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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 influence upon the functions of immunocompetent cells and inflammatory mediator gene expression and secretion. Particularly in cancer patients, immunosuppression attributable to anesthetics, such as dysfunctions of natural killer cells and lymphocytes, might accelerate the growth and metastases of residual malignant cells, thereby worsening prognoses. Alternatively, anti-inflammatory effects of anesthetics might be beneficial in distinct situations involving ischemia and reperfusion injury or the systemic inflammatory response syndrome (SIRS). Regarding the respective long-term mortalities, morbidities, and the optimal prognoses, clinical anesthesiologists should select anesthetics and choose anesthetic methods with careful consideration of the clinical situation and immunity status of critically ill patients.

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Title page

Title: Anesthetics, immune cells and immune responses

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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 influence upon the functions of immunocompetent cells and inflammatory mediator gene expression and secretion. Particularly in cancer patients, immunosuppression attributable to anesthetics, such as dysfunctions of natural killer cells and lymphocytes, might accelerate the growth and metastases of residual malignant cells, thereby worsening prognoses. Alternatively, anti-inflammatory effects of anesthetics might be beneficial in distinct situations involving ischemia and reperfusion injury or the systemic inflammatory response syndrome (SIRS). Regarding the respective long-term mortalities, morbidities, and the optimal prognoses, clinical anesthesiologists should select anesthetics and choose anesthetic methods with careful consideration of the clinical situation and immunity status of critically ill patients.

Introduction

The possible effects of anesthesia on the immune system have been discussed from the early 20th century. Studies reported by Graham in 1911 [1] and Gaylord in 1916 [2] respectively describe the influence of ether anesthesia on bacteriolysis and phagocytosis in human, 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 21st century, anesthesiologists 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]. Immunological effects affect the long-term outcomes of patients after surgery. Therefore, awareness of immunological properties in the surgical area is helpful for daily anesthetic management.

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

The main causes of immunocompromised responses in surgical patients are well known to be related to the neuroendocrine stress 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 primarily result from surgical trauma and neuroendocrine responses. Surgical-stress-induced releases of hormones such as catecholamines (norepinephrine and epinephrine), adrenocorticotropin hormone (ACTH), and cortisol via the autonomic nervous system and the HPA mediate inhibitory effects on immune functions because monocytes and macrophages and T cells have both β2-adrenoreceptors and glucocorticoid receptors, which promote cellular signaling to inhibit the production of representative helper T cell 1 (Th1) cytokines such as IL-12 and interferon (IFN)-γ, and to produce Th2 cytokines, so-called anti-inflammatory cytokines such as interleukin (IL)-4 and IL-10 [7]. Although these Th2 cytokines act intrinsically to limit the exaggerated inflammatory responses induced by surgical trauma, excessive or uncontrolled secretion of Th2 cytokines engenders immunosuppression. Pro-inflammatory 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, the pro-inflammatory cytokines and anti-inflammatory cytokines, synergistically augment their suppressive effects in the perioperative immune system. Indeed, this immunosuppressive network by the activated neuroendocrine system and hypercytokinemia during the perioperative period might adversely affect long-term clinical outcomes. For example, Younes *et al*. demonstrated in their high-impact study that the number of hypotensive episodes during an operation was associated with a shorter disease-free interval after liver resection for metastatic colorectal carcinoma as the single most significant risk factor [8]. The precise mechanism by which the intraoperative hypotension accelerated the recurrence and/or metastases of malignant tumor after surgery remains unclear, but the activation of the neuroendocrine system induced by intraoperative hypotension might engender inhibitory effects on anti-tumor immunity, especially on natural killer cells and lymphocyte functions in the patients.

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 by hypothermia and postoperative pain probably are 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 HPA [17,19]. Hyperglycemia during perioperative periods increases the risk of bacterial infections because of the glycosylation of

circulating immunoglobulin [20] and the impaired phagocytic capacity of neutrophils, of which respiratory burst (i.e. explosive secretion of reactive oxygen species) is dependent on nicotinamide adenine dinucleotide phosphate hydroxide (NADPH) from the hexose monophosphate metabolism. Particularly for diabetes, NADPH is less available for neutrophil functions because the polyol pathway, which is a great consumer of NADPH, is activated to reduce excess glucose into sorbitol [21, 22]. The mechanism underlying the immunosuppression associated with allogenic blood transfusion remains elusive. It was recently 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 the 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 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 might 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 3–4-fold increase in retention of tumor metastases when compared to groups in which anesthesia was combined [4]. An immunosuppressive effect of approximately 20% normally might not have greater consequences for a patient. However, the patient is already compromised, *e.g.*, because of aging, tumor burden, diabetes mellitus and malnutrition, immunosuppressive effects of anesthetics might play a salient role in postoperative morbidity and mortality [3]. On the other hand, immunosuppressive effects of anesthetics, which lead to anti-inflammatory responses, might be therapeutically beneficial in distinct situations such as ischemia and reperfusion injury or the systemic inflammatory response syndrome [25]. Therefore, anesthetics impart 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

1) Innate and acquired immunity

The Latin term *immunis*, meaning "exempt", gave rise to the English word *immunity*. The primary purpose of the immunity is to distinguish "self" from "nonself" and to clear "nonself" antigens from the body. The two major components of immune response are non-specific innate immunity and specific acquired immunity. Innate immunity is the first line defense against "non-self" invaders. Innate immunity response is rapid, non-specific 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, macrophages and polymorphonuclear neutrophils, which use primitive non-specific recognition systems to bind micro-organisms, 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 activation of lymphocytes and the synthesis of proteins such as cytokines and antibodies. Acquired immunity is classified into humoral or cell-mediated immunity. The humoral immunity is mediated by B cells, which produce antibody. Other cells, T cells, are responsible for cell-mediated immunity and recognize an antigen only in the presence of MHC using the antigen-specific T cell receptors [28]. Actually, T cells comprise helper T cells (Th cells) and cytotoxic T cells (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 that 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.

2) 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 lymphocytes population, NK cells account for approximately 5%–15% of peripheral lymphocytes in human. It is common sense among tumor immunologists that NK cells, Tc cells and Th1 cells play

a crucial role for powerful elimination of tumor cells [32]. Particularly, NK cells function not only as a surveillant in the early stage of tumor development, including metastasis, and function through their capacity of killing activity; they also function as a helper 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 fact implies that anti-inflammatory cytokines produced by immune cells through activation of neuroendocrine system or blood transfusion play a potent role in suppressed NK-cell-mediated tumor immunity. Therefore, a surgically mediated decrease in NK cell functions has been implicated as the major contributing factor associated with an increase in tumor metastases and recurrence. Indeed, Ben-Eliyahu *et al*. have shown in an animal study that metastatic colonization of a lung tumor after surgery sensitively reflects *in vivo* activity levels of NK cell function [40].

3) Neutrophils and ischemia-reperfusion injury

Neutrophils are present in much larger numbers than any other inflammatory cell in circulation or in tissue. Neutrophils are viewed as phagocytes that rapidly accumulate at the site of infection or tissue damage; they serve a pivotal role in the antimicrobial immunity at the early stage of infection by ingesting and killing invading microorganisms [41]. By contrast, other pathogens that cause chronic infections are thought of as being dependent on a distinct phagocyte, monocyte/macrophage following activation by T cells for their elimination [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 as called by the term of neutrophil polarization and chemotaxis. Most neutrophils die by apoptosis while still in circulation because of their short life span; apoptotic neutrophils are ingested by macrophages. Neutrophils produce the enzyme-rich granules containing myeloperoxidase, elastase, and protease 3, aside from the respiratory burst being able to secrete reactive oxygen species (ROSs) by the NADPH oxidase system; ROSs are toxic to microorganisms [41]. 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 primary mediators of injury after reperfusion to coronary vascular endothelium and cardiomyocytes because neutrophils respond to myocardial ischemia-reperfusion in a manner similar to a bacterial invasion and ischemic stress-induced ROSs from activated neutrophils impart direct injury to endothelium and cardiomyocytes [44].

Effects of volatile anesthetics on immune cells

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

1) Neutrophil function

In the past, neutrophils were widely studied in the fields 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 reported halothane-induced "reversible" inhibition of human neutrophil bacterial killing function *in vitro* [45]. The 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 the ROS production by activated neutrophils was inhibited by halothane, enflurane, isoflurane, and sevoflurane [46–47]. The mechanism by which volatile anesthetics inhibit the ROSs' 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 ROSs' release by volatile anesthetics results in suppression of initial inflammatory responses through the reduced adherence of neutrophils to the endothelial cells because ROSs from neutrophils provide a stimulus for upregulation of endothelial adhesion molecules such as P-selectin and 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, inhibitory effects of volatile anesthetics on neutrophil functions not only reduce the ability to kill microorganisms but also reduce the available information to initiate the inflammatory responses because tissue injury by activated neutrophils is a 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 might provide a therapeutically beneficial effect on ischemia-reperfusion injury. Abundant evidence substantiates the role of neutrophils in ischemia-reperfused myocardium as a progenitor of primary inflammatory damage leading to reperfusion injuries, followed later by the extension of the infarcted zone and myocardial stunning, ultimately resulting in prolonged depression of post-ischemic contractile function [44]. The key elements that induce ischemia-reperfusion injury are ROSs that are released by neutrophils and adherence of neutrophils to the vascular endothelium *via* the 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 patients have suggested that isoflurane and sevoflurane might provide protective effects on ischemia-reperfusion injury by reducing both ROS production from neutrophils and postischemic adhesion of neutrophils to

endothelial cells [52]. These inhibitory actions of volatile anesthetics might be associated with the anesthetic preconditioning of the ischemic myocardium [53].

2) Monocyte and macrophage functions

Most *in vivo* and *in vitro* studies about the effects of volatile anesthetics on monocyte and macrophage functions are based on investigations into the functions of the 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]. *In vivo* study using rat endotoxemia showed that inhalation of isoflurane reduced the release of proinflammatory cytokine, IL-1β in bronchoalveolar lavage fluid (BALF) [56]. This finding suggests the 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 inhalation of isoflurane increased the release of nitric oxide (NO) and expression of inducible nitric oxide synthase (iNOS) proteins from alveolar macrophages, which were completely inhibited by beta adrenoceptor antagonist propranolol. In this connection, Tschaikowsky *et al*. showed that the expression of iNOS by murine macrophage cell line was increased by volatile anesthetics (halothane, enflurane, isoflurane, and desflurane) when the cell line was stimulated with the combination of lipopolysaccharide (LPS) and IFN-γ [57]. Although the role of NO release from macrophages by volatile anesthetics remains unknown, NO might have several protective roles in the inflammatory response because NO-induced vasodilation might prevent accumulation of injurious mediators at the endothelium and might scavenge free radicals and prevent up-regulation of neutrophil CD11b/CD18 adhesion molecules [51, 58–59]. Indeed, the anti-inflammatory properties of volatile anesthetics in the endotoxin-challenged acute lung injury have been demonstrated previously [60–61]. In contrast, we also obtained conflicting results to those of previous studies using murine or rat macrophages, which indicated inhibited LPS-induced iNOS expression by volatile anesthetics (halothane, enflurane, isoflurane, and desflurane) [57] and 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 presenting by monocytes and macrophages (and dendritic cells) as APC.

3) NK cell function

The 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. The 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 found that volatile anesthetics, halothane and enflurane, reversibly inhibited NK cell activity dose-dependently *in vitro*. One hour after removing the NK cells from exposure to the volatile anesthetics, full recovery of NK cell activity was apparent [67]. Halothane and isoflurane inhibit the augmentation of splenic NK cell cytotoxicity by interferon treatment in mice both *in vivo* and *in vitro* [68]. In addition, a study using an animal model indicated that 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, volatile anesthetics might induce $CD8^+$T cells, which suppress activation of NK cell cytotoxicity, because *in vitro* depletion of $CD8^+$ T cells from splenocytes derived from anesthetized mice restored the ability of NK cells to respond to interferon stimulation [70]. In addition, perioperative depression of NK cell cytotoxicity might be associated with the activation of neuroendocrine system because changes in serum cortisol showed an inverse relationship with NK cell cytotoxicity during and after surgery [71].

4) Lymphocyte function

Various studies have shown inhibitory effects of volatile anesthetics on lymphocyte proliferation [72–77] and suppressive effects in cytokine releases in peripheral blood mononuclear cells (PBMC) [78–79]. Splenic T cells derived from rats anesthetized by 1% halothane for 5 h *in vivo* reduced the proliferative capacity and impaired their ability to express CD25 (IL-2) receptor in response to mitogens [77]. *In vitro* study using human PBMC demonstrated that exposure of 1% halothane for 60 min impaired both the immunoglobulins and concanavalin A-surface bindings 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 PBMC, including lymphocyte and NK cells, in response to tumor cells [79]. The inhibitory effects of volatile anesthetics on lymphocyte functions might reduce the immunocapacity against microorganisms and tumor cells. However, they might contribute to anti-inflammatory responses by regulating secretion of pro-inflammatory cytokines implicated in the pathophysiology of systemic inflammatory response syndrome (SIRS) [25].

Although the mechanisms by which volatile anesthetics inhibit the lymphocyte functions remain elusive, lymphocyte apoptosis induced by volatile anesthetics might be involved to some degree. Isoflurane and sevoflurane directly induce apoptosis in human peripheral lymphocytes *in vitro* in a dose-dependent and time-dependent manner [80]. The induction of apoptosis is accompanied by the increased caspase-3-like activity in lymphocytes [80]. In accordance with the results, Loop *et al*. found that sevoflurane and isoflurane induce apoptosis in human T lymphocytes

dose-dependently through the apoptotic signaling pathway involving disruption of the mitochondrial membrane potential and release of cytochrome c from mitochondria to the cytosol [81]. The authors have surmised that cytochrome c released by the volatile anesthetics, the component of the electron transfer chain, engenders a failure to maintain the mitochondrial membrane potential and adenosine triphosphate (ATP) synthesis in lymphocytes, which results in caspase activation to induce 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*. reported that sevoflurane inhibits activation of the transcription factor activator protein-1 (AP-1) in human T lymphocytes and that the suppression of AP-1 is associated with interference of the p38 MAPK cascade *via* increased phosphorylation of the p38γ /p38δ isoforms[87]. Therefore, the decrease of mitochondrial transmembrane potential, the release of cytochrome c from mitochondria, and interference with the MAPK cascade might provide possible mechanisms for volatile anesthetics-induced inhibitory or anti-inflammatory effects on lymphocytes (Fig. 4). In contrast to the toxic (apoptotic) or inhibitory effects of volatile anesthetics on the lymphocytes, volatile anesthetics impart a protective effect on the myocytes: anesthetic preconditioning in the ischemic heart [88]. Although this article does not specifically refer to anesthetic preconditioning, the mitochondrial membrane appears to play important roles in anesthetic preconditioning as well as the toxic (apoptotic) or inhibitory effects on the lymphocytes. However, there might appear to be discrepancies in mitochondrial functions between myocytes and lymphocytes. Briefly, as in lymphocytes, volatile anesthetics induce the attenuation of mitochondrial membrane potential in myocytes, which enhances the production of ROSs. The enhanced production of ROSs leads to activation of PKC and p38 MAPK, which opens the mitochondrial adenosine triphosphate-sensitive K^+(K_{ATP}) channel in myocytes. Consequences of mitochondrial K_{ATP} channel opening reduce cytosolic and mitochondrial calcium loading and improve myocardial oxygen efficiency during myocardial ischemia, which might lead to anesthetic preconditioning (Fig. 4). 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 might appear that the balance between the sarcoplasmic and mitochondrial K_{ATP} channels, the regulation of cytosolic Ca^{2+}, and/or NADH dehydrogenase activity which is a powerful generator of ROSs in cardiomyocytes, differ from those in lymphocytes.

Effects of propofol on immune cells

Propofol, which belongs to the phenolic hydroxyl group, chemically resembles the antioxidant α-tocopherol [90]. The accumulated data indicate that propofol has inhibitory effects on neutrophils and monocyte and macrophage functions of the innate immunity, but not on NK cells and lymphocytes functions. These effects of propofol might 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.

1) Neutrophil function

In vitro propofol dose-dependently inhibits N-formyl-methionyl-leucyl-phenylalanine-stimulated neutrophil chemotaxis and ROS production [92]; it also impairs neutrophil phagocytosis of *Escherichia coli* and *Staphylococcus aureus* at clinically achievable concentrations [93, 94]. The reduction of the intracellular calcium concentration ([Ca]i) in neutrophils might be responsible for the functional inhibition by propofol [92]. However, other studies have found that propofol has no effect on phagocytosis of *E. coli* [95] or *S. aureus* [96] at clinically relevant concentrations. Neutrophil polarization [97] and respiratory burst [91, 92] are reduced by clinical concentration of propofol *in vitro*. *Ex vivo* human studies in critically ill patients indicated no remarkable effect on neutrophil respiratory burst [98]. Propofol decreases the release of IL-8 from lipopolysaccharide (LPS)-stimulated neutrophils, although intracellular IL-8 and mRNA levels remain increased [99]. That fact suggests that the decrease of IL-8 release by propofol occurs at the post-translational level without altering mRNA. In another study, of intracellular signaling molecules, propofol inhibited phosphorylation of p42 MAPK in neutrophils [100]. This finding might explain the inhibitory effects of propofol on neutrophil functions.

2) 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, LPS-induced expression of IFN-γ mRNA in murine macrophages is blocked by propofol [102]. The reduction of the membrane potential of macrophage mitochondria and ATP synthesis in macrophages might be responsible for 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 would cause arrest of the cell cycle in G1/S phase, increase lactate dehydrogenase release and lead to cell death [102]. In contrast to the cell death-induction of

macrophages by a high concentration of propofol, another study demonstrated that propofol (30 μM) protects murine macrophages from NO-induced apoptosis as well as cell death [103]. In addition, propofol suppresses NO biosynthesis by inhibiting iNOS expression in LPS-activated murine and human macrophages at a clinically relevant concentration [104,105]. The production of proinflammatory cytokines, TNF-α, IL-β, and IL-6 in LPS-activated human macrophages are inhibited by propofol at a pre-translational 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].

3) NK cell function

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

4) Lymphocyte function

Propofol has no effect on *in vitro* lymphocyte proliferation from healthy volunteers [108, 109]. Nevertheless, in surgical intensive care patients, it apparently inhibits lymphocyte proliferation in response to pokeweed mitogen [108]. This result suggests that B lymphocyte proliferation in critically ill patients might be inhibited by propofol. *In vitro* T lymphocyte proliferation in response to phytohaemagglutinin is unaffected in healthy volunteers [109]. Furthermore, the Th1/Th2 ratio, as measured by IFN-γ (produced by Th1 cells) and IL-4 (produced by Th2 cells) accumulation in human PBMC, is 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 increased Th1/Th2 ratio by propofol, which is contributing to the maintenance of cell-mediated immunity, might be beneficial for immunocompromised patients. Propofol does not induce lymphocyte apoptosis in human in clinically acceptable concentrations (1–10 μg/ml) but not in high concentration (50 μg/ml) [111]. In this context, K^+channels might be associated with the induction of apoptosis at a high dose of propofol because propofol blocks voltage-gated K^+channels in human T lymphocytes [112]. In addition, 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 does not impair cytokine release in

response to endotoxin in a whole blood culture medium from healthy volunteers [114]. Collectively, propofol appears to impart only minor effects on lymphocyte functions at clinically relevant concentrations.

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 immunomodulations are mediated by opioid receptors [115] and by the participation of both the autonomic nervous system [116] and the hypothalamic-pituitary-adrenal axis (HPA) [117]. The activation of opioid receptors can regulate the peripheral immune system throughout the stimulation of HPA [117] and the sympathetic nervous system [116]. The activation of opioid receptors in 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 nervous system, including HPA, but also in immunocompetent cells. Neutrophils and NK cells express μ and δ receptors, and monocytes and macrophages and T cells are expressing μ , δ and κ receptors [120]. A classical μ opioid receptor is thought to be involved in morphine-related immunomodulations because the effects of morphine can be blocked by the antagonist naloxone [121].

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

Morphine suppresses neutrophil functions such as phagocytosis, respiratory burst, and complement receptors expression by stimulating NO release via μ3 receptors [123]. The inhibitory production of ROSs through the respiratory burst by neutrophils is reversible 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 with peritoneal, alveolar and splenic macrophages, indicating a general down-regulation of innate immunity. It appears from results of all these studies that morphine acts to decrease host defenses against various infectious diseases.

Furthermore, NK cell is very sensitive to morphine-induced modulation *in vivo*. *In vivo* administration of morphine depresses NK cell activity [126].

The T lymphocyte functions and B lymphocyte functions are also suppressed by morphine *in vivo*. The mitogenic response [127] and induction of antibody-forming by B lymphocytes [121] are suppressed by morphine administration *in vivo*. Moreover, T lymphocyte proliferation is decreased by both acute and chronic morphine administrations [125, 128]. Production of IFN-γ and IL-2 (*i.e.*

Th1 cytokines) by T lymphocytes is inhibited by morphine *in vivo* [121]. However, the results reported of morphine modulation of IL-4 production (*i.e.* Th2 cytokine) are contradictory. *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 demonstrated that morphine can trigger T lymphocyte apoptosis by modulating the Fas-Fas ligand system *in vitro*; this effect is also mediated by opioid receptors present on immune cells themselves [131].

In contrast to the morphine-induced inhibitory effects on immune cells, synthetic opioids such as fentanyl and remifentanil seem to have no effect to attenuate immune cell responses through reduced interaction of synthetic opioids with specific opioid receptors. Fentanyl, remifentanil, and alfentanil do not impair the function of neutrophils such as respiratory burst [132] and phagocytosis [133]. Indeed, fentanyl has no effects on cytokine releases from whole blood cells [114]. Although one experiment using an animal model indicated that a relative high dose of fentanyl suppresses NK activity and resistance to tumor metastases [134], the clinical relevant dose of fentanyl augments NK activity and increases the number of NK cells and $CD8^+$cytotoxic T lymphocytes in healthy volunteers [135]. On the other hand, the quantities of circulating B and T lymphocytes remain unchanged [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 then 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 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 afferent neural blockade by extradural anesthesia can decrease the intra-operative and post-operative neuroendocrine stress responses [140]. Such decreased lymphocyte proliferation and lymphokine production 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 accompanying laparotomy attenuates the suppression of tumoricidal function of liver mononuclear cells by preserving Th1/Th2 cytokine balance and NK cell/NK-T cell functions, resulting in the reduction of tumor metastases [143]. These effects of extradural or spinal anesthesia on immunosuppression by surgery and general anesthesia might protect patients from post-operative development of infectious complications or tumor metastases [144].

Implications of *in vivo* studies comparing anesthetic-induced immunomodulation between 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 less effect on immunocompetent cells. In addition, attenuation of stress responses by the combination of the extradural anesthesia with general anesthesia protects surgical patients from further immunosuppression during the perioperative periods. In this context, a general anesthesia using propofol and fentanyl with epidural/spinal anesthesia might be optimal for immunocompromised hosts to prevent tumor metastases or postoperative nosocomial infections, and the general anesthesia using volatile anesthetics might be useful for patients with ischemia/reperfusion injury involving cardiopulmonary bypass or SIRS. Indeed, *in vivo* studies comparing perioperative immunomodulation between inhalation anesthesia and intravenous anesthesia have indicated more suppressive effects of inhalation anesthesia on the immune system than those of total intravenous anesthesia (TIVA). The number of T lymphocytes and expression of HLA-DR decrease more in response to surgery after inhalation anesthesia when compared with TIVA [145]. The plasma level of IL-6, which is important to stimulate the neuroendocrine system, significantly increases during and after abdominal surgery with inhalation anesthesia [146]. A lower level of serum cortisol has been reported in patients undergoing TIVA compared to 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 long-term prognosis of surgical patients because the direct immunomodulatory effects of anesthetics are a double-edged sword: immunosuppression might be both beneficial and harmful. Unfortunately, insufficient attention to long-term prognosis has been directed to the perioperative period, even by anesthesiologists. The negative consequences associated with perioperative immunosuppression, such as an increased risk of tumor metastasis and postoperative infections, might be decreased by the optimal selection of anesthetics and anesthetic techniques. In contrast, anti-inflammatory effects of anesthetics might be therapeutically beneficial in some situations such as ischemia and reperfusion injury and SIRS. In the future, it will become necessary to differentiate the different applications of anesthetics with careful regard to the immunological status of the surgical patients.

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Figure Legends

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 TNF-α, IL-1, and IL-6 released from surgical stress-activated immune cells stimulate the corticotrophin-releasing hormone (CRH) and activate both the HPA and SNS. Catecholamines and glucocorticoids derived from the HPA and SNS drive a Th2 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.

Fig. 2. Scheme showing possible modulators of immune competence during anesthesia and surgery. Anesthetics impart direct effects on the immune system.

Fig. 3. Interactions between NK cells, Th cells, Tc cells, and APC in anti-tumor immunity. Particularly, NK cells function not only as a surveillant in the early stage of tumor development but also as a helper in priming process of APC, tumor-specific Tc cells and Th1 cells by producing IFN-γ: *NK cells*, natural killer cells; *Th cells*, helper T cells; *Tc cells*, cytotoxic T cells; *APC*, antigen-presenting cells; and *MHC*, major histocompatibility complex.

Fig. 4. Possible pathways leading to volatile anesthetic-induced apoptosis and anti-inflammatory responses in lymphocytes and the preconditioning in cardiac myocytes. The key and shared element of the volatile anesthetic-induced modulations of cellular functions 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; and *AP-1*, activator protein-1.

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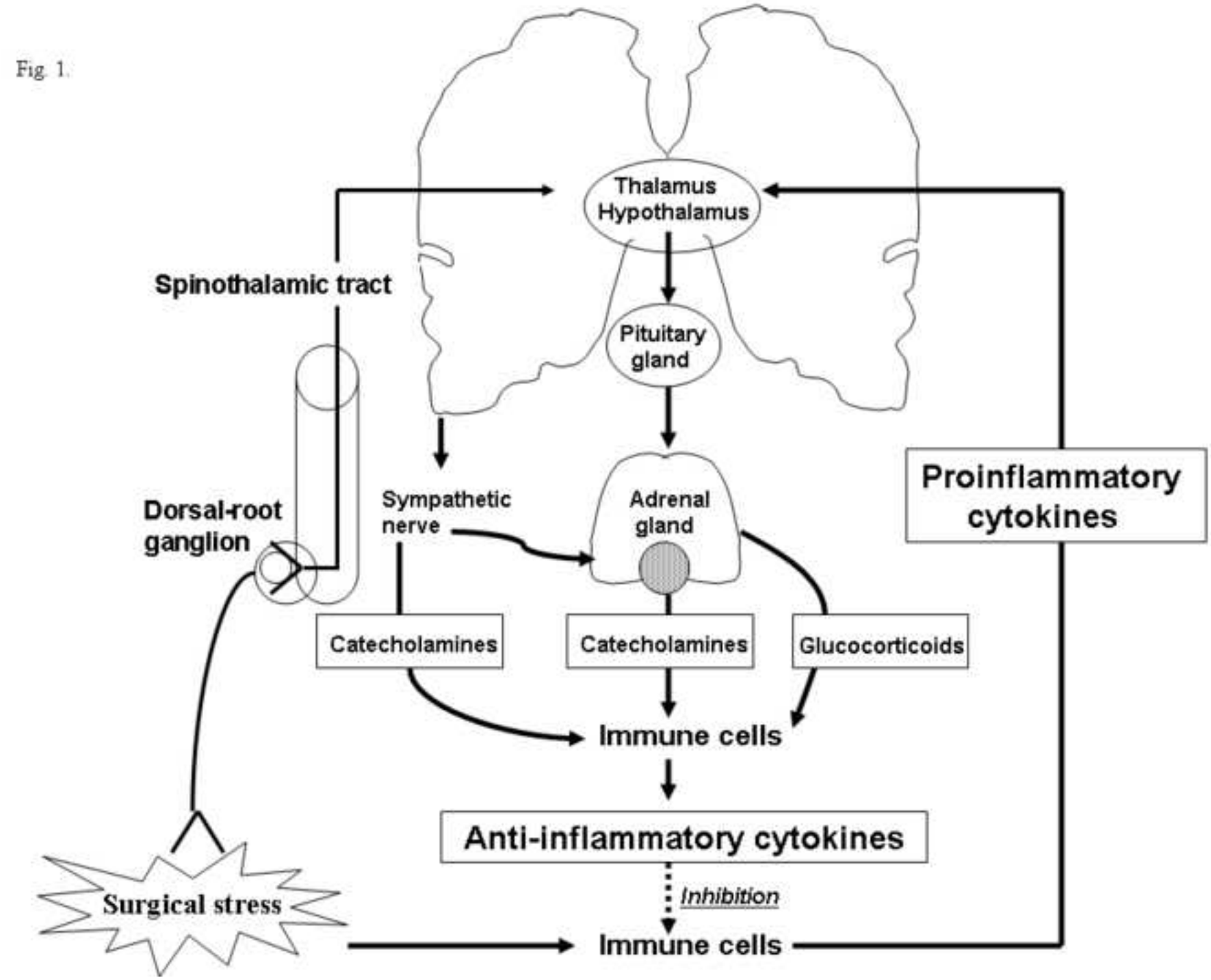

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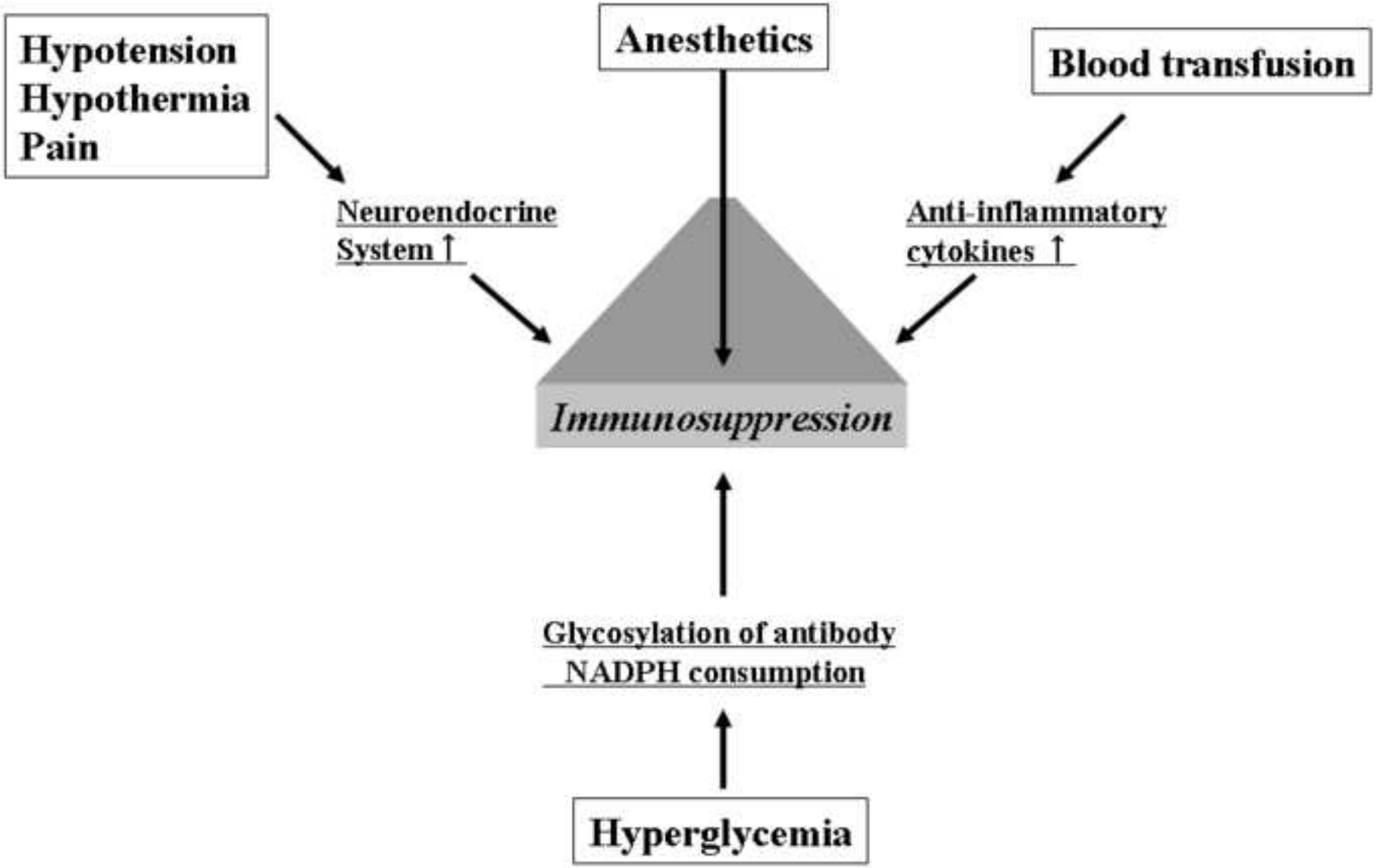

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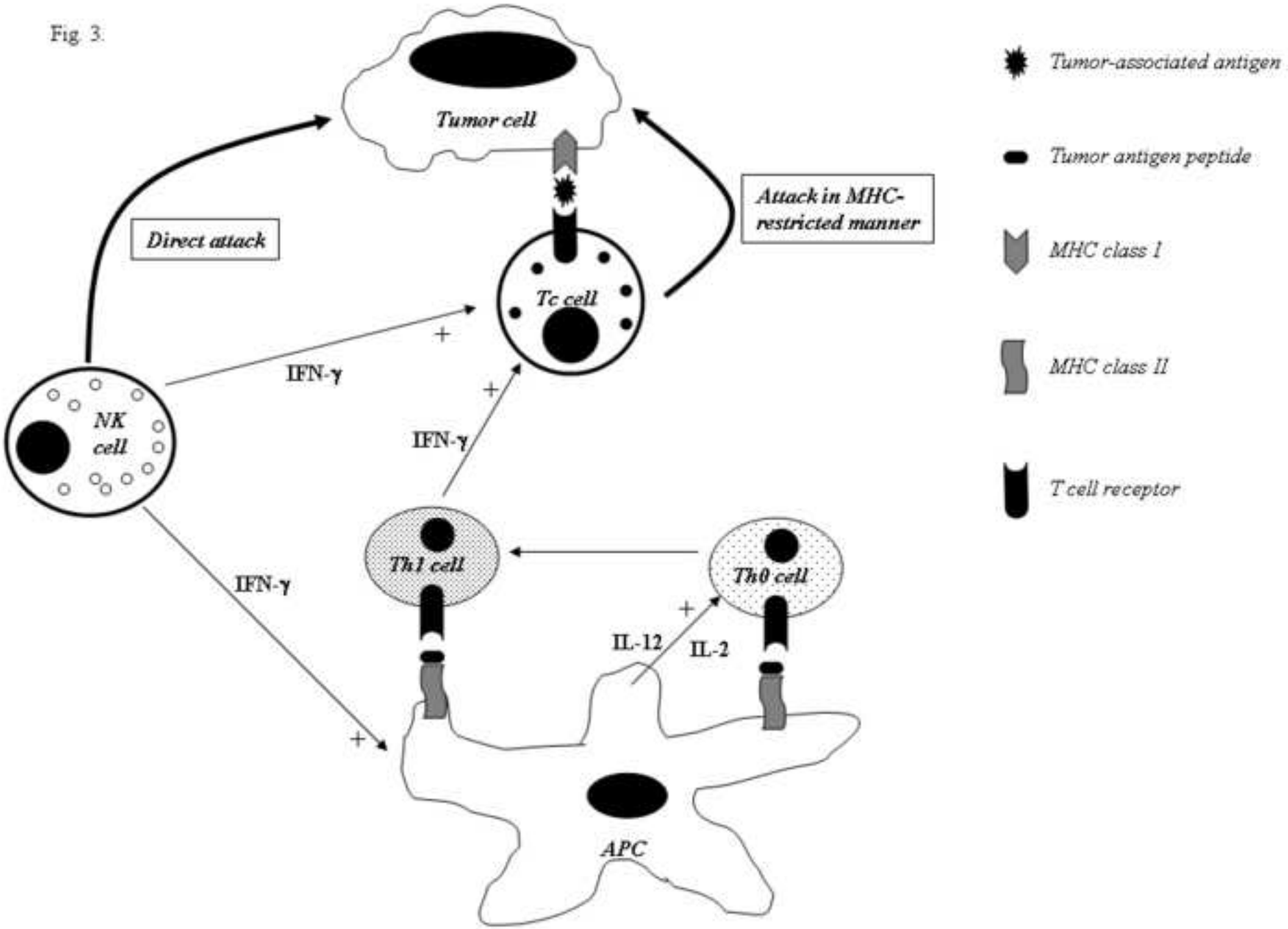

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