Immune Function following Major Spinal Surgery and General Anesthesia

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- anesthesia
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There are reported differences in the effects that general anesthetics may have on immune function after minor surgery. To date, there are no prospective trials comparing total intravenous anesthesia (TIVA) with a volatile agent-based technique and its effects on immune function after major spinal surgery in adolescents. Twenty-six adolescents undergoing spinal fusion were randomized to receive TIVA with propofol-remifentanyl or a volatile agent-based technique with desflurane-remifentanyl. Immune function measures were based on the antigen-presenting and cytokine production capacity, and relative proportions of cell populations. Overall characteristics of the two groups did not differ in terms of perioperative times, hemodynamics, or fluid shifts, but those treated with propofol had lower bispectral index values. Experimental groups had relatively high baseline interleukin-10 values, but both showed a significant inflammatory response with similar changes in their respective immune functions. This included a shift toward a granulocytic predominance; a transient reduction in monocyte markers with significant decrease in antigen-presenting capacity and cytokine production capacity. Anesthetic choice does not appear to differentially impact immune function, but exposure to anesthetics and surgical trauma results in reproducibly measurable suppression of both innate and adaptive immunity in adolescents undergoing posterior spinal fusion. The magnitude of this suppression was modest when compared with pediatric and adult patients with critical illnesses. This study highlighted the need to evaluate immune function in a broader population of surgical patients with higher severity of illness.

Introduction

Adolescent idiopathic scoliosis (AIS) is a common disease with a prevalence as high as 5.2%.¹ In extreme cases, patients may suffer from physical deformities that cause undue

pressure on the lungs, heart and abdomen. Even if AIS is treated before its progression becomes too severe, the primary surgical intervention is posterior spinal fusion (PSF), which in itself is a major procedure with its own risks. In addition to the intraoperative distraction of the vertebral

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column that may lead to inadvertent spinal cord injury or ischemia, it has been shown that the severity of surgery has a direct correlation with immune response and peak serum concentration of specific cytokines and stress hormones.²

Evidence has suggested that the choice of anesthetic agents may have differential effects on immune function,^{3,4} which when combined with the immuno-depressant effects of major trauma and surgical procedures, may impact the short and long-term outcome following major surgical procedures.⁵ In the context of minor surgical procedures, the variable immunomodulatory effects of different anesthesia procedures are reportedly significant such that cell counts and activation markers of adaptive immunity decrease more in response to inhaled anesthetics as compared with TIVA.⁶ This is important to our working model, which proposes that major trauma or surgery may have an overriding influence on immune function relative to the choice of anesthetic. While the goal of our current study was to evaluate the effects of anesthetic technique on immune function following a major orthopedic surgery such as PSF, it also serves as a proof of concept that immunological assays are feasible in a pediatric population using minimal volumes of blood.

Materials and Methods

Study Design

We conducted this prospective study following approval and in accordance with the local institutional review board requirements (ethics approval protocol IRB11-00727). After obtaining parental consent and assent from patients scheduled to undergo PSF for the treatment of AIS, enrolled subjects were randomized to receive anesthetic regimens that included either desflurane-remifentanyl (DES) or propofol-remifentanyl (TIVA). Collected data included both demographic (age, weight, height, body mass index, and sex) and orthopedic/surgical (Lenke grade, pre-/post-operative Cobb angle, levels fused) factors, as well as intraoperative parameters, and postoperative pain scores based on a previously described visual analog scale on which patients self-report pain intensity represented by a mark on a line.⁷

Anesthetic Management

Midazolam was administered as needed, but no dexamethasone and labetalol or anticholinergic agents were administered perioperatively. Routine American Society of Anesthesiologists' (ASA) monitors, plus the bispectral index (BIS) monitor, and an arterial cannula were used intraoperatively. All patients had a peripheral intravenous cannula for baseline blood sampling. Subsequent induction of anesthesia was based on the treatment group: DES sevoflurane followed by desflurane, and TIVA 2 to 3 mg/kg bolus followed by infusion of intravenous propofol. Both groups were treated with fentanyl (2–3 µg/kg) and rocuronium (0.3 mg/kg) to facilitate endotracheal intubation and dosing of the respective general anesthetic agents were titrated to maintain a BIS value of 40 to 60 during the surgical procedure. Additional doses of rocuronium (0.2 mg/kg) were administered as needed during dissection of the paraspinal muscles. A remifentanyl infusion was administered starting at

0.1 µg/kg/minute and increased up to 1 µg/kg/minute, in combination with hydralazine (0.1–0.2 mg/kg) as needed, to maintain the mean arterial pressure (MAP) at 55 to 65 mm Hg as a means to limit intraoperative blood loss.

Clinical data were collected every 30 minutes during the case. When neurophysiological monitoring was no longer required based on the surgical procedure, the propofol and remifentanyl infusions were discontinued. Desflurane was then used in both groups to maintain the BIS value at 50 to 70, and hydromorphone (HM) was administered in increments of 0.2 mg based on the respiratory rate. Acetaminophen (15 mg/kg) to a maximum dose of 1,000 mg and ondansetron (4 mg) were administered intravenously.

Blood sampling for immune function testing was performed at three time points: immediately before the induction of anesthesia (preoperative), at the end of surgery (postoperative), and on the morning of postoperative day 1 (POD1). These samples were then analyzed as follows:

Flow Cytometry

We evaluated leukocyte proportions and quantitated expression of the class II major histocompatibility complex molecule human leukocyte antigen (HLA)-DR on the cell surface of circulating monocytes. In brief, 100 µL aliquots of whole blood were collected in ethylenediamine tetraacetic acid-coated tubes to prevent clotting and blocked with human IgG before primary antibody (Ab) labeling, red blood cell lysis (BD Biosciences, Cat 349202), and subsequent fixation in 4% paraformaldehyde. Primary labeling was done with the following conjugates: fluorescein isothiocyanate (FITC)-isotype control, allophycocyanin anti-CD14 Ab, and FITC-anti-HLA-DR Ab. Samples were acquired using a BD LSR II flow cytometer and analyzed using FlowJo (Tree Star) software. Human lymphocytes, monocytes, and granulocytes may be identified based on their relative cell size and internal complexity, which contribute to respective forward scatter (FSC) and side scatter (SSC) using flow cytometry. In this gating strategy, lymphocytes were identified based on relatively low FSC and SSC compared with the larger monocytes (high FSC) and more complex granulocytes (high SSC) before being manually gated by fluorescence.

Ex Vivo Stimulation Assays

We performed ex vivo stimulation assays to quantify the responsiveness of circulating leukocytes as a measure of immune function. For the purposes of this study, we used the capacity of whole blood samples to produce cytokines in response to ex vivo incubation with lipopolysaccharide (LPS) or phytohemagglutinin (PHA) as measures of innate and adaptive immune function, respectively. In this case, a reduced cytokine production capacity signifies immune suppression.^{8–10} Within 30 minutes of initial collection into heparinized tubes on ice, 50 µL aliquots of whole blood were treated either with 500 pg/mL LPS or 10 µg/mL PHA at 37°C for either 4 hours (LPS) or 24 hours (PHA). Stimulation solutions were manufactured bimonthly under rigorous quality control (<10% intrabatch variation) in the Immune Surveillance Laboratory at The Research Institute at Nationwide Children's Hospital (NCH) as previously described.⁹ After stimulation,

samples were centrifuged for 5 minutes at $1,000 \times g$ and frozen at -80°C for batch analysis. We quantified tumor necrosis factor- α (TNF- α) in LPS-stimulated supernatants using the Immulite 1000 automated chemiluminometer (Siemens Healthcare Diagnostics), and quantified interferon- γ (IFN- γ), and interleukin (IL)-10 in PHA-stimulated supernatants using a Bio-plex MAGPIX multiplex analyzer (Bio-Rad). Reduced cytokine production capacity of TNF- α or IFN- γ and IL-10 is characteristic of innate or adaptive immune suppression, respectively. We also used the Immulite 1000 automated chemiluminometer to measure IL-6 levels in unstimulated plasma, which is directly related to the degree of systemic inflammation.

Healthy Controls

We recruited healthy age- and gender-matched control subjects from the outpatient phlebotomy area at NCH. These volunteers did not receive anesthesia or undergo surgery, and were screened to exclude those with underlying conditions or medications that are known to influence immune function.

Statistical Analysis

Data were analyzed using Prism 7 (GraphPad Software; La Jolla California, United States). Two-way analysis of variance was used to assess differences between and within the groups. Mann-Whitney U test was applied to compare patient characteristics and control values. A p -value less than 0.05 was considered as statistically significant.

Results

We enrolled 26 subjects (13 per group) into the spinal surgery arm of the study, along with 13 healthy control subjects. ▶ **Table 1** shows the general demographic information and perioperative characteristics of the scoliosis cohort. Subjects were predominately females with most graded II on the ASA Physical Status Classification System. While the DES group had slightly taller subjects, it did not differ significantly from the TIVA group. None of the subjects developed postoperative infection.

Intraoperative Management

Both treatment groups had similar surgery and anesthesia times. In line with the controlled hypotension (target MAP 55–65 mm Hg) to minimize blood loss, both groups showed a decrease in the MAP over time with an associated increase in heart rate (▶ **Fig. 1**). All patients received a similar amount of intraoperative crystalloid, while none received colloid or allogeneic blood product transfusions. Although BIS values in both groups were maintained in the target 40 to 60 range, the TIVA group had notably lower BIS values compared with the DES group ($p = 0.0017$). These treatment groups did not differ in terms of administered amounts of perioperative remifentanyl or HM.

Flow Cytometric Analyses

We observed a significant shift in favor of granulocytes with a corresponding proportional decrease in lymphocytes

Table 1 Patient demographics and perioperative characteristics

	DES (n = 13)	TIVA (n = 13)	p-Value
Sex (F:M)	12:1	12:1	
ASA (1:2:3:4)	3:9:1:0	2:11:0:0	
Age (y)	15.5 (12.2–20.3)	14.7 (11.8–19.3)	0.355
Weight (kg)	55.7 (37.1–71.8)	53.8 (43.3–88.3)	0.671
Height (cm)	165.7 (153.0–186.0)	158.8 (145.0–168.0)	0.047
BMI (kg/m ²)	20.3 (15.2–29.3)	21.3 (15.9–32.6)	0.550
Duration of anesthesia (min)	330 (208–508)	352 (211–570)	0.555
Duration of surgery (min)	249 (122–412)	261 (142–470)	0.719
Spinal levels fused	11 (7–13)	10 (5–13)	0.603
Intraoperative Remi dose (mg)	6.2 (1.9–14.1)	6.7 (2.6–14.6)	0.707
Intraoperative HM dose (mg)	1.3 (0.4–2.2)	1.0 (0.6–2.4)	0.121
HM dose in 24 h (mg)	16.5 (6.9–30.8)	12.6 (4.1–18.8)	0.109
Intraoperative fluids (mL)	2,034 (600–3750)	2,266 (1,375–4,000)	0.542
EBL (mL)	418 (75–800)	369 (150–700)	0.558

Abbreviations: ASA, American Society of Anesthesiologist; BMI, body mass index; DES, desflurane-remifentanyl; EBL, estimated blood loss; F, female; HM, hydromorphone; Remi, remifentanyl; TIVA, total intravenous anesthesia.

Note: Excepting Sex and American Society of Anesthesiologists' status ratios, values are expressed as mean (range).

(▶ **Fig. 2A**, $p < 0.0001$) in both the DES and TIVA groups. There was a transient reduction CD14 (a monocyte marker) expression at the end of surgery in both groups, though this normalized by POD1. Among CD14⁺ cells, however, there was a statistically significant reduction in HLA-DR expression that was greatest in both groups on POD1 (▶ **Fig. 2B**; DES, $p = 0.0102$ and TIVA, $p = 0.0149$ vs. preoperative samples).

Cytokine Production Capacity and Systemic Inflammation

There was a trend ($p = 0.06$) toward lower LPS-induced TNF- α production capacity over the study period in both treatment groups, though not significantly different than healthy controls (▶ **Fig. 3**). The DES group exhibited a significant reduction in PHA-induced cytokine production capacities for both IFN- γ ($p = 0.009$) and IL-10 ($p = 0.025$) compared with preoperative values, with trends for the same in the TIVA group. By POD1, subjects in the DES group had lower IFN- γ responses compared with the healthy control group ($p = 0.038$). Interestingly, both groups had higher PHA-induced IL-10 production capacities compared with healthy controls throughout the study period, including at their preoperative baseline. Healthy control subjects had undetectable plasma IL-6 levels (< 5 pg/mL), while IL-6

Fig. 1 Intraoperative characteristics. Box plots represent the median values and interquartile ranges of mean arterial pressure, heart rate, and bispectral index values recorded every 30 minutes after induction of anesthesia. Whiskers represent ranges. Significant changes in the respective values for each of measure within each group were noted across time points ($p < 0.05$). * $p < 0.01$ for a comparison of bispectral index values between the subjects treated with desflurane versus those who were treated with total intravenous anesthesia.

levels increased significantly over time in the both surgical groups ($p < 0.0001$ for both).

Discussion

In our study, we evaluated immune function in a predominantly female adolescent population undergoing a major orthopedic surgery, comparing the effects of two different general anesthetic techniques. There is existing evidence that immune function is suppressed during various phases of the menstrual cycle,¹¹ which could make a majority female population a confounding variable. Of note, the randomized assignment in this study yielded an identical sex ratio between experimental groups, which were also compared with a matched healthy control group to balance any possible confounding influence related to the sex of the subjects. We demonstrate that children with AIS who undergo PSF often experience a relative decrease in circulating lymphocytes with an associated reduction in their cytokine production capacity. We similarly showed a reduction in monocyte antigen presenting capacity with minimal reduction in innate cytokine production capacity. While differences in immune function between anesthetic groups were modest and not statistically different, we could not exclude a

limited difference due to the low number of patients in this study.

The immune response to major surgery is known to be highly dynamic in adults.^{12–14} Local tissue injury, potentially complicated by concurrent systemic illnesses or trauma, often results in a systemic inflammatory response syndrome (SIRS) that is characterized by high levels of circulating pro-inflammatory cytokines. More recently, a compensatory anti-inflammatory response (CARS) was described which occurs nearly concurrently with the SIRS response.¹⁵ While there is no clinical phenotype for the anti-inflammatory response, it can be quantified in the laboratory through the measurement of both innate and adaptive immune cell function. This response is poorly understood in children.

Innate immune function in this setting can be quantified through the measurement of antigen presenting capacity and cellular responsiveness to ex vivo stimulation. Monocytes are key innate immune cells which survey for infection and migrate to the tissues where, as macrophages, they combat infection and participate in wound healing. Greater than 90% of circulating healthy monocytes strongly express the antigen-presenting HLA-DR molecules, which are internalized in the setting of a CARS response. A reduction in this measure of antigen presenting capacity is associated with

Fig. 2 Flow cytometry data. Proportions of leukocyte populations are presented in (A) as a percentage of the live cell gate at the following perioperative time periods: preoperative, immediately postoperative, and the morning of postoperative day 1. The gating hierarchy in (B) is as follows: live cells > monocytes > CD14⁺ cells > human leukocyte antigen-DR⁺ cells. The proportion of each listed subset is presented as a percentage of its respective parent population based on the aforementioned gating strategy. Box plots represent median values with interquartile ranges. Whiskers represent ranges. Each of the represented populations showed a significant shift in proportional representation over time ($p < 0.05$ in each case).

adverse outcomes from acute illness and injury in adults and children.^{8,13,16,17} Severe reduction in HLA-DR expression has been associated with increased risks for the development of nosocomial infection and death in adults after major surgery, severe trauma, and sepsis.^{12,13,16,18} Ex vivo stimulation of healthy whole blood should produce robust amounts of TNF- α when stimulated with LPS, while a reduction in this response has been associated with adverse outcomes in adult surgical, trauma, and sepsis patients.^{19–21} The TNF- α response was also studied in children with sepsis,^{8,9} trauma,²²

Fig. 3 Ex vivo stimulated and plasma cytokine data. Ex vivo LPS-induced tumor necrosis factor- α production capacity and phytohemagglutinin-induced interferon- γ and IL-10 production capacities are shown along with unstimulated plasma IL-6 levels at each perioperative time period. Included on the left-hand side of each plot are data from healthy control patients who did not undergo anesthesia. Box plots represent median values with interquartile ranges. Whiskers represent ranges. ND denotes undetectable values. Groups that showed significance across time points included the following: DES/IFN- γ , DES/IL-10, DES/IL-6 and TIVA/IL-6. Significant differences relative to the control are marked with an asterisk ($p < 0.05$). DES, desflurane-remifentanyl; IFN, interferon; IL, interleukin; TIVA, total intravenous anesthesia.

and cardiopulmonary bypass,^{23,24} but little is known about this immune function following major, albeit elective orthopedic surgery such as PSF.

Lymphopenia, when seen in the setting of adult or pediatric sepsis and pediatric burn injury, is a known risk factor for nosocomial infection and death.^{25–27} Further, the ability of lymphocytes to produce cytokines upon ex vivo stimulation with agents like PHA is known to be impaired in the setting of critical illness-induced immune suppression.^{10,28} Our group recently described this phenomenon in the setting of pediatric severe sepsis, but lymphocyte function after pediatric surgery is poorly understood as well. In addition to the immunomodulatory effects of the surgery itself, surgical patients have the potential to experience immunologic effects from anesthetics. Several in vitro studies suggest an immunosuppressive effect

of propofol^{29–39} and volatile anesthetics,^{40–47} but this is in contrast to other observations of propofol not impairing lymphocyte proliferation^{48,49} or cytokine release^{50,51} and an increase in pro-inflammatory cytokines with volatile anesthetics.⁵²

Ours is the first study to directly compare the immunologic effects of desflurane- and propofol-based anesthetic regimens *in vivo* in children. While our data demonstrated limited differences between the treatment groups, contrary to a report that anesthetic technique may have differential immunomodulatory effect in the context of minor surgery,⁶ our study is consistent with that of Pirttikangas et al in which no differences in immunological effects were seen between propofol and isoflurane during hysterectomy in adults.⁵³ The observations in our study were in alignment with previously published adult data showing a perioperative upregulation of acute phase proteins and a subsequent immunosuppressive profile.^{54–57}

In our cohort of children with AIS who underwent PSF, we identified innate and adaptive immune suppression that was typically most evident on POD1. Studies of adult critical illness and injury typically identify a monocyte HLA-DR expression level <30%+ as being associated with adverse outcomes.^{18,58,59} Our operative subjects, all of whom experienced uncomplicated postoperative courses, showed more modest declines in HLA-DR expression with values typically in the 60 to 80%+ range on POD1. While reductions in lymphocyte cytokine production capacity—at the mRNA and protein levels—have been associated with increased infection, organ failure, and mortality risks in adults and children with critical illness,^{28,60,61} specific thresholds of cytokine production have not been established. Our group has previously reported that PHA-induced IFN- γ values below a threshold of 8.6 pg/mL were associated with prolonged multiple organ dysfunction syndrome in children with severe sepsis.¹⁰ IFN- γ response values in the current cohort were substantially higher than that, typically between 200 and 400 pg/mL, even on POD1. We have also previously shown that reductions in lymphocyte IL-10 production capacity are associated with infectious complications from severe sepsis in children.⁶¹ It is unclear why subjects in the current study with AIS had significantly higher PHA-induced IL-10 production capacity at baseline compared with healthy controls. Despite this, we saw marked reductions in the production capacity for this cytokine over the postoperative period as well. These reductions may be due to impairment of lymphocyte function, but may also be due to relative lymphopenia as the differential was skewed toward the granulocytic lineage.

We note that the subjects in this study were relatively healthy at baseline, presented for elective surgery, and that the degree of systemic inflammation experienced by these subjects (plasma IL-6 levels ~100–400 pg/mL on POD1) was much less than many subjects with more critical illness in whom IL-6 values >1,000 pg/mL are not uncommon.^{9,22} This likely explains the relatively modest decrement in immune function seen in the study. The fact that statistically significant reduction in immune function was seen, however, lends strong impetus to the future study of the immune effects of major

surgery in children with acute and chronic comorbidities, and those with greater severity of illness in whom the additional immunologic “hit” of surgery may result in more clinically relevant immune suppression. Further, the current study establishes the feasibility of performing immune function screening across innate and adaptive arms of the immune system using a small blood volume testing approach.

We recognize that this pilot study has some important limitations. First, its small sample size may have left it underpowered to detect differences in immune function between the anesthetic groups. The randomized nature of group assignment and protocolized nature of the treatment regimens is, however, a strength. Further, the immune profiles between the anesthetic groups was so similar that even with a larger sample size, it is unclear that differences would be appreciated. Second, the demographics of the cohort, focusing on adolescent females undergoing a highly specific surgical intervention, likely limits the generalizability of our findings to other pediatric surgical populations. As noted above, however, the fact that we were able to consistently observe immune suppression in this relatively healthy cohort highlights the need to undertake additional study of the effects of anesthesia and surgery in more acutely ill pediatric surgical populations. Given that a wider range of tests is warranted, we are working to include these in subsequent studies.

Conclusion

This is among the first reports of the impact of major surgery on immune function in children. While the effects in our study did not differ based on anesthetic technique, the data indicate that the surgical insult, including exposure to anesthetics combined with surgical trauma, results in reproducibly measurable suppression of both innate and adaptive arms of the immune system in adolescents undergoing elective PSF for AIS. The data highlight the need to evaluate immune function in a broader population of pediatric surgical patient with higher severity of illness.

Authors' Contributions

L.M.R. processed the experimental data, performed the analysis, drafted the manuscript, and designed the figures. M.H. and J.D.T. conceived and planned the experiments and supervised the work. D.P.M., A.B., W.S., J.K. and J.D.T. provided clinical care and collected tissue samples. M.H., J.N., L.H., and J.M. performed the experiments. All authors discussed the results and commented on the manuscript.

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Conflict of Interest

None declared.

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