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RADIATION INDUCED MEMBRANE CHANGES AND PROGRAMMED CELL DEATH: POSSIBLE INTERRELATIONSHIPS

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Abstract

A short review of the evidence that lymphocyte membranes are a target for the initiation of irradiation induced programmed cell death (PCD) is given. It is assumed that for lymphocytes PCD represents an essential physiological mechanism in order to prevent degeneration of the biological system involved. Initiation of PCD can be obtained by a pharmacological activation as well as with irradiation. In both cases, protein kinase-C (PKC) is involved in the signal transduction from the cellular membrane to the nucleus where, by means of a metabolically active process, DNA fragmentation is induced. It is hypothesized that processes connected to lipid peroxidation in the cell membrane constitute a primary effect of irradiation induced PCD, where membrane fluidization or a compensatory process aimed to the maintenance of membrane fluidity (membrane homeoviscosity hypothesis) are likely to be involved.

Key Words: X-irradiation, lymphocyte programmed cell death, surface IgG modulation, protein kinase C, protein kinase A.

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Introduction

For about 100 years ionizing radiation has been known to cause cell necrosis (burning), impairment of cell mitosis that leads to clonal truncation or depending on dose and target cell, strand breaks in the DNA which in some cases end up in cell mutations. In the last decade, another radiation effect has been investigated which finally results in a regular breakage of the cell DNA [7, 21, 24]. Since such DNA breakage can be inhibited by blockers of either protein or RNA biosynthesis [22, 23], this type of cell death appears to be a kind of active cell death, called programmed cell death (PCD) or apoptosis [23]. In opposition to necrosis, PCD may also be induced by chemical or biological cell activators [6, 8, 9] and it appears to represent an intrinsic cellular regulation mechanism for cell growth and differentiation.

PCD is easily induced in lymphocytes [2, 6], thus playing an important role in building a repertoire in the immune system, especially in the thymus. This explains the fact that PCD in lymphoid cells has attracted so much attention in the last years. Since it is possible to trigger programmed cell death at cell membrane level in lymphocytes and as irradiation induced programmed cell death is similar to the former, both resulting in DNA fragmentation, we propose that the radiation induced PCD also starts at the membrane level.

Our working hypothesis on the interrelationships of radiation induced membrane changes and PCD in lymphocytes (Fig. 1) assumes that radiation induced membrane lipid radical production leads to lipid peroxidation. The lipid peroxidation itself induces membrane fluidization, and subsequently a membrane bound reaction takes place in order to restore the physiological membrane fluidity, causing an imbalance of the second messenger systems. This disturbance initiates a chain reaction that ends up in an activation signal for endonucleases which leads to the characteristic end point of PCD. For lymphocytes at least, teleologically speaking, a cell activation without clear instruction for cell growth or differentiation would preferentially lead to cell death preventing a cell degeneration or a system adverse activity [3].

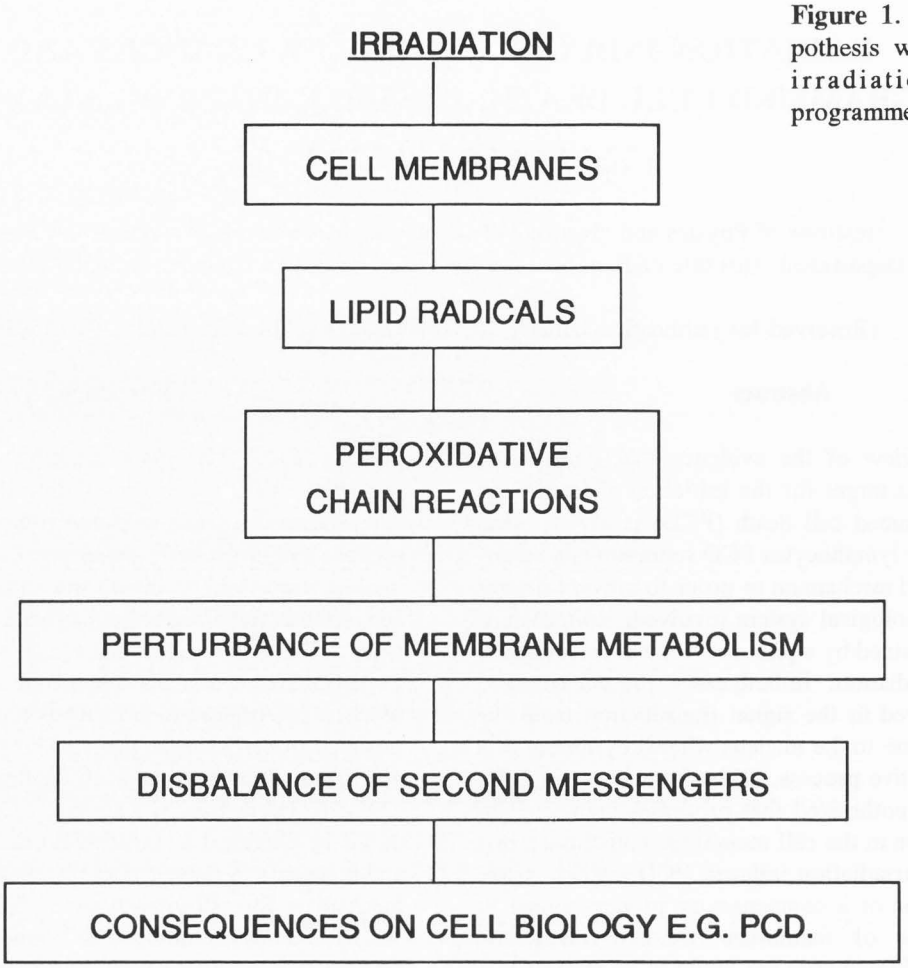

Figure 1. Working hypothesis with regards to irradiation induced programmed cell death.

Results and Discussion

Irradiation effects at isolated membrane level

The classical work of Petkau and Chelak [18] demonstrates that in lipidic preparations, ionizing radiation produces and intense lipid peroxidation. Konings [11] showed that liposomal lipid peroxidation occurs together with a decrease in fluidity. Both effects are dose proportional and they are inhibited by the radical scavenger α-tocopherol. These facts suggest the participation of a radical induced lipid peroxidation mechanism. It is characterized by a self-quenching effect which increases with dose rates, since radicals combine with each other to form a non-radical non-reactive lipid peroxidation product. Therefore, an inverse dose-rate effect should be expected for the membrane effects produced by irradiation induced lipid peroxidation.

When we investigated the radiation effect on microsomes, we found however, that at low doses of irradiation the lipid peroxidation appears together with an increase of membrane fluidity. This indicates that proteins interact with lipids, at least at low doses of X-rays and ultraviolet light in the wavelength range 200-280 nm (UV-C), so that the bulk lipid peroxidation itself occurs as in liposomes, but the fluidization change seen in the whole membrane is determined by the membrane proteins (Fig. 2) [12]. Microsomes represent a protein rich natural membrane, in which gradients of membrane fluidity exist [4].

Irradiation effects on membranes of intact cells

Lymphocytes are known to be very radiosensitive cells. We investigated the effect of irradiation on the IgG membrane expression of lymphocytes obtained from mouse lymph nodes [16]. As can be seen in Figure 3, irradiated lymphocytes show a clear dose dependent loss of Ig expression. In order to find out if the membrane is also the primary target here, we studied the dose rate dependency of the observed effects. Figure 3 shows that the irradiation efficiency increases with decreasing dose rate. These observations led us to the hypothesis that, again, in this experimental situation a chain of radical reactions connected to membrane lipid peroxidation is the bottle-neck in the modification of s-IgG receptor expression in irradiated lymphocytes. However, even though the membrane is the primary target of irradiation

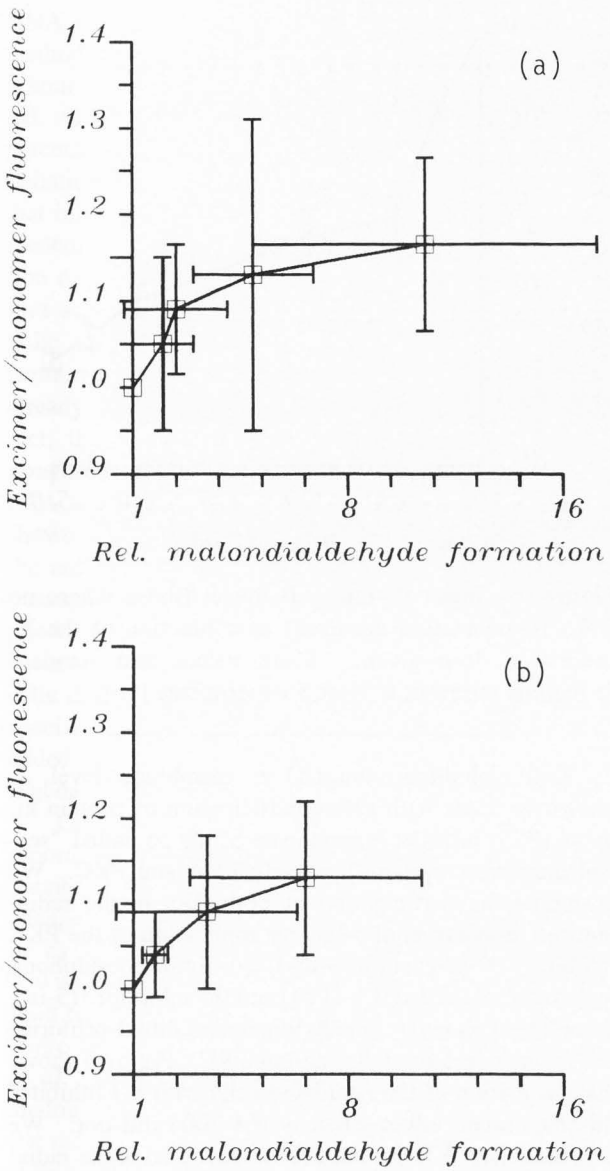

Figure 2. Microsomal membrane fluidity measured as the pyrene excimer to monomer ratio, normalized to un-irradiated samples plotted as function of lipid peroxidation (measured as malondialdehyde formation) and also normalized to un-irradiated samples. Microsomal protein concentration 0.11 mg ml. Each point represents one dose. (**a**) X-irradiation, dose rate 0.67 Gy min^{-1}, doses (from left to right): 0, 13.3, 28, 69.3, and 120 Gy. (**b**) UV-C irradiation exposure rate 1.62 J cm^{-2} min^{-1}, exposures (from left to right): 0, 0.23, 0.49, 0.73 J cm^{-2} [12].

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in the induction of this phenomenon, the participation of cytoplasmic reactions in the s-IgG modulation must also be considered. Figure 4 shows that almost no s-IgG modulation appears when blockers of the cellular energy metabolism like 2,4-dinitrophenol are added to the cells

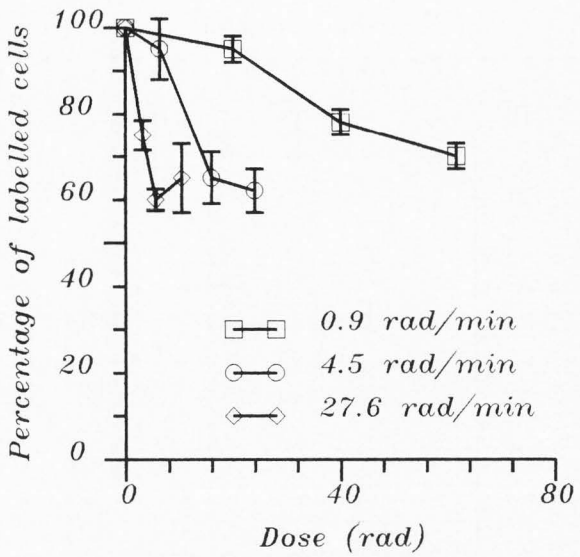

Figure 3. Lymphocyte s-IgG expression measured as fluorescence from FITC-labeled anti IgG bound to lymphocytes as a function of radiation dose. Results are referred to the non-irradiated control values. Parameter: Dose rate (1 rad = 1 cGy). Cells were incubated for 10 minutes at 37°C. Mean values and standard deviations are from 3-7 experiments. Control assays (non-irradiated) contained 19.7 ± 1.5% labeled cells [17].

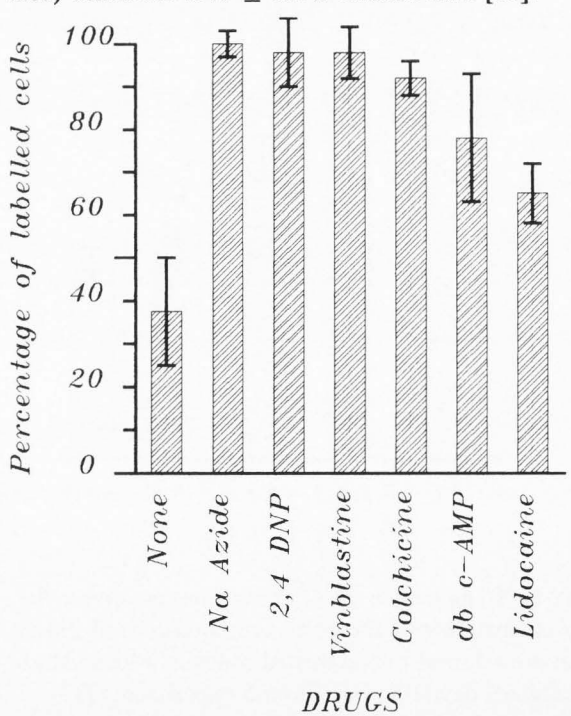

Figure 4. Lymphocyte s-IgG expression under X-irradiation of 38 cGy for 10 minutes at 37°C and incubated with the drugs named. Results are referred to the non-irradiated controls. Mean values and standard deviations are from at least 3 experiments [5].

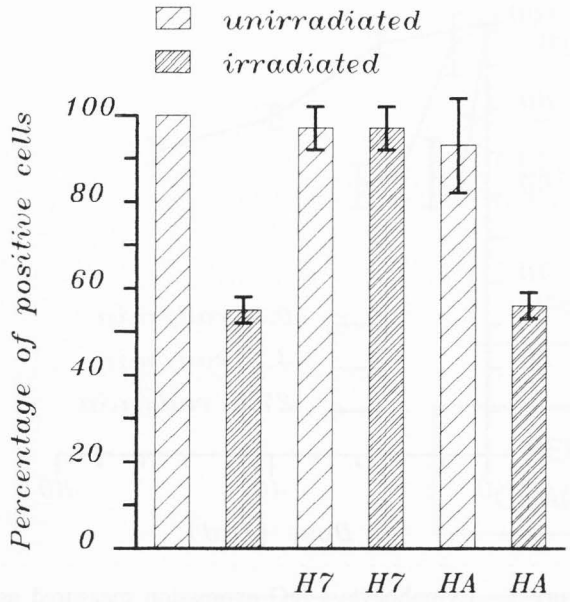

Figure 5. Lymphocyte s-IgG expression (positive cells) under X-irradiation of 25.5 cGy, controls, and in the presence of H-7 (50 mM) or HA-1004 (50 mM). Values are referred to the non-irradiated controls. Mean values and standard deviations are from 4 or 5 experiments [13].

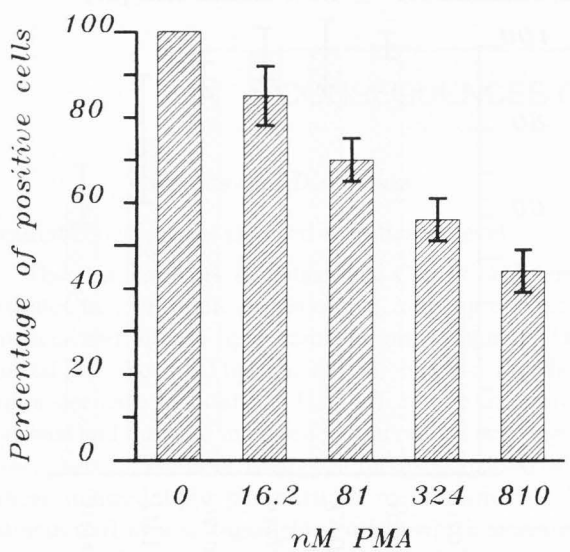

Figure 6. Lymphocyte s-IgG expression (positive cells) under incubation with the given concentrations of PMA. Values are referred to non treated controls. Mean values and standard deviations are from 5 experiments [13].

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during irradiation [5]. Therefore, it is clear that different cell compartments participate in radioinduced changes of the membrane and that signalling between these compartments must occur.

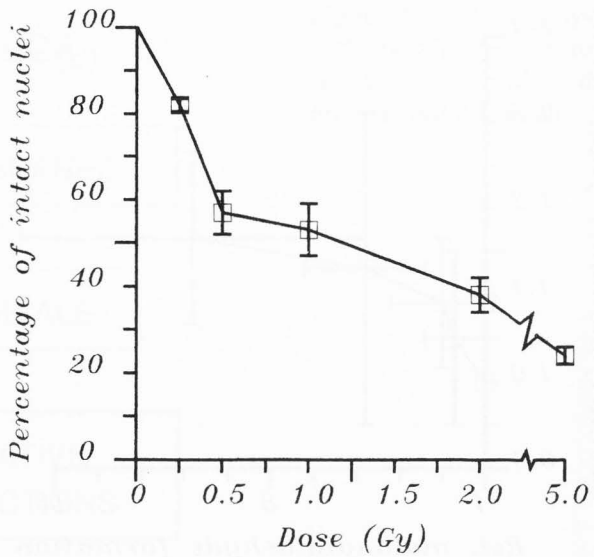

Figure 7. Intact thymus cell nuclei (those where no DNA fragmentation occurred) as a function of the X-irradiation dose given. Mean values and standard deviations are from at least 5 experiments [15].

Cell signalling, assigned at membrane level, is known to occur with active participation of protein kinases (PK), intrinsic components of the so called "second messenger systems", namely PKA and PKC. We examined the participation of both PKs in the radioinduced modulation of s-IgG by application of the PKA inhibitor N-(2-guanidinoethyl)-5-isoquinilinesulphonamide hydrochloride (HA-1004) and the inhibitor 1(5-isoquinolinesulphonyl)-2-methylpiperazine dihydrochloride (H7) which preferentially inhibits PKC. Figure 5 shows that incubation of the irradiated cells with H7 inhibited this membrane effect whereas HA-1004 did not. We conclude that PKC but not PKA is involved in the radiation induced membrane effect in lymphocytes. The radiation effect can be mimicked by chemically activating the PKC with phorbol myristate acetate (PMA). Figure 6 shows that PMA effectively induces a dose dependent loss of s-IgG positive cells, which clearly indicates the involvement of PKC in this phenomenon [13].

Radiation induced programmed cell death

In lymphocytes, some hours after irradiation the interphase death is known to occur, long after the radiation induced membrane effects have taken place. This special way of dying, mainly seen in lymphoid cells, has many features similar to PCD and for low irradiation doses it seems to be the final manifestation of the same process.

In our laboratory, we measured the extent of PCD by a flowcytometrical counting of the content of DNA remaining in the nuclei after allowing the fragmented

DNA to diffuse out from the nuclei [14]. With this method, the percentage of intact nuclei of thymocytes, 8 hours after irradiation with different doses, was studied. The results presented in Figure 7 show that the percentage of intact nuclei decreases with increasing irradiation dose, a fact that is in line with the assumption that interphase death is actually PCD. The DNA fragmentation may be either a consequence of direct irradiation damage on the nuclear chromatin, or a more complex active phenomenon with participation of the metabolic cell machinery, which may originate from the membrane level in a similar way to the other processes already described. Should the second alternative be correct, then PKC is likely to be involved. To test this possibility, irradiated cells were exposed again to the PKA and PKC inhibitors HA-1004 and H7. Figure 8 shows that inhibition of PKC results in an inhibition of the radiation induced DNA fragmentation. DNA fragmentation can be induced by the activation of PKC with phorboldibutyrate [15] (Fig. 9). In this process, PKA seems not to be involved since HA-1004 added to the cells does not interfere with the phenomenon, and the specific PKA activator 5,6 sp-cAMPS (a c-AMP agonist) added to normal cells is not able to induce the effect (Ojeda *et al.*, in preparation).

On the other hand, Ormerod *et al.* [17] recently reported the association of programmed cell death with membrane modifications as measured by permeability changes towards fluorescein and some of its analogues, or by determining the binding ability of merocyanine 450 which reveals an augmentation of the disorder of the lipid bilayer of the membrane of those cells which undergo PCD. In line with these findings, Ramakrishnan *et al.* [20] recently reported that Trolox, a vitamin E analog which is known to protect membranes, inhibits the radiation induced PCD in thymocytes. All these results speak in favor of the membrane participation in the first steps of the chain of events leading to PCD. However, against this general conclusion, the results of Afanasyef *et al.* [1] demonstrate, using ethidium bromide, that permeability changes in the membrane start 6 to 8 hours after irradiation, whilst DNA fragmentation had already occurred. This discrepancy may be explained due to the high dependency of the results from the method and probe used, when permeability is evaluated.

In addition, Posokhov *et al.* [19] reported that in thymocytes under cholesterol enrichment, the radiation-induced lipid peroxidation as well as the enzymatic chromatin degradation and PCD are inhibited. Moreover, the results of Konings [10, 11], who demonstrated the inverse dose rate effect with respect to hemoglobin and K^+ release from erythrocytes and the interphase death in bovine lymphocytes, support to assume the participation of membranes as primary target for irradiation.

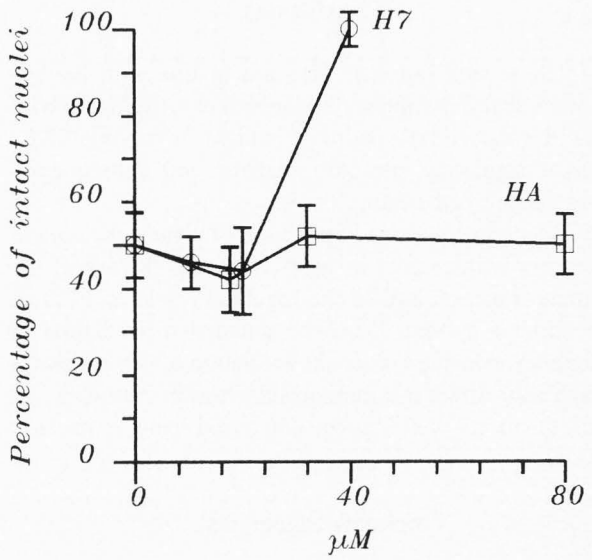

Figure 8. Intact thymus cell nuclei after X-irradiation of 100 cGy and incubated for 6 hours with the given concentrations of H-7 or Ha-1004. Mean values and standard deviations are from at least 5 experiments [15].

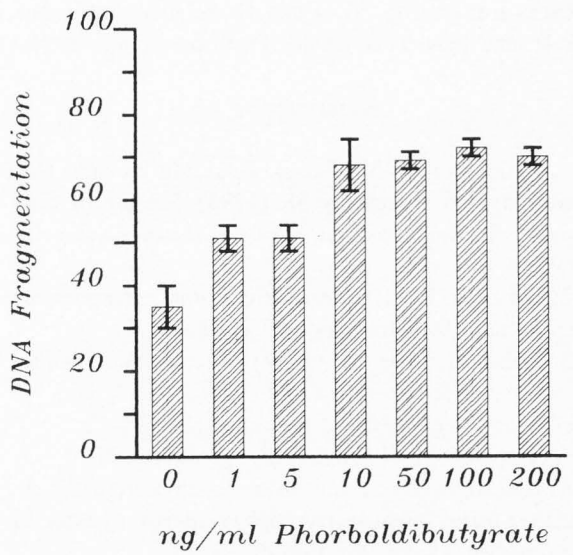

Figure 9. Thymus cell nuclei DNA fragmentation following incubation with phorboldibutyrate for 6 hours at 37°C. Mean values and standard deviations from 3 experiments [15].

Finally, we think that there is much evidence to postulate that lymphocyte membranes are the essential target for the initiation of processes in which lipid membrane peroxidation, PKC and cell metabolic processes are involved, and which end up with the radiation induced programmed cell death.

Conclusions

The results reported here are in line with the hypothesis that in lymphocyte membranes, irradiation triggers a biochemical pathway which, involving PKC, causes membrane receptor changes and subsequently programmed cell death.

Due to the extreme importance of plasma membrane receptors with respect to many central pathways of the immune function and to the regulatory role of PCD in the immune system, it can be stated that irradiation in lymphocytes could induce the activation of cell pathways which may affect the immune function in mammals, in addition to its well known deleterious and mutagenic effects.

Acknowledgements

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Discussion with Reviewers

Z. Somosy: In my opinion the proposed mechanism of apoptotic cell death is incomplete. As suggested by some authors [Zhivotovsky *et al.* (1993) Exp Cell Res **207**, 163; Somosy *et al.* (1993) Scanning Microsc **7**, 961; Story *et al.* (1992) Int J Radiat Biol **61**, 243; Baffy *et al.* (1993) J Biol Chem **268**, 6511; Ramakrishnan *et al.* (text ref. 20); Sando *et al.* (1992) Cell Signaling **4**, 595; Hallahan *et al.* (1992) Radiat Res **129**, 345; Hallahan *et al.* (1992) Cancer Res **51**, 4565] the changes in cellular calcium homeostasis play an essential role in the apoptotic process via regulation of nuclear and membrane bound enzyme(s) and signal transduction system(s), as well as in the effect of radiation on these systems and enzymes. Therefore I suggest to include the role and importance of calcium in the authors' proposed scheme.

Authors: We agree that calcium appears to be involved in radiation-induced apoptosis of thymocytes, but this does not appear to be a general rule for lymphocytes since in splenocytes, Zhivotovsky *et al.* [26] did not find an increase of Ca, following irradiation, in contrast with the clearcut increase in thymocytes. On the other hand, Ca rise does not appear before 1 to 3 hours following irradiation [20, 26] in contrast to the very fast calcium rise typically observed in calcium mobilization after cell stimulation. Moreover, in the work of Ramakrishnan *et al.* [20], the membrane protector Trolox prevents apoptosis when administered until 1 hour after irradiation. Two hours after irradiation, Trolox has no effect. Under the same conditions, they found the first calcium rise at 3 hours after irradiation. Under these conditions, it is difficult to use the calcium involvement in apoptosis as a support of membrane participation.

T.M. Seed: Inverse dose-rate effects have been reported in other (non-apoptotic) types of biological responses under different types of biologic stresses [e.g., cell transformation with high linear energy transfer (LET) particles]. Would the authors speculate whether these responses, like apoptosis, might well reflect a generic "initializing" event(s) by ionizing radiations and the subsequently induced peroxidation an increased fluidity of lipidic regions of the targeted plasma membrane?

Authors: In the case of low doses of high LET radiation, mainly single hits per cell are expected, thus, if a dose rate effect is observed this cannot be interpreted in terms of a decreased self quenching of radical chain reactions by free radicals. For that reason, in the case of high LET radiation, the dose rate effect gives no support to assume whether or not membranes are participating in the radiation effect. We think that such effects must be interpreted in terms of intercellular "cross-talk", which is of essential importance to be considered in the effect of irradiation on whole organs or whole body. Since the immune system essentially comes in at this point, and since this system is highly membrane dependent due to the intercellular signal transmission, we think it is worthwhile to pay additional attention to the possible membrane effects of irradiation. In this context, recent experiments of irradiation of the salivary gland have been reported [25], where an inverse dose-rate effect gives support to the importance of membranes as primary target structures.

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