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γ-RAY ENHANCED REACTIVATION OF γ-IRRADIATED ADENOVIRUS IN HUMAN CELLS

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Summary - An enhanced reactivation of γ-irradiated human adenovirus type 2 (Ad 2) was detected following the infection of normal human fibroblasts which had been pre-irradiated with γ-rays. γ-irradiated or non-irradiated fibroblasts were infected with either non-irradiated or γ-irradiated Ad 2, and at 48 hours after infection cells were examined for the presence of viral structural antigens (Vag) using immunofluorescent staining. Pre-irradiation of the cells with 1 Krad immediately prior to infection resulted in a 5 to 15 fold increase in the survival of this viral function following different γ doses to the virus up to 3 Mrad. For a fixed γ dose of 2 Mrad to the virus this enhancement increased with pre-irradiation dose to the cells up to a maximum factor of 5 to 30 for a dose of 2 Krad. When infection was delayed until 48 hours after irradiation of the cells, this enhancement was reduced to about half the level found for immediate infection.

Enhanced reactivation of a UV-irradiated mammalian virus by pre-treatment of susceptible host cells with physical or chemical agents has now been found to exist for a number of different systems (1-6). In particular, UV-enhanced reactivation (UVER) of UV-irradiated Herpesvirus has been shown to be mutagenic in monkey kidney cells (7,8). This observation appears to be analogous to the phenomenon observed for UV-irradiated λ phage many years ago by Weigle (9), following pre-treatment of bacterial cells. The effect in bacterial cells has been shown to involve the induction of an 'error-prone' DNA repair mode that is highly mutagenic and is one of a number of metabolically diverse so-called "SOS" functions (10). It has been reported that an enhanced reactivation of γ-ray-damaged phage λ exists in *E. coli* cells (11). The magnitude of reactivation for γ-irradiated phage was found to be less than (about one half) that for UV-irradiated phage. More recently, low levels of enhanced reactivation for X-irradiated Herpesvirus have been reported to exist in monkey kidney cells (12).

Lytle *et al.* have shown that UVER of UV-irradiated Herpesvirus occurs in human fibroblasts from several different XP complementation groups as well as normal fibroblasts (4). We have shown in a previous report that γ-ray enhanced reactivation (γRER) of UV-damaged human adenovirus type 2 (Ad 2) exists in a variety of normal human fibroblast lines (13). The magnitude

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of enhanced reactivation for γ- and X-irradiated virus in human cells has not to our knowledge been reported.

In this report, we have investigated the possible existence of γ-ray enhanced reactivation of γ-irradiated human adenovirus type 2 (Ad 2) in 3 different normal human fibroblast lines. Our results indicate that γ-irradiation of host cells, immediately prior to infection with γ-irradiated virus, results in a marked increase in the survival of Vag formation, as compared to the survival obtained on unirradiated cells. If the infection was delayed until 48 hours after irradiation of the cells, however, this enhancement was reduced to about half the level observed for immediate infection after 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 Eagle's α-minimal essential medium (α-MEM) supplemented with 10% fetal bovine serum together with antibiotics. Strains A1 and A2 were kindly supplied by Dr. Samuel Goldstein, Departments of Medicine and Biochemistry, McMaster University, Hamilton, Ontario, Canada. Strain CRL 1221 was obtained from the American Type Tissue Culture Collection, Rockville, Maryland, USA. All 3 lines were obtained from apparently normal individuals. Cell cultures were generally confluent by 7-9 days following a split ratio of 1:3.

Ad 2 was prepared as previously described (14). Stock virus containing 2.2×10^{12} particles/ml was suspended in TBS (15) plus 2% glycerol and stored at -70°C.

The method of gamma irradiation of the virus has also been described (16). Two ml samples of stock Ad 2 were kept at dry ice temperature (-75°C) during irradiation at a dose rate of 1-6 Mrad/hr using a Cobalt 60 source. Cell monolayers, in 8-well chamber slides, were irradiated separately with γ-rays using a Cs^{137} source at a dose rate of about 5 rad/sec.

UV-irradiation of the virus was performed using the germicidal lamp (General Electric G8T). Stock virus was diluted 2-3 fold in α-MEM and 1 ml of viral suspension was irradiated in a 35 mm diameter petri dish (Falcon Plastic), kept on ice, with constant swirling during the irradiation. Under these conditions, the incident dose rate was about 6.2 J/m sec as determined using a J-225 short wave UV meter (Ultraviolet Products, San Gabriel, California, USA).

In the experiments, non-irradiated and irradiated suspensions of Ad 2 were assayed for their ability to form viral structural antigens (Vag) in UV-, γ-, and non-irradiated human fibroblast cells infected in monolayer. Monolayers of cells grown in 8-well chamber slides (Lab Tek Products, Naperville, Ill.) were infected with either γ-irradiated or non-irradiated Ad 2. Three serial dilutions of the virus were used to infect each slide. Duplicate wells were used for each viral dilution with the 2 additional wells serving as uninfected controls. Following viral adsorption for 2 hours, infected cells were incubated in growth medium. At 48 hours after infection, the monolayers were fixed in a cold acetone-ethanol mixture (1:1), incubated for 30 min at 37°C, and then incubated for the same time with fluorescein-conjugated anti-rabbit globulin. For each slide, the number of fluorescing centres was counted in duplicate wells at 3 serial dilutions of the virus, and the data points fitted to a straight line using least squares analysis. The slope of the line was then used as quantitative measure of Vag formation. In this way, the production of viral antigen under different conditions of irradiation of cells and virus was compared for infection both immediately, and 48 hours after pre-irradiation of the host cells.

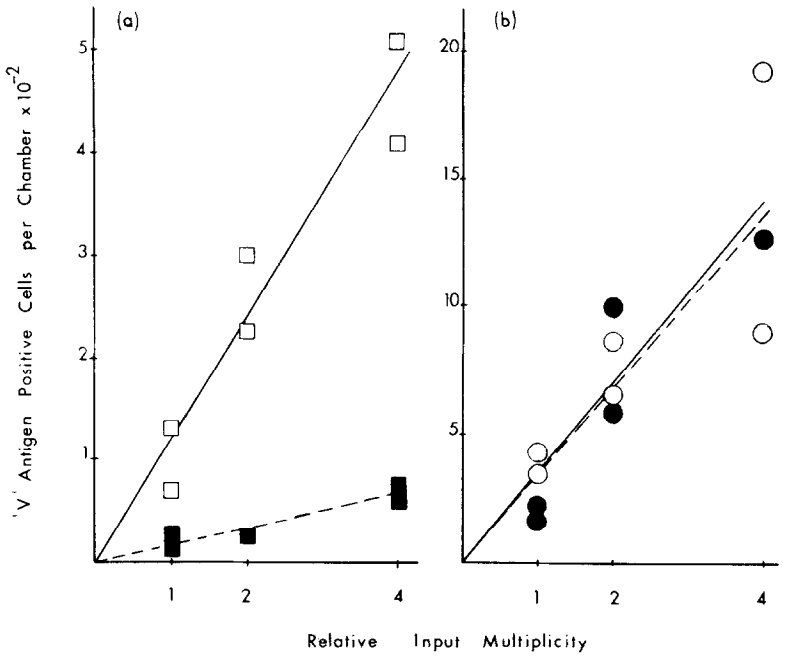

Figure 1. The frequency of Vag positive cells following infection of the normal fibroblast line CRL 1221. The dilution series for non-irradiated and irradiated virus were prepared separately: non-irradiated virus was diluted 25-fold more than γ-irradiated virus. The total number of cells in each well was around 4 x 10^4. Virus was adsorbed for 2 hr and samples were taken 48 hr post infection and scroed for Vag positive cells. Fig. 1(A) shows a marked decrease in Vag positive cells for infection with non-irradiated virus of pre-γ-irradiated cells compared to non-irradiated cells, while Fig. 1(B) shows no such decrease for γ-irradiated virus. □, no γ dose to cells, no γ dose to virus; ■, 1 Krad to cells, no γ dose to virus; o, no γ dose to cells, 1 Mrad to virus; ●, 1 Krad to cells, 1 Mrad to virus.

RESULTS

Typical results for Vag production in non-irradiated and pre-γ-irradiated fibroblasts following infection with unirradiated or γ-irradiated Ad 2 are shown in Figure 1. It can be seen that γ-irradiation of the host cells prior to infection with unirradiated virus (Figure 1A) leads to a substantial decrease in the frequency of Vag positive cells, compared to that in non-irradiated cells. This decrease is much reduced or absent for infection of γ-irradiated cells with γ-irradiated virus (Figure 1B). For some experiments (not shown), γ-irradiation of the cells stimulated an absolute enhancement of Vag production for γ-irradiated virus. Thus, the survival of γ'd virus was greater following infection of pre-irradiated cells.

Figure 2 shows results for the survival of Ad 2 Vag production at 48 hours post infection in non-irradiated and pre-γ-irradiated host cells for 3 different normal fibroblast lines. It can be seen that pre-irradiating the cells with 1 Krad of γ-rays immediately (Figure 2A) prior to infection (within 2 hours)

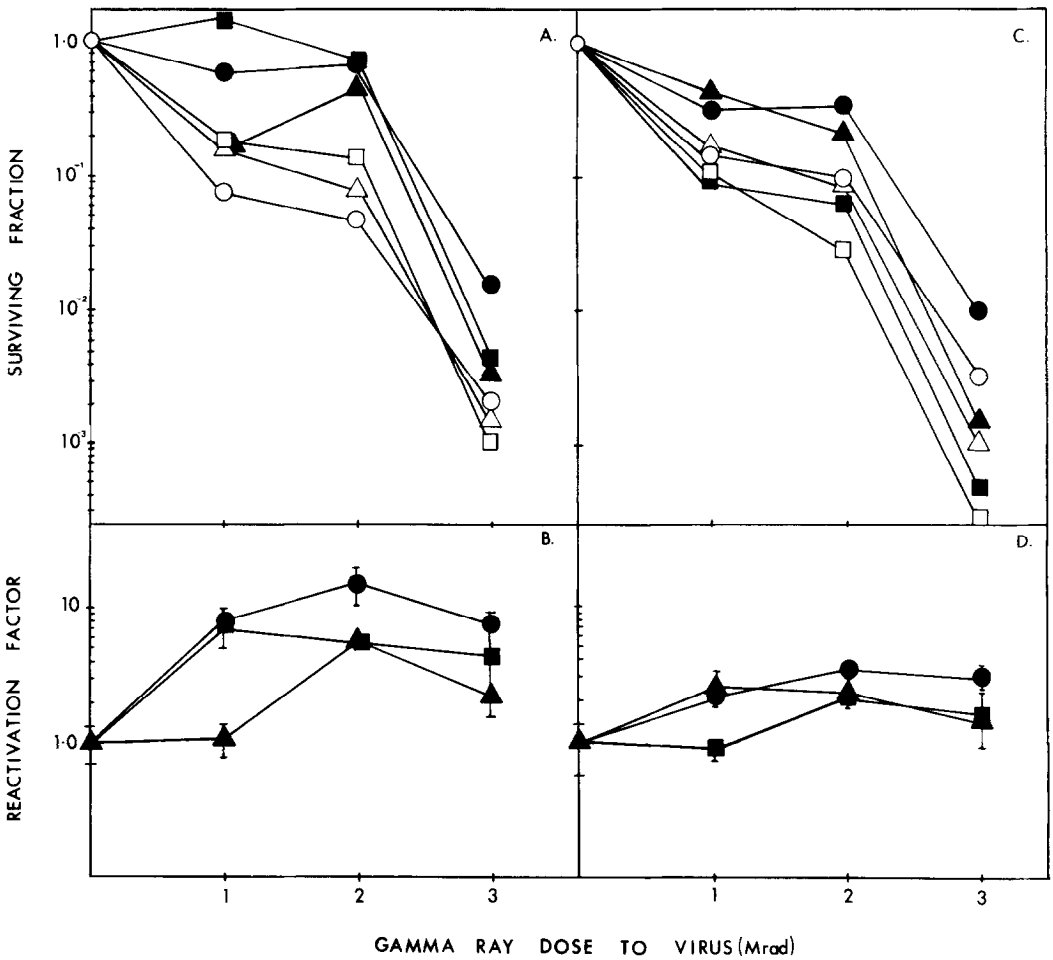

Figure 2. The effect of pre-γ-irradiation on the γ-survival curve for adenovirus type 2 Vag in 3 different normal human fibroblast lines. The frequency of Vag positive cells was determined in duplicate at 3 serial dilutions for each dose to the virus. The data points were fitted to a straight line using least squares analysis in order to obtain each survival point. Fig. 2A shows results for infection immediately after irradiation of the cells, Fig. 2C for infection at 48 hours after irradiation. o, □, Δ, A1, 1221, A2, respectively, no γ dose to cells; o, ■, ▲, A1, 1221, A2, respectively, 1 Krad to cells. Figs. 2B and 2D show the γRER factors, the normalized ratios of survival of γ-irradiated virus in γ-irradiated to that in non-irradiated cells. ●, A1; ■, 1221; ▲, A2.

with γ'd virus, results in an enhanced survival of Vag production for all 3 normal lines examined. The normalized ratio of survival of γ-irradiated virus in irradiated cells to that in non-irradiated cells, that is, the gamma ray enhanced reactivation (γRER) factor, is shown in Figure 2B. The γRER factor reaches a value of between 5 and 15 for a γ does of 2 Mrad to the virus. If infection of the host cells is delayed by 48 hours after irradiation of the

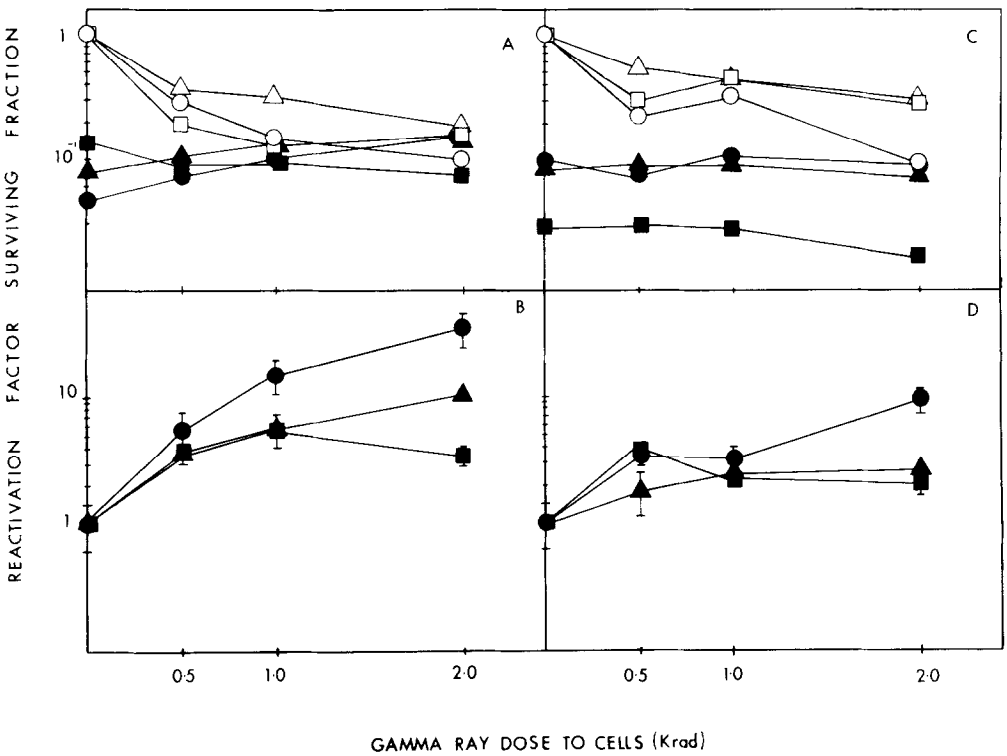

Figure 3. Survival curves of Ad 2 Vag in the 3 different normal fibroblast lines at 48 hr after infection, as a function of γ-ray dose to the cells. Fig. 3A shows results for infection immediately after irradiation and Fig. 3C for infection at 48 hr after irradiation. o, □ , Δ, A1, 1221, A2, respectively, no γ dose to virus; ●, ■, ▲, A1, 1221, A2, respectively, 1 Mrad γ-rays to virus. Figs. 3B and 3D show the γRER factors, the normalized ratios of survival of γ-irradiated virus to non-irradiated virus at each γ-ray dose to the cells. ●, A1; ■ , 1221; ▲ , A2.

cells, the results shown in Figures 2C and 2D are obtained. It is clear that for a 48 hour delayed infection, the γRER factor has been reduced by at least half of its value seen for immediate infection.

Figure 3 shows results for survival of Vag production for unirradiated and γ-irradiated (2 Mrad) virus, as a function of γ-ray dose to the cells (Figure 3A). It can be seen that pre-irradiation of the fibroblasts results in a marked decrease in Vag production for non-irradiated virus, whereas the Vag production for γ-irradiated virus remains essentially the same over the range of γ dose to the cells examined. Figure 3B shows the γRER factor, that is, the normalized ratio of the survival curve for γ-irradiated virus to that for non-irradiated virus. It can be seen that for a fixed dose of 2 Mrad to the virus, the γRER factor increases with increasing γ dose to the cells, to a value of between 5 and 30. Figures 3C and 3D show the results obtained if infection is delayed until 48 hours after γ-irradiation of the cells. It is evident that for 48

hour delayed infection, the γRER factor has been reduced by at least half its value seen for immediate infection over the entire range of γ dose to cells examined.

Previously we have reported the existence of γRER of UV-irradiated Ad 2 in normal human fibroblasts (13). It was, therefore, considered of interest to compare the level of γRER of UV'd virus in the 3 fibroblast lines examined in the present report. Figures 4A and 4B show results for immediate infection of γ-irradiated cells with UV-irradiated Ad 2, and they are to be compared with Figures 2A, B and 3A, B, respectively, for γ-irradiated virus. It is evident that γ-irradiation of the cells prior to infection enhances the survival of both γ- and UV-irradiated Ad 2, and to comparable levels.

DISCUSSION

A positive γ-ray reactivation effect for γ-irradiated adenovirus type 2 was detected in 3 normal human fibroblast lines. For a fixed dose of 2 Mrad to the virus, the γRER factor reaches an average value of 16 at 2 Krad to the cells for the 3 normal fibroblasts examined. Delay of infection until 48 hours after irradiation of the cells results in about a 50% reduction in the magnitude of the γRER.

Gamma-irradiation of adenovirus at -75°C as described above, introduces several different types of DNA lesion in the viral DNA, such as single- and double-strand breaks (17) as well as alkaline labile bonds (18). UV light produces a somewhat different spectrum of lesions, including cross-linking, thymidine dimers, and alkaline labile damage (19). At least some proportion of the lesions produced by UV light and γ-rays are therefore qualitatively different.

The results of this report indicate that γ-irradiation of normal human fibroblasts prior to infection leads to an enhanced reactivation of both UV- and γ-irradiated Ad 2. This enhanced reactivation may result from the enhancement of a pre-existing repair mechanism and/or the induction of some new mode of repair. The reactivation is of a similar level for both UV- and γ-irradiated virus. This suggests that the enhanced/induced mechanism is capable of repairing both UV and γ-ray induced lesions. The fact that 48 hour delayed infection results in a significantly reduced level of γRER for irradiated Ad 2 suggests that these phenomena result from a γ-ray induced/enhanced repair mechanism, in normal human fibroblasts, which reaches a maximum within 2-3 days post irradiation.

In contrast to the pronounced reactivation of γ-irradiated adenovirus in human cells reported here, the enhanced reactivation of X-irradiated herpesvirus in monkey kidney cells has been reported to exist only at low levels (12). This may result from a true species difference between simian

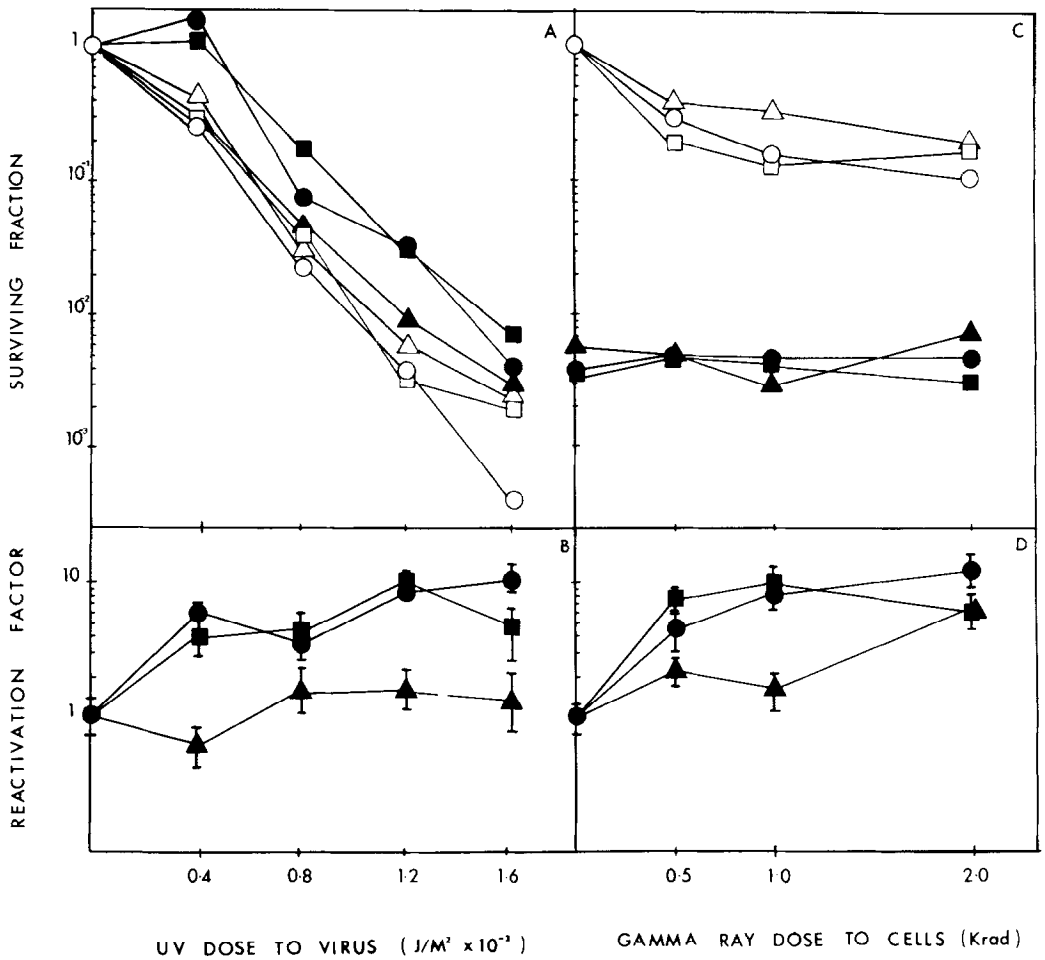

Figure 4. The effect of pre-γ-irradiation on the UV survival curves for adenovirus type 2 Vag in 3 different normal human fibroblast lines. Fig. 4A shows UV survival curves as a function of UV dose to virus for infection immediately after γ-irradiation of the cells. o, □ , Δ , A1, 1221, A2, respectively, no γ dose to cells; ●, ■ , ▲ , A1, 1221, A2, respectively, 1 Krad to cells. Fig. 4B shows the γRER factors, the normalized ratios of survival of UV-irradiated virus in γ-irradiated to that in non-irradiated cells. ●, A1; ■ , 1221; ▲ , A2. Fig. 4C shows UV survival curves as a function of γ-ray dose to the cells, for infection immediately after γ-irradiation of the cells. o, □ , Δ , A1, 1221, A2, respectively, no UV to virus; ●, ■ , ▲ , A1, 1221, A2, respectively, 1.2 x 10^3 J/m^2 UV to virus. Fig. 4D shows the γRER factors, the normalized ratios of survival of UV-irradiated virus to non-irradiated virus at each γ-ray dose to the cells. ●, A1; ■ , 1221; ▲ , A2.

and human cells, the difference in virus assay employed, the conditions of virus irradiation, or a combination of these factors.

The results of this report indicate a substantial gamma-ray enhanced reactivation of Vag expression for γ-irradiated adenovirus in normal human cells. It remains to be seen whether this results from the expression of a radiation induced mutagenic repair mechanism in human cells.

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