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Anesthetics, immune cells, and immune responses

Shin Kurosawa[1] and Masato Kato[2]

[1] Department of Anesthesiology and Intensive Care Medicine, Tohoku University Hospital, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan
[2] Department of Anesthesiology and Perioperative Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

Abstract
General anesthesia accompanied by surgical stress is considered to suppress immunity, presumably by directly affecting the immune system or activating the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. Along with stress such as surgery, blood transfusion, hypothermia, hyperglycemia, and postoperative pain, anesthetics per se are associated with suppressed immunity during perioperative periods because every anesthetic has direct suppressive effects on cellular and neurohumoral immunity through influencing the functions of immunocompetent cells and inflammatory mediator gene expression and secretion. Particularly in cancer patients, immunosuppression attributable to anesthetics, such as the dysfunction of natural killer cells and lymphocytes, may accelerate the growth and metastases of residual malignant cells, thereby worsening prognoses. Alternatively, the anti-inflammatory effects of anesthetics may be beneficial in distinct situations involving ischemia and reperfusion injury or the systemic inflammatory response syndrome (SIRS). Clinical anesthesiologists should select anesthetics and choose anesthetic methods with careful consideration of the clinical situation and the immune status of critically ill patients, in regard to long-term mortality, morbidity, and the optimal prognosis.

Key words Anesthetics · Immunosuppression · Immune cells · Prognosis · Hypothalamic-pituitary-adrenal axis

Introduction

Possible effects of anesthesia on the immune system have been discussed from the early twentieth century. Studies reported by Graham in 1911 [1] and Gaylord in 1916 [2] describe, respectively, the influence of ether anesthesia on bacteriolysis and phagocytosis in humans, and the effects of anesthetics on tumor growth in an animal model. During recent decades, rapid development has occurred in the fields of immunology and anesthesia. In the early twenty-first century, anesthesiologists have acknowledged that dysregulation or suppression of the immune system during the perioperative period provokes postoperative complications, e.g., wound-healing disturbances and infections leading to sepsis, followed by multiple organ failure and death [3]. Particularly in cancer patients, immunosuppression after surgery accelerates the development of residual cancer cells and promotes the establishment of new metastases [4]. The immunological effects of surgery and anesthetics affect the long-term outcomes of patients after surgery. Therefore, awareness of these immunological properties is helpful for daily anesthetic management.

Factors and possible mechanisms of immunosuppression during the perioperative period—implications for long-term outcomes in immunocompromised patients

The main causes of immunocompromised responses in surgical patients are well known to be related to the neuroendocrine stress exerted through activation of the autonomic nervous system and the hypothalamic-pituitary-adrenal axis (HPA; Fig. 1) [5–6]. Apparently, many immune changes occurring in surgical patients result primarily from surgical trauma and neuroendocrine responses. The surgical-stress-induced release of hormones such as catecholamines (norepinephrine and epinephrine), adrenocorticotropic hormone (ACTH), and cortisol, via the autonomic nervous system and the HPA, mediates inhibitory effects on immune functions, because monocytes and macrophages and T cells have

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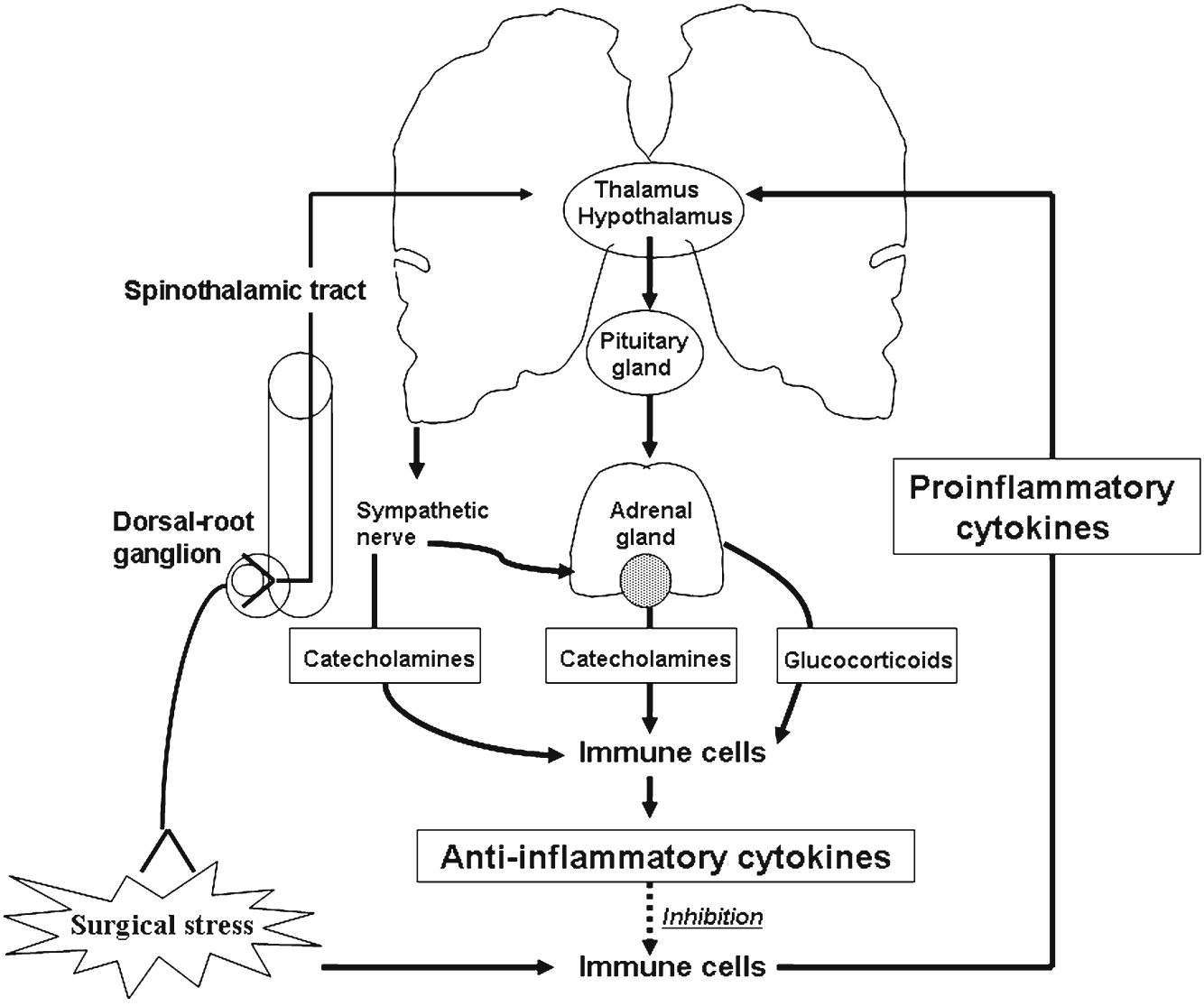

Fig. 1. Neuro-immune-endocrine interactions during surgical stress. The hypothalamic-pituitary-adrenal axis (HPA), sympathetic nervous system (SNS), and cytokines represent the peripheral limbs of the stress system. The central components of this system are located in the hypothalamus and the brain stem. Proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and IL-6 released from surgical stress-activated immune cells stimulate corticotropin-releasing hormone (CRH) and activate both the HPA and SNS. Catecholamines and glucocorticoids derived from the HPA and SNS drive a T-helper (Th)2 shift at the level of both antigen-presenting cells (APC) and helper T cells to produce anti-inflammatory cytokines such as IL-4 and IL-10. These anti-inflammatory cytokines suppress cell-mediated immune responses, resulting in immunosuppression. *Solid lines* represent stimulation; *dashed lines* represent inhibition

both β2-adrenoreceptors and glucocorticoid receptors, which promote cellular signaling to inhibit the production of representative helper-T-cell 1 (Th1) cytokines such as interleukin (IL)-12 and interferon (IFN)-γ, and to produce Th2 cytokines, the so-called anti-inflammatory cytokines, such as IL-4 and IL-10 [7]. Although these Th2 cytokines act intrinsically to limit the exaggerated inflammatory responses induced by surgical trauma, the excessive or uncontrolled secretion of Th2 cytokines engenders immunosuppression. Proinflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor (TNF)-α from monocytes and macrophages and lymphocytes activated by surgical stress can stimulate the HPA [5]. Therefore, the neuroendocrine system, and proinflammatory cytokines and anti-inflammatory cytokines, synergistically augment their suppressive effects in the perioperative immune system. Indeed, this immunosuppressive network manifested by the activated neuroendocrine system and hypercytokinemia during the perioperative period may adversely

affect long-term clinical outcomes. For example, Younes et al. [8] demonstrated, in their high-impact study, that the number of hypotensive episodes during an operation was the single most significant risk factor associated with a shorter disease-free interval after liver resection for metastatic colorectal carcinoma. The precise mechanism by which the intraoperative hypotension accelerated the recurrence and/or metastases of malignant tumors after surgery remains unclear, but activation of the neuroendocrine system induced by intraoperative hypotension may have inhibitory effects on anti-tumor immunity, especially on natural killer cells and lymphocyte functions.

In addition to the management of intraoperative blood pressure, blood transfusion [8–10], hyperglycemia [11,12], hypothermia [13–15], and postoperative pain [16–18], which are managed by anesthesiologists during operations, cause perioperative immunosuppression (Fig. 2). Immunosuppression induced by hypothermia and postoperative pain is probably mediated through activation of the neuroendocrine system, because perioperative hypothermia impairs the oxidative killing function of neutrophils by triggering thermoregulatory vasoconstriction under the control of the autonomic nervous system [5]; also, postoperative pain activates the HPA [17,19]. Hyperglycemia during perioperative periods increases the risk of bacterial infections because of the glycosylation of circulating immunoglobulin [20] and because of the impaired phagocytic capacity of neutrophils, in which respiratory burst (i.e., the explosive secretion of reactive oxygen species) is dependent on nicotinamide adenine dinucleotide phosphate (NADPH) arising from hexose monophosphate metabolism. Particularly in patients with diabetes, there is less NADPH available for neutrophil functions, because the polyol pathway, which is a great consumer of NADPH, is activated, reducing excess glucose to sorbitol [21,22]. The mechanism underlying the immunosuppression associated with allogenic blood transfusion remains elusive. It has recently been suggested that allogenic blood transfusion probably promotes host immune cells to produce immunosuppressive Th2 cytokines such as IL-10 and IL-4 [23,24].

However, even when the anesthetic technique and the surgery are managed adequately, certain patients undergoing surgery for malignant tumors later succumb to tumor progression with multiple metastases, resulting in death. This clinical situation in cancer patients following surgery is now thought to be mediated in part by the direct immunosuppressive effects of anesthetics and analgesic agents. Recently, along with immune suppression caused by surgical stress, numerous studies have shown that anesthetics and analgesic agents commonly used in surgery and in intensive care may directly affect the functions of immune-competent cells. In comparison to surgical stress, anesthetics probably have a minor effect on the immune system in patients undergoing surgery, because surgery—by itself—is reported to cause a three- to fourfold increase in the retention of tumor metastases when compared to findings in groups in which the effects of anesthesia and surgery were combined [4]. An immunosuppressive effect of approximately 20% might not normally have great consequences for a patient. However, if the patient is already compromised, e.g., because of aging, tumor burden, diabetes mellitus, or malnutrition, the immunosuppressive effects of anesthetics might play a salient role in postoperative morbidity and mortality [3]. On the other hand, the immunosuppressive effects of anesthetics that lead to anti-inflammatory responses may be therapeutically

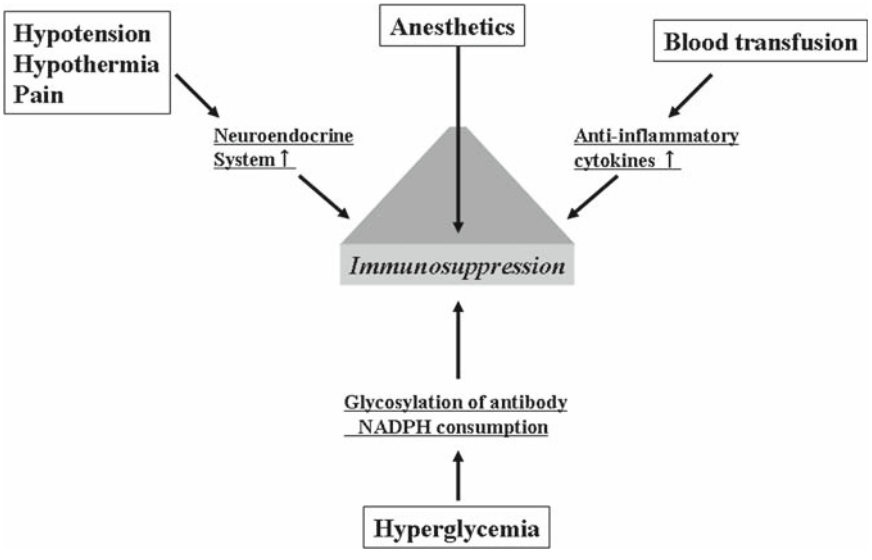

Fig. 2. Scheme showing possible modulators of immune competence during anesthesia and surgery. Anesthetics have direct effects on the immune system. *NADPH*, Nicotinamide adenine dinucleotide phosphate

beneficial in distinct situations such as ischemia and reperfusion injury or the systemic inflammatory response syndrome (SIRS) [25]. Therefore, anesthetics have not only adverse effects but also beneficial effects on the perioperative immune system. Investigations of the immune effects of anesthetics have been derived mostly from in vitro studies, because clinical human studies are more complex in their findings, involving the type of surgery procedure, length of surgery, and patients' complications. Although it is difficult to distinguish the relative contributions of surgical stress, anesthetics, and analgesic agents to a patient's immune system, anesthesiologists must not ignore the immunosuppressive effects of anesthetic drugs on perioperative immunity, because modern anesthesia now makes it possible to anesthetize immunocompromised patients.

Overview of the immune system

Innate and acquired immunity

The Latin term *immunis*, meaning "exempt", gave rise to the English word *immunity*. The primary purpose of immunity is to distinguish "self" from "nonself" and to clear "nonself" antigens from the body. The two major components of the immune response are nonspecific innate immunity and specific acquired immunity. Innate immunity is the first-line defense against "nonself" invaders. The innate immunity response is rapid, nonspecific for the antigen, and requires no prior exposure to the antigen target to activate nonspecific immune system components. Innate immune responses are mediated by natural killer (NK) cells and phagocytic cells such as monocytes and macrophages and polymorphonuclear neutrophils, which use primitive nonspecific recognition systems to bind microorganisms, then neutralize and destroy them [26]. In addition, monocytes and macrophages and dendritic cells play an important role as "professional" antigen-presenting cells (APC) to present the processed exogenous antigen in the groove of major histocompatibility complex (MHC) class II to helper T cells [27].

Acquired immunity is more specialized than innate immunity. It supplements the protection provided by innate immunity. Acquired immunity came into play late in evolutionary terms: it is present only in vertebrates. The initial contact with the foreign antigen triggers a chain of events that leads to the activation of lymphocytes and the synthesis of proteins such as cytokines and antibodies. Acquired immunity is classified as humoral immunity or cell-mediated immunity. Humoral immunity is mediated by B cells, which produce antibodies. Other cells, T cells, are responsible for cell-mediated immunity, and they recognize an antigen only in the presence of MHC, using antigen-specific T-cell receptors [28]. Actually, T cells comprise helper T (Th) cells and cytotoxic T (Tc) cells. The particular type of Th cell is determined by the differentiation of precursor helper T cells (Th0) into Th1 or Th2 cells. The Th1 cells produce IFN-γ and favor cell-mediated immune responses. The Th2 cells produce IL-4 and/or IL-10 and favor humoral immunity in the control of antibody production, leading to the suppression of cell-mediated immune responses, i.e., immunosuppression. For this reason, IL-4 and IL-10 are also called anti-inflammatory cytokines. The Th1 responses are considered most beneficial in terms of an appropriate and effective response to trauma and infection [29,30]. The Tc cells recognize and destroy tumor cells and virus-infected cells.

The roles of NK cells in anti-tumor immunity

Especially useful in the early phases of host immune responses, NK cells are a distinct subpopulation of lymphoid lineage that can "naturally" kill certain tumor cells and virus-infected cells without prior sensitization or MHC restriction [31]. Considered as the third major lymphocyte population, NK cells account for approximately 5%–15% of peripheral lymphocytes in humans. It is common knowledge among tumor immunologists that NK cells, Tc cells, and Th1 cells play a crucial role in the powerful elimination of tumor cells [32]. Particularly, NK cells function not only as surveillants in the early stage of tumor development (including metastasis) and through their killing activity; they also function as helpers in the priming process of APC, tumor-specific Tc cells, and Th1 cells, by producing IFN-γ (Fig. 3) [33–36]. Anti-tumor-specific Tc cells are considered to be the final and most important effectors against tumors. Therefore, NK cells are the main effectors responsible for the early anti-tumor defense [37]. Anti-inflammatory cytokines, IL-4 and IL-10; i.e., Th2 cytokines, are known to depress NK cell activities [38,39]. This finding implies that anti-inflammatory cytokines produced by immune cells through the activation of the neuroendocrine system or blood transfusion play a potent role in suppressed NK-cell-mediated tumor immunity. Therefore, a surgically mediated decrease in NK cell function has been implicated as the major contributing factor associated with an increase in tumor metastases and recurrence. Indeed, Ben-Eliyahu et al. [40] have shown, in an animal study, that the metastatic colonization of a lung tumor after surgery sensitively reflects the in vivo activity levels of NK cell function.

Neutrophils and ischemia-reperfusion injury

Neutrophils are present in much larger numbers than any other inflammatory cell in the circulation or in

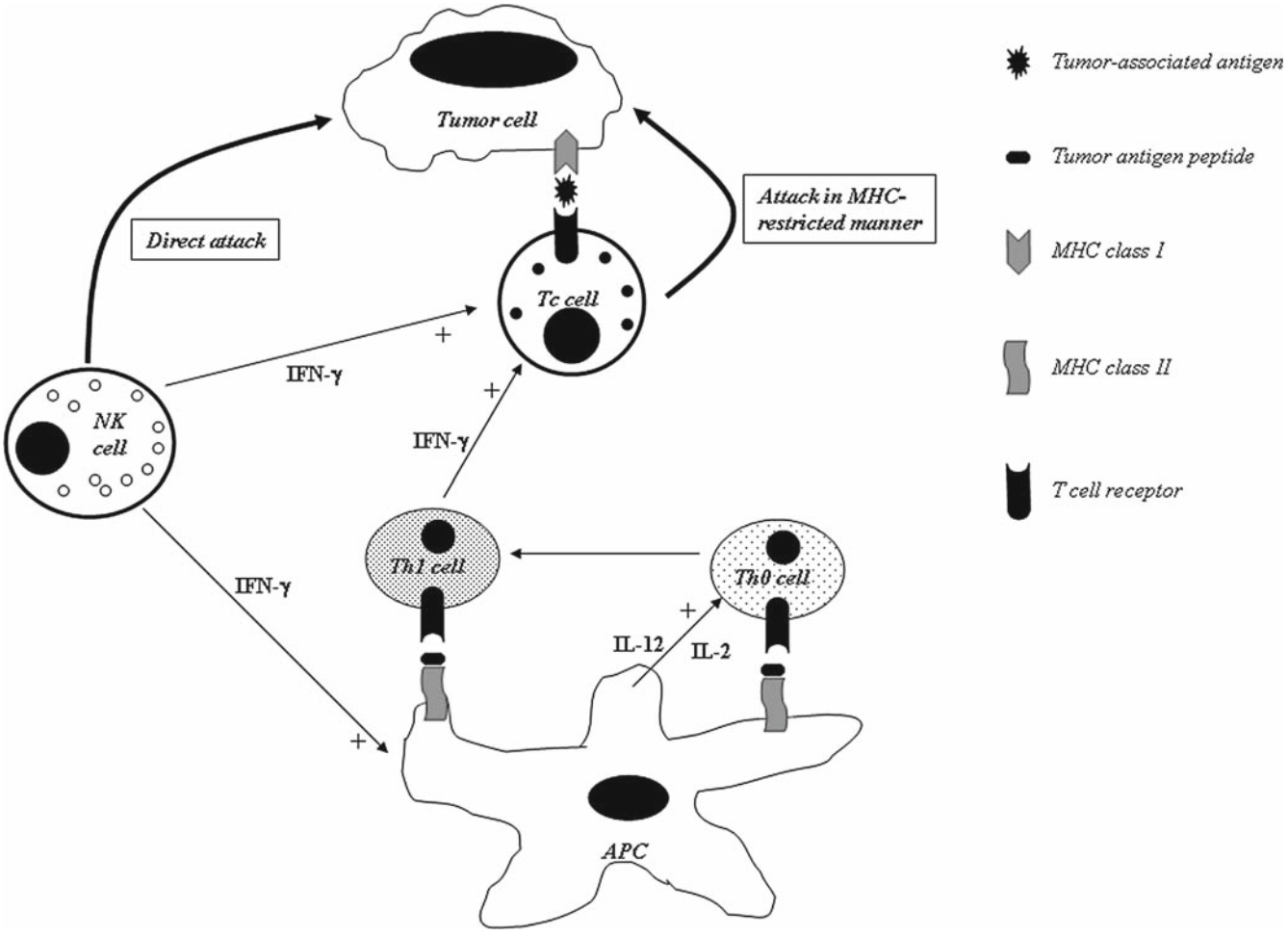

Fig. 3. Interactions between natural killer (*NK*) cells, helper T (*Th*) cells, cytotoxic T (*Tc*) cells, and antigen-presenting cells (*APC*) in anti-tumor immunity. In particular, NK cells function not only as surveillants in the early stage of tumor development but also as helpers in the priming process of APC, tumor-specific Tc cells, and Th1 cells, by producing interferon-γ (*IFN-γ*). *MHC*, Major histocompatibility complex

tissue. Neutrophils are viewed as phagocytes that rapidly accumulate at the site of infection or tissue damage; they serve a pivotal role in antimicrobial immunity at the early stage of infection, by ingesting and killing invading microorganisms [41]. By contrast, the elimination of other pathogens that cause chronic infections is thought of as being dependent on the action of a distinct phagocyte, monocyte/macrophage, following activation by T cells [42]. Neutrophils are continuously produced by bone marrow and circulate in the blood until recruited to inflamed tissues through the cooperation of neutrophil surface adhesion molecules and endothelial cells, termed "neutrophil polarization and chemotaxis". Most neutrophils die by apoptosis while still in the circulation because of their short life span; apoptotic neutrophils are ingested by macrophages. Neutrophils produce enzyme-rich granules containing the proteins such as myeloperoxidase, elastase, and protease 3, aside from the respiratory burst that secretes reactive oxygen species (ROSs) induced by the NADPH oxidase system; ROSs are toxic to microorganisms [41]. However, these proteins and ROSs are also harmful to the cells and tissues of the host if released inappropriately [43]. In this context, neutrophils have been implicated as the primary mediators of injury to the coronary vascular endothelium and cardiomyocytes after reperfusion, because neutrophils respond to myocardial ischemia-reperfusion in a manner similar to their response to a bacterial invasion, and ischemic stress-induced ROSs from activated neutrophils cause direct injury to the endothelium and cardiomyocytes [44].

Effects of volatile anesthetics on immune cells

Many in vitro investigations have elucidated the dose-dependent and time-dependent immunosuppressive effects of volatile anesthetics on various immune cells.

Neutrophil function

In the past, neutrophils were widely studied in the field of anesthesiology, not only because these cells are important for the immune system but also because this cell type is easy to study. More than two decades ago, Welch [45] reported halothane-induced "reversible" inhibition of human neutrophil bacterial killing function in vitro; this author suggested that the mechanism of inhibitory bacterial killing might be attributable to a deleterious effect of halothane on the oxidative microbicidal activity of human neutrophils. The suggestion was examined and confirmed by other investigations, which indicated that ROS production by activated neutrophils was inhibited by halothane, enflurane, isoflurane, and sevoflurane [46,47]. The mechanism by which volatile anesthetics inhibit ROS release from neutrophils is suggested to be either a direct inhibitory effect on NADPH oxidase or an inhibitory effect at some site in the signal transduction pathway regulating NADPH oxidase, such as protein kinase C [47,48]. Inhibition of ROS release by volatile anesthetics results in the suppression of initial inflammatory responses through the reduced adherence of neutrophils to endothelial cells, because ROSs from neutrophils provide a stimulus for the upregulation of endothelial adhesion molecules such as P-selectin and intercellular adheion molecule (ICAM)-1, which, respectively, mediate the initial rolling and slowing of neutrophils along the endothelial surface and the subsequent firm adherence of neutrophils to the endothelial cell surface [49,50]. Therefore, the inhibitory effects of volatile anesthetics on neutrophil functions not only reduce their ability to kill microorganisms but also reduce the available information necessary to initiate inflammatory responses, because tissue injury by activated neutrophils is the main source of "alarm" information that launches inflammation, which, in turn, launches immunity.

On the other hand, these inhibitory effects of volatile anesthetics on neutrophil functions may provide a therapeutically beneficial effect on ischemia-reperfusion injury. Abundant evidence substantiates the role of neutrophils in the ischemia-reperfused myocardium as a progenitor of primary inflammatory damage leading to reperfusion injury, followed later by the extension of the infarcted zone and myocardial stunning, ultimately resulting in the prolonged depression of postischemic contractile function [44]. The key elements that induce ischemia-reperfusion injury are ROSs that are released by neutrophils, and the adherence of neutrophils to the vascular endothelium via adhesion molecules such as CD11b/CD18 and L-selectin on neutrophils and P-selectin and ICAM-1 on endothelial cells [51]. Recent findings in various animal models and in patients have suggested that isoflurane and sevoflurane may exert protective effects on ischemia-reperfusion injury by reducing both ROS production by neutrophils and the postischemic adhesion of neutrophils to endothelial cells [52]. These inhibitory actions of volatile anesthetics may be associated with the anesthetic preconditioning of the ischemic myocardium [53].

Monocyte and macrophage functions

Most in vivo and in vitro studies of the effects of volatile anesthetics on monocyte and macrophage functions are based on investigations of the functions of alveolar macrophages. For example, halothane inhibits the intraalveolar recruitment of macrophages in response to influenza virus infection in mice [54]. Isoflurane decreases the phagocytotic capacity of human alveolar macrophages during surgery [55]. An in vivo study using rat endotoxemia showed that the inhalation of isoflurane reduced the release of the proinflammatory cytokine, IL-1β in bronchoalveolar lavage fluid (BALF) [56]. This finding suggests an inhibitory effect of isoflurane on proinflammatory cytokine release from alveolar macrophages, because the main source cells of proinflammatory cytokines in BALF in endotoxemia are alveolar macrophages. In addition, the study demonstrated that the inhalation of isoflurane increased the release of nitric oxide (NO) and the expression of inducible nitric oxide synthase (iNOS) proteins from alveolar macrophages, which phenomena were completely inhibited by the beta adrenoceptor antagonist propranolol. In this connection, Tschaikowsky et al. [57] showed that the expression of iNOS by a murine macrophage cell line was increased by volatile anesthetics (halothane, enflurane, isoflurane, and desflurane) when the cell line was stimulated with a combination of lipopolysaccharide (LPS) and IFN-γ. Although the role of NO release from macrophages induced by volatile anesthetics remains unknown, NO may have several protective roles in the inflammatory response; NO-induced vasodilation may prevent the accumulation of injurious mediators in the endothelium and may scavenge free radicals and prevent the upregulation of neutrophil CD11b/CD18 adhesion molecules [51,58,59]. Indeed, the anti-inflammatory properties of volatile anesthetics in endotoxin-challenged acute lung injury have been demonstrated previously [60,61]. In contrast, there are

results conflicting with those of previous studies using murine or rat macrophages, which indicated the inhibition of LPS-induced iNOS expression by volatile anesthetics (halothane, enflurane, isoflurane, and desflurane) [57] and the inhibition of NO release by isoflurane or sevoflurane [62,63]. Furthermore, no data in the literature describe the effects of volatile anesthetics on the antigen-processing capacity or the presenting of monocytes and macrophages (and dendritic cells) as APC.

NK cell function

NK cells are of primary importance in the elimination of tumor target cells at the early stage of tumor development, up to and including tumor metastasis. Decreased NK cell function during the perioperative period is associated with an increased risk of mortality in cancer patients [4,64–66].

Many studies monitoring in vitro cell responses after surgery and anesthesia have reported decreased NK cell cytotoxic activity. Two decades ago, Woods and Griffiths [67] found that the volatile anesthetics, halothane and enflurane, reversibly inhibited NK cell activity dose-dependently in vitro. One hour after the removal of NK cells from exposure to the volatile anesthetics, the full recovery of NK cell activity was apparent [67]. Halothane and isoflurane inhibited the augmentation of splenic NK cell cytotoxicity induced by IFN treatment in mice both in vivo and in vitro [68]. In addition, a study using an animal model indicated that the halothane-induced suppression of NK cell activity increased tumor metastases in vivo [69]. Although the precise mechanism underlying the direct inhibitory effect of volatile anesthetics on NK cell activity remains unclear, it is possible that volatile anesthetics may induce $CD8^+$ T cells, which suppress the activation of NK cell cytotoxicity, because it was found that the in vitro depletion of $CD8^+$ T cells from splenocytes derived from anesthetized mice restored the ability of NK cells to respond to IFN stimulation [70]. In addition, the perioperative depression of NK cell cytotoxicity may be associated with the activation of the neuroendocrine system, because changes in serum cortisol were found to show an inverse relationship with NK cell cytotoxicity during and after surgery [71].

Lymphocyte function

Various studies have shown inhibitory effects of volatile anesthetics on lymphocyte proliferation [72–77] and suppressive effects of these agents on cytokine release in peripheral blood mononuclear cells (PBMC) [78,79]. Splenic T cells derived from rats anesthetized with 1% halothane for 5 h in vivo showed reduced proliferative capacity and impaired ability to express CD25 (IL-2) receptor in response to mitogens [77]. An in vitro study using human PBMCs demonstrated that exposure of the cells to 1% halothane for 60 min impaired both immunoglobulin and concanavalin A-surface binding to lymphocytes; this phenomenon was reversible after 24 h [76]. Exposure to halothane depressed the secretion of IFN-γ by human lymphocytes in response to a mitogen [78]. Other volatile anesthetics, sevoflurane, isoflurane, and enflurane, also suppress the release of IL-1β and TNF-α from human PBMCs, including lymphocytes and NK cells, in response to tumor cells [79]. The inhibitory effects of volatile anesthetics on lymphocyte function may reduce the immunocapacity of these cells against microorganisms and tumor cells. However, these inhibitory effects may contribute to anti-inflammatory responses, by regulating the secretion of proinflammatory cytokines implicated in the pathophysiology of SIRS [25].

Although the mechanisms by which volatile anesthetics inhibit lymphocyte function remain elusive, lymphocyte apoptosis induced by volatile anesthetics may be involved to some degree. Isoflurane and sevoflurane directly induced apoptosis in human peripheral lymphocytes in vitro in a dose-dependent and time-dependent manner [80]. The induction of apoptosis was accompanied by increased caspase-3-like activity in lymphocytes [80]. In accordance with these results, Loop et al. [81] found that sevoflurane and isoflurane induced apoptosis in human T lymphocytes dose-dependently through the apoptotic signaling pathway involving the disruption of mitochondrial membrane potential and the release of cytochrome C from mitochondria to the cytosol. Some authors have surmised that cytochrome C, a component of the electron transfer chain, released by volatile anesthetics, engenders failure to maintain mitochondrial membrane potential and adenosine triphosphate (ATP) synthesis in lymphocytes, which results in caspase activation, inducing apoptosis and cell death [82]. In addition, the decrease of mitochondrial transmembrane potential reportedly induces superoxides and other ROSs [83,84], which activate protein kinase C (PKC) and mitogen-activated activated protein kinases (MAPK) [85,86]. Loop et al. [87] reported that sevoflurane inhibited the activation of transcription factor activator protein-1 (AP-1) in human T lymphocytes and that the suppression of AP-1 was associated with interference in the p38 MAPK cascade *via* the increased phosphorylation of the p38γ/p38δ isoforms. Therefore, the decrease of mitochondrial transmembrane potential, the release of cytochrome C from mitochondria, and interference with the MAPK cascade may provide possible mechanisms for the volatile anesthetic-induced inhibitory or anti-inflammatory effects on lymphocytes (Fig. 4). In con-

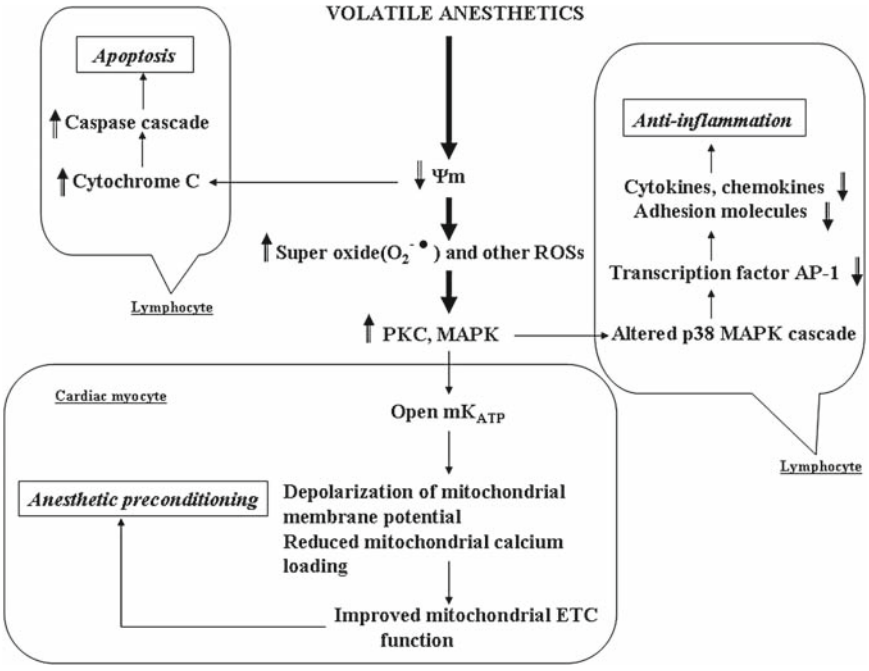

Fig. 4. Possible pathways leading to volatile anesthetic-induced apoptosis and anti-inflammatory responses in lymphocytes, and preconditioning in cardiac myocytes. The key and shared element of the volatile anesthetic-induced modulation of cellular function is the attenuation of mitochondrial membrane potential. *ψm*, Inner mitochondrial membrane potential; *ETC*, electron transport chain; *ROSs*, reactive oxygen species; mK_{ATP}, mitochondrial adenosine triphosphate-sensitive K^+ channel; *PKC*, protein kinase C; *MAPK*, mitogen-activated protein kinases; *AP-1*, activator protein-1

trast to the toxic (apoptotic) or inhibitory effects of volatile anesthetics on lymphocytes, volatile anesthetics have a protective effect on myocytes: i.e., producing anesthetic preconditioning in the ischemic heart [88]. Although we do not specifically examine anesthetic preconditioning in this article, it appears that the mitochondrial membrane plays important roles in anesthetic preconditioning, as well as in the toxic (apoptotic) or inhibitory effects on lymphocytes. However, there may be differences in mitochondrial functions between myocytes and lymphocytes. Briefly, in myocytes, as in lymphocytes, volatile anesthetics induce the attenuation of mitochondrial membrane potential, which enhances the production of ROSs. The enhanced production of ROSs leads to the activation of PKC and p38 MAPK, which opens the mitochondrial adenosine triphosphate-sensitive $K^+(K_{ATP})$ channel in myocytes. The consequences of mitochondrial K_{ATP} channel opening are the reduction of cytosolic and mitochondrial calcium loading and the improvement of myocardial oxygen efficiency during myocardial ischemia, which may lead to anesthetic preconditioning (Fig. 4). The volatile anesthetic-induced protection of mitochondria energetics in myocytes, but not in lymphocytes, would result in the reduction of cytochrome C release from mitochondria [89]. It appears that, in cardiomyocytes, the balance between sarcoplasmic and mitochondrial K_{ATP} channels, the regulation of cytosolic Ca^{2+}, and/or the activity of NADH dehydrogenase which is a powerful generator of ROSs, differ from these factors in lymphocytes.

Effects of propofol on immune cells

Propofol, which belongs to the phenolic hydroxyl group of drugs, chemically resembles the antioxidant α-tocopherol [90]. Accumulated data indicate that propofol has inhibitory effects on neutrophil and monocyte and macrophage functions in innate immunity, but not on NK cell or lymphocyte functions. These effects of propofol may be related, in part, to its lipid carrier vehicle [91]. Propofol appears to have anti-inflammatory and anti-oxidative actions through its inhibitory effects on innate immunity.

Neutrophil function

In vitro, propofol was shown to dose-dependently inhibit N-formyl-methionyl-leucyl-phenylalanine-stimulated neutrophil chemotaxis and ROS production [92]; it also impaired neutrophil phagocytosis of *Escherichia coli* and *Staphylococcus aureus* cells at clinically achievable concentrations [93,94]. A reduction in intracellular calcium concentration ([Ca]i) in neutrophils may be responsible for the inhibition of their function by propofol [92]. However, other studies have found that propofol has no effect on the phagocytosis of *E. coli* [95] or *S. aureus* [96] at clinically relevant concentrations. Neutrophil polarization [97] and respiratory burst [91,92] were reduced by clinical concentrations of propofol in vitro. Ex vivo human studies in critically ill patients indicated no remarkable effect of propofol on neutrophil respiratory burst [98]. Propofol decreased

the release of IL-8 from lipopolysaccharide (LPS)-stimulated neutrophils, although intracellular IL-8 and mRNA levels remained increased [99]. This finding suggests that the decrease of IL-8 release induced by propofol occurs at the post-translational level without altering mRNA. In another study, of intracellular signaling molecules, propofol inhibited the phosphorylation of p42 MAPK in neutrophils [100]. This finding may explain the inhibitory effects of propofol on neutrophil functions.

Monocyte and macrophage functions

Propofol has been shown to impair monocyte and macrophage functions, including chemotaxis [101,102], oxidative burst [93,102], and phagocytosis [93,102]. The suppressive effects of propofol on murine macrophage chemotaxis and oxidative burst are reversed 6–24 h after the removal of propofol [102]. In addition, the LPS-induced expression of IFN-γ mRNA in murine macrophages is blocked by propofol [102]. A reduction in the membrane potential of macrophage mitochondria and ATP synthesis in macrophages may be responsible for the propofol-induced inhibitory effects on macrophages [101,102]. Exposure of murine macrophages to propofol at a low concentration (3–30 μM) did not affect cell viability. However, a high concentration (300 μM) of propofol caused arrest of the cell cycle in G1/S phase and an increase in lactate dehydrogenase release, and led to cell death [102]. In contrast to the induction of cell death in macrophages by a high concentration of propofol, another study demonstrated that propofol at 30 μM protected murine macrophages from NO-induced apoptosis as well as cell death [103]. In addition, propofol, at a clinically relevant concentration suppressed NO biosynthesis by inhibiting iNOS expression in LPS-activated murine and human macrophages [104,105]. The production of the proinflammatory cytokines, TNF-α, IL-β and IL-6 in LPS-activated human macrophages was inhibited by propofol at a pretranslational level [105]. However, conflicting data have been reported related to whether or not propofol directly stimulates human monocytes to release TNF and IL-1α [106].

NK cell function

Little information is available related to the effects of propofol on NK cell function in vivo or in vitro. The results of an in vivo animal study suggest that propofol has no effects on NK cell activity in whole blood or on the susceptibility to tumor metastasis in nonoperated rats after anesthesia [69]. The results of an in vivo human study showed a remarkable decrease in circulating NK cell numbers in patients anesthetized with propofol and fentanyl after the induction of anesthesia [107].

Lymphocyte function

Propofol had no effect on the in vitro proliferation of lymphocytes from healthy volunteers [108,109]. Nevertheless, in surgical patients in intensive care, propofol apparently inhibited lymphocyte proliferation in response to pokeweed mitogen [108]. This result suggests that B-lymphocyte proliferation in critically ill patients may be inhibited by propofol. The in vitro proliferation of T lymphocytes from healthy volunteers in response to phytohemagglutinin was unaffected by propofol [109]. Furthermore, the Th1/Th2 ratio, as measured by IFN-γ (produced by Th1 cells) and IL-4 (produced by Th2 cells) levels in human PBMCs was increased by propofol [110]. The cytokines produced by Th1 cells activate cells involved in cell-mediated immunity, such as NK cells, monocytes and macrophages, and $CD8^+$ cytotoxic T cells. In contrast, the cytokines produced by Th2 cells trigger B cells to synthesize immunoglobulins. Therefore, the increase in the Th1/Th2 ratio induced by propofol, which is contributing to the maintenance of cell-mediated immunity, may be beneficial for immunocompromised patients. Propofol, at clinically acceptable concentrations (1–10 μg/ml), does not induce lymphocyte apoptosis in humans but it does induce the apoptosis at a high concentration (50 μg/ml) [111]. In this context, K^+ channels may be associated with the induction of apoptosis by a high dose of propofol, because propofol was shown to block voltage-gated K^+ channels in human T lymphocytes [112]. In addition, the results of a recent study investigating the activation of human T lymphocytes suggest that propofol does not inhibit the activation of nuclear factor kappa B (NK-κB), a transcription factor involved in the expression of many genes, including IFN-γ, IL-2, IL-6, and IL-8 [113]. This finding is in accordance with a previous report indicating that propofol did not impair cytokine release in response to endotoxin in a whole-blood culture medium prepared from healthy volunteers [114]. Collectively, propofol, at clinically relevant concentrations, appears to have only minor effects on lymphocyte fuctions.

Effects of opioids on immune cells

The immunosuppressive effects of opioids have been known for more than a century. Although the precise mechanisms remain unidentified, opioid-induced immunomodulation is mediated by opioid receptors [115] and by the participation of both the autonomic nervous system [116] and the HPA [117]. The activation of

opioid receptors can regulate the peripheral immune system through stimulation of the HPA [117] and the sympathetic nervous system [116]. The activation of opioid receptors in the HPA elicits the production of ACTH from the pituitary, which, in turn, elicits the release of glucocorticoids, which suppress the immune system [117,118]. Activation of the sympathetic nervous system by opioids elicits the release of catecholamines, which have been demonstrated to suppress lymphocyte, NK cell, and macrophage functions [119]. Four major classes of opioid receptors have been identified: δ, κ, μ, and σ. These opioid receptors are present not only in the nervous system, including the HPA but also in immunocompetent cells. Neutrophils and NK cells express μ and δ receptors, and monocytes and macrophages and T cells express μ, δ, and κ receptors [120]. A classical μ opioid receptor is thought to be involved in morphine-related immunomodulation, because the effects of morphine can be blocked by the morphine antagonist naloxone [121].

Morphine stimulates μ3 receptors on immune cells to increase intracellular calcium transients ([Ca]i), which may, in turn activate constitutive nitric oxide synthase (cNOS), liberating NO. NO, in turn, stabilizes inhibitory kappa Bα (IκBα), by preventing its degradation, and inhibits NF-κB binding to the representative DNA promoter region and the subsequent expression of proinflammatory cytokines and adhesion molecules, resulting in an anti-inflammatory effect [122].

Morphine suppresses neutrophil functions such as phagocytosis, respiratory burst, and complement receptor expression by stimulating NO release via μ3 receptors [123]. The inhibitory production of ROSs through the respiratory burst by neutrophils is reversed by naloxone. In vivo studies demonstrate that morphine inhibits the proliferation and differentiation of macrophage progenitor cells [124], phagocytosis by monocytes and macrophages [125], and IL-10 and IL-12 production from monocytes and macrophages [121]. These impairments were evident in peritoneal, alveolar, and splenic macrophages, indicating a general downregulation of innate immunity. It appears from the results of all these studies that morphine acts to decrease host defenses against various infectious diseases. Furthermore, NK cells are very sensitive to morphine-induced modulation in vivo. The in vivo administration of morphine depresses NK cell activity [126].

T lymphocyte functions and B lymphocyte functions are also suppressed by morphine in vivo. The mitogenic response of B lymphocytes plasma cells [127] and the induction of antibody-forming [121] are both suppressed by morphine administration in vivo. Moreover, T-lymphocyte proliferation is decreased by both acute and chronic morphine administration [125,128]. The production of IFN-γ and IL-2 (Th1 cytokines) by T lymphocytes is inhibited by morphine in vivo [121]. However, the results reported for the action of morphine in modulating the production of IL-4 (Th2 cytokine) are contradictory. The in vivo administration of morphine increased IL-4 production by T lymphocytes in one experiment [129] and decreased it in another experiment [130]. In addition, an interesting study has demonstrated that morphine can trigger T-lymphocyte apoptosis by modulating the Fas-Fas ligand system in vitro; this effect was also mediated by opioid receptors present on immune cells themselves [131].

In contrast to the inhibitory effects induced by morphine in immune cells, synthetic opioids, such as fentanyl and remifentanil, seem to have no effect in attenuating immune cell responses because of a reduced interaction of synthetic opioids with specific opioid receptors. Fentanyl, remifentanil, and alfentanil do not impair functions of neutrophils such as respiratory burst [132] and phagocytosis [133]. Indeed, fentanyl has no effects on cytokine release from whole blood cells [114]. Although one experiment using an animal model indicated that a relatively high dose of fentanyl suppressed NK activity and resistance to tumor metastases [134], a clinically relevant dose of fentanyl augmented NK activity and increased the number of NK cells and $CD8^+$ cytotoxic T lymphocytes in healthy volunteers [135]. In contrast, the quantities of circulating B and T lymphocytes remained unchanged in the presence of fentanyl [136]. Fentanyl has no ability to bind to μ3 receptors. Therefore, it does not influence NO release and cellular adhesion [137]. As a result, fentanyl appears to lack the ability to downregulate the inflammatory responses associated with surgery.

Effects of local anesthetics on immune cells

In surgical patients, extradural anesthesia with local anesthetics reduces the activation of the neuroendocrine system and thus prevents immunosuppression during surgery. In patients undergoing hysterectomy, the depression of NK cell cytotoxic activity in patients receiving general anesthesia was abrogated when patients received both general and extradural anesthesia. The inhibitory effect on the depression of NK cell activity was associated with the suppression of the cortisol response [138]. In patients undergoing total hip replacement, cortisol levels were lower during surgery in the regional-anesthesia group than in the general-anesthesia group [139]. These results imply that surgery-related increases in serum cortisol are attenuated by extradural analgesia. Therefore, it is clear that the afferent neural blockade induced by extradural anesthesia can decrease intraoperative and postoperative neuroendocrine stress responses [140]. The decreased

lymphocyte proliferation and lymphokine production seen in patients under general anesthesia were not seen in patients undergoing extradural anesthesia [141]. In addition, spinal anesthesia prevented the depressed mitogen-induced lymphocyte proliferation in patients undergoing general anesthesia for prostate surgery [142]. Recently, in vivo experiments, using a murine model, revealed that the addition of spinal block to sevoflurane-general anesthesia used with laparotomy attenuated the suppression of liver mononuclear cell tumoricidal function by preserving the Th1/Th2 cytokine balance and NK cell/NK-T cell functions, resulting in a reduction of tumor metastases [143]. These effects of extradural or spinal anesthesia on the immunosuppression induced by surgery and general anesthesia may protect patients from the postoperative development of infectious complications or tumor metastases [144].

Implications of in vivo studies comparing the anesthetic-induced immunomodulation of volatile and intravenous anesthetics

The accumulated evidence described above suggests that immunocompetent cells seem to be more sensitive to volatile anesthetics than to propofol or synthetic opioids, because propofol and synthetic opioids have fewer effects on immunocompetent cells. In addition, the attenuation of stress responses by a combination of extradural anesthesia with general anesthesia protects surgical patients from further immunosuppression during the perioperative period. In this context, general anesthesia using propofol and fentanyl combined with epidural/spinal anesthesia may be optimal for immunocompromised hosts to prevent tumor metastases or postoperative nosocomial infections, and general anesthesia using volatile anesthetics may be useful for patients with ischemia/reperfusion injury involving cardiopulmonary bypass or SIRS. Indeed, in vivo studies comparing the perioperative immunomodulation exerted by inhalation anesthesia and that exerted by intravenous anesthesia have indicated that inhalation anesthesia has more suppressive effects on the immune system than total intravenous anesthesia (TIVA). The number of T lymphocytes and the expression of HLA-DR decrease more in response to surgery after inhalation anesthesia when compared with findings after surgery with TIVA [145]. The plasma level of IL-6, a cytokine which is important for stimulating the neuroendocrine system, significantly increased during and after abdominal surgery with inhalation anesthesia [146]. A lower level of serum cortisol has been reported in patients undergoing TIVA compared to the cortisol level in those with isoflurane, anesthesia [146,147]. Isoflurane anesthesia reduces the bactericidal activity of macrophages more effectively than does propofol anesthesia [148]. In addition, the Th1/Th2 ratio decreases significantly after isoflurane anesthesia, but it does not change after propofol anesthesia [149].

Conclusion

The perioperative period is crucial for the long-term prognosis of surgical patients because the direct immunomodulatory effects of anesthetics are a double-edged sword: immunosuppression may be both beneficial and harmful. Unfortunately, insufficient attention has been directed to the perioperative period in regard to long-term prognosis, even by anesthesiologists. The negative consequences associated with perioperative immunosuppression, such as an increased risk of tumor metastasis and postoperative infection, could be decreased by the optimal selection of anesthetics and anesthetic techniques. In contrast, the anti-inflammatory effects of anesthetics may be therapeutically beneficial in some situations, such as ischemia and reperfusion injury and SIRS. In the future, it will be necessary to differentiate the different applications of anesthetics with careful regard to the immunological status of surgical patients.

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