Recognition of Hyaluronan Released in Sterile Injury Involves a Unique Receptor Complex Dependent on Toll-like Receptor 4, CD44, and MD-2*§

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Inflammation under sterile conditions is not well understood despite its importance in trauma and autoimmune disease. To investigate this process we established mouse models of sterile injury and explored the role of hyaluronan in mediating inflammation following injury. The response of cultured monocytes to hyaluronan was different than the response to lipopolysaccharide (LPS) despite both being dependent on Toll-like receptor 4 (TLR4). Cultured cells exposed to hyaluronan showed a pattern of gene induction that mimics the response seen in mouse skin after sterile injury with an increase in molecules such as transforming growth factor-β2 and matrix metalloproteinase-13. These factors were not induced by LPS despite the mutual dependence of both hyaluronan and LPS on TLR4. Explanation for the unique response to hyaluronan was provided by observations that a lack of TLR4 or CD44 in mice diminished the response to sterile injury, and together with MD-2, was required for responsiveness to hyaluronan in vitro. Thus, a unique complex of TLR4, MD-2, and CD44 recognizes hyaluronan. Immunoprecipitation experiments confirmed the physical association of TLR4 and CD44. Taken together, our results define a previously unknown mechanism for initiation of sterile inflammation that involves recognition of released hyaluronan fragments as an endogenous signal of tissue injury.

Inflammation, as defined by changes in vascular permeability and leukocyte recruitment, is an essential step for the control of microbial invasion. Specific microbial products trigger this process through a diverse array of innate immune pattern recognition receptors. However, an inflammatory response is also important for processes independent of infection. For example, normal wound healing requires a controlled inflammatory response to enable the recruitment of monocytes and the subsequent release of pro-inflammatory mediators. Identification of the triggers of sterile inflammation represents an important goal with immediate diagnostic and therapeutic significance.

Among the best-studied microbial elements responsible for triggering the host inflammatory response is lipopolysaccharide (LPS). This is a major component of Gram-negative bacteria and is an extrinsic pathogen-associated molecule recognized by host Toll-like receptor 4 (TLR4) (1). The ability of LPS to signal through TLR4 requires the presence of several accessory molecules, including LPS-binding protein (2), CD14 (3, 4), and MD-2 (5–7). Following engagement of TLR4 with an appropriate co-accessory molecule, signaling occurs through Toll/interleukin-1 receptor-like domain-containing MyD88 (8), Mal (9), TRIF/TSCAM-1 (10, 11), or TRAM/TICAM-2 (10, 12). These signaling pathways lead to the activation of a variety of components vital for resistance to infection and associated with inflammation, including antimicrobial peptides, interleukins, cytokines, chemokines, and tumor necrosis factor (13–17).

Recently, the glycosaminoglycan hyaluronan (HA) has also been identified as an inducer of TLR4 activation, capable of causing the release of pro-inflammatory cytokines (18–21). It has been suggested that trauma can release small molecular weight HA fragments (sHA) from the extracellular matrix, thus potentially presenting HA as an intrinsic signal of inflammation. In support of this hypothesis are observations that inflammation following chemical injury by bleomycin is altered in mice deficient in either MyD88 or TLR4/TLR2 (21). This model results in release of HA but also is available to respiratory microbes, therefore complicating interpretations and analysis of the elements involved in the initial trigger and response systems.

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1 The on-line version of this article (available at http://www.jbc.org) contains supplemental Table S1 and Fig. S1.

2 The abbreviations used are: LPS, lipopolysaccharide; GAG, glycosaminoglycan; WFGAG, wound fluid GAG; HA, hyaluronan; sHA, small molecular weight HA fragment; PBS, phosphate-buffered saline; TGF, transforming growth factor; MMP, matrix metalloproteinase; IL-8, interleukin-8; EGFR, epidermal growth factor receptor; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; TBS, Tris-buffered saline; MIP-2, macrophage inflammatory protein-2.

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We sought to develop mouse models of sterile injury to determine innate elements that recognize and mediate sterile inflammation. Our results demonstrate that the initiation of a sterile intrinsic inflammatory process is dependent on TLR4 activation and resembles signaling events that are activated by HA. Furthermore, a novel alternative recognition complex is defined, involving the contribution of TLR4, MD-2, and CD44. Additionally, this unique receptor complex and downstream signaling events specific to the injury response alter the host's ability to respond to subsequent sepsis induced by LPS activation. These observations provide new insight into the mechanisms responsible for sterile inflammation.

EXPERIMENTAL PROCEDURES

Cells, Media, and Reagents

The mouse alveolar macrophage cell line MH-S was purchased from American Type Culture Collection (ATCC, catalog CRL-2839). Cells were maintained in RPMI 1640 media supplemented with 1-glutamine, 10% heat-inactivated fetal calf serum, penicillin/streptomycin (100 units/ml and 50 μg/ml, respectively), and 0.05 mM 2-mercaptoethanol. The human monocyte THP-1 cell line was purchased from ATCC (catalog TIB-202). Cells were grown in RPMI 1640 media supplemented with 1-glutamine, 10% fetal calf serum, penicillin/streptomycin (100 units/ml and 50 μg/ml, respectively), 0.05 mM 2-mercaptoethanol, 1 mM sodium pyruvate, and 2.25 g of glucose. Non-transfected HEK293 cells were purchased from ATCC (CRL-1573) and grown in Dulbecco’s modified Eagle’s medium supplemented with 1-glutamine, 10% fetal calf serum, and penicillin/streptomycin (100 units/ml and 50 μg/ml, respectively). HEK293 cells transfected with TLR4 and TLR4/MD-2 were a generous gift from Dr. Douglas Golenbock (University of Massachusetts, School of Medicine) and have been described previously (22). Briefly, transfected HEK293 cells were maintained in Dulbecco’s modified Eagle’s medium (plus 1-glutamine and 4.5 g/liter glucose) supplemented with 10% fetal calf serum, 0.5 μg/ml G-418, and 10 μg/ml ciprofloxacin. Bone marrow-derived macrophages were isolated from the tibia and femur of euthanized mice by culturing bone marrow cells in RPMI 1640 media supplemented with 1-glutamine, 20% heat-inactivated fetal calf serum, penicillin/streptomycin (100 units/ml and 50 μg/ml, respectively), 0.05 mM 2-mercaptoethanol, and 5 ng/ml macrophage-colony-stimulating factor. Selection media were changed every other day for a total of 7 days. Experiments on bone marrow-derived macrophages were performed on day 7.

Human umbilical cord hyaluronan was purchased from Sigma. Small 1-ml HA batches were boiled for 1 h to inactivate any enzyme or protein contamination. Samples were then run on two endotoxin-removal columns (Associates of Cape Cod) sequentially to remove any possibility of endotoxin contamination. HA preparations were free of DNA contamination, as preparations showed no peaks at 260 and 280 nm using a Spectrometer. LPS was obtained from LIST Biologics, Inc., and Sigma.

In Vitro Cell Stimulation Experiments (MH-S Cells, HEK293 Cells, and THP-1 Cells)

Cells were grown to confluence in a 96-well flat bottom plate (Costar). If experiments were performed in low serum media conditions as indicated, then their growth medium was removed and replaced the night before the experiment with low serum-containing medium. For the experiment, medium was removed from the cells and replaced with medium containing either HA or LPS at the indicated concentrations. Cells were allowed to incubate with the described condition for a specific time point, medium was then removed and spun down at 1000 rpm for 10 min at 4 °C to remove any debris. Cell medium was stored at −20 °C until analysis.

In Vivo Mouse Experiments

Models of Sterile Inflammation—Balb/c, TLR4−/− (C3H/HeJ), and TLR4+/+ controls, and CD44−/− (Cd44tm1Hbg) and CD44+/+ controls were all purchased from Jackson Laboratories. For bead injection, 0.5 μg of Cytodex microcarrier beads (Sigma) was placed in 25 ml of sterile, endotoxin-free PBS, maintained in sterile conditions, and autoclaved. Mice were anesthetized with isoflurane, and fur was plucked from the back area and wiped with an ethanol pad. 250 μl of Cytodex bead slurry was injected subcutaneously into the back space using a sterile 25-gauge needle and sterile 1-ml syringe, creating a large bubble in the skin. After a specified time point, mice were euthanized by over-anesthesia with halothane, and an 8-mm punch biopsy was used to isolate skin regions, either injured sites or non-injured sites on the backs of the mice. The skin sections were placed in a tube with 500 μl of 1× radioimmunoprecipitation assay buffer (and protease inhibitors) and 2.4-mm Zirconia beads (BioSpec Products, Inc., Bartlesville, OK) and were beaten in a mini-bead beater apparatus (BioSpec Products, Inc.) for 50 s on full speed. Extracts were then sonicated for 5 min on ice and spun down at 14,000 rpm for 10 min at 4 °C. Supernatant was removed and kept at −20 °C until analysis by MIP-2 enzyme-linked immunosorbent assay.

For the liquid nitrogen sterile inflammation model, mice were anesthetized with isoflurane, and back fur was plucked. A CRY-AC liquid nitrogen dispenser with a 1-mm nozzle (Brymill Cryogenic Systems, Ellington, CT) was used to administer a steady liquid nitrogen 1-cm diameter circle to the mouse back for 15 s. This provided a frozen injury that was kept frozen for a full 60 s. After a specified time point, mice were euthanized by over-anesthesia with halothane, and an 8-mm punch biopsy was used to isolate skin regions, either injured sites or non-injured sites on the backs of the mice. Skin extracts were processed exactly as described above for the bead model of sterile injury. Animal procedures were approved by the Veterans Affairs San Diego Healthcare System subcommittee on animal studies, protocol 02-037.

HA Staining following Sterile Skin Injury—To determine if HA accumulates following injury, mice were anesthetized, and injury was induced by injecting 250 μl of Cytodex beads as described earlier. After the specified time, mice were euthanized, and an 8-mm punch biopsy was used to remove both non-injured and injured skin. Skin was embedded in OCT compound and frozen at −20 °C. Sections (7 μm thick) were cut, and staining was carried out according to the protocol using Pep-1 described by Zmolik et al. (23). Pep-1 was synthesized as previously described (19), and fluorescein isothiocyanate-streptavidin was obtained from Jackson ImmunoResearch.
Immunofluorescence was observed using an Olympus BX41 fluorescence microscope (Scientific Instrument Co., Temecula, CA). Confocal images were obtained with a Zeiss LSM510 laser scanning confocal microscope (Carl Zeiss Light Microscopy, Göttingen, Germany) coupled with an Axiovert 100 (Carl Zeiss) inverted stage microscope.

**RNA Quantification following Sterile Skin Injury**

To determine the expression of MMP-13 and TGF-β2 following injury, mice were anesthetized and 250 μl of Cytodex beads was injected into a site on their backs as described above. After an indicated time, the mouse was euthanized, and an 8-mm punch biopsy was used to remove both a non-injured skin site and the bead-induced injury site. Each skin section was placed in 1 ml of TRizol reagent (Invitrogen) and 2.4-mm Zirconia beads as described above. Each sample was beaten in a mini-bead beater apparatus, as described above, for 50 s on full speed. To extract RNA, 200 μl of chloroform was added to each tube and was mixed vigorously by hand for 15 s, and the mixture was vortexed, and then centrifuged at 7500 rpm for 15 min, 12,000 /g. The samples were centrifuged for 10 min at 4 °C. The aqueous phase was transferred to a new tube, 500 μl of 100% isopropanol was added, and the mixture was incubated for 10 min at room temperature. The samples were centrifuged for 10 min at 12,000 × g, 4 °C. The RNA pellet was washed with 75% ethanol, vortexed, and then centrifuged at 7500 × g for 5 min at 4 °C. The pellet was dried and then dissolved in RNase-free H2O, and concentration was determined by spectrometry at 260 nm.

Real-time PCR was used to determine the induction of Mmp13 and Tgfb2 following sterile injury. cDNA was synthesized from RNA by the iScript cDNA Synthesis Kit (Bio-Rad) as described by the manufacturer’s protocol. TaqMan Gene Expression Assays (Applied Biosystems) were used to analyze for Mmp13 and Tgfb2 expression as described by the manufacturer. Gapdh or 18 S ribosomal RNA controls was used to normalize for each sample.

**WFGAG Isolation and Digestion**

Wound fluid GAG was isolated as previously described by anion exchange from human wound fluid (24). However, where previous elutions of GAG have occurred at 2 m NaCl, WFGAG in these experiments was eluted from 0.75 to 1 m NaCl as to enrich for less sulfated GAG populations that would include HA. The presence of HA and approximate concentration in wound fluid was determined by dot blot using a known HA standard for comparison and HA-binding protein (Seikagaku Corp.) for detection.

To digest WFGAG preparations, as well as stock HA, chondroitin sulfate, and dermatan sulfate for comparison, all GAG samples were lyophilized and resuspended in digestion buffer (50 mM Tris, pH 6.8, 60 mM sodium acetate, and 0.02% bovine serum albumin). The chondroitinase ABC enzyme at pH 6.8 will preferentially digest HA over chondroitin sulfate and is a relatively neutral pH for further *in vitro* cell culture experiments. 50 milliunits of chondroitinase ABC (Sigma) was added to each digest, and buffer was added to no-digest controls. Samples were digested overnight at 37 °C, and then boiled for 3 min to inhibit enzyme activity. Samples were reconstituted with cell media to achieve the desired concentration and added to cells to determine activity as described in cell stimulation experiments *in vitro*.

**Microarray Analysis**

MH-S macrophages were incubated with 25 μg/ml HA, 50 ng/ml LPS, or vehicle control for 6 h in 100-mm dishes at 37 °C. After incubation, total RNA was prepared using the Qiagen RNeasy protocol and reagents. The experiment was repeated on three different occasions, isolating RNA for each condition to have triplicate RNA samples. RNA was submitted to the SDVA Microarray Core Facility for biochemistry, and image analysis was carried out according to Affymetrix Standards and the MIAME checklist. Affymetrix mouse 2.0A arrays were utilized where vehicle control chips were compared with triplicate HA- and LPS-treated chips. Data were analyzed using Affymetrix GeneChip Operating Software and Agilent Technologies GeneSpring 7.0. Experimental HA and LPS chips were normalized to vehicle control chips, and data were analyzed using a one-way analysis of variance comparing treatment type, with ρ value cutoff of 0.05. Data were also analyzed using GeneSifter (VizX Lab) where the mean ± S.E. was determined for all three treatment types. HA and LPS were then compared with control, the t test was used to determine significance, and ρ values are listed with -fold change. Shown in Table 1 are data analyzed using GeneSifter, although both methods of analysis provided similar results.

**Chemokine Analysis**

Cell culture media samples as well as Balb/c c<sup>++</sup> mouse serum were analyzed for the presence of cytokines and chemokines by enzyme-linked immunosorbent assay for human IL-8 and mouse MIP-2 (R & D Systems), and analysis was carried out following kit protocols or by LINCOplex panel analysis at Linco Research, Inc. for analysis using the cytokine 22-multiplex panel.

**Immunoprecipitation**

MH-S macrophage cells were stimulated with 25 μg/ml HA, 50 ng/ml LPS, and vehicle control for 10 min and extracted in 1× radiolmmune precipitation assay buffer containing protease inhibitors (Roche Applied Science). Cells were placed on ice, scraped, and transferred to a 1.5-ml tube. Cells were sonicated for 20 min on ice and then spun down at 10,000 rpm for 10 min at 4 °C. Cell lysate was transferred to a clean tube and stored at −20 °C. Protein concentration was determined by BCA assay (Pierce) according to the manufacturer’s instructions. 500 μg of total protein was pre-cleared with 0.25 μg of control IgG antibody and 20 μl of Protein A/G-Plus agarose (Santa Cruz Biotechnology, Santa Cruz, CA) for 30 min at 4 °C while rotating. Beads were pelleted by centrifuging at 2500 rpm for 30 s at 4 °C, and lysate was removed and placed in a new tube. To the pre-cleared cell lysate, 2 μg of rat anti-mouse CD44 primary antibody (Santa Cruz Biotechnology) was added, and the mixture was incubated at 4 °C rotating for 2 h. Twenty microliters of Protein A/G-Plus agarose was added, and the mixture was incubated overnight at 4 °C while rotating. Beads were pelleted by centrifuging at 2500 rpm for 30 s at 4 °C. Beads
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**Figure a**

![Graph showing concentration of MIP-2 in non-injured vs. injured samples with beads and nitrogen.](image)

**Figure b**

![Graph showing MIP-2 concentration in TLR4-/- and TLR4+/- mice with beads and nitrogen.](image)

**Figure c**

![Images comparing injured and non-injured tissue sections.](image)

**Figure d**

![Graph showing MIP-2 concentration in WPGAG with and without enzyme.](image)

**Figure e**

![Graph showing MIP-2 concentration with 10ug/ml HA, 10ug/ml DS, and 10ug/ml CS, with and without enzyme.](image)

**Figure f**

![Graph showing MIP-2 concentration at various HA and LPS concentrations.](image)
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were washed three times with ice-cold PBS, spinning down as described after each wash. After the last wash, the supernatant was removed and beads were resuspended in 40 μl of sample buffer for Western blotting. For control experiments, IgG antibody (Pierce) and EGFR antibody (BD Biosciences) were used at 2 μg to verify that the immunoprecipitation using CD44 and probing for TLR4 was specific.

Samples were boiled for 3 min and loaded onto a 10% Tris-Tricine gel (BioExpress) along with 5 μl of biotinylated protein ladder (Cell Signaling Technology). Gels were run for 2 h at 60 V and then transferred onto a polyvinylidene difluoride Immobilon-P membrane (Millipore) overnight at 4 °C. Membranes were blocked with 5% nonfat milk/3% bovine serum albumin in 1× PBS for 1 h at room temperature, and then incubated with goat anti-mouse TLR4 antibody (dilution 1:200, Santa Cruz Biotechnology) in block solution for 1 h at room temperature.

Following primary antibody, cells were washed three times with TBST (0.3% Tween 20 in TBS) and incubated with donkey anti-goat IgG horseradish peroxidase (dilution 1:5000, Santa Cruz Biotechnology) in block solution for 30 min at room temperature. Membranes were washed three times with TBST and then developed with Western Lightning Chemiluminescence (PerkinElmer Life Sciences).

Alternatively, the same protocol described above was done in the reverse. Following a pre-clear with IgG antibody, 2 μg of TLR4/MD-2 (Santa Cruz Biotechnology) antibody was added to the lysate, and the mixture was incubated for 2 h at 4 °C and then with 20 μl of Protein A/G-Plus agarose overnight at 4 °C. As described above, 2 μg of antibody control as well as 2 μg of EGFR antibody were used as controls to verify that the immunoprecipitation was specific. Gels were run as described above, and Western blot was carried out using rat anti-mouse CD44 antibody (dilution 1:200, Santa Cruz Biotechnology). The secondary antibody used for detection was a goat anti-rat IgG horseradish peroxidase (dilution 1:1000, Santa Cruz Biotechnology).

To evaluate the effect of MD2 on the interaction between CD44 and TLR4, non-transfected HEK293 cells, TLR4-transfected HEK293 cells, or TLR4/MD2-transfected HEK293 cells (22) were cultured and subjected to immunoprecipitation as described above. Following a pre-clear with IgG antibody, 2 μg of TLR4 (Santa Cruz Biotechnology) antibody was added to lysates, the mixture was incubated for 2 h at 4 °C, and then 20 μl of Protein A/G-Plus agarose was added overnight at 4 °C. Gels were run as described above, and Western blotting was performed using a rat anti-mouse CD44 antibody (dilution 1:200, Santa Cruz Biotechnology). The secondary antibody used for detection was a goat anti-rat IgG horseradish peroxidase (dilution 1:1000, Santa Cruz Biotechnology). The University of California San Diego MS-Isotope analysis program was then used as a tool for calculating the predicted molecular weight of known CD44 splice variants to verify the corresponding band.

Immunostaining

MH-S macrophage cells were grown to sub-confluency on 8-well chamber slides (Nunc International). Cells were rinsed once with media and then incubated with 25 μg/ml HA, 50 ng/ml LPS, or vehicle control for indicated time points at 37 °C. Media was removed, and cells were rinsed once with PBS and then fixed with 2% paraformaldehyde for 5 min at room temperature. Cells were washed three times with PBS and blocked with appropriate serum for 30 min at room temperature. Cells were incubated with primary antibodies rat anti-mouse CD44 (clone IM7) and goat anti-mouse TLR4 (L-14) from Santa Cruz Biotechnology at the recommended concentrations (1:50 dilution) at room temperature for 1 h in a humidified chamber. Antibodies were diluted in 3% bovine serum albumin in PBS. Following primary antibody, cells were washed three times with PBS, and two secondary antibodies, anti-goat-IgG-fluorescein isothiocyanate and anti-rat-IgG-R (1:1000 dilution, Santa Cruz Biotechnology), were diluted in 3% bovine serum albumin in PBS, and the mixture was incubated for 30 min at room temperature in a dark humidified chamber. Cells were washed three times with PBS, the chamber was removed from the slide, and cells were fixed with Prolong anti-fade reagent (Invitrogen) and a coverslip. Immunofluorescence was observed using an Olympus BX41 fluorescence microscope (Scientific Instrument Co.). Confocal images were obtained with a Zeiss LSM510 laser scanning confocal microscope (Carl Zeiss Light Microscopy, Göttingen, Germany) coupled with an Axiovert 100 (Carl Zeiss) inverted stage microscope.

Statistical Analysis

Results are generally expressed as the mean ± S.D. and are representative of at least three separate experiments. A paired t test was used to determine significance unless otherwise stated. For the mouse in vivo experiments, a two-way analysis of variance was used for behavioral experiment and one-way analysis of variance was used for sterile injury; these were analyzed by Prism 4 (GraphPad Software, Inc.).

RESULTS

Induction of MIP-2 by Sterile Injury and HA Is Dependent on TLR4—To study both the triggers and recognition elements of sterile injury, it was first necessary to develop sterile models that could be rapidly evaluated following trauma. Two comple-

FIGURE 1. Sterile inflammation induces MIP-2 and requires TLR4. a, sterile injury induced by injection of sterile Cytodex beads or liquid nitrogen treatment results in up-regulation of MIP-2 in the injured skin (solid bars) after 4 h (***, p < 0.0001). Control, non-injured skin has low baseline levels of MIP-2 expression (open bars). b, the injection of 250 μl of sterile beads (left panel) and the stream of liquid nitrogen (right panel) induced MIP-2 expression in injured skin of TLR4 WT mice (TLR4+/+), which was significantly diminished in the injured skin of TLR4-deficient mice (TLR4−/−). c, HA expression increased in sterile injury following injection of sterile beads. Shown is HA expression at 24 h after injury using labeled Pep-1 (left panels), nuclei were stained with 4',6-diamidino-2'-phenylindole (right panels). Arrows indicate location of beads. d, WFGAG (0.01 μg) stimulated MH-S macrophages to release MIP-2 (solid bars) after 24 h incubation. Digestion of WFGAG with chondroitinase ABC (open bars) resulted in a 57.8% decrease in activity (*, p = 0.06). e, only HA, and not dermatan sulfate (DS) or chondroitin sulfate (CS), was capable of stimulating MIP-2 release from MH-S macrophages (solid bars) after a 24 h incubation. HA, dermatan sulfate, and chondroitin sulfate were digested with chondroitinase ABC (open bars), and digestion of HA resulted in a 56.2% decrease in activity (***, p = 0.0003). f, a concentration curve of HA (squares, solid line) and LPS (circles, dotted line) stimulation on MIP-2 release by MH-S cells after 24 h.
mentory models were developed in mice to accomplish this with minimal risk from extrinsic microbial stimuli. In the first, 0.25 ml of sterile Cytodex beads were injected subcutaneously in the skin, thereby disrupting the subcutaneous tissue by physical expansion, but leaving the overlying epidermis intact. In a second model, freeze injury was induced by applying a targeted stream of liquid nitrogen to the skin for 15 s and keeping the injury site frozen for 60 s. This model did not result in acute disruption of the epidermis. Both of these models produced inflammation at the site within hours as measured by an increase in the pro-inflammatory cytokine MIP-2 (Fig. 1a). In the bead injection model, skin samples adjacent to the injection site, and thus equally susceptible to any small microbial inoculation accidentally introduced by the injection, did not show a significant elevation in MIP-2. Inflammation only occurred in the region physically disrupted by tissue expansion generated by the introduction of sterile beads. To confirm that the beads used for tissue disruption were not contaminated or themselves capable of initiating a similar response, beads were also directly added to a sensitive indicator cell line (MH-S macrophages). This did not result in chemokine release (data not shown).

To next determine if the increase in MIP-2 observed following sterile injury could be attributed to the activation of TLR4, sterile inflammation was initiated in mice without functional TLR4 (TLR4−/−). Compared with control mice, TLR4−/− mice had a significantly diminished response to sterile injury induced by both beads injection and liquid nitrogen in terms of local MIP-2 expression (Fig. 1b). No difference was detected between the knock-out and control groups in the absence of injury.

To determine if the TLR4-dependent effect observed in Fig. 1b could be due to changes in the HA composition at the site of injury, biopsies of non-injured and injured sites skin were first examined histologically by staining with the HA-binding peptide Pep-1 (23, 25). As expected, HA could be detected throughout normal non-injured skin. Following injury, HA staining greatly increased and was localized in the areas surrounding Cytodex beads (Fig. 1c). To confirm that wound fluid contains HA and wound fluid HA is active and capable of inducing an inflammatory response, human wound fluid was processed to purify glycosaminoglycans (GAGs), such as HA. Human wound fluid was used in these experiments due to the inability to collect large quantities of mouse wound fluid required for these assays. This material induced an increase in MIP-2 secretion by assays. This material induced an increase in MIP-2 secretion by

**TABLE 1**

| Genes induced by HA | ID | -Fold change | p value |
|---------------------|----|--------------|---------|
| Mmp13               |    | 17.40        | 0.008   |
| Rest                |    | 12.20        | 0.04    |
| Tgfb2               |    | 10.94        | 0.007   |
| Tfna                 |    | 10.87        | 0.008   |
| Frk                  |    | 7.07         | 0.01    |
| Socs3                |    | 5.56         | 0.05    |
| Col6a2               |    | 3.89         | 0.001   |
| Mmp3                 |    | 3.03         | 0.04    |

Genes suppressed by HA

| Genes suppressed by HA | ID | -Fold change | p value |
|------------------------|----|--------------|---------|
| CD79B                  |    | −8.34        | 0.0006  |
| Mgl1                   |    | −6.73        | 0.0006  |
| N-acetylgalactosamine-specific lectin 1 |    | −6.45        | 0.02    |
| Ccnd3                  |    | −6.09        | 0.02    |
| Cdhr5                  |    | −4.17        | 0.0006  |
| Traf3                  |    | −3.91        | 0.03    |
| Socs7                  |    | −3.54        | 0.03    |

Genes induced by LPS

| Genes induced by LPS | ID | -Fold change | p value |
|----------------------|----|--------------|---------|
| Kcng5                 |    | 26.71        | 0.03    |
| Ccr6                  |    | 9.55         | 0.003   |
| Defb15               |    | 3.54         | 0.0009  |
| Ext1                 |    | 3.00         | 0.01    |

Genes suppressed by LPS

| Genes suppressed by LPS | ID | -Fold change | p value |
|-------------------------|----|--------------|---------|
| Ccne2                  |    | −7.39        | 0.0008  |
| Gpc3                   |    | −5.92        | 0.002   |
| Il6ra                  |    | −5.40        | 0.008   |
| Adam3                  |    | −4.25        | 0.02    |

**The Inflammatory Response to HA Is Distinct from LPS—**

Having established that TLR4 participates in the sterile wound response, and that the TLR4 ligand HA is released after sterile injury, we next sought to determine if the TLR4-dependent response to HA could be distinguished from the response to LPS. In this way intrinsic and microbial inflammatory triggers may be distinguished and more clearly separated for study. Microarray analysis was performed on the MH-S macrophage cell line treated with either HA or LPS. Several pro-inflammatory genes such as IL-6 and tumor necrosis factor-α (in addition to MIP-2 shown in Fig. 1) were induced by LPS and HA (supplemental Table S1). However, HA induced several genes that LPS did not. These included matrix metalloproteinase 13 (Mmp13), Tgfb2, and suppressor of cytokine signaling 3 (Socs3) (Table 1). To extend and partially validate this microarray analysis, media samples from cells treated with HA or LPS were analyzed for cytokine release. After 6, 24, and 48 h some cytokines such as tumor necrosis factor-α, monocyte chemoattractant protein, and RANTES (regulated on activation normal T cell expressed and secreted), were equally induced by LPS and HA, whereas others, such as granulocyte macrophage-colony stimulating factor were significantly different (Fig. 2). To determine if these responses resembled the sterile inflammatory response seen after injury, HA-induced genes, Mmp13 and Tgfb2 were also examined in tissue samples collected following injury as described in Fig. 1. These molecules were also induced in vivo and were dependent on TLR4 for induction (Fig. 3).
HA Signaling Requires TLR4 and MD-2 but Not CD14—Differences in the response to HA and LPS, despite a shared dependence on TLR4, suggested that distinguishable receptor activation states might be induced by the two ligands. The adapter molecules CD14 and MD-2 are absolutely required for TLR4 to signal in response to smooth (fully glycosylated) LPS. However, CD14 is not required for TLR4 to respond to lipid A in a MyD88-dependent fashion. Rather, it is only required for MyD88-independent signaling. These findings suggest that TLR4 has more than one possible mode of signaling (26). To determine whether MD-2 and/or CD14 were essential for TLR4 signaling induced by HA, HEK293 cells transfected with TLR4 (HEK293/TLR4) (22), and with or without soluble MD-2, were stimulated with HA or LPS at various concentrations for 24 h. MD-2 was required for LPS or HA to stimulate IL-8 (functional equivalent to MIP-2 in human cells) release from HEK293/TLR4 cells (Fig. 4a). In accordance with previous experiments, it was confirmed that HEK293 cells without TLR4 were unable to respond to HA (supplemental Fig. S1).

In contrast, a distinct difference in the requirement for CD14 was observed in HA recognition. The human monocytic cell line THP-1 express low levels of CD14 and, as previously reported (27), did not release IL-8 even at high LPS concentrations (Fig. 4b). HA was capable of stimulating IL-8 release in the absence of supplemental CD14 (Fig. 4b). Soluble CD14 in serum could partially restore LPS responsiveness in this system but had no additional effect on the HA response (data not shown), thus indicating that HA recognition is not dependent on CD14.

CD44 Participates in HA-induced Signaling and Associates with TLR4—Because CD14 was not required for HA signaling, we next asked whether other HA receptors are involved in facilitating the HA response. The contribution of CD44 was investigated because previous studies have suggested a role for HA-induced CD44 in cytokine signaling (28). CD44+/+ and CD44−/− mice were injected with HA, and serum MIP-2 levels were determined after 2 h. CD44−/− mice were deficient in their capacity to respond to HA (Fig. 5a). To verify that CD44 was involved in HA-mediated signaling, bone marrow-derived macrophages were isolated from both CD44+/+ and CD44−/− mice and treated with HA, and serum MIP-2 levels were determined after 24 h. CD44−/− mice were deficient in their capacity to respond to HA (Fig. 5b).

The partial dependence on CD44 and requirement for TLR4 to recognize HA prompted us to directly
CD44 was immunoprecipitated from HEK293/TLR4 cell lysates compared with non-transfected HEK293 cells, observed as a 40-kDa product (Fig. 5f). However, HEK293 cells transfected with both TLR4 and MD2 were able to immunoprecipitate substantially more CD44 in contrast to either non-transfected or TLR4 transfected HEK293 cells.

**DISCUSSION**

The mechanisms responsible for initiating inflammation in the absence of microbial stimuli are not understood. We describe for the first time that response to cutaneous sterile injury is dependent on TLR4 and CD44. This observation, combined with prior observations that TLR4 recognizes HA released following injury, suggests that HA serves as an intrinsic trigger for inflammation. Further experiments reveal that activation of TLR4 by this system depends on a unique signaling complex consisting of TLR4, MD-2, and CD44. This leads to the induction of a unique set of molecules that differ (in part) from those induced when TLR4 is stimulated by LPS. Taken together these observations show that an innate immune receptor best known for its capacity to detect microbial invasion also can initiate a response to sterile injury.

Our observations suggest a model whereby HA is broken down following injury and initiates events necessary for inflammation (Fig. 6). Evidence supporting the role for HA in this process was indirect but included direct observation of its accumulation at the site and an ability to extract active HA from sterile wounds. Additional support for the role of HA in sterile injury was also obtained by enzymatically neutralizing the ability of GAG extract derived from wounds to stimulate a cytokine response. For these experiments shown in Fig. 1, chondroitinase ABC was selected over hyaluronidase due to its ability to digest HA to disaccharides, a process necessary to inactivate the oligosaccharide. However, because this enzymatic reaction was incomplete, as seen by the partial inactivation of purified HA preparations, the enzyme only partially inactivated material derived from wounds. Despite this incomplete response, the selectivity of this enzyme for GAG clearly showed that HA is at least part of the response and diminished the potential for undetected lipid or protein contaminants to contribute to these observations.

In addition to observations that HA is released in wounds and can stimulate a cytokine response, additional support for HA as a trigger of sterile inflammation came from similarities in the products induced by HA in culture and in wounds. These included molecules such as TGF-β2 and MMP-13 that accumulate after sterile injury and are induced by HA but not LPS. The ability of HA to induce MMP-13 expression has recently been independently confirmed (29).

Following the release of HA to initiate a response, our data show that a previously unrecognized cooperation between TLR4 and CD44 is necessary for response to HA in culture and optimal inflammation in sterile injury and suggests that the presence of the TLR4 accessory molecule, MD2, enhances the binding interaction between TLR4 and CD44. However, the presence of CD44 is not essential. Unlike deletion of TLR4 or MD2 that abolished all HA activity, loss of CD44 resulted in a significant but incomplete decrease in the ability of HA to stimulate MIP-2 release.
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Therefore, CD44 may act to enhance or stabilize the interaction between HA and TLR4.

CD44 has several other functions that could influence these observations, including facilitating leukocyte adhesion (30, 31), bacterial adhesion (32, 33), and mediating the uptake and degradation of HA (34, 35). The present observations cannot be entirely attributed to these other functions of HA on adhesion of leukocytes, because our findings showed rapid chemokine response in culture systems that did not depend on cell recruitment. Furthermore, complex alternatively spliced CD44 transcripts have been described as a consequence of particular cellular responses under both normal and pathological conditions (36 –38). CD44 transcripts are subject to alternative splicing of its 10 variable exons. Thus, depending upon a sterile or non-sterile injury, the presence of particular isoforms of CD44 may dictate the specific response to HA, as well as the interaction between TLR4 and CD44.

As more work emerges on HA signaling, the more complicated HA recognition appears. Early experiments performed by Noble and colleagues (35, 39, 40) suggested a role for CD44 in binding HA and inducing a variety of downstream inflammatory mediators in macrophages. However, work by Termeer et al. (18) found that TLR4 was required for recognizing HA fragments (4-, 6-, and 8-mers) in dendritic cells, resulting in inflammatory cytokines and dendritic cell maturation. In addition, Termeer found that CD44, TLR2, and RHAMM (receptor for HA-mediated motility) were not involved in recognition of HA fragments (18, 41). In 2002, Noble and colleagues (35) showed that CD44 is required for the uptake of HA leading to lung injury resolution. Recent work by Noble and colleagues (21, 35) has suggested a role for TLR4 and TLR2 in non-infectious lung injury models. More recently, TLR2 has been suggested to be the sole receptor for HA (42) and RHAMM shown to be a positive regulator of macrophage influx after injury (43). The reason for the participation of different receptor molecules in different studies might be the size and structure of HA, as well as the cell type under investigation.

Future work needs to address what elements contribute to HA activity. Is it strictly a size issue, and, if so, what size is “active” and in what capacity? Are there other components of the HA molecule that contribute to its functionality? Are there structural differences in HA that distinguish synthesized HA and degraded HA? Current HA data suggest that the contribution of multiple HA receptors can lead to multiple downstream effects. Due to limited availability of pure HA at defined sizes, the use of commercial preparations of HA of multiple sizes has been a useful reagent to identify elements responsible for detection of HA and downstream signaling consequences of this detection. The use of mixed preparations has contributed to difficulties in determining what defines HA as active or inactive. In the current study identical mixed HA preparations have been previously characterized to eliminate the potential for contamination (19). However, future work to define maximal bioactive HA structural characteristics remains to be done.

Fully understanding the components of HA recognition and signaling may provide other candidates that contribute to the downstream effects of HA. For example, MyD88 was shown to signal downstream of TLR4/TLR2 in the mouse lung injury model (21). The contribution of other adaptor molecules remains to be explored, including the roles of those associated with TLR signaling such as MyD88, Mal, TRIF/TICAM-1, and TRAM/TICAM-2, or those associated with CD44, such as ERM proteins (44 – 46). Studies to determine the involvement of...
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![Diagram of HA signaling pathways](image)

**FIGURE 6. Model of proposed HA signaling.** Injury leads to extracellular matrix breakdown, including the degradation of HA and resulting oligosaccharides. Lower molecular weight HA signals through TLR4, requiring co-accessory molecules MD-2 and CD44. In contrast, LPS requires CD14 and LPS-binding protein to signal through TLR4. Shown in this figure are proposed signaling pathways and components based on work published by several groups, including this study.

these signaling molecules would be of great interest in further understanding how HA signaling occurs and what effects it has downstream.

There are many immediate clinical implications and important questions that result form the current observations. Does injury-induced soluble HA alter the ability of the host to respond to infection? Do other receptors thought to act in recognition of microbes also respond to GAGs? What tissues and cell types are responding to HA when present systemically? Prior work has associated polymorphisms or mutations in sensors such as TLR and NOD (nucleotide-oligomerization domain family) with human diseases without obvious microbial causes such as asthma, inflammatory bowel disease, and neutrophilic urticarial disorders (13, 47–49). HA has been shown to circulate in the blood of patients with rheumatoid arthritis and scleroderma (50, 51), and HA injection is currently used to treat osteoarthritis. These diseases are not defined by their triggers, but rather are described by the subsequent release of pro-inflammatory mediators. Such observations have led to development of therapies for sterile inflammatory disorders that rely on targeting the inflammatory response broadly (e.g. glucocorticoids) or more specifically (e.g. with tumor necrosis factor-α antagonists). Exploring the triggers of sterile inflammation provides potential new information to dissociate this form of inflammation from that initiated by microbial elements. The ability to recognize that endogenous components of the extracellular matrix such as HA are also triggers of inflammation has immediate diagnostic and therapeutic significance.

REFERENCES

1. Poltorak, A., He, X., Smirnova, I., Liu, M. Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenberg, M., Ricciardi-Castagnoli, P., Layton, B., and Beutler, B. (1998) *Science* **282**, 2085–2088
2. Schumann, R. R., Leong, S. R., Flagg, G. W., Gray, P. W., Wright, S. D., Mathison, J. C., Tobias, P. S., and Ulevitch, R. J. (1990) *Science* **249**, 1429–1431
3. Moore, K. J., Andersson, L. P., Ingalls, R. R., Monks, B. G., Li, R., Arnaout, M. A., Golenbock, D. T., and Freeman, M. W. (2000) *J. Immunol.* **165**, 4272–4280
4. Haziot, A., Ferrero, E., Kontgen, F., Hijiya, N., Yamamoto, S., Silver, J., Stewart, C. L., and Goyert, S. M. (1996) *Immunity* **4**, 407–414
5. Shimazu, R., Akashi, S., Ogata, H., Nagai, Y., Fukudome, K., Miyake, K., and Kimoto, M. (1999) *J. Exp. Med.* **189**, 1777–1782
6. Visintin, A., Mazzoni, A., Spitzer, J. A., and Segal, D. M. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98**, 12156–12161
7. Schromm, A. B., Lien, E., Henneke, P., Chow, J. C., Yoshimura, A., Heine, H., Latz, E., Monks, B. G., Schwartz, D. A., Miyake, K., and Golenbock, D. T. (2001) *J. Exp. Med.* **194**, 79–88
8. Kawai, T., Adachi, O., Ogawa, T., Takeda, K., and Akira, S. (1999) *Immunity* **11**, 115–122
9. Yamamoto, M., Sato, S., Hemmi, H., Sanjo, H., Uematsu, S., Kaisho, T., Hoshino, K., Takeuchi, O., Kobayashi, M., Fujita, T., Takeda, K., and Akira, S. (2002) *Nature* **420**, 324–329
10. Hoebe, K., Du, X., George, P., Janssen, E., Tabeta, K., Kim, S. O., Goode, J., Lin, P., Mann, N., Mudd, S., Crozat, K., Sovath, S., Han, J., and Beutler, B. (2003) *Nature* **424**, 743–747
11. Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., Takeuchi, O., Sugiyama, M., Okabe, M., Takeda, K., and Akira, S. (2003) *Science* **301**, 640–643
12. Yamamoto, M., Sato, S., Hemmi, H., Uematsu, S., Hoshino, K., Kaisho, T., Takeuchi, O., Takeda, K., and Akira, S. (2003) *Nat. Immunol.* **4**, 1144–1150
13. Beutler, B. (2004) *Nature* **430**, 257–263
14. Beutler, B., Hoebe, K., George, P., Tabeta, K., and Du, X. (2005) *Adv. Exp. Med. Biol.* **560**, 29–39
15. Palsson-McDermott, E. M., and O’Neill, L. A. (2004) *Immunology* **113**, 153–162
16. Hoebe, K., and Beutler, B. (2004) *J. Endotoxin. Res.* **10**, 130–136
17. Fitzgerald, K. A., Rowe, D. C., and Golenbock, D. T. (2004) *Microbes Infect.* **6**, 1361–1367
18. Termeer, C., Benedix, F., Sleeman, J., Fieber, C., Voith, U., Ahrens, T., Miyake, K., Freudenberg, M., Galanos, C., and Simon, J. C. (2002) *J. Exp. Med.* **195**, 99–111
19. Taylor, K. R., Trowbridge, J. M., Rudsill, J. A., Termeer, C. C., Simon, J. C., and Gallo, R. L. (2004) *J. Biol. Chem.* **279**, 17079–17084
20. del Fresno, C., Otero, K., Gomez-Garcia, L., Gonzalez-Leon, M. C., Soler-Ranger, L., Fuentes-Prior, P., Escoll, P., Baos, R., Caveda, L., Garcia, F., Arnalich, F., and Lopez-Collazo, E. (2005) *J. Immunol.* **174**, 3032–3040
21. Liang, D., Liang, J., Fan, J., Yu, S., Chen, S., Luo, Y., Prestwich, G. D., Mascarenhas, M. M., Cargueira, G., Doughty, R. L., Buca, R., Lee, P. J., Medzhidov, R., and Noble, P. W. (2005) *Nat. Med.* **11**, 1173–1179
22. Latz, E., Visintin, A., Lien, E., Fitzgerald, K. A., Monks, B. G., Kurt-Jones, E. A., Golenbock, D. T., and Espevik, T. (2002) *J. Biol. Chem.* **277**, 47834–47843
23. Zmolik, J. M., and Mummert, M. E. (2005) *J. Histochem. Cytochem.* **53**, 745–751
24. Penc, S. F., Pomahac, B., Winkler, T., Dorschner, R. A., Eriksson, E., Hern-
don, M., and Gallo, R. L. (1998) J. Biol. Chem. 273, 28116–28121
25. Mummert, M. E., Mohamadzadeh, M., Mummert, D. I., Mizumoto, N., and Takashima, A. (2000) J. Exp. Med. 192, 769–779
26. Jiang, Z., Georgel, P., Du, X., Shamblin, L., Sovath, S., Mudd, S., Huber, M., Kalis, C., Keck, S., Galanos, C., Freudenberg, M., and Beutler, B. (2005) Nat. Immunol. 6, 565–570
27. Ma, W., Lim, W., Gee, K., Aucoin, S., Nandan, D., Kozlowski, M., Diaz-Mitoma, F., and Kumar, A. (2001) J. Biol. Chem. 276, 13664–13674
28. Sampson, P. M., Rochester, C. L., Freundlich, B., and Elias, J. A. (1992) J. Clin. Invest. 90, 1492–1503
29. Ohno, S., Im, H. J., Knudson, C. B., and Knudson, W. (2006) J. Biol. Chem. 281, 17952–17960
30. DeGrendele, H. C., Estess, P., Picker, L. J., and Siegelman, M. H. (1996) J. Exp. Med. 183, 1119–1130
31. DeGrendele, H. C., Estess, P., and Siegelman, M. H. (1997) Science 278, 672–675
32. Schrager, H. M., Alberti, S., Cywes, C., Dougherty, G. J., and Wessels, M. R. (1998) J. Clin. Invest. 101, 1708–1716
33. Cywes, C., and Wessels, M. R. (2001) Nature 414, 648–652
34. Kaya, G., Rodriguez, I., Jorcano, J. L., Vassalli, P., and Stamenkovic, I. (1997) Genes Dev. 11, 996–1007
35. Teder, P., Vandivier, R. W., Jiang, D., Liang, J., Cohn, L., Pure, E., Henson, P. M., and Noble, P. W. (2002) Science 296, 155–158
36. Stern, R., Shuster, S., Wiley, T. S., and Formby, B. (2001) Exp. Cell Res. 266, 167–176
37. Srebrow, A., and Kornblitt, A. R. (2006) J. Cell Sci. 119, 2635–2641
38. Oertl, A., Castein, J., Engl, T., Beecken, W. D., Jonas, D., Melamed, R., and Blaha, R. A. (2005) World J. Gastroenterol. 11, 6243–6248
39. McKee, C. M., Lowenstein, C. J., Horton, M. R., Wu, J., Bao, C., Chin, B. Y., Choi, A. M., and Noble, P. W. (1997) J. Biol. Chem. 272, 8013–8018
40. McKee, C. M., Penno, M. B., Cowman, M., Burdick, M. D., Strieter, R. M., Bao, C., and Noble, P. W. (1996) J. Clin. Invest. 98, 2403–2413
41. Termeer, C. C., Hennis, J., Voith, U., Ahrens, T., Weiss, J. M., Prehm, P., and Simon, J. C. (2000) J. Immunol. 165, 1863–1870
42. Scheibner, K. A., Lutz, M. A., Boodo, S., Fenton, M. J., Powell, J. D., and Horton, M. R. (2006) J. Immunol. 177, 1272–1281
43. Zaman, A., Cui, Z., Foley, I. P., Zhao, H., Grimm, P. C., Delisser, H. M., and Savani, R. C. (2005) Am. J. Respir. Cell Mol. Biol. 33, 447–454
44. Bourguignon, L. Y., Lokeshwar, V. B., Chen, X., and Kerrick, W. G. (1993) J. Immunol. 151, 6634–6644
45. Tsukita, S., Oishi, K., Sato, N., Sagara, J., Kawai, A., and Tsukita, S. (1994) J. Cell Biol. 126, 391–401
46. Tsukita, S., Yonemura, S., and Tsukita, S. (1997) Trends Biochem. Sci. 22, 53–58
47. Rifkin, I. R., Leadbetter, E. A., Busconi, L., Viglianti, G., and Marshak-Rothstein, A. (2005) Immuno. Rev. 204, 27–42
48. Sutterwala, F. S., Ogura, Y., Szczepanik, M., Lara-Tejero, M., Lichtenberger, G. S., Grant, E. P., Bertin, J., Coyle, A. J., Galan, J. E., Askernase, P. W., and Flavell, R. A. (2006) Immunity 24, 317–327
49. Stojanov, S., and Kastner, D. L. (2005) Curr. Opin. Rheumatol. 17, 586–599
50. Laurent, T. C., and Fraser, J. R. (1992) Faseb J. 6, 2397–2404
51. de la Motte, C. A., Hascall, V. C., Draza, J., Bandyopadhyay, S. K., and Strong, S. A. (2003) Am. J. Pathol. 163, 121–133

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