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Genetics

Hypothesis: "Rogue cell"-type chromosomal damage in lymphocytes is associated with infection with the JC human polyoma virus and has implications for oncopenesis

(viral chromosome damage/JC viral oncogenesis)

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ABSTRACT **The hemagglutination inhibition antibody titers against the JC and BK polyoma viruses (JCV and BKV, respectively) are significantly elevated in individuals exhibiting "rogue" cells among their cultured lymphocytes. However, the elevation is so much greater with respect to JCV that the BKV elevation could readily be explained by cross reactivity to the capsid protein of these two closely related viruses. The JCV exhibits high sequence homology with the simian papovavirus, simian virus 40 (SV40), and inoculation of human fetal brain cells with JCV produces polyploidy and chromosomal damage very similar to that produced by SV40. We suggest, by analogy with the effects of SV40, that these changes are due to the action of the viral large tumor antigen, a pluripotent DNA binding protein that acts in both transcription and replication. The implications of these findings for oncogenesis are briefly discussed.**

Cultured lymphocytes exhibiting extreme chromosomal damage in the absence of any known cause, which we have termed "rogue" cells, were first encountered in significant numbers in 1968 in studies on two villages of Yanomama Amerindians. Twenty-one among 4875 cultured lymphocytes derived from 49 individuals and examined at first mitotic division (1 in 232 cells scored) exhibited the phenomenon (1). Subsequently, similar cells have been reported in mixed nationality North Sea divers (2), as well as in the inhabitants of England (3), Japan (4), Ukraine (5, 6), Lithuania (7), Russia (8), and Byelorussia (9, 10). The studies in which these cells were detected usually examined the lymphocyte cultures at a time that for most metaphases would correspond to the first cell division in culture, because of an interest in the detection of unstable chromosome aberrations.

Our past speculations concerning the agent(s) responsible for this phenomenon have centered on the effect of some unidentified exogenous virus or the activation of latent retroviral elements (6). With respect to the former possibility, there is a large literature from several decades ago on virally induced chromosomal damage (for reviews, see refs. 11 and 12). Especially relevant in the present context is the demonstration more than 30 years ago by Koprowski *et al.* (13), Yerganian *et al.* (14), Cooper and Black (15), and Moorhead and Saksela (16) of extensive cytogenetic abnormalities in human cell lines transformed by a papovavirus, simian virus 40 (SV40) (17). Wolman *et al.* (18) and Lehman (19) found that the chromosomal damage began to appear within a few days of the inoculation of a human fibroblast culture with SV40. Ray *et al.* (20) and Stewart and Bacchetti (21) demonstrated that the SV40 large tumor antigen (TAg) alone can drive the karyotypic instability that precedes the neoplastic transformation of human diploid fibroblasts, and Ray and colleagues (22, 23) suggested that the TAg by the process of iterative mutation is responsible for all the steps in the neoplastic transformation of fibroblasts by SV40.

These findings with respect to a simian papovavirus (SV40) have led us to test the hypothesis that the rogue cells observed in the various studies cited above might result from human infection with one or the other of the two papovaviruses to which human populations commonly exhibit antibodies, namely, BK virus (BKV) or JC virus (JCV), each of which possesses a gene with high homology to the gene responsible for the large TAg of SV40 (for reviews, see refs. 24–26). In this communication we will present the result of studies that strongly suggest that the rogue cell phenomenon does indeed result from infection with the JCV. We have previously speculated that the rogue cell phenomenon may play a role in carcinogenesis, teratogenesis, and the chromosomal rearrangements of evolution. The current findings lend support to that speculation.

SEROLOGICAL STUDIES

Materials and Methods

Sources of Plasma. In the course of a search for transmitted cytogenetic damage among the children of parents exposed to the atomic bombings of Hiroshima and a suitable and approximately equal group of control children (total of 9818 subjects), 24 individuals each exhibiting one rogue cell were encountered (4). The protocol specified that 10 cells be examined from each subject, but because of minor departures from protocol, a total of 102,170 cells were scored, an average of 10.4 cells per subject. The rogue cell phenomenon was not related to the radiation history of the parents. The studies were performed between 1967 and 1984. When cytogenetic preparations were made, if available, an aliquot of plasma from the same blood sample was placed in storage at −196°C. These samples constitute the F_1 plasma bank. When for some reason an aliquot of plasma was not available from blood obtained at the time of the cytogenetic studies, an effort was made to obtain one at the time of any subsequent contact with the individual.

In this study, plasma samples were available from 11 (8 males and 3 females) of the 24 persons who had exhibited rogue cells in the course of the previously described study. [Of the 24 persons in whom rogue cells were detected in the Japanese study, 16 were male and 8 were female, an excess of males that does not achieve statistical significance ($\chi^2 = 3.30$; df = 1; 0.05

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Abbreviations: SV40, simian virus 40; TAg, tumor antigen; HA unit, hemagglutination unit; JCV, JC virus; BKV, BK virus.

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$< P < 0.10$).] Eight of these samples were obtained at the time of the cytogenetic studies; three samples were obtained 4–8 months after the cytogenetic studies. The mean age of these 11 individuals was 24.2 ± 6.1 years, with a range of 15–35 years. Three types of control samples were analyzed. For control set 1, samples from 8 of the 11 persons exhibiting rogue cells from whom plasma was available were matched by age, sex, and time of collection, as nearly as possible, with 3 other samples in the F_1 plasma bank from persons in whom rogue cells had not been encountered. The mean age of this group was 23.0 ± 5.1 years, with a range of 16–34 years. For control set 2, 100 plasma samples distributed over the entire time frame of the cytogenetic study (1967–1984) were pulled from the F_1 plasma bank. We emphasize that these latter samples are not temporally matched to individuals exhibiting rogue cells (unlike the first set) but may be considered representative of a Japanese population aged 15–33 years (average age, 23.9 ± 4.54 years), sampled over a period of 18 years. The individuals in control sets 1 and 2 were all born subsequent to the atomic bombings as were the study subjects. Finally, for control set 3, 100 plasma samples were available from parents of children in the genetic study, parents from whom blood samples had also been obtained earlier and aliquots stored in the F_1 plasma bank. These individuals were all alive at the time of the atomic bombs, some being exposed to the effects of the bombs, some not. Their ages at the time the samples were drawn ranged from 39 to 81 years, with an average age of 56.0 ± 8.8 years.

Serologic Procedures. The presence of antibodies to JCV and BKV was determined by a hemagglutination assay using human type O erythrocytes. Unlike the simian papovavirus, SV40, both JCV and BKV have the ability to agglutinate human type O erythrocytes. The major capsid protein, VP1, is the predominant structural protein of the icosahedral virion particle and is responsible for attachment to cells and for erythrocyte agglutination (27). The property of hemagglutination has been the standard method of quantitation of JCV antibodies.

Human type O erythrocytes were centrifuged at 1800 × *g* for 30 min at 4°C, washed twice in Alsever's buffer (20 mM sodium citrate/72 mM NaCl/100 mM glucose, pH 6.5 adjusted with glacial acetic acid), and suspended in Alsever's buffer at a final concentration of 0.5%. Serial 1:2 dilutions of virus suspensions were made in Alsever's buffer. An equal volume of red blood cells was added to each dilution of virus and incubated at 4°C for 3–6 hr. The final dilution of virus suspension that agglutinates red blood cells was considered the end point of the titration and read as the reciprocal of that dilution. The end point dilution is considered 1 hemagglutination (HA) unit. The estimated ratio of infectious particles to HA units is approximately 10^4. The assay is performed in microtiter wells at a final volume of 100 μl.

Hemagglutination inhibition was used to measure the presence of serum antibody to JCV or BKV. The serum was treated with potassium periodate (0.05 M) for 30 min at room temperature to inactivate nonspecific inhibitors of erythrocyte agglutination. After periodate treatment, glycerol is added to a final concentration of 1%. The samples were incubated for 1 hr at 56°C. The treated serum samples were prepared as serial 1:2 dilutions in Alsever's buffer in microtiter wells. Each well then received an equal volume of 4 HA units of virus and an equal volume of human type O erythrocytes. The highest dilution of serum that prevents agglutination, indicated as a button of red blood cells in the bottom of the well, is considered the antibody titer against the virus. The assays were performed in duplicate and repeated several times to eliminate the effects of nonspecific reactions and errors in dilutions or inconsistencies in end-point analysis.

Results

Anti-JCV and -BKV Titers in Japanese in Whom Rogue Cells Were Detected and Controls. The distribution of hemagglutination titers to JCV and BKV in the Japanese data set is given in Table 1. Titers for both viruses in the three individuals with rogue cells from whom plasma was collected up to 8 months after the rogue cells were encountered appear similar to those in individuals in whom plasma was obtained at the time of the original study, and we will combine these two sets of observations in the analysis to follow. The results of our analyses are given in Table 2. Because of the nature of the distributions, nonparametric statistics have been employed, using the SAS statistical system. With respect to the distribution of the titer values, two other tests (Wilcoxon two-sample test and Savage two-sample test) yielded significances quite similar to the Kolmogorov–Smirnov test, and we present only the results of the latter test.

As Table 2 shows, with respect to JCV, there is no difference between the titers encountered in control sets 2 and 3, and they can be combined. There is a borderline difference between controls 2 and 3 vs. control 1, but not such as to preclude combining this data set with the other two control sets. The difference between the titers for the combined controls and the titers from those in whom rogue cells were detected is highly significant. We note, however, that some 32% of individuals in control series 1, 2, and 3 exhibit titers charac-

Table 1. Hemagglutination inhibition titers against the JCV and BKV in Japanese exhibiting rogue cells and in three sets of controls

	Time, month(s)		JCV titer			Time, month(s)		BKV titer		
Titer	0	1–8	Control 1	Control 2	Control 3	0	1–8	Control 1	Control 2	Control 3
<1:20			4	20	14			7	23	34
1:20			6	18	13			6	10	24
1:40			5	9	11			6	10	17
1:80			2	9	6	3	2	3	14	7
1:160	1			7	5	4	1	2	15	11
1:320			1	9	13	1			15	2
1:640	4	1	4	6	8				8	1
1:1,280	2			6	9				2	2
1:2,560		1		4	9				2	
1:5,120		1	2	6	5				1	
1:10,240	1			4	3					
1:20,480				1	2					
1:40,960				1	1					
Σ	8	3	24	100	99	8	3	24	100	98

Table 2. Results of a statistical analysis (*P* values) of the data of Table 1

Virus	Comparison	Median value (Mood's test)	Distribution (Kolmogorov–Smirnov)
JCV	Control 2 × control 3	0.2204	0.3244
	Controls 2 and 3 × control 1	0.0662	0.3244
	Controls 1, 2, and 3 × R	0.0007	0.0013
BKV	Control 2 × control 3	0.0002	0.0001
	Control 1 × control 3	0.6089	0.9997
	Control 1 × control 2	0.0015	0.0126
	Control 1 × R	0.0024	0.0002
	Control 2 × R	0.0057	0.0512
	Control 3 × R	0.0191	0.0001

R, rogue cell sample.

teristic of persons in whom rogue cells have been encountered (or even greater), a finding to which we return later.

On the other hand, the BKV titers differ significantly in two of the three pairwise comparisons of the controls, precluding an analysis based on combining the three sets of controls. Accordingly, Table 2 presents separate comparisons of the titers of individuals with rogue cells with the three sets of controls. Two of the contrasts reveal significant differences, the other yields a borderline difference. However, inspection of Table 2 reveals that although for both JCV and BKV there are no titers ≤1:40 in persons with rogue cells, 10 of the 11 persons with rogue cells exhibit an anti-JCV titer ≥1:640, whereas this is not true of any of the anti-BKV titers. We accordingly attribute the elevation in the anti-BKV titers to cross-reactivity of antibodies against the capsid protein of JCV or to the concurrent appearance of antibodies to both viruses. We suggest the data strongly implicate a role for JCV in the etiology of rogue cells. We must note, however, that contrary to expectation with this hypothesis, for both control series 2 and control series 3, the Spearman rank correlation within individuals between JCV and BKV titers was insignificant: 0.0084 ± 0.101 and −0.0717 ± 0.101, respectively.

There is a further inference to be drawn from Tables 1 and 2. Note that whereas for the anti-JCV titers, "young" (control 2) and "old" (control 3) agree in the distribution of titers, with respect to the anti-BKV titers, in the "young" group, 28 of 100 titers are ≥1:320, but in the "old" group, only 5 of 98 titers are ≥1:320. This suggests continuing activity of the JCV throughout life, either as reinfection or activation of a latent virus, whereas this is less the case for BKV.

An Estimate of the Frequency of Individuals Exhibiting Rogue Cells in the "Young Adult Japanese Population." The present data, combined with the results of certain previous studies, permit a preliminary estimate of the prevalence of persons with rogue cells in a Japanese population between ages 15 and 33, with an average age of 23.9 ± 4.5, an estimate that can be compared with the data on anti-JCV titers. In the study of Awa and Neel (4), whenever a rogue cell was encountered among the 10 cells routinely scored in each preparation, additional cultured lymphocytes were scored on the slide of that individual that yielded the rogue cells. Altogether 2138 cells from 20 persons were scored, and 7 additional rogue cells were encountered. Thus, whereas in the original material the frequency of rogue cells was 24/102,170 (1/4257), in this enriched sample the frequency was 7/2138 (1/305), and we will adopt this latter figure as the current best estimate of the frequency of rogue cells in a population of young Japanese in whom the rogue cell phenomenon is occurring. With this frequency, the probability of detecting a rogue cell in an individual exhibiting the phenomenon when only 10 cells are scored is $[1 - (304/305)^{10}]$ or 0.032. Thus for each person in this population of young adults in whom rogue cells were detected, there should be some 30.25 additional persons exhibiting the phenomenon in whom the cells were not detected. This leads to an estimate of 24 × 31.25, or 750 persons with circulating rogue cells in the total sample of 9818 individuals in the study (7.64%). Although there are several approximations in this calculation that preclude deriving an error, the calculation is sufficiently accurate to remove the rogue cell phenomenon from a somewhat exotic happening to a rather commonplace event in this age group of this population.

CYTOGENETIC STUDIES

Materials and Methods

A set of experiments was conducted to test directly whether JCV causes chromosome damage in human cells *in vitro*. The host cells for these experiments were cultured primary human fetal brain cells, so chosen because of their established susceptibility to JCV (28). Cultured cells were infected with 500 HA units of JCV, Mad-1 strain, and grown for an additional period of 5–21 days, depending on the experiment. In a pilot study (experiment 1), cells were subcultured 24 hr prior to harvesting for chromosome analysis 5, 6, or 7 days after infection. In the more extensive study (experiment 2), cells were subcultured approximately 48 hr prior to harvesting, and cells cultured for more than 7 days after infection were also subcultured on days 7 and 14, to allow for cell division. Chromosome preparations were made after 2–3 hr of Colcemid treatment. Metaphases were trypsin G-banded and analyzed for gross chromosomal changes. Control cultures were treated identically to infected cultures except that they were not inoculated with virus.

Results

Results are shown in Table 3. In the analysis of these data, we note, first, that the mitotic indices are low in both control and virus-treated fetal brain cell cultures. However, in the total material, the mitotic index (percent of mitotic cells) is about twice as high in the treated cultures, a significant difference ($P = 0.010$; all probabilities in this section are derived by Fisher's exact one-tailed test for a 2 × 2 table). SV40 also stimulates mitosis in cultured fibroblasts (e.g., ref. 17). In experiment 2, there is a significant increase in polyploid cells at day 7 ($P = 0.00000015$). Neither the increase in cells with structural abnormalities nor endoreduplication (as a percent of the polyploids) is significant (analysis not shown). It should be noted, however, that the metaphases observed in these cultured brain cells were of unusually poor quality, in consequence of which we believe the recorded frequency of damage is a minimum estimate. The findings of the small pilot study (experiment 1) with respect to an excess of polyploidy in the virus-treated cells at days 5–7 are marginally significant ($P = 0.050$), but the actual differences between control and treated are even more pronounced than the findings at day 7 in experiment 2. Since these two sets of early observations were

Table 3. Cytogenetic findings on days 7, 14, and 21 after inoculation of a human fetal brain cell line with JCV

Exp.	Day(s)	Treatment	No. chromosomes: Diploid cells Total	Diploid cells Abn	Polyploid cells Total	Polyploid cells Endo	Polyploid cells Abn	Grand total	Mitotic index (1000 cells)
1	5–7	Control	9	0	2	0	0	11	
	5–7	JCV	20	1	21	3	10	41	
2	7	Control	90	5	10	3	0	100	0.7
		JCV	86	5	56	18	10	142	1.4
	14	Control	48	6	2	1	0	50	0.1
		JCV	134	6	19	3	6	153	0.6
	21	Control	41	1	5	0	0	46	0.3
		JCV	146	8	8	0	2	154	0.6
	Total	Control	179	12	17	4	0	196	0.4
	(exp. 2)	JCV	366	19	83	21	18	449	0.9

Number of chromosomes in the diploid cells ranged from 30 to 48, with the mode at 46. The number of chromosomes in the polyploid cells varied between 58 and ≈200, with the mode at 92. Abn, cells with structural abnormality (gaps, breaks, fragments, dicentrics, and radial figures) but not including telomere associations, centromere separation, or questionable dicentrics. Cells were not completely analyzed for structural rearrangements; Endo, endoreduplication.

made under similar experimental conditions, we have elected to combine the results. In the combined data set, the increase in polyploidy in the JCV-inoculated cells is even more significant ($P = 0.0000000035$), and the increase in cells with structural abnormalities is now also significant, either as a percent of total cells ($P = 0.0065$) or as a percent of the polyploid cells ($P = 0.037$). A cell illustrating several types of chromosome damage is pictured in Fig. 1. In this connection, immunofluorescent staining for JCV TAg in the infected cell nucleus demonstrated that, depending on the sample, 42–48% of the cultured human fetal brain cells had been infected. The excess of polyploidy in the combined data set is 31.3%. This suggests that the majority of infected cells have become polyploid by day 7, thus accounting for the concentration of structural aberrations in the polyploid cells. Proof of this hypothesis awaits a direct analysis of JCV presence in polyploid cells.

It is notable that both treated and untreated cultured fetal brain cells exhibited a relatively high level of endoreduplication, a rare cytogenetic finding but common in SV40-infected cells (18). We also observed numerous metaphases characterized by unusual centromere separation or "splaying," reminiscent of that seen in Roberts syndrome (29). The basis for these findings is unknown. Numerous examples of telomere association between two or more chromosomes were also noted in the JCV-infected cultures (data not shown).

We did not observe the very complexly damaged pseudodiploid cells we have termed rogue cells, illustrated in refs. 1, 4, and 6. However, the results clearly show that in this experimental system, JCV has a pronounced effect on ploidy, and, to a lesser extent, on structural aberrations. These preliminary results closely parallel those observed at early stages after SV40 infection of human fibroblasts (18, 19), which infection at later stages is also characterized by the induction of cells with chromosomal instability and multiple chromosome aberrations reminiscent of rogue cells.

In view of the foregoing findings, it was of some interest to determine the frequency of rogue cells in the "young" Japanese of control series 2 with the most grossly elevated anti-JCV titers. For the 11 individuals with the highest anti-JCV titers in control series 2 (all ≥1:5120) (none of whom were found to exhibit rogue cells among the 10 cells scored in the previous study), repository cytogenetic preparations were available for 10, prepared as described in Awa and Neel (4). As many cells as possible were scored for each person. There were two rogue cells among a total of 12,927 metaphases scored. This frequency, of 1/6464 metaphases, does not differ from the frequency of 1/4257 observed in the original survey (see above).

We suggest that this apparent paradox, of a "normal" frequency of rogue cells in persons with high anti-JCV titers, can be explained by the foregoing experimental studies on the production of cytogenetic effects by JCV plus the usual course of antibody production after a viral infection. In the experimental material, cytogenetic changes were at a maximum in 1 week. However, in most viral infections, antibody titers typically continue to increase for weeks to months after infection (or reinfection or reimmunization). We, therefore, suggest that in this Japanese population, following JCV infection (or reactivation), by the time the anti-JCV titers reached a maximum, the extreme cytogenetic consequences of the infection (rogue cells) had already largely cleared from the circulating lymphocytes. The fact that the modal titer in persons exhibiting rogue cells was 1:640 reinforces the suggestion that rogue cells appear (and disappear) before maximum anti-JCV titers are reached.

Discussion

The seroepidemiological data presented in this paper strongly implicate JCV in the etiology of rogue cells. The data also suggest that the "rogue cell phenomenon" is not uncommon in a Japanese population of young adults (average age, 23.9 ± 4.5 years), by our calculation involving some 7.6% of the population. Direct confirmation of the hypothesis that JCV produces cytological damage has been provided by JCV inoculation of cultured human fetal brain cells that are highly permissive for viral multiplication. Although no typical rogue cells were seen in these experiments (which involved human fetal brain cells rather than the lymphocytes in which rogue cells have been detected), the damage was almost identical (although less dramatic) to that produced by SV40 in human fibroblasts in the early period after inoculation (18); in preparations of SV40-infected fibroblasts later in infection, typical rogue cells were seen (20). It should be noted that the cytological effects of the SV40 appear to be due entirely to the gene encoding the large TAg (20) that, at the amino acid level, is 72% homologous to the JCV large TAg (30). We also note that there is 75% sequence homology between the gene encoding the SV40 large TAg and the corresponding gene in BKV and recognize that we have not yet excluded the possibility that BKV may sometimes be involved in the production of rogue cells.

The Epidemiology of JCV. In Japan, the source of all the serum/plasma samples employed in this study, seropositivity to JCV (hemagglutination inhibition titers, ≥1:20) was observed in 24.7% of 146 children under 1 year of age living in Tokyo, but this frequency may to some extent reflect passive

transmission of maternal antibody (31). In the age group 1–5 years, seropositivity was 45%, rising to 65% in the age interval 21–30, and 80% in persons >40 years of age (see also ref. 32). These latter percentages are in good agreement with our data based on a Hiroshima population (control 2, age 23.9 ± 4.5 years, 80%; control 3, age 56.0 ± 8.8 years, 86%). Similar findings characterize all urban populations studied to date (for review, see ref. 26). We note that grossly elevated anti-JCV titers are just as common in the older-age as in the younger-age controls. As noted earlier, this observation raises the question of whether rogue cells occur only once, at the time of the primary infection, or whether a latent infection may periodically be activated (or reinfection occurs), each time with a new shower of rogue cells. The observations of Kitamura *et al.* (33) concerning the absence of mixed JCV strain infections in Japanese can be interpreted as indicating that the high titers in later life indicate reactivation of the virus rather than reinfection.

The Possible Role of JCV in Oncogenesis. These new data enable us to further our earlier speculations concerning the role of the "rogue cell phenomenon" in oncogenesis (6). The potential role depends on at least three aspects of the action of the virus, each of which we discuss briefly.

Spectrum of cytogenetic damage and frequency of rogue cells in individuals exhibiting the phenomenon. The average rogue cell is so damaged that it could not undergo a successful meiosis. However, we have observed a considerable spectrum in the degree of damage and have postulated that some very small fraction of the least damaged rogue cells might undergo mitosis (6). Support for this suggestion derives from the small increase in "minor" cytogenetic damage we have recorded in persons exhibiting rogue cells (6). This range in damage could be related to the multiplicity of the viral infection of the cell. Furthermore, the ability of the large TAg of SV40 to drive the neoplastic transformation through its successive steps (23) suggests that some "rogue" cells are capable of reproduction. In this connection, we have calculated that at the time of a "shower" of rogue cells, there may be as many as 1.3×10^8 such lymphocytes in circulation (6). No data are available for other tissues, but if only 1 in 10,000 of these lymphocytes (or other cell types) were capable of reproduction, the possibilities for the establishment of abnormal clones are obvious.

Periodicity of the phenomenon. Padgett (24) from the distribution of JCV antibody titers in human populations suggested that "these viruses do persist and provide a constant antigenic stimulation" (p. 345). We would modify this suggestion, as noted earlier, to include the hypothesis that the virus replicates in infected persons when antibody titers fall below some critical level and that these cycles of viral activity are accompanied by bursts of rogue cells. By inference, the same episodic production of cytogenetic abnormality in lymphocytes could be true for other tissues. The pressure the rogue cell phenomenon exerts on oncogenesis should be directly proportional to the periodicity of the phenomenon. Studies on both individuals and cohorts that would clarify this postulated periodicity are urgently needed.

Spectrum of host cell infection. If the rogue cell phenomenon, *sensu strictu*, is confined to lymphocytoid tissue, already known to be a cell type susceptible to JCV (27, 34), then its role in oncogenesis could be limited to generating some portion of the specific chromosomal rearrangements seen in the various leukemias (for reviews, see refs. 35 and 36). However, the frequent demonstration by various techniques of the presence of the virus in brain in the progressive multifocal leukoencephalopathy (PML) of immunocompromised individuals (37) and the excretion of the virus in the urine of such persons and normal pregnant women (for reviews, see refs. 38 and 39) suggest two other complex tissues on which the virus might commonly exert its cytogenetic effects. Further, virus has been detected in the bone marrow and spleen of AIDS patients with PML (see references in ref. 40). The occurrence in peripheral lymphocytes is especially relevant to our thesis of the origin of rogue cells. The relevance of these findings may, however, be challenged because they so often involve immunocompromised individuals.

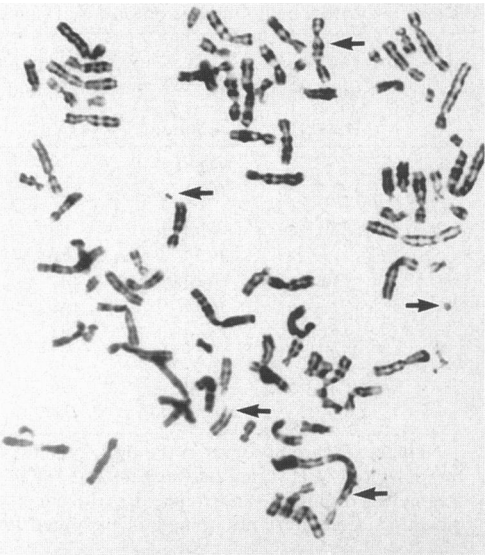

FIG. 1. JCV-infected fetal brain cell 7 days after infection. Pseudopolyploid cell (r = 87–88) with structural aberrations (arrows), including breaks and fragments, a probable dicentric, and a telomere association.

Concern with the oncogenic potential by JCV dates almost from the recognition of the virus (for review, see ref. 26). By virtue of what is now known concerning JCV, but also by analogy with knowledge concerning the action of SV40, there are at least three pathways through which JCV could produce the genetic changes contributing to oncogenesis. (*i*) JCV inserts in the host cell's genome (41), leading to the possibility of insertional mutagenesis. (*ii*) The large TAg of SV40 exhibits helicase activity in an experimental setting (for review, see ref. 42). Should the large TAg of JCV exhibit the same type of activity, this would constitute a mechanism for chromosomal breakage (and recombination). Since there is no established specificity for these insertion points, the chromosomal damage is presumably random, with subsequent *in vivo* selection for those clones in which the damage (and misrepair) results in oncogene activation (for review, see ref. 43). (*iii*) The protein product of the TAg of SV40 is known to interact with no less than seven proteins of the host cell, including the pRb and p53 proteins (see references in ref. 44). Should this also be true of the JCV TAg, these interactions point the way to another possible avenue of cell transformation, since in the mouse deficiency of p53 is associated with the development of tetraploidy *in vivo* (45).

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