Chromosomal radiosensitivity of lymphocytes from Alzheimer's disease patients

S E Tobi, J E Moquet, A A Edwards, D C Lloyd, R F Itzhaki

Abstract
We have examined chromosome aberrations in $\gamma$ irradiated (3 Gy) lymphocytes from five patients with Alzheimer's disease (AD). In each case, the number of dicentrics was significantly higher than the number in irradiated lymphocytes from five age matched normal subjects, the mean value for AD cells being about 25% higher. There was no significant difference in number of acentrics between AD and normal cells. Examination of the number of first, second, and third division metaphases, using fluorescence plus Giemsa staining, indicated that there was no difference in cycling time between AD and normal cells, and that after irradiation both groups showed the same mitotic delay. The similarity of our findings to those of others with irradiated Down's syndrome cells (from adult patients) is discussed.

We have examined DNA repair in Alzheimer's disease (AD). The rationale for this is that lymphoblastoid cells from AD patients have been shown after x irradiation to be less viable than those from age matched normal controls.1 Further, hypersensitivity of cells to certain DNA damaging agents has been detected in a number of neurological diseases; one of these, Down's syndrome (DS), is particularly relevant since all middle aged patients show the neuropathological characteristics of AD. Recently, fibroblasts from AD patients have been found to be more sensitive than those from normal subjects to the alkylating agents N-methyl-N'-nitro-N-nitrosoguainidine (MNNNG)$^2$ and methyl methanesulphonate (MMS),$^3$ as assayed by unscheduled DNA synthesis (UDS) or rate of strand rejoining. However, in the case of MMS, another study has indicated that the rate of rejoining in AD and normal cells is similar.$^4$

Our previous studies on $\gamma$ irradiated peripheral lymphocytes have shown that cells from AD patients and age matched normal controls are equally proficient in UDS,$^5$ in replication after PHA stimulation,$^6$ and in rate of repair of single strand breaks (Smith and Itzhaki, submitted for publication). However, we decided to examine chromosome aberrations in AD cells after irradiation, because aberrations are probably a more sensitive reflection of inadequate or inaccurate repair of DNA, and because x irradiated lymphocytes from DS patients show more chromosome aberrations than those from normal people.

Material and methods
PATIENTS
There were two males, aged 66 and 75, and three females, age range 63 to 71, who fulfilled the NINCDS–ADRSA (National Institute of Neurological and Communicative Disorders, Stroke-AD and Related Disorders Association) criteria for 'probable Alzheimer's disease'. In addition, they all had amnesia, visuospatial disorientation, and aphasia. The Hachinski ischaemia score was less than 4 in all cases. Single photon emission tomography showed posterior hemisphere reductions in uptake. No patient had familial AD (the criterion being that at least one close relative was affected). Medication for four patients consisted of: gammamimil-proxaline-carnitine; naproxin-prolaxine-digoxin-folic acid; diuretics-fibagel; dithiopene-hydergene. The other patient received no medication.

Normal controls were three males, age range 62 to 70, and two females, aged 61 and 76. These were University colleagues, none of whom was receiving medication.

PREPARATION OF SPECIMENS AND SCORING
Slides were prepared according to standard procedures$^7$ from triplicate samples of irradiated whole blood (3 Gy of Cobalt–60 $\gamma$ radiation) and of unirradiated controls. In each case, some cultures were terminated at 51 hours to score chromosomal aberrations and the
remainder at 72 hours to assess cell cycle delay by measuring the number of first, second, and third division metaphases (M1, M2, and M3). Each slide was coded and stained by the fluorescence plus Giemsa method. For each subject at 0 and 3 Gy, 200 first division metaphases from the 51 hour cultures were scored by the same person for dicentrics, centric rings, and excess acentic aberrations, and numbers of M1 and M2 metaphases were recorded also. Two hundred metaphases per subject were examined also from the 72 hour cultures to ascertain the numbers of M1, M2, and M3 cells.

Results
Table 1 gives the age and sex of each normal person and AD patient. The numbers of dicentrics, centric rings, and excess acentics scored in 200 cells are given for irradiated (3 Gy) and unirradiated samples. In all the latter, a total of 12 acentics (six in the ADs and six in the normals), but no dicentrics or rings were seen. These background levels of aberrations are consistent with normal levels previously published.

Similarly, the background (and irradiated) incidence of chromatid type aberrations was insignificant.

The distribution of radiation induced dicentric aberrations approximated closely to a Poisson distribution. Standard errors of the mean were, therefore, calculated on this basis (that is, for a Poisson, the mean is equal to the variance). However, acentrics showed overdispersion; their average ratio of variance to mean was 1.33. Average values of yield for the AD patients and the normal controls are also shown in table 1. It is clear that every AD patient has a higher sensitivity to dicentric induction than the normal controls, the mean AD value being greater by a factor of about 1.25. The difference between the means is significant (p<0.001). However, AD patients do not seem to be more sensitive to the induction of acentrics: the ratio of the means is 1.05 and the difference between them is not statistically significant. Also, the total numbers of centric rings observed in the two groups were similar.

On the 51 hour slides, very few (average 3.6%) unirradiated M2 cells were seen for either AD or normal cells. An effect of radiation induced mitotic delay was discernible in both groups but since only 0.85% of cells were in M2, analysis of the data was not taken further. Ratios of M1 to M2 suggest that cycling times of AD and normal cells are similar.

For the 72 hour cultures, table 2 shows the expected inter-donor variation in M1, M2, and M3 values within both AD and normal cells; however, the average numbers of M1 do not differ significantly between AD and normal cells for either unirradiated or 3 Gy irradiated cells, suggesting again that the rate of cell cycling, and of entry into cycle, is similar for AD and normal cells. After irradiation, mitotic delay is quite evident and the average difference in number of M1 cells (38 for AD and 44 for normal controls) between unirradiated and irradiated is not significantly different, that is, radiation induced mitotic delay is similar in AD and normal cells.

Discussion
Our main finding is that γ irradiated AD lymphocytes from each patient display significantly more dicentric chromosomal aberrations than cells from age matched, normal controls. On average, the increased yield after 3 Gy is about 25%. In contrast, the levels of excess acentric fragments in the two groups are not significantly different. Our other finding (table 2) is that there appears to be no difference in cycling times between AD and normal cells, and after irradiation.

Table 2 Numbers of cells at first, second, and third metaphase, at 72 hour post-stimulation (200 cells assessed per subject per dose).

| Subjects (sex, age) | Dicentrics | Centric rings | Excess acentics
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>AD1 F 66</td>
<td>122</td>
<td>7</td>
<td>136 (3)</td>
</tr>
<tr>
<td>AD2 F 63</td>
<td>115</td>
<td>3</td>
<td>108 (2)</td>
</tr>
<tr>
<td>AD3 M 66</td>
<td>103</td>
<td>4</td>
<td>90 (1)</td>
</tr>
<tr>
<td>AD4 M 76</td>
<td>100</td>
<td>6</td>
<td>69 (0)</td>
</tr>
<tr>
<td>AD5 M 75</td>
<td>103</td>
<td>1</td>
<td>67 (0)</td>
</tr>
</tbody>
</table>

Mean (SEM) 108.6 (4.6) 94.0 (10.0)

*200 unirradiated cells per subject yielded only excess acentics, shown in parentheses.

*Table 1 Chromosomal aberrations in G0 lymphocytes exposed to 3 Gy γ irradiation (200 cells scored per subject).

<table>
<thead>
<tr>
<th>Subjects (sex, age)</th>
<th>Dicentrics</th>
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*Table 2 Numbers of cells at first, second, and third metaphase, at 72 hour post-stimulation (200 cells assessed per subject per dose).

100 metaphases were examined on each of two replicate slides from the 72 hour cultures to ascertain the numbers of M1, M2, and M3 cells; since no significant difference was found within any pair of slides, values were combined.
both groups show the same mitotic delay. Thus, the observed difference in aberration yields is unlikely to be the result of a difference in time of entry into cycle. Previous studies on spontaneous chromosome aberrations in AD cells have yielded conflicting results, some showing levels similar to those of normal cells, and others higher levels. However, a definitive, large scale study showed that in female AD patients, but not in males, there was increased aneuploidy compared to age matched, normal controls, abnormalities being broadly similar to those seen in 20 year older normal persons. In the present study we have not examined aneuploidy; however, our unirradiated AD cells do not show significantly more spontaneous aberrations than age matched, normal controls. For DS lymphocytes also, the general consensus appears to be that the spontaneous aberration level resembles that of normal cells.

A few previous studies have used chemical agents rather than radiation to induce chromosome aberrations, specifically sister chromatid exchanges (SCE), in AD cells. After ethyl methanesulphonate treatment, no significant difference was found in SCE yields between AD and normal lymphocytes; after mitomycin C (MMC), two studies found no difference in SCE yields, but another claimed to find an increase in AD lymphocytes.

A study published while the present work was in progress has detected a higher level of aberrations in AD (and DS) lymphoblastoid cell lines than in normal ones after \( \gamma \) irradiation; however, the cells were irradiated in exponential phase, that is, at different stages of the cell cycle, with consequent variation in radiosensitivity, and so interpretation is difficult.

The increase of approximately 25% above normal in numbers of induced dicentrics in AD cells reported here is similar to that detected in cells from adult DS patients (and to those in two DS studies where ages were not given) after \( \alpha \) irradiation, and also after the radiomimetic, bleomycin. In the case of DS children, the number of dicentrics after irradiation is about two fold greater than in normals. Irradiation in \( G_0 \) does not increase the number ofacentric aberrations in DS lymphocytes just as we find for AD cells. The yields of centric rings were similar in the two groups and this conforms also with observations on DS lymphocytes. However, the numbers are low and so carry considerable error. Rings are observed in irradiated human lymphocytes typically at about 5% of the frequency of dicentrics, and so it is rarely possible to make statistical comparisons for this type of exchange aberration.

A similar rise in the number of dicentrics, but not acentrics, has been reported in lymphocytes from other radiosensitive syndromes such as ataxia telangietasia, Bloom’s and Fanconi’s syndromes (although doubts have been raised about this for ataxia). Further, in DS cells from adults, as in our AD cells, the proportion of cells at M1, M2, and M3 is similar to that of normal cells, and after treatment (with bleomycin) progression to mitosis decreases to a similar extent.

Our previous studies have shown no significant difference in radiosensitivity between AD and normal lymphocytes. Thus, our findings parallel those on DS Down’s syndrome lymphocytes, in which there is no conclusive evidence of a defect at the molecular level, although a higher level of chromosome aberrations has been detected after \( \alpha \) irradiation. Our present study therefore points to yet another similarity between AD and DS, in addition to the neuro-pathological changes and the involvement of chromosome 21.

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