Patient-specific Immune States before Surgery Are Strong Correlates of Surgical Recovery

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ABSTRACT

Background: Recovery after surgery is highly variable. Risk-stratifying patients based on their predicted recovery profile will afford individualized perioperative management strategies. Recently, application of mass cytometry in patients undergoing hip arthroplasty revealed strong immune correlates of surgical recovery in blood samples collected shortly after surgery. However, the ability to interrogate a patient’s immune state before surgery and predict recovery is highly desirable in perioperative medicine.

Methods: To evaluate a patient’s presurgical immune state, cell-type–specific intracellular signaling responses to ex vivo ligands (lipopolysaccharide, interleukin [IL]-6, IL-10, and IL-2/granulocyte macrophage colony-stimulating factor) were quantified by mass cytometry in presurgical blood samples. Selected ligands modulate signaling processes perturbed by surgery. Twenty-three cell surface and 11 intracellular markers were used for the phenotypic and functional characterization of major immune cell subsets. Evoked immune responses were regressed against patient-centered outcomes, contributing to protracted recovery including functional impairment, postoperative pain, and fatigue.

Results: Evoked signaling responses varied significantly and defined patient-specific presurgical immune states. Eighteen signaling responses correlated significantly with surgical recovery parameters ($|R| = 0.37$ to $0.70$; false discovery rate < 0.01). Signaling responses downstream of the toll-like receptor 4 in cluster of differentiation (CD) 14+ monocytes were particularly strong correlates, accounting for 50% of observed variance. Immune correlates identified in presurgical blood samples mirrored correlates identified in postsurgical blood samples.

Conclusions: Convergent findings in pre- and postsurgical analyses provide validation of reported immune correlates and suggest a critical role of the toll-like receptor 4 signaling pathway in monocytes for the clinical recovery process. The comprehensive assessment of patients’ preoperative immune state is promising for predicting important recovery parameters and may lead to clinical tests using standard flow cytometry. *(Anesthesiology 2015; 123:1241-55)*

Anesthesiologists play a sentinel role in multidisciplinary efforts to improve perioperative care by reducing the incidence of postoperative complications, shortening the recovery period, and optimizing the allocation of healthcare resources.1 Outcomes that are meaningful to patients are at the very core of such efforts as ultimately the aim is to provide the best possible value to patients.2–3 Time to recovery and return to normal activities are patient-centered outcomes of high priority in the perioperative context.4 Major factors that determine the speed of recovery include fatigue, pain, and functional impairment.5–6 Postoperative recovery is highly variable among patients undergoing similar surgical interventions.7–8 The ability to risk-stratify patients based on their predicted recovery profile would be predictive of recovery across several domains, with toll-like receptor 4 signaling in cluster of differentiation (CD) 14+ monocytes accounted for 50% of observed variance.

What We Already Know about This Topic
- Recovery from functional impairment, pain, and fatigue after surgery is highly variable
- Examination of immune cell-type–specific states and activation after surgery accounted for much of the interindividual differences in these outcomes, but whether preoperative samples would also be predictive is unknown

What This Article Tells Us That Is New
- In an analysis of the same group of orthopedic surgery patients from the postoperative sample study, preoperative immune state as assessed by mass cytometry of blood samples was predictive of recovery across several domains, with toll-like receptor 4 signaling in cluster of differentiation (CD) 14+ monocytes accounted for 50% of observed variance
is of significant interest as it would enable patient-tailored and cost-conscious approaches to perioperative management. For example, patients would be better able to make appropriate arrangements for postoperative needs, and patients at risk for protracted recovery could be stratified to resource-intensive interventions such as prehabilitation programs to accelerate their recovery.6

A recent application of single-cell mass cytometry at the “bedside” revealed strong immune correlates of surgical recovery in patients undergoing primary hip arthroplasty.7 Mass cytometry allows for the simultaneous phenotypic and functional characterization of all major immune cell subsets in peripheral blood at unparalleled single-cell resolution.8,9 Surgery-induced signaling changes in monocyte subsets within 24 h after surgery were strongly associated with the speed of recovery from fatigue, pain, and functional impairment and accounted for 40 to 60% of observed variance. The identification of strong immune correlates shortly after surgery is an important advancement in understanding the biology that drives recovery. The ability to interrogate a patient’s immune state before surgery and accurately predict recovery would have a major impact on the practice of perioperative medicine.

This study was built on the premise that patients undergoing surgery differ in their presurgical immune state, which then affects their immune response to surgery and determines the speed of their clinical recovery. To evaluate a patient’s presurgical immune state, cell-type–specific intracellular signaling responses to ex vivo ligands were quantified by mass cytometry in whole-blood samples collected before surgery. This experiment was designed to mimic the surgical stress on a patient’s immune system by use of ligands known to modulate signaling processes in specific immune cells that are perturbed by surgery.8 The major hypothesis tested in this study was that patient-specific presurgical immune states predict the speed of recovery from fatigue, pain, and functional impairment in patients undergoing primary hip arthroplasty.

Materials and Methods

Study Design

This study was registered at ClinicalTrials.gov on March 23, 2012 (NCT01578798) and was conducted from March 2012 to July 2013. The study produced two distinct and large molecular datasets in patients undergoing hip arthroplasty. An initial analysis addressed the question of whether specific immune responses to surgery correlated with the clinical recovery profile of individual patients. Strong immune correlates were identified in postsurgical blood samples, and the results have been published in a separate article.8 The analysis described here addressed whether evoked immune responses in presurgical blood samples correlate with the clinical recovery profile of individual patients. The workflow of this study is illustrated in figure 1. Some portions of the Materials and Methods and Results sections, including the description of subjects, aspects of the study protocol, and clinical outcomes, are included in both articles to ensure completeness and accessibility for the reader.

Subjects

Patients scheduled for primary hip arthroplasty for nontraumatic osteoarthritis were recruited from the Arthritis and Joint Replacement Clinic in the Department of Orthopedic Surgery at Stanford University School of Medicine. The study was approved by the Institutional Review Board of Stanford University School of Medicine (Stanford, Calif.). All patients gave written informed consent before being enrolled in the study. Inclusion criteria were (1) age between 18 and 90 yr, (2) fluency in English, and (3) willingness and ability to sign informed consent and the Health Insurance Portability and Accountability Act authorization. Exclusion criteria were (1) any systemic disease or medication that might compromise the immune system; (2) diagnosis of cancer within the last 5 yr; (3) autoimmune, psychiatric, or neurological conditions interfering with data collection and interpretation; (4) pregnancy; and (5) other conditions that, in the opinion of the investigators, may have compromised a participant’s safety or the integrity of the study, such as a history of substance abuse, chronic opioid therapy (> 30 mg/ day), infectious disease within 1 month, or renal, hepatic, cardiovascular, or respiratory disease resulting in clinically significant functional impairment.

Surgery and Anesthesia

Hip arthroplasties were performed by one of three surgeons using a standard lateral approach, wound drains, and compression dressings. Patients were mobilized on the day of surgery, their bladder catheter was removed on postoperative day 1 or 2, and hospital discharge was planned for postoperative day 3 or 4. Captured surgery-related data included duration of surgery, intraoperative blood loss, intravenous fluid administration, and time to hospital discharge.

Patients were premedicated with midazolam (NOVAPLUS, USA). General anesthesia was induced with propofol (NOVAPLUS), fentanyl (Hospira, USA), and rocuronium (NOVAPLUS) and was maintained with the volatile anesthetics sevoflurane (AbbVie, USA) or desflurane (Baxter, USA). Use of nitrous oxide (Praxair Technology, USA) was at the discretion of the anesthesiologist as was the choice of using either fentanyl (Hospira) or hydromorphone (Perdue, USA) for subsequent dosing of an opioid. Toward the end of surgery, muscle relaxation was reversed with neostigmine (Éclat, USA) and glycopyrrolate (American Regent, USA). Patients received ondansetron (NOVAPLUS) for prophylaxis of postoperative nausea and vomiting. All patients were offered adjuvant spinal anesthesia, which was performed in the sitting position using a midline approach at the lumbar level (L2/L3 or L3/L4). Typically, a 25-gauge Whitacre spinal...
needle was used (Portex; Smiths Medical, USA), but on rare occasion, a 22-gauge Quincke needle (Becton-Dickinson, USA) was chosen instead. The intrathecal space was identified by the return of cerebrospinal fluid through the spinal needle. Single-shot administration of 1.2 to 1.4 ml of hyperbaric 0.75% bupivacaine in dextrose (Portex) established spinal anesthesia. Addition of 100 to 200 μg morphine (Astramorph; AstraZeneca, USA) was at the discretion of the anesthesiologist. Postoperative pain was treated with intravenous patient-controlled analgesia using bolus administration of hydromorphone (Perdue). Adjuvant analgesic included intravenous or per-or acetaminopen (Cadence, USA, or Gemini, USA) according to the surgeons’ preference. Captured anesthesia-related data included average alveolar concentrations of the volatile anesthetic and nitrous oxide, intraoperative opioid dose, intrathecal dose of bupivacaine and morphine in patients with adjuvant spinal anesthesia, cumulative postoperative opioid dose during in-hospital stay, and postoperative opioid dose after hospital discharge (assessed every third day for up to 6 weeks).

Clinical Outcomes

The three major clinical outcomes—fatigue and resulting functional impairment, function of the operated hip, and pain—were evaluated before surgery, daily during hospitalization, and every third day for up to 6 weeks after hospital discharge. Fatigue and resulting global functional impairment were assessed with aid of the surgical recovery scale (SRS).12 The SRS is a categorical questionnaire that (1) assesses how energetic, lively, vigorous, worn-out, fatigued, and physically tired a patient feels and (2) quantifies the level of daily activities, restrictions in daily activity, and the ability to read/watch TV, dress, socialize, perform leisurely or recreational activities, and shop or do errands. A score of 100 indicates no fatigue and typical daily functioning; 17 is the worst possible score. Function of the operated hip was assessed with a modified Western Ontario and McMaster Universities Arthritis Index scale (WOMAC) that evaluates impairment when lying in bed, rising from bed, sitting, raising from sitting, standing, and walking on a flat surface on six separate 11-point numerical rating scales.8 A score of 60 indicates maximum impairment, and a score of 0 indicates no impairment. Postoperative pain was captured on a modified WOMAC scale assessing pain at rest, during the night, when bearing weight, and when walking on a flat surface on four separate 11-point numerical rating scales. A score of 40 indicates maximum pain, whereas a score of 0 indicates no pain. In addition, daily opioid consumption was captured and expressed as intravenous hydromorphone equivalents using an opioid conversion table.13

Fig. 1. Flowchart summarizing the experimental approach. Whole blood was obtained 1 h before surgery from patients undergoing primary hip arthroplasty (A). Separate whole-blood aliquots were stimulated ex vivo with extracellular ligands (B) (lipopolysaccharide [LPS], interleukin [IL]-6, IL-10, or a combination of IL-2 and granulocyte colony-stimulating factor [GMCSF]) or left untreated (U) (C). Using mass cytometry, the expression of 23 cell surface markers and the phosphorylation states of 11 intracellular signaling proteins were measured in single cells from blood samples (D). Unsupervised hierarchical clustering and manual gating strategies were applied to visualize and quantify patient-specific signaling responses in immune cell subsets spanning the entire immune system. Shown is a visual representation of a cluster hierarchy plot (E). Signaling responses that correlated significantly with clinical recovery parameters were identified by significance analysis of microarrays (F). Arrows indicate results for the two hypothetical patients (A). CD = cluster of differentiation; NK cells = natural killer cells.
Parameterization of Clinical Data
Parameterization was performed as previously described. Recovery from postoperative fatigue and resulting functional impairment was quantified as the time required to half-maximum recovery (SRS-$t_{1/2}$), where maximum recovery is defined by the presurgical SRS score. SRS-$t_{1/2}$ was chosen as the outcome because the time to full recovery was affected by ceiling effects. Recovery of hip function was quantified as the time required to regress to a score 18 or less on the WOMAC scale. A score of 18 is indicative of the transition from moderate to mild impairment. Recovery from pain was quantified as the time required to regress to a score of 12 or less on the WOMAC scale. A score of 12 is indicative of the transition from moderate to mild pain.

Specimen Collection and Stimulation with External Ligands
Whole blood was collected in heparin-containing tubes 1 h before surgery (fig. 1). Whole-blood samples were processed within 30 min of collection. Samples were divided into 1-ml aliquots and incubated at 37°C for 15 min with phosphate-buffered saline (PBS) (control), 100 ng/ml interleukin (IL)-6 (BD Biosciences, USA), 100 ng/ml IL-10 (BD Biosciences), a combination of 100 ng/ml IL-2 (BD Biosciences) and 2 ng/ml granulocyte macrophage colony-stimulating factor (GMCSF; PeproTech, USA), or 1 µg/ml lipopolysaccharide (LPS; LPS-EK Ultra Pure; InvivoGen, USA). Blood samples were resuspended in 1.4 ml stabilizing buffer (Smarttube Inc., USA), incubated for 10 min at room temperature for fixation, cooled to 4°C, and stored at -80°C until further processing for barcoding and antibody staining.

Selection of Stimulation Conditions
Previous work identified cell-type–specific signaling responses that were significantly modulated by surgery. Three of these signaling responses strongly correlated with clinical recovery parameters. External ligands were selected to specifically target pathways containing these signaling proteins (table 1). LPS was chosen to activate downstream effectors of the toll-like receptor 4 (TLR4) signaling pathway in cluster of differentiation (CD) 14+ monocytes, IL-6 and IL-10 were chosen to evoke signal transducer and activator of transcription 1 (STAT1) and STAT3 phosphorylation in CD14+ monocytes and T cell subsets, and a combination of 100 ng/ml lipopolysaccharide (LPS; LPS-EK Ultra Pure; InvivoGen, USA) to block nonspecific Fc binding. Cells were washed once with CSM then incubated overnight at 4°C with an antibody cocktail for 30 min at room temperature for fixation, cooled to 4°C, and stored at -80°C until further processing for barcoding and antibody staining.

Sample Barcoding
Reagents for barcoding were prepared as previously described. Palladium isotopes were chelated by isothiocyanobenzyl-EDTA in 0.02% saponin in PBS. Each well of a barcoding plate contained a distinct combination of three chelated palladium isotopes dissolved in dimethyl sulfoxide. After thawing and erythrocyte lysis in a hypotonic buffer, cells were barcoded as previously described. In brief, cells were transferred into a deep-well block and washed once with cell staining media (CSM, PBS with 0.5% bovine serum albumin, 0.02% NaN3), once with PBS, and once with 0.02% saponin in PBS. The barcoding plate was thawed, and each well of barcode reagent was diluted in 1 ml 0.02% saponin in PBS. Diluted barcode reagent was transferred to cells, and samples were incubated at room temperature for 15 min, washed twice with CSM, and then pooled for staining.

Antibody Staining
The panel included 23 antibodies for the comprehensive phenotypic characterization of immune cells, and 11 antibodies (phosphorylated cyclic adenosine monophosphate response element-binding protein [pCREB], phosphorylated extracellular regulated kinase [pERK], phosphorylated mitogen-activated protein kinase [pMAPK], phosphorylated nuclear factor κ-light-chain-enhancer of activated B cells [pNFκB, pP65], phosphorylated phospholipase C gamma 2 [pPLCG2], phosphorylated ribosomal S6 kinase [pRPS6], phosphorylated ribosomal protein S6 [pRPS6], phosphorylated STAT1 [pSTAT1], pSTAT3, and pSTAT5] directed toward phosphorylated forms of intracellular signaling proteins for the functional characterization of each immune cell (see table 1, Supplemental Digital Content 1, http://links.lww.com/ALN/B198, which is a table that lists the antibodies used in the study). Panel development and validation have previously been described in detail.

Cells were washed once with CSM and then incubated for 10 min at room temperature with one test of FeC block (Biolegend, USA) to block nonspecific Fc binding. Cells were stained with all surface antibodies for 30 min and washed once with CSM. Cells were permeabilized with 1 ml of methanol for 10 min on ice. Cells were then washed twice with PBS and once with CSM and incubated with the intracellular antibody cocktail for 30 min at room temperature. Cells were washed once with CSM then incubated overnight at 4°C with an iridium-containing intercalator (Fluidigm, USA) in PBS with 1.6% formaldehyde. Cells were then washed twice with CSM, once with water, and resuspended in a solution of normalization beads as previously described. Cells were filtered through a 35-µm membrane before analysis by mass cytometry.

Mass Cytometry
Barcoded and antibody-stained cells were analyzed on a mass cytometer (CyTOF; Fluidigm) at an event rate of 400 to 500 cells per second. The data were normalized using Normalizer v0.1 MATLAB Compiler Runtime (MathWorks, USA). Files were debarcoded using a single-cell MATLAB Debarcoder Tool. Gating was performed using the Cytobank platform as previously described.
**Table 1. Ligand Selection and Evoked Signaling Responses**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Cell Type</th>
<th>Signaling Protein*</th>
<th>Arcsinh Ratio (mean ± SD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>CD14+ monocyte</td>
<td>pSTAT1</td>
<td>0.171 ± 0.092</td>
<td>2.25 × 10^{-10}</td>
</tr>
<tr>
<td>IL-6</td>
<td>CD14+ monocyte</td>
<td>pSTAT3†</td>
<td>0.437 ± 0.136</td>
<td>2.40 × 10^{-14}</td>
</tr>
<tr>
<td>IL-10</td>
<td>CD14+ monocyte</td>
<td>pSTAT3†</td>
<td>0.502 ± 0.173</td>
<td>2.27 × 10^{-13}</td>
</tr>
<tr>
<td>GMCSF</td>
<td>CD14+ monocyte</td>
<td>pSTAT5</td>
<td>1.855 ± 0.253</td>
<td>1.41 × 10^{-22}</td>
</tr>
<tr>
<td>LPS</td>
<td>CD14+ monocyte</td>
<td>pNFKB†</td>
<td>0.249 ± 0.089</td>
<td>4.95 × 10^{-15}</td>
</tr>
<tr>
<td>LPS</td>
<td>CD14+ monocyte</td>
<td>pCREB†</td>
<td>0.483 ± 0.126</td>
<td>1.22 × 10^{-15}</td>
</tr>
<tr>
<td>LPS</td>
<td>CD14+ monocyte</td>
<td>pP38</td>
<td>2.001 ± 0.221</td>
<td>8.96 × 10^{-25}</td>
</tr>
<tr>
<td>LPS</td>
<td>CD14+ monocyte</td>
<td>pMAPKAPK2</td>
<td>1.552 ± 0.241</td>
<td>3.03 × 10^{-21}</td>
</tr>
<tr>
<td>LPS</td>
<td>CD14+ monocyte</td>
<td>pP90RSK</td>
<td>0.515 ± 0.192</td>
<td>1.21 × 10^{-15}</td>
</tr>
<tr>
<td>LPS</td>
<td>CD14+ monocyte</td>
<td>prpS6</td>
<td>0.625 ± 0.235</td>
<td>1.45 × 10^{-15}</td>
</tr>
<tr>
<td>LPS</td>
<td>CD14+ monocyte</td>
<td>pERK</td>
<td>0.970 ± 0.205</td>
<td>3.95 × 10^{-14}</td>
</tr>
<tr>
<td>IL-6</td>
<td>CD4+ T cell</td>
<td>pSTAT3</td>
<td>0.224 ± 0.095</td>
<td>2.05 × 10^{-11}</td>
</tr>
<tr>
<td>IL-10</td>
<td>CD4+ T cell</td>
<td>pSTAT3</td>
<td>0.264 ± 0.128</td>
<td>2.46 × 10^{-10}</td>
</tr>
<tr>
<td>IL-2</td>
<td>CD4+ T cell</td>
<td>pSTAT5</td>
<td>1.606 ± 0.284</td>
<td>5.93 × 10^{-20}</td>
</tr>
<tr>
<td>IL-6</td>
<td>CD8+ T cell</td>
<td>pSTAT3</td>
<td>0.069 ± 0.045</td>
<td>5.36 × 10^{-28}</td>
</tr>
<tr>
<td>IL-10</td>
<td>CD8+ T cell</td>
<td>pSTAT3</td>
<td>0.293 ± 0.138</td>
<td>1.41 × 10^{-10}</td>
</tr>
<tr>
<td>IL-2</td>
<td>CD8+ T cell</td>
<td>pSTAT5</td>
<td>1.677 ± 0.262</td>
<td>3.50 × 10^{-21}</td>
</tr>
</tbody>
</table>

* Signaling responses previously shown to be significantly modulated by surgery. † Signaling response to surgery previously shown to correlate significantly with clinical recovery parameters.

CD = cluster of differentiation; GMCSF = granulocyte macrophage colony-stimulating factor; IL = interleukin; LPS = lipopolysaccharide; pCREB = phosphorylated cyclic adenosine monophosphate response element-binding protein; pERK1/2 = phosphorylated extracellular regulated kinase 1/2; pMAPKAPK2 = phosphorylated mitogen-activated protein kinase-activated protein kinase-2; pNFkB = phosphorylated nuclear factor κ-light-chain-enhancer of activated B cells; pP38 = phosphorylated p38 MAP kinase; pP65RSK = phosphorylated ribosomal S6 kinase; prpS6 = phosphorylated ribosomal protein S6; pSTAT1/3/5 = phosphorylated signal transducer and actuator of transcription 1/3/5.

**Phenotyping of Immune Cells**

Major immune cell subsets including granulocytes, CD14+ monocytes, natural killer cells, classical and plasmacytoid dendritic cells (pDCs), CD4+ and CD8+ T cells, and B cells were identified with a manual gating strategy as previously described. Hierarchical clustering using Ward’s linkage and Euclidean distance in R was used as a second phenotyping strategy for the detailed characterization and visual representation of CD45+CD66− cells. Cells were clustered based on the expression of CD7, CD19, CD11b, CD4, CD8, CD127, CCR7, CD123, CD45RA, CD33, CD11c, CD14, CD16, FoxP3, CD25, CD3, HLA-DR, and CD56. Ten thousand events were sampled from each patient sample. The cluster hierarchy plots and histograms were created in R. Clusters containing at least 1% of all clustered cells are graphically displayed. Cell clusters are colored based on the median arc sinh ratio of the phosphorylated protein level in a stimulated condition (IL-6, IL-10, IL-2/GMCSF, and LPS) relative to the control condition (PBS). The arc sinh ratio is the median difference between the median arc sinh value in control and stimulated cell subsets. Protein phosphorylation data were transformed to arc sinh values by taking the inverse hyperbolic sine of the raw data.

**Statistical Analysis**

Data are expressed as means ± SDs or as medians with interquartile ranges (IQRs) if interindividual differences were of particular interest. Alternatively, the mean or median and corresponding 95% CIs are reported if confidence in the mean or median was of particular interest.

The sample size was based on a power calculation pertinent to the detection of surgery-induced alterations in cell signaling responses rather than the detection of immune correlates of surgical recovery. Considering all signaling responses with arc sinh ratios equal or greater than 0.2, the response associated with the highest variance (pp38 in CD14+ monocytes) was used to determine power. A sample size of 24 patients allows detecting signaling response with a power of 90% at an α level of 0.001.

**Cell-type–specific Signaling Responses to Stimulation with External Ligands.** To verify that selected ligands changed the phosphorylation state of signaling proteins altered by surgery, a two-class paired t test was used to compare arc sinh-transformed raw data between control and stimulated conditions at a Bonferroni-adjusted P value less than 8.92 × 10^{-4} (56 conditions: 14 signaling proteins in specific cell subsets and four stimulations).

**Correlation Analyses between Molecular and Clinical Parameters.** Significant correlations between the three major clinical recovery parameters and ligand-evoked changes in the phosphorylation state of intracellular signaling proteins in hand-gated cell subsets were detected by significance analysis of microarrays using the “samr” package in R. Significance analysis of microarray is a nonparametric test specifically designed for multiple comparison analysis of high-dimensional datasets. Two approaches were used to infer statistical significance. The first approach was most stringent and used a Bonferroni-corrected P value less than 1.42 × 10^{-4} (352 conditions: 4 stimulations, 11 signaling proteins, and 8 cell subsets). This approach may miss...
biologically important results in high-dimensional datasets. Therefore, a more inclusive approach was used by setting the statistical threshold for inferring significance at a false discovery rate less than 1% ($q < 0.01$). Statistically significant correlations were further filtered by applying two effect-size criteria. Results were only considered significant if the arcsinh ratio passed the threshold of 0.5 in at least one patient and the 95% interval of the arcsinh ratio did not include 0.

**Additional Correlational Analysis.** Previously reported postsurgical immune correlates (pMAPKAPK2, pCREB, and prpS6) were correlated with the same presurgical immune correlates, and functional recovery outcomes using $R$ (Bonferroni-adjusted $P < 1.16 \times 10^{-2}$; three conditions). Similarly, correlational analysis was performed for the three major clinical outcome variables ($P < 1.16 \times 10^{-2}$).

**Covariate Analysis.** Partial correlation analysis was performed to test whether a correlation between an immune parameter and a clinical recovery parameter remained significant when controlling for a particular covariate. This analysis correlated residuals that resulted from correlating clinical covariates and immune parameters with residuals resulting from correlating clinical covariates and clinical recovery parameters. Clinical covariates included age, sex, body mass index, estimated intraoperative blood loss, duration of surgery, use of spinal anesthesia, and presurgical values for major clinical outcomes. Analysis was performed in $R$. Correlations of residuals were considered significant for a Bonferroni-adjusted $P$ value less than $2.5 \times 10^{-3}$ (20 conditions: 10 covariates and 2 immune parameters). A significant partial correlation indicated that the correlation between an immune parameter and a clinical recovery parameter remained significant when adjusting for a particular covariate.

**Multilinear Regression Analysis.** The analysis was performed in SPSS (IBM SPSS Statistics version 20, USA) to determine whether age contributed to the correlation between immune parameters and clinical outcomes that passed Bonferroni correction. The age range of patients was wide enough to warrant such analysis.

**Results**

**Subjects**

Two hundred fifty-one patients from the Arthritis and Joint Replacement Clinic in the Department of Orthopedic Surgery at Stanford University School of Medicine were screened. One hundred five patients did not meet inclusion criteria, 31 declined to participate, 65 could not be enrolled due to failed barcoding. The analysis set included complete datasets of 25 patients: 24 Caucasian and 1 African American, 16 men and 9 women, median age of 59 yr (IQR, 54 to 67), and median body mass index of 24.4 kg/m$^2$ (IQR, 26.4 to 28.0). Comorbidities included controlled arterial hypertension (eight subjects), stable coronary artery disease (two subjects), hyperlipidemia (eight subjects), controlled asthma (two subjects), controlled diabetes mellitus type 2 (one subject), controlled hypothyroidism without evidence for an autoimmune disease (two subjects), and asymptomatic hyperparathyroidism (one subject). Over the course of the 6-week study period, none of the patients experienced cardiac, pulmonary, neurological, infectious, thromboembolic, or bleeding complications or required a second operation for surgical complications.

**Surgery and Anesthesia**

The median duration of surgery was 97 min (IQR, 85 to 113), median blood loss was 225 ml (IQR, 200 to 315), and median intraoperative crystalloid load was 1,500 ml (IQR, 1,000 to 2,000). Nineteen of 25 patients received an adjuvant spinal anesthetic with a median bupivacaine dose of 11.3 mg (IQR, 10.5 to 12.0) and a median morphine dose of 0.1 mg (IQR, 0.1 to 0.2). The median minimal alveolar concentration of the volatile anesthetic was 0.5% (IQR, 0.5 to 0.7), and the median minimal alveolar concentration of nitrous oxide was 0.4% (IQR, 0.4 to 0.6) in the 10 patients who were exposed to nitrous oxide. The median opioid doses were 2.6 mg (IQR, 1.5 to 3.8) intravenous hydromorphone equivalents during surgery, 16.5 mg (IQR, 13.1 to 27.4) after surgery and during hospital stay, and 9.0 mg (IQR, 5.3 to 16.9) for up to 6 weeks after hospital discharge. The median time to hospital discharge was 3.1 days (IQR, 3.0 to 3.8).

**Clinical Outcomes**

Clinical outcomes were previously reported in detail. The median time required to half-maximum recovery from postoperative fatigue and resulting functional impairment was 10 days (IQR, 6 to 15); this time varied widely among patients with a range of 0 to 36 days. The median time required to regress to mild functional impairment of the operated hip was 15 days (IQR, 11 to 21); this time also varied widely from 2 to 42 days. The median time required to regress to mild pain was 10 days (IQR, 6 to 18) with a range from 2 to 36 days. The median time required to half-maximum recovery from postoperative fatigue and resulting functional impairment did not correlate significantly with the time required to regress to mild functional impairment of the hip or the time required to regress to mild pain. The time required to regress to mild functional impairment of the hip and the time required to regress to mild pain were significantly correlated ($R = 0.54$, $P = 5.00 \times 10^{-2}$).

**Evoked Signaling Responses in Presurgical Blood Samples**

Ligands selected for *ex vivo* stimulation of presurgical blood samples were those that were previously shown to evoke the...
Variability of Evoked Signaling Responses in Presurgical Blood Samples

Although evoked signaling responses in samples collected before surgery were directionally consistent, the magnitude of these signaling responses varied three- to five-fold among patients (tables 1 and 2, Supplemental Digital Content 1, http://links.lww.com/ALN/B198). This is illustrated in figure 3 in plots of the arcsinh ratios of CD14+ monocyte responses to stimulation in individual patient samples. The results are reminiscent of the variability reported for pSTAT3, pCREB, and pNFκB in presurgical samples with IL-6 or LPS are graphically displayed in figure 2. The results confirm that stimulation of presurgical samples with the selected ligands interrogated signaling pathways that were altered by surgery and correlated with clinical recovery parameters.

Evoked Signaling Responses in Presurgical Blood Samples Correlate with Clinical Recovery

Patient-specific immune states before surgery were characterized by measuring 352 different immune parameters, that is, response of 11 intracellular signaling proteins to 4 different ex vivo ligands separately assessed for 8 different immune cell types (fig. 4). Each immune parameter was regressed against the three clinical recovery parameters. Two correlates passed the stringent Bonferroni correction (fig. 5A). The pMAPKAPK2 signaling responses to LPS in classical dendritic cells ($R = 0.70$) and in CD14+ monocytes ($R = 0.69$) strongly correlated with the clinical recovery parameter “time to regress to mild functional impairment of the operated hip” ($P < 0.001$) and age ($P = 0.032$) both correlated significantly with the clinical recovery parameter “time to regress to mild functional impairment of the operated hip.” pMAPKAPK2 signaling in CD14+ monocytes ($P < 0.001$) but not age ($P = 0.053$) was significantly correlated with this clinical outcome. The overall contribution of age relative to the pMAPKAPK2 signaling was small as indicated by $R$ coefficients of 0.2 and 27.3 for dendritic cells and 0.2 and 21.3 for CD14+ monocytes.

Eighteen significant correlations were identified when using a false discovery rate less than 1%, a less stringent approach commonly applied to large datasets ($R = 0.36$ to 0.70, $q < 0.01$; table 3). Significant findings were limited to specific immune cell subsets, signaling proteins, and stimulation conditions. Importantly, these correlations were restricted to innate immune cells (CD14+ monocytes, dendritic cells, and granulocytes) and concerned only 5 of the 11 examined signaling proteins. Significant correlations were identified between signaling activities in CD14+ monocytes, dendritic cells, and granulocytes and the two clinical recovery parameters (1) time required to regress to mild functional impairment of the operated hip and (2) time required to regress to mild pain (table 3). Two themes evolved. First, LPS-evoked signaling responses in CD14+ monocytes and classical dendritic cells, including pMAPKAPK2, pCREB, and pprS6, were correlated with a prolonged functional recovery of the operated hip and a slower resolution of postoperative pain. These findings are supported by intermediate to strong correlations ($R = 0.39$ to 70). Second, IL-2/GMCSF-evoked signaling in pDCs and granulocytes, including pERK, p90RSK, and pMAPKAPK2, were correlated with shortened functional recovery of the operated hip. These findings are supported by correlations of intermediate strength ($R = 0.36$ to 0.54).

Taken together, these data are consistent with the view that the interrogation of a patient’s immune state before surgery has significant predictive potential regarding a patient’s clinical recovery after surgery.

Discussion

The results of this study in patients undergoing primary hip arthroplasty suggest that patient-specific immune states before surgery are a key determinant of surgical recovery. A patient’s presurgical immune state was interrogated in peripheral blood samples by assessing ligand-evoked signaling responses across all major immune cell subsets with mass cytometry at single-cell resolution.10 The analysis revealed patient-specific immune states that contained strong correlates of surgical recovery. Immune correlates accounted for 15 to 50% of observed patient variability in functional recovery and regression of pain.
The study interrogated cell-specific intracellular signaling pathways implicated in the endogenous immune response to surgery, a subset of which—namely pSTAT3, pCREB, and pNFkB—measured in CD14+ monocytes shortly after surgery—have previously been shown to correlate strongly with surgical recovery. In the current analysis, 18 presurgical signaling responses correlated significantly with clinical recovery parameters; two particularly strong correlates were the pMAPKAPK2 response to LPS in CD14+ monocytes and classical dendritic cells (table 3). Each correlate accounted...
cyte monocyte colony-stimulating factor (GM-CSF). The
charide (LPS), or the combination of IL-2 and granulo-
response element-binding protein (pCREB) signal in CD14 +
(signaling response varied 3.5-fold among patients.

Differentiation (CD) 14+ monocytes increased significantly
activator of transcription 3 (pSTAT3) signal in cluster of
conditions. (perbolic sine) obtained in unstimulated and stimulated
difference between arcsinh-transformed values (inverse hy-
subsets. Values reflect the arcsinh ratio, that is, the dif-
variations of evoked signaling responses in pre-
surgical whole-blood samples. Cell-type–specific sig-
responses were quantified in manually gated cell
(A) The phosphorylated signal transducer and
activator of transcription 3 (pSTAT3) signal in cluster of
differentiation (CD) 14+ monocytes increased significantly
response to interleukin (IL)-6 ($P = 2.40 \times 10^{-14}$) and IL-10
($P = 2.27 \times 10^{-14}$), but not in response to lipopolysac-
charide (LPS), or the combination of IL-2 and granulo-
cyte colony-stimulating factor (GM-CSF). The signaling
response varied 3.5-fold among patients. (B) The phosphorylated cyclic adenosine monophosphate
response element-binding protein (pCREB) signal in CD14+
monocytes increased in response to LPS ($P = 1.22 \times 10^{-15}$),
the combination of IL-2 and GMCSF ($P = 4.80 \times 10^{-14}$),
and IL-6 ($P = 3.03 \times 10^{-14}$), but not in response to IL-10.
The signaling responses varied by 3.5- to 5.5-fold
among patients. (C) The phosphorylated nuclear factor
-light-chain-enhancer of activated B cells (pNFkB) signal
in CD14+ monocytes increased in response to LPS
($P = 4.95 \times 10^{-13}$), the combination of IL-2 and GMCSF
($P = 1.65 \times 10^{-9}$), and IL-6 ($P = 1.87 \times 10^{-9}$), but not in
response to IL-10. The signaling responses varied five-fold
among patients. * Statistical significance based on the
Bonferroni-adjusted $P$ value ($P = 8.92 \times 10^{-4}$).

The findings integrate with the well-established role of
the TLR4 signaling pathway in the innate immune response
to traumatic injury. MAPKAPK2 is a component of the
p38 MAP kinase pathway, which mediates TLR4 priming
in injury. Injured cells release danger-associated molecular
pattern molecules that alert the immune system to the
presence of tissue damage. Certain danger-associated
molecular pattern molecules, including high-mobility group
protein B1 and heat shock proteins, act principally on innate
immune cells by binding to TLR4, the primary receptor for
LPS. Upon TLR4 activation, p38 phosphorylates MAP-
KAPK2, mitogen- and stress-activated kinases 1/2, and ribo-
sonal S6 kinase. This signaling cascade converges on the
phosphorylation of CREB and ribosomal protein S6, which
regulate transcriptional and translational mechanisms that
are essential for the survival, proliferation, and differentia-
tion of innate immune cells.

Recovery from functional impairment of the operated
hip was correlated not only with the pMAPKAPK2 response
to LPS in CD14+ monocytes but also with the prpS6 and
cREB responses in CD14+ monocytes (table 3). These
findings are consistent with previous results documenting
that levels of pMAPKAPK2, prpS6, and pCREB in CD14+
monocytes at 1 h after surgery correlated with recovery from
functional impairment of the hip (table 4). Furthermore,
the pMAPKAPK2, prpS6, and pCREB responses to LPS
in presurgical samples correlated with respective pMAP-
KAPK2, pCREB, and prpS6 signals measured 1 h after
surgery (table 4). These results suggest that the LPS-evoked
activation of a TLR4-dependent signaling network engaging
MAPKAPK2, CREB, and rpS6 in CD14+ monocytes reca-
pitulates aspects of surgery-evoked mobilization of the same
signaling network. As pMAPKAPK2, CREB, and prpS6
injured presurgical and postsurgical samples corre-
lated with the same clinical functional parameter of recovery,
the activation of an entire signaling network downstream of
TLR4, rather than isolated signaling activities, may deter-
mine functional recovery (fig. 6).

The analysis revealed other interesting though somewhat
less robust immune correlates. In particular, the pMAP-
KAPK2, pCREB, and prpS6 responses to LPS in CD14+
monocytes correlated with the speed at which postoperative
pain resolved and accounted for 16% of observed variance.
Such association may be expected, as the resolution of pain
for almost 50% of the variability associated with the speed at
which patients recovered from functional impairment of the
operated hip. Thus, among all measured immune responses
in adaptive and innate immune cells, evoked signaling
responses in innate cells of myeloid lineage were the strongest
indicators of the recovery process. In postsurgical samples,
the strongest immune correlates of surgical recovery were
also signaling events in monocyte subsets. Current results
dovetail with a large body of work implicating a critical
role of monocyte subsets and dendritic cells in the immune
response to traumatic injury and surgical recovery.

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lated with the same clinical functional parameter of recovery,
the activation of an entire signaling network downstream of
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mine functional recovery (fig. 6).

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KAPK2, pCREB, and prpS6 responses to LPS in CD14+
monocytes correlated with the speed at which postoperative
pain resolved and accounted for 16% of observed variance.
Such association may be expected, as the resolution of pain
and the recovery from functional impairment of the operated hip are interdependent outcomes. This finding is also reminiscent of the strong relation between the pNFkB signal measured 1 h after surgery in CD14+ monocytes and the resolution of pain as all signaling molecules are components of the TLR4 signaling pathway (fig. 6).8 The pERK, p90RSK, and pMAPKAPK2 responses to IL-2/GMCSF stimulation in pDCs and granulocytes were correlated with the speed at which patients recovered from functional impairment of the operated hip. GMCSF promotes survival and proliferation in pDCs and granulocytes via several interconnected pathways including the ERK/MAPK, phosphoinositide 3-kinase, NFkB, and JAK2/STAT5 pathways.38 The suggestion that a predisposition for pDCs and granulocytes to mount a robust response to GMCSF may hasten the recovery process warrants further investigation.

Identified immune correlates provide the mechanistic framework for developing a diagnostic test that will predict the speed of functional recovery after hip arthroplasty. In a next step, present findings will need to be validated in a larger and independent patient cohort and the response elements within the TLR4 signaling pathway in CD14+ monocytes that best predict the speed of functional recovery will need to be defined. This will then allow reducing the number of parameters required in a clinical test by only including the most predictive parameters and permit switching from the complex 50-parameter mass cytometry platform to a standard 4 to 6 parameter fluorescence flow cytometry platform readily available in clinical laboratories. A clinical test could then be performed fairly quickly in freshly collected whole blood without further requirements for sample freezing, storing, or barcoding. Although samples in this study were collected 1 h before surgery, in a clinical setting sampling would likely occur during a preoperative assessment visit days before surgery to guide perioperative management.

The study has certain limitations. First, the clinical indices of surgical recovery are derived from the same patient cohort as in our first study.8 Second, this patient cohort had minimal comorbidities and underwent the same surgical procedure to eliminate many confounding variables and enable the identification of immune correlates. As such results may not extrapolate to other types of surgeries or patient cohorts with complex comorbidities. Third, although convergent findings in the pre- and postsurgical analyses provide validation for reported immune correlates of functional recovery and resolution of pain, all clinical outcomes were questionnaire based. Future studies will need to broaden clinical assessments and include more objective outcome tools to better understand the overall impact of the current findings on short- and long-term function and pain, as well as other outcome domains including cognitive function and sleep. For example, WOMAC-based functional results and sleep. For example, WOMAC-based functional results and sleep.

Fig. 4. Patient-specific evoked signaling responses in myeloid and lymphoid cell subsets. Patient-specific immune states before surgery were characterized along three axes including 8 cell types, 11 signaling proteins, and the activity of these signaling proteins in response to 4 different extracellular ligands. Signaling activity is indicated by the color-coded arcsinh ratio, that is, the difference between arcsinh-transformed values (inverse hyperbolic sine) obtained in unstimulated and stimulated conditions. A total of 352 parameters described each patient’s immune state. Shown are the three-dimensional heat maps characterizing and differentiating the immune states of two exemplary patients. CD = cluster of differentiation; cDCs = classical dendritic cells; GMCSF = granulocyte monocyte colony-stimulating factor; IL = interleukin; LPS = lipopolysaccharide; NK cells = natural killer cells; pCREB = phosphorylated cyclic adenosine monophosphate response element-binding protein; pDCs = plasmacytoid dendritic cells; pERK1/2 = phosphorylated extracellular regulated kinase 1/2; pMAPKAPK2 = phosphorylated mitogen-activated protein kinase-activated protein kinase-2; pNFkB = phosphorylated nuclear factor κB signaling and the recovery from functional impairment of the operated hip. GMCSF promotes survival and proliferation in pDCs and granulocytes via several interconnected pathways including the ERK/MAPK, phosphoinositide 3-kinase, NFkB, and JAK2/STAT5 pathways.38 The suggestion that a predisposition for pDCs and granulocytes to mount a robust response to GMCSF may hasten the recovery process warrants further investigation.

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quantification of pain during standardized physical tasks may provide more robust outcomes data that could actually strengthen the correlation between immune parameters and clinical outcomes. Fourth, none of the evoked signaling responses correlated significantly with recovery from fatigue and resulting functional impairment. In our previous study, changes in STAT3 signaling between 1 and 24 h after surgery strongly correlated with recovery from fatigue. The signaling differences between time points after surgery may be more challenging to recapitulate in a presurgical in vitro...
assay. Finally, although mass cytometry currently enables the simultaneous detection of up to 50 antigens per cell, the technology nonetheless requires an a priori selection of a subset of intracellular signaling proteins. Only a fraction of the downstream effectors of TLR4 signaling were therefore analyzed in the current study. There is the possibility that relevant intracellular signals remained undetected. LPS or trauma-induced TLR4 agonists such as high-mobility group protein B1 modulate the function of more than 30 intracellular proteins in innate immune cells.41 Future studies will expand on this first report and comprehensively assess the first report thoroughly and account for less than 10 to 15% of observed patient variability.

Despite significant interest in identifying predictors of surgical recovery ahead of surgery, current clinical and molecular variables with predictive potential typically account for less than 10 to 15% of observed patient variability.22,42–49 Most studies have focused on metrics that may predict hospital length of stay and the incidence of postoperative complications and mortality; patient-centered outcomes, including functional impairment, pain, and fatigue, have received less attention.2 Our findings suggest that the comprehensive assessment of a patient's preoperative immune state may add significant value to predict important surgical recovery parameters as reported immune correlates accounted for up to 50% of observed patient variability. The a priori identification of patients at risk for a protracted and/or complicated recovery will allow for patient-tailored and cost-effective strategies to mitigate such risks. Mitigation might include postponing procedures to coincide with a more favorable recovery profile and tailoring intervention to improve the "readiness" to recover from surgical trauma to the individual patient. Examples of interventions requiring further validation include preoperative exercise and/or stress-reduction programs, dietary supplementation, and administration of immune-modulating medications.9,50–55

Table 3. Significant Immune Correlates

<table>
<thead>
<tr>
<th>Cell Types</th>
<th>Ligand</th>
<th>Signaling Protein</th>
<th>Arcsinh Ratio (Median and 95% CI)</th>
<th>Clinical Outcome</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>cDCs</td>
<td>LPS</td>
<td>pMAPKAP2</td>
<td>1.336 (1.293–1.453)</td>
<td>Impaired hip function</td>
<td>0.702</td>
</tr>
<tr>
<td>CD14+ monocytes</td>
<td>LPS</td>
<td>pMAPKAP2</td>
<td>1.500 (1.453–1.652)</td>
<td>Impaired hip function</td>
<td>0.689</td>
</tr>
<tr>
<td>pDCs</td>
<td>IL-2/GMCSF</td>
<td>pERK</td>
<td>1.159 (0.947–1.203)</td>
<td>Impaired hip function</td>
<td>−0.544</td>
</tr>
<tr>
<td>pDCs</td>
<td>IL-2/GMCSF</td>
<td>pP90RSK</td>
<td>0.485 (0.388–0.569)</td>
<td>Impaired hip function</td>
<td>−0.517</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>IL-2/GMCSF</td>
<td>pMAPKAP2</td>
<td>0.574 (0.516–0.647)</td>
<td>Impaired hip function</td>
<td>−0.498</td>
</tr>
<tr>
<td>CD14+ monocytes</td>
<td>LPS</td>
<td>prpS6</td>
<td>0.576 (0.528–0.722)</td>
<td>Impaired hip function</td>
<td>0.467</td>
</tr>
<tr>
<td>CD14+ monocytes</td>
<td>LPS</td>
<td>pCREB</td>
<td>0.442 (0.411–0.514)</td>
<td>Impaired hip function</td>
<td>0.445</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>IL-2/GMCSF</td>
<td>pERK</td>
<td>1.053 (0.886–1.114)</td>
<td>Impaired hip function</td>
<td>−0.442</td>
</tr>
<tr>
<td>CD14+ monocytes</td>
<td>LPS</td>
<td>pMAPKAP2</td>
<td>1.500 (1.453–1.652)</td>
<td>Pain</td>
<td>0.415</td>
</tr>
<tr>
<td>pDCs</td>
<td>IL-2/GMCSF</td>
<td>prpS6</td>
<td>0.875 (0.725–0.924)</td>
<td>Impaired hip function</td>
<td>−0.410</td>
</tr>
<tr>
<td>CD14+ monocytes</td>
<td>LPS</td>
<td>pCREB</td>
<td>0.442 (0.411–0.514)</td>
<td>Pain</td>
<td>0.409</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>IL-2/GMCSF</td>
<td>pP90RSK</td>
<td>0.269 (0.211–0.306)</td>
<td>Impaired hip function</td>
<td>−0.408</td>
</tr>
<tr>
<td>pDCs</td>
<td>IL-2/GMCSF</td>
<td>pMAPKAP2</td>
<td>0.361 (0.292–0.389)</td>
<td>Impaired hip function</td>
<td>−0.400</td>
</tr>
<tr>
<td>CD14+ monocytes</td>
<td>LPS</td>
<td>prpS6</td>
<td>0.576 (0.528–0.722)</td>
<td>Pain</td>
<td>0.392</td>
</tr>
<tr>
<td>pDCs</td>
<td>IL-2/GMCSF</td>
<td>pCREB</td>
<td>0.635 (0.552–0.735)</td>
<td>Impaired hip function</td>
<td>0.381</td>
</tr>
<tr>
<td>CD14+ monocytes</td>
<td>LPS</td>
<td>pP90RSK</td>
<td>0.397 (0.301–0.432)</td>
<td>Impaired hip function</td>
<td>0.376</td>
</tr>
<tr>
<td>cDCs</td>
<td>IL-2/GMCSF</td>
<td>pERK</td>
<td>0.172 (0.119–0.212)</td>
<td>Impaired hip function</td>
<td>0.372</td>
</tr>
<tr>
<td>cDCs</td>
<td>IL-2/GMCSF</td>
<td>pERK</td>
<td>0.467 (0.392–0.623)</td>
<td>Impaired hip function</td>
<td>0.367</td>
</tr>
</tbody>
</table>

Table 4. Immune Correlates Pre- and Postsurgery

<table>
<thead>
<tr>
<th>Signaling Protein</th>
<th>Presurgery Signaling vs. Hip Function</th>
<th>Postsurgery Signaling vs. Hip Function</th>
<th>Presurgery vs. Postsurgery Signaling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P Value</td>
<td>R</td>
</tr>
<tr>
<td>pMAPKAP2</td>
<td>0.689</td>
<td>1.39 × 10−4</td>
<td>0.465</td>
</tr>
<tr>
<td>pCREB</td>
<td>0.445</td>
<td>2.59 × 10−2</td>
<td>0.569</td>
</tr>
<tr>
<td>prpS6</td>
<td>0.467</td>
<td>1.86 × 10−2</td>
<td>0.591</td>
</tr>
</tbody>
</table>

* Values for presurgery signaling are arcsinh ratios in cluster of differentiation (CD) 14+ monocytes for lipopolysaccharide-stimulated and control samples, and values for postsurgery signaling are arcsinh ratios in CD14+ monocytes for samples collected before surgery and 1 h after surgery. LPS = lipopolysaccharide; pCREB = phosphorylated cyclic adenosine monophosphate response element-binding protein; pDCs = plasmacytoid dendritic cells; pERK1/2 = phosphorylated extracellular regulated kinase 1/2; pMAPKAP2 = phosphorylated mitogen activated protein kinase-activated protein kinase-2; pP90RSK = phosphorylated ribosomal S6 kinase; prpS6 = phosphorylated ribosomal protein S6; R = correlation coefficient.
The ability to detect strong immune correlates was due to the application of a recently developed mass cytometry protocol, which enabled functional interrogation of precisely phenotyped cells with single-cell resolution. Previous studies probing for molecular markers of recovery may have missed strong correlates as these studies relied on bulk analysis, precluded detailed identification of cell subsets, or could not measure functional attributes of cell subsets. Although a high-dimensional mass cytometry assay enabled the detection of strong correlates of surgical recovery, identified immune correlates can readily be assessed with traditional and widely available clinical flow cytometry platforms. This prospect holds significant promise for the development and validation of a diagnostic test that will help predict surgical recovery.

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Fig. 6. A model summarizing the toll-like receptor 4 (TLR4)-dependent signaling responses implicated in surgical recovery. In response to tissue damage, damage-associated molecular patterns (DAMPs) molecules including alarmins such as high-mobility group protein B1 (HMGB1) and heat shock proteins (HSPs) bind to and activate the TLR4 on the surface of innate immune cells. The TLR4 signaling pathway is also induced by binding of lipopolysaccharide (LPS) to the TLR4 receptor and to coreceptors cluster of differentiation 14 (CD14) and myeloid differentiation factor 2 (MD2). The TLR4 receptor engages a complex signaling cascade (only partially represented) leading to the activation of the phosphorylated P38 mitogen-activated protein kinase (p38) and extracellular regulated kinase and mitogen-activated protein kinase (ERK/MAP) and the phosphorylated nuclear factor κ-light-chain-enhancer of activated B cells (NFκB) signaling pathways. Signaling proteins that changed significantly in response to surgery are indicated by blue circles. A subset of signaling proteins modulated after surgery (mitogen-activated protein kinase-activated protein kinase 2 [MAPKAPK2], ribosomal protein S6 (rpS6), cyclic adenosine monophosphate response element-binding protein [CREB]) overlapped with presurgical signaling responses that correlated with surgical recovery parameters and are indicated by red circles. IKK = IkB kinase; IkB = inhibitor of NFκB; MEKKs = mitogen-activated protein kinase kinase kinase; p90RSK = phosphorylated ribosomal S6 kinase.
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Competing Interests
Dr. Nolan has a personal financial interest in Fluidigm (South San Francisco, California), the manufacturer of the mass cytometer used in this article. The other authors declare no competing interests.

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PERIOPERATIVE MEDICINE