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Response patterns of cytokines/chemokines in two murine strains after irradiation

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Purpose: To determine the plasma concentrations of acute responding cytokines/chemokines following 9-Gy ionizing radiation in C57BL/6 (radiation tolerant) and C3H/HeN (radiation sensitive) murine strains.
Methods and materials: Mice (5/group) received 9-Gy total body irradiation (TBI), and the plasma from each mouse was collected at 6 h or 1, 2, 4, or 10 days after TBI. A multiplex bead array was used to assess the levels of 32 cytokines/chemokines in plasma to determine their common and strain-specific temporal responses.
Results: The plasma levels of five cytokines/chemokines (Axl, FasL, ICAM-1, TARC, and TSLP) were beyond the detectable level. Five (VEGF, IL-2, IL-5, IL-17, and CD30) were unaffected by irradiation in either strain. Temporal patterns were similar in both murine strains for 10 of the cytokines tested, including G-CSF, IL-6, TCA-3, MCP-1, MIP-1γ, KC, CXCL 13, CXCL 16, MDC, and TIMP-1; the other 12 molecules (GM-CSF, IL-3, SCF, IL-1β, IL-4, IL-10, IL-12p70, MIP-1α, Eotaxin, TNF-α, sTNF-R1, and CD40) showed strain-specific response patterns. While a number of cytokines had temporal response patterns following TBI, the strains exhibited quantitatively different results.
Conclusions: The levels of 27 of the 32 plasma cytokines measured indicate the following: (1) different cytokine concentrations and temporal patterns in the two strains may partly explain different radiation sensitivities and sequelae following irradiation; (2) many of the cytokines/chemokines exhibit similar temporal responses in the two strains. These responses suggest the potential value of using a panel of cytokine/chemokine temporal patterns for radiation dosimetry. Although radiation doses will be difficult to quantitate due to the large variation in levels and temporal responses exhibited in the two murine strains, serial measurements of cytokines might help identify subjects exposed to radiation.
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1. Introduction

When wild-type mice are exposed to total body irradiation (TBI), the dose that causes 50% of the population to die within 30 days ($LD_{50/30}$) varies by strain [1,2]. For example, the $LD_{50/30}$ of C57BL/6 mice in our experiment was ≈8.5 Gy, while the $LD_{50/30}$ of more sensitive murine strains (such as BALB/c, C3H/HeN, and NIH Swiss) varies from 5.8 to 7.6 Gy. While C57BL/6 is more tolerant to whole body exposure, it is also more prone to fibrosis than other strains, including C3H/HeN [3]. The mechanism underlying this difference in sensitivity is not completely understood, but it occurs, at least in part, through inflammatory mechanisms governed by cytokines, particularly in the transforming growth factor beta (TGF-β) and interleukin 1 (IL-1) pathways.

Abbreviations: IR, ionizing radiation; TBI, total body irradiation; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony stimulating factor; IGF-1, insulin-like growth factor-1; PDGF, platelet-derived growth factor; SCF, stem cell factor; sTNF-R1, soluble TNF receptor type 1; TIMP-1, metallopeptidase inhibitor 1; TARC/CCL17, thymus and activation-regulated chemokine; TSLP, thymic stromal lymphopoietin; TCA-3/CCL1, T-cell activation 3; VEGF, vascular endothelial growth factor; MMPs, metalloproteinases; $LD_{50/30}$, lethal dose of radiation that causes the death of half of the exposed population within 30 days.

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The cytokine response to radiation exposure, first described by Talas et al. [4], generally begins with a "storm"; however, the details of this response have not been fully characterized. The cytokine storm is a self-limited, all-or-nothing response that reaches its peak within a few hours and resolves within 24 h. This apparently non-specific acute reaction, which involves a multitude of cytokines acting as general acute responders, occurs after even a minimally toxic dose of radiation. Many investigators theorize that the cytokine/chemokine reaction is a significant element in the radiation toxicity experienced by different murine strains [5]. Currently, the complexity of the cytokine/chemokine response is being studied for patterns that can be used for radiation biodosimetry [6].

We hypothesize that cytokine-response levels and patterns induced by ionizing radiation are related to the radiation tolerance of a murine strain. Cytokines play many roles in the regulation of a host's response to radiation [7]. Among such cytokines are those that help to regenerate critical elements of the hematopoietic system, such as granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony stimulating factor (GM-CSF) [8]. Other cytokines, such as interleukin 4 (IL-4) and IL-5, regulate and augment immune function [9]. Others, such as fibroblast growth factor (FGF) and keratinocyte growth factor (KGF), aid in healing and regenerating epithelial tissues [10].

However, the intrastrain differences in cytokine responses to TBI have not been studied. In order to fill this gap, we examined the ability of murine strain-specific cytokine expression patterns to help us better understand the differences in acute responses to irradiation.

2. Materials and methods

2.1. Animals

Male C57BL/6 and C3H/HeN mice (8 weeks old) were purchased from the National Cancer Institute (NCI, Frederick, MD) and kept under specific pathogen-free conditions. Animals were divided into groups (5 mice/group), maintained on a 12-h light schedule, and fed a standard diet. The University Committee on Animal Research at the University of Rochester (Rochester, NY) approved all protocols, and we carried out all experiments in accordance with United States Public Health Service guidelines.

2.2. Irradiation

Control mice were immobilized but not irradiated; other groups were subjected to 9-Gy TBI at a dose rate of 1.84 Gy/minute, which was carried out with a Shepherd Mark-I unit (JL Shepherd and Associates, San Fernando, CA) featuring a cesium-137 source. Mice were immobilized with a plastic restrainer during the exposure period.

2.3. Plasma collection and storage

Mice were sacrificed at 6 h or 1, 2, 4, or 10 days after irradiation. Blood was collected in a tube containing ethylenediaminetetraacetic acid disodium salt (EDTA-Na_2), and the plasma was aliquoted and stored at −70 °C until analysis. Before analysis, the plasma was thawed and centrifuged at 6000 rpm for 5 min to remove precipitated proteins.

2.4. Bead array reagents

We purchased antibody pairs for 32 analytes from R&D Systems (Minneapolis, MN) and carboxyl microspheres from Luminex Corporation (Austin, TX). The pairs of antibodies were directed against different epitopes of individual cytokines and purified by affinity column. The capture antibodies were conjugated on appropriate microspheres according to the manufacturer's protocol [11]. Coupling controls were examined to confirm immobilization efficiency.

2.5. Multiplex bead arrays

First, a singleplex assay was run to determine the optimal second antibody concentration for each analyte. Next, a multiplex assay was set up by mixing beads coated with captured antibodies for all 32 analytes and then incorporating them with an optimal concentration of their second antibodies. To create a standard curve, a known concentration of 32 recombinant murine proteins corresponding to each analyte was mixed together. Cross reactivity was tested to avoid interactions between different analytes [12]. Control and calibration were achieved by measuring the concentration of the cytokines spiked into the mixed samples.

2.6. Murine plasma cytokine/chemokine analysis

Twenty-five microliters of murine plasma were added to the capture beads, and they were incubated at 4 °C and gently shaken overnight. This process was followed by incubation with the second antibody and streptavidin-PE for signal amplification [13]. Using the Luminex 200 System (Luminex Corp., Austin, TX) for measurements, we applied a 5-parametric fitting curve to convert fluorescence intensity into concentration values. In addition, the cytokine concentration for each analyte was exported by xPonent software (Luminex Corp.). The cytokines/chemokines included in this assay were grouped into five categories: (1) growth factors: G-CSF, GM-CSF, stem cell factor (SCF), IL-3, vascular endothelial growth factor (VEGF), thymic stromal lymphopoietin (TSLP); (2) interleukins: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, and IL-12p70; 3) chemokines: keratinocyte chemoattractant (KC), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory proteins 1α (MIP-1α), macrophage inflammatory proteins 1γ (MIP-1γ), B lymphocyte chemoattractant (CXCL13), CXC chemokine ligand 16 (CXCL 16), CC chemokine ligand 11 (Eotaxin), T-cell activation 3 (TCA-3), thymus and activation regulated chemokine (TARC), and macrophage-derived chemoattractant (MDC); (4) tumor necrosis factor (TNF) super family members: TNF-α, cluster of differentiation 30 (CD30), CD40, fas ligand (FasL), and soluble TNF receptor type 1 (sTNF-R1); and (5) others: type I transmembrane receptor tyrosine kinase (Axl), intercellular adhesion molecule-1 (ICAM-1), and metallopeptidase inhibitor 1 (TIMP-1).

2.7. Statistical analysis

The plasma level of each analyte was plotted against the observation time following irradiation. Data was represented as group mean ± standard error of the mean (SEM from five mice in each group). To help us better understand the behavior of individual cytokines, we used the Bonferroni correction that controls the family-wise error rate (FWER) with a *t*-test to compare the control group with all other groups. A P-value <0.05 was regarded as statistically significant; a P-value <1 was selected to indicate a trend.

3. Results

3.1. Setup of multiplex bead array assay

Sandwich immunoassays were set up on the bead-based Luminex platform to analyze 32 cytokines/chemokines. The cross-reactivity test showed good specificity (data not shown). The correlation coefficient of the standard curve for each analyte was ⩾0.98. The intra-assay coefficient of variation (CV) for the tested analytes ranged from 1.94–17.67%, while the inter-assay CV was between 5.66% and 25.65%. Except for the relatively low recovery

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