Impact of Anesthetics on Human Neutrophil Function

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Anesthetics are widely used drugs administered in a multitude of clinical settings. Their impacts on various functions of the immune system have been studied but are still not fully understood. Neutrophil granulocytes are a critical first-line host defense mechanism against infections and contribute to the inflammatory phase of wound healing, but dysregulated neutrophil activation can also precipitate perioperative organ injury. A better understanding of the interactions between common anesthetics and neutrophils may reveal considerations toward optimizing treatment of our most vulnerable patients in the intensive care unit and in the perioperative setting. (Anesth Analg XXX;XXX:00–00)

Anesthetics are among the most commonly administered drugs. The American Society of Anesthesiologists estimates that 40 million anesthetics are administered annually in the United States alone. Most anesthetic treatments accompany a surgical procedure, where proper immune system function is critical for recovery because patients stand at risk for postoperative infections and must heal their wounds. Anesthetics are also administered as sedatives in intensive care units, where our most vulnerable and critically ill patients are treated, several of whom are at risk for deep seated or opportunistic infections, nosocomial pneumonia, and sepsis. Evidence is accumulating that various classes of anesthetics impact the immune system in differing but important ways.

Neutrophils (also known as neutrophilic granulocytes or polymorphonuclear granulocytes [PMNs][1]) are the most abundant type of white blood cell and serve a critical first-line role in innate immune defense against invading pathogens. Neutrophils undertake a variety of functions to protect normal host physiology (Figure 1), and their regulation is multifaceted (Figure 2). Individuals who lack neutrophils or have deficiencies in key neutrophil functions can experience life-threatening infections, and chemotherapy-induced neutropenia renders patients highly susceptible to invasive bacterial or fungal disease.[2] Conversely, neutrophils are involved in the pathophysiology of organ injury associated with significant perioperative morbidity and mortality[3] including in the pathophysiology of acute respiratory distress syndrome,[4] myocardial infarction,[5] and stroke.[3,6] Tight regulation of neutrophil function is therefore necessary in order for neutrophils to effectively protect against invading pathogens while limiting accompanying tissue damage and organ dysfunction. The present succinct review summarizes what is known about the impact of different anesthetic classes on human neutrophil function (Figure 3). While the clinical significance of these phenomena remains underexplored, in vitro evidence of a significant impact is accumulating.

This review focuses principally on studies performed using human neutrophils and close to realistic concentrations of anesthetics achieved in human plasma. Selected animal studies are included when they have provided unique mechanistic insight and/or placed anesthetic effects on neutrophil function in an experimental disease context in vivo.

INHALATIONAL ANESTHETICS AND NEUTROPHIL FUNCTION

Isoflurane and Related Agents

Inhalational anesthetics, with the exception of nitrous oxide and xenon, are halogenated alkanes (halothane) or halogenated ethers (isoflurane, enflurane, sevoflurane, and desflurane). Inhalational anesthetics are widely used to induce hypnosis, amnesia, and immobility in the setting of painful stimuli. Most commonly, this transpires as an adjunct to another procedure[7] such as surgery, electroconvulsive therapy, or endoscopy, but such agents can also be used in other clinical scenarios such as treatment of refractory asthma.[8]

Two distinct effects of inhalational anesthetics, immobility and amnesia, can be attributed to the action of these drugs on different parts of the nervous system. While the immobility effect of isoflurane is considered to be mediated through the spinal cord, hypnosis and amnesia are thought to be exerted directly through the brain. The exact mechanisms through which inhalational anesthetics exert their pharmacological effects are not fully elucidated. Once prevalent "lipid theories" such as the Meyer-Overton rule (the assumption that volatile anesthetics act nonspecifically on lipid layers of cells) have largely been abandoned in favor of theories based on lipid–protein interactions.[7] For example, inhalational anesthetics interact with ion channels including the family of neurotransmitter receptors.[9,10] Recently, an important role of hyperpolarization-activated cyclic nucleotide-gated subtype 1 channels in mediating inhalational anesthetic effects has been demonstrated,[11] and their impact on cytoplasmic signaling proteins including protein kinase C (PKC) has also been described.[7,12] Inhalational anesthetics increase the sensitivity of gamma butyric acid A (GABA) receptors[13] by prolonging the inhibitory current resulting from their stimulation,[14] potentiate

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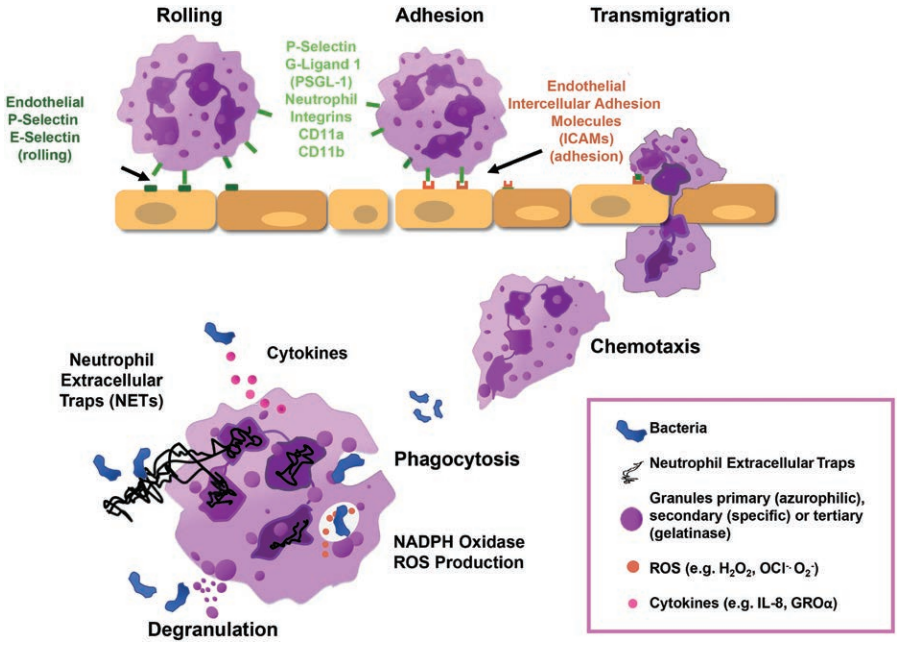
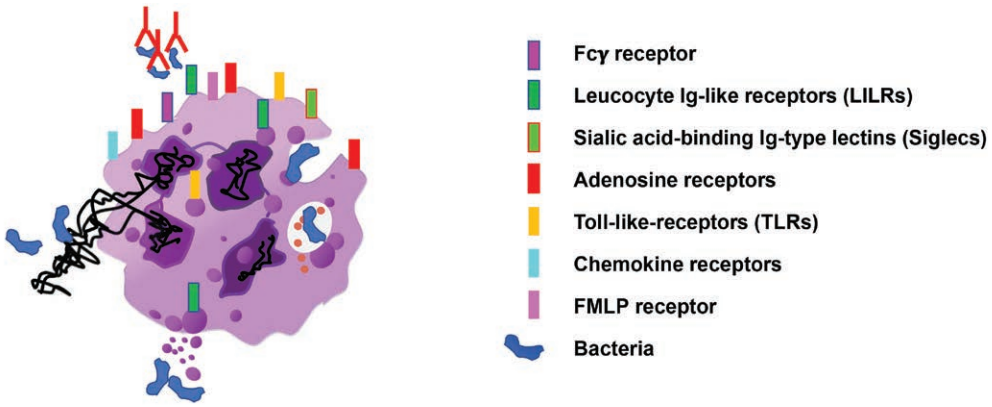


Figure 1. Neutrophils and their functions. H_2O_2 indicates hydrogen peroxide; ICAM, intercellular adhesion molecule; IL, interleukin; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; NET, neutrophil extracellular trap; O_2^-, superoxide anion; OCl^-, hypochlorite ion; PSGL, P-selectin G-ligand 1; ROS, reactive oxygen species.



Fc receptors	FcγRI, FcγRII, FcγIIIb, FcαR	FcγRI FcγRII activating, FcγIIIb unclear, FcαR binds IgA	DJ van Rees et al. (Semin Immunol 2016)
Leucocyte Ig-like receptors (LILRs)	LILRB2, LILRB3	Inhibitory	B Favier (Immunol Rev 2016)
Sialic acid binding Ig-like lectins (Siglecs)	Siglec-3, Siglec-5, Siglec-9 Siglec-14	Inhibitory or activating (14)	MS Macauley et al. (Nat Rev Immunol 2014)
Adenosine receptors	A_1, A_{2A}, A_{2B}, A_3	Activating or inhibitory	KE Barletta et al. (Arterioscler Thromb Vasc Biol 2012)
Toll-like receptors (TLRs)	TLR1-10	Activating	LR Prince et al. (Curr Opin Pharmacol 2011)
Chemokine Receptors	CXCR1, CXCR2	Activating	O Bonavita et al. (Cytokine Growth Factor Rev 2016)
N-formyl-l-methionyl-l-leucyl-phenylalanine (FMLP) receptor	FPR1-3	Activating	K Chen et al. (J Autoimmun 2017)

Figure 2. Regulation of neutrophil function-selected immune receptors. CXCR indicates chemokine receptor; fMLP, *N*-formyl methionyl-leucyl-phenylalanine; FPR, formyl peptide receptor; LILR, leukocyte Ig-like receptor; TLR, toll-like receptor.

the effects of glycine on glycine receptors,[15] and inhibit α4β2 nicotinic acetylcholine receptors.[16]

The effects of inhalational anesthetics on the immune system are manifold and have been recognized for decades. However, in the case of human neutrophils, most studies have focused narrowly on 1 particular aspect of neutrophil function, and no comprehensive analysis exists to date. The preponderance of these focused studies has evaluated neutrophil adhesion molecule expression. One minimum alveolar concentration of isoflurane led to reduced neutrophil expression of CD11a (also known as "integrin alpha L"), which plays a key role in cellular adhesion and costimulatory signaling.[17] Volatile anesthetics also blocked upregulation of neutrophil CD11b, leading to impaired adhesion to human endothelial cell when the endothelial cells were activated with hydrogen peroxide (H_2O_2)or the neutrophils stimulated with the chemotactic peptide *N*-formyl methionyl-leucyl-phenylalanine (fMLP).[18] Isoflurane treatment of

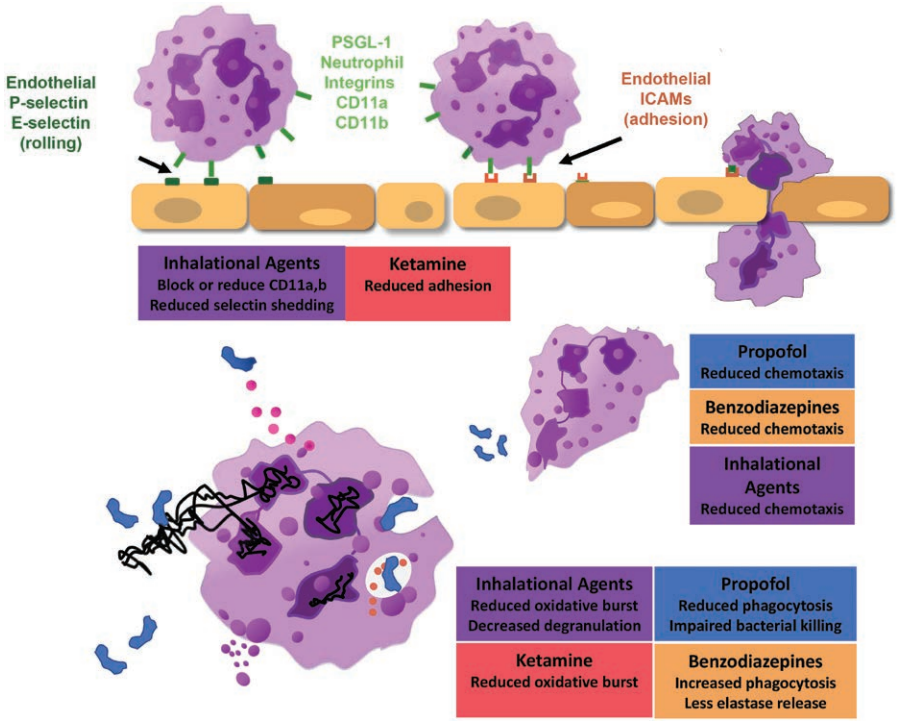


Figure 3. Described effects of anesthetics and sedatives on human neutrophil function. ICAM indicates intercellular adhesion molecule; PSGL, P-selectin G-ligand 1.

fMLP-stimulated neutrophils was associated with reduced shedding of L-selectin, a molecule that promotes initial interactions of neutrophils with endothelium and plays a role in priming of degranulation. When these neutrophils were stimulated with phorbol myristate acetate (PMA), a PKC agonist, isoflurane once again blocked depletion of L-selectin from the neutrophil surface while expression of β2-integrins was unchanged.[17] In another study, desflurane and isoflurane also interfered with neutrophil adhesion molecules and migration: volatile anesthetics blocked the interleukin-8–induced upregulation of the leukocyte adhesion molecule CD11b and inhibited the CD11b-dependent migration of human neutrophils via a mechanism involving chemokine receptor-2 signaling.[19] Furthermore, indirectly mediated, but influencing neutrophil function, isoflurane exposure upregulated the expression of P-selectin on activated platelets, increasing their binding to neutrophils; the opposite effect was seen after halothane exposure.[20] Another indirect effect of isoflurane on neutrophil function was observed due to increased phagocytosis of apoptotic neutrophils by macrophages after isoflurane exposure resulting in reduced lipopolysaccharide-induced inflammatory lung injury.[21]

With respect to oxidative burst function, 1 study reported that fMLP-induced activation of neutrophil H_2O_2 production was impaired in the presence of sevoflurane, halothane, and enflurane, but not isoflurane.[22] In contrast, PMA-induced H_2O_2 production was not influenced by any of the tested inhalational anesthetics. Because PMA is a direct activator of PKC while fMLP interacts with specific receptors on the cell surface, the inhibitory effects of volatiles appear to be exerted upstream of PKC. Finally, one study showed that in vitro exposure of blood drawn from term women to isoflurane for 90 minutes did not impact neutrophil phagocytosis of fluorescently labeled *Escherichia coli (E coli)* bacteria.[23]

INTRAVENOUS ANESTHETICS AND NEUTROPHIL FUNCTION

Propofol

Propofol (2,6-diisopropylphenol) is a widely used hypnotic both in and outside the operating room, due to its rapid onset and short duration of action in combination with its minimal side effect profile. Propofol is the most commonly used anesthetic for induction in the adult population undergoing general anesthesia, and the most common sedative used long term in the adult intensive care unit, where it can be administered for days and even weeks.[24] As an alkylphenol derivate, propofol is not soluble in an aqueous solution and is therefore formulated as an emulsion.[25] Propofol acts on the $GABA_A$ receptor potentiating GABA-elicited currents.[26] At high concentrations, it can directly open the chloride channel on the $GABA_A$ receptor and inhibit glutamate release through presynaptic voltage-gated sodium (Na^+) channels[27]; additional mechanisms of propofol action have been proposed.[28]

Different aspects of human neutrophil function are potentially influenced by propofol in clinically relevant concentrations, but studies have yielded contradictory results. Propofol inhibited neutrophil phagocytosis and killing of *E coli* and *Staphylococcus aureus* in one study,[29] and while impaired neutrophil phagocytosis of *E coli* was corroborated in another examination, the investigators observed no difference in bacterial clearance from whole human blood.[30] Still another group more recently concluded that propofol affected neither granulocyte phagocytosis nor recruitment,[31] and, while impaired neutrophil phagocytosis of *E coli* was corroborated in another examination, the investigators observed no decrease in bacterial clearance from whole human blood when propofol in clinical concentrations was present.[32] Importantly, human neutrophils do not express $GABA_A$,[33,34] so impacts of propofol on neutrophils would have to be mediated by either its lipid carrier or off-target effects.

Interestingly, a study from Taiwan found that propofol inhibited chemotaxis, superoxide production, and elastase release in fMLP-stimulated human neutrophils by blocking the formyl peptide receptor.[35] In another study, propofol inhibited fMLP-stimulated neutrophil chemotaxis at least in part via inhibition of p44/42 mitogen-activated protein kinase phosphorylation.[36] While a comprehensive functional analysis of propofol effects on human neutrophils still awaits, in vivo data in the mouse suggest that propofol sedation exacerbates bloodstream dissemination of *Staphylococcus aureus* infections[37] consistent with a potentially significant adverse impact on innate immunity warranting further investigation.

Ketamine

The cyclohexanone derivative ketamine is a commonly used anesthetic that has gained increasing popularity due to pain relieving and antidepressant properties and its lack of respiratory depressant side effects. Ketamine is used as an induction or sole anesthetic agent or as an adjunct to opioids in the setting of general anesthesia or postoperative pain to achieve better pain control without depressing the respiratory drive. For its anesthetic and analgesic properties, ketamine is thought to mainly act as a noncompetitive antagonist on *N*-methyl-D-aspartate receptors[38] with additional effects on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors, mechanistic target of rapamycin,[39] and sigma receptors.[40] However, additional studies suggest broad promiscuity in ketamine's receptor engagement because the anesthetic has also been found to signal through the $GABA_A$ receptor,[41] the hyperpolarization-activated cyclic nucleotide-gated subtype 1 receptor,[42] α- and β-adrenergic receptors,[43] muscarinic receptors, and opioid receptors.[38]

Zilberstein et al[44] described that the addition of ketamine to the general anesthesia regimen in the setting of coronary artery bypass graft surgery attenuated superoxide anion production of human neutrophils for up to 6 days in the setting of PMA, fMLP, or zymosan stimulation. Ketamine suppression of fMLP-induced neutrophil oxidative burst was subsequently linked to decreased phosphorylation of p47 (PHOX), a key step in the activation of nicotinamide adenine dinucleotide phosphate oxidase in the phagosome membrane. Ketamine treatment of human neutrophils ex vivo also reduced PMA- or fMLP-induced upregulation of CD18, a key neutrophil adhesion molecule that heterodimerizes with CD11b, as well as shedding of L-selectin.[45] These inhibitory effects occurred in a concentration-dependent manner and were similar for racemic ketamine and its S(+) or R(−) isomers, suggesting that suppression of neutrophil function is likely not attributable to a specific receptor interaction.

Benzodiazepines

Benzodiazepines (eg, midazolam, lorazepam, and clonazepam) are commonly used as sedatives, anxiolytics, as anticonvulsants, and in the setting of alcohol withdrawal. Structurally, this family of drug molecules consists of a benzene ring and a diazepine ring with varying side chains. To induce their sedative actions, benzodiazepines potentiate the inhibitory effect of GABA at the postsynaptic site. Their allosteric modulation of the $GABA_A$ receptor, a member of the pentameric, ligand-gated ion channel superfamily,[46] increases $GABA_A$-mediated chloride channel activity,[47] resulting in inhibition of neuronal activity.[48] Of note, some, but not all, benzodiazepines also bind to a peripheral benzodiazepine receptor (PBR).

Studies on the effects of benzodiazepines on human neutrophil function have yielded mixed results, with application of varying model systems and frequent treatments using supraphysiological concentrations of the agents. One group observed that diazepam, acting through a peripheral benzodiazepine binding site, inhibited fMLP-induced human neutrophil chemotaxis and superoxide production[49] although PMA-induced superoxide production was unaffected in the same analysis. Additional studies reporting suppressive effects on human neutrophils include a finding that diazepam and midazolam reduced oxidative burst measured by a chemo luminescence–based assay in fMLP- but non zymosan-stimulated cells,[50] and reduced CD11b/CD18 expression and p38 mitogen-activated protein kinase phosphorylation in neutrophils treated with midazolam, albeit at supratherapeutic concentrations.[51] In contrast, Marino et al[52] found that using isolated human neutrophils that diazepam induced human neutrophil migration and phagocytosis via a direct interaction with the PBR, a property not shared by clonazepam, a benzodiazepine known not to bind the PBR. In these studies, diazepam appeared to enhance phagocytosis through PBR by increasing intracellular calcium, while the stimulatory effect on migration was not calcium, dependent.

Central α_2-Adrenergic Receptor Agonists

The agents dexmedetomidine, an imidazole derivative that stimulates α_2-adrenergic receptors, and clonidine, an imidazoline that stimulates both α_2 and central imidazoline receptors, are anesthetic agents of increasing clinical importance. Dexmedetomidine is commonly used for sedation in the intensive care unit and operating room, while clonidine can be used to lower blood pressure or to ease withdrawal-related symptoms.

While human neutrophils do not express the main receptors that other intravenous anesthetics such as propofol classically interact with, neutrophils do express adrenergic receptors including the α_2-adrenoreceptor.[53] However, whereas a synthetic agonist of α_2-adrenoceptors was shown to desensitize human neutrophils to cytokine activation,[53] a study examining dexmedetomidine and clonidine did not find any impact of these α_2-adrenoceptor agonist drugs themselves on neutrophil phagocytosis, chemotaxis, or superoxide production at clinically relevant concentrations.[54] One additional study that did report a significant impact of dexmedetomidine on human neutrophil respiratory burst, inducible nitric oxide synthase production, and nitric oxide production in response to *E coli* challenge tested only drug concentrations that were significantly supratherapeutic.[55]

Local Anesthetics

Local anesthetics are widely used to either prevent or relieve pain[56] and may be administered intravenously, intrathecally, epidurally, subcutaneously, or perineurally. These drugs block Na+ current by targeting specific sited in voltage-gated Na+ channels[57] and are represented in 2 classes: amino amides such as bupivacaine lidocaine, mepivacaine, prilocaine, or ropivacaine, and amino esters such as benzocaine, chloroprocaine, or tetracaine. Clinical applications include spinal or epidural anesthesia during childbirth or surgery, targeted nerve blocks,

regional infiltration, and when given intravenously, pain control and cardiac arrhythmia treatment. The side effect profile of local anesthetics can include cardiac toxicity and central nervous system toxicity, but adverse events do not occur frequently unless the maximal recommended dose is exceeded, or an accidental large volume intravascular injection occurs.

Most effects on neutrophil function from local anesthetics have been demonstrated only at concentrations unlikely to be achieved during clinical use and effects on systemic immunity may be limited in the setting of regional anesthesia. For example, while there was no impact of clinically relevant concentrations of lidocaine, chloroprocaine, bupivacaine, or ropivacaine on reactive oxygen species production by human neutrophils,[58,59] measurable effects on oxidative burst, chemotaxis, and bacterial phagocytosis were observed at much higher concentrations.[60,61] Hoffman et al[62] did report an effect of clinically relevant concentrations of ester and amide local anesthetics on neutrophil priming induced not by fMLP, but rather by platelet activating factor, which may reflect key in vivo settings. Interestingly, human neutrophils do not express voltage-gated Na+ channels,[63] so the interaction of local anesthetics with neutrophils may lie in the platelet activating factor priming pathway upstream of PKC.[62]

SUMMARY

Many anesthetics suppress various aspects of human neutrophil function, but the underlying mechanisms have not been fully elucidated. The regulation of neutrophil function is complex because both "too much" and "too little" can cause result in significant patient injury and adverse clinical outcomes. Studies revealing the composite of such regulations, including neutrophil regulation through hypoxia-inducible factor, adenosine receptors, microRNAs, and other mechanisms (Figure 2), are providing new insights into the neutrophil's roles and ability to respond to injury and infection.[64–67] Importantly, studies that systematically analyze many key aspects of neutrophil functions taking into consideration, current concepts on how neutrophils are regulated and directly comparing multiple anesthetic agents and their alternatives have not been published.

Anesthetics are administered in situations where intact yet not overstimulated immune responses are critical, including the perioperative setting where impaired wound healing and surgical site infections dramatically impact patient well-being, or in the intensive care unit where patients often are fighting severe infection. Future studies should be directed at fully elucidating the effect of anesthetics on neutrophil function and their comparative impact of patient outcome in relation to infection, sepsis, organ damage, and wound healing. If anesthetic effects at pharmacologically achieved concentrations will counteract or work in concert with other factors that perioperatively may impact immune function, such as surgical incision or overwhelming infection, is yet to be elucidated.

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Name: Angela Meier, MD, PhD.
Contribution: This author helped write the manuscript and draw the figure.
Conflicts of Interest: A. Meier previously received consulting fees from Millennium Health unrelated to this work.
Name: Victor Nizet, MD.
Contribution: This author helped write and edit the manuscript, edit the figure, and provide crucial input and mentorship.
Conflicts of Interest: V. Nizet has consulted for InhibRx Therapeutics, Cidara Therapeutics, SutroVax, Inc, and Cellular Approaches, Inc, on subjects unrelated to this work.
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