Abstract

In addition to the directly mutagenic effects of energy deposition in DNA, ionizing radiation is associated with a variety of untargeted and delayed effects that result in ongoing bone marrow damage. Delayed effects are genotype dependent with CBA/Ca mice, but not C57BL/6 mice, susceptible to the induction of damage and also radiation-induced acute myeloid leukemia. Because macrophages are a potential source of ongoing damaging signals, we have determined their gene expression profiles and we show that bone marrow–derived macrophages show widely different intrinsic expression patterns. The profiles classify macrophages derived from CBA/Ca mice as M1-like (pro-inflammatory) and those from C57BL/6 mice as M2-like (anti-inflammatory); measurements of NOS2 and arginase activity in normal bone marrow macrophages confirm these findings. After irradiation in vivo, but not in vitro, C57BL/6 macrophages show a reduction in NOS2 and an increase in arginase activities, indicating a further M2 response, whereas CBA/Ca macrophages retain an M1 phenotype. Activation of specific signal transducer and activator of transcription signaling pathways in irradiated hematopoietic tissues supports these observations. The data indicate that macrophage activation is not a direct effect of radiation but a tissue response, secondary to the initial radiation exposure, and have important implications for understanding genotype-dependent responses and the mechanisms of the hematotoxic and leukemogenic consequences of radiation exposure. [Cancer Res 2008;68(2):450–6]

Introduction

The dogma that genetic alterations are restricted to directly irradiated cells has been challenged by observations in which effects of ionizing radiation, characteristically associated with the consequences of energy deposition in the cell nucleus, arise in nonirradiated cells. These so-called nontargeted effects (1, 2) are shown in cells that have received signals produced by irradiated cells (radiation-induced bystander effects) or that are the descendants of irradiated cells (radiation-induced genomic instability). Radiation-induced genomic instability is characterized by a number of delayed adverse responses, including chromosomal abnormalities, gene mutations, and cell death days and months postexposure. Similar effects, as well as responses that may be regarded as protective, have been attributed to bystander mechanisms (3, 4). The majority of studies of nontargeted effects use in vitro model systems and it is far from clear whether such mechanisms operate in vivo. However, plasma from X-irradiated patients can cause chromosome damage in cultured lymphocytes and similar activities have been obtained from atomic bomb survivors, Chernobyl liquidators, and from patients with chromosome instability syndromes and inflammatory disorders (2, 5). These effects are attributed to indirect acting mechanisms that generate clastogenic factors (6). These factors may be produced at sites of irradiation, often represent inflammatory-type tissue responses, and can mediate late tissue injury (7). These observations imply that the target for the biological effects of radiation is larger than the directly irradiated cell (8). Thus, after exposure in vivo, a case can be made for the tissue microenvironment contributing to delayed cell damage as a consequence of responses that are secondary to the initial radiation-induced injury. Because the responses of the hemopoietic system are major determinants of outcome following therapeutic, occupational, or accidental radiation exposures, we have been interested to determine the contribution of both direct and indirect effects to hemopoietic cell and tissue responses and have conducted studies to compare responses in CBA/Ca and C57BL/6 mice that differentially express nontargeted effects and are also, respectively, susceptible or resistant to radiation-induced acute myeloid leukemia (9).

In previous studies of the in vivo response to a potentially leukemogenic dose of ionizing radiation, we showed the expected p53 signaling pathway responses in the first few hours after irradiation, albeit with some genotypic differences in the amount and timing of apoptosis; however, at 24 h, a marked genotype-dependent macrophage activation was consistent with indirect mechanisms resulting in a potentially damaging inflammatory-type microenvironment in the CBA/Ca hemopoietic system (10, 11). Inflammatory macrophages are a potent source of microenvironmental reactive nitrogen and oxygen species that can damage bystander cells and initiate tumor formation (12, 13). Macrophages are important components of the hemopoietic microenvironment that, in addition to their involvement in immunoregulatory and inflammatory processes, control tissue architecture dynamics and cell mobilization by the secretion of cytokines and chemokines and through cell-cell and cell-matrix interactions (14). More generally, they are versatile cells that respond to environmental cues and different subpopulations of tissue macrophages show differences in expression of a variety of receptors and regulatory cytokines (15, 16). Macrophages are increasingly recognized as contributing to tumorigenesis through the production of stimulatory or inhibitory molecules that affect tumor cell growth, formation of blood vessels, cellular adhesion, and tissue architecture (16). Moreover, tumorigenic agents often act not on the target...
cell for tumor initiation, but affect stromal cells that make up the microenvironment (17–20). Tumor susceptibility is strongly influenced by genetic composition, and, in model systems, part of the genetic component lies in differences in inflammatory responses (21–24), including differences in the genetics of macrophage function (25). Therefore, macrophages are likely to be major determinants of changes in tissue microenvironment that occur after the initial cellular damage responses and result in the potential for ongoing bystander-mediated damage in CBA/Ca but not in C57BL/6 hemopoietic tissues. To further investigate the phenotypes of macrophages in normal and irradiated hemopoietic tissues, we have determined gene expression profiles of C57BL/6 and CBA/Ca bone marrow–derived macrophages before and after irradiation in vitro, conducted confirmatory gene and protein expression studies, and used these in vitro results to guide in vivo experiments. We found that macrophages do not change their phenotypes as a result of direct irradiation but do so in the context of a tissue response: C57BL/6 macrophages have an intrinsic anti-inflammatory M2-like phenotype that is enhanced in irradiated tissue, whereas CBA/Ca macrophages are intrinsically M1-like and express a pro-inflammatory phenotype after irradiation in vivo. Overall, our data are consistent with macrophages having the potential for producing ongoing damage in CBA/Ca but not in C57BL/6 bone marrow and therefore contributing to the differential expression of nontargeted and delayed radiation effects.

Materials and Methods

Macrophage culture and irradiation. CBA/Ca and C57BL/6 mice were bred in-house under conventional conditions. Experiments were approved by local ethical review and followed the guidelines of the Medical Research Council and the Home Office (PPL 60/2841). To produce sufficient mature macrophages for analyses and to reduce random variation in cultures derived from different individuals, femoral bone marrow cells obtained from five individuals were pooled and grown in 10 T25 tissue culture flasks containing 10 mL modified Eagle’s medium (Invitrogen) supplemented with 25% pretested horse serum and 25% pretested conditioned medium from the L929 cell line as a source of CSF-1. For each experiment, five flasks were exposed to 4 Gy γ-irradiation at a dose rate of 0.5 Gy/min at room temperature using a CIS Bio International 637 cesium irradiator and five were sham irradiated. Cohorts of mice were whole body irradiated with the same source (dose rate 0.45 Gy/min) or were sham irradiated. Cohorts of mice were irradiated with the temperature using a CIS Bio International 637 cesium irradiator and five

Reverse transcription-PCR. Reverse transcription-PCR (RT-PCR) was performed in duplicate from bone marrow–derived macrophages prepared independently from those used for microarray analysis. cDNA synthesis used SuperScript III reverse transcriptase with oligo(dT) (Invitrogen). cDNA was diluted 1:5 with sterile water and 1 μL was used for PCR with varying numbers of cycles. All primer pairs amplify across at least one intron to ensure specificity. All reactions contained additional primers to amplify Gapdh as a housekeeping gene to allow normalization of samples for input cDNA.

Immunohistochemical analysis. Immunohistochemical identification of antigens was performed on frozen sections of spleen fixed for 10 min in 50:50 methanol/aceton, or on 4-μm sections of formalin-fixed, paraffin-embedded femurs that had been decalcified in EDTA (pH 7.0), taken from C57BL/6 and CBA/Ca mice with or without previous whole-body exposure to 4 Gy γ-irradiation. Antibodies were identified using peroxidase avidin-biotin-complex (Vector Elite, Vector Labs) and 3,3'-diaminobenzidine as chromogen. Polyclonal rabbit serum to murine Nos2 was obtained by immunizing with COOH-terminal peptide (VFSYGAKKGSALEEPKATRL) conjugated to keyhole-limpet hemocyanin with glutaraldehyde (Moravians Biotechnology). Specificity for this control included preimmune serum applied at the same dilution and addition of peptide (1 μg/mL) to diluted serum before use for immunostaining. Both controls showed an absence of staining. Antibodies to CD14, Lcn2, Mfge8, and SR-A (Santa Cruz Biotechnology); goat anti-Aif1 (Abcam); rabbit anti–3-nitrotyrosine (Upstate Biotechnology); rat monoclonal antibodies to Dectin-1 and C1q (Serotec); and CD68 (Hyctul BioClone); rabbit monoclonal antibodies to phospho-Stat1 (Tyr701) and phospho-Stat3 (Tyr705; Cell Signalling); and to phospho-Stat5 (Tyr885), signal transducer and activator of transcription 6 (STAT6), and phospho-STAT6 (Tyr641; Abcam) were also used.

Macrophage isolation. Macrophages were isolated from the femurs of cohorts of 4 to 10 CBA/Ca or C57BL/6 mice with or without 4 Gy wholebody γ-irradiation 24 h previously. Femurs were gently blown out with air pushed through 23G needles and cell clumps were digested with medium containing liberase (1.67 Wunsch units/mL) and 0.2 mg/mL DNase (Roche) for 30 min at 37°C. Cells were collected into lipopolysaccharide-free PBS containing 2 mmol/L EDTA and 0.5% bovine serum albumin (Invitrogen). Macrophages were positively selected using F4/80 followed by antirat microbeads (Miltenyi Biotec).

Arginase activity. We used an adapted method from ref. 28 to increase sensitivity and allow measurements of urea production from primary macrophages, F4/80-positive macrophages (2 × 10^5) isolated from cohorts of mice were lysed in 50 μL 0.1% Triton X-100 for 10 min on ice and 50 μL 50 mmol/L Tris-HCl (pH 7.4) containing 10 mmol/L MnCl2 were added. After enzyme activation at 55°C for 10 min, 25 μL of lysate were added to

---

1 http://geneservice.co.uk
2 http://biosun1.harvard.edu/comlab/gosurfer/
3 http://www.affymetrix.com/analysis/netaffx/
4 http://www.bioconductor.org/
25 µL of 0.5 mol/L L-arginine (pH 9.7) and incubated at 37°C for 2 h. The reaction was acidified with 200 µL of H₂SO₄/H₃PO₄/H₂O (1/3/7 v/v), and 25 µL of 5% α-isonitrosopropiophenone (Sigma) were added and heated to 95°C for 1 h. Urea production was measured at 540 nm. Five thousand cultured macrophages were used and incubated for 1 h at 37°C.

Results

Genotype-dependent expression profiles and response to irradiation in vitro. Bone marrow–derived macrophages from both genotypes had the typical microscopic appearance of macrophages and expressed the macrophage marker F4/80. Contamination with other cell types was assessed by microarray analysis of lineage-specific transcripts that are highly expressed in B cells (CD19), T cells (CD3), granulocytes (myeloperoxidase), megakaryocytes (Itga2b/CD41), and erythrocytes (glycophorin A; ref. 29). All transcripts were either not expressed or expressed at very low levels, with <10% variation between strains or after irradiation. Thus, contaminating cell populations do not contribute to the expression profiles observed. Affymetrix analysis also showed no significant differences in cell cycle–related gene expression between genotypes, indicating that differences in cell cycle stage do not influence the results.

Duplicate cultures from the two genotypes were either sham-irradiated or exposed to 4 Gy γ-irradiation and used for microarray analysis 24 h later, a time when in vivo alterations to macrophage activities are seen in hematopoietic tissues (10). Using ANOVA tests with Benjamini and Hochberg false-discovery correction for multiple testing (P < 0.05), no genes were identified as significantly different between irradiated and unirradiated macrophages in either strain, or when data from the two strains were combined. In contrast, 243 probe sets were identified as differentially expressed between genotypes regardless of irradiation status (~1.5% of the expressed genes; Supplementary Table S1). Using the available Gene Ontology annotations, differentially expressed genes were found in all biological processes and cellular components and in each of the major categories of molecular function. Statistically significant associations were calculated (P < 0.001) for the 136 genes with annotation. Gene products involved in cell adhesion or phosphate transport, or that have lyase activity, function in the immune response or have scavenger receptor activities that were statistically significant (Supplementary Table S2). Expression differences were confirmed by RT-PCR of two independent sets of bone marrow–derived macrophages from the two strains with and without irradiation 6 or 24 h before collection, the 6-h time point representing the early response to radiation and the time at which in vivo macrophage responses are first observed (10). These data confirmed that expression levels are determined by the genotype of the macrophages and are not significantly altered by irradiation in vitro (Fig. 1A). We also did microarray analysis of macrophages collected 6 h after irradiation to examine earlier radiation-induced responses. Using ANOVA with false discovery rate, no genes were statistically significantly changed and the Cdkn1a inhibitor of cyclin-dependent kinases that is induced in a p53-dependent manner after genotoxic stress was the only gene induced by at least 2-fold at 90% confidence in both genotypes. Expression values for genes involved in p53-mediated apoptosis were unaffected by radiation at either 6 or 24 h (Supplementary Table S3). These data indicating a lack of transcriptional activation of apoptotic pathways are in keeping with the radioresistant nature of bone marrow macrophages (cell counts showed >90% survival 24 h after irradiation).

Genotype-dependent expression of genes involved in immune response, cellular adhesion, and oxidative stress. Gene Ontology analysis showed significant overrepresentations of genes that are involved in immune/defense responses or in regulating cell adhesion. Sixteen genes whose products are involved in cell motility or adhesion and 16 cytokines, genes regulated by cytokines, or cytokine receptors were identified as differentially expressed between the two strains (Fig. 2); expression differences were confirmed for selected genes. The expression of these genes

Figure 1. RT-PCR and immunohistochemical verification of Affymetrix data. A, RT-PCRs were performed on cDNAs prepared from two independent sets of cultured macrophages from the two genotypes. Cells were either sham irradiated or exposed to 4 Gy γ-irradiation and collected 6 or 24 h later. B, immunohistochemistry of spleen frozen sections confirms genotype-dependent expression levels of the selected proteins.
was not altered by irradiation in vitro (Fig. 1A). Ten genes involved in the production, removal, or response to reactive oxygen and/or nitrogen species were expressed in a genotype-dependent manner and were unchanged following irradiation in vitro (Figs. 1A and 2).

**Genotype-dependent expression of scavenger receptors and opsonins.** Genes with scavenger receptor activity were overrepresented (P = 0.000036). Retrieval of expression values for other scavenger receptors revealed that CD14 was 2.72-fold altered in CBA/Ca than C57BL/6 by immunohistochemistry (Fig. 1). Elevated expression of CD14 and SR-A by CBA/Ca macrophages was confirmed by RT-PCR and immunohistochemical staining of frozen sections of spleen (Fig. 1). Of other opsonic cell ligands, macrophages from both strains express high levels of Gas6 and moderate levels of serum amyloid-P (Fig. 2).

**Genotype-dependent M1/M2 phenotype.** Arginase 1 is expressed at 40-fold higher levels in C57BL/6 than CBA/Ca macrophages in vitro and is a reliable marker of M2 macrophage polarization in murine macrophages (30). Other markers of the M2 phenotype, Dectin1 (CLECSF12), IL-1Ra, and chitinase-like proteins (Ym1/2 and Chi3l3), are also elevated in C57BL/6 compared with CBA/Ca. Levels of Dectin1 protein were measured by immunostaining (Fig. 1B) and on macrophages in vitro by fluorescence-activated cell sorting (FACS) analysis (not shown) and were higher in C57BL/6 than CBA/Ca in both assays. In contrast, CD14 and Marco show higher expression in CBA/Ca and are associated with innate activation phenotypes. Antibodies to Marco were insufficiently sensitive to identify this receptor in bone marrow macrophages by FACS, but CD14 is more highly expressed in CBA/Ca and were higher in C57BL/6 than CBA/Ca in both assays. These microarray and protein expression data indicate that macrophages from C57BL/6 have phenotypic characteristics of M2 alternatively activated macrophages, whereas the same cells derived from CBA/Ca have a phenotype in common with classically activated M1 macrophages. Irradiation of macrophages in vitro did not alter the expression of these phenotypic markers (Supplementary Table S1; Fig. 1A).

**Macrophage responses to radiation in vivo.** Although cultured macrophages do not change their gene expression profiles after irradiation in vitro, they possess intrinsic genotype-specific differences in their M1/M2 phenotypes. Previous investigations had shown macrophage activation 24 h postirradiation in vivo; therefore, to examine any alterations to macrophage phenotypes and inflammatory responses that are elicited as a consequence of irradiation in vivo, we measured Arginase 1 activities in purified macrophages freshly isolated from bone marrow of control and irradiated mice (Fig. 3A). In keeping with the microarray and RT-PCR data, C57BL/6 cells from control mice show higher arginase activity than CBA/Ca (P = 0.01687). Macrophages isolated from bone marrow of C57BL/6 mice that were irradiated 24 h previously show 3.2-fold higher arginase activity compared with cells isolated at 24 h from sham-irradiated mice (P = 0.0014, control versus 4Gy) but there is no increase in arginase activity in macrophages isolated from irradiated compared with nonirradiated CBA/Ca bone marrow (P = 0.11628; Fig. 3A). This leads to an increase in the difference in arginase activity between irradiated CBA/Ca and C57BL/6 (2.6-fold higher in macrophages from unirradiated C57BL/6 mice compared with unirradiated CBA/Ca, increasing to an 8.4-fold higher activity in irradiated C57BL/6 compared with irradiated CBA/Ca).
Nitric oxide (NO) is an effector of the innate immune system and its production is a characteristic feature of M1 macrophages. The normal method for measuring NO is the Griess reaction for nitrite but we found this method insufficiently sensitive to measure nitrite concentrations in primary bone marrow. Therefore, we assessed differences in NOS2 (the enzyme that synthesizes NO) by immunocytochemistry and found higher expression of this M1-associated enzyme in CBA/Ca than in C57BL/6. After whole-body irradiation, NOS2 is strongly repressed in C57BL/6 but not in CBA/Ca bone marrow (Fig. 3B). Immunohistochemistry also showed preferential activation of STAT1 (a signal transducer and activator of transcription characteristic of pro-inflammatory M1 responses) in irradiated CBA/Ca bone marrow, whereas STAT6 (characteristic of M2 responses) showed increased activation in C57BL/6 but not in CBA/Ca (Fig. 3C and D).

Discussion

Because cultured macrophages retain the essential properties of primary cells (14), we have been able to use whole-genome microarrays to define the transcriptome of bone marrow–derived macrophages from two inbred strains of mice previously shown to be differentially susceptible to ongoing bone marrow damage and radiation-induced acute myelogenous leukemia and also to exhibit differences in macrophage activation 6 to 24 h after whole-body radiation (10). The microarray data of in vitro bone marrow–derived macrophages reveal widely different intrinsic expression profiles that reflect genetically determined differences and guided subsequent phenotypic studies in vivo. Notably, there are limited transcriptional responses of in vitro bone marrow–derived macrophages to direct irradiation. These data contrast with many previous studies of more radiosensitive primary cells, where even low doses of ionizing radiation exposure induce p53-mediated responses associated with induction of genes involved in growth arrest and/or apoptosis (31). However, radiation responses, including p53 stabilization, transcriptional activation, and apoptosis are dependent on cell and tissue type (reviewed in ref. 32). Importantly, even high-dose radiation does not induce p53-regulated proapoptotic genes in bone marrow–derived macrophages, consistent with their relatively radioresistant nature (33).

The genotypic differences classify CBA/Ca mice as an M1-like strain and C57BL/6 as an M2-like strain. M1 and M2 functional subsets display pro-inflammatory versus anti-inflammatory and

![Figure 3](https://cancerres.aacrjournals.org/)

**Figure 3.** Changes to M1 and M2 phenotypes of CBA/Ca and C57BL/6 mice after irradiation in vivo. **A,** arginase activity in macrophages purified from bone marrows of CBA/Ca or C57BL/6 24 h after whole-body 4 Gy γ-irradiation or sham irradiation. Data are shown relative to the level of sham-irradiated CBA/Ca mice. Macrophages from C57BL/6 have increased arginase activity after irradiation, whereas CBA/Ca do not. **B to D,** immunoperoxidase staining with the indicated antibodies in sections of bone marrow from sham-irradiated (0 h) mice or those irradiated 24 h previously (24 h). Positive staining is seen as a brown product and cell nuclei are counterstained blue.
reparative patterns of function (15, 16) and after in vivo, but not after in vitro irradiation, C57BL/6 macrophages show enhanced M2 activities and those from CBA/Ca mice enhanced M1 activities. Changes in macrophage activity after irradiation in a tissue context in vivo but not as isolated cells in vitro is consistent with a lack of a direct radiation effect and in vivo responses being due to some kind of signal(s) derived secondarily from other cells in response to radiation exposure. Previous studies had shown that macrophage activation in irradiated hemopoietic tissues was associated with phagocytic clearance of apoptotic cells, indicating that apoptotic cell engulfment is one of these signals (10). A plausible explanation for genotype-dependent differences in macrophage activation following engulfment of radiation-induced apoptotic cells is the expression levels of certain scavenger receptors and opsonins. The strain-dependent expression of these molecules implies that the engulfing cells in the two genotypes use different pathways for recognition and clearance. Although little is known about the signaling pathways activated or repressed by distinct receptors with or without particular opsonins, differential responses have been described (30).

A major finding of our study is the genotypic differences that classify macrophages obtained from bone marrow of CBA/Ca or C57BL/6 mice as, respectively, M1-like or M2-like. In M1 macrophages, metabolism of arginine through NOS2 produces citrulline and NO, a powerful inflammatory mediator. Conversely, in M2 macrophages, metabolism of arginine through Arginase 1 reduces NOS2 activity and simultaneously produces polyamines and proline, which act as antioxidants and stimulate tissue regeneration (16, 30). Thus, the balance between NOS2 versus arginase activity is a key determinant of the pro-inflammatory M1 phenotype or the anti-inflammatory and protective environment associated with M2 macrophages. Hemopoietic tissues of CBA/Ca have more NOS2 protein than C57BL/6, whereas arginase activity is higher in C57BL/6, consistent with CBA/Ca macrophages having intrinsic M1 properties and the same cells from C57BL/6 exhibiting an M2 phenotype. These observations may be regarded as surprising, because C57BL/6 is considered a prototypic Th1/M1 strain (34, 35). However, those classifications are based on the responses of C57BL/6 macrophages to classic pro-inflammatory stimuli such as lipopolysaccharide and IFN, and in fact those same studies show that C57BL/6 macrophages in vitro in the absence of stimuli exhibit M2 characteristics (34). Similarly, analysis of microarray data provided in a study of the transcriptional responses of macrophages to lipopolysaccharide (35) reveals M2 features in unstimulated C57BL/6 macrophages. Importantly, our data obtained by studying bone marrow macrophages in vivo show that genetically determined default M1/M2 phenotypes are independent of Th1/Th2 characteristics, because C57BL/6 are intrinsically Th1 yet their bone marrow macrophages are intrinsically M2 in vivo. Similarly, although T-cell activities are regulated through interactions with macrophages and other antigen-presenting cells, primary Th1/Th2 phenotypes are determined by the genotype of the T cells and not the antigen-presenting cell (36). Therefore, although pro-inflammatory/anti-inflammatory M1/M2 and the corresponding Th1/Th2 phenotypes are regulated through interdependent cytokine signaling pathways (34), each phenotype is also independently determined by genotypic influences. Thus, macrophage phenotypes are generated by complex interactions between intrinsic characteristics and responses to external stimuli that differ according to different genetic components.

Most notably for our studies relating to delayed and indirect effects of irradiation, M2 phenotypes associate with reduced inflammation and improved tissue repair after injury, whereas the M1 phenotype associates with pro-inflammatory conditions (15, 16, 30). Although these characteristics are not altered by direct irradiation of bone marrow–derived macrophages in vitro, there are genotype-dependent alterations in vivo, where CBA/Ca bone marrow macrophages maintain NOS2 levels and arginase activity, whereas C57BL/6 lose NOS2 and increase arginase activity after irradiation. These radiation-induced changes therefore enhance the differences between genotypes, with C57BL/6 becoming more M2-like than previously whereas CBA/Ca retain an M1 phenotype within the bone marrow and do not induce M2 characteristics. We also show phosphorylation of STAT1 but not STAT6 in hemopoietic tissues of CBA/Ca mice in response to radiation and STAT6 but not STAT1 in C57BL/6 mice. STAT1 activation is characteristic of pro-inflammatory M1 responses, whereas STAT6 activation is characteristic of M2 responses (16, 30). These observations provide further evidence for tissue-dependent responses following irradiation and indicate genotype-dependent differences in the overall production of potentially damaging (CBA/Ca) and protective (C57BL/6) hemopoietic microenvironmental responses. These responses can be considered in terms of “danger” signals that mobilize the innate and acquired immune system to maintain the integrity of the body following exposure to a variety of pathologic, chemical, or physical agents and have been suggested to act to mediate local tissue recovery or mediate damaging bystander effects (reviewed in ref. 37).

Taken together, our data show altered macrophage activity after irradiation in vivo but not in vitro. The genotype-specific expression profiles indicate intrinsic differences in bone marrow macrophage activities, including M1/M2 phenotypes that govern their regulatory functions in immunity and hemopoiesis. Although macrophages do not respond to direct irradiation, their activities are altered in vivo after irradiation, with CBA/Ca hemopoietic tissues showing an M1-like phenotype that associates with potentially damaging inflammatory-type responses, compared with the induction of an anti-inflammatory phenotype and tissue reparative response seen in C57BL/6 tissues. These complex differences in macrophage function are likely to contribute to the medium and long-term outcomes of radiation exposure in the hemopoietic system by their involvement in the delayed and nontargeted effects exhibited in vivo (38–40). Important, a genotype-dependent, radiation-induced genomic instability phenotype in vivo need not necessarily be a reflection of intrinsically unstable cells but the responses to ongoing production of damage as a consequence of a persisting inflammatory-type response secondary to the initial radiation-induced injury.

Acknowledgments


Grant support: Leukaemia Research Fund specialist programme grant 0214.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Tom Freeman and the LRF microarray service for advice on gene expression profiling.
References


Indirect Macrophage Responses to Ionizing Radiation: Implications for Genotype-Dependent Bystander Signaling

Philip J. Coates, Jana K. Rundle, Sally A. Lorimore, et al.


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/68/2/450

Supplementary Material

Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2008/01/11/68.2.450.DC1

Cited articles

This article cites 39 articles, 16 of which you can access for free at:
http://cancerres.aacrjournals.org/content/68/2/450.full#ref-list-1

Citing articles

This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/68/2/450.full#related-urls

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.