Long-lived inflammatory signaling in irradiated bone marrow is genome dependent

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Running title: Genotype-dependent inflammatory radiation signaling

Key words: ionizing-radiation, chromosomal instability, bystander-effects, bone marrow, inflammation

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Abstract

Ionizing radiation is carcinogenic but genotype is a key determinant of susceptibility. Mutational DNA damage is generally attributed to cause disease, but irradiation also affects multicellular interactions as a result of poorly understood bystander effects that may influence carcinogenic susceptibility. In this study, we demonstrate that the bone marrow of irradiated mice will retain the ability to kill hemopoietic clonogenic stem cells and to induce chromosomal instability for up to 3 months after irradiation. Chromosomal instability was induced in bone marrow cells derived from CBA/Ca mice, a strain that is susceptible to radiation-induced acute myeloid leukemia (r-AML), but not in C57BL6 mice which are resistant to r-AML. Similarly, clonogenic cell lethality was exhibited in CBA/Ca mice but not C57BL6 mice. Mechanistic investigations revealed that these genotype-dependent effects involved cytokine-mediated signaling and were mediated by a cyclooxygenase-2-dependent mechanism. Thus, our results suggested that inflammatory processes were responsible for mediating and sustaining the durable effects of ionizing radiation observed on bone marrow cells. Since most exposures to ionizing radiation are directed to only part of the body, our findings imply that genotype-directed tissue responses may be important determinants of understanding the specific consequence of radiation exposure in different individuals.
**Introduction**

The risk of an individual developing a malignant disease after exposure to ionizing radiation is currently estimated by extrapolation of epidemiological data correlating malignancy with exposure dose (1). Estimates of risk derived from these data assume the radiation response is identical in all exposed individuals. However, for any given level of exposure, it is evident that only a proportion of exposed individuals will develop a malignancy and inherited cancer susceptibility and resistance genes together with genetic influences on various tissue microenvironmental and immunological factors contribute to an individual’s susceptibility (2-6). The major adverse consequences of radiation exposures, including initiation of malignancy, are conventionally attributed to DNA damage in irradiated cells induced at the time of exposure that has not been correctly restored by metabolic repair processes. Such damage may be regarded as a targeted effect. However, a number of studies have demonstrated damaging effects in non-irradiated cells as a consequence of communication between irradiated and non-irradiated cells and indicate additional mechanisms. The findings, generally termed non-targeted effects, are of two broad categories: effects in the unirradiated descendants of irradiated cells, collectively regarded as radiation-induced genomic instability and effects in unirradiated cells that have received signals produced by neighbouring irradiated cells, collectively regarded as radiation-induced bystander effects (7-14).

Because the responses of the hemopoietic system are major determinants of outcome following exposure to ionizing radiation we are conducting studies to compare radiation responses in CBA/Ca mice and C57BL/6 mice that are, respectively, susceptible or
resistant to the development of radiation-induced acute myeloid leukemia (15). In the present study, we have shown that up to 3 months after radiation exposure there are ongoing signaling processes where the bone marrow of irradiated C57BL/6 mice secrete factors that are toxic to C57BL/6, but not CBA/Ca, hemopoietic clonogenic cells (demonstrated as reduction in colony-forming efficiency). Additionally, the bone marrow of irradiated CBA/Ca mice secretes factors able to induce chromosomal instability (demonstrated as non-clonal cytogenetic aberrations) in CBA/Ca, but not C57BL/6 bone marrow cells. These long-lived effects result from responses to pro-inflammatory cytokine signaling. The findings provide evidence that radiation-induced non-targeted effects in hemopoietic cells are genotype-dependent and associated with the microenvironment contributing ongoing secondary cell damage as a consequence of pro-apoptotic and pro-inflammatory cytokine signaling in response to the initial radiation damage.

Materials and Methods

Irradiation. CBA/Ca and C57BL/6 mice were bred in-house under conventional conditions. Suspensions of femoral bone marrow were obtained from 8-16-week-old male mice that had been \(\gamma\)-irradiated (or sham-irradiated) at a dose rate of 0.4Gy/min using a CIS Bio International 637 Cesium irradiator to a total dose of 4Gy; a potentially leukaemogenic dose for CBA strains of mice (16). Experiments were approved by local ethical review and carried out in compliance with Home Office Project Licences PPL 60/2841 and 60/3564.
Clonogenic assays: Bone marrow cells were obtained from the femora of 5 normal mice and the clonogenic CFU-A assay was used to obtain clones of cells derived from members of the hemopoietic stem cell compartment. For each experiment, 1.5 x 10^4 cells were plated in each of nine 45mm culture dishes containing 2 ml growth medium supplemented with colony-stimulating activities and 0.3% low melting point agarose and incubated at 37°C in a humidified atmosphere of 5% CO₂ in air. Colony-forming efficiency was determined by the number of colonies generated at 10-11 days of incubation. Cytogenetic preparations were obtained from individual CFU-A-derived colonies 7–9 days (~10-13 cell divisions) after initiation of culture using a previously published method for karyotyping hemopoietic colonies. For each experiment, individual colonies from 9 replicate culture dishes were examined and ten colonies containing 5 or more well-spread metaphases with no overlapping chromosomes were analysed for cytogenetic aberrations. Typically in an individual experiment, 5-7 colonies met this criterion and pooled data was obtained from up to 5 replicate experiments.

Co-culture and media transfer assays (Figure 1). Cell suspensions of bone marrow were obtained from 5 normal or 5 irradiated mice for co-culture or media transfer experiments. For media transfer experiments the irradiated cells were centrifuged and filtered through a 0.45µm membrane to obtain irradiated bone marrow medium (IBMM) that would contain factors secreted by the marrow and/or sequestered in the extracellular matrix of the marrow cells. Because we were interested in signals generated in vivo, using the in vitro system only as a means of assaying such signals, experiments were conducted to investigate effects of cell-free medium obtained directly from post-
irradiation bone marrow, *i.e.* the cells were not incubated *in vitro* prior to testing.

Accordingly the terminology bone marrow medium rather than bone marrow conditioned medium has been used; *i.e.* CBMM or IBMM for control or irradiated bone marrow medium, respectively. Responder bone marrow cells obtained from non-irradiated mice were exposed to such medium or co-cultured with irradiated producer bone marrow cells and the colony-forming efficiency of CFU-A determined. The protocol is based on one previously used successfully for studying hemopoietic regulatory molecules produced *in vivo* (19). Cells or cell-free media were added to the same volume of medium containing unirradiated bone marrow cells based on cell ratios *e.g.* cells or medium from a cell suspension of $5 \times 10^6$ cell/ml added to cells suspended at $5 \times 10^6$ cells /ml to produce a final concentration of $2.5 \times 10^6$/ml assay cells is a 1:1 concentration. From this mixture, $60\mu$l ($1.5 \times 10^5$ cells) was added to 20 ml of supplemented growth medium and 2ml of the resultant mixture ($1.5 \times 10^4$ cells) added to each of nine 45mm culture dishes.

In the case of co-culture, mixing normal and irradiated cells, the dose of irradiation was sufficient to significantly reduce the CFE such that any irradiated survivors would make negligible contribution (<1 colony per 100 colonies) to overall colony-formation. Using the media transfer method, bone marrow cells obtained from non-irradiated mice were exposed to medium obtained from mice irradiated 3 months previously or from age-matched controls. Co-culture was not an option for these time points as, unlike the situation immediately post-irradiation, 3 months post-exposure the bone marrow cells obtained from irradiated mice were fully viable. Data from ‘n’ replicate experiments were decoded, pooled, tested for normality using the Shapiro-Wilk Test and differences
between control and experimental groups analyzed by the Fisher’s exact test for
cytogenetics and the Student t test for other endpoints.

**Signaling studies.** Antibodies (R &D Systems) directed against TNF-α (0 - 0.5 \( \mu \)g/ml final concentration) or FasL (0 - 5 \( \mu \)g/ml final concentration) or a scavenger of reactive oxygen species or nitric oxide, di-methyl sulphoxide (DMSO, 0-0.1%) and 2-(4-carboxyphenyl)-4,4,5,5- tetramethyylimidazole-1-oxyl-3-oxide (c-PTIO, 0-50\( \mu \)M) (Sigma), respectively, were added to the clonogenic cultures such that any changes, relative to controls, could be attributed to inhibition of specific molecules. Similarly, small molecule inhibitors (Calbiochem, UK) of mitogen activated protein (MAP) kinase signalling SB-203580 (p38 MAP kinase inhibitor, 0 - 10 \( \mu \)M final concentration), PD-98059 (MAP kinase kinase 1 / MEK1 inhibitor, 0 -0.5\( \mu \)M final concentration), the stress-activated protein kinase SP-600125 (c-Jun N-terminal kinase / JNK inhibitor 0 - 5 \( \mu \)M final concentration) and of cyclooxygenase-2 / COX-2 (NS-398, Cayman Chemical UK, 0 - 10 \( \mu \)M final concentration) were used to investigate signal transduction pathways.

Toxicity studies for each inhibitor/blocking agent were performed using the CFU-A assay and non-toxic concentrations producing significant effects are shown in the Results section. For each experiment, 60\( \mu \)l (1.5 x 10^5 cells from the 1:1 mixture of assay cells plus irradiated cells) was added to 20 ml of supplemented growth medium which included the antibody or inhibitor) and 2ml of the resultant mixture (1.5 x 10^4 cells) added to each of nine 45mm culture dishes.
Results

**Radiation-induced bystander-mediated reduction in colony-forming efficiency**

Within the first hour post-irradiation there are cells present in the bone marrow of irradiated C57BL/6 mice that in a co-culture situation reduce the colony forming efficiency (CFE) of primary unirradiated clonogenic C57BL/6 cells by 12% (p<10^{-4}, n = 20) (Table 1). Using cells from CBA/Ca mice the reduction is 4% and not significant (p=0.2827, n = 20). To determine whether signals that reduce CFE are present late after radiation exposure, bone marrow medium prepared from mice 3 months post-irradiation (IBMM) was added to non-irradiated bone marrow and colony forming efficiency determined. As shown in Table 2, the CFE of C57BL/6 bone marrow exposed to C57BL/6 IBMM was significantly reduced by 22% relative to the control (p = 0.0010 , n =7) unlike the 2 % reduction in CFE of CBA/Ca bone marrow exposed to CBA/Ca IBMM which was not significant (p =0.4361, n = 7). Because of the genotype-dependent difference at both time points, we investigated the potential for irradiated cells from one genotype to reduce the CFE of bone marrow obtained from the other genotype. The CFE of C57BL/6 bone marrow exposed to CBA/Ca IBMM prepared from bone marrow 3 months post-irradiation was not significantly reduced relative to control (3%, p = 0.8687, n = 7) and the CFE of CBA/Ca bone marrow exposed to 3 month C57BL/6 IBMM was also not significantly reduced (2%, p = 0.7541, n = 7).

**Signalling processes mediating reduction in colony-forming efficiency**

TNF-α and FasL were shown to be implicated in the mechanism underlying the reduction in CFE by the addition of neutralizing antibodies to co-cultures of cells obtained within one hour of irradiation with unirradiated cells (Figure 2). Addition of anti-TNF-α or...
anti-FasL resulted in CFE values indistinguishable from those of the control cocultures in the presence of antibody and the reduction in CFE was also reduced using small molecule inhibitors (SB-203589 or PD-98059) of the MAP Kinase pathway and of the stress-activated kinase pathway (SP-600125). Involvement of reactive oxygen/nitrogen species was shown by treatment with DMSO or c-PTIO (respectively, a scavenger of reactive oxygen species and nitric oxide) also reducing the reduction in colony formation. In antibody studies conducted using IBMM obtained 3 months post-irradiation, TNF-α and FasL were confirmed as contributing to the long-lived mechanism underlying the reduction in CFE (Figure 3). As all the data were consistent with pro-inflammatory responses mediated through NFκB, ERK/MAPK and JNK/SAPK pathways, all of which converge on cyclooxygenase 2 (COX 2), which plays important roles in modulating inflammatory processes we investigated the effect of a non-cytotoxic concentration of NS-398, a specific inhibitor of COX-2 activity. Using both co-culture (Figure 2) and media transfer (Figure 3) protocols, addition of NS-398 restored the CFE to values not significantly different from those of control values.

**Radiation-induced bystander-mediated chromosomal instability**

The proportion of cells with non-clonal aberrations, characteristic of chromosomal instability, in CFU-A-derived colonies initiated from normal CBA/Ca bone marrow exposed to IBMM obtained from CBA/Ca mice within one hour of radiation exposure (Table 3) was significantly increased relative to colonies derived from CBA/Ca bone marrow exposed to control bone marrow medium (CBMM), *i.e.* obtained from the bone marrow of non-irradiated CBA/Ca mice (18.5% *versus* 9.2%; *p* = 0.0385). Using cells
obtained from C57BL/6 mice, the proportion of cells exhibiting instability in response to C57BL/6 IBMM was not significantly increased relative to that in colonies derived from C57BL/6 bone marrow exposed to CBMM (10.8% versus 9.8%; p = 0.4825). Thus, the chromosomal instability was expressed in colonies initiated from CBA/Ca bone marrow exposed to CBA/Ca IBMM but not in colonies initiated from C57BL/6 bone marrow exposed to C57BL/6 IBMM. As shown in Table 3, the proportion of cells expressing instability in colonies initiated from CBA/Ca bone marrow exposed to C57BL/6 IBMM (8.6%) was not greater than that in colonies initiated from CBA/Ca bone marrow exposed to CBA/Ca CBMM (9.2%, p = 0.6528) and the proportion of cells expressing instability in colonies initiated from C57BL/6 bone marrow exposed to CBA/Ca IBMM (9.7) was not greater than that in colonies initiated from C57BL/6 bone marrow exposed to C57BL/6 CBMM (9.8%, p = 0.5593). As signals present in the bone marrow of irradiated C57BL/6 mice significantly reduced the colony-forming efficiency of primary unirradiated bone marrow via a COX-2-dependent mechanism (Figures 2 and 3) the role of COX-2 in mediating the instability phenotype was investigated by use of the specific inhibitor NS-398 (Table 3). The proportion of cells with non-clonal aberrations in colonies initiated from CBA/Ca bone marrow exposed to CBA/Ca IBMM plus NS-398 (7.8%) was not greater than that in colonies initiated from CBA/Ca bone marrow exposed to CBA/Ca CBMM (9.2%, p = 0.8118) and significantly less than the proportion of cells with non-clonal aberrations in colonies initiated from CBA/Ca bone marrow exposed to CBA/Ca IBMM (18.5%, p = 0.0248). When the time to obtaining the IBMM was increased to 100 days post-irradiation (Table 4), the proportion of cells expressing instability in colonies initiated from normal CBA/Ca bone marrow exposed to such
IBMM was also significantly increased relative to that in colonies derived from CBA/Ca bone marrow exposed to age-matched control bone marrow medium (CBMM), *i.e.* obtained from the bone marrow of non-irradiated CBA/Ca mice (13.1% *versus* 6.9 2%; *p* = 0.0173). The effect of addition of neutralizing antibodies directed against TNF-α or FasL to IBMM (8.2% and 6.8%, respectively) produced effects that were not significantly different from those of CBMM plus antibody (7.3% and 7.7%, *p* = 0.3067 and 0.2974 respectively) and significantly reduced relative to IBMM alone (13.1%, *p* = 0.03115 and 0.0322, respectively). The proportion of cells expressing instability in colonies initiated from CBA/Ca bone marrow exposed to CBA/Ca IBMM plus NS-398 (7.7%) was not greater than that in colonies initiated from CBA/Ca bone marrow exposed to CBA/Ca CBMM (8.5%, *p* = 0.3209) and significantly less than that in colonies initiated from CBA/Ca bone marrow exposed to CB/Ca IBMM (13.1%, *p* = 0.0241).

**Discussion**

Radiogenic malignancy emerges in the context of host genetics and physiology as a result of a complex interplay between targeted and non-targeted radiation effects and this study was designed to investigate the potential of bone marrow exposed to radiation *in vivo* to produce factors able to induce non-targeted effects, demonstrated as a reduction in colony-forming efficiency (a clonogenic cell death endpoint) or as non-clonal cytogenetic aberrations (a characteristic of the chromosomal instability phenotype). The bone marrow cells studied were obtained from CBA/Ca and C57BL/6 mice, strains that are susceptible and resistant to radiation-induced acute myeloid leukaemia, respectively (16). It is evident that induction of the non-targeted effects is strongly genotype-dependent and
reflects a long-lived microenvironmental change after the radiation exposure. C57BL/6 bone marrow, but not CBA/Ca bone marrow was characterized by clonogenic cell death (Tables 1 and 2) and the reductions in CFE are comparable to reported radiation-induced bystander effects (1). The lethality is significant and equivalent to the effect of ~0.3-0.6 Gy direct irradiation on the survival of bone marrow clonogenic cells (17). A cross-genetics experiment, using cells obtained 3 months post-irradiation (Table 2) demonstrated that both producer and responder need to be of the ‘susceptible’ C57BL/6 genotype to express the CFE reduction phenotype. Although there was no evidence of a significant CFE reduction using CBA/Ca bone marrow, CBA/Ca cells clearly demonstrated a chromosomal instability phenotype after exposure of clonogenic cells to IBMM prepared within one hour of irradiation (Table 3). Chromosomal instability was not exhibited in colonies initiated from C57BL/6 bone marrow exposed to signals produced by irradiated CBA/Ca mice or in CBA/Ca bone marrow exposed to signals produced by irradiated C57BL/6 mice. Thus, CBA/Ca-type, but not C57BL/6-type, signalling and response allows expression of this instability phenotype. Previously, we demonstrated that in response to the same whole body dose of radiation there is a greater incidence of apoptotic cells at 6 hours post-exposure correlating with a greater reduction in tissue cellularity at 24h post-exposure in C57BL/6 than in CBA/Ca hemopoietic cells. This could be attributed to genetic differences in the sectoring between growth arrest and apoptotic pathways (20). Thus, in the current study, the greater bystander-induced CFE reduction in the C57BL/6 genotype may be reflecting the genetic background that produces the more effective apoptotic response to genotoxic insults. This more effective apoptotic response would result in a more effective elimination of unstable and
potentially malignant cells and may contribute to the genotype dependency of the relative susceptibility (CBA/Ca) and resistance (C57BL/6) to expressing a radiation-induced chromosomal instability phenotype. However, the cross-genetics study revealed that although the genotype of the responder cells was an important determinant of outcome, both producer and responder cells need to be of the same genotype to elicit either the CFE reduction (C57BL/6) or the instability phenotype (CBA/Ca).

The mechanism underlying both the bystander-mediated reduction in CFE and the chromosomal instability phenotype can be attributed to a complex signaling process (Figs 2, 3 and Table 3) including the activities of pro-apoptotic /pro-inflammatory cytokines FasL and TNF-α. These cytokines are ligands for the Fas (CD95/Apo) and TNFR1 receptors, respectively, both belonging to the Tumor Necrosis Factor (TNF) superfamily of receptors involved in proliferation, differentiation and apoptosis (21, 22). FasL is an important mediator of apoptotic cell death but can also trigger a non-apoptotic caspase-independent form of cell death (23) and has non-apoptotic functions in activating the NF-κβ pathway and promoting inflammatory processes (22, 24). TNF-α also has important pro-inflammatory properties including the ability to induce DNA strand breaks at concentrations that are not acutely toxic (25). TNF-α signaling can alter the redox status of cells and induce nitric oxide production with the downstream products of NF-κβ-mediated signaling contributing to TNF-α cytotoxicity (26). Thus, involvement of reactive oxygen and nitrogen species may be attributed to pro-inflammatory cytokine signaling. The reduction of CFE in studies of C57BL/6 bone marrow implicates signal transduction processes involving the ERK/MAPK and JNK/SAPK pathways (Figure 2).
Activation of, and interaction of, multiple signalling pathways has been shown to occur after exposure of cells to ionizing radiation (27) resulting in transcriptional induction of genes encoding inflammatory and immunomodulatory factors (28). Previous studies implicated pro-inflammatory cytokine signalling with the in vivo chromosomal instability phenotype (29) and involvement of COX-2 in cell line bystander responses in vitro (30), the present study demonstrated both the non-targeted reduction in CFE and the chromosomal instability phenotype in bone marrow cells are mediated by signals converging on COX-2, the initial and rate-limiting enzymatic step in the metabolism of arachidonic acid to prostaglandins which play important roles in modulating inflammatory processes (31-33). Thus the non-targeted effects studied may be considered to result from long-lived complex stress response signaling and their persistence for months post-irradiation is associated with a genotype-dependent response to ongoing inflammatory processes. The genotype-dependent responses (Table 2 and Table 3) cannot be simply attributed to the type of signal produced as the same blocking agents reduced the two different effects in the two strains (Figure 2). As it is known that one cytokine may induce the expression of other cytokines and cytokine receptors (34) it is possible that such downstream signaling effects in the clonogenic cells influence the genotype dependency of the response.

Pro-inflammatory processes have previously been implicated as components of the poorly characterized mechanisms underlying the toxicity of clastogenic factors and in vivo abscopal effects of radiation (10, 35) and a case can be made for common mechanisms underlying the various manifestations of post-radiation cellular interactions.
having properties in common with inflammatory processes (14, 36, 37). It is of interest that in addition to the well-documented increases in malignancy in the Japanese A-bomb survivors there are also reports of increases in cardiovascular, gastrointestinal and respiratory system diseases (38, 39) associated with persisting inflammation (40, 41).

Considerable progress has been made in understanding the cellular and molecular events that are involved in the acute inflammatory response to infection but responses to tissue injury are less well understood (42). However, the biology of normal tissue responses to moderate and clinically relevant doses of radiation is inextricably connected to innate immunity (43) and, in addition to the well-characterized immediate responses to irradiation, mechanism common to both radiation and immune signaling are activated that contribute to tissue damage, regeneration and repair. The findings of the present study are consistent with radiation-induced in vivo non-targeted effects being long-lived and resulting from genotype-dependent responses to pro-inflammatory cytokine signaling. Such effects demonstrate the importance of genetics and of studying ongoing tissue responses, as well as the immediate effects of irradiation, when considering the mechanisms underlying the consequences of radiation exposure.

References


Table captions

**Table 1** The reduction in colony-forming efficiency (CFE) of bone marrow cells cocultured with irradiated bone marrow cells obtained from mice within one hour of exposure to 4 Gy γ-rays. Data are presented as mean ± sem, n = 20.

**Table 2** The reduction in colony-forming efficiency (CFE) of bone marrow cells exposed to cell-free irradiated bone marrow medium (IBMM) obtained from mice 3 months post-exposure to 4 Gy γ-rays. Data are presented as mean ± sem, n = 7.

**Table 3** Non-clonal aberrations in clonal progeny of short-term repopulating stem cells derived *in vitro* from non-irradiated mouse bone marrow exposed to cell-free bone marrow medium (BMM) prepared from normal mice (CBMM) or from irradiated mice (IBMM) within one hour of exposure to 4 Gy γ-rays. Pooled data obtained from 3 replicate experiments are shown.

**Table 4** Non-clonal aberrations in clonal progeny of short-term repopulating stem cells derived *in vitro* from non-irradiated mouse bone marrow exposed to cell-free bone marrow medium (BMM) prepared from normal mice (CBMM) or from irradiated mice (IBMM) 100 days after exposure to 4 Gy γ-rays. Pooled data obtained from 5 replicate experiments are shown.
Figure legends

Figure 1. A schematic view of the experimental protocol in which normal unirradiated primary bone marrow cells co-cultured with irradiated bone marrow cells or exposed to medium obtained from bone marrow irradiated in situ are cultured in standard semi-solid clonogenic cultures. The cultures are incubated for 9 days before harvesting individual colonies for clonal cytogenetic analysis and incubated for 12 days to determine the colony-forming efficiency.

Figure 2 The colony-forming efficiency of normal bone marrow co-cultured with irradiated bone marrow obtained from mice within one hour of exposure to 4 Gy γ-rays or with irradiated bone marrow in the presence of 0.1μg/ml anti-FasL, 0.01 μg/ml anti-TNF-α, an inhibitor of cyclooxygenase-2 (1 μM NS-398), small molecule inhibitors of mitogen activated protein kinase signalling (5 μM SB-203580, 1 μM PD-98059) stress-activated protein kinase (0.1 μM SP-600125), a scavenger of reactive oxygen species (0.01% DMSO) or nitric oxide (1 μM c-PTIO). Data are presented as mean ± sem, n = 10, expressed as percent of control colony-forming efficiency. CFE data for co-cultures supplemented with antibodies or inhibitors are not significantly different (p>0.05) from control CFEs but significantly increased (p<0.05) by comparison with data obtained from non-supplemented co-cultures.

Figure 3 The colony-forming efficiency of normal bone marrow exposed to cell-free bone marrow medium prepared from normal mice or from irradiated mice 100 days after exposure to 4 Gy γ-rays (IBMM) or from age-matched controls (CBMM) in the presence
of 0.01 μg/ml anti-TNF-α 0.1μg/ml anti-FasL, or an inhibitor of cyclooxygenase-2 (1 μM NS-398). Data are presented as mean ± sem, n = 10, expressed as percent of control colony-forming efficiency. CFE data for unirradiated cells cultured with IBMM supplemented with antibodies or inhibitors are not significantly different (p>0.05) from CFEs of unirradiated cells cultured with CBMM plus antibody but significantly increased (p<0.05) relative to IBMM alone.
Table 1.

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<th>Surviving fraction</th>
<th>% CFE reduction</th>
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<tr>
<td>Irradiated CBA/Ca</td>
<td>CBA/Ca + NS-398</td>
<td>15 / 193 (7.7)</td>
<td>178 14 1 0</td>
<td>0.083</td>
</tr>
</tbody>
</table>
Figure 3

CFE (% of control)

IBMM  
IBMM + Anti-TNFα  
IBMM + Anti-FasL  
IBMM + NS-398
Long-lived inflammatory signaling in irradiated bone marrow is genome dependent

Sally A Lorimore, Debayan Mukherjee, Joanne I Robinson, et al.

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