Perioperative Innate Immunity and Its Modulation

Takashi KAWASAKI and Takeyoshi SATA

Department of Anesthesiology, School of Medicine, University of Occupational & Environmental Health, Japan. Yahatanishi-ku, Kitakyushu 807-8555, Japan

Abstract: Innate immunity plays a pivotal role in host defense. The trauma of major surgery induces a variety of immunologic alterations in patients, which can lead to subsequent increased susceptibility to postoperative infections. Surgical stress inhibits innate immunity from the time of incision until about the first postoperative day. This is an alert period with susceptibility to bacterial infections. Normal patients regain innate immunity in the first postoperative day. The absence of recovery of innate immunity may cause susceptibility to infection and eventually lead to postoperative complications. Surgical stress causes catabolism, pain, ileus, nausea and vomiting, immunosuppression, increase of cardiac demands and coagulation cascade, and pulmonary dysfunction. Perioperative treatment or remedy will affect these surgical responses. Prevention of surgical site infections (SSI) is very important for clinicians. In the past, we worked with sterilization techniques, prophylactic antibiotics and environmental support such as maintaining body temperature and supplying a high concentration of oxygen. The goal of further clinical studies must be to establish the immunomodulating property of the individual, which is very important for controlling innate immunity and inflammation in each patient. The strategy for minimizing post-surgical infections is to optimize the immune response by maintaining homeostasis through nutritional support, and to reduce the surgical trauma by using minimal invasive surgery, which consequently reduces the stress response and immunosuppression.

Key words: innate immunity, surgical stress, immunosuppression, perioperative, immunomodulation.

(Received 15 November 2010, accepted 14 February 2011)
Introduction

The human immune system is divided into two systems: innate immunity and adaptive immunity [1–4]. Recently, it has become increasingly clear that the innate immune system has a much more important and fundamental role in host defense. In addition, innate immunity also plays an important role in initiating adaptive immunity. The onset of innate immunity is immediately after infection. Therefore, it is the first line of host defense. Pathogen associated molecular pattern (PAMP), such as lipopolysaccharide (LPS), peptidoglycan, bacterial DNA, or RNA binds the pattern recognition receptor and causes the activation of innate immunity. PAMP is essential for the pathogenicity of microorganisms. PAMP’s structure is invariant and shared by entire classes; for example, all gram negative bacteria have the same PAMP, such as LPS. In contrast to adaptive immunity, innate immunity has a simple mechanism. Macrophages, monocytes, neutrophils, and dendritic cells are the main functioning cells of innate immunity. Their main functions are phagocytosis, complement activation, antimicrobial peptide and cytokine production.

In 1999, Centers for Disease Control and Prevention (CDC) presented the guideline for prevention of surgical site infection [5]. Surgical site infections (SSIs) are the third most frequently reported nosocomial infection, accounting for 14% to 16% of all nosocomial infections among hospitalized patients. During 1986 to 1996, hospitals conducting SSI surveillance reported 15,523 SSIs following 593,344 operations. Of these SSIs, two thirds were confined to the incision, and one third involved organs or spaces accessed during the operation. When surgical patients with nosocomial SSI died, 77% of the deaths were reported to be related to the infection. A 1992 analysis showed that each SSI resulted in 7.3 additional postoperative hospital days, adding $3,152 in extra charges. SSIs have occurred in which anesthesia personnel were implicated as the source of the pathogen.

In patients’ bodies, an operation or incision triggers two pathophysiological processes. One is the vanishing of the skin barrier which protects against bacteria invasion. This leads to susceptibility to infection. The second pathophysiological process is inflammation. In these situations, innate immunity plays an important role in the first line of host defense during an operation.

In this review, we focus on innate immune responses associated with surgery. The effect of surgical stress on innate immune responses will be discussed. In addition, strategies to modulate innate immune systems will also be discussed in this review.

Surgical stress and innate immunity

Surgical stress induces systemic responses, such as sympathetic nervous system activation, endocrine response, and immunological changes [6–11]. Activation of the hypothalamic-pituitary-axis (HPA) by corticotrophin releasing factors results in an increase of adrenocorticotropic hormone (ACTH) and beta-endorphin. ACTH stimulates the cortical secretion of glucocorticoids. After incision, plasma levels of ACTH and cortisol increase immediately [11]. Surgical stress is one of the most potent activators of ACTH and cortisol secretion. In normal settings, there is a feedback mechanism between ACTH and cortisol. Therefore, increased plasma concentration of
cortisol inhibits further secretion of ACTH. However, in a surgical setting, this feedback system does not work, so that concentrations of both hormones remain increased. Surgical stress also evokes increased endogenous secretion of catecholamines, which can be measured by the increases in plasma adrenaline and noradrenaline levels.

There are complex interactions between stress hormones and the immune system. The sympathetic nerve block induced by epidural anesthesia reduces the surgical stress responses of plasma catecholamines and cortisol levels and improves some immune responses, such as natural killer (NK) cell cytotoxicity in patients undergoing lower abdominal surgery [12]. Nevertheless, some investigators have reported that epidural anesthesia had no effect on surgical stress in patients undergoing upper abdominal surgery [13, 14]. Conflicting results of the effects of the sympathetic nerve block induced by epidural anesthesia on surgical stress and the immune response still exist. In patients undergoing total hip replacement, previous studies [15, 16] suggested that lymphocyte and monocyte functions were suppressed under general anesthesia, but maintained under epidural anesthesia. These studies also showed that epidural anesthesia suppressed the increase in serum cortisol concentration during the operation [15, 16]. In contrast, some studies reported that epidural anesthesia does not improve immunosuppression or the stress response in patients undergoing upper abdominal surgery. Tonnesen et al. [13] showed that NK cell activity drops significantly during general anesthesia and general-plus-epidural anesthesia in patients undergoing upper abdominal surgery. Norman and Fink [14] also showed that epidural anesthesia did not improve the neuroendocrine response during abdominal aortic replacement. Supporting the results of these studies, our previous study demonstrated that epidural blockade to T4 dermatome did not prevent immunosuppression and the increase of plasma cortisol concentration during upper abdominal surgery [17]. These findings imply that the advantageous effect of epidural anesthesia on the innate immune system is still doubtful, especially in patients undergoing upper abdominal surgery. Regarding this issue, Loick et al. [18] reported that high thoracic epidural anesthesia (an esthetized dermatomes C6-T10) prevented the increase of plasma adrenaline but did not affect plasma cortisol concentration. Segawa et al. [19] also demonstrated that the epidural blockade up to C8-T2 dermatome suppressed stress response after skin incision; however, it had no affect during an intraabdominal procedure. Up to C3-4 dermatome epidural blockade could suppress the surgical stress response during the intraabdominal procedure. In upper abdominal surgery, these studies indicate that nociceptive neural information is conveyed by the sensory fibers and by the phrenic nerves that innervate the diaphragm.

LPS-induced proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, play a crucial role in activating macrophages and neutrophils. Hyporesponsiveness to LPS may contribute to the high mortality rates in septic disease and trauma [20–23]. Previously, we demonstrated that surgical stress rapidly induced transient hyporesponsiveness of whole blood to endotoxin from 2 hours after the incision, and that plasma IL-10, which increases during surgery, participates in this hyporesponsiveness [24]. We also demonstrated that the levels of expression of monocyte mCD14 and human leukocyte antigen (HLA)-DR, major histocompatibility complex (MHC) class 2 proteins that have central roles in antigen presentation to lymphocytes and initiation of the adaptive immune response, were reduced early in sur-
urgery under general anesthesia [25]. CD14, a molecule expressed on the surface of macrophages/monocytes and neutrophils, is not only the LPS receptor that activates cytokine production, but is also an important receptor that activates the phagocytosis of *E. coli* [26]. In addition, we have shown that epidural anaesthesia failed to prevent LPS hyporesponsiveness and the suppression of monocyte mCD14 expression in patients undergoing partial gastrectomy [17]. Taken together, LPS hyporesponsiveness and the suppression of monocyte mCD14 may explain the innate immune dysfunction via the deactivation of macrophages/monocytes and neutrophils during the perioperative period. As well as CD14, recent studies demonstrated that surgical stress also influences the expression of toll-like receptors (TLRs) on leukocytes. Adib-Conquy *et al.* [27] demonstrated decreased TLR2 and TLR4 expression on monocytes in trauma patients. They also showed that LPS-induced TNF-α production decreased, whereas LPS-induced IL-10 production increased, in those patients. Furthermore, Dybdahl *et al.* [28] demonstrated that monocyte surface expressions of CD14, TLR2, and TLR4 decrease after open heart surgery. TLRs play an important role in host defense via chemokine/cytokine production and activation of phagocytosis [29]. The above suggest that the inhibition of TLR expression causes surgical stress-induced immunosuppression.

It is well known that cortisol inhibits TNF-α production and potentiates IL-10 production [30, 31]. Previous studies [17, 25] showed that plasma cortisol concentration increases during upper abdominal surgery, such as in partial gastrectomy or partial hepatectomy. We also showed that epidural blockade fails to suppress surgical-stress induced immunosuppression, such as low phagocytic activity, LPS hyporesponsiveness, decreased monocyte HLA-DR expression, and increased IL-10 concentration [17]. Increased serum cortisol impairs polymorphonuclear (PMN) functions [9], and that increased IL-10 is correlated with a decrease in monocyte HLA-DR expression [32]. Therefore, the increase of both cortisol and IL-10 may be the mechanisms that suppress immune functions during upper abdominal surgery. In contrast, Dybdahl *et al.* [28] demonstrated that heat shock protein (HSP)-70 induces TNF and IL-6 production. HSP-70 induced IL-6 production was inhibited by anti-TLR-4 antibody, which suggests that HSP-70 signaling is transduced through TLR-4. Roger *et al.* [33] also demonstrated that macrophage migration inhibitory factor (MIF) regulates innate immune responses through modulation of TLR4. They showed that MIF upregulates the basal expression of TLR4 in the macrophage. MIF promotes the recognition of endotoxin-containing particles and gram-negative bacteria by the innate immune system. These studies suggest that HSP-70 and MIF may restore the decrease of TLR expressions and immunosuppression during perioperation. Figure 1 shows the balance between pro-inflammatory and anti-inflammatory mediators. Proinflammatory cytokines such as Interferon (IFN)-γ, IL-1, IL-2, TNF, MIF and HSPs lead to the activation of innate immunity and inflammation. On the contrary, anti-inflammatory cytokines such as IL-4, IL-10, transforming growth factor (TGF)-β or cortisol lead to inactivation of innate immunity and inflammation (Fig.1).

Major surgery is associated with neutrophil dysfunction, as indicated by reduced chemotactic ability, phagocytic ability, and superoxide anion production [34, 35]. Consistent with the results of these studies, we demonstrated that the phagocytic ability of neutrophils was suppressed rap-
Perioperative Innate Immunity and Its Modulation

Balance Between
Proinflammatory and Anti-inflammatory Mediators

- Th1: Proinflammatory
cytokines
(IFN-γ, IL-1, IL-2, TNF, MIF)
- Heat shock proteins
- Th2: Anti-inflammatory
cytokines
(IL-4, IL-10, TGF-β)
- Cortisol

\[ \text{Innate Immunity} \quad \text{Inflammation} \]

**Fig. 1.** The balance between pro-inflammatory and anti-inflammatory mediators. Proinflammatory cytokines and heat shock proteins (HSPs) lead to activation of innate immunity and inflammation. On the contrary, anti-inflammatory cytokines or cortisol lead to inactivation of innate immunity and inflammation.

Perioperative Innate Immunity

\[ \text{Incision} \quad \text{Normal response} \]

**Fig. 2.** The perioperative innate immunity. Surgical stress inhibits innate immunity from the incision until about the first post-operative day. Absence of the recovery of innate immunity may cause susceptibility to infection. OPE: operation.

idly by surgical stress [17]. There were no significant differences between the general anesthesia group and general anesthesia with epidural anesthesia group in the suppression of phagocytic ability. Furthermore, we suggested that neutrophil respiratory burst activity did not change during the operation. However, some investigators have shown that phagocytic ability and production of reactive oxygen intermediates by circulating neutrophils increased during the postoperative period [36]. An excessive neutrophil activation may be responsible for post-operative organ dysfunction. However, the reason for these differences remains unclear.
Sex and age also affect innate immune response during the perioperative period. Ono et al. [37] found that excessive TNF-α and suppressive IFN-γ production of peripheral blood mononuclear cells occur more often in men than in women after gastrectomy. Monocyte HLA-DR expression also decreases in men. In addition, they showed that LPS-induced TNF-α production by monocyte and CD11b/CD18 expression on monocyte are significantly higher in elderly patients than in young patients [38]. These results may contribute to systemic inflammatory response syndrome (SIRS) and increased susceptibility to postoperative complications in male and elderly patients.

Figure 2 summarizes perioperative innate immunity. Surgical stress inhibits innate immunity from the incision until about the first postoperative day. This is an alert period and susceptible to bacterial infections. Normal patients regain innate immunity from the first postoperative day. The absence of the recovery of innate immunity may cause susceptibility to infection and eventually lead to postoperative complications.

**Strategies against surgical stress induced immunosuppression**

Figure 3 shows the strategies against surgical stress responses. Surgical stress causes catabo-

![Treatments against surgical stress responses](image)

**Fig. 3.** The strategies against surgical stress responses. Surgical stress causes many unfavorable responses. Perioperative treatment or remedy will affect these surgical responses. PONV: post operative nausea and vomiting, GM-CSF: granulocyte macrophage-colony stimulating factor.
Fig. 4. Prevention of SSI from past to future.
In the past, we worked with sterilization techniques, prophylactic antibiotics and environmental support. In the future, we need to move toward individual immunoregulation. SSI: surgical site infection.
Immunonutrition during perioperative period

A range of nutrients, including several amino acids, omega-3 fatty acids, antioxidant vitamins and minerals, and nucleotides, are able to modulate innate immune function. Mayer et al. [43, 44] demonstrated that omega-3 fatty acids have anti-inflammatory effects, including lower blood leukocyte counts, serum C-reactive protein (CRP) concentration, and production of inflammatory cytokines by isolated endotoxin-stimulated mononuclear cells. Glutamine is the most abundant free amino acid in the cytosol. A previous study [45] demonstrated that the provision of parenteral glutamine for 48 hours after major abdominal surgery maintains HLA-DR expression on monocyte. They also demonstrated that glutamine may enhance HSP expression and reduce inflammatory cytokine release [45]. Glutamine administration decreases the length of hospital stay and ventilator time in critically ill patients [46]. Novak et al. [47] showed that glutamine decreases infections and the length of hospital stay in surgical patients. As well as glutamine, arginine reveals an immunoprotective effect on innate immunity. A previous study [48] demonstrated that the depletion of arginine reduces wound healing and Kupffer cell function. Arginine supplementation increases NK cell cytotoxicity and macrophage tumor toxicity. However, a recent report [49] demonstrated its toxic effect as a substrate for inducible nitric oxide synthase (iNOS). iNOS increases the production of nitric oxide, which contributes to organ dysfunction. In addition, one study [50] also demonstrated that arginine supplementation increases mortality in septic patients. The use of arginine in perioperative periods still remains controversial.

Consensus recommendations of the use of enteral immunonutrition were shown in the US summit in 2001 [51]: 1) There is a clearly established benefit for patients undergoing elective gastrointestinal surgery or surgery secondary to blunt or penetrating torso trauma. 2) There is a probable benefit to patients undergoing elective major surgery, as well as severe head injury, burn (30% body surface area), and ventilator-dependent non-septic intensive care unit (ICU) patients. 3) No benefit was observed in patients able to resume oral intake within 5 days or in patients who were in the ICU for monitoring only.

The European Society for Clinical Nutrition and Metabolism (ESPEN) also made guidelines for the use of enteral immunonutrition [52, 53]: 1) Use enteral nutrition with immuno-modulating substrates (arginine, nucleotides, and omega-3 fatty acids) perioperatively in: a. Patients undergoing major neck surgery for cancer, b. Patients undergoing major abdominal surgery for cancer. 2) The guidelines for patients in the ICU stated that immune-modulating formulae (solutions enriched with arginine, nucleotides, and omega-3 fatty acids) are superior to standard enteral formulae for: a. Elective upper gastrointestinal surgical patients, b. Patients with mild sepsis, c. Patients with trauma, d. Patients with acute respiratory distress syndrome (formulae containing omega-3 fatty acids and antioxidants). 3) No recommendations for the use of immune-modulating solutions can be provided for burn patients due to insufficient data. 4) ICU patients with very severe illness who can not tolerate more than 700 ml of enteral formula per day should not receive an immune-modulating formula enriched with arginine, nucleotides, and omega-3 fatty acids. 5) Glutamine should be added to standard enteral formula in burns and trauma patients.

Recent studies [54–56] suggest that malnourished patients should undergo preoperative artifi-
cial nutrition administration for at least 10 days prior to major surgery and 7 days after surgery. Enteral nutrition is the best support for these patients. The benefit of immune-enhancing diets in severely malnourished patients remains to be proven; however, preoperative oral immunonutrition is indicated for non-malnourished patients. Impaired immune function associated with malnutrition is a common feature in surgical patients. Immunonutrition is one of the strategies for improving patient outcome during the perioperative period.

The immunologic advantage of minimally invasive surgery

To reduce the stress response to surgical stress, minimally invasive surgery such as laparoscopic surgery and video-assisted thoracic surgery (VATS) is widely used. Many studies demonstrated that there are significant differences in immunologic response between minimally invasive surgery and conventional open surgery [57–61].

Laparoscopy is commonly associated with less generation of circulating IL-6 and CRP [58]. Maruszynski and Pojda [62] reported an up to four fold increase in circulating IL-6 levels in patients after open as compared with laparoscopic cholecystectomy. Studies [63–65] also demonstrated that serum IL-6 concentration increases significantly in patients after open colectomies compared to laparoscopic surgery. Joris et al. [66] demonstrated a marked increase in CRP levels with open cholecystectomy, as compared with laparoscopic cholecystectomy. The laparoscopic approach also reduced plasma CRP levels in patients undergoing Nissen fundoplication [67] or colectomy [64]. Some studies [68–70] demonstrated that IL-6 and CRP levels were lower in patients that underwent VATS compared to conventional open lobectomy patients.

Significantly higher levels of IL-1 during and 6 hours after conventional cholecystectomy than after the laparoscopic procedure have been reported [71]. It is believed that IL-1ra, a polypeptide antagonistic to IL-1, is a sensitive marker of the anti-inflammatory response to surgical trauma. Plasma levels of IL-1ra were found to be significantly lower after laparoscopic approaches, indicating less inflammation [72].

Minimally invasive surgery affects perioperative neutrophil functions. Carey et al. [73] showed that neutrophil production of hypochlorous acid, a product of surperoxide anion production which is involved in microbial killing, decreases after laparotomy, whereas no decrease is observed in laparoscopic patients. Phagocytosis by neutrophils was significantly lower after open Nissen fundoplication compared to laparoscopic approach [67].

Kloosterman et al. [74] found that HLA-DR expression on monocytes was unimpaired in laparoscopic cholecystectomy patients. In addition, they reported that a significant reduction in HLA-DR expression was observed 1 day after open cholecystectomy, whereas no reduction was observed after laparoscopy. Schwenk et al. [75] also demonstrated that HLA-DR expression decreased within 2 hours after both conventional and laparoscopic approaches; however, it returned to normal within 1 day in laparoscopic patients.

In conclusion, these results indicate that laparoscopic surgery or VATS attenuates immunosuppression during the early postoperative period, which may be critical in minimizing postoperative infection. Less traumatic surgery may offer a simple strategy to improve innate immune
function further in the perioperative period.

**Summary**

Impaired innate immune function is a common feature in surgical patients, and it influences recovery from surgery. Prevention of SSI is very important for clinicians. Recently, cases of major surgery for immunocompromised patients has been increasing. Therefore, the goal of further clinical studies must be to establish the immunomodulating property of individuals, which is important for controlling innate immunity and inflammation in each patient.

**Acknowledgement**

This work was supported in part by the Clinical Research Foundation, Japan.

**References**

Scand 28: 654–660


周術期自然免疫と免疫制御

川崎 貴士，佐多 竹良

産業医科大学 医学部 麻酔科学教室

要旨：自然免疫は感染防御に重要な役割を果たしている。外科的侵襲はさまざまな免疫能変化を惹起するが、術後の感染性合併症発生のリスク上昇に宿主の免疫能低下が関与している可能性がある。手術侵襲により執刀直後から術1日後までの間、自然免疫能低下が起こる。正常な患者では術1日後から自然免疫能は回復してくれるが、自然免疫能の回復の遅れ、欠如は感染性合併症発生の原因となる。われわれは今まで、予防的抗生剤投与や患者の体温保持、高濃度の酸素投与などで手術部位感染を予防してきた。これから将来に向けては、患者個人の自然免疫能、炎症反応を制御すること、患者個々の免疫制御法を確立することが重要となる。この総説では、手術侵襲による自然免疫能変化を概説し、術後感染性合併症発生を最小限にするための戦略として1. 周術期免疫栄養により免疫能を維持する方法、2. 低侵襲手術により手術侵襲によるストレス反応を抑制する方法を紹介する。

キーワード：自然免疫、手術侵襲、免疫抑制、周術期、免疫制御。

JUOEH（産業医大誌）33（2）：123 - 137（2011）