RESPONSES TO LOW DOSES OF IONIZING RADIATION IN BIOLOGICAL SYSTEMS

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□ *Biological tissues operate through cells that act together within signaling networks. These assure coordinated cell function in the face of constant exposure to an array of potentially toxic agents, externally from the environment and endogenously from metabolism. Living tissues are indeed complex adaptive systems.*

To examine tissue effects specific for low-dose radiation, (1) absorbed dose in tissue is replaced by the sum of the energies deposited by each track event, or hit, in a cell-equivalent tissue micromass (1 ng) in all micromasses exposed, that is, by the mean energy delivered by all microdose hits in the exposed micromasses, with cell dose expressing the total energy per micromass from multiple microdoses; and (2) tissue effects are related to cell damage and protective cellular responses per average microdose hit from a given radiation quality for all such hits in the exposed micromasses.

The probability of immediate DNA damage per low-linear-energy-transfer (LET) average microdose hit is extremely small, increasing over a certain dose range in proportion to the number of hits. Delayed temporary adaptive protection (AP) involves (a) induced detoxification of reactive oxygen species, (b) enhanced rate of DNA repair, (c) induced removal of damaged cells by apoptosis followed by normal cell replacement and by cell differentiation, and (d) stimulated immune response, all with corresponding changes in gene expression. These AP categories may last from less than a day to weeks and be tested by cell responses against renewed irradiation. They operate physiologically against nonradiogenic, largely endogenous DNA damage, which occurs abundantly and continually. Background radiation damage caused by rare microdose hits per micromass is many orders of magnitude less frequent. Except for apoptosis, AP increasingly fails above about 200 mGy of low-LET radiation, corresponding to about 200 microdose hits per exposed micromass. This ratio appears to exceed approximately 1 per day for protracted exposure. The balance between damage and protection favors protection at low cell doses and damage at high cell doses. Bystander effects from high-dosed cells to nonirradiated neighboring cells appear to include both damage and protection.

The senior author especially acknowledges with deep appreciation the long and fruitful collaboration and discussions on biological consequences of low-dose irradiation with his early mentors Dr. V.P. Bond and the late Dr. E.P. Cronkite, the late Dr. K.I. Altman, and Drs. J. Booz, T.M. Flidner, M. Frazier, and R.D. Neumann. The authors are grateful for editorial help by Mrs. J. Rocheck, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

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Regarding oncogenesis, a model based on the aforementioned dual response pattern at low doses and dose rates is consistent with the nonlinear response data and contradicts the linear no-threshold dose-risk hypothesis for radiation-induced cancer. Indeed, a dose-cancer risk function should include both linear and nonlinear terms.

Keywords. radiation low-dose effects, hormesis

INTRODUCTION

The function of biological tissues depends on concerted contributions of tissue constituent cells. These are embedded in networks of signaling for the coordination of cell responses and maintenance of tissue structure. The latter serves the former and does so at a homeostatically controlled level and in response to an array of potentially toxic agents that derive exogenously from the environment and endogenously from metabolism. Living tissues are indeed complex adaptive systems (Gell-Mann, 1994). Because of the immense complexity of mammalian tissues, cellular and tissue responses to potentially toxic agents are certainly not simple.

To comprehend tissue alterations such as those occurring in the course of cancer development, the consequences of all cellular responses within the signaling networks in a tissue system need to be considered. This appears to be especially crucial in the case of exposure to low doses and dose rates of ionizing radiation, because different types of radiation cause different numbers of energy deposition events of different sizes per unit absorbed dose at the level of cells and corresponding tissue micromasses. In fact, at low doses and dose rates, the mean value of absorbed dose in the tissue is usually very different from the individual values of absorbed dose in micromasses such as the cells of the exposed tissue. Moreover, low absorbed doses of low-linear-energy-transfer (LET) radiation may evoke a spectrum of biochemical and functional cell and tissue responses not seen at high doses, as discussed later in this paper.

Biological and health effects of low doses of ionizing radiation are currently broadly debated and studied by both experimental research and epidemiological methods and are being reassessed for radiation protection by administrative and regulatory agencies. The primary reason for this is the fact that epidemiological investigations on radiation-induced cancer incidence from low doses of low-LET radiation are limited by statistical constraints and thus rely on various models, whose scientific basis needs justification (UNSCEAR, 1994). In addition, over the past two decades the increasing evidence that is summarized and reviewed in this paper has shown that cellular responses per unit dose at low doses cannot be predicted by linear extrapolation of cell responses observed per unit dose at high doses. Despite its widespread misinterpretation by users of ionizing radiation, regulators and the public

alike, it has nevertheless been argued that the linear no-threshold (LNT) dose-risk approach remains a simple and convenient method to optimize procedures and regulations in radiation protection (Trott and Rosemann, 2000).

Radiation-induced cell and tissue effects, especially those for DNA, are rather well known from studies on single cells, tissues, and whole organisms largely following high-dose exposure (Bond *et al.*, 1966; Hall, 2000). Investigation of the effects of low doses, especially of low-LET radiation, has arisen only over the past two decades, made possible mainly because of the advent of refined and sensitive analytical procedures. Responses of complex biological systems to low doses from different types of ionizing radiation have been found to differ in both type and degree from the responses expected per unit dose based on data obtained at high doses (Sugahara *et al.*, 1992; UNSCEAR, 1994; Academie des Sciences, 1995; DOE/NIH, 2000). It has become increasingly clear that low doses of ionizing radiation may evoke dual responses in the exposed mammalian tissue and its cells (Feinendegen *et al.*, 1995, 1996, 1999, 2001). One response entails damage mainly to DNA which is also observed after high radiation doses. The other response is not seen at high doses and expresses various categories of adaptive protection against an array of endogenous and exogenous toxins such as reactive oxygen species as well as ionizing radiation, as discussed later. Both response patterns not only arise in directly irradiated cells but also appear to involve unirradiated cells receiving signals from neighboring irradiated cells, that is, through bystander effects and from tissue matrix (Nagasawa and Little, 1992; Mothersill and Seymour, 1997; Barcellos-Hoff and Brooks, 2001).

There are inherent difficulties with conventional dosimetry when applied to the study of the heterogeneously distributed energy deposition events in tissues that frequently occur at low-dose exposures and are nearly always encountered in nuclear medicine. This difficulty has been extensively discussed and put into a practical framework with recommendations for nuclear medicine (ICRU, 2002). Expressing overall risks as a function of the doses delivered to individual cells and equivalent tissue micromasses by heterogeneous distribution of energy deposition events at the cellular level requires a dosimetry at the microscopic level. Because acute as well as late effects of ionizing radiation such as cancer arise at the cellular level of biological organization, absorbed doses to cell-equivalent micromasses may reveal more than does the absorbed dose to whole tissue, especially under conditions of low-dose exposure. Dosimetry at the micromass level of tissue may of course be linked to conventional tissue dosimetry by summing the energies absorbed per micromass over all the exposed micromasses and dividing by their total mass, thus obtaining the expectation value of the microdose, which equals the average tissue dose.

It thus appears justified to approach both absorbed dose and radiation-induced responses in tissues by whatever mechanisms, including signaling at the cellular level of biological organization. To do so requires attention to

1. the relationship between absorbed dose in tissue and microdose in micromasses;
2. the spectrum of cellular responses to doses in tissue micromasses; and
3. the contribution of various cell responses to the generation of tissue effects, including cancer.

This type of analysis is unconventional but, on the one hand, remains in concordance with the fundamental quantities and units for ionizing radiation (ICRU, 1998) and, on the other, offers the possibility of assessing biological tissue effects as influenced by intercellular signaling and the efficiency at which the signaling is carried out at various levels of absorbed dose.

RELATIONSHIP BETWEEN ABSORBED DOSE TO TISSUE AND DOSE TO ITS MICROMASSES

Absorbed Dose

The absorbed dose is defined as the energy deposition per unit mass (ICRU, 1998), but its effects also depend on the total mass considered. The SI unit of absorbed dose (D) is the gray: $1 \text{ Gy (100 rad)} = 1 \text{ J/kg}$. At a sufficiently high value of absorbed dose from external irradiation, absorbed dose in a large exposed mass is identical to the absorbed dose in any small exposed mass or micromass; but the total energy absorbed in either mass is not the same. The assessment of effects as a function of absorbed dose may thus demand adjustments regarding the definition of the mass critical to effect development.

In this presentation, the principal gross-sensitive tissue micromass is defined to be that of an average mammalian cell. The cell is the basic unit of life with structural and functional components interacting through signaling networks and in mutual signal exchanges with other cells and tissue matrix throughout tissue. Although various shapes of micromasses may be defined, for the present level of microdosimetric modeling, the reference average mammalian cell is taken to have a spherical volume of 1 ng mass (Feinendegen *et al.*, 1994).

Penetrating ionizing radiation causes the deposition of energy from particle tracks that arise stochastically throughout the exposed mass (ICRU, 1983). The energy deposited by a single particle track in traversing a tissue micromass of 1 ng will be denoted in this paper by the term “microdose” and the event delivering this microdose is referred to as a “microdose hit.” Large absorbed doses D in the tissue create large numbers of microdose hits per

exposed micromass and the microdose-distribution weighted sum of energies delivered by multiple microdoses divided by the sum of micromasses, here denoted average cell dose, is then identical to D . As D decreases, the number of microdose hits per exposed micromass is reduced. When the number of microdose hits falls far enough below an average value of 1 per micromass, the dose to each micromass becomes either 0 or the microdose from a single track traversing the micromass, and only some fractional number of micromasses experience a microdose hit.

Individual microdose values conform to a spectrum that depends on the radiation type and quality. The individual single microdose from a single track may be formally equated to the specific energy z_1 , and its fluence derived mean is $\bar{z}_F$, here denoted by $\bar{z}_1$ (ICRU, 1983). The number of $\bar{z}_1$ -sized events (i.e., of average-sized microdose hits N_H , per number of exposed micromasses N_E) is then $(D/\bar{z}_1)$. The values of N_H may be obtained from measurements of D and microdosimetric measurements of $\bar{z}_1$ (ICRU, 1983). Absorbed dose D conforms to $\Sigma z_1/N_E$. Multiplying both numerator and denominator of the quotient by N_H results in (Bond *et al.*, 1995)

$$D = \bar{z}_1 (N_H/N_E) \quad (1)$$

Obviously, because $\bar{z}_1$ is constant for a given radiation quality, the quantity that varies with D is N_H/N_E , in which N_E is constant. Therefore, when a high-LET radiation produces larger values of $\bar{z}_1$ (i.e., mean microdoses), then fewer hits per exposed micromasses (i.e., lower N_H/N_E , will deliver the same D as does a low-LET radiation with its lower values of $\bar{z}_1$ and larger N_H/N_E (Feinendegen *et al.*, 1985). Thus, $\bar{z}_1$ and N_H/N_E are inversely related to each other, and the ratios between $\bar{z}_1$ and different values of D are specific to different radiation qualities.

For dosimetry at low-dose and low-dose rate exposures, presenting D in terms of $\bar{z}_1 N_H/N_E$ permits the expression of energy concentration per unit mass to be made in terms of number of average microdose hits per number of exposed micromasses in any given field of radiation of a certain quality with its specific value of $\bar{z}_1$. The denominator N_E may be a value adapted to a chosen experimental or observational constraint in that any chosen tissue mass can serve to link an effect to the energy absorbed in that mass. This approach has opened long-overlooked analytical possibilities. For instance, divergent cellular response probabilities may be related to the number of average microdose hits in a number of exposed micromasses as well as to different radiation qualities. In fact, the ratio N_H/N_E may be seen as a derived quantity for ionizing radiation of a given quality and one might propose to name it the “rossi” in honor of the founder of microdosimetry (Rossi, 1967; Rossi and Kellerer, 1972; ICRU, 1983).

When an average of five or more microdose hits from a uniform exposure to penetrating stochastically absorbed radiation occurs per micromass, more

than 99% of the exposed micromasses have been hit and the total dose to a given micromass (i.e., the cell dose), becomes equal to the tissue dose. For an average 1 hit per micromass, 37% of the micromasses receive 1 hit, 26% have more than 1 hit, 2% receive 4 hits, and 37% have no hits (Feinendegen and Graessle, 2002). When the fraction of exposed micromasses that are hit is less than or equal to 0.2, it is unlikely that more than a single hit occurs in any individual micromass (ICRU, 1983). Equation (1) also indicates this to be true when the absorbed dose D is less than 0.2 of the value of $\bar{z}_1$. This level sometimes serves to define a “low” dose. Thus, an absorbed dose D in tissue of 1 mGy of 250 kVp X-rays with $\bar{z}_1 = 0.9$ mGy would generate an average of about 1 microdose hit per exposed micromass. D would need to be below approximately 0.2 mGy to generate not more than 1 microdose hit per exposed micromass (ICRU, 1983). In contrast, D as high as 70 mGy would meet the “not more than 1 hit” criterion for 4 MeV alpha particles, where $\bar{z}_1 = 350$ mGy. Irrespective of these considerations, a value of D below about 200 mGy is generally considered a low dose.

Dose Rate

The microdose approach shows that dose rate (i.e., D per unit time t), in tissue expresses repeated hits to its micromasses. Thus, using Eq. (1),

$$\frac{D}{t} = \bar{z}_1 \left(\frac{N_H}{N_E} \right) \left(\frac{1}{t} \right)$$

or

$$\frac{D}{t} = \frac{\bar{z}_1}{(tN_E/N_H)}$$

and with t_x for (tN_E/N_H) ,

$$\frac{D}{t} = \frac{\bar{z}_1}{t_x} \quad (2)$$

The denominator t_x in Eq. (2) is equal to $(t\bar{z}_1/D)$; see also Eq. (1). The denominator expresses the average time interval between two consecutive microdose hits in a given micromass for a given radiation quality (ICRU, 1983; Feinendegen *et al.*, 1985).

Varying the time interval between two consecutive microdose hits per exposed micromass may either enhance or limit the full expression of radiation-induced responses of any type in the affected cells. It may be long enough for a microdose hit not to interfere with a response to a preceding hit. This would likely be the case, for instance, at continuous and uniform exposure to 1 mGy of 100 kVp X-rays per year, which would cause on average one mean microdose of 1 mGy per micromass per year. A chronic uniform exposure

to 100 mGy of 250 kVp X-rays per year would give an average time interval of 3.29 days between two consecutive microdose hits per exposed micromass and each hit would result in a mean microdose of 0.9 mGy; 31 such hits would occur per 100 micromasses per day—an important consideration in view of potential bystander effects. A chronic uniform exposure to 330 mGy of 250 kVp X-rays per year would bring on average one microdose hit per exposed micromass per day. For ^{137}Cs γ -radiation, an annual chronic exposure to 150 mGy would cause on average one microdose hit of 0.4 mGy per day per exposed micromass.

The aforementioned values of average time intervals and mean microdoses, of course, include two different types of distributions. One is for the stochastic incidence of microdose hits per micromass from penetrating radiation, as discussed earlier. The other distribution expresses the probability of microdose value per hit according to the measured particle spectrum from a given radiation quality (Feinendegen and Graessle, 2002).

THE SPECTRUM OF CELLULAR RESPONSES TO MICRODOSES

Endogenously Caused Damage and Signaling Effects

In the context of low-dose effects, it appears appropriate first to consider cell responses to potentially toxic agents from nonradiation sources (Feinendegen *et al.*, 1995; Lindahl, 1996; Pollycove and Feinendegen, 2003). Such toxic agents constantly arise from oxidative metabolism in the form of reactive oxygen species (ROS), and may come from micronutrient deficiencies as well as from various toxic compounds in the environment (Beckman and Ames, 1998; DOE/NIH, 2000).

As discussed in more detail elsewhere (Pollycove and Feinendegen, 2003), ROS arise from mitochondria and other reactions in cytoplasm of mammalian cells *in vivo*. The average generation rate is close to 10^9 ROS per cell per day. In addition, minimal ROS bursts frequently occur in the cytoplasm from various metabolic reactions (Beckman and Ames, 1998). The ROS cause many oxidative reactions throughout the cell and attack renewable molecules, lipids, and proteins with concomitant temporary changes in intracellular signaling (Stadtman and Berlett, 1998; Finkel and Holbrook, 2000; Sen *et al.*, 2000). On average about 10^6 oxidative DNA damages, DNA oxyadducts, are estimated to occur per cell per day: on average about 10 per second (Pollycove and Feinendegen, 2003). The calculation of 0.1 double-strand break (DSB) being produced per cell per day from endogenous sources (Pollycove and Feinendegen, 2003) closely agrees with an experimental value of 0.04 to 0.06 steady-state DSB per cell at any time in cultures of human fibroblasts from the lung (MRC-5) (Rothkamm and Löbrich, 2003). Despite an overall highly effective physiologic repair system, some injuries escape repair and leave some permanent DNA alterations. Such calculations lead to the

estimate that, on average, one permanent DNA alteration or mutation is likely to occur from endogenous sources per mammalian cell every day (Pollycove and Feinendegen, 2003). Most cells with such damage accumulation exit their tissue through cell differentiation and senescence and others may be removed by apoptosis or immune responses, which is referred to later. In surviving cells, these DNA alterations likely contribute to both aging and spontaneous carcinogenesis (Harman, 1956, 1992; Beckman and Ames, 1998; Finkel and Holbrook, 2000). The probability of non-radiation-induced (i.e., spontaneous) oncogenic transformation of a human hemopoietic stem cell combined with the probability that it will eventually lead to lethal leukemia has been estimated nevertheless to be only about 10^{-11} (Feinendegen *et al.*, 1995).

The various types of ROS potentially also change intracellular signaling and gene expression. The different categories of cellular adaptations to toxic agents and corresponding protection such as ROS detoxification, DNA repair, and damage removal including apoptosis may be stimulated by ROS because they occur at various rates in the course of normal metabolism (Ramana *et al.*, 1998; Bauer, 2000; Chandra *et al.*, 2000; Finkel and Holbrook, 2000; Sen *et al.*, 2000). These publications suggest that cellular ROS may directly or indirectly produce or suppress DNA alterations depending on ROS concentration.

Endogenously Caused and Radiation-Induced Damage

Primary radiation-induced DNA damage appears to be proportional to absorbed dose within a certain dose region down to low doses and the corresponding dose-effect function is linear. This statement relies on measurements that come from isolated DNA as well as DNA extracted from cell populations in culture and in complex tissues (Hall, 2000). DNA damage from low-dose irradiation adds to the damage from nonradiation and endogenous sources. For instance, a Compton electron track of about 6 keV from 100 kVp X-rays ranges over less than 1 μm in tissue and creates about 150 ROS with considerable clustering along its track. The ROS from low-LET radiation in hit cells are estimated to be responsible for some 60–70% of the resulting DNA damage, with about 30–40% of the damage coming from direct electron interactions with the DNA (Hall, 2000). In all, a 10 mGy average cell dose from low-LET radiation causes at least 10 base changes, approximately 10 single-strand breaks (SSBs), an average of about 0.4 DSBs and less than 5 intermolecular cross-links (Ward, 1988).

A continuous uniform exposure to a tissue-absorbed dose of about 2 mGy of low-LET radiation per year produces on average one microdose hit per exposed micromass at an approximate rate of two to six times a year, depending on radiation quality with its particular mean microdose. Thus, a tissue dose of 2 mGy per year from Co^{60} γ -radiation brings one hit per exposed micromass

of a mean microdose of about 0.3 mGy on average every 2 months, whereas a tissue dose of 2 mGy per year from 100 kVp X-rays brings one hit per exposed micromass of a mean microdose of about 1 mGy on average every 6 months. These microdose hits each generate, correspondingly, on average some 50 to 150 ROS in less than a microsecond (Feinendegen, 2002). Because of the low hit frequency from background radiation, the resulting persistent DNA damages per average cell per day are many orders of magnitude lower than the incidences of DNA damage from nonradiation sources, as discussed earlier.

The preponderance of endogenously caused DNA damages also holds regarding damage severity. Endogenous ROS primarily cause single DNA oxyadducts at a relatively low rate, on average of about 10 adducts per second per cellular genome. On the other hand, background-radiation-induced DNA damage is relatively rare. Microdose hits per given micromass occur at average time intervals of months. Direct microdose hits in cells expose their DNA to frequently clustered ROS and direct particle interactions. This causes a relatively large fraction of the total DNA alterations to be a multiple-damage-site type in the hit cells (Ward, 1988). Indeed, the probability of a DSB per primary DNA alteration from directly interacting radiation is estimated to be 10^5 times higher than that per ROS attack on DNA from endogenous sources (Pollycove and Feinendegen, 2003). Nevertheless, the daily high incidence of endogenously generated DNA oxyadducts alone in all cells is thought to generate so many DSB-type lesions per average tissue cell that they outnumber the DSB incidence per day caused by background radiation by a factor up to 10^3 (Pollycove and Feinendegen, 2003).

It thus appears that DNA damages from endogenous nonradiation sources outnumber even the more severe DNA damages from radiation exposure at background levels.

Cellular Defense, Repair, and Damage Removal

The enormous vulnerability of the mammalian genome to endogenously and environmentally generated toxins other than radiation may be the evolutionary cause for highly effective biochemical mechanisms that provide cell and tissue protection (Lindahl, 1996). Both damage and protection may be induced by endogenous toxins such as ROS as well as by low-dose irradiation. The cell in all likelihood does not distinguish between ROS produced endogenously and by low-dose irradiation even though their topography of ROS generation in the cell is different. The type and extent of both damage and protection apparently vary with species, cell type, cell cycle, and metabolism (Alberts *et al.*, 1994; Hall, 2000).

The physiological protective mechanisms lead to (a) elimination of toxic agents, especially ROS, by different biophysical and biochemical scavenging systems, (b) repair of DNA damage of various kinds such as base changes, SSBs, and DSBs; and (c) removal of cells with certain types and degrees of

damage, for example, by apoptosis, necrosis, premature differentiation, or by competent immune responses.

Following irradiation, cells apparently initiate these protective mechanisms in at least two ways. One of these leads to quick responses, for instance, to apoptosis or repair of cellular damage, such as DNA repair, which may take from minutes to hours after exposure depending on the type of damage (Friedberg *et al.*, 1995; Hall, 2000). The recent report on lack of DSB repair following low doses of low-LET radiation in human lung fibroblasts in culture does not appear relevant here because this observation was limited only to nonproliferating cells, and such cells with DSBs were quickly eliminated when they were permitted to proliferate (Rothkamm and Löbrich, 2003). The other mechanism appears with a delay of several hours following low-but not high-dose exposure. It elicits stress-response-like reactions that may last up to several weeks and provide improved protection in a manner that is also called an adaptive response (Olivieri *et al.*, 1984; Wolff, 1998), as further discussed later.

Immediate Repair and Damage Removal

In regard to immediate removal of cellular damage, the repair of DNA base changes, SSBs, and DSBs has been studied extensively after high-dose irradiation (Alberts *et al.*, 1994; Friedberg *et al.*, 1995; Hanawalt, 1995; Ohyama and Yamada, 1998; Wallace, 1998; Hall, 2000; Wood *et al.*, 2001). DNA repair begins almost immediately after the damage has occurred. Different base changes are repaired within about 10 min to one h (Jaruga and Dizdaroglu, 1996). SSBs are usually repaired with a half-time of less than 10 min, whereas the repair half-times for DSBs are longer than 30 min (Frankenberg-Schwager, 1990). Damage removal by signal-induced cell death, or apoptosis, is readily seen within hours after irradiation (Potten, 1977; Yamada and Hashimoto, 1998). Misrepaired and unrepaired damage may make viable daughter cells more susceptible to oncogenic transformation (i.e., may cause “genomic instability”), even after many cell divisions (Little, 2000, 2002). These cells may also be more vulnerable to renewed toxic attacks and become more susceptible to removal by immune responses and apoptosis. Induction of apoptosis of transformed fibroblasts in culture also occurs through signaling from activated nontransformed cells; this involves various ROS and nitrous oxide species triggered by the transformed cells. Intercellular induction of apoptosis appears to represent a general signaling concept in several natural antitumor systems (Bauer, 2000). The probability of an oncogenic transformation of a human hemopoietic stem cell *in vivo* with lethal consequences is very low, about 10^{-13} to 10^{-14} per microdose hit of 1 mGy of low-LET radiation such as 100 KVp X-rays (Feinendegen *et al.*, 1995, 2000). The corresponding risk per microdose hit of 1 mGy per cell for a DSB is about 10^{-2} and that for chromosomal aberrations is 10^{-4} . This large ratio of chromosomal aberrations to

oncogenic transformation and lethality, about 10^9 to 10^{10} , suggests the existence of effective defense systems that prevent development of lethal tumors in humans.

Adaptive Protection Response

Increasing evidence in the literature on adaptive protection indicates that corresponding responses occur in mammalian cells *in vivo* and *in vitro* after single as well as protracted exposures to X- or γ -radiation (Sugahara *et al.*, 1992; UNSCEAR, 1994; Academie des Sciences, 1995; DOE/NIH, 2000; Feinendegen *et al.*, 2000, 2001; Pollycove and Feinendegen, 2003). Through direct and indirect cellular exposure to low-dose induced bursts of ROS from microdose hits, consecutive intra- and intercellular signaling effects may at least in part be responsible for low-dose induced adaptive protection, which except for apoptosis is not seen at high doses. The resulting biochemical reactions develop relatively slowly within a few hours, may last for several weeks, and resemble physiological stress responses that protect against DNA damage from any source, whether it originates endogenously or from renewed irradiation (Wolff *et al.*, 1988). Such protective responses occur in various ways and appear to depend on mammalian species, types of tissue, cell types, and the cell cycle. Adaptive protection categories after single low-dose, low-LET irradiation, are as follows:

- Stimulation of the radical detoxification system appears to reach a maximum at about 4 hr after irradiation and lasts for several hours to even weeks, depending on tissue and cell type. In mouse bone marrow *in vivo*, the effect slowly declined over a period of about 6 hr. There was a delayed and temporary reduction of the incorporation of DNA precursors and of thymidine kinase activity to some 70% of control in relation to a concomitant rise of free glutathione (Zamboglou *et al.*, 1981; Feinendegen *et al.*, 1984, 1987, 1995). In other low-dose irradiated rodent tissues, increased levels of superoxide dismutase occurred in parallel with decreased lipid peroxidation lasting for weeks (Yamaoka, 1991; Yamaoka *et al.*, 1992) and an elevated level of glutathione up by a factor of close to five in spleen cells was involved in an increase in natural killer cell activity (Kojima *et al.*, 2002). ROS detoxification was also linked to gene activation. Thus, mRNAs for glutathione-synthesis-related proteins in the mouse liver became elevated after low-dose γ -irradiation (Kojima *et al.*, 1998). The low-dose-caused increase in intracellular glutathione in RAW-264.7 cells with its maximum between 3 and 6 hr after exposure was mediated by transcriptional regulation of the γ -glutamylcysteine synthetase gene, predominantly through the AP-1 binding site in its promoter (Kawakita *et al.*, 2003).
- Protection against high-dose induced chromosomal aberrations in human lymphocytes increased to a maximum about 4 hr after a conditioning

low-dose low-LET irradiation; the protection also operated against other DNA damaging agents (Olivier *et al.*, 1984; Wolff *et al.*, 1988). This protection of up to about 30% of nonconditioned controls varies between individuals and in other cells types or is absent; it is probably determined genetically (Wojcik *et al.*, 1992; Raaphorst and Boyden, 1999). In case it operates, it appears to last up to about 3 days, as reported for various human cells *in vivo* as well as in culture (UNSCEAR, 1994). This adaptive response probably involves a several-fold enhancement of the DNA repair rate (Ikushima *et al.*, 1996; Le *et al.*, 1998). A similar adaptive response appeared regarding micronuclei formation in human fibroblasts (Azzam *et al.*, 1994). In these cells, conditioning doses from 1 to 500 mGy were equally effective; this also indicates that at the lowest dose, when approximately 40% of the cells do not have a microdose hit, a bystander effect is involved in causing the adaptive protection (Broome *et al.*, 2002). The degree of inhibition of DNA synthesis and cell growth in rat glial cells in culture by a high dose of X-rays was reduced by about one-fourth to one-third following a conditioning low-dose exposure, when the cells were obtained from young rats; the adaptive response decreased with age of the donor rats. This adaptive response involved protein-kinase C (PCK), DNA-dependent protein-kinase (DNA-PK), and phosphatidylinositol 3-kinase (PI3K), as well as the activity of the ataxia-telangiectasia gene (ATM) (Miura *et al.*, 2002).

- Damaged cells may disappear through intra- and intercellular cellular signaling, for instance, by signal-induced cell death (i.e., apoptosis). This usually occurs within hours after high-dose irradiation. Low-dose induced apoptosis of predamaged cells with replacement by healthy cells may be a major route of *in vivo* removal of oncogenically transformed cells (Potten, 1977; Kondo, 1988, 1993, 1999; Norimura *et al.*, 1996; Yamada and Hashimoto, 1998; Ohyama and Yamada, 1998). This route is supported by the finding of intercellular induction of apoptosis generated by transformed cells in culture (Bauer, 2000). Nongrowing human fibroblasts in culture with DSBs from low-dose low-LET irradiation readily lost this damage to the level of DSBs in nonirradiated control cells after induction of proliferation; this damage removal was mainly due to apoptosis (Rothkamm and Löbrich, 2003). Damaged cells also may exit the system by premature differentiation and maturation to senescence (Trott and Rosemann, 2000). This was also observed to follow low-dose irradiation via bystander effect in microbeam experiments directed to single cells in complex tissue (Belyakov *et al.*, 2002). The various mechanisms of protection may be directly or indirectly linked to transient changes in the activity of the G₁ cell-cycle checkpoint (Boothman *et al.*, 1996). Another mechanism in this category of damage removal is known to occur in a number of tissue culture cell types by way of hypersensitivity to low-dose radiation that disappears at higher doses (Joiner *et al.*, 1996, 1999). This hypersensitivity in

some cells was linked to the cell cycle (Short *et al.*, 2003). This hypersensitivity disappeared in a number of culture cells about 4 hr, but not immediately, after a single low-dose, low-LET irradiation (M.C. Joiner, personal communication, 2002). Radiation-induced predisposition to genetic instability in culture cells also declined following low-dose irradiation (Suzuki *et al.*, 1998). These data indicate prevention of damage removal by way of low-dose induced DNA repair. Low-dose induced enhancement of DNA repair may be responsible for the observation in rat thymocytes, where the incidence of radiation-induced apoptosis first declined at low doses and only rose with higher doses (Liu *et al.*, 1996). The induction of apoptosis apparently requires a certain level of DNA damage.

- Removal of damaged cells occurred *in vivo* by way of a low-dose induced immune competence (James and Makinodan, 1990; Anderson, 1992). This was, in another study, associated with a reduction in the incidence of cancer metastases to less than one-third of control concomitantly with an increased number of circulating cytotoxic lymphocytes (Hashimoto *et al.*, 1999). Such response had its maximum *in vivo* at about 0.2 Gy (Sakamoto *et al.*, 1997). Low-dose induced immune competence may last for several weeks (Makinodan, 1992).

The coordinated action of these protective responses, in one form or another, may be responsible for the observation of a reduction of spontaneously occurring cancers. In fact, single low doses of low-LET radiation in tissue culture cells initiated with a delay of 1 day, but not immediately, a significant reduction of spontaneous clonogenic transformation to about one-third of control (Azzam *et al.*, 1996; Redpath and Antoniono, 1998; Redpath *et al.*, 2001). In mice heterozygous for the Trp-53 gene, a single low dose of low-LET radiation given at the age of about 2 months significantly delayed the appearance of “spontaneous” lymphoma and spinal osteosarcoma later in life (Mitchel *et al.*, 2003). A review of tumor development following low-dose, low-LET irradiation in rodents showed the existence of a threshold dose (Tanooka, 2001). This is supported by a recently published study of induction of lymphomas, solid tumors, and ovarian tumors in BC3F1 female mice that at the age of 1 month or 3 months received single whole-body doses up to 32 cGy of low-LET radiation; the threshold dose was 4 cGy (Di Majo *et al.*, 2003).

The preceding listed categories of adaptive protection involve changes in gene expression (Kojima *et al.*, 1998; Amundson *et al.*, 1999; DOE/NIH, 2000; Miura *et al.*, 2002; Kawakita *et al.*, 2003). An example for DNA repair gene activation refers to the telangiectasia gene (Miura *et al.*, 2002). Human fibroblasts in culture showed DNA repair in the course of adaptive protection against micronucleus formation following acute high-dose irradiation; the repair was more effective in the gene-poor chromosome than in the gene-rich chromosome of the cells (Broome *et al.*, 1999). Another recent presentation

showed that exposure of human skin fibroblasts in culture to a single dose of 20 mGy γ -radiation caused more than 100 genes to change their expression within 2 hr. This gene group included stress response genes and was different from the group of genes in parallel cultures that concomitantly responded to 500 mGy (Golder-Novoselsky *et al.*, 2002).

Except for apoptosis, all the aforementioned protective responses to single exposures tend to be expressed maximally after less than 0.1 and not more than 0.5 Gy X- or γ -radiation (Shadley and Wiencke, 1989; Feinendegen *et al.*, 1995) and to increasingly fail with higher doses depending on type of adaptive protection, cell type, and species, as summarized previously (Feinendegen *et al.*, 2000, 2002). In most mammalian cells so far examined, the expression of adaptive protection had a maximum above 5 mGy and below about 200 mGy. One might speculate that DNA damage accumulation from any source eventually conditions a cell to become susceptible to apoptosis.

Adaptive protection appears as physiological expression of cellular capabilities to maintain integrity of tissue structure and function in the face of various exposures to potentially toxic agents including ROS, be they from endogenous sources or from ionizing radiation including that from background radiation exposure (Feinendegen *et al.*, 1983, 1987; Pollycove and Feinendegen, 2003). Despite the disparity of the examined systems and responses, there appears to be a common pattern in the data. In fact, adaptive protection following low doses of low-LET radiation appears to be the consequence of changed cellular signaling and to be ubiquitous.

It follows that (1) at background radiation exposure levels, DNA damage in mammalian cells comes mainly from nonradiation sources; (2) induction of adaptive protection outweighs damage at doses well below 200 mGy low-LET radiation; (3) the delayed and temporary protective responses at low doses appear to operate primarily against DNA damage from nonradiation sources; and (4) at higher absorbed doses in tissue and the corresponding cell doses, cell and DNA damage appear increasingly to overrule, negate, or annihilate the more subtle signaling effects seen after low doses that lead to adaptive protection. Protection against DNA damage from any source presumably brings more benefit than damage to the entire organism.

CELL RESPONSES IN THE GENERATION OF TISSUE EFFECTS

Tissue System with Cellular Elements

The dual cell response to low-dose irradiation, as discussed earlier (i.e., lasting DNA damage on the one hand and delayed appearance of temporary protection on the other), can be depicted in a model aiming to elucidate the pathogenesis of tissue effects. The various components of the dual response, as presented earlier, have become known predominantly from measurements

made after single low-LET irradiation rather than chronic irradiation of tissues or multicellular systems in culture.

Single cells in culture systems have been separately irradiated using microbeams of different LET types and effects were registered throughout the cell population. Clearly, unirradiated cells being contact neighbors to irradiated cells and in some cells through culture medium showed responses as bystander effects, which proved to be a source of DNA damage (Brooks *et al.*, 1974; Nagasawa and Little, 1992; Mothersill and Seymour, 1997, 1998; Azzam *et al.*, 1998; Barcellos-Hoff and Brooks, 2001; Brenner *et al.*, 2001; Sawant *et al.*, 2001a, 2001b). At very low doses, this damaging bystander effect will cause greater harm than expected under the assumption that damage comes only from directly irradiated cells. This bystander-induced damage amplification in tissue, however, needs to be viewed in the context of both radiation quality with its different microdose values and of tissue-absorbed dose. At high microdose values and low tissue-absorbed dose, the damaging bystander effect may become more visible than at low microdose values at similar tissue-absorbed dose and may significantly outweigh the effects from single cells. Some of the reported experiments on bystander effects following single-cell α -particle irradiation (Brenner *et al.*, 2001), however, have used technical approaches that only allow the observation of damage from bystander effects and preclude the observation of protective bystander effects (Feinendegen and Pollycove, 2003). In fact, there is evidence that bystander effects induce protective responses in nonirradiated neighboring cells (Lyng *et al.*, 2000; Matsumoto *et al.*, 2001; Sawant *et al.*, 2001b; Belyakov *et al.*, 2002; Broome *et al.*, 2002; Lehnert and Iyer, 2002) and that normal cells in culture may signal for the apoptosis of transformed neighboring cells (Bauer, 2000). Moreover, physiological intercellular signaling also involving tissue matrix function appears to be less disrupted by low doses than high doses and at low doses rather modulates than destroys the balance between damage and repair in tissues (Barcellos-Hoff and Brooks, 2001). Other experiments used transfer of culture medium from low-dose irradiated cells to test cell survival in recipient cultures growing in the transferred medium; there was cell damage but no initiation of adaptive protection against a single low-dose γ -irradiation (Mothersill and Seymour, 2002). This set of data, however, may not apply to measuring adaptive protection. First, it rather expresses toxicity in the recipient culture cells from long-term exposure to the damaging factor in the transferred medium. Second, this factor concentration under these conditions may have been too high to initiate adaptive protection, since the conditioned medium from more than 10^5 irradiated culture cells was added to only about 300 recipient cells that received a single low dose of γ -radiation prior to growing clones in testing for cell survival.

In principle, data on cellular responses that are stochastically generated and summarily measured in multicellular systems and not single cells, of

course, include not only intercellular bystander effects of both damaging and protecting types, but also responses to any extracellular signaling that may affect the ensuing results. It is increasingly clear that radiation effects in cell populations and tissues are always the consequence of all cell responses in the exposed system (Feinendegen, 1991). In this way, tissue as a whole should be seen as a system being composed of elements with different radiation sensitivities and responses, but reacting as a whole (Barcellos-Hoff and Brooks, 2001; Gössner, 2003).

Regarding the generation of data so far, observations of cell systems rather than single cells readily registered one or more categories of adaptive protection at low doses of low-LET radiation, where damaging effects are hardly or not at all measurable. As explained earlier, when tissue doses of low-LET radiation are high enough to cause on average more than five microdose hits per exposed micromass, average cell doses eventually become equal to tissue doses. With high-LET radiation, individual microdoses have a relatively high value. In this case, therefore, a low tissue dose cannot be equated with an average cell dose. Instead, overall tissue effects may be related to the number of microdose hits in the number of micromasses exposed to a given radiation quality.

From Dose-Risk Function to Microdose-Hit-Number Effectiveness—Function for Oncogenesis

To display the principle of the approach, oncogenesis at a low tissue dose of penetrating ionizing radiation of a given type is here expressed as the consequence of several cellular effect probabilities that vary independently with the ratio of the number of average microdose hits to the number of exposed micromasses (i.e., N_H/N_E). It is here assumed that the probability of radiation-induced oncogenic transformation is proportional to the number of microdose hits where the measured cancer incidence appears to be linearly proportional to absorbed dose. Taking this assumption to apply down to the lowest dose region and taking α to be the proportionality constant yields the well-known relationship between risk R and tissue dose D :

$$R = \alpha D \quad (3)$$

This equation expresses the frequency of oncogenic transformation as a function of D at low doses. Whether it may also apply to the subsequent appearance of cancer will be further examined.

Equation (3) may be expressed in microdosimetric terms (ICRU, 1983; Bond *et al.*, 1995) as follows. Let R be the risk of oncogenic transformation in an irradiated tissue. R gives the ratio of the number of transformed cells, from which observable radiation-induced cancer may arise, N_q , to the number of exposed micromasses, N_E . Thus, substituting for R the ratio N_q/N_E and for

D using Eq. (1): $D = \bar{z}_1 (N_H / N_E)$, the following equation results:

$$(N_q / N_E) = (\alpha \bar{z}_1) (N_H / N_E) \quad (4)$$

This equation expresses for a given radiation quality with constant $\bar{z}_1$ the incidence of oncogenic transformation, not as a function of energy absorbed per unit mass but as a linear function with proportionality α of the number of average microdose hits per number of exposed micromasses. The conventionally used dose-risk function has thus been transformed into a microdose-hit-number effectiveness function or “hit-number-effectiveness function” for a given radiation quality in the exposed system (Bond *et al.*, 1995).

This function also may be used to relate observed tissue effects such as cancer to the probabilities of cellular responses per average microdose hit and the number of such hits per number of micromasses exposed to a given radiation quality. By rearranging Eq. (4) to

$$\alpha = \frac{N_q}{(\bar{z}_1 N_H)}$$

and regarding the term α as a sum of probabilities, as discussed in the following section, the term N_q can be redefined as the number of oncogenically transformed cells, which cause a clinically observable malignancy. The expression $\bar{z}_1 N_H$ denotes the total energy deposited by multiple numbers of $\bar{z}_1$ -sized hits. It will be shown below that at a given $\bar{z}_1$ the value of α cannot be regarded as remaining constant with decreasing values of N_H / N_E in the low-dose region (i.e., below about 200 mGy low-LET radiation).

It is understood that replacing the conventional dose-risk function by the microdose-hit-number-effectiveness function for a given radiation quality may require attention to possible physical and biological constraints that may arise from the gross heterogeneity in the distribution of microdose hits and from characteristics of particle track structures in tissues as well as from bystander effects. The term microdose in tissue, on the other hand, offers the advantage of being consistent with the conventional quantities and units for ionizing radiation (ICRU, 1998).

Summing All Cellular Response Probabilities per Microdose Hit over All Hits

Low-LET Microdoses

The present approach is a first approximation and involves considerable complexity, but it leads to a model that does not rely on the specific steps in the process of oncogenic transformation of a cell that result in its becoming the seed of a malignant tumor. Instead, the probability of a cancer to appear is

put into the context of balance between probabilities per average microdose hit of both oncogenic transformation with potential cancer development and various protective responses acting against oncogenic transformation and tumor development, whether the latter stems from nonradiation toxins or ionizing radiation.

The probability of a radiation-induced malignant transformation in a cell causing tumor development derives from the measured incidence of a malignant tumor in the observed organism. The following assumptions are made: (1) that a malignant tumor arises from a single cell potentially in interaction with the tissue matrix (Barcellos-Hoff and Brooks, 2001); and (2) that cancer incidence obtained at higher doses in the linear region of the dose-risk function indicates the incidence of radiation-induced oncogenic cell transformation per unit dose down to single average microdose values. On these assumptions, the probability of radiation-induced oncogenic cell transformation with potential cancer development per average microdose hit of a given radiation quality is denoted by p_{ind} . In case a radiation-induced enhancement of p_{ind} occurs, such as through genomic instability of the progeny of an affected cell, or through bystander effects and other cancer-enhancing effects on that cell, the fractional enhancement of p_{ind} per average microdose hit is described here by the probability p_{enh} . The probability of oncogenic cell transformation with potential cancer development caused by nonradiation toxins such as endogenous ROS (i.e., spontaneous carcinogenesis) per oncogenically transformable cell at a given age during its lifetime is denoted by p_{spo} .

The adaptive protection responses have already been defined. The cumulative probability of all operating protection against any oncogenic transformation and tumor development is expressed here by p_{prot} . Because protection depends on both dose D , that is, on N_{H} conditional on D because $D = \bar{z}_1 (N_{\text{H}}/N_{\text{E}})$, and the duration of such protection after exposure, t_{p} , the term p_{prot} becomes $p_{\text{prot}} f(N_{\text{H}} t_{\text{p}})$.

The relation between the number of microdose hits per number of micromasses exposed to a given radiation quality and the various cellular response probabilities is first analyzed here for single low-dose exposures (Feinendegen *et al.*, 1995; 2000). With

$$\begin{aligned}
 p_{\text{spo}} &= \text{lifetime probability of spontaneous oncogenic cell} \\
 &\quad \text{transformation with potential cancer development, per cell} \\
 &\quad \text{at a given age} \\
 p_{\text{ind}} &= \text{probability of radiation-induced oncogenic cell} \\
 &\quad \text{transformation with potential cancer development, per} \\
 &\quad \text{average microdose hit}
 \end{aligned}$$

p_{enh} = fractional enhancement of p_{ind} , per average microdose hit
 $p_{\text{prot}} f(N_{\text{H}}, t_{\text{p}})$ = cumulative probability of adaptive protection that operates
 against oncogenic cell transformation and the occurrence of
 cancer, that is, against p_{spo} , p_{ind} , and p_{enh} , per average
 microdose hit

and with $N_{\text{q}}/N_{\text{E}}$ and $N_{\text{H}}/N_{\text{E}}$ as explained earlier,

$$N_{\text{q}}/N_{\text{E}} = [p_{\text{ind}} + p_{\text{ind}} p_{\text{enh}} - p_{\text{prot}} f(N_{\text{H}}, t_{\text{p}}) p_{\text{spo}} - p_{\text{prot}} f(N_{\text{H}}, t_{\text{p}}) \\ \times p_{\text{ind}} - p_{\text{prot}} f(N_{\text{H}}, t_{\text{p}}) p_{\text{ind}} p_{\text{enh}}] (N_{\text{H}}/N_{\text{E}})$$

or

$$N_{\text{q}}/N_{\text{E}} = [p_{\text{ind}}(1 + p_{\text{enh}}) - p_{\text{prot}} f(N_{\text{H}}, t_{\text{p}})(p_{\text{spo}} + p_{\text{ind}} + p_{\text{ind}} p_{\text{enh}})] \\ \times (N_{\text{H}}/N_{\text{E}}) \quad (5)$$

Combining Eqs. (4) and (5) gives

$$\alpha = [p_{\text{ind}}(1 + p_{\text{enh}}) - p_{\text{prot}} f(N_{\text{H}}, t_{\text{p}})(p_{\text{spo}} + p_{\text{ind}} + p_{\text{ind}} p_{\text{enh}})]/\bar{z}_1 \quad (6)$$

The positive and negative terms contained in Eq. (6) determine the value of α at various absorbed doses in tissues and thus the shape of the dose–risk function for tissue effects. The average values of the components of Eq. (6) may arise from multiple individual measurements. However, Eq. (6) primarily only applies to individual risk of cancer without addressing the variability in susceptibility of different individuals to cancer induction. For a heterogeneous population of humans of different ages that includes both sexes, individually different α values will express different susceptibilities for cancer induction. As discussed earlier, whereas low-LET radiation-induced oncogenic cell transformation with potential cancer development increases over a certain dose range proportional to the number of microdose hits per number of exposed micromasses, adaptive protection appears specifically at low numbers of microdose hits per exposed micromass and approaches zero with increasing numbers of microdose hits. It is justified to assume that the radiation-induced adaptive protection at low numbers of instantaneous microdose hits of the low-LET type, $[p_{\text{prot}} f(N_{\text{H}}, t_{\text{p}})(p_{\text{spo}} + p_{\text{ind}} + p_{\text{ind}} p_{\text{enh}})] (N_{\text{H}}/N_{\text{E}})$, reduces the generation and accumulation of DNA damage from any source. Because DNA damage comes overwhelmingly from endogenous sources, the adaptive protection is expected to operate mainly against endogenous DNA damage. If the probability of cancer induction were equal to the probability of protection

at a given number of microdose hits per number of exposed micromasses, the value of α under this condition would become zero. It would be negative if the probability of adaptive protection attains a larger value than the probability of cancer induction. In the latter case, a hormetic tissue effect would appear (Feinendegen *et al.*, 1995, 1999, 2000). This implies that the use of the empirical LNT model for low-dose risk assessment is questionable at best and needs reevaluation.

As an example, neglecting any enhancement, Eq. (5) can be simplified to

$$N_q/N_E = [p_{\text{ind}} - p_{\text{prot}} f(N_H, t_p)(p_{\text{spo}} + p_{\text{ind}})](N_H/N_E)$$

and, letting N_q/N_E be zero, then

$$(N_H/N_E) p_{\text{prot}} f(N_H, t_p) = (N_H/N_E) p_{\text{ind}}/(p_{\text{spo}} + p_{\text{ind}}) \quad (7)$$

With aforementioned values of $p_{\text{ind}} = 10^{-13}$ – 10^{-14} per average microdose hit N_H of low-LET type, and of $p_{\text{spo}} = 10^{-11}$, the value of $p_{\text{prot}} f(N_H, t_p) = 10^{-2}$ to 10^{-3} would result. In other words, the coefficient α would already be zero at a protection probability of as little as 10^{-2} to 10^{-3} per average microdose hit from low-LET radiation (Feinendegen *et al.*, 1995, 2000). As indicated earlier in the section on adaptive protection response, $(N_H/N_E) p_{\text{prot}} f(N_H)$ can attain the value of 0.3 and last an average of 30 days, for instance as an expression of a low-dose-stimulated immune response. If the average time span between cellular oncogenic transformation and tumor occurrence (also referred to as lag time) is 5 years (i.e., 1825 days), (Leenhouts, 1999), the time-corrected value of $(N_H/N_E) p_{\text{prot}} f(N_H)$, now denoted by $(N_H/N_E) p_{\text{prot}} f(N_H, t_p)$, becomes $0.3(30/1825) = 5 \cdot 10^{-3}$. With the preceding assumptions, this degree of protection would closely compensate for the value of $(N_H/N_E) p_{\text{ind}}$.

Indeed, the value of α only becomes constant at higher doses of low-LET radiation and consequently with higher numbers of microdose hits per number of micromasses exposed to this radiation, when the probability of adaptive protection is zero. It therefore appears likely that the value of α is not constant at the ranges of D in which protection operates. In addition to experimental results, some epidemiological data also support this reasoning (Pollycove and Feinendegen, 2001).

High-LET Microdoses

Concerning the situation with high-LET radiation, the corresponding relatively high mean microdose values, $\bar{z}_1$, for instance, 350 mGy for 4 MeV α -particles, may be ineffective with regard to $p_{\text{prot}}(D, t_p)$ in the hit cells. This

statement is based on the observation that adaptive protection except for apoptosis increasingly fails at doses above about 200 mGy. In fact, low-dose α -irradiation of C3H 10T1/2 cells in culture increased the rate of oncogenic transformation (Bettega *et al.*, 1992) rather than decreased it as was seen with low-LET low-dose irradiation (Azzam *et al.*, 1996; Redpath and Antoniono, 1998). However, p_{ind} , $p_{\text{ind}}p_{\text{enh}}$ and p_{spo} in exposed tissues may be offset by $p_{\text{prot}}(p_{\text{prot}}f(N_{\text{H}}, t_{\text{p}}))$ if protective mechanisms are initiated in nonhit cells through bystander effects and tissue-specific extracellular signaling. Indeed, bystander effects should be considered to operate between hit and nonhit cells to induce both damage and signaling for protection. The available data are scanty and mostly from tissue culture studies; in principle, the data do not contradict but rather support the occurrence of protective effects as well as damaging effects in nonirradiated cells that are neighbors of high-LET irradiated cells (Matsumoto *et al.*, 2001; Sawant *et al.*, 2001b; Lehnert and Iyer, 2002; Belyakov *et al.*, 2002). It needs to be seen to what degree bystander effects operate in either way—that is, to cause damage and/or protection—in tissues that are exposed to very low doses and dose rates of low- or high-LET radiation.

Microdoses at Low Dose Rate

With respect to low dose rates, the time interval between consecutive microdose hits per exposed micromass at a given radiation quality, as seen in Eq. (2), is obviously crucial in comparison with both the time required for acute DNA repair and the relatively longer time t_{p} over which the adaptive protection operates. If the value of t_{x} is large compared with t_{p} , Eq. (5) remains valid. If t_{x} becomes shorter than t_{p} , yet remains longer than the time of acute DNA repair, the effects of a microdose hit may interfere with the protective responses that are elicited by the preceding hit. If t_{x} becomes even shorter, the immediate DNA repair may be affected. Thus, Eq. (5) demands adjustment. Accordingly, a factor ($F(t_{\text{x}})$) may serve to correct for dose-rate-dependent interference with acute DNA repair and adaptive protection, as discussed elsewhere (Feinendegen and Graessle, 2002).

For example, the factor can be evaluated to correct for a relatively short t_{x} in case it reduces protection and thus increases risk, or when, by increasing protection, it may reduce risk, for instance, through repetitive protection or induction of apoptosis of predamaged cells. Increased protection also may cause increased risk, such as when a protected cell survives and transforms oncogenically. As stated earlier in the section on low-LET microdoses, the corresponding probabilities would be expressed by either p_{ind} or p_{enh} . Despite the complexities that are here involved in assessing tissue risk from a low dose-rate exposure to a given radiation quality, the present approach in principle allows experimental verification, new experimental avenues, and interpretations. Thus, as was presented in detail elsewhere (Feinendegen

and Graessle, 2002) and in line with Eq. (5),

$$N_q/N_E = [p_{\text{ind}}(1 + p_{\text{enh}}) - p_{\text{prot}}f(N_H, t_p)(p_{\text{spo}} + p_{\text{ind}} + p_{\text{ind}}p_{\text{enh}})\{F(t_x)\}] \times (N_H/N_E) \quad (8)$$

By substituting here the term D for N_H for a given radiation quality, and expressing D in microdosimetric terms at low-dose rate, as seen in Eq. (2),

$$N_q/N_E = [p_{\text{ind}}(1 + p_{\text{enh}}) - p_{\text{prot}}f[\bar{z}_1 t/t_x], t_p)(p_{\text{spo}} + p_{\text{ind}} + p_{\text{ind}}p_{\text{enh}}) \times \{F(t_x)\}](N_H/N_E) \quad (9)$$

This equation expressing risk from low-dose, low-dose-rate exposure is in line with a critical review of the literature on radiation-induced lung cancer from low-dose-rate exposure to a large total absorbed dose. This has led to the conclusion that protracted exposure to low-LET radiation to a total dose of less than 2 Gy does not appear to cause lung cancer; in contrast, evidence has been found for a reduction of natural incidence of lung cancer at such exposures (Rossi and Zaider, 1997).

As another example, a unique set of recent experiments addressed life shortening and appearance of thymic lymphoma in C57BL/6N and C3H/He mice that were on lifelong supply of tritiated water (Yamamoto *et al.*, 1995, 1998). Since 1 mGy whole-body dose from tritium beta particles corresponds to a mean microdose per exposed micromass of 1 mGy, (i.e., about 6 keV/ng), the listed dose rate can be easily converted, for example, to microdose hits per exposed micromass occurring at average time intervals of t_x . Life shortening and tumor development were only observed if the dose rate increased to beyond 1 mGy per day. This then amounts to repetitive microdose hits per exposed micromass, each with a mean microdose of about 1 mGy within an average t_x of less than 1 day. When the average t_x was longer than about 1 day, no tumors appeared and no life shortening was observed. On the other hand, a recent life-span study on specific-pathogen-free B6C3F1 mice used chronic exposure to ^{137}Cs γ -radiation at 0.05, 1.1, and 21 mGy per day beginning at 8 weeks for 400 days (Tanaka *et al.*, 2003). No lengthening of life span was found. However, significant life shortening only occurred when the dose rate was 21 mGy per day. It is not clear to what extent the mouse strain being specific pathogen free influenced this set of data.

Another analysis reviewed published data on the mutagenic effects of low-LET radiation at increasing dose rates beginning with 0.01 mGy per minute. This study first revealed a reduction of mutagenesis with increasing dose rates: the minimum was at the range of 1 to 10 mGy per minute in various mammalian cell lines in culture, and at the range of 0.1 to 1 mGy per minute in mouse spermatogonia. Mutagenesis then increased with higher dose rates (Vilenchik and Knudson, 2000). The authors attribute the observed

phenomenon of inverse dose-rate effect to an optimal induction of error-free DNA repair at the observed mutation locus in a dose-rate region of minimal mutability. The diminished activation of repair at the lowest dose rates was considered to reflect a low ratio of induced to spontaneous DNA damage, much of which stems from endogenous ROS. In fact, the results suggest a genetically programmed optimization of response to radiation in the minimal mutability dose-rate region, possibly depending on cell replication.

In the preceding experiments (Vilenchik and Knudson, 2000), the average time interval between consecutive microdose hits per exposed micromass is in the range of minutes in the dose-rate region of minimal mutability in spermatogonia. Thus, the data express a specific protective response that obviously keeps operating throughout repetitive hits with their relatively large, i.e., suprabasal) bursts of ROS and deserve further examination. These data also agree in principle with the observation that the induction of adaptive protection appears to depend on the dose rate of the conditioning dose in experiments where the protection is tested by repeated irradiation at higher doses (Shadley and Wienke, 1989; Broome *et al.*, 2002).

CONCLUSION

Low doses of low-LET radiation evoke a spectrum of biochemical and functional cell and tissue responses in various mammalian cells that express both damage and adaptive cell protection. The latter appears with a delay of up to several hours and may last for weeks. Except for apoptosis, adaptive protection is not observed at high doses. The protective responses vary according to species, cell type, cell metabolism, and cell cycle.

The probability of low-dose induced cancer arises from a balance between low-dose induced oncogenic transformation and various types of adaptive protection against oncogenic transformation and tumor development, whether induced by nonradiation toxins or by ionizing radiation.

To dissect the factors at a given radiation quality that contribute to low-dose-induced tissue effects including cancer, it appears necessary to pay attention to (1) the relationship between the absorbed dose to tissue and the corresponding energy deposition values and events from particle tracks in defined tissue micromasses (i.e., microdose values and numbers of microdose hits per number of exposed micromasses of the tissue); (2) the probabilities of various cellular responses per average microdose hit of a given radiation quality at increasing numbers of microdose hits per number of exposed micromasses; and (3) the contribution of all cellular response probabilities over all microdose hits in the exposed tissue to the generation of tissue effects including cancer. The analysis for the case of penetrating ionizing radiation, that is, of a stochastic distribution of microdose hits in the

exposed tissue micromasses at a given radiation quality, leads to the following conclusions:

1. Ionizing radiation causes DNA damage in the exposed tissue in proportion to the number of microdose hits per number of exposed micromasses, by both direct and indirect or “bystander” cell effects.
2. Acute low doses of low-LET radiation and thus low instantaneous numbers of microdose hits per number of exposed micromasses can initiate various categories of protective responses. Except for apoptosis, these increasingly fail and then disappear as the number of microdose hits per number of exposed micromasses rises to a level that is equivalent to a tissue dose of about 200 mGy. High microdoses at low tissue doses can initiate both damage and protective responses by direct and bystander cell effects.
3. The protective response categories involve
 - cellular defenses such as radical detoxification,
 - DNA repair involving various pathways,
 - cell removal by immune response, and
 - cell removal through intracellular signaling, such as by cell differentiation and apoptosis, which also occurs at high doses.
4. Non-radiation-induced DNA damage far outweighs radiation-induced damage at relatively low numbers of microdose hits per number of exposed micromasses depending on radiation quality, so that adaptive protection induced by these microdoses should operate mainly against non-radiation-induced DNA damage.
5. Cancer induction appears to be proportional to the degree of DNA damage; thus, radiation induced adaptive protection mainly against non-radiation-induced DNA damage may reduce the “spontaneous” cancer incidence. This finds support from experimental observations and some epidemiological data.
6. Regarding dose-rate, the spacing in time between consecutive microdose hits from a given radiation quality per number of exposed micromasses influences cellular responses. With increasing dose rates (i.e., decreasing time intervals between microdose hits per micromass), the incidence of DNA damage may first decrease, then rise, and eventually accumulate to a level sufficient to cause tissue failure.
7. In view of the limited power of epidemiological data on radiation-induced cancer and the clear broad evidence of low-dose-induced adaptive protection alongside with the relatively low probabilities of damage at low doses, the LNT hypothesis on radiation-induced cancer appears to be invalid. It needs further reexamination and replacement by a scientifically more rigorous model that includes both linear and nonlinear response probabilities.

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