Radioresponsiveness at low doses: hyper-radiosensitivity and increased radioresistance in mammalian cells

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Abstract

The rationale for and importance of research on effects after radiation at ''low doses'' are outlined. Such basic radiobiological studies on induction of repair enzymes, protective mechanisms, priming, and hypersensitivity are certainly all relevant to treatment of cancer (see Section 1, Studies at low doses — relevance to cancer treatment). Included are examples from many groups, using various endpoints to address the possibility of an induced resistance, which has been compared to the adaptive response [M.C. Joiner, P. Lambin, E.P. Malaise, T. Robson, J.E. Arrand, K.A. Skov, B. Marples, Hypersensitivity to very low single radiation doses: its relationship to the adaptive response and induced radioresistance, Mutat. Res. 358 (1996) 171–183.]. This is not intended to be an exhaustive review — rather a re-introduction of concepts such as priming and a short survey of molecular approaches to understanding induced resistance. New data on the response of HT29 cells after treatment (priming) with co-cultured activated neutrophils are included, with protection against X-rays ($S > 1$). Analysis of previously published results in various cells lines in terms of increased radioresistance (IRR)/intrinsic sensitivity are presented which complement a study on human tumour lines [P. Lambin, E.P. Malaise, M.C. Joiner, Might intrinsic radioresistance of human tumour cells be induced by radiation?, Int. Radiat. Biol. 69 (1996) 279–290].

It is not feasible to extrapolate to low doses from studies at high doses. The biological responses probably vary with dose, LET, and have variable time frames. The above approaches may lead to new types of treatment, or additional means to assess radioresponsiveness of tumours. Studies in many areas of biology would benefit from considerations of different dose regions, as the biological responses vary with dose. There may also be some implications in the fields of radiation protection and carcinogenesis, and the extensions of concepts of hyper-radiosensitivity (HRS)/IRR extended to radiation exposure are considered in Section 2, Possible relevance of IRR concepts to radiation exposure (space). More knowledge on inducible responses could open new approaches for protection and means to assess genetic predisposition. Many endpoints are used currently — clonogenic survival, mutagenesis, chromosome aberrations and more direct — proteins/genes/functions/repair/signals, as well as different biological systems. Because of scant knowledge of the relevant aspects at low doses, such as inducible/protective mechanisms, threshold, priming, dose–rate effects, LET within one system, it is still too early to draw conclusions in the area of radiation exposure. Technological advances may permit much needed studies at low doses in the areas of both treatment and protection. © 1999 Elsevier Science B.V. All rights reserved.

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1. Studies at low doses — relevance to cancer treatment

Ionizing radiation is used to treat approximately 60% of Canadian cancer patients. A better understanding of agents which affect cell kill by radiation is needed, particularly at doses used in the clinic. Since many patients will also have chemotherapy, the interactions between the two modalities also require better understanding — again at clinically relevant doses and concentrations. We touch upon some of the approaches used in various groups to understand effects at low doses, which may be an indicator of *intrinsic* radioresistance as distinct from *hypoxic* resistance or *drug* resistance, and possibly *cross-resistance*. Such knowledge may lead to new approaches for improvements in radiotherapy.

1.1. Mammalian cell survival

The original clonogenic assay [1] lends itself to studies of the effects of ionizing radiation on mammalian cells at relatively high doses, but extrapolations based on various theoretical and empirical models to dose regions of clinical interest may be invalid. This problem was considered years ago [2,3]. With improved technology providing methods to accurately measure effects at low doses, anomalies at low doses were confirmed:

· The unexplained decrease in sensitization by oxygen at low doses [4] was measured directly [5]. Subsequently, other electron affinic modifiers were shown to have less effect at low doses [3,6] while cisplatin and other platinum agents affect low does response to a greater extent [7,8].

· Hyper-radiosensitivity (HRS) at low doses in vitro [9] (Fig. 1) confirmed in vivo results (reviewed in Ref. [10]) and earlier indication of anomaly in mammalian cell response [11].

It is not yet known whether there is a relationship between these two deviations at low doses (effect of modifiers, and hypersensitivity/IRR). Fig. 1 shows an idealistic version of the shape referred to as HRS/IRR in papers in [12]. It is also reflected in colony size [13]. The possibility of induced or increased radioresistance (IRR) cannot be explained by mixed populations and has been the subject of several reviews [14–17] and specific [12] or related conferences, e.g., Ref. [18]. Some attempts to see
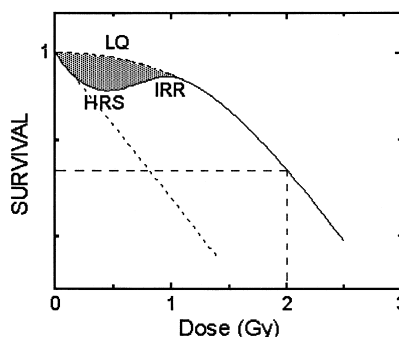

Fig. 1. IRR/HRS-stylized structure. The anomalous structure at low doses: HRS (to about 0.25 Gy) is followed by increased radioresistance (IRR, to about 1 Gy) a stylized curve representing a mammalian cell line with a large IRR. *LQ*: The linear quadratic fit (LQ: $-\ln S = \alpha D + \beta D^2$) is shown by the dashed line. The ''deficit'' from the LQ fit [21] used in Fig. 2 is shaded. The SF_2 is the survival at 2 Gy, used by many groups to describe intrinsic radiosensitivity. The dotted line indicates the survival in cells where there is no IRR (sensitive lines, neutrons, development of IRR inhibited by sensitizer), following the initial HRS. *Priming*: Cells which have been ''primed'', for example, by a small dose of radiation, followed by a time to produce the response (usually 4–6 h), have lost the HRS, i.e., response to the challenge doses is similar to the LQ fit. Prime and insult need not be the same agents. See text for references.

HRS in vivo have not been successful [19,20], but it must be noted that even in vitro, not all lines show this structure, i.e., more sensitive lines have no IRR [21]. It is interesting to note that the in vivo results in the 1980s (skin, kidney, lung in Ref. [10] with dose fractions as low as 0.25), led to the search for HRS in mammalian cell survival curves [9]. Many of these studies in the Gray Laboratory and in the author's employ a device called ''DMIPS'' [22] to record location of cells to improve studies at high survival levels.

1.2. Priming, an additional aspect of HRS/IRR

In the 1980s, Wolff's group described a phenomenon in lymphocytes, called the adaptive response, e.g., Refs. [23–25], whereby certain treatments primed the cells, making them more radioresistant, as recently reviewed [26]. In the case of HRS/IRR, priming was first observed after treatment with the same agents used in the early studies on the adaptive response: hydrogen peroxide or X-rays (0.2 Gy), which remove the HRS portion [27]

such that the curve follows the LQ prediction. This led to comparison of the adaptive response with priming the IRR [15] — e.g., protein synthesis seems required, as cycloheximide prevents development of resistance in both phenomena. However, our ongoing studies on IRR show some different responses to certain priming agents, as well as some similarities to the adaptive response. For example, tritium primes in both cases [24,28] whereas bleomycin only primes for the adaptive response [30] and not for HRS/IRR (Skov and Zhou, unpublished). However, peroxide priming suggests a role for hydroxyl radicals, as previously suggested [29]; and, finally, neutrons do not prime for the adaptive response [25] but do remove HRS [31]. (Another difference is their relationships to intrinsic radiosensitivity, Section 1.4.) Note that our studies use cell survival as an endpoint, and many others are used for the adaptive response: lower yields of micronuclei, chromosome damage, mutations, larger/smaller colony size and others, e.g., Refs. [32–41]. Within one study, protection was not seen with survival but was seen with other endpoints (MN, transformation — low dose rate (LDR) gamma priming for X-rays) [32]. Investigation of the possibility that an induced factor responsible for resistance could be transferable gave negative results. [1]

1.3. Other systems, other agents

Historically, there have been other examples of anomalous shapes of curves in response to low radiation doses (insect cells, pollen, protozoa, algae, and fern) as summarized [14–17] and Joiner (pp. 5–8 in Ref. [12]) as well as priming results or adaptive responses in other systems, e.g., yeast [29]. Despite this evidence from many fields, there has been some reluctance to accept that there is in some cases a hypersensitive region, and/or that cells can be made resistant to X-rays by various priming agents. However, there is a realistic biological basis as the cell must be prepared to deal with environmental insult, if spontaneous and natural DNA damage *each hour* is equivalent to 50–100 cGy [42], which approaches

the acute priming dose for IRR in mammalian cells (~ 0.25 Gy). Furthermore, non-linear behavior or resistance after priming has been observed for UV [43]; chemicals [44], drugs [17,30,45], inhibitors [46], and other agents (many of which also prime for each other, as suggested by Wolff a decade ago for the adaptive response and recently reviewed [26]).

1.4. Clinical relevance of HRS / IRR

It is important to understand the nature of the effects of low doses of radiation on biological systems, even if a non-linear dose dependent radiosensitivity is difficult to accept. This is interesting basic science, but has clinical relevance as follows. One aspect is the radiation response of normal tissue (late or early effects) and possible subsequent consequences. The possibility that there could be hypersensitivity in the penumbra which would lead to more significant responses certainly bears consideration [47]. In fact, there is now also clinical evidence for HRS in human skin response after radiotherapy [48,49] and subsequent lack of confidence in the LQ model [50]. A second aspect concerns the effects of increased dose fractionation as in the rodents [10,51] and, thus, also the effect of LDRs and brachytherapy for which there is again clinical evidence of a problem [52]. A third factor, which may also be relevant to fractionation [53] is the variable OER (oxygen enhancement ratio), since the HRS region exists in cells irradiated in hypoxia such that the OER is a complicated function of dose in this region [54]. Evidence for priming by hypoxia has been presented which could further complicate the aspect of OER. [2]

Finally, the fourth area of relevance of IRR to the clinic has to do with its potential application in predictive assays. It is known that different cell types and indeed different patients have variable intrinsic radiosensitivity and the SF_2 (surviving fraction at 2 Gy) is being examined as a predictor of response [47,55–58] and references therein. This is technically very demanding [55,58]. An alternate predictive assay might be developed once the IRR phenomenon is understood (Section 1.5), because *SF_2 may be re-*

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[1] Wojcik et al., presented at Radiat. Res. Ann. Mtg., 1996, pp. 14–264.

[2] Skov et al., 10th Chemical Modifiers Meeting, Clearwater, FL, 1998; manuscript in preparation.

lated to the extent of IRR shown in a study by Lambin et al. [21] in human lines. Fig. 2 shows data from additional cell lines, including ours in hamster lines [59] and some from Wouters et al. [60]. The calculation (area of deficit as a percentage of total area to 2 Gy) is markedly affected by the fit used for the data, as in UV20, a repair deficient line, which is shown twice as (+), once using the fit with a slight IRR (upper), the other assuming no IRR (lower), as well as average. That there was no relationship found between radiosensitivity and magnitude of adaptive response in a panel of human cell lines [41] suggests again (Section 1.2) differences between IRR and the adaptive response.

1.5. Possible mechanisms

What is IRR due to? What is turned on/off? What is the trigger? Several groups have become interested in the possibility of induced repair at low radiation doses, which follows HRS (typically to ∼ 0.25 Gy). Direct molecular approaches include searches for increased or decreased levels of proteins, genes turned on or off, etc. after doses less than 1 Gy. DNA damage is often suggested as the trigger of IRR with induced repair (DNA repair implied) suggested to cause IRR. The inhibition of development of protection in both IRR and adaptive responses by an inhibitor of poly (ADP-ribose) poly-
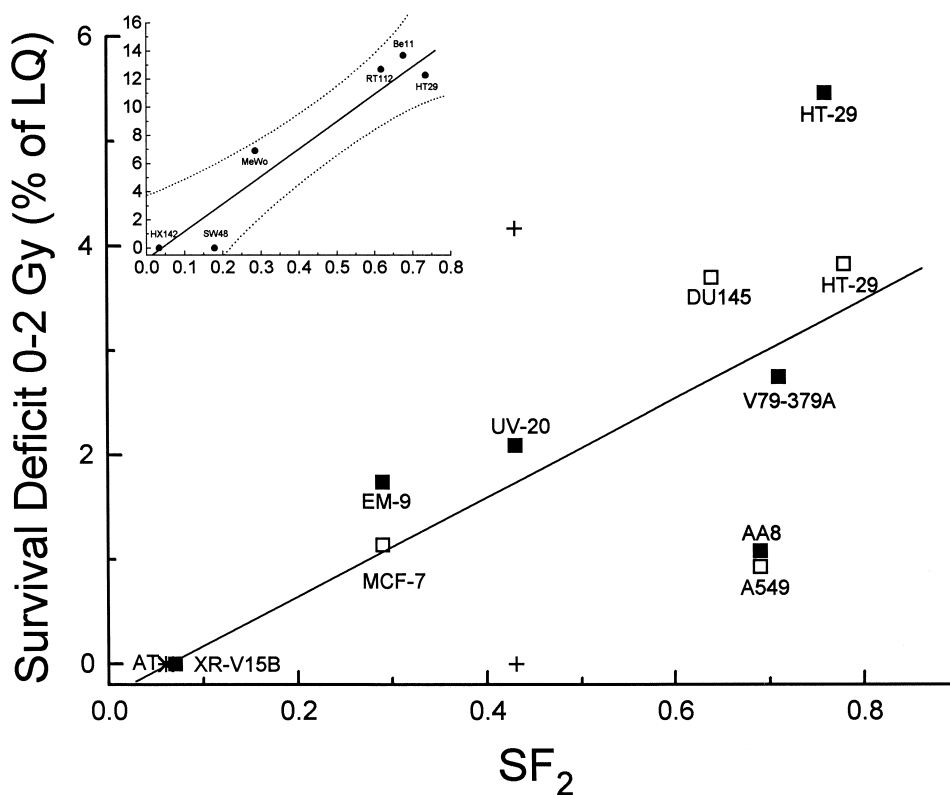

Fig. 2. Intrinsic radioresistance (SF_2) and extent of hypersensitivity (deficit). SF_2 is the surviving fraction after a dose of 2 Gy, used to compare cell sensitivity, and being explored as a predictive assay (see text, Section 1.4). The relationship between intrinsic radioresistance (SF_2) and extent of hypersensitivity, measured as the ''deficit'' from the linear quadratic fit (LQ, Fig. 1), as presented by Lambin et al. [21]. Results in hamster lines [59] (see text for explanation of (+) points), V79 and HT29 cells (from our lab, ■) and AT (∗) using DMIPS as well as and those from the literature using a flow cytometric assay [60] (□).

(AT data used were in the Gray Laboratory report, 1993, p. 46 and M.C. Joiner, personal communication. The flow cytometric assay used by Skarsgard et al. was compared in Ref. [6] with DMIPS, the microscopic localization procedure (used in the Gray Lab and by the author's groups).) These are more varied in origin of cells and have been calculated differently from Lambin et al. [21] but show the same trend (fit by eye). [The insert ● shows the results in various human tumour lines replotted from the latter study, with permission.]

merase [15,17,41] supports involvement of repair of DNA damage in the protective response (Section 1.5.2). DNA strand breaks are usually the initial class of damage considered which are caused by radiation and which might trigger such a response, e.g., Refs. [29,61]. Damage due to a non-DNA target/trigger was considered but data support ''an oxidation product which is rapidly repaired'' such as SSB (single-strand breaks) [61] at least for low LET (see also Section 2.4.2).

1.5.1. Approaches in author's group

In our studies, using colony forming ability as an indirect method as well as direct measurement of DNA damage (SSB), we have found no support for a straightforward correlation between the number of strand breaks and priming, but rather have suggested a role for DNA protein crosslinks or excision repair to explain effects at low doses [62] (Skov et al., manuscript in preparation), as suggested elsewhere [63,64]). A second approach is to examine the effects of modifiers of radiation response (low doses) [to understand trigger/triggered] such as oxygen (Section 1.4), topoisomerase inhibitors, radiosensitzers [16,65] and other agents such as cisplatin. Cisplatin is an important chemotherapeutic agent which forms crosslinks, and which appears to have an effect at low doses [7]. It is interesting in that it can remove/prevent IRR, i.e., sensitize, or protect-prime, removing HRS-depending on the treatment [17]. This may be related to confusion with this agent in that it sometimes appears to give beneficial results with radiation, yet there is cross-resistance between the two modalities. The possibility that a drug such as cisplatin could prime IRR at the clinically relevant doses is noteworthy, as it is used often as an adjunct to radiotherapy.

Finally, in our third approach, the hypothesis that apoptosis could be responsible for the HRS portion, being turned off at a critical damage level resulting in IRR, has been investigated using a morphological endpoint [66]. There is a higher cumulative frequency of apoptosis after 0.25 than 0.5 Gy [67] (Matthews et al., manuscript submitted).

1.5.2. Molecular approaches

If a molecular mechanism for IRR were found, it might be possible to find a probe for intrinsic ra-

diosensitivity to assist with treatment planning. Knowledge of the effects at the molecular level caused by ionizing radiation (and by other stresses) has exploded: genes turned off and on; apoptosis; signal transduction; cell cycle/checkpoints/cyclins, effects on nuclear proteins, etc. [15,26,40,68–72]. Some studies have focused on DNA repair and the dose region of interest here [63,64,71]. For example, SSB rejoining was increased (LDR) but this seemed distinct from the adaptive response in its requirement for protein synthesis [73]. Other examples include a report of increased efficiency of DSB repair in V79 cells 4 h after 5 cGy [74]; more rapid removal of thymine glycols 4 h after a priming dose of 0.25 Gy [75];[3] and 2–4 more recombinational events 6 h after 0.25 Gy prime (optimal time and dose) in relatively resistant human cells [76]. Many of these studies were responses to low LET. There is the possibility that direct DNA damage is not required for α particles to turn on p53 expression ([77], Section 2.4.2).

Other studies are not so directly related to DNA damage or repair. For example, factors produced in response to one agent turn up in response to another, a classic case being ''heat shock proteins'' (HSP), now known to be chaperones of protein folding. Thus, it is exciting but perhaps not surprising (if fidelity of repair is a factor) that a HSP70 is induced by low doses (30 min), and possibly related to the HRS/IRR structure [78]. It is also interesting that LDR led to increased HSP production and enhanced proliferation in mice [79]. In other cases, down regulation after low doses is observed, e.g., 4 × lower level of ''clone 8.6'' after 0.25 Gy (T. Robson, p. 112 in Refs. [18,15]) has been found which is being studied for relevance to IRR. During the preparation of this summary, a paper appeared on priming doses which protected testes (mice) against subsequent challenge (γ) and which also increased lipid peroxidation and related enzyme levels [39]. As suggested in the abstract, this unfortunately is not a complete list, but a sampling of different approaches to mechanisms of protection against high doses.

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[3] This is a very sensitive assay which could be automated, then possibly used for dosimetry or a predictive assay. Many other biological dosimetry methods have been developed (MN, FISH) as discussed elsewhere in this issue.

1.6. Summary of implications for treatment

Consideration should be given to normal tissue response (penumbra). There are also implications of IRR for fractionated doses, brachytherapy, pulsed brachytherapy and for the OER. Radiation modifiers, including some drugs/sensitizers may have unexpected effects — preventing IRR (to sensitize) or priming (to remove HRS — which would be useful for normal tissue) as shown in Fig. 1. The relationship to intrinsic radiosensitivity (Section 1.4) remains to be exploited. Experiments in different cell lines with different priming and challenge doses, timing, LET, etc., make it difficult to compare and to attain a general perspective. To summarize, it was perhaps naive to think that there would be one single response to a given priming dose, and there appears to be many responses — genes/proteins affected (up or down). This is probably the situation at still lower doses, and at higher doses. The biological response is dose dependent. The timing and duration of the responses can vary. All aspects are further compounded by cell type, cycle stage, LET, etc. Many of these considerations are also relevant to radiation exposure. Comparison is made in the table at the end of Section 2.

2. Possible relevance of IRR concepts to radiation exposure (space)

The above has been directed mainly to tumours and their treatment with ionizing radiation. Here, we comment briefly on certain aspects that may be relevant to radiation exposure in the workplace: normal tissues, genetics, protection, LET, priming and dose rate. The possibilities of priming for resistance at low dose are relevant to guidelines for those working in hazardous conditions where the concept of a threshold dose and the adaptive response are areas of constant investigation despite a statement that induced responses ''will never be of importance in practical radiation protection'' [80]. In fact, the possibility of non-linearity of dose response is being addressed by the NCRP.[4] As well, Dr. Wolff [26] has

been recognized for this area by the Radiation Research Society [23] and more recently by the BELLE Society (devoted to non-linear phenomena including hormesis).[5] The HRS/IRR phenomenon (mainly, survival and some molecular endpoints) has many similarities to the adaptive response (mutations, micronuclei, and aberrations), compared and discussed in Ref. [15] and in Section 1.2.

2.1. Responses in normal tissue / cells

Normal cell lines also exhibit hypersensitivity or inducible resistance [35,40,41,81] and references in Sections 1.2 and 1.5.2 on studies in lymphocytes by Wolff's group. Earlier animal studies had suggested HRS in normal organs [10,51] at doses per fraction up to ~ 1 Gy. There are also examples of priming in vivo, e.g., the protective effect of LDR irradiation in vivo [79] or pre-exposure to $^{16}O^{8+}$ or γ-rays [39] (Section 1.5.2 above) protects against subsequent high dose. Implications of HRS in cancer treatment by radiotherapy include fringe areas near the irradiated tumour which could experience greater damage than predicted [47] and LDR effects (brachytherapy) because of the HRS, as suggested in Section 1.4, with direct evidence for both aspects [48,49,52]. Again, the simple LQ model is not adequate [50]. It does not apply to responses in mammalian cells in vitro, nor to in vivo responses, and requires a correction, e.g., Refs. [9,10,59,82].

2.2. Individual sensitivity

Many persons with genetic diseases involving DNA repair e.g., Ref. [83], suffer from increased sensitivity to ionizing radiation, such as ataxia telangiectasia AT. An AT cell line does not show a LDR effect (while normal fibroblasts show higher survival after LDR irradiation) [84]. AT cells did not appear to exhibit HRS or IRR (M.C. Joiner, personal communication see Fig. 2 caption). Note, however, that the magnitude of the adaptive response in AT and normal cells was the same (0.01 Gy prime, 0.5 Gy γ challenge, chromatid breaks) [41]. A DNA

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[4] Warren Sinclair comments in ''rrs news'', the Radiation Research Society Newsletter (Nov. 1997, issue XXX, p. 11, regarding NCRP held April 1998).

[5] BELLE Newsletter, Vol. 7, 1998, p. 36 — W. Morgan on Leonard Sagan award.

binding protein relocalizes from the cytoplasm to the nucleus in normal cells after γ or neutron irradiation, but in AT cells it is constuitively located in the nucleus [70] (see Section 2.4.2). *Xermodosa pigmentosum* (XP) patients, who are sensitive to UV, can also be radiosensitive. We have not examined XP cells for existence of IRR, but note that the radiosensitive UV20 hamster line (defective in a step in excision repair [83]) does not have a ''normal'' IRR response [59]. We have not yet examined for structure the UV5 line, which lacks a different excision repair component [83].

2.3. Protectors

At the ISLSSPWG radiation workshop (CSA/ASC), it was noted by Dr. Setlow that chemoprotection research was not among the highest priorities for the space program. Furthermore, in a recent review, it was concluded that there is not much hope for radiation protectors [85]. But before we abandon this approach, we should consider

· the increasing knowledge about the many steps in carcinogenesis [86], to exploit fully intervention at stages other than initiation
· the concept that certain chemical agents prime to protect against ionizing radiation (Sections 1.3 and 1.4) as opposed to mechansims such as scavenging of radicals
· priming by yet other stresses than radiation or chemicals (Fig. 3)
· protection by certain natural products [87] vs. priming vs. possible sensitization by factors in diet (e.g., caffeine which prevents certain repair, and induction of a relevant protein [68])
· development of agents akin to antisense bax (or antisense bcl2) to suppress (or enhance) apoptosis to protect against (or sensitize to) the insult [88]. Such protection might have application in acute exposure, but this does not likely have chronic application.

2.4. LET, priming and dose rate considerations

2.4.1. Structures and thresholds

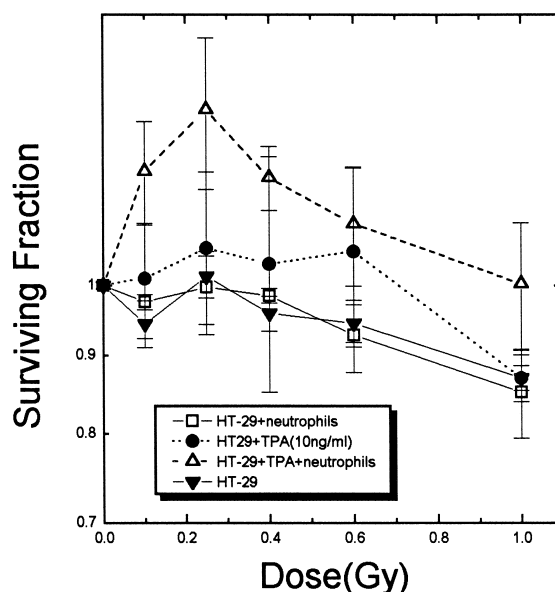(i) **Does response to high LET have structure?** While the initial study indicated no structure in

Fig. 3. HT29 cells primed by activated neutrophils. HT29 cells were irradiated with X-rays with and without exposure to neutrophils, ±TPA. (Results from three experiments with standard deviations.)

(Procedure for co-culture. HT29 cells were grown as monolayer cultures 2 days prior to the experiment, then trypsinized (0.1% trypsin) and resuspended at 2×10^6/ml in RPMI[###] medium (without serum).) For co-incubation, neutrophils (2×10^6) were added to TPA solution[###] at final concentration 10 ng/ml in 15 ml RPMI for 1 h at room temperature in dark. After centrifugation (1100 rpm) at room temperature, 10 min, the pellet (neutrophils) was resuspend in 1 ml RPMI, and added to 14 ml RPMI containing 2×10^6 HT29 cells. Following incubation for 1 h at 37°C, ~4000 HT29 cells were plated in 25 cm 2/40 ml flasks (NUNCLON), which were filled after attachment with fresh RPMI (plus 10% FBS, Gibco). Cells were scanned by DMIPS at 37°C to locate cells and irradiated in flasks 4 h after plating. Controls: HT29 cells were incubated with either TPA or neutrophils. The treatment was somewhat toxic and thus data were normalized (some plating efficiencies are in Table 1). Neutrophils were prepared according to Ref. [94].

[###] Methods and products from Sigma (St. Louis, MO). Briefly, 6 ml of heparanized human blood were placed onto a gradient of 3 ml Histopaque #1077 overlaid on 3 ml Histopaque #1119 which was centrifuged (2300 rpm) at room temperature for 30 min. The separated neutrophils were washed twice in PBS and resuspended at 2×10^6/ml (total neutrophils about 5.5×10^6).

response to low doses of neutrons unlike the X-ray response [9], it may be that the survival assay is limited in sensitivity and that response to high LET radiation also could exhibit structure at low doses. Pions have also been studied, and produce intermedi-

ate responses [89]. Considering other endpoints: mutagenesis data after γ irradiation could be fitted by two lines (or D^2) with a break at 2 Gy while higher LET had no such break [90]. This is usually considered to be the shoulder, but the shoulder may be related to IRR (Fig. 2, Ref. [21]). (ii) **Are there thresholds?** Induction of p53 by α particles had no threshold dose, whereas there was a threshold for X-ray induction (10 cGy) and different kinetics [77]. In a study on β vs. γ tumorigenesis in mice, it was noted that the threshold dose rate for γ (Co) was greater than for β (^{3}H) [91]. (iii) **Is there a dose rate effect?** An inverse dose rate effect was noted for mutagenesis endpoints [34,92] and also in a clinical situation [52]. This controversial area is beyond the scope of this paper (see Williams, this issue) and we cite only those articles already incorporated herein. As noted in Section 2.2, AT homozygotes do not exhibit the LDR effect seen in normal cells (protection-higher survival) [84] and these cells have no IRR. At lower dose rates, the shapes of the survival curves are reminiscent of HRS/IRR structure (Fig. 3A,B of Ref. [92]). (iv) **Additional relevant areas** also beyond this summary include different responses of transformed vs. non-transformed cells and cell cycle effects [92]. Very recently, a method which can detect cell cycle effects following doses as low as 0.25 Gy in a sensitive line has been described [93] which will be useful to considerations of thresholds, LET and priming.

2.4.2. Priming and proteins (protection / adaptive response)

Small doses of various kinds of radiation protect against challenge doses of low LET (γ, X). Beta particles from tritiated thymidine incorporated into the DNA primed hamster cells to protect against X-rays and also produced the adaptive response, as outlined in Section 1.2. On the other hand, neutrons do not prime for the adaptive response but do remove HRS in the X-ray response (Table 1); pions also remove the HRS; protons do not cause the adaptive response [31]. It has been noted that the proteins turned on by neutrons differ from those after γ irradiation [72].

The marked protection against X-rays seen due to priming with neutrons with "survival greater than 1" has only been superseded in our hands by priming with activated neutrophils (Fig. 3 and Table 1). This response suggests further studies on the effects of other stresses on IRR and perhaps of nitric oxide (Birnboim, this issue).

Far more cells expressed p53 at doses as low as 0.6 cGy of α particles than would be expected (only 0.45% should have had a particle through the nucleus, yet > 10% expressed p53), suggesting a mechanism other than exclusively direct DNA damage [77]. In this context, it is interesting that a specific DNA binding protein moves to the nucleus from the cytoplasm after neutron irradiation [70]. One wonders whether, like UV, higher LET can cause protective responses to damage outside the nucleus. This may not be the case for low LET damage [61]. Further studies are needed on the roles of direct vs. indirect damage in priming for such responses. The molecular spectrum, as well as frequency, of mutations both in vivo and in vitro depends on LET [90].

2.4.3. Cytotoxic vs. mutagenic effects at low doses

It could be suggested that cells with a small extent of damage (< 0.25 Gy in mammalian cells) are

Table 1
Summary of neutron (n) and X-ray (X) priming data (V79 cells, see Ref. [31] for details, errors, controls) and comparison with priming by activated neutrophils (HT29 cells, additional data in Fig. 3)

Prime, time, challenge	Plating efficiency	Survival	Control
(0 Gy, 4 h) 1 Gy X	0.68 ± 0.06	0.82 ± 0.07	*V79*
0.2 Gy X, 4 h, 1 Gy X	0.74 ± 0.04	0.94 ± 0.05	*PE = 0.83*
0.2 Gy n, 4 h, 1 Gy X	0.75 ± 0.03	**1.08** ± 0.04	
Control HT29, 0.25 Gy X	0.84 ± 0.005	1.02 ± 0.03	*HT29 PE = 0.85*
TPA and neutrophils, 4 h, 0.25 Gy X	0.49 ± 0.13	1.30 ± 0.16	*With TPA and neutrophils, PE = 0.37*
Control HT29, 0.1 Gy X	0.78 ± 0.04	0.94 ± 0.02	
TPA and neutrophils, 4 h, 0.1 Gy X	0.44 ± 0.05	**1.19** ± 0.08	

Table 2
Areas of overlap

	Treatment (Section 1)	Radiation exposure (Section 2)
Sensitivity	IRR/HRS (Section 1.1, Fig. 1) deficit from LQ and IRR (Section 1.4, Fig. 2)	Normal cells have IRR (Section 2.1) intrinsic and genetic sensitivity (Section 2.2)
Prime/protect	Priming (Section 1.2) and mechanisms (Section 1.5)	LET and threshold considerations (Section 2.3)
Implications	Fractionation, normal tissues	Hormesis vs. hypersensitivity
Genes	New predictive assays for tumor response (Sections 1.4, 1.5.2)	Genetic predisposition (Section 2.2) and and individual sensitivity
Apoptosis	''Anti-sense bcl2''	''Anti-sense bax''
Chemical agents	New sensitizers (prevent IRR in tumour) or protectors (prime normal)	Chemoprevention (Section 2.3) nutritional factors (protect or prime)
Dose rate	Brachytherapy (Section 1.4)	Inverse? LET? (Section 2.4)
Required info w.r.t. IRR, prime	Mutations vs. survival, dose rate effects, LET, chronic vs. acute, is there a threshold, what is trigger, is DNA damage essential, what is triggered (Sections 2.4, 3)?	

programmed to die, and that while priming may increase survival, such primed cells might have a higher mutation frequency. This needs to be measured directly. However, many studies have indicated that priming decreases mutations [34,37,38] as well as chomososmal damage, micronucleus formation etc. (references in Section 1.2). Few studies compare both endpoints (survival and mutagenesis, usually hprt) after low doses because of technical difficulties. Of the studies cited above, we note those which compare survival and mutagenesis:

(i) Albertini et al. [90] found high LET more toxic and more mutagenic (0.2 vs. 0.8 Gy doubling dose) but the lowest dose was 0.5 Gy, whereas a study on dose rate (inverse) included survival and mutagensis results after much lower doses [92].

(ii) No correlation was found between survival (extent of HRS/IRR) of two cell lines and their frequency of translocations [95].

(iii) As for priming results, Azzam et al. [32] found no improvement of clonogenic survival, while decreased frequency of micronuclei and neoplastic transformation were obtained.

We intend to examine the frequency of mutagenesis in rodent cells (primed vs. unprimed) in comparison with survival. It will be of interest in the context of radiation protection to determine whether the nature of the priming agent (LET, stress, chemical, etc.) affects the extent and nature of mutagenesis.

2.4.4. Summary

It is too early for definitive comment on the relevance of HRS/IRR in mutagenesis/carcinogenesis. Most studies have been carried out in cellular systems, although the possible relevance is encouraged by the comparable results obtained in vitro (0.8 Gy) vs. in patients (1.0 Gy) on the dose to double the yield of mutations at low LET [90]. The possibility that high LET may induce relevant protein expression without direct nuclear damage is fascinating [77] and suggests alternate approaches for protection against high LET. Finally, as recently summarized,[6] there may be accumulating unexpected evidence for protection (hormesis) coming from analysis of the Hiroshima and Chernobyl results. Some areas of common interest in cancer treatment and carcinogenesis in these contexts are summarized in Table 2.

3. Future directions

It is important to determine whether a certain type of DNA damage turns on the protective response

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[6] Roger Macklis in ''rrs news'', the Radiation Research Society Newsletter (Nov. 1997, issue XXX, p. 3).

after ionizing radiation; whether this is LET dependent; whether other stresses can protect; whether DNA damage is an absolute requirement for such responses; whether HRS reflects apoptosis; and whether it is fidelity of repair that matters as well as or in addition to the rate of repair, excision repair, repair of breaks, etc. These questions are of interest both to treatment of cancer and to carcinogenesis (Table 2). The frequency of mutagenesis may be higher than expected in the HRS region, or lower because damaged cells are dying to avoid mutations (e.g., by higher apoptosis, Section 1.5.1). Similarly, the primed cells may have different mutation frequencies (fidelity of repair?) than predicted. Much more work is needed to understand thresholds in radiation damage and the possibility of hormesis. There is not yet enough information on the effects of LET in these contexts, nor of the differences that might result from acute vs. chronic exposure. Dietary substances should be considered with respect to protection or priming at low doses for possible use in exposure situations. There is a need for further study on relationship(s) between survival and mutatgenesis following low doses of different types of insult.

4. Conclusions

It is not possible to predict responses at low doses from experiments at high doses — extrapolation is not appropriate, for example using the standard LQ model, because certain biological responses are dose dependent. Technologies continue to improve, permitting more sensitive assays. As our understanding of the effects of low radiation doses increases, there is less tendency to assume that we can learn ''everything'' from studies at high doses. Clinically relevant results are more likely to be attained if clinically relevant doses are studied; occupational exposure is more difficult, but should not be assumed from the other ranges. This applies to cell survival, mutagenesis, molecular biology, signal transduction and many other areas relevant to radioresponsiveness.

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