Am. J. Hum. Genet. 63:489–497, 1998

An Association, in Adult Japanese, between the Occurrence of Rogue Cells among Cultured Lymphocytes (JC Virus Activity) and the Frequency of "Simple" Chromosomal Damage among the Lymphocytes of Persons Exhibiting These Rogue Cells

James V. Neel

Department of Human Genetics, University of Michigan, Ann Arbor

Summary

Data from a previous study of the cytogenetic effects, in cultured lymphocytes, of exposure to the atomic bomb in Hiroshima have been reanalyzed to determine the relationship between the occurrence of "rogue" cells in an individual and the frequency of "simple" chromosomal damage in the nonrogue cells of the same individual. Rogue cells are cells with complex chromosomal damage, currently believed to be a manifestation of the activity of a human polyoma virus termed "JC." Among a total of 1,835 persons examined, there were 45 exhibiting rogue cells. A total of 179,599 cells were scored for simple chromosomal damage. In both the exposed and the control populations, there was an absolute increase of ~1.5% in the frequency of simple chromosomal damage in the nonrogue cells of those exhibiting rogue cells, when compared with the frequencies observed in those not exhibiting rogue cells, which is a statistically significant difference. It is argued that this phenomenon, occurring not only in lymphocytes but possibly also in other cells/tissues, may play a contributory role in the origin of malignancies characterized by clonal chromosome abnormalities. Unexpectedly, among those exhibiting rogue cells, there was a disproportionately greater representation of persons who had received relatively high radiation exposures from the bomb. The reason for this is unclear, but it is tempting to relate the finding to some lingering effect of the exposure (or the circumstances surrounding the exposure) on immunocompetence.

Received December 3, 1997; accepted for publication June 3, 1998; electronically published July 29, 1998.

Address for correspondence and reprints: Dr. James V. Neel, Department of Human Genetics, Box 0618, M4708 Medical Science II, University of Michigan, Ann Arbor, MI 48109-0618. E-mail: cgaffney@umich.edu

© 1998 by The American Society of Human Genetics. All rights reserved.

0002-9297/98/6302-0024$02.00

Introduction

In recent years, studies of the somatic-cell genetics of cancer have been dominated by considerations of mutation in so-called proto-oncogenes and tumor suppressor genes. However, clonal cytogenetic abnormalities are also characteristic of malignancies, for which the break points of the translocations, inversions, and deletions often involve the site of known proto-oncogenes or tumor suppressor genes. Current genetic thought tends to regard the chromosome breaks preceding these rearrangements as random events inherent in the complex molecular structure of DNA. From time to time, however, the possibility that the necessary breaks might be, to some extent, virus driven has been raised. In this article I examine this hypothesis on the basis of epidemiological evidence concerning a common human viral infection.

The genesis of this study may be dated to 1968, when, in the course of cytogenetic studies of two villages of Yanomama Amerindians, Bloom et al. (1970) observed that, of 4,875 scored lymphocytes, 21 cultured lymphocytes exhibited a picture of extreme chromosomal damage. Similar cells were encountered subsequently—usually in a much lower frequency—in cytogenetic studies of English persons (Fox et al. 1984; Tawn et al. 1985), of Japanese (Awa and Neel 1986), and of the members of various countries formerly part of the Soviet Union, namely, Lithuania (Lazutka 1996), Russia (Bochkov and Katosova 1994; Salomaa et al. 1997), the Ukraine (Neel et al. 1992; Scheid et al. 1993; Sevankaev et al. 1993), and Belorussia (Verschaeve et al. 1993). The heavy representation of members of the former Soviet Union results from studies of the cytogenetic effects of the Chernobyl disaster. My colleagues and I termed these cells "rogue cells," arbitrarily defined as containing five or more exchange-type aberrations for which precise karyotypic identification of the origin of the aberrant chromosomes usually was impossible (Awa and Neel 1986). Similar cells have been produced by the inoculation of human fibroblast cultures with a simian poly-

oma virus, simian virus 40 (SV40), with the chromosomal damage demonstrated to be due to the gene encoding the T antigen of the virus (Ray et al. 1990, 1992; Stewart and Bacchetti 1991; Ray and Kraemer 1993).

These facts led us to search for elevated antibody titers against the two best-known human polyoma viruses, JC virus (JCV) and BK virus (BKV), in humans exhibiting rogue cells. At maturity, ⩾70%–90% of individuals in the urbanized countries of the world thus far studied exhibit significant seropositivity (titers ≥ 1/40) for these two viruses, which have a high homology (~70%) with each other and with SV40 (reviewed in Hogan et al. 1984; Walker and Frisque 1986). The serological studies revealed significantly elevated titers, compared with matched controls, against both JCV and BKV in individuals exhibiting rogue cells, with the elevation against JCV being more striking (Neel et al. 1996). Inoculation of cultured human fetal brain cells with JCV resulted in chromosomal damage (Neel et al. 1996) that was very similar to that encountered in the early stages of infection with SV40 of a human fibroblast line (Wolman et al. 1964; Lehman 1974), which created the strong presumption that JCV infection was at least one cause of the rogue cells, although participation of BKV in the rogue-cell phenomenon was not excluded rigorously. (Of course, the demonstration that JCV infection is associated with the rogue-cell phenomenon does not preclude a role for other, nonpolyoma viruses in this phenomenon.)

From the outset of our studies, we have been interested in the possibility that "simple" chromosomal damage was elevated in the non–rogue cell lymphocytes of persons exhibiting rogue cells. Such simple damage consists of stable translocations and inversions and unstable multicentric chromosomes, free fragments, centric and acentric rings, and double minutes; when such damage is observed, it usually involves only one or two chromosomes per cell. There are extensive reports of such simple chromosomal rearrangements resulting in clones that play a significant role in oncogenesis (reviewed in Sandberg 1990; Heim and Mitelman 1995; Rowley 1996). In our previous studies, the evidence that an elevation of this baseline might be characteristic of persons exhibiting rogue cells has been somewhat erratic. Thus, in the original Yanomama study, 4.10% ± 0.28% of all cells scored (200 of 4,875 cells) showed simple damage of the types enumerated, whereas in follow-up studies in the same two villages 2 years later, when the frequency of rogue cells had fallen to 0.01% (1 of 9,849 cells), the corresponding percentage was 1.30% ± 0.13% (128 of 9,849 cells) (Bloom et al. 1970, 1973). Although this difference is highly significant ($\chi^2 = 117.62$, $P < .0001$ [one-tailed]), the validity of the comparison is diminished by the 2-year interval between the observations. Among the eight persons in a Ukrainian village who exhibited rogue cells, the frequency of simple damage was 1.52% ± 0.30% (24 of 1,580 cells scored), whereas among the 16 persons not found to exhibit rogue cells, the corresponding figure was 1.03% ± 0.18% (33 of 3,200 cells scored) ($\chi^2 = 2.14$, $P = .095$ [one-tailed]; Neel et al. 1992). Finally, in a study in Lithuania of simple chromosomal damage in 33 workers occupationally exposed to low doses of ionizing radiation and of 11 control persons, a multivariate regression analysis revealed a significant relationship of simple damage to JCV titers ($P = .0204$) and to BKV titers ($P = .0202$) (Lazutka et al. 1996).

Two observations by other groups are important in this respect. From the various studies of English persons (Tawn et al. 1985; Tawn 1987; Tawn and Binks 1989), one can conclude that, in the control individuals in these series, the frequency of cells with asymmetrical exchange-type aberrations (i.e., dicentrics and centric rings) was 12/1,141 (three persons) among individuals in whom rogue cells were observed but was 25/16,550 (114 persons) among individuals in whom rogue cells were not observed ($\chi^2 = 41.4$, df 1, $P < .001$). (These studies are based on the use of both banding and nonbanding techniques, which detect exchange-type aberrations with equal efficiency.) In a study of chromosome aberrations in persons residing in Lithuania who had been involved in the Chernobyl cleanup operation and in suitable controls, Lazutka (1996) found that, for the total sample, the frequency of simple damage (all types of chromosomal damage in nonrogue cells) per 100 cells was 3.57% ± 0.39% among the 31 persons exhibiting rogue cells but was 3.40% ± 0.16% among the 179 persons in whom rogue cells were not observed, which is an insignificant difference. The relatively high frequency of cells with damage in both groups reflects the radiation exposures sustained by the cleanup workers.

The possible relevance of these findings to the origin and role of clonal cytogenetic abnormalities in oncogenesis is such that further observations on this point seemed highly desirable. For this study, we returned to an extensive data set collected in connection with studies of the cytogenetic effects of exposure to the atomic bombs. It will be shown that control individuals not exposed to the atomic bombs in whom lymphocyte rogue cells were encountered, as well as such individuals with relatively low exposures to atomic radiation, do indeed exhibit a significant elevation of simple chromosomal damage in their nonrogue lymphocytes. An unexpected finding is that in these cytogenetic studies, which were conducted 23–40 years after the bombing, persons with relatively heavy radiation exposures from the atomic-bomb detonation appear to exhibit rogue cells more frequently than persons experiencing lesser or no radiation exposures. This observation raises the

possibility of lingering effects of exposure to the bomb on the immune system.

Subjects and Methods

The data used in this study were obtained during the years 1968–85 in an effort to evaluate the cytogenetic damage resulting from exposure to the atomic bombs (Awa et al. 1971, 1978; Awa 1991; Stram et al. 1993). The exposed subjects were all atomic-bomb survivors residing in Hiroshima who were selected from among the participants in an adult-health study, for each of whom detailed dosimetry studies had been conducted and organ doses assigned by the so-called DS86 algorithm. The control group consisted of distally exposed survivors with estimated surface exposures at the time of the bombings (ATB) of <0.005 Sievert (Sv) equivalents, whereas the exposed group all had estimated exposures of $\geqslant$0.005 Sv equivalents. In the previous analysis of these data, the presence of what we now term "rogue cells" was recognized (Awa et al. 1978, p. 131), but it was not included in the statistical analysis. (I am deeply indebted to Dr. A. A. Awa, who was in charge of the cytogenetic studies during the period of these studies, for making these data available in their present form.)

Between 1968 and 1974, peripheral blood was cultured by use of the method of Moorhead et al. (1960), with minor modifications (see Awa et al. 1978). In view of the findings, it should be stated that the media were not thought, at any time during the study, to be folate deficient. In 1974, after extensive tests, a modified technique was introduced that continues to be used currently. For this study, 2 ml of heparinized whole blood was added to a mixture of 8 ml of RPMI (Roswell Park Memorial Institute) culture medium, 0.5 ml of an L-glutamine solution (3 mg of L-glutamine), and 0.2 ml of phytohemagglutinin solution, and total culture time was reduced to 48 h. The heparinized whole blood was refrigerated up to a maximum of 2 d before culture. At the outset, 200 μl of colcemid solution (10 μg/ml; GIBCO) was added to the culture, for a final concentration of 0.2 μg/ml. Other procedures were as described previously. The ratio of control to exposed persons being processed through the laboratory at any one time was held constant throughout the study, to minimize observer and technical bias.

Data Analysis

The data from the previous cytogenetic study that are relevant to the present treatment are presented in table 1 and were tabulated by dose and by detection of one or more rogue cells. (Given the previous demonstration of elevated anti-JCV titers in persons exhibiting rogue cells, the presence of rogue cells in peripheral lymphocytes were equated with JCV activity in that person.) The estimated bone-marrow radiation dose ATB is given in Sieverts, with neutron dose assigned a relative biological effectiveness of 10. For the following analyses, dose intervals among those exposed to the atomic-bomb blast have been arbitrarily categorized in the fashion customary at the Radiation Effects Research Foundation (RERF)—namely, controls (exposure <0.005 Sv equivalents) and exposed in three categories (0.005–0.49 Sv equivalents, 0.50–0.99 Sv equivalents, and 1.0 Sv equivalents). For the following analysis, subjects who had a malignancy at the time of the study were excluded because of the possibility that they had received clastogenic therapy. Also excluded were persons to whom it has proved difficult to assign a precise radiation exposure ATB. The protocol for this study called for the examination of 100 cells/person. In a few cases this was not possible, so that, although a total of 1,835 persons were examined, only 179,599 cells were scored (average of 97.9 cells/person). Rogue cells were encountered in 45 persons. In 44 persons, only a single rogue cell was observed, but one individual exhibited two such cells among 100 cells scored. The frequency of rogue cells was 0.026% ± 0.0038%.

A preliminary tabulation and analysis of the data in table 1 by sex (data not shown) revealed no noteworthy differences between sexes, with respect to the items in the table, so the data on males and females have been combined. The combined results are derived from 753 males and 1,082 females. The excess of females reflects the higher proportion of females among the survivors of the atomic bombings. The relatively advanced ages (~59 years) of the study subjects at the time of the examinations reflect the fact that the primary purpose of the original study was to determine the cytogenetic effects of the atomic-bomb exposures in 1945.

The data in table 1 have been analyzed by a series of $2 \times n\chi^2$ contrasts with, unless otherwise noted, 1 df. The χ^2 tests of independence (see Snedecor and Cochran 1989) were computed by use of the SAS software, release 6.07 (SAS Institute). All numbers used in the χ^2 tests can be derived directly from table 1 but, for expository purposes, also are sometimes repeated in the text. Because in these statistical tests the data sets are not always independent of one another, the χ^2 values also are not independent of one another.

The fact of an increase in simple chromosomal damage in the cultured lymphocytes of those exposed to the atomic bomb, which is evident in the table, has been treated adequately elsewhere (Awa et al. 1971, 1978; Awa 1991) and will not be analyzed further here. Proceeding to matters more relevant to the present study, we tested first for the relative frequency of cells with simple stable and unstable chromosomal damage in the

Table 1

The Occurrence in Japan of Simple Chromosomal Damage in Cultured Lymphocytes, in Relation to Both Exposure to the Atomic Bomb and Detection of Rogue Cells

Subject Category	No. of Subjects	Mean Age ATB (years)	Mean Age at Exam (years)	No. (%) of Cells				Mean Dose (Sv)
				Total Scored	With Simple Stable Damage	With Simple Unstable Damage	Total with Simple Damage	
Rogue cells detected:								
Control	13	23.92 ± 11.40	54.31 ± 11.61	1,284	26 (2.02)	16 (1.25)	42 (3.27)	.00
Exposed, by Dose (in Sv):								
.005–.49	1	36.0	62.0	100	0 (.00)	1 (1.00)	1 (1.00)	.16
.50–.99	6	28.67 ± 12.07	63.00 ± 9.04	600	60 (10.00)	10 (1.67)	70 (11.67)	.76
1.0+	25	27.56 ± 14.20	55.96 ± 14.43	2,450	530 (21.63)	23 (.94)	553 (22.57)	2.41
Total	32	28.03 ± 14.12	57.47 ± 13.58	3,150	590 (18.73)	34 (1.08)	624 (19.81)	2.03
Rogue cells not detected:								
Control	640	25.47 ± 12.64	58.61 ± 11.76	62,147	906 (1.46)	538 (.87)	1,444 (2.32)	.00
Exposed, by Dose (in Sv):								
.005–.49	414	22.45 ± 10.95	6.40 ± 10.50	40,797	1,014 (2.50)	461 (1.13)	1,475 (3.62)	.17
.50–.99	290	23.66 ± 11.78	59.38 ± 10.40	28,564	2,243 (7.85)	324 (1.13)	2,567 (8.99)	.75
1.0+	446	23.22 ± 12.93	57.57 ± 12.44	43,657	7,288 (16.69)	482 (1.10)	7,770 (17.80)	2.04
Total	1,150	23.05 ± 11.99	59.05 ± 11.33	113,018	10,545 (9.33)	1,267 (1.11)	11,812 (10.45)	1.04

controls and also in the exposed, in relation to rogue-cell presence. (The operational distinction between stable and unstable damage was stated in the Introduction.) Both for rogue cells absent and for rogue cells present, there is a significant difference ($\chi^2_{\text{rogue cells absent}} = 770.01$, $P < .0001$; $\chi^2_{\text{rogue cells present}} = 60.40$, $P < .0001$). In both contrasts, the proportion of unstable damage is much less in the exposed than in the controls. For example (see table 1), for the sample in which rogue cells were not detected, the proportion of simple unstable damage among all damage was [0.87/(1.46 + 0.87)] = 0.37 for the data on controls, whereas the corresponding proportion was [1.11/(9.33 + 1.11)] = 0.11 for the data on the exposed.

This striking finding is attributed primarily to the fact that the unstable damage induced by the atomic-bomb exposure mostly has been eliminated by now in the exposed; that is, a higher proportion of the simple damage in the controls is expected to be of relatively recent origin and, in the course of the cell divisions following the occurrence of the damage, to not have been eliminated to the same extent that such damage has been eliminated from subjects experiencing damage following the bomb exposures. We assume that this "elimination factor" has operated similarly in those exposed who were found to have rogue cells and in those who were not found to have rogue cells. Although this time-dependent loss of the unstable chromosomal damage induced by the atomic bombs blurs the comparisons being made, there is no way to compensate for it, since the cytogenetic studies were not initiated until 23 years after the bombings; therefore, we worked with the total simple damage.

To test for an increase in simple chromosomal damage (stable and unstable combined) in persons in whom rogue cells were detected, it is necessary to demonstrate that, among those exposed to the bomb, the radiation exposures were comparable for those with and without rogue cells. Table 1 suggests that this is not the case, since those exhibiting rogue cells received an average estimated bone-marrow dose of 2.03 Sv equivalents, whereas those not found to have rogue cells received an average dose of 1.04 Sv equivalents (SDs are not given for the six mean doses, because dose distribution within categories is very asymmetrical.) An inspection of table 1 reveals that this finding is due in part to a higher average dose for those in the highest dose category. However, among those exposed who exhibited rogue cells, the proportion of individuals who fall into the highest dose category is higher than that for those not exhibiting rogue cells (rogue cells absent: 446/1150 = 38.8%; rogue cells present: 25/32 = 78.1%; $\chi^2 = 20.11$, $P < .0001$.) Furthermore, although there is no statistically significant difference between the frequency of rogue cells in the control cell population and that in the total exposed cell population ($\chi^2 = 0.814$, $P = .230$), the frequency of rogue cells appears to be relatively higher in the highest dose category. Thus, in the three dose categories, the frequencies of persons with rogue cells are 1/415 = .002, 6/296 = .020, and 25/471 = .053. The heterogeneity χ^2 value for this 3 × 2 table is 22.20, with 2 df and $P \leqslant .0001$. However, for the two lower dose categories, the frequency of rogue cells is not significantly different from that in the controls.

For now, I therefore will confine my analysis of the relationship between the presence of rogue cells (as indicators of JCV activity) and the frequency of simple chromosomal damage to the controls and the two lower dose categories, returning to the findings for the highest dose category in the Discussion. Because of the small number of rogue cells, it is desirable to consolidate categories for statistical tests, but this is appropriate only if, for the exposed, the average exposures, by category, are comparable for those with and those without rogue cells. By pooling the data from the controls and the two lower exposure categories, we found that, for those in whom rogue cells were not detected, the percentage of cells with simple damage was 4.17% (5486/131,508), whereas, for those in whom rogue cells were detected, the percentage of simple damage was 5.70% (113/1984) ($\chi^2 = 11.30$, $P < .001$). (The high frequency of simple damage reflects the radiation histories of some of the subjects.) For this study, we concluded that, in the presence of rogue cells, there is an absolute increase of ~1.5% in the frequency of simple chromosomal damage in the controls and in persons exposed to relatively low doses of radiation ATB. The data do not exclude a similar relationship at higher levels of exposure, but, for this latter group, the higher average radiation-exposure levels for persons exhibiting rogue cells, compared with those not exhibiting rogue cells, precludes a direct comparison. Since most of these data were collected at a time when chromosome-banding techniques were not available, no effort has been made to analyze chromosomal breakage patterns.

Discussion

Simple Chromosomal Damage

The present analysis would seem to establish that in Japanese persons exposed 52 years ago to the atomic bombs, as well as in those not so exposed, simple chromosome damage was shown, in cytogenetic studies conducted during the years 1968–85, to be significantly higher in those persons found to be exhibiting rogue cells than in those in whom rogue cells were not observed. This confirms the general thrust of the earlier observations cited in the Introduction. The differences that we observed in the present study are minimal estimates of the true difference. This is because, under our

research design, only a fraction of the persons in the population who currently exhibit rogue cells was detected when the sample of cells scored was limited to 100. In a previous study of the children of atomic-bomb survivors, for each of whom 10 cultured lymphocytes had been scored for the presence of transmitted chromosomal abnormalities (Awa and Neel 1986), 7 rogue cells were found among 2,138 additional cells scored (1 of 305 cells), when as many additional cells as possible (beyond the standard 10) were scored from 20 persons in whom rogue cells were detected. Although this estimate probably is biased upward because of a tendency to detect the presence of rogue cells in those in whom they are most frequent, we adopted this finding as the best current estimate of the frequency of rogue cells in Japanese in whom such cells were detected during this survey. Then, in the present study, the probability of detecting a rogue cell in an individual exhibiting the phenomenon was $1 - (304/305)^{98}$, or 0.275, when an average of 98 cells were scored per person. Thus, each person in this population in whom one or more rogue cells were detected represents 1.00/0.275, or 3.64, persons with rogue cells. Confining the calculation to the control data from 653 persons leads to an estimate of $13 \times 3.64 = 47.3$ such persons in the present Japanese control data set. Expressed as a percentage, it thus can be estimated that 7.2% of the control population exhibited rogue cells during the period over which the present study extended. This is a rather rough estimate, to which it would be unwise to attach an error term. If these additional persons with rogue cells have the same frequency of simple damage as those who in fact were found to have rogue cells and if allowance were made for this fact, then the magnitude of the differences in simple chromosomal damage, between the exposed and the controls, in the "rogue-cell effect" can only increase.

It is instructive to compare the results of the present analysis of the frequency of persons with rogue cells with the results of the above-referenced similar analysis of a younger sample of Japanese (children of survivors and controls, with an average age of 23.4 ± 6.3 years), carried out over approximately the same time frame (Awa and Neel 1986). The frequency of rogue cells in that sample was 24/102,170 (0.024% ± 0.005%), which is in striking agreement with the 0.026% ± 0.004% observed in the present study. With respect to the frequency of persons exhibiting rogue cells during the previous study, the calculation was 7.6%, compared with the current calculation of 7.2%. Previously, we suggested, on the basis of the similarity in anti-JCV titers in young (23.9 ± 4.54 years of age) and older (56.0 ± 8.8 years of age) Japanese (Neel et al. 1996), "continuing activity of the JCV throughout life, either as reinfection or activation of a latent virus" (p. 2692). This suggestion now has been reinforced by the present calculation of the proportion manifesting rogue cells at any one time, with no suggestion that reactivation might be less common in the older age group.

Potential for Oncogenesis through Cytological Damage

Neel et al. (1996) previously had raised the question of whether the chromosomal damage thought to be produced by JCV (and possibly by BKV) can contribute to the clones of cytogenetically abnormal cells so often encountered in malignant tissues and, in some instances, demonstrated to be accompanied by specific alterations of oncogenes and tumor suppressor genes (reviewed in Sandberg 1990; Heim and Mitelman 1995; Rowley 1996). No clastogenic site specificity of viral action was implied; rather, the thought was that, among the thousands and perhaps millions of chromosomal breaks induced by JCV in susceptible tissues, over the infected individual's lifetime, some would involve loci critical to oncogenesis. In this situation, the overall potential significance of JCV will be determined primarily by its host cell specificities and its pattern of activity in susceptible tissues.

With respect to tissue and/or cell specificity, the human cell best known for its sensitivity to JCV infection is the oligodendrocyte, in which viral activity results in the progressive multifocal leukoencephalopathy encountered in the immunosuppressed, especially those with acquired immunodeficiency syndrome. In such patients, viral DNA also can be found in lymphocytes, bone marrow, liver, spleen, and lung (Grinnel et al. 1983). Recently, Rencic et al. (1996) described the presence of JCV DNA in an oligoastrocytoma from an immunocompetent person. JCV DNA also has been found, by the PCR technique, in a small fraction of the lymphocytes of apparently normal persons, as well as in hematopoietic stem cells (Tornatore et al. 1992). Viral involvement of the renal system, first suggested by JCV DNA detection in the urine (Coleman et al. 1980), now is thought to be very probable, as suggested by the recovery of JC sequences from the normal renal medulla of 13 (40.6%) of 32 individuals who have undergone surgery for renal cancer (Tominaga et al. 1992). However, whether the virus normally is resident in renal cellular tissue or whether its presence in urine and in the renal medulla is the result of contamination of the kidney, by virus-bearing lymphocytoid cells, is not yet clear. Finally, we recently reported the presence of JCV DNA in normal and malignant colons. DNA was isolated from 37 resected colon cancers and matched normal tissues and was examined for the presence of three JCV T-antigen sequences, by use of the PCR technique. Sequences were found in 73% of normal samples and 97% of colon cancers (Laghi et al. 1997). That this viral presence is not (always) due to transitory cells of the lymphocytoid

line is suggested strongly by the fact that viral fragments were detected in 5 of 10 colon-cancer xenografts.

With respect to the temporal pattern of activity in these tissues, primarily on the basis of the finding that the distribution of anti-JCV titers was not significantly different in a sample of 100 Japanese 23.9 ± 4.5 years of age and a second sample of 99 Japanese 56.0 ± 8.8 years of age (Neel et al. 1996), we previously endorsed the suggestion made by Padgett (1981) that, following the primary infection in youth, the virus is episodically active in infected persons, much like herpes simplex, and, presumably for the same reason, fluctuations in the infected person's immunocompetence (Neel et al. 1996). In this article, the calculation that persons exhibiting rogue cells are approximately as frequent among older adults as among younger adults, tends to confirm that suggestion, but, at present, there is no basis for estimating how many cycles of viral activity characterized by the appearance of rogue cells in peripheral blood are to be anticipated in an individual infected in the 1st or 2d decade of life.

Potential of JCV for Oncogenesis through Other SV40-Like Effects

Thus far, this article has emphasized the clastogenic possibilities of infection with the polyoma viruses, presumably primarily through helicase activity on the part of the large T antigen, as documented for the large T antigen produced by SV40 (reviewed in Walker and Frisque 1986; Shah 1996). However, the T antigen of SV40 also has been shown to complex with no less than seven proteins in host human cells, including two proteins playing key roles in oncogenesis, namely, p53 and the pRb family of proteins (reviewed in Fanning and Knippers 1992). The highly homologous T antigen of BKV also has been shown to react with both p53 and the pRb family of proteins, as well as with several other cellular proteins (see references in Dyson et al. 1990; Harris et al. 1996). Although studies of the JCV T antigen have not been as numerous, recent experiments have revealed on the JCV T-antigen functional domains similar to those in the BKV T antigen (and the SV40 T antigen) (see references in Major et al. 1992; Swenson et al. 1996).

Loeb (1991) and Jackson and Loeb (1998), arguing from existing knowledge of somatic-cell mutation rates, have suggested that these rates are not sufficiently high enough to drive the multihit hypothesis of oncogenesis and have postulated the existence of a currently unidentified "mutator phenotype" acquired during the course of oncogenesis. It is now a legitimate hypothesis that this "phenotype" may be the reoccurring activation of the human polyoma virus, especially with reference to mutations associated with chromosome damage.

High Radiation Exposures ATB and the Occurrence of Rogue Cells

Finally, I return to the unexpected finding that rogue cells were relatively more common in those receiving the larger doses of radiation ATB. In other words, whereas at the two lower exposure categories recognized in this study the mean exposure doses were quite similar for those in whom rogue cells were and were not detected, at the highest dose category this was not the case, the mean exposure dose for those who exhibited rogue cells being substantially greater than that for those who did not.

The implication of this observation is that a relatively heavy exposure to the radiation of the atomic bombs 23–40 years prior to the cytogenetic studies (or some circumstance associated with the atomic-bomb exposure) has altered the exposed individuals' long-term susceptibility to the activity of JCV and the rogue-cell phenomenon. As previously noted, our present interpretation of the rogue-cell phenomenon is that it is a recurrent event in persons who, for the most part, acquired the JCV infection early in life. Thus, viral expression as rogue cells in apparently normal individuals may be presumed to reflect periodic fluctuations in the immunocompetence of these persons. Pursuing this line of thought, we suggest that the observation under discussion implies a departure from the usual manifestations of immunocompetence in some of those persons previously exposed to the atomic bombs.

Several earlier studies have suggested that the immunocompetence of survivors of the atomic bombings who had received significant amounts of radiation might have been altered by that experience (reviewed in Akiyama 1995). The most notable observation has been an alteration of the balance/interaction between the T- and B-cell subsets of lymphocytes—specifically, a decrease in the T-cell population and an increase in the B-cell population, among lymphocytes in the peripheral circulation. Interestingly, as found in the present study, this effect seemed to be confined mostly to those experiencing the higher doses. In addition, an *increased* level of anti–Epstein-Barr virus antibody titer has been observed in the exposed; it will be of interest to determine whether the same is true for the JCV antibody titer.

The etiology of the increase in cancer incidence among atomic-bomb survivors (reviewed in Schull 1995; Pierce et al. 1996) undoubtedly is complex, but the well-documented increase in chromosomal damage in the survivors (reviewed in Awa 1991) surely is a major player in the phenomenon. The present observations suggest that this chromosomal damage could be both a direct and an indirect result of the exposure to the bomb, the latter mediated through an increased susceptibility to the periodic clastogenic effects of reactivated polyoma (and

other possible) viruses. Current knowledge does not permit an evaluation of the relative impact of the direct and indirect components in this equation over an extended period of time.

Many of the observations in population surveys of rogue cells, cited in the Introduction, involve populations exposed to very low levels of radioactive fallout following the Chernobyl disaster. In view of the observations just discussed, we must ask whether the exposure to the fallout is related, directly or indirectly, to the occurrence of these rogue cells. Inasmuch as rogue cells have been observed in numerous populations with no history of an increased exposure to radiation (e.g., Amerindians, English Caucasians, and Japanese), it is clear that radiation exposure is not a necessary prerequisite to their appearance. However, it is not possible at this time to state categorically that, in populations receiving low levels of increased radiation, radiation exposure plays no role in their appearance, but, because of the very low radiation exposures involved in the studies of populations exposed to the Chernobyl fallout, we view this possibility as highly unlikely.

Acknowledgments

The author wishes to thank all the professional and technical staff members at the Cytogenetics Laboratory, Department of Genetics, RERF Hiroshima Laboratory, and, in particular, Dr. A. A. Awa, for their devotion to the long-range cytogenetic study program, a portion of which has been the basis for this report. This laboratory is an activity of RERF, Hiroshima and Nagasaki, Japan, a private nonprofit foundation funded by the Japanese Ministry of Health and Welfare and the U.S. Department of Energy, through the National Academy of Sciences. I acknowledge current support from National Institutes of Health grant CA26803. I also am indebted to Dr. Janet Tawn for a reanalysis of her publications, to obtain data on simple chromosome damage in English subjects exhibiting rogue cells.

References

Akiyama M (1995) Late effects of radiation on the human immune system: an overview of immune response among the atomic-bomb survivors. Int J Radiat Biol 68:497–508

Awa AA (1991) Persistent chromosome aberrations in the somatic cells of A-bomb survivors, Hiroshima and Nagasaki. J Radiat Res Suppl 32:265–274

Awa AA, Honda T, Sofuni T, Neriishi S, Yoshida M, Matsui T (1971) Chromosome-aberration frequency in cultured blood-cells in relation to radiation dose of A-bomb survivors. Lancet 2:903–905

Awa AA, Neel JV (1986) Cytogenetic "rogue" cells: what is their frequency, origin, and evolutionary significance? Proc Natl Acad Sci USA 83:1021–1025

Awa AA, Sofuni T, Honda T, Itoh M, Neriishi S, Otake M (1978) Relationship between the radiation dose and chromosome aberrations in atomic bomb survivors of Hiroshima and Nagasaki. J Radiat Res 19:126–140

Bloom AD, Neel JV, Choi KW, Iida S, Chagnon N (1970) Chromosome aberrations among the Yanomama Indians. Proc Natl Acad Sci USA 66:920–927

Bloom AD, Neel JV, Tsuchimoto T, Meilinger K (1973) Chromosomal breakage in leukocytes of South American Indians. Cytogenet Cell Genet 12:175–186

Bochkov NP, Katosova LD (1994) Analysis of multiaberrant cells in lymphocytes of persons living in different ecological regions. Mutat Res 323:7–10

Coleman DV, Wolfendale MR, Daniel RA, Dhanjal NK, Gardner SD, Gibson PE, Field AM (1980) A prospective study of human polyoma virus infection in pregnancy. J Infect Dis 142:1–8

Dyson N, Bernards R, Friend SH, Gooding LR, Hassell JA, Major EO, Pipas JM, et al (1990) Large T antigens of many polyomaviruses are able to form complexes with the retinoblastoma protein. J Virol 64:1353–1356

Fanning E, Knippers R (1992) Structure and function of simian virus 40 large tumor antigen. Annu Rev Biochem 61:55–85

Fox DP, Robertson FW, Brown T, Whitehead AR, Douglas JDM (1984) Chromosome aberrations in divers. Undersea Biomed Res 11:193–204

Grinnel BW, Padgett BL, Walker DL (1983) Distribution of nonintegrated DNA from JC papovavirus in organs of patients with progressive multifocal leukoencephalopathy. J Infect Dis 147:669–675

Harris KF, Christensen JB, Imperiale MJ (1996) BK virus large T antigen: interactions with the retinoblastoma family of tumor suppressor proteins and effects on cellular growth control. J Virol 70:2378–2386

Heim S, Mitelman F (1995) Cancer cytogenetics. John Wiley & Sons, New York

Hogan TF, Padgett BL, Walker DL (1984) Human polyomaviruses. In: Belshe RB (ed) Textbook of human virology. PSG Publishing, Littleton, MA, pp 969–995

Jackson AL, Loeb LA (1998) The mutation rate and cancer. Genetics 148:1483–1490

Laghi L, Chauhan DP, Marra G, Major EO, Neel JV, Boland CR (1997) Amplification of JC virus (JCV) sequences from human colorectal cancers. Gastroenterology 110:A548

Lazutka JR (1996) Chromosome aberrations and rogue cells in lymphocytes of Chernobyl clean-up workers. Mutat Res 350:315–329

Lazutka JR, Neel JV, Major EO, Dedonyte V, Mierauskine J, Slapsyte G, Kesminiene A (1996) High titers of antibodies to two human polyoma viruses, JCV and BKV, correlate with increased frequency of chromosomal damage in human lymphocytes. Cancer Lett 109:177–183

Lehman JM (1974) Early chromosome changes in diploid Chinese hamster cells after infection with simian virus 40. Int J Cancer 13:164–172

Loeb LA (1991) Mutator phenotype may be required for multistage carcinogenesis. Cancer Res 51:3075–3079

Major EO, Amemiya K, Tornatore C, Houff SA, Berger JR (1992) Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus–induced demyelineating disease of the human brain. Clin Microbiol Rev 5:49–73

Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA (1960) Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res 20: 613–616

Neel JV, Awa AA, Kodama Y, Nakano M, Mabuchi K (1992) "Rogue" lymphocytes among Ukrainians not exposed to radioactive fall-out from the Chernobyl accident: the possible role of this phenomenon in oncogenesis, teratogenesis, and mutagenesis. Proc Natl Acad Sci USA 89:6973–6977

Neel JV, Major EO, Awa AA, Glover T, Burgess A, Traub R, Curfman B, et al (1996) Hypothesis: "rogue cell"–type chromosomal damage in lymphocytes is associated with infection with the JC human polyoma virus and has implications for oncogenesis. Proc Natl Acad Sci USA 93:2690–2695

Padgett B (1981) Human papovaviruses. In: Tooze J (ed) DNA tumor viruses, pt 2, rev. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 339–370

Pierce DA, Shimizu Y, Preston DL, Vaeth M, Mabuchi K (1996) Studies of the mortality of atomic bomb survivors. Report 12, Part I. Cancer 1950–1990. Radiat Res 146:1–27

Ray FA, Kraemer PM (1993) Iterative chromosome mutation and selection as a mechanism of complete transformation of human diploid fibroblasts by SV40 T antigen. Carcinogenesis 14:1511–1516

Ray FA, Meyne J, Kraemer PM (1992) SV40 T antigen induced chromosomal changes reflect a process that is both clastogenic and aneuploidogenic and is ongoing throughout neoplastic progression of human fibroblasts. Mutat Res 284: 265–273

Ray FA, Peabody DS, Cooper JL, Cram LS, Kraemer PM (1990) SV40 T antigen alone drives karyotype instability that precedes neoplastic transformation of human diploid fibroblasts. J Cell Biochem 42:13–31

Rencic A, Gordon J, Otte J, Curtis M, Kovatich A, Zoltick P, Khalili K, et al (1996) Detection of JC virus DNA sequence and expression of the viral oncoprotein, tumor antigen, in brain of immunocompetent patient with oligoastrocytoma. Proc Natl Acad Sci USA 93:7352–7357

Rowley JD (1996) Leukemias, lymphomas, and other related disorders. In: Rimoin DL, Connor JM, Pyeritz R (eds) Principles and practice of medical genetics. Churchill Livingstone, London, pp 1687–1701

Salomaa S, Sevankaev AV, Zhloba AA, Kumpusalo E, Mäkinen S, Lindholm C, Kumpusalo L, et al (1997) Unstable and stable chromosome aberrations in lymphocytes of people exposed to Chernobyl fallout in Bryansk, Russia. Int J Radiat Biol 71:51–59

Sandberg AA (1990) The chromosomes in human cancer and leukemia. Elsevier Science Publishing, New York

Scheid W, Weber J, Petrenko S, Traut H (1993) Chromosome aberrations in human lymphocytes apparently induced by Chernobyl fallout. Health Phys 64:531–534

Schull WJ (1995) Effects of atomic radiation: a half-century of studies from Hiroshima and Nagasaki. Wiley-Liss, New York

Sevankaev AV, Tsyb AF, Lloyd DC, Zhloba AA, Moiseenko VV, Skrjabin AM, Climov VM (1993) "Rogue" cells observed in children exposed to radiation from the Chernobyl accident. Int J Radiat Biol 63:361–367

Shah KV (1996) Polyomaviruses. In: Fields BN, Knipe DM, Howley PM, Chanock RM, Melnick JL, Monath TP, Roizman B, et al (eds) Fields virology, 3d ed. Lippincott-Raven, Philadelphia, pp 2027–2043

Snedecor GW, Cochran WG (1989) Statistical methods. Iowa State University Press, Ames, IA

Stewart N, Bacchetti S (1991) Expression of SV40 large T antigen, but not small T antigen, is required for the induction of chromosomal aberrations in transformed human cells. Virology 180:49–57

Stram DO, Sposto R, Preston D, Abrahamson S, Honda T, Awa AA (1993) Stable chromosome aberrations among A-bomb survivors: an update. Radiat Res 136:29–36

Swenson JJ, Trowbridge PW, Frisque RJ (1996) Replication activity of JC virus large T antigen phosphorylation and zinc finger domain mutants. J Neurovirol 2:78–86

Tawn EJ (1987) The frequency of chromosome aberrations in a control population. Mutat Res 182:303–308

Tawn EJ, Binks K (1989) A cytogenetic study of radiation workers: the influence of dose accumulation patterns and smoking. Radiat Prot Dosim 28:173–180

Tawn EJ, Cartmel CL, Pyta EMT (1985) Cells with multiple chromosome aberrations in control individuals. Mutat Res 144:247–250

Tominaga T, Yogo Y, Kitamura T, Aso Y (1992) Persistence of archetypal JC virus DNA in normal renal tissue derived from tumor-bearing patients. Virology 186:736–741

Tornatore C, Berger JR, Houff SA, Curfman B, Meyers K, Winfield D, Major EO (1992) Detection of JC virus DNA in peripheral lymphocytes from patients with and without progressive multifocal leukoencephalopathy. Ann Neurol 31:454–462

Verschaeve L, Domracheva EV, Kuzuetsov SA, Nechai VV (1993) Chromosome aberrations in inhabitants of Byelorussia: consequence of the Chernobyl accident. Mutat Res 287:253–259

Walker DL, Frisque RJ (1986) The biology and molecular biology of JC virus. In: Salzman NP (ed) The papovaviridae. Vol 1: The polyomaviruses. Plenum Press, New York, pp 327–377

Wolman SR, Hirschhorn K, Todaro G (1964) Early chromosomal changes in SV40 infected human fibroblast cultures. Cytogenetics 3:45–61