Comparison of Type and Frequency of Chromosome Aberrations by Conventional and G-staining Methods in Hiroshima Atomic Bomb Survivors

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Somatic chromosomes derived from lymphocytes of 23 Hiroshima A-bomb survivors were analyzed to determine the type and frequency of radiation-induced chromosome aberrations, using both the ordinary staining method (O-method) and the trypsin G-banding method (G-method). Of 896 cells examined, 342 had aberrations, including 31 unidentifiable cells even by the G-method. The number of aberrations detected was 376 in 311 cells. The majority of them were intra- or inter-chromosomal symmetric exchanges, while only 24 were found to be asymmetric exchanges (dicentrics, rings and interstitial deletions). Further, 28 aberrations included acentric fragments and terminal deletions, and the remaining 36 were complex intra- and inter-chromosomal exchanges showing insertions and double translocations.

An analysis of the same metaphases examined by sequential O- and G-methods was carried out independently on 361 aberrations. It was found that 78 were detectable only by the G-method; among these were 14 paracentric inversions, 48 reciprocal interchanges of chromosome segments with either equal or unequal length, 14 minor deletions and 2 complex rearrangements, all of which were judged as the normal variation by the O-method. In contrast, 25 aberrations detected by the O-method were found to show normal banding patterns by the G-method.

INTRODUCTION

Radiation-induced chromosome aberrations are known to persist in circulating lymphocytes of Hiroshima and Nagasaki A-bomb survivors for more than three decades after radiation exposure. The frequency of these aberrant cells proved to be proportional to the estimated dose received by each individual1,2. Furthermore, symmetric aberrations, such as reciprocal translocations and pericentric inversions, were found to predominate over asymmetric exchanges (dicentrics and rings), and the former aberrations were the major components contributing to the dose-aberration relationship.
Heretofore, the usefulness of asymmetric aberrations was considered to be a more sensitive indicator for evaluating the dose-aberration response than symmetric aberrations, because the latter were not easily detectable, and the experience of the microscopist may further influence the observed results.

Recently developed banding methods for identifying individual chromosomes have enabled us to detect more accurately and objectively a variety of radiation-induced structural rearrangements, and some types of symmetric exchanges, previously undetectable by the O-method, can now be identified by these new methods; these include paracentric inversions and intra- and inter-chromosomal symmetric exchanges of chromosomal segments of equal length.

The present report describes the radiation-induced chromosome aberrations in the somatic cells of Hiroshima A-bomb survivors, by comparing the results derived from G-method with those from O-method in the same metaphases. Results of comparative analysis of aberrant cells between the two methods have already been reported elsewhere, and thus the present report concerns the type and frequency of induced symmetric aberrations, which could not be detectable by the O-method.

MATERIALS AND METHODS

Twenty-three Hiroshima A-bomb survivors, in whom radiation-induced chromosome aberrations were observed in more than 10% of cultured lymphocyte metaphases by previous cytogenetic examinations, were selected and reexamined cytogenetically using the G-method. Subjects studied here were participants in the RERF Adult Health Study sample who received biennial physical examination, and whose estimated exposure dose was over 100 rad of mixed gamma rays and neutrons. None had received either radiation therapy at any time in the past or any extensive diagnostic irradiation within the year preceding blood drawing.

Peripheral lymphocytes were cultured using the whole blood culture method of Hungerford, with an incubation time of 52 hours, during the last 2 hours of which colchicine was added. Slides were prepared for microscopic examination by hypotonic pretreatment with a solution of 0.075M KCl and 1% sodium citrate, followed by 3:1 alcohol-acetic acid fixation and flame-drying, and finally stained for 20 minutes with 5% Giemsa solution.

Well-spread metaphases were first selected and photographed without mounting the coverslip on the slide. The exact location of each photographed metaphase on the microscopic stage was recorded for the later examination of the same metaphases. After microscopic observations, the slides were soaked with tetrachloroethylene to remove immersion oil from the slide, and then in an acetic acid-methanol mixture to destain the cells, washed by tap water, and dried.
The G-banded preparations were prepared by a minor modification of the trypsin technique of Seabright; the slide were treated in a 0.2% trypsin solution (1:250, Difco) for 1 to 15 sec at room temperature, then washed with running tap water and restained with a 5% Giemsa solution for 10 to 15 minutes.

The banded metaphases, which had been previously photographed, were then relocated, reexamined, and again photographed for karyotype analysis. All of the cells with definite or suspected structural rearrangements detected by either or both the O- and G-methods were karyotyped separately using the printed photographs to determine the type and frequency of radiation-induced chromosome aberrations by both methods.

Chromosome aberrations were classified into the following two groups according to the number of breaks involved in the formation of aberrations: simple aberrations produced by one or two breaks, and complex aberrations, involving three or more breaks.

RESULTS

Of a total of 896 metaphases analyzed by both methods, 342 cells (38.2%) were found to show radiation-induced chromosome aberrations. In 31 cells of these 342, aberrations were so complicated that even the banding method could not specify the types of aberrations (unidentifiable cells). Therefore, an analysis of the type and frequency of chromosome aberrations was restricted to the remaining 311 identifiable aberrant cells. Among these aberrant cells, 376 aberrations were detected, the majority of which were classified as simple aberrations (340 or 90.4%). The remaining 36 (9.6%) were identified as complex aberrations (Table 1).

Among the simple aberrations, the term “acentric fragment” is used when the acentric material was present in the complement, and “terminal deletion” indicates the loss of an acentric part from the broken chromosome in the complement, perhaps being eliminated through the preceding mitoses. There were only 28 (7.4%) aberrations produced by a simple break, consisting of 8 acentric fragments and 20 terminal deletions (Table 1). In the present study, four minute fragments, which also could be acentric rings, were detected and tentatively classified as acentric fragments.

The majority of chromosome aberrations observed were exchanges involving two breaks (312 or 83.0% of total aberrations). There were only 24 asymmetric exchanges; 4 dicentrics, 2 rings, 1 acentric ring, and 17 interstitial deletions (Table 1). The remaining 288 (76.6%) were symmetric exchanges among which were 234 reciprocal translocations (62.2%), 40 pericentric inversions (10.6%), and 14 paracentric inversions (3.7%). Of the 234 translocations, 5 were characterized as incomplete exchanges owing to the loss of one of the two chromosome segments participating in the exchange.
### Table 1. Type and frequency of chromosome aberrations identified by G-method in 311 aberrant cells* from 23 heavily exposed A-bomb survivors of Hiroshima

| Type                     | Number (%) |
|--------------------------|------------|
| Simple aberration:       | 340 (90.4) |
| 1) One break             |            |
| Acentric fragment        | 8 (2.1)    |
| Terminal deletion        | 20 (5.3)   |
| 2) Two breaks            | 312 (83.0) |
| a) Asymmetric exchange   | 24 (6.4)   |
| Dicentric                | 4 (1.1)    |
| Ring                     | 2 (0.5)    |
| Acentric ring            | 1 (0.3)    |
| Interstitial deletion    | 17 (4.5)   |
| b) Symmetric exchange    | 288 (76.6) |
| Reciprocal translocation | 234 (62.2) |
| Pericentric inversion    | 40 (10.6)  |
| Paracentric inversion    | 14 (3.7)   |
| Complex aberration:      | 36 (9.6)   |
| Insertion                | 13 (3.5)   |
| Complex translocation    | 13 (3.5)   |
| Complex exchange         | 10 (2.7)   |
| Total                    | 376 (100.0)|

*Excluding 31 unidentifiable cells

Complex aberrations described here were further divided into three groups; 1) either direct or inverted “insertion” within a chromosome or between two chromosomes due to three-break rearrangements, 2) “complex translocations” such as sequential exchanges and double reciprocal translocations due to rearrangements with at least three breaks, and 3) three- or more-break rearrangements by a combination of a translocation and an insertion, an inversion and a translocation, and so on, referred to as “complex exchanges”.

Of the 36 complex aberrations, 13 insertions, 13 complex translocations, and 10 complex exchanges were observed (Table 1). Partial karyotypes of representative complex aberrations are shown in Figure 1. In two complex exchanges, partial deficiency of a chromosome segment was observed, whereas in the remaining 34 complex aberrations, neither deficiency nor duplication of a chromosome segment could be seen.
Excluding 15 asymmetric aberrations from the total of 376 aberrations observed, the remaining 361 were classified into the following three groups; 1) aberrations identical by both methods, 2) aberrations not identical though detectable by both methods, and 3) aberrations detected only by the G-method. Of the 361 aberrations, 188 (52.1%) were classified into the first group: 153 translocations, 23 inversions, and 12 deletions (Table 2). The second group included 95 (26.3%) aberrations: 41 translocations, 9 inversions, 11 deletions, and 34 complex aberrations. The majority of reciprocal translocations in this group were identified as pericentric inversions or terminal deletions by the O-method, since the abnormal counterpart could not be identified by this method. For the same reason, complex aberrations identified by the G-method were classified as simple type aberrations such as translocations, inversions, or deletions by the O-method.

Seventy-eight (21.6%) aberrations in the third group were detected exclusively by the G-method, and they were anticipated a priori to be either paracentric inversions or reciprocal translocations of chromosome segments of equal length. In fact, only 14 (17.9%) were identified as paracentric inversions, and 11 (14.1%) as translocations of chromosome segments of equal length (Table 3). Among the remaining 53 aberrations, 39 (50.0%) were intra- and inter-changes of chromosome segments of unequal length, and 14 (17.9%) were deletions with a small segment at the distal end which were judged to be within the normal limits of variation by the O-method.

39 exchanges between chromosome segments of unequal length in the third group were further divided into two subgroups: a) both abnormal chromosomes
were normal in appearance as judged by the O-method, and b) both excess and deficiency of the chromosome material due to exchanges of the segments with unequal length were so subtle that there was no apparent morphological change in the chromosome constitution as observed by the O-method (Figure 2). The former comprised 17 translocations and 5 inversions, while the latter included 12 translocations, 3 inversions, and 2 complex aberrations.

There were 25 aberrations detected only by the O-method among which 21 aberrant chromosomes were found to be either overcontracted or unusually twisted, while by the G-method the banding patterns proved to be normal. In the remaining four aberrations observed, exchanges (or breaks), could conceivably have occurred at the negative regions of the distal part of the chromosomes. This indicates that even the G-method fails to detect structural aberrations if they occur at the negative bands of the chromosome.

| Classification | Aberrations by G-method |
|----------------|-------------------------|
| O-method       | G-method                | O : G |
| Abnormal : Abnormal | Identical | 153 | 23 | 12 | 0 | 188 | 52.1 |
| Abnormal : Abnormal | Not identical | 41 | 9 | 11 | 34 | 95 | 26.3 |
| Normal : Abnormal | Not identical | 40 | 22 | 14 | 2 | 78 | 21.6 |
| Total          |                         | 234 | 54 | 37 | 36 | 361 | 100.0 |

*Excluding 15 asymmetric aberrations (8 acentric fragments, 4 dicentrics, 2 rings, and 1 acentric ring).

Table 3. Classification of 78 aberrations detected exclusively by G-method

| Type               | Aberration |
|--------------------|------------|
|                    | t | inv | del | complex | Total | %  |
| Paracentric inversion | — | 14 | —   | —      | 14    | 17.9 |
| Equal length exchange | 11 | 0  | —   | 0      | 11    | 14.1 |
| Unequal length exchange | 29 | 8  | —   | 2      | 39    | 50.0 |
| Minor deletion      | —   | —  | 14  | —      | 14    | 17.9 |
| Total               | 40  | 22 | 14  | 2      | 78    | 99.9 |
DISCUSSION

The present study of the somatic chromosomes of 23 Hiroshima survivors using the G-method has confirmed our previous findings\(^1,2\) in which asymmetric exchanges were found to be less frequent than symmetric aberrations (6% vs. 77% of the total aberrations observed). This suggests that the symmetric exchanges may be the more useful indicators for evaluating the relationship between chromosome aberrations and radiation dose, particularly for those who were exposed many years before the cytogenetic examination.

Until banding techniques for identifying individual chromosomes were developed, the identification of symmetric exchanges by the conventional staining method was technically limited, since only those showing either an unusual shift in the position of the centromere or abnormal arm length were recognizable as abnormal monocentrics and thus constitute only a small proportion of the true symmetric exchanges. Paracentric inversions and some of the reciprocal translocations where the exchanged segments are equal in length are undetectable by the O-method, and it is estimated that the efficiency of scoring symmetrical rearrangements in cultured human lymphocytes following irradiation may be as low as 20\(^\%\)\(^8,9,10\).

In the present study, it was found that 22% of G-method symmetric exchanges (62 of 288) were undetectable by the O-method, in other words, about 78% of the symmetric exchanges identified by the G-method were also detected by the O-method. This efficiency of scoring symmetric exchanges by the O-
method is higher than expected, assuming that all the symmetric exchanges are detectable by G-method analysis.

One-third (25 of 78) of the aberrations by G-method showed paracentric inversions and reciprocal translocations of the chromosome segments with equal length. No pericentric inversion due to breaks at equidistant points from the centromere and subsequent rejoining were observed in the present study.

Nevertheless some of the unequal length exchanges could not be detected by the O-method. One possible explanation for this is that both of the abnormal chromosomes thus produced fell within the normal range of variation for the corresponding chromosome groups in terms of the length and the position of centromere, as shown in Figure 2 (37 of the 78 undetectable-type aberrations belonged to this category). Further, 14 minor terminal deletions and 2 complex aberrations were also undetected by the O-method.

It is worth noting that complex exchange aberrations involving three or more breaks observed by the G-method were present in the lymphocytes of A-bomb survivors with a frequency of about 10% of total aberrations so far detected.

Seabright reported, from in vitro irradiation experiments of human lymphocytes using the G-method, that 8 complex exchanges, including double reciprocal translocations between three chromosomes and simultaneous production of one dicentric and one translocation chromosome, were observed in a total of 131 aberrations. Complex exchanges were also identified by Buckton in X-irradiated peripheral lymphocytes in vitro, in which 8 translocations between three chromosomes and 3 dicentrics with the acentric fragments translocated onto another chromosome were detected by sequential R- and G-banding methods.

In the present examination, however, there were no complex aberrations involving dicentrics or rings, suggesting that cells with unstable complex aberrations would have been eliminated from the lymphocyte population due to mitotic disturbance in the lapse of time that has occurred after in vivo exposure to A-bomb irradiation.

Of the 342 aberrant cells detected, there were 31 unidentifiable cells in which a total of 145 chromosomes were found to show abnormal banding patterns, and thus considered to have participated in exchange formations. There were already 36 cells with complex but identifiable exchanges, yielding a total of 67 cells with complicated structural rearrangements of chromosomes which have persisted to date in the circulating lymphocytes of A-bomb survivors.

In the present study, no attempt was made to analyze the chromosome aberration frequency by estimated radiation dose for individual survivors because of the paucity in the number of cases as well as in the number of cells per case.
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