1995).

The RS-1 assay eliminates the need for fixing and immunostaining protocols and the need for equipments such as a flow-cytometer is avoided. The assay can now be performed with just the antibodies and simple Heamagglutination plates.

CONCLUSION

In the studies that we carried out using the RS-1 assay, we could find out the nature of distribution of the GPA protein molecules in the Heterozygous MN individuals in a quantitative approach. This quantitative approach can be employed to look at the variant erythrocytes in a normally co dominantly expressed system.

The RS-1 Assay developed to assess mutations at the GPA locus can be used primarily to construct a base line frequency of the mutation in the human population with MN-heterozygous blood group. The same can also be used for constructing a Dose-Response for mutagen insults and can be a simple and yet effective tool for Biodosimetry. This makes the RS-1 assay

a simple, rapid and yet a sensitive technique, which requires blood samples as low as 0.5 ml.

ACKNOWLEDGMENTS

This work was done as a part of a research project funded by The Atomic Energy Regulatory Board, Government of India.

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