Does Uninjured Skin Release Proinflammatory Cytokines Following Trauma and Hemorrhage?

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Hypothesis: Uninjured skin contributes to the elevation in circulating levels of proinflammatory cytokines seen following severe injury.

Design: Male C3H/HeN mice underwent trauma, trauma-hemorrhage and resuscitation, or closed long-bone fracture. Serum, skin, and liver samples were harvested at designated times after experimental treatment.

Main Outcome Measures: Levels of interleukin (IL) 1β, IL-6, and tumor necrosis factor α (TNF-α) were determined in serum and skin cultures at 1, 8, and 24 hours after trauma-hemorrhage. The RNA was isolated from liver and skin samples at 1, 2, 4, 8, and 24 hours from all 3 experimental groups, and gene expression of the cytokines was determined.

Results: Remote (nontraumatized) skin from trauma-hemorrhage animals released significantly more IL-6 and TNF-α into culture supernatants at 1 and 24 hours and significantly more IL-1β at 1, 8, and 24 hours than did skin from sham animals. Serum levels of all 3 cytokines were significantly elevated at 1 and 24 hours after trauma-hemorrhage relative to sham animals. Gene expression of all 3 cytokines was detected in skin and liver following trauma-hemorrhage. Furthermore, gene expression of all 3 cytokines was detected in uninjured skin after soft tissue trauma and closed long-bone fracture.

Conclusions: Proinflammatory cytokine gene expression is up-regulated in uninjured skin following trauma, trauma-hemorrhage, and long-bone fracture. This increase in gene expression correlates with increased cytokine production by cultured skin as well as increased circulating cytokine levels. These results suggest that uninjured skin may also contribute to the rise in circulating cytokine levels seen after injury.

Arch Surg. 1999;134:368-374

Severe injury, particularly with concomitant hemorrhagic shock, predisposes patients to the development of infectious complications and multiple organ failure. In particular, hypotension induces a decline in cell-mediated immunity that is more profound in the presence of concurrent soft tissue trauma or long-bone fracture. Our laboratory has previously demonstrated that Kupffer cells are primed following hemorrhagic shock, and that unlike other macrophage populations, they produce increased amounts of proinflammatory cytokines under those conditions. In combination with activation of neutrophils, the systemic release of inflammatory mediators such as interleukin (IL) 1, IL-6, and tumor necrosis factor α (TNF-α) is believed to have a negative impact on the function of many organ systems.

The body’s largest immune organ, the skin, is composed of multiple cell types that are also capable of producing cytokines. Specifically, keratinocytes, fibroblasts, dermal endothelial cells, Langerhans cells, dendritic cells, and inflammatory cells infiltrating the skin are able to release cytokines under various conditions. Previous investigations have established that thermally injured skin produces detectable levels of several cytokines, including IL-1, IL-6, and TNF. Since early burn wound excision has been shown to restore the depressed cellular immune response seen after thermal injury, this suggests that skin-derived mediators are capable of modulating systemic immune function. Interestingly, cytokines produced by the skin in response to topical irritants have been shown to be measurable in draining lymph, indicating that skin-derived cytokines can reach the systemic circulation.

Direct injury to the skin is known to incite a vigorous immune response associated with wound healing. Furthermore, after the onset of severe shock, the skin is known to be one of the first organs from which blood is shunted to preserve central perfusion. Given the known ability of skin to produce cytokines in response to topical injury, and given the sustained cutaneous hypoperfusion that occurs...
MATERIALS AND METHODS

EXPERIMENTAL MODEL

We used inbred male C3H/HeN mice (Charles River Laboratories, Wilmington, Mass), 7 weeks of age. Mice in the trauma-hemorrhage groups were lightly anesthetized with methoxyflurane and restrained in a supine position, and a 2.5-cm midline laparotomy (ie, trauma induction) was performed. The incision was then closed aseptically using 6-0 monofilament nylon (Ethilon; Ethicon Inc, Somerville, NJ) sutures. Next, both femoral arteries were aseptically cannulated with polyethylene-10 tubing (Clay-Adams, Parsippany, NJ). Blood pressure was constantly monitored using a commercially available arterial blood pressure analyzer (DigiMed; Micro-Med Inc, Louisville, Ky). On awakening, the animals were bled rapidly through the other catheter to a mean (±SEM) arterial blood pressure of 35 ± 5 mm Hg (prehemorrhage mean ±SEM arterial blood pressure, 93 ± 5 mm Hg), which was maintained for 90 minutes. After hemorrhage, the animals were resuscitated with lactated Ringer solution (4 times the shed blood volume for 30 minutes), the catheters were removed, the vessels were ligated, and the groins incisions were closed. Animals undergoing sham operation (sham controls, group for trauma-hemorrhage) underwent anesthesia and the same groin dissection, which included ligation of both femoral arteries, but no hemorrhage or fluid resuscitation. Mice in the trauma group underwent anesthesia and midline laparotomy as described above; mice in the fracture group underwent anesthesia and had a unilateral closed tibial fracture induced as described previously. In the fracture group, the fractured limb was splinted to prevent further soft tissue injury or conversion to an open fracture. Control animals for the trauma and fracture groups underwent anesthesia and restraint (sham trauma) or splinting (sham fracture). Animals were killed at 1, 2, 4, 8, or 24 hours for RNA isolation or 1, 8, or 24 hours for skin culture. Different groups of animals were used for the experiments involving RNA isolation and skin culture. There was no mortality in any group within the 24 hours. All procedures were performed in accordance with the guidelines set forth in the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health. This project was approved by the Institutional Animal Care and Use Committee of Rhode Island Hospital and Brown University, Providence.

RNA ISOLATION

Animals were killed with an overdose of methoxyflurane, and skin and liver samples were harvested. The tissues were homogenized in thiocyanate and phenol in monophase solution (Tri-Reagent; Sigma-Aldrich Corporation, St Louis, Mo) using a Dounce homogenizer. To clarify the samples, they were centrifuged at 10 000g for 10 minutes, and the liquid phase was used. Chloroform was added, and the samples were shaken vigorously for 15 seconds and then allowed to stand at room temperature for 15 minutes. The samples were centrifuged at 12 000g for 15 minutes at 4°C, and the aqueous phase was transferred to a fresh tube to which isopropanol was added. The samples were again shaken and allowed to stand overnight at −20°C. Following centrifugation at 12 000g for 10 minutes at 4°C, the pellets were washed in 75% ethanol. The resultant pellets were resuspended in a minimal volume of diethylpyrocarbonate (DEPC)–treated water. The RNA was run on a 1% agarose gel using morpholino propane sulfonic (MOPS) running buffer to ensure there was no RNA degradation or contaminating genomic DNA.

REVERSE TRANSCRIPTASE–POLYMERASE CHAIN REACTION

A commercially available kit (RETROscript; Ambion, Austin, Tex) was used for the reverse transcription (RT) reaction as described by the manufacturer. Briefly, 2 µg of RNA was reversed transcribed to cDNA using a kit. The cDNA was then used in a polymerase chain reaction (PCR) amplification using the following primers: for IL-1β, forward primer 5’-GCTGGGTCTTCAGTCCCTG-3’ and reverse primer 5’-TCTTGCCTGCCTTCTTGG-3’; for IL-6, forward primer 5’-GGTGGGAGATGCTTGTTCT-3’ and reverse primer 5’-TTCCAGTCCCTGCTCTTCT-3’; and for TNF-α, forward primer 5’-GCTGGGTCTTCAGTCCCTG-3’ and reverse primer 5’-TCTTGCCTGCCTTCTTGG-3’.

RESULTS

GENE EXPRESSION FROM UNINJURED SKIN AND LIVER FOLLOWING TRAUMA-HEMORRHAGE

The results shown in Figure 1 investigated the expression of IL-1β, IL-6, and TNF-α in uninjured skin and liver samples harvested following trauma-hemorrhage and resuscitation. Skin samples (Figure 1, A) were obtained from dorsal uninjured skin that was remote from the site of laparotomy. Proinflammatory gene expression was also noted in the liver (Figure 1, B).

PROINFLAMMATORY CYTOKINE PRODUCTION IN ISOLATED SKIN

The results presented in Figure 2 demonstrate release of IL-1β, IL-6, and TNF-α from skin placed in culture media following trauma-hemorrhage and resuscitation. Production of IL-1β by skin was significantly greater in trauma-hemorrhage animals relative to sham animals at all times assayed. Production of IL-6, although greater in trauma-hemorrhage animals at all times, was only found to be elevated to a significant degree at 1 and 24 hours after resuscitation. Production of TNF-α similarly was elevated in trauma-hemorrhage animals relative to sham animals at all times; however, statistically significant increases were seen only at 1 and 24 hours.

CIRCULATING LEVELS OF PROINFLAMMATORY CYTOKINES

Serum levels of IL-1β, IL-6, and TNF-α were elevated in trauma-hemorrhage animals relative to sham animals at 1 and 24 hours. Maximal circulating levels were seen at...
was added to the supplied deoxynucleotidetriphosphate (dNTP) mix and first-strand primers (Random Decamers; Ambion) in a final volume of 20 mL. The cocktail was heated to 85°C for 3 minutes and placed on ice. To this, 10× RT-polymerase chain reaction (PCR) buffer, plasmid RNase inhibitor, and Moloney-murine leukemia virus RT were added and incubated at 42°C for 1 hour. The mixture was incubated at 92°C for 10 minutes to inactivate the RT, and the resultant complementary DNA was used in the PCR. A DNA Taq polymerase kit (SuperTaq; Ambion) was used to perform PCR as described by the manufacturer. Briefly, 4 mL of RT product was added to the reaction master mix (containing 10× reaction buffer and magnesium chloride, dNTP mix, and 3′- and 3′-primers) in a final volume of 24 mL. The mixture was vortexed and heated to 95°C for 3 minutes, followed by 58°C for 5 minutes. Samples were then placed at 72°C, and 1 mL (0.5 U) of SuperTaq enzyme was added. The samples were run through a 30-cycle amplification on a programmable thermocycler gradient 96 (RoboCycler; Stratagene, La Jolla, Calif) set up as follows: 95°C for 1.5 minutes, 58°C for 2.5 minutes, and 72°C for 3 minutes. The primers for murine IL-1, IL-6, and TNF-α were obtained from Clontech, Palo Alto, Calif, and used according to the manufacturer’s specifications. To control for RT activity and ensure uniform sample concentration, a control primer (S15 rRNA) was run with all samples. Resultant amplified DNA was run on a 2% agarose gel containing ethidium bromide, and the gels were photographed using commercially available film (Polaroid ISO 3000/36; Polaroid Corp, Cambridge, Mass). Images were captured using commercially available image analysis software (Mocha; Jandel Scientific, San Rafael, Calif). Results shown represent repeated experiments (n = 4).

**SKIN CULTURE**

At the appropriate time after the experimental procedure, animals were killed using methoxyflurane overdose. The dorsal surface of the animal was shaved and rinsed with 70% ethanol. A 2×2-cm sample of skin was dissected free from the underlying fascia and minced using surgical scissors. The tissue was placed in 2 mL of RPMI 1640 media supplemented with 10% fetal bovine serum, 10-mg/mL gentamycin, and 100-U/mL penicillin. The samples were incubated under standard tissue culture conditions (37°C, 5% carbon dioxide, and 85% humidity) for 8 hours. Following incubation, the supernatants were harvested and centrifuged at 10 000g and frozen at −80°C until assayed for cytokine concentration.

**SKIN SUPERNATANT CYTOKINE PRODUCTION**

The levels of IL-1β, IL-6, and TNF-α present in skin supernatants were determined using commercially available murine enzyme-linked immunosorbent assay (ELISA) kits. Six animals were included for each time. The IL-1β and TNF-α ELISA kits (DuoSet) were obtained from Genzyme Diagnostics (Cambridge, Mass). The IL-6 ELISA kit (OptEIA) was obtained from PharMingen (San Diego, Calif). The kits were used as described by the manufacturer.

**CIRCULATING CYTOKINE LEVELS**

Whole blood samples were obtained from sham and trauma-hemorrhage groups by cardiac puncture (n = 6) at the appropriate time after experimental treatment and methoxyflurane overdose. The samples were centrifuged at 10 000g for 10 minutes in serum separator tubes (Microtainer; Becton Dickinson, Franklin Lakes, NJ). The serum was removed and frozen at −80°C until assayed for cytokine concentration. Cytokine levels were determined using the ELISA kits described above.

**STATISTICS**

Differences between experimental groups were considered to be statistically significant if P<0.05 as determined using 1-way analysis of variance and Tukey test.

1 hour for all 3 cytokines. These results are presented in the Table.

**GENE EXPRESSION FROM INJURED AND UNINJURED SKIN FOLLOWING SOFT TISSUE TRAUMA**

The results in *Figure 3A,* demonstrate that IL-1β, IL-6, and TNF-α gene expression is up-regulated in injured and uninjured skin following laparotomy (ie, soft tissue trauma) alone, in the absence of hemorrhagic shock. *Figure 3B,* also demonstrates proinflammatory cytokine gene expression in uninjured remote skin following unilateral closed tibial fracture.

**COMMENT**

Investigators have long sought the mechanisms responsible for the depression in organ function noted after injuries. The discovery and characterization of soluble mediators of inflammation, including cytokines and prostanoids, has greatly facilitated this study. Specifically, the proinflammatory cytokines IL-1β, IL-6, and TNF-α have been implicated in the mediation of organ dysfunction following hemorrhage and/or injury. The prevailing hypothesis regarding cytokine production following trauma and hemorrhage holds that the liver is the organ responsible for elaboration of these mediators. Ayala et al17 demonstrated that production of Kupffer cell IL-1 and TNF was significantly increased following trauma and hemorrhage. Furthermore, O’Neill et al18 reported that hepatic Kupffer cells were responsible for IL-6 release following trauma-hemorrhage and resuscitation and that depleting or inhibiting Kupffer cells with gadolinium chloride reduced the circulating levels of IL-6 by 80%. The effect of gadolinium chloride on the skin cytokine expression remains unknown, as does the effect of gadolinium chloride treatment on IL-1 and TNF production; thus it is not clear whether the liver is the sole organ affected by gadolinium chloride treatment. Using a model of hemorrhagic shock in rats, Deitch et al19 found that portal vein levels of IL-1 and TNF were significantly higher than the concentration of these cytokines in cardiac (ie, systemic) blood, allowing them to con-
clude that the gut becomes a cytokine-liberating organ following hemorrhagic shock as well.

Our results indicate that proinflammatory gene expression is induced in the liver following trauma and hemorrhage. We also report an increase in IL-1β, IL-6, and TNF-α gene expression in uninjured skin following trauma-hemorrhage and resuscitation. Skin from trauma-hemorrhage animals released significantly more IL-1β, IL-6, and TNF-α at 1 and 24 hours after resuscitation, indicating that the increase in gene expression correlates with an increased elaboration of these cytokines. Since the skin is the largest immune organ in the body, and since it is capable of producing proinflammatory cytokines following trauma-hemorrhage, we suggest that skin may be another important source of circulating cytokines following injury. Evidence supporting the ability of skin to produce systemic elevations in cytokine levels is present in the literature. Skin injured by UV radiation is known to release histamine, kinins, cytokines, and cyclooxygenase- and lipoxygenase-derived products of

Circulating Levels of Proinflammatory Cytokines After Trauma-Hemorrhage and Resuscitation

Table: Circulating Levels of Proinflammatory Cytokines After Trauma-Hemorrhage and Resuscitation

<table>
<thead>
<tr>
<th>Time of Harvest, h</th>
<th>Sham</th>
<th>Trauma-Hemorrhage</th>
<th>Sham</th>
<th>Trauma-Hemorrhage</th>
<th>Sham</th>
<th>Trauma-Hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-1β, pg/mL</td>
<td>IL-6, pg/mL</td>
<td>TNF-α, pg/mL</td>
<td>IL-1β, pg/mL</td>
<td>IL-6, pg/mL</td>
<td>TNF-α, pg/mL</td>
</tr>
<tr>
<td>1</td>
<td>43.6 ± 13.4</td>
<td>129.6 ± 18.4†</td>
<td>16.5 ± 16.5</td>
<td>830.5 ± 150.1†</td>
<td>0</td>
<td>365.9 ± 49.7†</td>
</tr>
<tr>
<td>8</td>
<td>38.4 ± 9.3</td>
<td>75.4 ± 27.0</td>
<td>24.8 ± 15.7</td>
<td>172.6 ± 67.9</td>
<td>0</td>
<td>88.8 ± 51.3</td>
</tr>
<tr>
<td>24</td>
<td>20.3 ± 9.5</td>
<td>93.6 ± 20.0†</td>
<td>50.8 ± 18.8</td>
<td>457.1 ± 181.2†</td>
<td>8.4 ± 8.4</td>
<td>127.2 ± 59.4†</td>
</tr>
</tbody>
</table>

*IL indicates interleukin; TNF, tumor necrosis factor. Serum levels of IL-1β, IL-6, and TNF-α were determined using enzyme-linked immunosorbent assay at 1, 8, and 24 hours after completion of resuscitation. Data are represented as mean ± SEM and compared using 1-way analysis of variance and Tukey test. P<.05 compared with animals undergoing sham operation (sham animals).
ies from our laboratory have shown that hypoxemia, in production of proinflammatory cytokines. Previous stud-
fusion and regional hypoxia, skin cells respond with the tempting to speculate that under conditions of hypoper-
tokine gene expression in skin remains unknown. It is account for the elevation in circulating cytokine levels induce cytokine gene expression in remote skin may inhibit immune function. Thus, evidence suggests that bone fracture induces the release of mediators that
orrhagic shock than hemorrhage alone, suggesting that this question may be answered by using the tech-niques responsible for the up-regulation of proinflammatory cytokine production may allow us to tailor our therapy to prevent their release. Our data indicate that injured and uninjured skin are potential sources of circulating cytokines following hemorrhagic shock and severe injury. Further study aimed at elucidating the signals responsible for up-regulating skin cytokine production may allow us ultimately to block the production of proinflammatory cytokines after hemorrhagic shock, thus reducing the morbidity and mortality associated with severe injury.

Figure 3. Proinflammatory cytokine gene expression after soft tissue trauma (A) and closed long-bone fracture (B). Expression of interleukin (IL)-1β, IL-6, tumor necrosis factor α (TNF-α), and S15 rRNA (positive control for RNA quality) was determined using reverse transcriptase–polymerase chain reaction with RNA isolated from injured and uninjured skin (A) or remote, uninjured skin (B). Samples were harvested 1, 2, 4, 8, or 24 hours after injury. The sham control (S) corresponds to 1 hour. The lane at the far right (plus sign) is a positive control indicating molecular weight of the amplified polymerase chain reaction product.

arachidonic acid metabolism,20 with these agents mediating the local inflammatory reaction evident histopatho-logically as sunburn. It has also been demonstrated that circulating levels of TNF-α are elevated in subjects with severe sunburn.21 Furthermore, patients with primary cold urticaria, a skin condition in which a shocklike re-
response occurs after cold exposure, have been found to have elevated levels of TNF-α in effuent venous blood from hands immersed in cold water.22 Clearly, then, the literature supports the notion that skin is able to pro-
duce cytokines that enter the systemic circulation.

Our data support the additional hypothesis that after distant injury, uninjured skin is able to up-regulate proinflammatory genes. Cytokine expression was detectable using RT-PCR after laparotomy (ie, soft tissue injury) and unilateral closed tibial fracture. As anticipated, there was also a profound up-regulation of cyto-

ke expression noted in injured skin. Previous investiga-
tors have found that following thermal injury, unburned skin produces TNF, IL-6, and IL-8 at the mes-
senger RNA (mRNA) and protein levels.10 In addition, Wichmann et al23 have shown immune function to be more compromised after closed bone fracture and hem-
orrhagic shock than hemorrhage alone, suggesting that bone fracture induces the release of mediators that inhibit immune function. Thus, evidence suggests that soft tissue injury and long-bone fracture induce cytokine production in uninjured skin, and that the ability to induce cytokine gene expression in remote skin may account for the elevation in circulating cytokine levels associated with such injuries.

The mechanism mediating the up-regulation of cy-
tokine gene expression in skin remains unknown. It is tempting to speculate that under conditions of hypoper-
fusion and regional hypoxia, skin cells respond with the production of proinflammatory cytokines. Previous studies from our laboratory have shown that hypoxemia, in

the absence of hypotension or blood loss, is sufficient stimulus to induce the production of IL-1, IL-6, and TNF from Kupffer cells.24 However, this does not explain the expression of cytokines in truly uninjured tissue (ie, that obtained following simple laparotomy or distant long-
bone fracture). We propose that humoral factors are released at the site of injury and then travel through the circulation to the skin, or that impulses are transmitted via the central nervous system and ultimately act to up-regulate proinflammatory cytokine gene expression. To further elucidate these mechanisms, pretreatment of ani-
mals with anti-inflammatory drugs or nerve blocks would be illustrative.

Although this study provides evidence that the skin may be an important source of elevated cytokine levels following multiple types of injuries, many questions remain unanswered. We have not demonstrated the cellular source of cytokine expression in skin. As previously noted, skin contains numerous different cell populations known to produce cytokines. We propose that this question may be answered by using the technique of in situ hybridization. Despite the evidence that cytokines are spontaneously released from cultured skin after trauma-hemorrhage, we have not demonstrated that these cytokines in fact reach the circulation. In addi-
tion, the time course of gene expression and cytokine re-
lease from the tissue differed somewhat. Although IL-1β and TNF-α gene expression was elevated as early as 1 hour and as late as 24 hours after completion of resus-ci-tation, IL-6 gene expression was limited to earlier times (1 and 2 hours). This is in contrast to tissue IL-6 re-
lease, which was elevated as late as 24 hours. Finally, cir-
culating levels of IL-6, although elevated 24 hours after injury, were much higher at 1 hour, whereas IL-6 pro-
duction in tissue culture remained elevated through 24

Statement of Clinical Relevance

Sepsis syndrome and multiple organ failure are the leading causes of death after hemorrhagic shock. The pos-
tulated mechanism responsible for this is an uncon-
trolled systemic inflammatory response. Circulating levels of proinflammatory cytokines are elevated after injury and hemorrhagic shock, and the gut and liver have been postulated to be the organ systems responsible for releasing these soluble mediators. Clinical trials aimed at reducing the systemic inflammatory response using anti-
cytokine antibodies have shown promise in the experi-
mental setting, yet they have been disappointing in hu-
man clinical trials. A better understanding of the mechanisms responsible for the up-regulation of proin-
flammatory cytokine production may allow us to tailor our therapy to prevent their release. Our data indicate that injured and uninjured skin are potential sources of circulating cytokines following hemorrhagic shock and severe injury. Further study aimed at elucidating the signals responsible for up-regulating skin cytokine production may allow us ultimately to block the production of proinflammatory cytokines after hemorrhagic shock, thus reducing the morbidity and mortality associated with severe injury.
hours. Taken together, this suggests that skin is not the principal source of circulating IL-6 after injury.

Our study demonstrates that circulating proinflammatory cytokine levels are elevated following trauma-hemorrhage, and that this elevation correlates with enhanced gene expression and tissue culture production of these cytokines from uninjured skin. Furthermore, gene expression of IL-1β, IL-6, and TNF-α are up-regulated in uninjured skin after soft tissue trauma and closed bone fracture. Given the known deleterious effects of circulating proinflammatory cytokines on organ function after severe injury, therapies aimed at reducing or eliminating skin production of cytokines may be beneficial to the care of the trauma patient. Although extrapolation of these results to the human system is premature, given the accessibility of skin, reproduction of this study in humans should be possible.

This investigation was supported by grant R01 GM 37127 from the National Institutes of Health, Bethesda, Md (Dr Chaudry).


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REFERENCES


DISCUSSION

Herbert B. Hechtman, MD, Boston, Mass: These are superb testable hypotheses with crisp data, giving us the nicely presented results. Drs Catania and Chaudry have continued to educate us at these meetings with regard to the mechanisms of inflammation following trauma. The subject matter, that is, cytokines, is in step with their recent emphasis on Kupffer cells that we heard about last year.

There are 3 separate models that are presented, each of which stimulates cytokine production by skin. Following hemorrhage and resuscitation, one might argue that the cytokine response is secondary to the generalized as well as local stimulus of ischemia-reperfusion. One could state the obvious that the so-called sham group, those animals that had only their femoral arteries ligated, also expressed cytokine mRNA.

I had the opportunity of seeing the manuscript and studying the blots which are presented there, but might have escaped your scrutiny during this presentation. This is fascinating to me. Would the authors think that this rise in mRNA could be a remote tissue response to hind-limb ischemia and reperfusion much as occurs remotely in other organs that they've described, such as the liver and the lung?

Possible findings in another sham group are more perplexing. That a sham incision leads to message expression for TNF-α indicates that multiple variables may be operative. Is there any information available regarding the role of changes in skin temperature on cytokine production?

Your observation of the importance of a simple laparotomy incision in provoking remote cytokine gene expression was really unexpected. It's reminiscent of our experience in patients of prominent systemic prostacycline synthesis accomplished by a slight but significant fall in blood pressure following simple laparotomy. Could you speculate about the mechanism of your observation? You imply that circulating mediators are of primary importance, and I certainly agree.

Dr Catania: There are quite a few questions to answer. In terms of the up-regulation of IL-1 that was evident in the sham group, I agree with you there is something occurring during the sham procedure. Whether it is related to the hind-limb ischemia, which could result from our bilateral catheterization of the femoral arteries, or in the case of shams, tying off the femoral arteries, or whether it is due to the incision itself, I'm not entirely sure. What is clear is that in animals that undergo no surgical procedure, there is no up-regulation of IL-1 in skin, so there is some component to the sham procedure which does elevate gene expression.
In terms of the elevated TNF in sham incisions, TNF was a rather interesting gene to work with, both in the liver and in the skin. We did see TNF expression variably in our sham animals, usually at very low levels. Whether this has something to do with the change in tissue temperature, or whether it has something to do with the actual procedure of anesthesia and restraint which the sham animals all undergo, I’m not exactly sure.

I agree with you that the findings following simple laparotomy were somewhat unexpected. When we designed this experiment, we actually thought that laparotomy would be a negative control. We believed that the main driving force behind cytokine expression in skin following trauma-hemorrhage would be hypotension and tissue hypoxia or hypoperfusion. There are a few experiments we would like to perform to further elucidate the mechanisms responsible for this up-regulation in gene expression. A soluble factor, such as prostanoid, may mediate this effect, and pretreatment with either ibuprofen or indomethacin to block these pathways would be interesting to determine if this is the mechanism which regulates skin gene expression. However, given the fact that this up-regulation occurs so rapidly, I also think that there is a fair chance the central nervous system is playing a role, and as we all know, the skin is very densely innervated. We would like to try to denervate the area where we perform our laparotomy and see if that has any effect on gene expression.

Mark Callery, MD, Worcester, Mass: I’d like you to just talk to us about nature for a second if you would. It seems that nature put 2 of the largest macrophage populations in the body conveniently at an environmental interface, namely, the macrophages of the skin (air) and the macrophages of the liver (gut). So my question to you is whether this response is a protective response that nature has provided, or is it wrong just because you’re saying it’s producing cytokines in response to an injury?

Dr Catania: I would be hesitant to ever criticize nature on evolution, which seems to have done a pretty good job in most cases. In the case following trauma and hemorrhage, however, I don’t know that we were necessarily meant to survive some of the injuries that we now routinely survive, thanks to medical intervention. Whether or not a less vigorous response would be protective in a less significant injury, I can only assume that it would be. This is why we’re always hesitant to tinker with the cytokine release, because clearly if it didn’t have some beneficial role it would have evolved away.

Exactly what constitutes a good response and what constitutes an overactive response remains to be determined.

Brad Patterson, MD, Weybridge, Vt: For those of us who are not very familiar with cytokine levels, could you make some comment about whether these are important at a systemic level, the kind of thing we’re used to hearing about as side effects of cytokines?

Dr Catania: I believe most cytokines function in a microenvironment. When we start seeing high levels of cytokines systemically, that’s usually when the cytokines are implicated in having a negative role. What the levels of the cytokines are in the microcirculation around the area of injury is probably the important factor in things like wound healing. Clearly the levels of cytokines in a healing wound are well in excess of the systemic levels we see here. However, when systemic cytokines levels do increase significantly, their action becomes less predictable. Their influence on neutrophil function following gut ischemia, for example, has been fairly well described by Dr Hechtman’s group. Based on previous research, we presume that systemic elevations in cytokine levels have a detrimental effect.

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**Surgical Anatomy**

The branches of the thyrocervical trunk may be remembered by the acronym, STAT:

- S = suprascapular artery
- T = transverse cervical artery
- A = ascending cervical artery
- T = thyroid artery, inferior