DECREASED REPAIR OF GAMMA RAY DAMAGED DNA IN PROGERIA

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Summary - A sensitive host-cell reactivation technique was used to examine the DNA repair ability of fibroblasts from two patients with classical progeria. Fibroblasts were infected with either non-irradiated or gamma-irradiated adenovirus type 2 and at 48 hrs after infection cells were examined for the presence of viral structural antigens using immunofluorescent staining. The production of viral structural antigens was considerably reduced in the progeria lines as compared to normal fibroblasts when gamma-irradiated virus was used, indicating a defect in the repair of gamma ray damaged DNA in the progeria cells.

Progeria is a rare human disease characterized by the appearance of premature and accelerated aging (1). Fibroblasts from such patients show a reduced growth capacity *in vitro* (2, 3,4) suggesting that the phenotype of accelerated aging is also expressed at the cellular level. Epstein *et al*. (5,6) have used alkaline sucrose gradient sedimentation to show a reduced capacity for the rejoining of gamma ray-induced single strand DNA breaks in fibroblasts from patients with progeria. More recent results indicate that distinct genetic complementation groups may exist for progeria (7). These results suggest that an enzyme involved in DNA repair may be absent or deficient in cells from progeria patients. Using a similar, but not identical technique, Regan and Setlow (8) obtained results suggesting that there is no inherent defect in DNA repair in progeria cells. It has also been demonstrated that progeria cells contain a diversity of altered proteins: HL-A antigens (9,10), insulin receptors (11), a clotting factor (12) and other cytoplasmic enzymes (13).

Using a sensitive host-cell reactivation technique for irradiated virus we have found that fibroblasts from two patients with classical progeria have some deficiency in the repair of DNA damaged by gamma irradiation.

MATERIALS AND METHODS

Stock monolayer cultures of diploid human fibroblasts were grown in screw-cap bottles (Falcon plastic) and placed in a CO_2 incubator at 37°C and 90-100% humidity. The growth medium was Eagles α-minimal essential medium (α-MEM) supplemented with 10% fetal calf serum together with antibiotics. Strains AK, PG, RE and A2 were kindly supplied by Dr. Samuel Goldstein, Departments of Medicine and Biochemistry, McMaster University, Hamilton, Ontario, Canada. Strain CRL 1119 was obtained from the American Type Tissue Culture Collection, Rockville, Maryland, USA. Lines RE, A2 and CRL 1119 were all obtained from apparently normal individuals. Lines AK and PG were obtained from patients with Classical Progeria and have been described previously (13). At the time when experiments were performed both progeria lines were growing as well as the normals. Cell cultures were confluent at 7-9 days following a split ratio of 1:3. Unirradiated fibroblasts of line AK have been reported to display extensive DNA degradation when analyzed on sucrose gradients (14), possibly as a result of the incorporated radioactivity used for labelling the DNA. Consequently, although DNA repair studies were attempted no results have previously been obtained on the repair ability of this line.

Non-irradiated and irradiated suspensions of adenovirus type 2 (Ad 2) were assayed for their ability to form viral structural (V) antigens in human fibroblast cells infected in monolayer. Ad 2 is a double stranded DNA virus capable of infecting cultured human fibroblasts. It replicates in the nucleus forming large quantities of viral structural proteins (15) which can readily be detected by immunofluorescent staining. Ad 2 was prepared as described previously (16). Stock preparations, generally containing around 10^{12} particles/ml were suspended in TBS (17). The method of gamma irradiation has also been described (18). One ml samples of stock Ad 2 were kept at dry ice temperature (-75°C) during irradiation at a dose rate of 1-6 Mrads/hr using a Cobalt 60 source.

For the experiments, monolayers of cells, grown in 8 well chamber slides (Lab Tek Products, Naperville, Illinois) were infected with either irradiated or non-irradiated Ad 2. The total number of cells in each well was around 4×10^4. Following viral adsorption for 2 hrs, infected cells were incubated in growth medium. At 48 hrs after infection, cells were fixed in a cold acetone-methanol mixture (1:1) incubated in the presence of rabbit Ad 2 antiserum for 30 mins at 37°C, and then incubated for the same time with fluorescein-conjugated sheep anti-rabbit globulin. Positive cells were scored in duplicate wells at 3 serial dilutions for each determination of the ability of the virus to form V antigens.

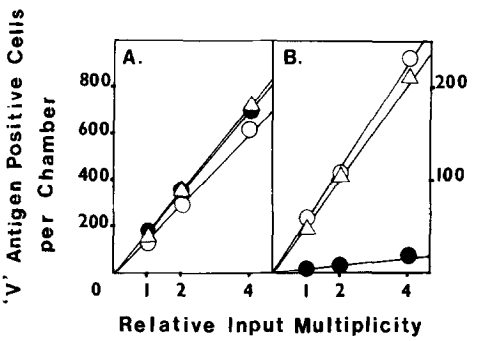

FIG. 1. The frequency of V antigen positive cells following adenovirus infection of a progeria and 2 normal fibroblast strains. Each point is the average of 2 chambers; the counts for each chamber generally being within 10% of each other. (A) unirradiated or (B) gamma irradiated virus (3 Mrads). ● AK progeria (passage 13) Δ RE normal (passage 7) o CRL 1119 normal (passage 14).

RESULTS

Typical results for the infection of normal and progeria fibroblasts with irradiated and non-irradiated virus are shown in Figure 1. It can be seen that the frequency of V antigen positive cells was similar for both the progeria and the two normal strains, when infected with non-irradiated virus (Figure 1A). However, the frequency of V antigen positive cells was considerably reduced in the progeria fibroblasts as compared to normal following infection with gamma-irradiated virus. The linear increase in V antigen positive cells with virus concentration for both unirradiated and irradiated virus indicates that survival curves obtained from these experiments were not subject to multiplicity reactivation. Typical survival data obtained following gamma irradiation are shown in Figure 2. It can be seen that the host cell reactivation of gamma-irradiated Ad 2 was similar for the 3 different normal fibroblasts but considerably reduced in both the progeria lines.

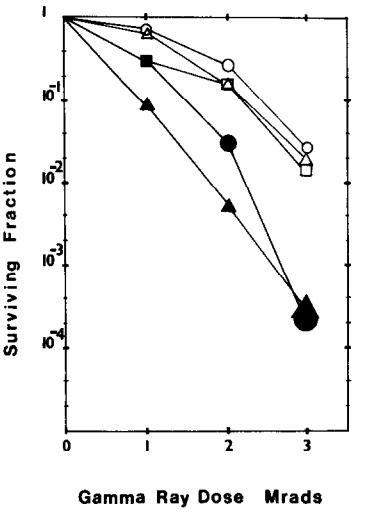

FIG. 2. Host cell reactivation of gamma-irradiated adenovirus in normal and progeria fibroblasts. The frequency of V antigen positive cells was determined in duplicate at three serial dilutions for each dose to the virus and the data points fitted to a straight line using least squares analysis. The standard errors obtained are contained within the symbol for each survival point. ● PG progeria (passage 11) ▲ AK progeria (passage 13) o CRL 1119 normal (passage 14) Δ RE normal (passage 7) A2 normal (passage 12).

DISCUSSION

A reduced host-cell reactivation of gamma-irradiated adenovirus was found for the 2 progeria strains AK and PG. These results indicate that these cells are unable to repair some type of gamma ray-induced DNA lesion. However, it does not indicate which repair process is defective. Little et al.(14) have reported a reduced capacity for the rejoining of gamma ray-induced single strand DNA breaks in several different progeria fibroblasts. The reduced host-cell reactivation reported here may be a reflection of this reduced capacity for single-strand DNA break rejoining found for progeria.

The detection of a wide diversity of proteins in progeria has been used to suggest that a qualitative defect in protein

turnover exists in progeria (11). However, such a diversity of abnormal gene production can also be explained on the basis of a decreased DNA repair capacity. The fact that SV_{40} transformed cells from these progeria lines have apparently normal repair ability has been used to suggest (13) that progeria may lack the control of genetic expression of a repair system rather than be carrying a specific gene defect.

Host cell reactivation of irradiated adenovirus has been used previously to determine the capacity for repair of xeroderma pigmentosum (17). It was found to be a sensitive assay for DNA repair in human cells, its greatest disadvantage being the long period required for the assay for plaque formation on human fibroblasts (16-18 days). The method using V antigen production by irradiated adenovirus in the infected cell allows the advantages of sensitivity without a prolonged assay period.

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