Detection of Somatic Mutations at the Glycophorin A Locus in Erythrocytes of Atomic Bomb Survivors Using a Single Beam Flow Sorter

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ABSTRACT

A modified method was developed for measuring the frequency of variant erythrocytes at the glycophorin A locus using a single beam cell sorter (SBS). Fluorescein- or phycoerythrin-labeled monoclonal antibodies specific for the M or N glycophorin A alleles were used for the SBS assay. To prevent contamination of nucleated cells in the sorting window, the nucleated cells in the fixed erythrocyte sample were stained with propidium iodide before flow sorting. Blood samples were obtained from atomic bomb survivors who were heterozygous for the MN blood type, and the frequencies of the hemizygous and homozygous variant of the M or N glycophorin A allele were measured by the SBS. For the three types of variants, hemizygotes for M and N allele (N0 and M0) and homozygotes for M allele (MM), the variant frequency measured by the SBS correlated well with that previously determined by a dual beam cell sorter. Variant frequencies of the N0, M0, and MM cell types in atomic bomb survivors determined by SBS measurements were found to increase with radiation dose (DS86, kerma) as well as with the frequency of chromosome aberrations in lymphocytes.

INTRODUCTION

It is well known that various environmental genotoxic substances including ionizing radiation induce mutational lesions as well as cancer in human somatic cells. Although the role of somatic cell mutations in carcinogenesis is still unclear, recent studies of oncogenes in somatic cells have shown that gene alterations such as point mutations, rearrangement, and amplification can facilitate some steps in carcinogenesis (for review, see Ref. 1). Therefore, the detection and measurement of somatic cell mutation in vivo is an important subject of research for the assessment of human cancer risk induced by environmental mutagens and ionizing radiation.

At present, only a few methods are available for measuring the frequency of in vivo mutants in human somatic cells. One such method is the HPRT assay to detect peripheral T-lymphocytes which have lost the expression of a functional chromosome aberrations in lymphocytes.

large volume of blood sample (20 ml) and long-term culture (2 wk). Further, it appears that the frequency of HPRT-mutant T-lymphocytes induced by genotoxic exposure found in the blood decreases during the many years after exposure, as suggested by our study of A-bomb-exposed people (7).

Recently, a flow cytometric assay for detecting mutant erythrocytes lacking surface protein, GPA, was developed and appears to have some advantages over the HPRT assay (8). Since the GPA mutation assay requires only a small volume of blood (less than 1 ml) and a short time (less than 2 days) for the measurement of mutant frequency, it is well suited for monitoring genotoxic exposure in large populations. In addition, mutations at the GPA locus (mapped to chromosome 4q) are not likely to be selected against in vivo because individuals totally lacking GPA on their erythrocytes show no clinical symptoms nor is the life span or function of the erythrocytes affected (9).

Moreover, this method can measure the frequency of erythrocytes of homozygous GPA phenotype, probably induced by somatic recombination, which is believed to be an important genetic event in the development of some human tumors (for review, see Ref. 10). Since GPA molecules are first expressed at the erythroblast stage (11, 12), a mutational lesion detected in mature erythrocytes should have occurred in early erythroid precursor cells at some stage including the pluripotent stem cell stage (8).

In our previous report, a flow cytometric GPA assay was used to measure the frequency of variant erythrocytes from A-bomb survivors in Hiroshima (13). It was found that the frequency of variant erythrocytes is significantly elevated in the exposed donors even 40 yr after a single exposure to A-bomb radiation, suggesting that the GPA system may provide a life-time cumulative dosimeter of past radiation exposure. Those data were obtained using a DBS (8). In the present report we will describe a modified method suitable using a SBS which is widely available in many laboratories.

MATERIALS AND METHODS

Blood Samples. Blood samples were obtained from 68 A-bomb survivors participating in the RERF Adult Health Study in Hiroshima and from 4 standard healthy donors. These donors were confirmed to be MN heterozygotes by a hemagglutination test using rabbit typing sera (Ortho Diagnostic Systems, Raritan, NJ). The exposure dose for each survivor was estimated by the recently developed DS86 system which is based on improved calculations of the yield of neutrons and γ rays from the bomb and shielding effects (14). One cohort of 43 donors was composed of proximally exposed survivors with DS86 dose estimates (total kerma) ranging from 0.11 Gy to 5.02 Gy. A second cohort consisted of 21 distally exposed survivors with assigned doses of less than 0.005 Gy, serving as age- and sex-matched controls to the proximally exposed group. The 4 standard healthy donors were sampled three or more times during this study to examine the reproducibility of the assay.

GPA variant frequencies for 54 of the donors examined in this study had also been measured by means of a DBS at LLNL, as reported...
previously (13). The frequency of peripheral blood lymphocytes bearing chromosome aberrations for 34 of the donors also had been measured in a previous study (15).

Monoclonal Antibodies, Cell Fixation, and Staining. The preparation and characterization of the MonAbs to GPA have been described previously (16-18). The following four MonAbs were used for this study: GPA (M)-specific MonAbs 6A7 (y1) and 9A3 (y1; GPA (N)-specific MonAb NN3 (μ); and GPA-specific MonAb 10F7 (y1) that binds equally well to both the M and N types of GPA. These MonAbs were labeled with appropriate fluorescent dyes according to methods described previously (18), modified to adapt the assay to SBS. Briefly, MonAbs 10F7 and 9A3 were directly conjugated with fluorescein (-F suffix on the antibody name), and MonAbs 6A7 and NN3 were conjugated with biotin allowing labeling with streptavidin-conjugated phycoerythrin (B-Av PE), which was substituted for the TR-avidin used at LLNL (18). PE-streptavidin was obtained from Becton Dickinson Immunocytometry Systems (Mountain View, CA).

Blood samples were fixed with formalin or DMS (Sigma Chemical Co., St. Louis, MO) to block antibody-induced agglutination of erythrocytes, as described previously (18). Briefly, formalin-fixed spherical erythrocytes were produced by diluting blood in a solution containing sodium dodecyl sulfate, followed by fixation with formalin. Formalin erythrocytes were produced by diluting blood in a solution containing 2W2), were developed using the DBS. In the present study, two assay: GPA (M)-specific MonAbs 6A7 (y1) and 10F7-F, but this method cannot be used with the MonAb NN3, as binding of NN3 to GPA is abolished by formalin fixation. Another fixation method using DMS which can cross-link proteins of erythrocytes was used after removing the buffy coat and plasma proteins. All four MonAbs were able to bind GPA on DMS-fixed erythrocytes.

Fixed erythrocytes (5 x 10^6) were suspended in phosphate-buffered saline (pH 7.2), containing 5 mg/ml of bovine serum albumin, 0.01% Nonidet P-40, and 1.5 mm NaN₃ (8), and incubated for 1 h with a mixture of the primary MonAbs (10 μg/ml each). After washing twice, cells were incubated for 1 h with PE-streptavidin (10 μg/ml). Before flow cytometry, PI (Sigma) was added at a final concentration of 10 μg/ml to gate out contaminating nucleated cells from the sorting windows, as described in “Results.”

Flow Cytometry and Cell Sorting. Flow cytometry and sorting were performed on a FACStar (Becton Dickinson) single beam flow sorter equipped with an argon ion laser (2 W). Both fluorescent dyes, fluorescein and PE, were excited with a 300-mW laser beam at 488 nm, and fluorescence was detected through a 530-nm band pass filter for fluorescein and a 585-nm band pass filter for PE. The spectral overlap of fluorescein and PE fluorescence was electronically compensated using a standard erythrocyte mixture, as described in “Results.” Linear amplification was used for precise intensity measurements, and logarithmic amplification with a range of four decades was used to display results from mutation analysis. Forward light scatter was also measured and was used to set the threshold to remove small debris. Flow speed for sorting of erythrocytes was approximately 1000 cells/s.

Cells with specific fluorescence intensities of hemizygous and homozygous variants were sorted simultaneously from each sample onto sorting windows, as described under a fluorescence microscope (Nikon, Tokyo, Japan) as described previously (8).

RESULTS

Characterization of Assay Systems Using SBS. Flow cytometric detection of variant erythrocytes was described in detail in a previous report (8). Three types of assays, namely, one-way assay (1W1) and two versions of the two-way assays (2W1 and 2W2), were developed using the DBS. In the present study, two of these assays, 1W1 and 2W2, were adapted for the SBS which uses fluorescein and PE as fluorescent dyes for two-color flow analysis.

The 1W1 assay uses formalin-fixed erythrocytes stained with MonAb 6A7-B-Av PE specific for GPA (M) and with MonAb 10F7-F that binds equally well to both GPA (M) and GPA (N). With the 1W1 assay, two variant cell types, hemizygous N0 and homozygous NN cells, can be detected simultaneously among erythrocytes from MN heterozygous donors. N0 cells, which have lost the expression of the M allele, lack 6A7-B-Av PE fluorescence and retain half the normal 10F7-F fluorescence, whereas NN cells, which have lost the expression of the M allele but express twice the normal amount of the N allele as MN heterozygous erythrocytes, display normal 10F7-F fluorescence.

The 2W2 assay uses DMS-fixed erythrocytes labeled with MonAb 9A3-F specific for GPA (M) and MonAb NN3-B-Av PE specific for GPA (N). With this assay, hemizygous M0 cells and homozygous MM cells from MN heterozygous donors can be measured simultaneously. M0 and MM variant cells lack expression of GPA (N) but express GPA (M) at a level equivalent to or twice that of normal MN heterozygous cells, respectively.

The relative fluorescence intensities of normal MM, MN, and NN erythrocytes stained with the four MonAbs were measured by SBS (Fig. 1). As shown in Fig. 1B, the PE fluorescence intensity of doubly stained NN cells in the 1W1 assay is greatly increased compared to that stained by PE alone (Fig. 1A). The same is true for MM cells in the 2W2 assay, and this is due to filter cross-talk. Correcting for the cross-talk using an electronic compensator eliminates the optical artifact introduced by PE fluorescence of homozygous cells and enhances the resolving power for detecting rare variant cells (Fig. 1C). Fluorescence window positions for sorting variant erythrocytes were defined using the corrected contour plots of the control cell mixtures.

The following are the ranges of fluorescence intensities that define variant windows (normalized to a value of 1 for the intensity of a single allele with each MonAb): for the N0 variant, Y = 0 to 0.03, X = 0.7 to 1.3; for the NN variant, Y = 0 to 0.03, X = 1.3 to 2.6; for the M0 variant, Y = 0 to 0.06, X = 0.7 to 1.3; and for the MM variant, Y = 0 to 0.06, X = 1.3 to 2.6. The window positions were nearly identical for all assays, but the upper limits in the Y axis for the window were slightly adjusted for each lot of antibody conjugates which differed in their resolution of normal MN cells and variant cells.

Detection of Variant Cells by SBS. For detection of N0 and NN variant cells, erythrocytes from heterozygous MN donors were stained with a pair of MonAb, and between 10^3 and 10^4 cells were analyzed by the SBS for each sample. All cells appearing in the two variant windows were sorted onto glass slides to determine if they were the erythrocytes having a fluorescein green fluorescence and no PE orange fluorescence. Fig. 24 shows the result from a 1W1 assay of 5 x 10^5 erythrocytes from a standard donor. The contour plot represents several hundred events near and in the area of the windows for N0 and NN cells. Microscopic examination of the sorted cells revealed that these events were mainly due to contamination of the erythrocytes by WBC which were nonspecifically stained with MonAb 10F7-F. Since WBC contamination of the sorted cells interferes with counting of the variant erythrocytes under a fluorescent microscope, it was necessary to remove the WBC from the window area of the variant cells. Labeling the WBC DNA with PI was found to move the WBC in the contour plot up above the peak PE fluorescence intensity of normal MN erythrocytes, while the intensity of the erythrocytes did not change, as shown in Fig. 2B. Sorting experiments confirmed that the WBC contamination in the sorted sample disappeared almost completely after PI staining, and the frequency of N0 or NN variants as enumerated microscopically remained constant or without PI staining. Fig. 2, C and D, shows results...
Fig. 1. Fluorescence distribution of a mixture of fixed erythrocytes from MM, MN, and NN individuals singly or doubly stained with anti-GPA MonAbs used for the 1W1 and 2W2 assay systems. Ordinates plot the logarithm of PE fluorescence intensity, and abscissae plot the logarithm of fluorescein fluorescence intensity. A, contour plot of a control cell mixture singly stained with PE-labeled MonAb without fluorescence compensation; B, doubly stained cell mixtures with a pair of PE- and fluorescein-labeled MonAbs without compensation; C, doubly stained cell mixtures with compensation for both PE and fluorescein fluorescence cross-talks. Compensated contour plots were used to define the positions of the variant cell windows for the 1W1 and 2W2 assays, respectively.

Fig. 2. Flow distributions obtained from analysis of about $5 \times 10^5$ cells from normal MN donors using 1W1 (A, B) and 2W2 (C, D) assay systems with (B, D) or without (A, C) PI staining. Contours differ by a factor of 10 in events per channel with the lowest contour representing one event per channel. The windows labeled No and Mo correspond to hemizygous variants, while windows labeled NN and MM correspond to homozygous variants. The arrows indicate clusters of white blood cells.

of a 2W2 assay of $5 \times 10^5$ MN-type erythrocytes stained without or with PI, respectively. Contamination of WBC in the sorted cells was not as frequent in the 2W2 assay as compared to 1W1 assay, probably because more WBC were removed by extensive washing of blood samples before DMS fixation. Flow sorting of samples stained with PI in addition to MonAbs revealed that 20 to 100% of the events occurring in the variant windows corresponds to erythrocytes with a variant cell phenotype. Other objects contaminating the sorted samples were missorted normal erythrocytes and fluorescent debris, but these objects could be microscopically differentiated from variant erythrocytes by their fluorescent staining pattern and/or morphology.

The efficiency of detection of variant cells in the assay system was examined by adding a small number of normal NN or MM cells to MN cells at an approximate frequency of $10^{-4}$ before staining. Cell staining and flow sorting were performed on samples with or without added homozygous cells. The efficiency of recovery of the added cells was then calculated from the difference between these measurements. As shown in Table 1, the average efficiency of recovery was approximately 100% in
### Table 1 Recovery of NN or MM cells added to MN cells in the 1W1 and 2W2 mutation assays

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of NN cells added/10^6</th>
<th>No. of sorted NN cells/10^6</th>
<th>No. of NN cells recovered/10^6</th>
<th>%</th>
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</thead>
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<td>42</td>
<td>88</td>
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<td>83</td>
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<tr>
<td>5</td>
<td>79</td>
<td>111</td>
<td>103</td>
<td>130</td>
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</table>

**1W1 assay**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of MM cells added/10^6</th>
<th>No. of sorted MM cells/10^6</th>
<th>No. of MM cells recovered/10^6</th>
<th>%</th>
</tr>
</thead>
<tbody>
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<td>0</td>
<td>36</td>
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<td>5</td>
<td>117</td>
<td>150</td>
<td>104</td>
<td>103</td>
</tr>
</tbody>
</table>

**2W2 assay**

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Erythrocytes enumerated by Coulter Counter.

* Difference in the frequency of sorted cells between samples with and without added homozygous cells.

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SOMATIC MUTATIONS IN ERYTHROCYTES OF A-BOMB SURVIVORS

Both the 1W1 and 2W2 assays, indicating that the variant frequency obtained from sorted erythrocytes did not require correction for recovery efficiency as was reported for the DBS (8).

**Variant Cell Frequencies in Normal Individuals and A-Bomb Survivors.** Fig. 3 summarizes the frequencies of variant cells in 4 standard healthy MN donors and 21 distally exposed A-bomb survivors whose DS86 dose estimates were less than 0.005 Gy (control donors). The standard healthy donors were sampled 3 or more times. For these donors hemizygous \( N_0 \) and \( M_0 \) cells and homozygous MM cells occurred at mean frequencies of \( 18 \times 10^{-6} \), \( 11 \times 10^{-6} \), and \( 11 \times 10^{-6} \), respectively, and standard deviations of these frequencies for each donor are less than \( 15 \times 10^{-6} \). In contrast, the frequency of homozygous NN variants varied considerably among multiple measurements on each of the standard healthy donors, indicating that the reproducibility of the measurement for NN variant cells is quite low compared with that for the other three types of measurements. The same kinds of results were obtained from the control donors, including the large deviation in NN variant frequency. These frequencies are in agreement with those obtained by the DBS measurements (8, 13). Although the exact reasons for variability in NN frequency are not known as yet, it is thought that the 1W1 assay is sensitive to alterations in glycosylation of GPA molecules occurring either in vivo or during sample preparations (8, 13).

Variant cell frequencies of 54 A-bomb survivors (15 control donors (<0.005 Gy) and 39 exposed donors (>0.11 Gy)) were measured by both the SBS and DBS. Table 2 shows the correlation of variant cell frequencies between the two types of cell sorter assays. Although blood samples were taken at different times for the SBS versus the DBS, \( N_0 \), \( M_0 \), and MM variant frequencies were highly significantly correlated between the SBS and DBS. On the other hand, the correlation of NN variant frequency was found not to be significant, probably due to the low reproducibility of measurement in either assay, as discussed above.

Fig. 4 shows the relationship between the DS86 dose estimates (total kerma dose of \( \gamma \) rays and neutrons) and variant cell frequencies of the \( N_0 \), \( M_0 \), and MM cell types. The trend was estimated by assuming a linear model \((y = a + bx)\), and significant linear correlations were obtained for all three types of variant cells. The fit parameters obtained were: \( a = 33 \times 10^{-6}, b = 0.63 \times 10^{-4}/\text{Gy} \) for \( N_0 \); \( a = 20 \times 10^{-6}, b = 0.32 \times 10^{-4}/\text{Gy} \) for \( M_0 \); and \( a = 15 \times 10^{-6}, b = 0.14 \times 10^{-4}/\text{Gy} \) for MM. As shown in Fig. 4, top, two donors had exceptionally high frequencies.
Table 2  Correlation between variant cell frequencies measured by SBS and DBS

<table>
<thead>
<tr>
<th>Variant cell type</th>
<th>No. of sample</th>
<th>Correlation coefficient (r)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>54</td>
<td>0.954</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NN</td>
<td>54</td>
<td>0.212</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>M(\phi)</td>
<td>33</td>
<td>0.828</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MM</td>
<td>33</td>
<td>0.726</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The correlation of erythrocyte variants with the frequency of lymphocytes bearing chromosome aberrations was examined for 34 A-bomb survivors. Both stable and unstable aberrations were included in the analysis, and significant linear correlations were obtained between aberration frequency and each of the variant erythrocyte frequencies of Na, M\(\phi\), and MM (Table 3). Fig. 5 shows the linear regression line for M\(\phi\) variant frequency versus chromosome aberration frequency with fit parameters of \(a = 11 \times 10^{-6}\) and \(b = 3.2 \times 10^{-4}\).

DISCUSSION

In this paper, we describe a flow cytometric method using SBS for enumerating mutant erythrocytes arising in vivo. The fundamental difference in the GPA assay between DBS and SBS is the choice of fluorescent dyes (fluorescein and TR for DBS, fluorescein and PE for SBS) used for labeling antibodies, as necessitated by the difference in optical systems of the two cell sorters. This difference required some modifications of flow cytometry in order to adapt the mutation assay to the SBS.

The recovery efficiencies of variant cells using SBS are much higher than those using DBS, which was especially noted for high Na variant frequencies (more than \(1 \times 10^{-3}\)), and these extreme values greatly affected the linear fit parameters. Eliminating these two values, the calculation of linear fit for Na variant frequency gave parameters of \(a = 27 \times 10^{-6}\), \(b = 0.34 \times 10^{-4}\)/Gy, closer to the parameters for M\(\phi\) frequency. Furthermore, Na and M\(\phi\) frequencies were significantly correlated in 44 survivors (29 exposed and 15 control donors), excluding the exceptional donors with variant frequency of more than \(10^{-3}\) (\(r = 0.530\), \(P < 0.001\)).
The induced rates per gray using bone marrow dose estimates are $b = 0.40 \times 10^{-4}/\text{Gy}$ for N0, $0.41 \times 10^{-4}/\text{Gy}$ for M0, and...
of the T-cell receptor (Cβ) and immunoglobulin genes (JH), indicating differentiation of lymphocytes from a monogenic mutant stem cell.

There is growing evidence that events leading to a homozygous state, including mitotic recombination and chromosome missegregation, occur in vivo and play an important role in the production of hereditary human tumors as suggested by RFLP analysis of tissues in retinoblastoma (10, 27, 28), Wilms' tumor (10, 29-31), and other tumors (32, 33). These studies also suggest that a new class of cancer gene may exist, defined as an antioncogene, in which mutation includes deletion of one allele followed by somatic recombination, which may result in carcinogenesis (10, 28). The somatic recombination of these mutated antioncogenes may also play an important role in nonhereditary oncogenesis. We have recently observed an extraordinarily high elevation of homozygous GPA variant frequency in Bloom's syndrome (1 to 2 × 10⁻⁷) known to be a cancer-prone hereditary disease. In these patients, it has been reported that the spontaneous mutation frequency is about 10 times higher than that of normal people (34, 35). Thus, the increased spontaneous frequencies of both mutation and somatic recombination may provide predisposing conditions for carcinogenesis. The observation that the frequency of homozygous MM variant cells significantly correlated with radiation dose may suggest that somatic recombination possibly contributes to radiation-induced oncogenesis in A-bomb survivors whose cancer incidences increase approximately linearly with DS86 dose (36). However, an assay method remains to be established to detect NN homozygous variants. Since somatic recombination should produce two kinds of daughter cells, MM and NN, it is expected that the two frequencies should be similar. Because the GPA assay cannot provide confirmation at the DNA level of somatic events, for MM variants. It is expected that the two frequencies should be similar. Because the GPA assay cannot provide confirmation at the DNA level of somatic events, for MM variants.

Finally, the frequency of chromosome aberrations in lymphocytes increased approximately linearly with increasing dose in Hiroshima A-bomb survivors (15), thereby providing valuable information as a biological dosimeter of past radiation exposure. Since the GPA variant frequency showed a significant correlation with chromosome aberration frequency, the erythrocyte GPA mutation can now be considered as a new biological parameter indicative of radiation exposure, and more precise dose estimation may be achieved by the combination of the two assays. A large-scale study of GPA mutation in A-bomb survivors using the SBS in conjunction with chromosome aberration frequencies will be undertaken at RERF.

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REFERENCES

2. Albertini, R. J., Castle, K. L., and Borchering, W. R. T-cell cloning to detect


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