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# Bystander effect and adaptive response in C3H 10T½ cells

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**Abstract.**
*Purpose*: To address the relationship between the bystander effect and the adaptive response that can compete to impact on the dose–response curve at low doses.
*Materials and methods*: A novel radiation apparatus, where targeted and non-targeted cells were grown in close proximity, was used to investigate these phenomena in C3H 10T½ cells. It was further examined whether a bystander effect or an adaptive response could be induced by a factor(s) present in the supernatants of cells exposed to a high or low dose of X-rays, respectively.
*Results*: When non-hit cells were co-cultured for 24 h with cells irradiated with 5 Gy α-particles, a significant increase in both cell killing and oncogenic transformation frequency was observed. If these cells were treated with 2 cGy X-rays 5 h before co-culture with irradiated cells, approximately 95% of the bystander effect was cancelled out. A 2.5-fold decrease in the oncogenic transformation frequency was also observed. When cells were cultured in medium donated from cells exposed to 5 Gy X-rays, a significant bystander effect was observed for clonogenic survival. When cells were cultured for 5 h with supernatant from donor cells exposed to 2 cGy and were then irradiated with 4 Gy X-rays, they failed to show an increase in survival compared with cells directly irradiated with 4 Gy. However, a twofold reduction in the oncogenic transformation frequency was seen.
*Conclusions*: An adaptive dose of X-rays cancelled out the majority of the bystander effect produced by α-particles. For oncogenic transformation, but not cell survival, radioadaption can occur in unirradiated cells via a transmissible factor(s).

## 1. Introduction

Current estimates of cancer risk at low doses (< 20 cGy) in the general population are generally derived using the linear, non-threshold (LNT) model which extrapolates to low doses data collected at higher doses from the Japanese atomic-bomb survivors. This model implies a linear relationship between cancer induction and dose in the low-dose region (Kellerer 2000). However, evidence has now emerged for a number of biological phenomena that may be important in determining the cellular response to low doses of radiation (Upton 2003). These include the bystander effect, adaptive response, genomic instability and low-dose hyper-radiosensitivity. These phenomena have been predominantly demonstrated with cell lines *in vitro* but if they were applicable *in vivo*, they may result in an overall risk which is a non-linear function of dose (Redpath *et al.* 2001, Rothkamm and Lobrich 2003). This would have implications for the applicability of the LNT model in extrapolating data into the low-dose region.

The bystander effect is defined as the observation of a biological response in cells that are not themselves traversed by ionizing radiation, but which can communicate with cells that are (reviewed in Morgan 2003a, b). This is in contrast to the adaptive response where a low priming dose of radiation (< 10 cGy) induces a protective adaptive response often against a high challenge dose (Azzam *et al.* 1996, Feinendegen and Pollycove 2001, Redpath *et al.* 2001, Broome *et al.* 2002).

Both phenomena have been demonstrated for numerous biological endpoints including alteration in gene expression, induction of micronuclei, clonogenic survival and neoplastic transformation (reviewed in Ballarini *et al.* 2002). However, the mechanisms by which they operate are still not fully understood. Two main processes are thought to underlie the bystander response depending upon the degree of cell-to-cell contact at the time of irradiation: direct communication between cells involving gap junctions (Azzam *et al.* 2001, Zhou *et al.* 2002a, Shao *et al.* 2003) and/or secretion of a cytotoxic factor into the surrounding medium (Mothersill and Seymour 1997, Narayanan *et al.* 1999). Any factor transferred through gap junctions would by necessity be small (< 2000 Da), whereas the cytotoxic factor(s) secreted into the media is thought to be a protein-like molecule. Considering the adaptive response, it is thought that a low priming radiation dose may enhance DNA repair ability through p53 and cellular antioxidant activity (Rigaud and Moustacchi 1996, Stecca and Gerber 1998, Sasaki *et al.* 2002).

Although both the bystander effect (via apoptosis and differentiation) and adaptive response may be protective mechanisms causing overestimation of the low-dose risk by the LNT model, the bystander effect may also increase the risk through the transmission of DNA damage and genomic instability (Ballarini and Ottolenghi 2002). Consequently, the bystander effect

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and adaptive response may operate in opposite directions to produce an overall biological effect, but to date there are only limited studies concerning their direct interaction (Sawant *et al.* 2001b, Zhou *et al.* 2003, Wang *et al.* 2004).

The present study used a novel radiation set-up to assess the interaction of these two potentially conflicting phenomena for the endpoints of clonogenic survival and oncogenic transformation.

Both a bystander effect (Mothersill and Seymour 1997) and adaptive response (Iyer and Lehnert 2002a, b) have been shown to be induced via the transfer of supernatant from irradiated cells onto unirradiated cells. In the present study, we set out to confirm whether such effects could be demonstrated in C3H 10T½ cells.

## 2. Materials and methods

### 2.1. *Cell culture*

C3H 10T½ cells (passages 9–12, received from Dr J. B. Little, Harvard School of Public Health, MA, USA) were routinely cultured in 75 $cm^2$ tissue culture flasks and grown in minimum essential medium (Eagle modified) (Mediatech Cellgro, Herndon, VA, USA) supplemented with 10% foetal calf serum (Hyclone Laboratories, Inc., South Logan, UT, USA) and penicillin/streptomycin (Gibco-BRL, Life Technologies, Gaithersburg, MD, USA) for all experimental procedures. All cell cultures were incubated at 37°C in humidified 5% $CO_2$/95% air. All plasticware used in the current study was purchased from Corning, Inc. (Corning, NY, USA).

### 2.2. *Adaptive versus bystander response*

The irradiation apparatus employed in this study consists of a metal outer and inner ring, which fit together as shown on the left in figure 1. The rings were designed and manufactured in the Design and Instrument Shop of the Center for Radiological Research. The outer ring (internal diameter 38 mm) has a base of 6 μm mylar (Steinerfilm, Inc., Williamstown, MA, USA) while the inner ring (internal diameter 35 mm) has seven, 2 mm strips of 38 μm mylar on the base. The mylar was fixed to the base of the metal rings using epoxy (EP21LV, Master Bond, Inc., Hackensack, NJ, USA) and baked for 2 h at 150°C until the adhesive was cured. Following sterilization with 70% ethanol, the rings are fitted together such that the mylar strips of the smaller ring sit directly on the mylar base of the larger ring. The cells can then be plated at the desired concentration. In this way, cells attach to both the 6 μm mylar and the top surface of the 38 μm mylar strips. The rings are irradiated from underneath using a track segment facility. The energy of the α-particles (5.3 MeV, stopping power 90 keV $\mu m^{-1}$) is such that they can penetrate the 6 μm mylar, irradiating cells attached to this layer but are unable to pass through the 38 μm mylar layer. Therefore, cells growing on the strips remain unirradiated but are in close physical proximity to irradiated cells. Following irradiation and further incubation, the rings can then be separated and the bystander cells removed from the strips and studied for several endpoints.

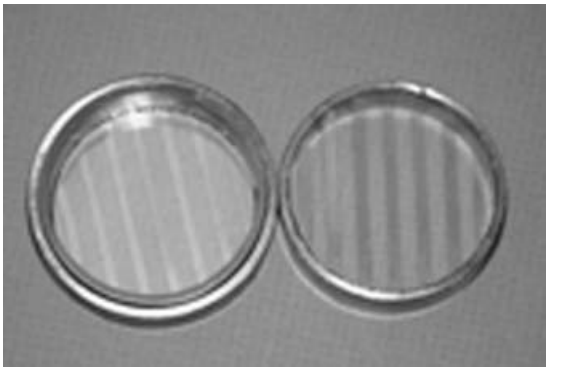

Figure 1. Double-ring apparatus used. The inner ring (internal diameter 35 mm) with 38-μm mylar strips (width 2 mm) is shown on the right with the complete double-ring apparatus on the left (outer ring diameter is 38 mm).

Eighteen hours before irradiation, C3H 10T½ cells were plated in 2 ml media at a concentration of $5 \times 10^5$ cells per ring and allowed to attach. At this concentration, the cells appeared confluent at the time of irradiation, with the bystander cells on the 38 μm mylar being in physical contact with the cells on the 6 μm mylar. Cells were exposed to either 0 or 2 cGy 250 kVp X-rays at 5 mA with 0.5 mm copper and 1 mm aluminium external filters. The absorbed dose rate was calculated to be 8.5 cGy $min^{-1}$. Five hours after the initial exposure, cells were either sham irradiated or irradiated with α-particles to a total dose of 5 Gy using the track segment mode of a 4 MeV Van de Graaff accelerator. The effect of irradiating medium alone was also examined to investigate the contribution of factors which may be generated in the irradiated medium. The rings were then returned to the incubator for either 24 or 48 h before assessment of clonogenic survival and oncogenic transformation (24 h only) as described in Section 2.5.

### 2.3. *Adaptive medium transfer*

C3H 10T½ cells were seeded into a series of 25 $cm^2$ flasks at an initial density of $5 \times 10^5$ cells per flask and maintained in culture until they reached

confluence, usually 24 h. Several flasks were then selected and either sham-irradiated (protocol A) or exposed to a 2 cGy dose of X-rays (protocol B) and returned to the incubator. Eighteen hours following irradiation, the supernatants were removed from these donor cultures and transferred onto unirradiated, confluent cell cultures from which the media had been aspirated. These recipient cell cultures were returned to the incubator for a further 5 h before being exposed to a 4 Gy dose of X-rays (at 15 mA). Following irradiation, cells were either trypsinized and plated immediately (IP) or incubated for a further 24 h at 37°C before trypsinization (delayed plating, DP). For comparison, a classic adaptive response protocol was also employed whereby confluent cell cultures were directly irradiated with 2 cGy and challenged 5 h later with 4 Gy (protocol C). Clonogenic survival and oncogenic transformation were assessed as described in Section 2.5.

### 2.4. *Bystander medium transfer*

The method employed has been described elsewhere (Mothersill and Seymour 1997). Briefly, 100-mm dishes containing viable cells for assessment of clonogenic survival were set up as detailed in Section 2.5. 150 $cm^2$ flasks used to generate donor medium were plated with $6 \times 10^5$ C3H 10T½ cells and 18 h later these flasks were irradiated with 5 Gy X-rays and returned to the incubator. The medium was then removed from these flasks 18 h after irradiation and filtered through a 0.22 μm filter to ensure that no cells could be present. Medium was removed from the dishes containing cells at cloning density and the filtered medium added. Filtered medium from unirradiated donor flasks was transferred to control dishes at the same time. All dishes were then returned to the incubator to allow for cell growth.

### 2.5. *Cell survival and oncogenic transformation*

To assess radiation-induced oncogenic transformation and clonogenic survival, approximately 300 or 100 viable cells were plated into 100 mm dishes respectively. For transformation studies, culture medium was changed at 12 day intervals during the 7-week incubation. The cells were then fixed in formalin, stained with Giemsa and transformed foci types II and III scored as described (Reznikoff *et al.* 1973). Cells plated for clonogenic survival were incubated for 2 weeks without medium change, stained with 2% crystal violet and colonies $>50$ cells scored.

Data from a minimum of three independent experiments were pooled. All data for clonogenic survival were presented as a mean together with standard error. The statistical significance of differences between groups was tested by Student's *t*-test.

## 3. Results

### 3.1. *Adaptive versus bystander response*

As shown in figure 2, a significant decrease in surviving fraction from control levels was observed in the non-hit bystander cells following both 24 and 48 h co-culture with cells irradiated with 5 Gy α-particles (24 h: $SF = 0.77 \pm 0.01$; $p < 0.0001$) using the double-ring irradiation apparatus. There was no significant difference in survival between the two time points studied. However culture of cells with irradiated medium alone had no effect on survival of the non-hit bystander cells at either time point (24 h: $SF = 1.00 \pm 0.02$). When cells were exposed to a 2-cGy priming dose 5 h before being co-cultured with irradiated cells, the majority of the bystander killing was lost and the surviving fraction was not significantly different from control levels at both time points (24 h: $SF = 0.96 \pm 0.02$).

Table 1 shows the oncogenic transformation frequencies obtained following 24 h of co-culture with irradiated cells. Bystander cells showed a significant increase in transformation frequency over spontaneous control levels ($p < 0.0001$). As was observed for clonogenic survival, cells pretreated with the priming dose showed a 2.7-fold significant decrease in transformation frequency from that observed in bystander cells ($p < 0.0001$) to a level that was not significantly different from control levels. Again, no significant

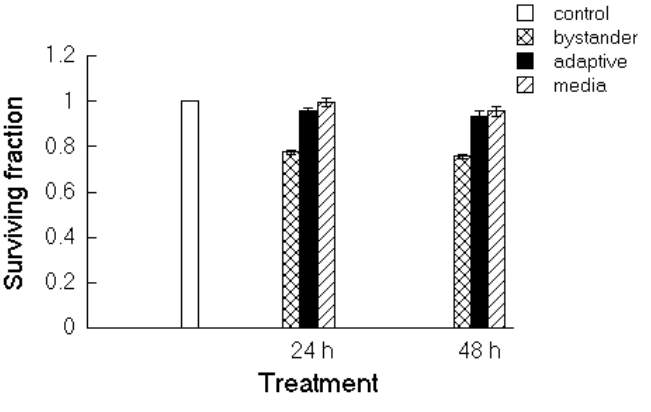


Figure 2. Surviving fraction of bystander C3H 10T½ cells co-cultured either with cells ('bystander') or culture media ('media') irradiated with 5 Gy α-particles. Results are also shown for bystander cells pretreated with a 2-cGy priming dose 5 h before co-culture with irradiated cells ('adaptive'). Data were pooled from at least three independent experiments (mean ± SEM).

Table 1. Clonogenic survival rates, numbers of viable cells exposed in transformation studies, number of transformed clones produced and transformation frequencies for bystander C3H 10T½ cells co-cultured for 24 h with cells ('bystander') or media ('media') exposed to 5 Gy α-particles. Results are also shown for cells irradiated with a 2-cGy priming dose 5 h before co-culture with irradiated cells ('adaptive'). Data were pooled from at least three independent experiments (mean ± SEM).

| Irradiation conditions | Clonogenic surviving fraction (plating efficiency) | Number* of viable cells exposed/$10^4$ | Number of transformants produced | Transformation frequency/$10^4$ surviving cells |
|---|---|---|---|---|
| 0 Gy | (0.45 ± 0.004) | 11.2 | 6 | 0.5 |
| Media | 1.0 ± 0.02 | 8.5 | 5 | 0.6 |
| Bystander | 0.77 ± 0.01 | 14.7 | 51 | 3.5 |
| Adaptive | 0.96 ± 0.02 | 11.2 | 14 | 1.3 |

*Estimated, accounting for plating efficiency and clonogenic survival.

increase in transformation frequency was seen following co-culture with irradiated medium only.

### 3.2. *Adaptive medium transfer*

The clonogenic survival and transformation results for the adaptive medium transfer assay are shown in tables 2A and B. Pretreatment of cells for 5 h with irradiated-conditioned medium (2 cGy X-rays) before the 4 Gy X-ray challenge dose (protocol B) had no significant effect on clonogenic survival compared with cells directly irradiated with 4 Gy or those treated with sham-irradiated medium and irradiated with 4 Gy (protocol A). This was true whether the cells were processed immediately after the acute exposure (table 2A) or held in plateau phase for 24 h before trypsinization (table 2B). However, following immediate plating (table 2A), an approximate twofold reduction in the oncogenic transformation frequency was observed in cells treated with irradiated supernatant (protocol B) compared with directly irradiated cells or those treated with sham-irradiated medium

Table 2A. Clonogenic survival rates, numbers of viable cells exposed in transformation studies, number of transformed clones produced and transformation frequencies for C3H 10T½ cells. Cells were either directly exposed to 4 Gy X-rays or: (1) supernatant from sham-irradiated cells (protocol A); (2) supernatant from cells exposed to 2 cGy X-rays (protocol B); or (3) a 2-cGy priming dose (protocol C). Following a further 5 h of incubation at 37°C, these cells were challenged with 4 Gy and processed immediately (IP).

| Irradiation conditions | Clonogenic surviving fraction (plating efficiency) | Number* of viable cells exposed/$10^4$ | Number of transformants produced | Transformation frequency/$10^4$ surviving cells |
|---|---|---|---|---|
| 0 Gy | (0.48 ± 0.01) | 7.2 | 3 | 0.4 |
| 4 Gy/IP | 0.34 ± 0.01 | 11.7 | 78 | 6.7 |
| Protocol A/IP | 0.35 ± 0.01 | 8.7 | 54 | 6.2 |
| Protocol B/IP | 0.33 ± 0.02 | 8.3 | 23 | 2.8 |
| Protocol C/IP | 0.34 ± 0.01 | 9.0 | 27 | 3.0 |

*Estimated, accounting for plating efficiency and clonogenic survival.

Table 2B. Clonogenic survival rates, numbers of viable cells exposed in transformation studies, number of transformed clones produced and transformation frequencies for C3H 10T½ cells. Cells were either directly exposed to 4 Gy X-rays or: (1) supernatant from sham-irradiated cells (protocol A); (2) supernatant from cells exposed to 2 cGy X-rays (protocol B); or (3) a 2-cGy priming dose (protocol C). Following a further 5 h of incubation at 37°C, these cells were challenged with 4 Gy and incubated for 24 h before processing (DP).

| Irradiation conditions | Clonogenic surviving fraction (plating efficiency) | Number* of viable cells exposed/$10^4$ | Number of transformants produced | Transformation frequency/$10^4$ surviving cells |
|---|---|---|---|---|
| 4 Gy/DP | 0.48 ± 0.01 | 11.6 | 24 | 2.1 |
| Protocol A/DP | 0.50 ± 0.01 | 11.9 | 25 | 2.1 |
| Protocol B/DP | 0.47 ± 0.01 | 11.0 | 12 | 1.1 |
| Protocol C/DP | 0.52 ± 0.01 | 11.3 | 11 | 1.0 |

*Estimated, accounting for plating efficiency and clonogenic survival.

(protocol A), although it did not quite reach statistical significance ($p = 0.06$). No increase in discrimination was seen when the cells were incubated for 24 h before processing (table 2B). A similar result was seen for both endpoints when cells were directly irradiated with 2 cGy before being exposed to the challenge dose (protocol C).

C3H 10T½ cells showed a marked repair of potentially lethal damage for all treatments (table 2B). When they were held in plateau phase for 24 h before processing a significant increase in survival ($p < 0.0001$) and an approximate threefold significant reduction in transformation frequency per viable cell was seen for all treatments ($p < 0.001$).

### 3.3. *Bystander medium transfer*

Figure 3 shows the clonogenic survival obtained when unirradiated cells were treated with either irradiated (5 Gy X-rays) or unirradiated medium taken from cells 18 h post irradiation. Growth in irradiated medium significantly reduced the clonogenic survival of the cells ($SF = 0.90 \pm 0.03$; $p < 0.002$). Cells treated with medium from unirradiated control flasks had a non-significant increase in survival ($SF = 1.08 \pm 0.04$).

## 4. Discussion

Epidemiological studies on the Japanese atomic-bomb survivors provide the best estimate of cancer risk over the dose range 20–250 cGy (Kellerer 2000). The risk of exposure to lower doses of radiation is currently estimated by extrapolating back from the definitive high-dose data using a linear, non-threshold (LNT) model.

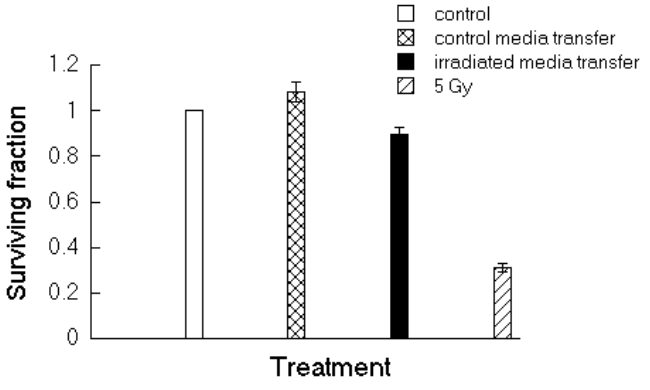


Figure 3. Surviving fraction of unirradiated C3H 10T½ cells cultured in media from either unirradiated donor cells or cells irradiated with 5 Gy X-rays 18 h before donation. Survival for cells directly irradiated with 5 Gy is also shown. Data were pooled from at least three independent experiments (mean ± SEM).

There is now a large body of experimental evidence both *in vitro* and to a lesser extent *in vivo* for a number of biological phenomena, which may have a role in modulating the shape of the dose–response curve below 20 cGy causing deviation from the LNT model (Redpath *et al.* 2001, Bonner 2003). These phenomena include, but are not limited to, the bystander effect and adaptive response (Ballarini *et al.* 2002, Morgan 2003b).

To date there are only limited data concerning the direct interaction of the bystander effect and adaptive response (Sawant *et al.* 2001b, Zhou *et al.* 2003, Wang *et al.* 2004). Two of these studies have made use of charged-particle microbeams whose precision is of particular importance for bystander studies as they allow charged particles to be targeted to individual cells within a population (Prise *et al.* 2002). However, although the double-ring irradiation protocol used in the present study cannot offer the same precision, it does offer some advantages over the previous microbeam-based studies. It allows only non-hit cells to be examined, in contrast to the previous studies where both the hit and the bystander cells contributed to the biological outcome. A further advantage is the greater number of cells which can be processed. Although the microbeam has an irradiation throughput of approximately 3000 cells/h (Randers-Pehrson *et al.* 2001), up to $10^5$ bystander cells/ring are available using the track segment protocol, making the experiments less labour intensive.

In the present study, a significant bystander effect was seen for both clonogenic survival and oncogenic transformation in non-hit, bystander cells after 24 h of incubation with targeted cells (table 1). This confirms previous microbeam-based studies where a significant bystander effect was observed for the same endpoints (Sawant *et al.* 2001a, b). At the density at which the cells were plated in the current study, the vast majority of cells were in close contact at the time of irradiation. Therefore, it is possible that the irradiated cells could transmit the bystander signal to non-hit cells either through the secretion of a soluble, extracellular factor into the medium and/or through direct cell-to-cell communication via gap junctions. However, no effect on either endpoint was observed when bystander cells were co-cultured in the presence of irradiated medium alone. This is in agreement with previous reports, suggesting that irradiation of medium alone does not produce any cytotoxic factors (Mothersill and Seymour 1997, Zhou *et al.* 2002b).

Exposure of bystander cells to a low dose of X-rays (2 cGy) cancels out the majority of the bystander effect generated by high-linear energy transfer α-particles, confirming the findings of previous microbeam-based experiments (Sawant *et al.* 2001b,

Zhou *et al.* 2003). In one such study also involving C3H 10T½ cells, a 2-cGy priming dose delivered 6 h before α-particle exposure cancelled out about half of the observed bystander effect for clonogenic survival (Sawant *et al.* 2001b). However, although a decrease in transformation frequency was observed in cells treated with the priming dose in this previous study, it did not reach statistical significance. This may reflect the protocol used as induced cell stress due to immediate trypsinization following the challenge dose may have interfered with the mechanism(s) underlying the adaptive response (Feinendegen and Pollycove 2003). In contrast, in the present study the cells were left undisturbed for 24 h following the challenge dose, allowing any adaptive response to be fully expressed.

Previous studies have shown that in comparison with untreated cells, unirradiated bystander fibroblasts treated with the supernatant from cells irradiated with 1 cGy α-particles or γ-rays, showed a significant increase in clonogenic survival following subsequent exposure to high or low-linear energy transfer radiation (Iyer and Lehnert 2002a, b). This is in contrast to the present study where no increase in cell survival was observed (tables 2A and B) although a similar protocol was followed and highlights the cell phenotype specific nature of the adaptive and bystander responses. However, a non-significant ($p = 0.06$) 2.4-fold reduction in the transformation frequency was observed in cells treated with irradiated supernatant (protocol B) compared with directly irradiated cells. This suggests that supernatant from cells exposed to 2 cGy X-rays may contain a factor(s) which acts on unirradiated, bystander cells, reducing their susceptibility to oncogenic transformation, but not cell killing.

A significant adaptive response has been demonstrated for oncogenic transformation following chronic exposure of cells to γ-rays at doses below 10 cGy (Azzam *et al.* 1996, Redpath and Antoniono 1998, Redpath *et al.* 2001). This may be due to selective killing of cells by low-dose hypersensitivity (Redpath *et al.* 2003). In the present study a non-significant ($p = 0.07$) 2.2-fold reduction was seen in the transformation frequency for cells directly irradiated with 2 cGy followed by a 4-Gy challenge dose (protocol C), in agreement with a previously published study (Azzam *et al.* 1994). As in the present study, they found no improvement in clonogenic survival and suggested that this may result from different endpoints being dependent upon unique pathways for their expression. Although both the amount of cell killing and transformation frequency were significantly lower following a further 24 h of incubation before processing (table 2B), the discrimination between the protocols was not enhanced by allowing extra time for adaptation as has been observed in other studies (Azzam *et al.* 1996, Redpath and Antoniono 1998).

It is interesting to note that in the present study, cells directly irradiated with a 2-cGy priming dose followed by a subsequent 4 Gy challenge dose (table 2A: protocol C) showed no increase in survival, in contrast to bystander cells in the double-ring experiments which were treated with a priming dose followed by co-culture with irradiated cells and which showed a significant adaptive response for survival (figure 2). Although it may be related to the size of the challenge dose, this suggests that following exposure to a priming dose of X-rays and consequent induction of the adaptive mechanism(s), C3H 10T½ cells are less sensitive to the deleterious effects of a bystander signal, but just as susceptible to damage from a direct, high-dose exposure to X-rays.

Medium removed from cells irradiated with 5 Gy X-rays was also able to induce a significant bystander effect in unirradiated cells as shown by an increase in cell killing (figure 3). This has been seen in previous studies and is suggestive of the fact that irradiated cells secrete a cytotoxic factor into the medium which is then able to elicit a bystander effect in unirradiated cells (Mothersill and Seymour 1997). It was shown to be cell-line specific with keratinocytes, but not fibroblasts, being able to induce the effect. However, the degree of cell killing in the present study was several-fold less than that observed in this previous study, where although using a similar protocol, up to 90% cell killing was seen in keratinocyte cultures as opposed to 10% in the present study. This may be due to the fibroblastic origin of C3H 10T½ cells and it would be of interest to see if the use of keratinocyte cultures as medium donors would be able to induce greater cell killing in C3H 10T½ bystander cells. Control cells, treated with medium from unirradiated cultures showed a non-significant increase in surviving fraction. This may be due to the medium becoming conditioned from the high-density cultures during the 18 h incubation period and then conferring a survival advantage on the cells to which it is transferred.

Although there are several differences in the protocols used making a direct comparison difficult, the amount of bystander cell killing seen in the medium transfer experiments was twofold less than that seen when using the double-ring protocol (figure 2 versus figure 3). This may be a result of the bystander signal being transmitted between cells via gap junctions in addition to the secretion of a cytotoxic factor into the medium in the high-cell density double-ring protocol. This may lead to a subsequent increase in cell killing confirming the

importance of cell-to-cell contact at the time of irradiation in transmitting the bystander response (Azzam *et al.* 2001, Zhou *et al.* 2002a).

In conclusion, the results indicate that the cellular response to radiation is dependent upon the interaction between several competing phenomena, the relative importance of which remains unclear. Although both the bystander effect and the adaptive response have been demonstrated *in vivo* their relevance at the tissue level is yet to be fully elucidated. Therefore the question of whether it is necessary to revise the LNT model to more accurately reflect the radiation risk at low doses remains unanswered (Preston 2003, Turesson *et al.* 2003).

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