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REVIEW ARTICLE

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# Nonhuman primate species as models of human bacterial sepsis

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**Sepsis involves a disordered host response to systemic infection leading to high morbidity and mortality. Despite intense research, targeted sepsis therapies beyond antibiotics have remained elusive. The cornerstone of sepsis research is the development of animal models to mimic human bacterial infections and test novel pharmacologic targets. Nonhuman primates (NHPs) have served as an attractive, but expensive, animal to model human bacterial infections due to their nearly identical cardiopulmonary anatomy and physiology, as well as host response to infection. Several NHP species have provided substantial insight into sepsis-mediated inflammation, endothelial dysfunction, acute lung injury, and multi-organ failure. The use of NHPs has usually focused on translating therapies from early preclinical models to human clinical trials. However, despite successful sepsis interventions in NHP models, there are still no FDA-approved sepsis therapies. This review highlights major NHP models of bacterial sepsis and their relevance to clinical medicine.**

Sepsis is a common clinical syndrome arising from a dysregulated host response to systemic infection leading to life-threatening end-organ damage[1]. While the incidence of sepsis varies[2], the substantial burden of critical illness reflects an aging and susceptible population with escalating comorbid conditions[3,4]. With the exception of early antibiotics and fluids[5–7], targeted new therapies for sepsis have remained elusive[8]. However, through research, our understanding of sepsis has evolved from being seen solely as an exuberant host immune response[9] to the intersection of pro- and anti-inflammatory, endothelial, coagulation, metabolic, endocrine, and neural pathways[10,11]. The foundation of such research has been the use of small animals in studies to unravel mechanisms of sepsis pathophysiology and develop novel therapeutics[12,13].

This review briefly presents the common nonhuman primate (NHP) models of bacterial sepsis and summarizes the contributions to our understanding of sepsis pathophysiology and drug discovery, and how they might impact on future targeted research.

## Finding an ideal animal model

An ideal animal model of sepsis must balance biological feasibility and practical considerations. The animal species should recapitulate the same hemodynamic changes to infection seen in humans, such as hypotension, low systemic vascular resistance (SVR), and a compensatory increase in cardiac output (CO) following fluid resuscitation[14–16]. Animals should also demonstrate similar signs and symptoms of infection, such as decreased activity or oral intake, change in body temperature, and change in white blood cell count[12]. The model should include provision of antibiotics[17,18], a standard of care treatment for patients[1] that may alter host cytokine responses[19] and significantly improves survival[20]. The species should also activate similar molecular pathways, such as cytokine release in response to circulating pathogen-associated molecular patterns (PAMPs)[11], and should lead to development of multiple organ dysfunction (MOD)[21,22]. Practically, the model must be economically reasonable, easy to support, and large enough for the collection of physiological data.

The earliest and most widely used animal species for sepsis studies has been rats and mice. Murines are attractive because they are inexpensive, easy to breed, versatile, and genetically modifiable[8,12,13]. The rat model of endotoxemic sepsis with fluid resuscitation relies on intravenous lipopolysaccharide (LPS) to mimic the physiological responses of Gram-negative sepsis in humans. Unlike human sepsis, which is a protracted response to live pathogens, LPS induces a rapid, transient inflammatory surge that causes mainly vascular injury[13]. The rat cecal-ligation and puncture (CLP) technique was developed to mimic human abdominal sepsis from live polymicrobial bacteria[12,23,24]. Though this model has been widely considered the gold standard for rodent studies, it is limited by variability in technique (e.g. number of punctures, size of puncture)[23] and pathogen (e.g. polymicrobial)[24]. As an alternative rat model, the monomicrobial *Staphylococcus aureus* impregnated clot model of peritoneal sepsis was developed to simulate post-operative abdominal wound infections[25]. Another model using intravenous injection of live bacteria simulates the bacteremia seen in up to 69% of human septic shock[24], but does so by artificially infusing a large inoculum of bacteria into the bloodstream rather than by slow release from an established nidus of infection[12].

Rodent models suffer from important technical limitations. Their small size makes invasive hemodynamic monitoring difficult, and their small total blood volume (approximately 2 ml in mice and 20 ml in rats) limits serial phlebotomy[23]. Mice are also more resistant to LPS and bacterial pathogens than are humans, requiring $10^4$- to $10^5$-fold higher doses to achieve shock[8,13,24]. Once shock develops, the hemodynamic and cytokine profiles of small rodents differ greatly from those of humans[8,24], though the inconsistent use of fluid resuscitation in these studies may have affected the results.

The sizable technical and biological challenges ultimately led to the use of larger mammals such as rabbits, sheep, dogs, and pigs, whose sizes permitted invasive and serial procedures[12,23]. Many large animals also exhibit sepsis physiology that is similar to humans. For instance, pigs are prone to sepsis-induced capillary leak and development of pulmonary edema, and sheep display similar hyperdynamic responses (i.e. elevated heart rate, cardiac output, and oxygen delivery with depressed systemic vascular resistance) to endotoxin

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as humans[23]. Nevertheless, notable physiological differences still exist between these large mammals and humans: Sheep and other ruminants have significantly more pulmonary intravascular macrophages compared to primates, which alters their susceptibility to sepsis-induced pneumonia and acute lung injury[26,27]. Pigs demonstrate a profound rise in pulmonary vascular resistance in response to bacterial sepsis which can be fatal and experiment-limiting[23]. And dog models of septic shock are confounded by significant intestinal mucosal congestion and hemorrhage[23].

## The nonhuman primate model of sepsis

To circumvent the limitations of rodent and large mammal models, researchers developed nonhuman primate (NHP) models of bacterial sepsis[20,28,29]. Baboons, macaques, and chimpanzees have historically been the most popular primates studied, though as of 2013 the National Institutes of Health has limited its funding support to monkeys, as great apes are widely agreed to be too sentient to ethically justify their use. Originating from a common phylogenetic ancestor, humans and monkeys have nearly identical anatomy and physiology. For example, the baboon and human tracheobronchial trees share a similar dichotomous branching pattern with a bronchiolar transition zone between the small airways and distal alveolar regions[30]. Moreover, the alveolar region of baboons and humans contain a prominent alveolar interstitial compartment[31]. These features are less prominent or less uniform in rodents, which may partly explain differing patterns of pneumonia-induced acute lung injury (ALI) compared with humans[30–32].

Humans and baboons also display almost identical hemodynamic and cytokine responses to intravenous endotoxin and live bacteria[29,33–35]. Following infusion of Gram-negative bacteria (*E. coli*), baboons develop tachycardia, increased cardiac output, decreased SVR, and hypotension similar to human sepsis[29].

Furthermore, the use of NHPs carries the same advantages seen in other large animals. For instance, the NHPs' large size allows for invasive monitoring, tissue collection, and serial phlebotomy[23]. Their use also satisfies the U.S. Food and Drug Administration (FDA) drug development requirement that investigational new drug (IND) applications include data from at least one non-rodent species[23]. As such, the NHP models are considered quite suitable for pre-clinical drug development studies.

**Endotoxemia model.** Endotoxin plays a central role in the development of Gram-negative sepsis. Located on the outer cell membrane of Gram-negative bacteria, LPS is a pathogen-associated molecular pattern (PAMP) recognized by the innate immune pathogen recognition receptor, toll-like receptor 4 (TLR4), and activates pro-inflammatory and coagulation cascades[36]. Healthy human volunteers given intravenous LPS (2–4 ng/kg) developed tachycardia, decreased SVR, and depressed left ventricular function[34]. These hemodynamic changes are associated with the release of pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), and activation of coagulation and fibrinolysis pathways[37] (Fig. 1a, b), similar to the responses of patients in septic shock.

NHPs are more resistant to endotoxin than humans. The baboon (*Papio ursinus* or *cynocephalus*) requires 0.1 mg/kg[33] and the rhesus macaque (*Macaca mulatta)* 0.1 mcg/kg[38] to achieve a hyperdynamic state. One exception appears in older studies of chimpanzees (*Pan troglodytes*) which, like humans, develop a mild and reversible inflammatory state at 4 ng/kg[39,40]. Nonetheless, NHP endotoxemia models are frequently employed to model Gram-negative sepsis without live pathogen inoculation, and they generally corroborate the hemodynamic and cytokine profiles seen in human sepsis[33].

Pharmacologic inhibitors are commonly used to test mechanism of immune pathways, and monoclonal antibodies have been used in

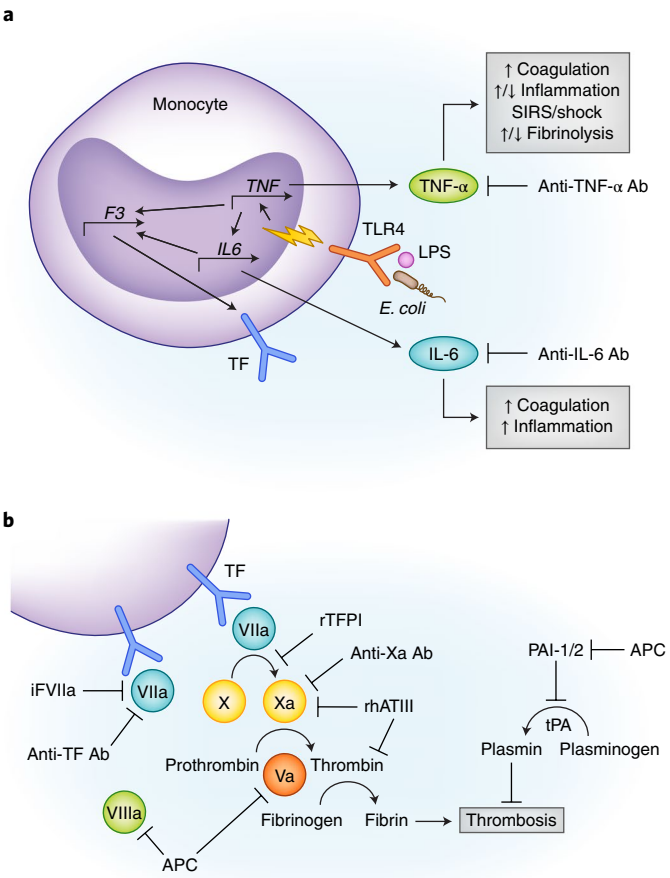


**Fig. 1 |** (**a**) Simplified rationale for performing studies in NHP sepsis models of antibody inhibition of TNF-α and IL-6. Lipopolysaccharide (LPS) or *E. coli* are shown binding to a TLR4 surface receptor of a monocyte which initiates downstream signal transduction (yellow lightning bolt) and transcriptional upregulation of TNF-α. TNF-α further activates other pro- and anti-inflammatory cytokines, including IL-6. Both cytokines promote hypercoagulability by upregulating tissue factor (TF) expression. TNF-α also simultaneously promotes and inhibits fibrinolysis via activation of tPA and PAI-1/2, respectively (see Fig. 1b). (**b**) Multiple therapeutics are shown inhibiting different components of the extrinsic coagulation pathway. These drugs were tested using LPS or *E. coli* NHP sepsis models. *Abbreviations*: Ab, antibody; APC, activated protein C; F3, factor 3; iFVIIa, site inactivated factor VIIa; IL6, interleukin 6; LPS, lipopolysaccharide; PAI-1/2, plasminogen activator inhibitor 1/2; rhATIII, recombinant human antithrombin III; SIRS, systemic inflammatory response syndrome; TF, tissue factor; TLR4, toll-like receptor 4; TNF, tumor necrosis factor.

NHP sepsis experiments to inactivate a variety of pro-inflammatory and coagulation mediators[38,40,41]. Chimpanzees given monoclonal anti-TNF-α antibodies (15 mg/kg) immediately following LPS injection had decreased downstream production of IL-6 and CXCL8[40], and within the lungs, had increased fibrinolysis[42] compared to LPS-only controls. Baboons and rhesus monkeys treated with anti-TNF-α antibodies had lower serum TNF-α levels, attenuated coagulation responses, and less severe organ dysfunction than control monkeys[38,43]. In the rhesus, treatment with anti-TNF-α antibodies significantly improved survival[38]. These findings suggest that TNF-α is not only a catalyst for other downstream inflammatory mediators, but also for sepsis-induced fibrinolysis and organ dysfunction (Fig. 1a).

Like TNF-α, IL-6 is also an early phase mediator (released within one hour) of LPS exposure[33,37]. In chimpanzees with mild endotoxemia, anti-IL-6 antibodies attenuate thrombin-antithrombin III (TAT) complex activity in both serum[44] and lungs[42], suggesting its role in the activation of sepsis-induced coagulation. Similarly, pretreatment with monoclonal antibody to tissue factor decreased TAT complex activity and coagulation response, but had no effect on cytokine release or fibrinolysis[41] (Fig. 1a).

IL-6 also influences the development of acute lung injury (ALI). In endotoxemic chimpanzees given anti-IL-6 antibody, procoagulant proteins were significantly reduced in bronchoalveolar lavage fluid (BALF) compared with untreated animals[42]. These studies also found that pro-coagulant proteins were elevated in the BALF without evidence of vascular permeability, suggesting that coagulation in ALI is a local response[42].

**E. coli bacteremia model**. Despite its ability to duplicate the initial hours of Gram-negative sepsis, the LPS model nonetheless cannot replace a live bacterial infection model. *E. coli* bacteremia in the baboon (*Papio cynocephalus*) is one of the earliest and most versatile models of NHP sepsis and has greatly expanded the understanding of sepsis-induced inflammation, coagulation, and organ injury[45–47].

**Disseminated intravascular coagulation.** A frequent complication of sepsis, disseminated intravascular coagulation (DIC) is a syndrome of dysregulated microvascular coagulation that can lead to MOD[45,47,48]. In the baboon with *E. coli* bacteremia, the degree of coagulopathy varies directly with the dose of pathogen. The three most common dosing models, lethal dose 100 ($LD_{100}$), $LD_{50}$, and $LD_{10}$, corresponding to a predicted mortality of 100%, 50%, and 10%, respectively[48], have been used to elucidate mechanisms of sepsis-induced DIC and cellular injury, and to test novel sepsis therapies.

The $LD_{100}$ model ($10^{10}$ CFU/kg *E. coli*) produces a highly virulent phenotype wherein all animals die within 24 to 32 hours[48,49]. Administration of intravenous *E. coli* triggers the release of TNF-α, IL-6, and CXCL8[48], activation of endothelial cells, and microvascular migration of leukocytes[50,51]. Activated endothelial cells also express tissue factor (TF), a stimulus for intravascular and extravascular fibrin deposition[45,47,48]. Figures 1a,b demonstrate the TF-dependent coagulation pathways culminating in fibrin clot formation. Moreover, fibrinolysis is simultaneously triggered and inhibited by upregulation of tissue plasminogen activator (tPA), TAT complexes, and plasminogen activator inhibitor (PAI)[45,46]. These competing processes result in unchecked and widespread microvascular clot formation and MOD[47].

NHP studies of coagulation cascade inhibitors further support DIC as a contributor to MOD (Fig. 1b, Table 1). Compared with control animals, baboons that received recombinant tissue factor-pathway inhibitor (TFPI) shortly after $LD_{100}$ infusion had an attenuated coagulation response, significantly reduced organ injury, and 100% survival at 7 days[52]. Likewise, $LD_{100}$*E. coli* animals given monoclonal antibodies against tissue factor[53] or inactivated factor VIIa[54] had attenuated coagulopathy and lethality. Moreover, treatment of *E. coli*-infected baboons with recombinant human antithrombin III (ATIII) reduced inflammation, coagulation, fibrinolysis, and mortality[55].

**Multi-organ dysfunction.** The $LD_{100}$ model demonstrated that inflammation and coagulation interconnect through tissue factor, and that derangement of coagulation pathways culminates in DIC and lethal organ failure. However, due to its high lethality, the $LD_{100}$ model was unable to define the cellular injury and repair that occurs in sublethal sepsis, prompting development of $LD_{10}$ and $LD_{50}$ models.

The baboon $LD_{50}$ model ($10^{9}$ CFU/kg *E. coli*) produced hypotension and progressive MOD that was fatal in 50% of animals. The baboon $LD_{10}$ model ($10^{7}$–$10^{8}$ CFU/kg *E. coli*) produced mild hypotension and non-progressive organ dysfunction with spontaneous resolution. Unlike the $LD_{100}$ model where animals expired rapidly, these sub-lethal models survived long enough to demonstrate that sepsis is a multistage process driven by distinct mechanisms[48]. Although these experiments preceded the current standard of fluid resuscitation in early sepsis[6], and ischemia-reperfusion is recognized and prevented clinically, these experiments show the natural course of under-resuscitated sepsis. The initial stage is a TNF-α- and tissue factor-dependent and PAMP-mediated intravascular innate immune activation leading to microvascular DIC and organ ischemia. The second stage is the extravascular (organ) sequelae of ischemia-reperfusion injury[48], with accumulation of free radicals and further release of inflammatory mediators even after pathogen clearance[56]. Thus, whereas the $LD_{100}$ animals expired from intravascular events (hemodynamic collapse and DIC) before reperfusion could develop, the $LD_{50}$ animals expired from extravascular tissue injury[48]. This host-mediated, pathogen-independent injury is due to widespread complement activation[48,56,57]. Specifically, serum C5b9 levels were significantly elevated in $LD_{50}$ non-survivors, comparable to that of $LD_{100}$ animals, but not in $LD_{50}$ survivors[48]. These findings provide a deeper understanding of the mechanisms of organ injury and rationale for modern clinical practice including early aggressive fluid resuscitation.

The role of complement activation in lethal MOD was demonstrated in greater detail by NHP complement inhibitor studies (Table 1). Administration of compstatin, a C3 convertase inhibitor, to $LD_{50}$ baboons inhibited complement activation, protected against organ injury, and attenuated sepsis-mediated coagulopathy[58]. Concurrent administration of *E. coli* with a C5 inhibitor similarly decreased the inflammatory and coagulation responses, leading to organ protection and improved survival[59].

**Acute respiratory distress syndrome.** Because of their similarities to human respiratory anatomy[32] and physiology during Gram-negative sepsis[60], baboon models are central to the study of sepsis-induced acute respiratory distress syndrome (ARDS), an ALI arising from systemic inflammatory responses[61]. ARDS is characterized by bilateral pulmonary infiltrates, reduced lung compliance, and impaired gas exchange, and in severe cases can lead to respiratory failure, MOD, and death[62–64]. The histopathologic hallmark is protein-rich alveolar edema and fibrin deposition due to breakdown of the alveolar-capillary barrier[61,63]. Compared with healthy controls, bronchoalveolar lavage fluid (BALF) from patients with ARDS demonstrates high factor X, extrinsic pathway inhibitor, and anti-plasmin activity, suggesting that a hypercoagulable environment develops within the lungs[65].

The $LD_{100}$ intravenous *E. coli* baboon model was further optimized to enhance ALI by the addition of a pre-inoculation bacterial priming step. Baboons pretreated with $10^{9}$ CFU/kg heat-killed *E. coli* (65°C water bath for 30 minutes) prior to inoculation with 9 x $10^{10}$ CFU/kg live *E. coli* developed endotoxin tolerance with less shock and renal injury than unprimed animals[66]. However, the tolerance did not extend to the lungs, as priming worsened alveolar inflammation and fibroblast proliferation compared to unprimed animals[66]. These findings suggest that prior activation of the systemic inflammatory system leads to more severe lung injury in subsequent infections.

Using this primed $LD_{100}$ *E. coli* model, the same tissue factor-dependent pathways involved in systemic endothelial activation were found to also mediate pulmonary microvascular injury (alveolar fibrin deposition) and contribute to ARDS[18,67,68]. Additionally, tissue factor is not only expressed on endothelial cells but also on pulmonary macrophages and alveolar epithelium, suggesting that these extravascular cells also regulate the procoagulant environment of the lung seen in ALI[67,69]. Further dysregulation comes from

**Table 1 | Summary of experimental therapies and their associated nonhuman primate models**

| Ref. | Species/Model | Pharmacological Target | Therapeutic | Outcome |
|---|---|---|---|---|
| 38 | Rhesus/i.v. LPS | TNF-α | Anti-TNF-α Ab | ↓ TNF-α, coagulation, MOF, death |
| 40-42 | Chimpanzee/i.v. LPS | TNF-α | Anti-TNF-α Ab | ↓ IL-6, CXCL8[40], & lung coagulation[42] |
| 43 | Baboon/i.v. LPS | TNF-α | Anti-TNF-α Ab | ↓ TNF-α, coagulation, MOF |
| 95-98 | Baboon/i.v. *E. coli* | TNF-α | Anti-TNF-α Ab | ↓ coagulation, MOF, death |
| 42-44 | Chimpanzee/i.v. LPS | IL-6 | Anti-IL-6 Ab | ↓ coagulation in lung/BAL[42], serum[44] |
| 52 | Baboon/i.v. *E. coli* | Xa, FVIIa/TF/Xa complex | rTFPI | ↓ coagulation, MOF, death |
| 53 | Baboon/i.v. *E. coli* | TF | Anti-TF Ab | ↓ coagulation, SIRS, death |
| 18-54 | Baboon/i.v. *E. coli* | TF | Anti-FVIIa Ab | ↓ coagulation, inflammation, MOF, death |
| 70 | Baboon/i.v. *E. coli* | TF | Anti-FX Ab | ↓ coagulation, MOF |
| 55 | Baboon/i.v. *E. coli* | Thrombin pathway | rhATIII | ↓ DIC, inflammation, death |
| 58 | Baboon/i.v. *E. coli* | C3 convertase | compstatin | ↓ coagulation, MOF |
| 72 | Baboon/i.v. *E. coli* | C3 convertase | compstatin | ↓ post-ARDS fibrosis |
| 59 | Baboon/i.v. *E. coli* | C5 | C5 inhibitor | ↓ coagulation, MOF, death |
| 94 | Baboon/i.v. *E. coli* | E- and L-selectins | Anti-selectin Ab | **Worsened** UOP, acidosis, mortality |
| 48-49 | Baboon/i.v. *E. coli* | FVa, FVIIIa, PAI-1 | APC | ↓ coagulation/TNF-α[48], MOF/death[49] |
| 109-111 | Baboon/i.b. *S. pneumoniae* | HO-1, SPM | Inhaled CO gas | ↓ ALI severity[109], ↑ SPM[110,111] |
| 88 | Cynomolgus/i.b. *B. anthracis* | 30S ribosomal subunit | TP-271 antibiotic | ↓ infection-related mortality |
| 89, 90-113 | Cynomolgus/i.b. *B. anthracis* | Host immunity | vaccines | ↓ infection-related mortality |

*Abbreviations*: Ref, references; i.v., intravenous; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha; Ab, antibody; MOF, multi-organ failure; IL, interleukin; BAL, bronchoalveolar lavage fluid; TF, tissue factor; rTFPA, recombinant tissue factor pathway inhibitor; TF, tissue factor; SIRS, systemic inflammatory response syndrome; rhATIII, recombinant human antithrombin III; DIC, disseminated intravascular coagulation; ARDS, acute respiratory distress syndrome; UOP, urine output; PAI-1, plasminogen activator inhibitor 1; APC, activated protein C; i.b., intra-bronchial; HO-1, heme oxygenase-1; SPM, specialized proresolving lipid mediators; CO, carbon monoxide; ALI, acute lung injury.

decreased expression of tissue factor pathway inhibitor (TFPI), a major checkpoint of the coagulation pathway[68] (Fig. 1b). Baboons treated with site-inactivated factor VIIa[18] or with factor X inhibitor[70] to block tissue factor binding had reduced lung injury and improved renal function. These experiments support the causative relationship between coagulation and MOD.

The lung pathology from patients at autopsy or from patients undergoing open lung biopsies show that ARDS is an acute inflammatory process followed by either resolution or fibrosis[63], but less is known about long term ARDS sequelae[61]. Keshari et al. used the $LD_{50}$*E. coli* model to characterize the evolution of ARDS-associated fibro-inflammatory changes for up to 27 months post-inoculation[71]. Lung histopathology within the first 24 to 48 hours after inoculation demonstrated an acute exudative process with increased alveolar-capillary permeability and intra-alveolar hemorrhage, fibrin, and edema. By day 7, a restorative phase had developed with proliferation of type 2 epithelial cells. In baboons surviving to six to 27 months, lung tissue universally showed fibroblast proliferation, collagen deposition, and significantly increased macrophage accumulation compared to controls, suggesting the long-term sequelae of ARDS is fibrosis and chronic inflammation[71]. Treatment of $LD_{50}$ baboons with the complement inhibitor compstatin, however, decreased expression of fibrosis mediators, such as transforming growth factor-beta (TGF-β) and connective tissue growth factor (CTGF), myofibroblast accumulation, and collagen deposition[72].

## Organ-specific NHP models of sepsis

In addition to the pathophysiology of sepsis, NHPs are also used to model organ-specific disease. The most common source of sepsis in adults is pneumonia followed by abdominal and urinary tract infections[21]. While no NHP model for urinary tract infections has been reported, abdominal sepsis has been modeled in baboons via intraperitoneal implantation of *E. coli*-laden fibrin clot at a dose of $10^{11}$ CFU[73]. This model produced coagulopathy, MOD, and a 42% mortality rate. However, the interpretation was limited because the animals did not receive antibiotics or surgical source control, while both are standard-of-care treatments for patients with peritonitis.

Necrotizing fasciitis has also been modeled in baboons by intramuscular injection with *Streptococcus pyogenes* (group A *Streptococcus*)[74]. The animals develop a multistage, suppurative, soft tissue infection similar to that of humans and characterized by abscess formation, violaceous skin discoloration, and bullae formation. Histopathology showed early intense neutrophilic influx at the site of infection followed by lymphoplasmacytic infiltration. Non-survivors, however, had a paucity of neutrophilic infiltration and a peripheral blood leukopenia, suggesting death may be linked to secondary immune paralysis. Neither of these surgical sepsis models has gained widespread use.

**Pneumonia.** The most extensively studied organ system is the lungs. The use of NHP to model pneumonia dates back to the 1970s, most commonly using *S. pneumoniae*, the leading cause of bacterial community-acquired pneumonia (CAP) in adults[75]. An inoculation is performed under direct visualization by bronchoscopic instilment of live bacteria into the airway[17,76,77]. Multiple species of NHP are susceptible to *S. pneumoniae* infection. Squirrel monkeys endotracheally inoculated with either influenza A or low-dose *Streptococcus pneumoniae* (770 CFU) develop a self-limited illness with cough, fever, and tachypnea much like in humans[76]. However, the two pathogens together produce severe bronchopneumonia, leading to death in three of four animals[76].

The rhesus macaque (*Macaca mulatta*) demonstrates a dose-dependent response to *S. pneumoniae* ranging from no clinical symptoms at $10^6$ CFU to fever, leukocytosis, and respiratory distress at $10^8$–$10^9$ CFU[77]. A more recent model of *S. pneumoniae* pneumonia was published in baboons (*P. cynocephalus*). Animals given $10^6$ CFU experienced spontaneous bacterial clearance without signs or symptoms of pneumonia, whereas animals given $10^9$

CFU consistently developed severe lobar pneumonia that closely mimics the human disease[17]. This novel model has since been externally validated[19].

The inflammatory responses are also consistent across primate species. In baboons infected with high dose *S. pneumoniae* ($10^9$ CFU), peripheral blood cytokines and chemokines such as IL-6, CCL2, G-CSF, IL-1ra, and IL-10 are significantly elevated by 24 to 48 hours post-inoculation, but return to normal or near normal levels with time (~ 1 week) and antibiotic therapy[17,19]. Reyes et al. found that antibiotic treatment may reduce cytokine and chemokine concentrations in the lung tissue itself, although differences in experimental duration between the antibiotic-treated and untreated groups (9–14 days vs. 4–9 days, respectively), could also account for this observation[19]. In the BALF of baboons and squirrel monkeys with *S. pneumoniae* pneumonia, TNF-α, IL-1β, and IL-6 levels are significantly elevated by 24 to 48 hours[17,77] and similarly decline during the resolution phase[17]. These plasma and BALF cytokine profiles corroborate observations in patients with pneumonia[78]. Lung histopathology at 168 hours also mirrors human disease and shows ALI with intra-alveolar fibrin, edema, neutrophilic infiltration, and hemorrhage, and in some cases, early organization with collagen deposition[17].

Models for methicillin-resistant *Staphylococcus aureus* (MRSA) pneumonia have also been developed in the cynomolgus macaque (*Macaca fasicularis*)[79] and rhesus macaque[80] to study virulence factors. Historically considered a nosocomial pathogen, MRSA is now a recognized cause of severe, necrotizing CAP[75,81], especially following influenza infection[82,83]. The MRSA cytotoxin Panton-Valentine leukocidin (PVL) has been implicated in necrotic *S. aureus* soft tissue infections and CAP[84], however in macaques inoculated with wild-type MRSA strain USA300 versus PVL deletion-mutant strain, no difference in disease severity was seen[79]. Influenza A with MRSA co-infection also did not potentiate morbidity in this NHP model[85], suggesting that other host factors must be present to predispose healthy individuals to highly morbid secondary bacterial pneumonia.

NHPs have also been used to develop models of *Bacillus anthracis* pneumonia[86]. Baboons were intravenously infused with *B. anthracis* at $10^5$–$10^9$ CFU and then treated with i.v. levofloxacin daily starting at 2 hours post-inoculation. Infected animals displayed a dose-dependent increase in severity of ALI, DIC, systemic inflammation, and capillary leak (vascular permeability and pleural effusions) that closely mirrored the human disease. At the highest doses ($\geq 10^8$ CFU), mortality was 100%[86]. Similar findings were seen in baboons infused with *B. anthracis*-derived peptidoglycan, the predominant carbohydrate of the Gram-positive bacterial cell wall, indicating this molecule is a pathogen-associated molecular pattern (PAMP) likely responsible for the inflammation and hypercoagulability seen with clinical anthrax infection[87]. Other studies using rhesus or cynomolgus macaques administered aerosolized *B. anthracis* spores via nose- or head-only exposure chambers[88–90]. Animals developed a moribund, disseminated anthrax infection with sepsis and respiratory failure over several days[88,89]. The inhalational model mimics the likely mechanism of exposure to *B. anthracis* (airborne dispersion) in a possible terrorist attack and is therefore invaluable to researchers developing novel anthrax therapies for such a public health emergency[90,91].

## NHP models in sepsis therapy

The *E. coli* baboon bacteremia model has been tested for proof-of-mechanism studies to test inhibitors of the inflammatory and coagulation pathways[8,92]. Some of these inhibitors, such as antibodies to the cell surface glycoproteins E- and L-selectin, which mediate leukocyte migration[93], worsened organ dysfunction and survival and never progressed beyond animal studies[94]. Other inhibitors did improve sepsis survival and/or organ dysfunction, and NHP models have been instrumental in translating these preclinical studies into clinical trials. Table 1 shows a summary of the experimental therapies and their associated NHP models.

One of the earliest inhibitor trials targeted TNF-α. Studies from the 1980s showed that $LD_{100}$ baboons given monoclonal antibodies against TNF-α had preserved organ function and reduced mortality[95,96]. Subsequent studies showed the drug attenuated the coagulopathy[97] and reduced mortality in recurrent $LD_{10}$ *E. coli* bacteremia[98]. These positive preclinical studies eventually led to the advancement of afelimomab, a murine monoclonal antibody against TNF-α, into phase III trials[99,100]. Similarly, recombinant TFPI reduced mortality in $LD_{100}$ baboons[52] and moved on to phase II[101] and phase III trials[102] in humans. However, neither afelimomab nor recombinant TFPI improved outcomes in patients and were ultimately abandoned.

One mediator-targeted sepsis therapy advanced as far as FDA-approval—activated protein C (APC). APC inhibits factors V and VIII and enhances fibrinolysis, providing negative feedback to the coagulation pathway[103] (Fig. 1b). Levels of protein C, however, are depleted during septic shock[104]. Administration of APC to $LD_{100}$ baboons prevented coagulopathy, hepatic injury, and lethality[49]. Conversely, administration of an antibody to protein C in $LD_{10}$ ($10^7$–$10^8$ CFU/kg *E. coli*) baboons turned sub-lethal sepsis into lethal septic shock[49]. In contrast to the other coagulation inhibitors, APC attenuated the early stage mediators of the inflammatory and coagulation pathways, including TNF-α and fibrinogen[48]. These positive studies eventually led to the phase III clinical trial (PROWESS) of recombinant human APC (drotrecogin alfa) for the treatment of sepsis[105], which showed a significant reduction in mortality in the treatment group. Drotrecogin alfa was approved by the FDA as a first-in-class drug for the treatment of sepsis. Subsequent phase IV trials however failed to demonstrate benefit[106,107], and actually showed increased risk of major bleeding, eventually prompting the removal of drotrecogin alpha from the market.

Despite decades of research, there are currently no inflammation- or coagulation-targeted therapies approved for the treatment of sepsis. One reason for the discrepant findings between the animal and human studies is a lack of concordance between experimental design and clinical practice. For example, in baboons the TNF-α inhibitor was given prior to[97] or immediately after[96] *E. coli* infusion. Similarly, the recombinant TFPI was more efficacious if administered 30 minutes (rather than 4 hours) after *E. coli* infusion[52]. These animal studies do not reflect the real-world initiation of sepsis treatment, which typically occurs a day or more after onset of infection. The negative human trials also demonstrate the complexity and redundancy of molecular pathways wherein blockade of one mediator is counteracted by activation of another.

Another promising potential therapy for sepsis-induced ARDS that has been tested in baboons with *S. pneumoniae* pneumonia ($10^8$–$10^9$ CFU) is low-dose inhaled carbon monoxide (CO) gas. In mice, low-dose inhaled CO exerts anti-inflammatory and anti-apoptotic effects, reducing severity of sepsis-induced organ injury[108]. In *S. pneumoniae*-infected baboons, the pharmacokinetics and delivery of inhaled CO were described and preliminary evidence reported for reduction of ALI severity in CO-treated animals compared with historical controls[109]. This was linked to CO-mediated induction of mitochondrial biogenesis in alveolar type 2 cells and generation of specialized proresolving lipid mediators[110,111]. The pharmacokinetics data generated from this NHP study was used to support an FDA IND application, and a multicenter phase I clinical trial[112].

The macaque anthrax model has also been used to develop novel anthrax therapies. Cynomolgus macaques inoculated with nebulized *B. anthracis* experienced significantly improved survival after receiving the novel tetracycline class antibiotic TP-271[88]. Survival of inhalational anthrax in this primate species was also significantly improved by administering a post-exposure prophylaxis vaccine, AV7909[89]. The combination of both post-exposure treatments was tested in rhesus macaques where antibiotics (ciprofloxacin x 14 days)

and vaccine therapy (anthrax vaccine adsorbed, AVA) significantly improved post-exposure survival compared to antibiotics alone, likely due to delayed germination of anthrax spores occurring after completion of antibiotics leading to anthrax infection[90]. Additionally, a pre-exposure vaccine study in rhesus macaques using a conjugated anti-capsule vaccine showed robust immune response and improved survival of anthrax infection compared to either component alone[113].

## Limitations of NHP models

Given the nearly identical pathophysiology amongst primates, it would appear that NHP can readily replace other animal models of sepsis in biomedical research. The reality, however, is that the use of NHPs in research is still subject to limitations[23]. As highly intelligent animals, NHPs require much more stimulation and environmental enrichment than rodents, and must be housed in spacious containment facilities subject to rigorous monitoring regulations[23]. Additionally, the cost of one NHP experiment, when taking into account housing, personnel, and equipment costs is equivalent to that of hundreds of mice[23]. Significant resources and expertise are especially essential for NHP experiments involving intravenous infusions, phlebotomy, or vital sign measurements; these procedures generally require sedation, which when administered to septic animals can worsen cardiopulmonary instability (similar to patients). While such sepsis experiments have been reported for chimpanzees[40], baboons[70], and rhesus macaques[114], they have required provision of ICU-level support (e.g., mechanical ventilation, vasopressors, fluids). An alternative strategy involves inserting and tethering indwelling intravascular catheters or vital sign recording devices to a harness worn by the animal[19,115]. Such devices can continuously administer fluids or medications, and measure telemetry, heart rate, blood pressure, and body temperature[19,115]. The implantation procedure itself is still a surgery that requires technical expertise, equipment, sedation, and a recovery period, but obviates the need for and inherent risks of sedation while the animal is septic. There can be device complications, however, such as catheter-associated infections[116], which could confound sepsis experiments. Cost aside, NHPs require greater skill and expertise to handle than small mammals. They are several times stronger than humans per pound and can carry communicable diseases, such as tuberculosis and herpes B virus[23].

While NHPs share much of the same physiology and cell biology, the clinical course of sepsis in experimental models can nonetheless be different from that of patients. Because animals receive direct inoculation of pathogen, sepsis tends to be quicker onset with either rapid death or resolution. In the clinical arena, however, sepsis can be indolent and develop slowly as the nidus of infection grows. Furthermore, the outcomes of certain studies may be confounded when standard of care treatments are withheld, such as antibiotics or surgery[8]. And the timing of therapies administered to animals (either before or immediately after inoculation) does not reflect clinical practice when patients present several days after the onset of symptoms.

NHP models also suffer from the same limitations in experimental design as all other animal models: experiments are tightly controlled and frequently performed on a population of young, typically male animals with no comorbid diseases[8,24]. Baboons are generally three to eight years of age at the time of experimentation[71,109], which is equivalent to an adolescent human. These conditions differ greatly from clinical sepsis, which is a notoriously heterogeneous disease affecting older adults of both sexes with multiple comorbidities. Thus the homogeneity that characterizes high quality experimental design becomes one of the biggest hindrances to integrating the findings into the clinical area.

Finally, there are ethical considerations that must be considered when discussing NHP sepsis research. The 2006 Weatherall Report commissioned by medical groups in the United Kingdom concluded that the use of NHPs in medical research is justified, provided that 1) there are no alternative means (e.g. lower animals or *in vitro* work) to answer the biological question; 2) the NHP model is representative of the human disease; 3) there are adequate regulations in place to ensure the welfare of NHPs; and 4) the work significantly advances human health[117]. The report further qualifies that each use of NHPs for medical research should be adjudicated on a case-by-case basis[117]. This ethical justification is predicated on the utilitarian principle that suffering by a small number of NHPs may be acceptable if the research leads to substantial benefits to many humans[118]. Applications of this principle include using NHP studies to prevent a harmful drug from going to clinical trial, or conversely, redirecting resources towards drugs that show significant benefits in NHPs[119]. Risks associated with this calculus can be attenuated by minimizing the number of animals used, and be ensuring adequate attention to animal welfare[118]. The former can be addressed by changes to experimental design, such as use of "rolling controls" where the control group changes by one or two animals every experiment, or "before-and-after" studies where an animal serves as its own control. Strategies to address the latter including providing the animals with adequate food, water, space, enrichment, and social interactions[117]. The latter can also be addressed by using humane endpoints, or predetermined changes in animal physiology, rather than experimental endpoints, such as mortality. Furthermore, distinctions between different NHP species are likely relevant. For instance, the use of use great apes (chimpanzees, bonobos, gorillas, orangutans) in medical research was banned in the European Union in 2011 and phased out in the U.S. in 2013[119]. These ethical and policy issues are far from settled, and the justification for using monkeys or lesser apes (siamangs and gibbons) in medical research going forward must be continually reassessed. At the very least, the authors propose that their use should be limited to therapeutic studies (rather than mechanistic ones), which are more likely to directly impact human health.

## Conclusions and Future Directions

NHP models mimic human sepsis more faithfully than any other species because of anatomic, molecular, and physiological similarities. They have helped to deeply shape our current understanding of sepsis pathophysiology. These models have evolved from discovery of biochemical pathways to drug development. The *E. coli* bacteremia model has translated dozens of therapies targeting the inflammatory and coagulation pathways from rodent experiments to human clinical trials. However, despite their initial promise in preclinical NHP studies, no pharmacologic therapy has yet demonstrated benefit in humans. Their lack of success may not be due to failure of the animal model itself, but rather to the need to identify crucial targets and pathways in sepsis. Owing to their high cost and intelligence, the use of NHPs in sepsis research today is reserved exclusively for preclinical drug and therapy development, rather than for mechanistic studies in order that novel sepsis treatments can either move to clinical testing or be abandoned before the expense of a clinical trial.

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## Additional information



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