Laparotomy in Mice Induces Blood Cell Expression of Inflammatory and Stress Genes

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Surgical trauma induces immune and stress responses although its effects on postsurgical inflammatory and stress gene expression remain poorly characterized. This study sought to improve current scientific knowledge by investigating the effects of laparotomy on mouse blood cell inflammatory and stress gene expression. Three-month-old male C57BL/6J mice were subjected to 2% isoflurane or 2% isoflurane with laparotomy and sacrificed 4 h postintervention. Blood was collected and blood cell expression of 158 genes central to inflammatory and stress responses was assayed using quantitative polymerase chain reaction arrays. Mice subjected to isoflurane with laparotomy, compared with mice receiving isoflurane alone, had >2-fold upregulation of genes in inflammation (*Osm*, *IL1rn*, *IL1b*, and *Csf1*), oxidative stress (*Hmox1*), heat shock (*Hspa1b*), growth arrest (*Cdkn1a*), and DNA repair (*Ugt1a2*). These genes demonstrated similar expression patterns by Pearson correlation and cluster analysis. Thus, laparotomy induces coordinated, postsurgical blood cell expression of unique inflammatory and stress genes whose roles in influencing surgical outcomes need further investigation.

Introduction

TRAUMA FROM SURGERY, including abdominal procedures, induces complex stress responses in immune cells that are pivotal in orchestrating tissue repair and immune defense. In the early postoperative period, macrophages and monocytes are activated and secrete proinflammatory mediators, including tumor necrosis factor alpha (TNF- α), interleukin (IL)-1 beta (IL-1 β), and IL-6, resulting in an early hyperinflammatory response (Gilliland and others 1997; Helmy and others 1999; Desborough 2000; Menger and Vollmar 2004). This early hyperinflammatory response peaks within hours to a few days depending on the type of trauma, and is subsequently terminated by compensatory cell-mediated immunosuppression through anti-inflammatory cytokines, including soluble TNF receptors (TNFsr-I and TNFsr-II), IL-1 receptor antagonist (IL-1RA), and IL-10 (Menger and Vollmar 2004). In addition to immune modulation, surgical trauma also induces oxidative bursts in lymphocytes, neutrophils, and macrophages, thus leading to alterations in oxidative stress equilibrium mediated by glutathione (GSH) and other reactive oxygen species (ROS) (Liu and others 1994; Kono and others 1995; Delogu and others 2001). While these early inflammatory and oxidative stress responses are important in facilitating tissue repair, uncontrolled early hyperinflammation, sustained compensatory cell-mediated immunosuppression, and prolonged oxidative stress activity predispose the host to

opportunistic infection, multiple-organ dysfunction, impaired tissue repair, and death in the postoperative period (Nathan and Shiloh 2000; Ni Choileain and Redmond 2006; Neher and others 2011; Wink and others 2011; Bryan and others 2012).

In immune cells, cellular stress induces concomitant release of inflammatory mediators and ROS. The complex interactions between these signaling pathways may constitute points of cross-talk between inflammatory and oxidative stress responses and result in feedback loops in the early postoperative period (Kregel and Zhang 2007). For instance, proinflammatory cytokines, such as colony stimulating factor-1 (CSF-1) and IL-1 β , can prime phagocytes thus resulting in a more rapid and amplified respiratory burst (Chitu and Stanley 2006; Bryan and others 2012). This disruption in intracellular redox balance induces expression of IL-1 β , IL-6, and TNF- α through the secondary messenger function of the TNF-nuclear factor-kappa B (NF- κ B) pathway (Reber and others 1998; Kregel and Zhang 2007; Naik and Dixit 2011). These inflammatory stimuli then further precipitate an increase in oxidative stress gene expression and mitochondrial ROS production and may contribute to an inflammation-ROS feed forward cycle (Laviano and others 2007; Moylan and Reid 2007; Zhou and others 2011). In addition to cross-talk between inflammation and oxidative stress signaling pathways, these responses may also influence other stress genes involved in cell cycle regulation and apoptosis (Song and others 2002; Chen and others 2009).

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Thus, cellular stress in immune cells impacts a number of stress genes with pleiotropic effects through complex interactions.

While inflammatory and oxidative stress responses after surgery have been investigated in a variety of surgical settings, most studies have focused on a small set of inflammatory and stress genes (Lin and others 2000; Ni Choileain and Redmond 2006). Moreover, interactions and points of cross-talk between various cellular stress signaling pathways in the postoperative period, which may be critical in maintaining cellular homeostasis during stress, remain under-investigated. Because of the important roles of tightly regulated cellular stress responses in optimizing postoperative outcomes, including tissue repair and immune functions, we sought to better characterize postoperative expression patterns of inflammatory and stress (oxidative stress, heat shock, growth arrest, and DNA repair) genes and the associations in their changes in expression in blood cells of mice subjected to a commonly utilized surgical procedure (laparotomy) and anesthesia (isoflurane) in mouse research. Here, we report a group of 8 genes across a range of stress responses, including inflammation (*Osm*, *Csf1*, *IL1rn*, and *IL1b*), oxidative or metabolic stress (*Hmox1*), heat shock (*Hspa1b*), growth arrest (*Cdkn1a*), and DNA repair (*Ugt1a2*), that are upregulated in a highly coordinated manner in blood cells after laparotomy.

Materials and Methods

Animals

All studies were approved by the institutional animal review board (Institutional Animal Care and Use Committee) at Icahn School of Medicine at Mount Sinai. All mice were obtained from The Jackson Laboratory and housed 5 per cage with free access to food and water under 12-h light and 12-h dark cycle (lights on at 07:00 h). Three-month-old male C57BL/6J mice were assigned, based on matched body weight, to receive 1 of 3 interventions: 2% isoflurane [Iso] ($n=8$), laparotomy with 2% isoflurane [Lap + Iso] ($n=8$), or intraperitoneal injection of normal saline (0.9% NaCl) solution [Sal] ($n=8$). Mice were euthanized by CO₂ 4 h postintervention. Blood was collected for blood cell reverse transcription-polymerase chain reaction (RT-PCR) with quantitative polymerase chain reaction (qPCR) arrays.

Interventions: isoflurane administration and laparotomy

Mice in the Iso and Lap + Iso groups were given vaporized inhaled isoflurane for a total duration of 15 min. Isoflurane (2%) was first delivered for 3 min in an induction chamber followed by its administration via face-mask for 12 min for maintenance.

Mice in the Lap + Iso group received laparotomy performed over 8 min. After adequate sedation with isoflurane, the mouse was placed in supine with limbs in full extension, hairs ventral to the midabdomen were clipped to the level of the skin, and abdominal skin was cleansed with alcohol prep pads. A 1-cm midline vertical marking was marked on the midabdomen and a superimposed 1-cm vertical midline incision traversing the skin, subcutaneous tissues, muscles, and peritoneum was made with a pair of fine surgical scissors. Abdominal organs were partially exposed for 2 min.

The abdominal wall was closed from cranial-to-caudal end by placing 4 interrupted sutures using 5-0 Sof silk™. Skin and subcutaneous tissue were then closed using the same approach.

Extraction of blood cell RNA and cDNA synthesis

After mice were euthanized by CO₂, trunk blood was immediately collected from the site of decapitation into ethylenediaminetetraacetic acid-containing tubes. Blood was then centrifuged and the resulting blood cell pellet (all cellular elements) was lysed with Buffer EL (Qiagen). Total RNA was extracted using an RNeasy mini kit (Qiagen) to obtain RNA for gene expression experiments by RT-PCR. Total RNA was quantified using a Biophotometer (Eppendorf). High-quality total RNA (1 µg) per blood sample was used for cDNA synthesis using RT² First Strand kit (Qiagen). All procedures were performed according to manufacturer's protocols.

RT-PCR with RT² Profiler™ PCR arrays

RT² Profiler PCR Array (Qiagen) technology for gene expression analysis provides the profiling capabilities of DNA microarray and the quantitative reliability and sensitivity of qPCR. Gene expression results from this technology are highly reproducible both within, and between, assay runs. Two cataloged RT² Profiler PCR Arrays (PAMM-150A Mouse Cytokines and Chemokines PCR Array and PAMM-003A Mouse Stress and Toxicity PCR Array) were used to simultaneously quantify mRNAs of 163 genes in 384-well plates according to the manufacturer's protocols. A full listing of these genes, categorized by functional grouping, consisted of chemokines (27 genes), ILs (25 genes), interferons (2 genes), growth factors (15 genes), TNF superfamily (10 genes), other inflammatory mediators (9 genes), oxidative or metabolic stress (27 genes), heat shock (10 genes), carcinogenesis (6 genes), growth arrest and senescence (6 genes), DNA damage and repair (11 genes), apoptosis signaling (10 genes), and housekeeping (5 genes). Gene symbols, descriptions, and Refseq of these 163 genes are provided in Supplementary Table S1 (Supplementary Data are available online at www.liebertpub.com/jir). The qPCRs were carried out using an ABI Prism 7900 thermocycler. Gene expression was determined by $\Delta\Delta C_t$ method using a web-based RT-PCR software provided by the manufacturer (Qiagen). Mean expression of 3 housekeeping genes (*Hprt*, *Hsp90ab1*, and *Actb*) on the array was used to provide a composite to normalize gene expression (ΔC_t). Subsequently, $\Delta\Delta C_t$ was calculated for each gene across 2 intervention groups using either Sal or Iso group as the control. Fold change for each gene was calculated as $2^{(-\Delta\Delta C_t)}$.

Data analysis

Body weight of groups of mice by intervention (Iso, Lap + Iso, or Sal), duration of isoflurane administration, and duration of laparotomy were presented as mean $\pm$ standard deviation. Differences in body weight of mice between intervention groups were tested by analysis of variance (ANOVA). Difference in duration of isoflurane administration was tested by 2-tailed *t*-test. Differences in gene expression between intervention groups were presented as fold change and tested by 2-tailed *t*-test. Associations of gene

expression levels between gene pairs (XY pairs of $\Delta\Delta Ct$) were tested by Pearson correlation. Distribution-insensitive cluster analysis for gene expression was performed using methods previously described (Tichopad and others 2006). Statistical analysis was performed using SAS (SAS Institute, Inc.). $P < 0.05$ indicated statistical significance. Scatter plots for gene pairs were generated by GraphPad Prism 4 for Macintosh.

Results

Isoflurane and laparotomy differentially affect expression of inflammatory and stress genes in blood cells

Groups of 3-month-old male C57BL/6J mice ($n=8$ per group) received either 2% isoflurane (Iso), laparotomy with 2% isoflurane (Lap+Iso), or intraperitoneal injection of 0.9% NaCl solution (Sal). The body weights were 26.16 ± 1.93 g, 26.86 ± 3.16 g, and 28.41 ± 1.83 g for the Iso, Lap+Iso, and Sal groups, respectively, and were not significantly different (ANOVA, $F=1.87$, $P=0.18$). The duration of isoflurane administration in mice was similar in Iso (15.13 ± 0.35 min) and Lap+Iso (15.38 ± 0.52 min) groups (t -test, $P=0.35$). The duration of laparotomy in mice in the Lap+Iso group was 8.13 ± 0.35 min.

The expression of 158 inflammatory and stress genes in blood cells 4 h postintervention (Iso, Lap+Iso, or Sal) was determined by RT-PCR arrays. A complete listing of the genes assayed is presented in Supplementary Table S1. The Mouse Cytokines and Chemokines PCR Array was used to quantify expression of inflammatory genes in the following functional groups: chemokines, ILs, interferons, growth factors, TNF superfamily, and other inflammatory mediators (except *Ccl21b*, *Nkfb1*, *Nos2*, and *Serpine1*). Gene expression for these inflammatory genes was calculated from groups of 7 mice for each intervention due to assay failure in 1 run of Mouse Cytokines and Chemokines PCR Array. The Mouse Stress and Toxicity PCR Array was used to quantify expression of stress genes in the following function groups: oxidative or metabolic stress, heat shock, carcinogenesis, growth arrest and senescence, DNA damage and repair, and apoptosis signaling, in addition to *Ccl21b*, *Nkfb1*, *Nos2*, and *Serpine1*. Gene expression for these stress genes was calculated from groups of 8 mice for each intervention.

The postintervention blood cell expression of inflammatory and stress genes was different in mice from the Iso and Lap+Iso groups (Table 1). Comparing to controls (Sal), mice from the Iso group had decreased gene expression of chemokines (*Ccl2* and *Cxcl10*), inflammatory mediator (*Serpine1*), and oxidative or metabolic stress mediators (*Mt2*, *Gstm1*, *Cyp1b1*, and *Fmo4*) by >2 -fold (Table 1, columns 6 and 7; $P < 0.05$). In contrast, mice from the Lap+Iso group compared with Sal had increased gene expression of ILs (*Il1rn*, *Il27*, and *Il1b*), growth factors (*Osm* and *Csf1*), oxidative or metabolic stress mediators (*Hmox1*), heat shock protein (*Hspa1b*), and apoptosis signaling (*Tnfrsf1a*), and decreased expression of ILs (*Il13* and *Il12b*), inflammatory mediators (*Serpine1* and *Nos2*), and oxidative or metabolic stress mediator (*Cyp4a10*), by >2 -fold (Table 1, columns 4 and 5; $P < 0.05$). Representative gene expression data for *Il1b*, *Il1rn*, *Csf1*, *Osm*, *Hmox1*, *Hspa1b*, *Cdkn1a*, and *Ugt1a2* expressed as mean relative gene ex-

pression (fold change) $\pm$ standard error of the mean are presented in Fig. 1 to provide a better sense of the variations among mice in each intervention group.

To evaluate the effects of laparotomy, correcting for effects of anesthesia, on postoperative expression of inflammatory and stress genes in blood cells, we compared gene expression results determined from mice in the Lap+Iso group with those in the Iso group (Table 2). Laparotomy significantly increased gene expression of chemokine (*Ccl2*), ILs (*Il1rn* and *Il1b*), growth factors (*Osm* and *Csf1*), inflammatory mediator (*Spp1*), oxidative or metabolic stress mediators (*Mt2*, *Hmox1*, and *Gstm1*), heat shock protein (*Hspa1b*), growth arrest and senescence (*Cdkn1a*), and DNA damage and repair (*Ugt1a2*), by >2 -fold ($P < 0.05$). In particular, the increased expression of 8 of these genes—*Il1rn*, *Il1b*, *Osm*, *Csf1*, *Hmox1*, *Hspa1b*, *Cdkn1a*, and *Ugt1a2*—was highly statistically significant ($P < 0.004$), and hence they were selected for subsequent Pearson correlation analysis.

Associations of inflammatory and stress gene expression in blood cells

Because of a consistent pattern of increased inflammatory and stress gene expression in blood cells of mice that received laparotomy, we further examined whether expression of these genes was correlated. To carry out this assessment, we compared gene expression levels ($\Delta\Delta Ct$) of 8 genes (*Il1rn*, *Il1b*, *Osm*, *Csf1*, *Hmox1*, *Hspa1b*, *Cdkn1a*, and *Ugt1a2*) as XY pairs by Pearson correlation (Table 3). These 8 genes were selected because of their highly significant ($P < 0.004$) increase in gene expression (>2 -fold) postlaparotomy. The expression of all 28 gene pairs was positively correlated (Pearson coefficient ρ , $0 < \rho < +1$) and the association of 27 of these gene pairs was significant ($P < 0.05$). In particular, 8 gene pairs (*Hmox1* vs. *Hspa1b*; *Hmox1* vs. *Osm*; *Ugt1a2* vs. *Csf1*; *Osm* vs. *Il1rn*; *Osm* vs. *Il1b*; *Il1rn* vs. *Il1b*; *Il1rn* vs. *Csf1*; and *Il1b* vs. *Csf1*) demonstrated high linear dependence ($\rho > 0.80$, $P < 0.0001$) (Fig. 2).

Cluster analysis is an analytical method that organizes observable data of similar kind into meaningful structures or groups (clusters). In an RT-PCR gene expression dataset, this type of analysis sorts genes into clusters in a way that the degree of association between 2 genes is maximal if they belong to the same cluster and minimal otherwise. To further determine meaningful gene structures in the expression data derived from the Mouse Cytokines and Chemokines and Mouse Stress and Toxicity PCR Arrays, we performed distribution-insensitive cluster analysis using methods previously described by Tichopad and others (2006). This analysis identified 29 clusters of genes with similar expression patterns, including clusters A and B shown in Table 4. Cluster A consisted of 10 genes and had high strength of associations within this cluster (R^2 with own cluster = 0.8962, $1 - R^2$ ratio = 0.3307). Of note, 7 of 10 genes (*Cdkn1a*, *Hspa1b*, *Hmox1*, *Ugt1a2*, *Osm*, *Il1b*, and *Il1rn*) from cluster A were highly expressed in blood cells of mice that received laparotomy (Lap+Iso) (Table 2). Similarly, cluster B consisted of 9 genes and also had high strength of associations within cluster (R^2 with own cluster = 0.8420, $1 - R^2$ ratio = 0.4063). The expression in 8 of 9 genes (*Spp1*, *Casp1*, *Il27*, *Gpi1*, *Gsr*, *Nfkb1a*, *Il15*, and *Csf1*) from cluster B was >1.4 -fold higher in mice from the Lap+Iso group.

TABLE 1. GENE DESCRIPTIONS AND EXPRESSION LEVELS IN BLOOD CELLS OF 3-MONTH-OLD C57BL/6J MICE 4 H POSTINTERVENTION, COMPARING LAPAROTOMY WITH ISOFLURANE (LAP+ISO) AND ISOFLURANE ALONE (ISO) TO SALINE-INJECTED CONTROL (SAL)

Functional grouping	Gene	Gene name	Lap + Iso:Sal		Iso:Sal	
			Fold change	P value	Fold change	P value
Chemokines	<i>Ccl2</i>	Chemokine (C–C motif) ligand 2	1.54	0.2202	0.40	0.0492
	<i>Cxcl10</i>	Chemokine (C–X–C motif) ligand 10	0.86	0.7774	0.46	0.0355
	<i>Cxcl9</i>	Chemokine (C–X–C motif) ligand 9	0.66	0.5420	0.53	0.0278
Interleukins	<i>Il1rn</i>	Interleukin 1 receptor antagonist	3.91	0.0091	0.91	0.3981
	<i>Il27</i>	Interleukin 27	2.62	0.0109	1.31	0.6038
	<i>Il1b</i>	Interleukin 1 beta	2.28	0.0078	0.87	0.5363
	<i>Il4</i>	Interleukin 4	0.67	0.0331	0.70	0.0718
	<i>Il13</i>	Interleukin 13	0.48	0.0014	1.56	0.3387
	<i>Il12b</i>	Interleukin 12B	0.48	0.0421	0.50	0.0525
Growth factors	<i>Osm</i>	Oncostatin M	3.53	0.0001	0.75	0.3354
	<i>Csf1</i>	Colony stimulating factor 1 (macrophage)	2.56	0.0115	1.27	0.9685
	<i>Gpi1</i>	Glucose phosphate isomerase 1	1.81	0.0034	1.29	0.3317
Other inflammatory mediators	<i>Nfkb1</i>	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, p105	1.59	0.0019	1.22	0.1825
	<i>Ctfl</i>	Cardiotrophin 1	0.60	0.0163	0.56	0.0215
	<i>Adipoq</i>	Adiponectin, C1Q and collagen domain containing	0.60	0.0177	0.63	0.0357
	<i>Serpine1</i>	Serine (or cysteine) peptidase inhibitor, clade E, member 1	0.46	0.0101	0.38	0.0031
	<i>Nos2</i>	Nitric oxide synthase 2, inducible	0.46	0.0024	0.57	0.0610
Oxidative or metabolic stress	<i>Hmox1</i>	Heme oxygenase (decycling) 1	3.18	0.0001	0.92	0.6512
	<i>Por</i>	P450 (cytochrome) oxidoreductase	1.86	0.0012	1.29	0.2654
	<i>Gsr</i>	Glutathione reductase	1.84	0.0010	1.20	0.4397
	<i>Hmox2</i>	Heme oxygenase (decycling) 2	1.68	0.0012	1.29	0.0615
	<i>Mt2</i>	Metallothionein 2	1.62	0.1536	0.38	0.0326
	<i>Fmo1</i>	Flavin containing monooxygenase 1	1.13	0.5852	0.59	0.0233
	<i>Gstm1</i>	Glutathione S-transferase, mu 1	0.98	0.8051	0.44	0.0359
	<i>Cyp1b1</i>	Cytochrome P450, family 1, subfamily b, polypeptide 1	0.53	0.0415	0.44	0.0005
	<i>Cyp4a10</i>	Cytochrome P450, family 4, subfamily a, polypeptide 10	0.48	0.0243	0.55	0.0526
	<i>Fmo4</i>	Flavin containing monooxygenase 4	0.36	0.0525	0.30	0.0331
Heat shock	<i>Hspa1b</i>	Heat shock protein 1B	2.87	0.0025	1.30	0.4560
	<i>Hspa8</i>	Heat shock protein 8	1.79	0.0013	1.19	0.2169
	<i>DnaJ1</i>	DnaJ (Hsp40) homolog, subfamily A, member 1	1.55	0.0009	1.26	0.0181
	<i>Hspe1</i>	Heat shock protein 1 (chaperonin 10)	1.34	0.0436	1.13	0.3863
	<i>Hspb1</i>	Heat shock protein 1	0.97	0.9187	0.65	0.0460
	<i>Cdkn1a</i>	Cyclin-dependent kinase inhibitor 1A (P21)	1.70	0.0299	0.67	0.1911
Growth arrest and senescence	<i>Ddit3</i>	DNA-damage inducible transcript 3	0.81	0.1064	0.67	0.0286
	<i>Rad50</i>	RAD50 homolog (<i>S. cerevisiae</i>)	1.46	0.1508	1.92	0.0130
DNA damage and repair	<i>Xrcc1</i>	X-ray repair complementing defective repair in Chinese hamster cells 1	0.79	0.0911	0.68	0.0242
	<i>Rad23a</i>	RAD23a homolog (<i>S. cerevisiae</i>)	0.66	0.0234	0.79	0.8773
Apoptosis signaling	<i>Tnfrsf1a</i>	Tumor necrosis factor receptor superfamily, member 1a	2.07	0.0149	1.15	0.8667
	<i>Nfkbia</i>	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	1.96	0.0051	1.26	0.5027
	<i>Casp1</i>	Caspase 1	1.75	0.0065	0.97	0.5898
	<i>Tradd</i>	TNFRSF1A-associated via death domain	1.62	0.0110	1.45	0.0688
	<i>Bax</i>	Bcl2-associated X protein	1.21	0.0134	0.95	0.7902
	<i>Bcl2l1</i>	Bcl2-like 1	0.65	0.0477	0.74	0.4302

Iso, isoflurane; Lap, laparotomy.

FIG. 1. Expression of *Il1b*, *Il1rn*, *Csf1*, *Osm*, *Hmox1*, *Hspa1b*, *Cdkn1a*, and *Ugt1a2* in blood cells of 3-month-old C57BL/6J mice 4h postintervention (Lap+Iso, Iso, or Sal) measured by quantitative polymerase chain reaction. Data for each gene were normalized to a panel of housekeeping transcripts and expressed as relative gene expression (fold change) compared with the Sal group. Data are mean $\pm$ standard error of the mean ($n=7-8$). * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Iso, isoflurane; Lap, laparotomy.

Discussion

In this study, we characterized blood cell stress responses to isoflurane and laparotomy in mice by determining expression of 158 genes central to inflammatory and stress responses using qPCR arrays. Isoflurane inhalation alone in mice decreased blood cell transcription of inflammatory (*Ccl2*, *Cxcl10*, and *Serpine1*) and oxidative or metabolic stress mediators (*Mt2*, *Fmo1*, *Fmo4*, *Gstm1*, and *Cyp1b1*). Laparotomy, after controlling for effects of isoflurane, increased postoperative blood cell expression levels of a

number of mRNA transcripts involved in mediating inflammation (*Ccl2*, *Il1rn*, *Il1b*, *Osm*, *Csf1*, and *Spp1*), oxidative or metabolic stress (*Mt2*, *Hmox1*, and *Gstm1*), heat shock (*Hspa1b*), growth arrest and senescence (*Cdkn1a*), and DNA damage and repair (*Ugt1a2*). Of these differentially expressed genes, 8 genes across a range of stress responses (*Il1rn*, *Il1b*, *Osm*, *Csf1*, *Hmox1*, *Hspa1b*, *Cdkn1a*, and *Ugt1a2*) were highly associated in expression pattern in response to surgical stress.

Isoflurane, a widely used inhalation anesthetic agent in veterinary and clinical medicine, has been shown to have anti-inflammatory immunomodulatory properties possibly mediated through inhibition of the NF- κ B pathway. Further, isoflurane may reduce expression of stress genes, such as *Hmox1* and *Hsp*, after lung injury in murine models (Faller and others 2012; Anderson and others 2014). Thus, our observations that isoflurane lowers the expression of inflammatory and stress genes are consistent with this body of literature. In contrast, laparotomy induced increased postoperative expression of a number of genes involved in immune regulation, including *Ccl2*, *Csf-1*, *Il1b*, *Il1rn*, *Il18*, *Osm*, *Il27*, *Tnfsf13b*, and *Spp1*. The recruitment of monocytes and macrophages in response to surgical trauma is a vital host response to tissue injury in order to eliminate invading pathogens by phagocytosis and to facilitate wound healing through trophic functions (Mouchemore and Pixley 2012). *Ccl2*, a member of the C-C chemokine family, is a potent chemotactic factor that regulates migration and infiltration of monocytes and macrophages from the blood stream across the vascular endothelium into site of tissue injury for immunological surveillance (Ajuebor and others 1998; Deshmane and others 2009). In addition, *Csf-1* is the primary growth factor for macrophages; it regulates progenitor proliferation and differentiation, and monocyte/macrophage survival and functions, such as cytokine production, cytotoxicity, and phagocytosis (Chitu and Stanley 2006; Mouchemore and Pixley 2012). In our study, expression of both *Ccl2* and *Csf-1* was increased after laparotomy, which may suggest an enhanced monocyte and macrophage involvement in the postoperative period. Moreover, CSF-1 induces monocyte expression of proinflammatory mediators, including IL-1 β , suggesting one explanation for the high degree of positive correlation between *Csf-1* and *Il1b* expression observed in this study (Chitu and Stanley 2006). Future investigation of intracellular protein levels of CCL2, CSF-1, and IL-1 β utilizing isolated monocytes is needed to better differentiate the effects of laparotomy on monocyte gene expression, proliferation, and migration.

IL-1 β , produced mainly by activated macrophages in response to injury and surgery, is one of the most important soluble mediators of inflammation and mediates production of IL-6 and other inflammatory mediators required to sustain an inflammatory response (Gilliland and others 1997; Helmy and others 1999; Desborough 2000; Menger and Vollmar 2004). IL-1RA, a protein encoded by *Il1rn*, inhibits IL-1 signaling and modulates IL-1 related immune response. In our study, expression of both *Il1b* and *Il1rn* was simultaneously increased and was highly associated after laparotomy, suggesting that IL-1 β activity may play an important role in postoperative stress response.

Glycoprotein 130 (gp130) is a ubiquitously expressed signal-transducing receptor on hematopoietic and non-hematopoietic cells that forms part of a receptor complex for

TABLE 2. GENE DESCRIPTIONS AND EXPRESSION LEVELS IN BLOOD CELLS OF 3-MONTH-OLD C57BL/6J MICE 4 H POSTINTERVENTION, COMPARING LAPAROTOMY WITH ISOFLURANE (LAP + ISO) TO ISOFLURANE ALONE (ISO)

Functional grouping	Gene	Gene name	Lap + Iso: Iso	
			Fold change	P value
Chemokines	<i>Ccl2</i>	Chemokine (C–C motif) ligand 2	3.82	0.0207
Interleukins	<i>Il1rn</i>	Interleukin 1 receptor antagonist	4.27	0.0000
	<i>Il1b</i>	Interleukin 1 beta	2.63	0.0004
	<i>Il27</i>	Interleukin 27	1.99	0.0123
	<i>Il18</i>	Interleukin 18	1.90	0.0479
	<i>Il15</i>	Interleukin 15	1.69	0.0025
Growth factors	<i>Osm</i>	Oncostatin M	4.72	0.0000
	<i>Csf1</i>	Colony stimulating factor 1 (macrophage)	2.01	0.0000
	<i>Gpi1</i>	Glucose phosphate isomerase 1	1.40	0.0298
TNF superfamily	<i>Tnfsf13b</i>	Tumor necrosis factor (ligand) superfamily, member 13b	1.93	0.0029
Other inflammatory mediators	<i>Spp1</i>	Secreted phosphoprotein 1	4.29	0.0440
	<i>Nfkb1</i>	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, p105	1.31	0.0434
Oxidative or metabolic stress	<i>Mt2</i>	Metallothionein 2	4.26	0.0275
	<i>Hmox1</i>	Heme oxygenase (decycling) 1	3.45	0.0000
	<i>Gstm1</i>	Glutathione S-transferase, mu 1	2.21	0.0133
	<i>Fmo1</i>	Flavin containing monooxygenase 1	1.92	0.0097
	<i>Gsr</i>	Glutathione reductase	1.53	0.0026
Heat shock	<i>Por</i>	P450 (cytochrome) oxidoreductase	1.44	0.0035
	<i>Hspa1b</i>	Heat shock protein 1B	2.21	0.0043
	<i>Hspa8</i>	Heat shock protein 8	1.50	0.0226
	<i>Dnaj1</i>	DnaJ (Hsp40) homolog, subfamily A, member 1	1.23	0.0464
Growth arrest and senescence	<i>Cdkn1a</i>	Cyclin-dependent kinase inhibitor 1A (P21)	2.54	0.0025
DNA damage and repair	<i>Ugt1a2</i>	UDP glucuronosyltransferase 1 family, polypeptide A2	2.76	0.0039
Apoptosis signaling	<i>Tnfrsf1a</i>	Tumor necrosis factor receptor superfamily, member 1a	1.81	0.0070
	<i>Casp1</i>	Caspase 1	1.80	0.0003
	<i>Nfkbia</i>	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	1.55	0.0119
	<i>Bax</i>	Bcl2-associated X protein	1.27	0.0369

several cytokines, including IL-6, IL-27, oncostatin M (OSM), and cardiotrophin 1 (Silver and Hunter 2010). In our study, laparotomy induced changes in expression of *Il27*, *Osm*, and *Ctfl* thus making gp130 and its downstream signaling pathways (JAK-STAT) a potential area of future investigation in surgery-induced immune response. OSM is a multifunctional cytokine of the IL-6 subfamily and can stimulate synthesis of IL-6 and CCL2 and contribute to immune cell recruitment (Brown and others 1991; Langdon

and others 1997). Thus, the robust expression of *Osm* and its strong association with *Il1b* and *Il1rn* after laparotomy makes it an interesting candidate gene to investigate in postoperative immune modulation.

Although TNF- α and IL-6 are potent mediators of inflammation that are frequently present in blood at elevated levels in the early postoperative period, *Tnf* and *Il6* expression was not upregulated in blood cells 4 h after laparotomy in our mouse model (Gilliland and others 1997; Helmy and

TABLE 3. ASSOCIATIONS OF INFLAMMATORY (*Osm*, *Il1rn*, *Il1b*, AND *Csf1*) AND STRESS (*Hmox1*, *Hspa1b*, *Cdkn1a*, AND *Ugt1a2*) GENES EXPRESSED IN BLOOD CELLS OF 3-MONTH-OLD C57BL/6J MICE 4 H POSTINTERVENTION (LAP + ISO, ISO, OR SAL) AS DETERMINED BY PEARSON CORRELATION

	<i>Hmox1</i>		<i>Hspa1b</i>		<i>Cdkn1a</i>		<i>Ugt1a2</i>		<i>Osm</i>		<i>Il1rn</i>		<i>Il1b</i>		<i>Csf1</i>	
	ρ	P value	ρ	P value	ρ	P value	ρ	P value	ρ	P value	ρ	P value	ρ	P value	ρ	P value
<i>Hmox1</i>			0.80	0.0000	0.55	0.0057	0.59	0.0024	0.81	0.0000	0.71	0.0003	0.79	0.0000	0.76	0.0001
<i>Hspa1b</i>	0.80	0.0000			0.61	0.0014	0.42	0.0401	0.73	0.0002	0.53	0.0127	0.54	0.0109	0.56	0.0077
<i>Cdkn1a</i>	0.55	0.0057	0.61	0.0014			0.39	0.0631	0.68	0.0007	0.61	0.0036	0.65	0.0015	0.67	0.0008
<i>Ugt1a2</i>	0.59	0.0024	0.42	0.0401	0.39	0.0631			0.55	0.0101	0.74	0.0001	0.78	0.0000	0.81	0.0000
<i>Osm</i>	0.81	0.0000	0.73	0.0002	0.68	0.0007	0.55	0.0101			0.89	0.0000	0.83	0.0000	0.71	0.0003
<i>Il1rn</i>	0.71	0.0003	0.53	0.0127	0.61	0.0036	0.74	0.0001	0.89	0.0000			0.85	0.0000	0.80	0.0000
<i>Il1b</i>	0.79	0.0000	0.54	0.0109	0.65	0.0015	0.78	0.0000	0.83	0.0000	0.85	0.0000			0.80	0.0000
<i>Csf1</i>	0.76	0.0001	0.56	0.0077	0.67	0.0008	0.81	0.0000	0.71	0.0003	0.80	0.0000	0.80	0.0000		

ρ , Pearson coefficient.

FIG. 2. Linear relationships between expression of inflammatory and stress genes in blood cells of 3-month-old C57BL/6J mice 4h postintervention (Lap+ Iso, Iso, or Sal). Each scatter plot shows gene expression measured in $\Delta\Delta Ct$ for a gene pair. Eight gene pairs (*Hmox1* vs. *Hspa1b*; *Hmox1* vs. *Osm*; *Ugt1a2* vs. *Csf1*; *Osm* vs. *Il1rn*; *Osm* vs. *Il1b*; *Il1rn* vs. *Il1b*; *Il1rn* vs. *Csf1*; and *Il1b* vs. *Csf1*) with high strength of linear dependence (Pearson correlation, $\rho > 0.80$, $P < 0.0001$) are shown.

others 1999; Desborough 2000; Menger and Vollmar 2004). In addition, with the exception of increased *Il1rn* expression, other markers of postoperative immunosuppression—such as increased *Il10* and decreased *Tnf*, *Il1b*, *Il12*, or *Ifng*—were not observed in our mice after laparotomy (Menger and Vollmar 2004). These differences may be accounted for by the time course of cytokine response (early hyperinflammation and subsequent immunosuppression) after surgery

(Partrick and others 1996; Ferguson and others 1997). Thus, future studies with more frequent perioperative blood sampling are needed to better characterize the changing trajectory of cytokine response after laparotomy. In addition, because the severity of tissue trauma endured during surgery may influence measurable cytokine levels, future studies that compare the effects of differing extent of surgical incisions on cytokine response are needed to better assess the impact

TABLE 4. CLUSTER LISTING FOR TWO CLUSTERS OF INFLAMMATORY AND STRESS GENES WITH SIMILAR EXPRESSION PATTERNS IN BLOOD CELLS OF 3-MONTH-OLD C57BL/6J MICE 4 H POSTINTERVENTION USING DISTRIBUTION-INSENSITIVE CLUSTER ANALYSIS

Cluster	Gene	R-squared with		
		Own cluster	Next closest	1 - R ² ratio
Cluster A	<i>Gstm1</i>	0.3138	0.3549	1.0636
	<i>Cdkn1a</i>	0.5371	0.4308	0.8133
	<i>Hspa1b</i>	0.5632	0.2348	0.5708
	<i>Tnfrsf1a</i>	0.7767	0.5900	0.5446
	<i>Hmox1</i>	0.7867	0.5457	0.4695
	<i>Ugt1a2</i>	0.8038	0.5992	0.4894
	<i>Osm</i>	0.8314	0.5857	0.4070
	<i>Por</i>	0.8469	0.7842	0.7098
	<i>Il1b</i>	0.8930	0.6022	0.2691
	<i>Il1rn</i>	0.8962	0.6862	0.3307
Cluster B	<i>Spp1</i>	0.5266	0.3132	0.6893
	<i>Casp1</i>	0.6199	0.4383	0.6768
	<i>Il27</i>	0.6934	0.4978	0.6104
	<i>Gpi1</i>	0.7480	0.4809	0.4855
	<i>Gsr</i>	0.7553	0.6335	0.6675
	<i>Nfkb1a</i>	0.7639	0.5211	0.4929
	<i>Il15</i>	0.7833	0.5313	0.4623
	<i>Hspa5</i>	0.7920	0.7069	0.7096
	<i>Csf1</i>	0.8420	0.6110	0.4063

of trauma severity on the immune response (Cruikshank and others 1990; Pape and others 2007).

Surgical trauma induces ROS production and oxidative bursts in immune cells (Liu and others 1994; Delogu and others 2001). In this study, isoflurane decreased expression of *Mt2*, *Fmo1*, *Fmo4*, *Gstm1*, and *Cyp1b1*, genes that encode for proteins that help cells maintain proper homeostasis of the redox system. In comparison, laparotomy increased expression of *Mt2*, *Fmo1*, *Gstm1*, *Gsr*, *Hmox1*, and *Por*, suggesting that inhaled anesthesia, such as isoflurane, and surgical procedure, such as laparotomy, may have vastly different postoperative effects on immune cell stress responses. Various types of surgical procedures cause a transient change in oxidative stress equilibrium. Some examples include increased lipid peroxidation and reduced liver GSH after abdominal surgery, increased lymphocyte ROS and reduced GSH after general surgery, elevated monocyte H₂O₂ after esophagectomy and gastrectomy, and increased serum malondialdehyde after cholecystectomy (Liu and others 1994; Kono and others 1995; Rajbala and others 1999; Delogu and others 2001; Bukan and others 2004). Moreover, surgery-induced oxidative stress may contribute to pathologic injury of tissues (Azevedo and others 2000; Delogu and others 2001; ten Raa and others 2006). Thus, the differential effects of laparotomy and isoflurane on oxidative stress in the postoperative setting may have potential clinical implications.

Reduced GSH is the most abundant free cellular thiol. It is essential in preserving a reduced intracellular environment that maintains intracellular redox homeostasis and protects cellular macromolecules against oxidizing damage (Circu and Aw 2010). An adequate intracellular level of reduced GSH is maintained through its regeneration catalyzed by glutathione reductase (GSR) and glutathione-S-

transferase (GST) (Meister and Tate 1976). In this study, laparotomy increased immune cell expression of *Gsr* and *Gstm1*, which therefore suggests that redox systems, such as GST, may participate in postlaparotomy stress response.

Heme oxygenase-1 (HO-1) provides another inducible defense mechanism that can be activated ubiquitously in cells in response to chemical and physical stress to confer cytoprotection against cellular injury (Ryter and others 2002). HO-1 functions as the rate-limiting step in the oxidative catabolism of heme to generate carbon monoxide, biliverdin-bilirubin, and ferrous iron (Fe²⁺), which provide cytoprotection through their anti-inflammatory, antioxidant, and antiapoptotic effects (Morse and others 2009). The transcription of *Hmox1* is induced by increased oxidative stress (increased ROS and reduced GSH) in activated immune cells under proinflammatory stress (Saunders and others 1991; Rizzardini and others 1994). Thus, in our study, the increased *Hmox1* expression observed in mice after laparotomy may be associated with the increased proinflammatory and oxidative stress state resulting from surgical trauma. This may be contributory to the high degree of associations between expression of *Hmox1* and inflammatory genes (*Il1rn*, *Il1b*, *Osm*, and *Csf1*) and constitute points of cross-talk between inflammation and oxidative stress pathways during the postoperative period (Tsuchihashi and others 2004).

Our data suggested that laparotomy affects immune cell expression of genes involved in apoptosis signaling, including *Tnfrsf1a*, *Tradd*, *Nfkb1a*, *Casp1*, and *Bcl2l1*. Extrinsic apoptosis signaling downstream of TNFRSF1A and TRADD occurs through activation of apoptosis signal-regulating kinase (ASK1) and executioner caspases (Circu and Aw 2012). In addition to proapoptotic signaling, TRADD also induces activation of NF- κ B, a transcriptional factor that mediates the transcription of a vast array of proteins involved in cell survival and proliferation, inflammatory response, and antiapoptotic factors (Circu and Aw 2010). The complex and often-conflicting effects mediated by these pathways suggest the existence of extensive cross-talk. Some examples of this cross-regulation include modulation of apoptosis signaling through GSH-dependent activation of ASK1 and the actions of HO-1 on NF- κ B and HSP70 (Frاند and Kaiser 2000; Song and others 2002; Morse and others 2009). Because NF- κ B is regulated by changes in intracellular redox balance and inflammatory state, it may serve as a convergence point for postoperative apoptotic, inflammatory, and oxidative stress responses in immune cells (Reber and others 1998; Song and others 2002; Kregel and Zhang 2007; Naik and Dixit 2011). Further investigations are needed to evaluate whether postoperative changes in cytokines and ROS affect the pleiotropic effects of NF- κ B and modify apoptosis regulation.

Aside from inflammatory and oxidative stress genes, laparotomy also increased postoperative blood cell expression of genes across a range of stress responses, including heat shock (*Hspa1b*), growth arrest and senescence (*Cdkn1a*), and DNA damage and repair (*Ugt1a2*). These genes have an overall protective effect in cells through their actions in maintaining a reduced cellular environment, inhibiting apoptosis, and reducing inflammation. Heat shock protein serves as intracellular chaperone to reduce protein denaturation due to oxidative stress, reduces ROS formation and preserves GSH levels, maintains mitochondrial membrane potential, inhibits apoptotic machinery, and inhibits NF- κ B signaling to reduce

inflammation (Guzhova and others 1997; Xu and Giffard 1997; Ouyang and others 2006; Giffard and others 2008). CDKN1A (p21) is a cyclin-dependent kinase that induces cell cycle arrest and DNA replication and repair in order to enhance cell survival in response to H₂O₂ oxidative damage and inhibits apoptosis through NF- κ B (Li and others 1994; Gartel and Tyner 2002; Chen and others 2009; Vitiello and others 2009). UDP glucuronosyltransferase 1 is a major phase II drug metabolizing enzyme that conjugates electrophiles and bilirubin into glucuronides to reduce accumulation of reactive intermediates that cause oxidative toxicity (Bock 2003). Thus, the high strength of associations of *Hspal1b*, *Cdkn1a*, and *Ugt1a* postoperative expression may reflect immune cells' compensatory mechanisms of maintaining redox homeostasis and limiting apoptosis in order to enhance survival in the setting of postsurgical stress.

While this study helped to identify a number of inflammatory and stress genes in immune cells that are upregulated in response to laparotomy, there were several limitations to our study. First, because cytokine trajectory changes after surgery, we were unable to determine whether the expression pattern of inflammatory genes 4 h postlaparotomy observed in our study was indicative of early hyperinflammatory response or compensatory cell-mediated immunosuppression (Partrick and others 1996; Ferguson and others 1997). Future investigations with increased frequency of perioperative blood sampling will likely better characterize immune cell inflammatory response after laparotomy. Second, there is evidence to suggest that the type of surgery, duration of surgery, and extent of musculoskeletal trauma may determine the severity of systemic inflammation and influence ROS release due to ischemic-reperfusion injuries and secondary infections (Cruickshank and others 1990; Moore 1999; Pape and others 2007; Neher and others 2011). However, the limited scope of surgical intervention (1-cm vertical midline incision without organ resection) and short duration of laparotomy (8 min) and postoperative monitoring period (4 h) used in our mouse model were unlikely to induce hypoperfusion or have sufficient time lapse to develop an infection. Moreover, the simplicity of the laparotomy procedure utilized in this study serves as a feasible and reproducible model to facilitate understanding of complex host stress responses induced by surgery. The RNA used in this study was extracted from a heterogeneous population of blood cells, including those in the myeloid and lymphoid lineages. Because T-helper cells may be involved in the development of immunosuppression after surgical trauma, isolation of specific subpopulations of leukocytes will likely further contribute to the understanding of Th1/Th2 balance and the resulting inflammatory response postsurgery (Brune and others 1999). Last, while this study provides novel characterization of laparotomy-induced inflammatory and stress responses at the mRNA level, additional studies that include protein expression determination and use of inhibitor or knockout mice are needed to gain further mechanistic understanding of surgery-induced stress responses.

In this report, we found a group of 8 genes across a range of stress responses, including inflammation (*Osm*, *Csfl*, *Il1rn*, and *Il1b*), oxidative or metabolic stress (*Hmox1*), heat shock (*Hspal1b*), growth arrest (*Cdkn1a*), and DNA repair (*Ugt1a2*), that are upregulated in a highly coordinated manner in blood cells in the early postoperative period after laparotomy. These associations, many of which are novel,

likely reflect the complexity of molecular interactions of the inflammatory, oxidative stress, and apoptotic pathways in maintaining cellular homeostasis and survival in the postoperative setting, and may suggest common mechanisms for coregulated gene expression or coordinated cell functions induced by surgical stress. While the clinical implications of these responses are currently unknown, further characterization of surgery-induced cross-talk between these biological pathways will likely lead to an improved understanding of some of the complex molecular mechanisms contributing to tissue repair, opportunistic infection, and multiple-organ dysfunction postsurgery.

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Authors' Contributions

F.K. and F.I. performed the experiments. F.K. wrote the article. All authors planned and designed the research study.

Author Disclosure Statement

The authors declare that no competing financial interests exist.

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