NOBLE LIFE SCIENCES

Non-Surgical Model of Sepsis & Inflammation

Introduction

The immune system is extraordinarily effective at eliminating small, local infections. However, when infection occurs systemically, such as in bacterial septicemia, a cascade of events is initiated in which potent mediators released by the immune system can endanger host survival. This whole body inflammatory state is referred to as sepsis. Sepsis can also occur in the absence of invading bacteria ("sterile sepsis"), for example, in cases of traumatic injury.

Sepsis is characterized by a system-wide dysregulation of the immune system referred to as Systemic Inflammatory Response Syndrome (SIRS). SIRS can eventually lead to Multi-Organ Dysfunction Syndrome (MODS) involving dysfunction of two or more major organs, hypoperfusion, or hypotension. Dysregulation of the coagulation system also can occur as a result of SIRS, leading to Disseminated Intravascular Coagulopathy (DIC).

SIRS is the leading cause of death in non-coronary intensive care units (ICUs) and the 10th leading cause of death in the United States. The incidence of severe sepsis in the United States is on the order of 700,000 cases per year and has been increasing in recent years. Mortality from MODS varies from 30% to 100% depending on the number of organs involved and this rate has not changed in the past 30 years. Therefore, a critical need exists to develop new diagnostics and therapeutics for sepsis/SIRS. Preclinical testing of new therapeutics requires suitable animal models. The gold standard has been the cecal ligation and puncture model, which is tedious and time consuming. For screening of new drugs, a model with a higher throughput is needed.

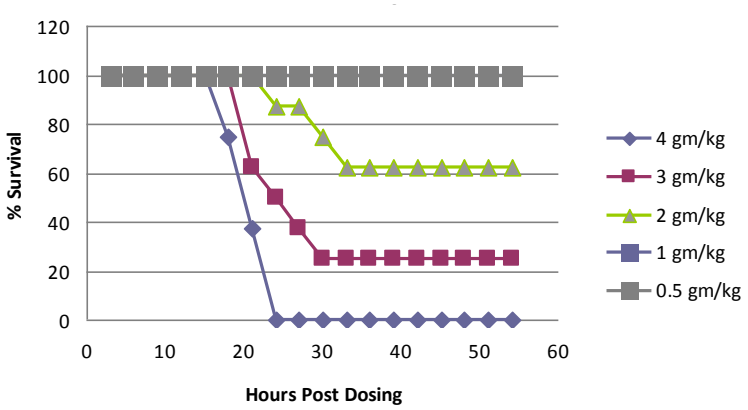
Non-Surgical Model of Sepsis and Inflammation

Noble Life Sciences has developed a robust and reproducible preclinical model of poly-microbial sepsis to facilitate drug screening. In this model, a cecal slurry is used to induce septic peritonitis resulting in systemic bacteremia, organ infection and eventual systemic release of cytokines. Because it is poly-microbial, involving both gram negative and gram positive bacteria, a wide range of pattern recognition receptors such as Toll-Like Receptors are activated on a variety of immune cells, mimicking sepsis. The model is highly reproducible, easy to monitor by a variety of endpoints and closely mimics clinical settings of abdominal sepsis in small animals. By titrating the amount of cecal slurry used, the severity of the septic response can be adjusted to the needs of the experimenter.

Because the inflammatory response and MODS characteristic of SIRS is similar whether induced by inflammation or by trauma, the model is useful for diagnostic and therapeutic studies of sepsis induced by pathogens, sterile sepsis or by trauma. If preferred, the model can be run using a modified Schwartzman reaction where a priming dose of lipopolysaccharide is given to the mice to sensitize them prior to administration of the cecal slurry, providing a useful DIC model in the context of SIRS.

Sample Protocol & Results

A cecal slurry was prepared as follows: 150 C57/BL6 mice were euthanized and their ceca removed. Cecal contents were extracted, diluted with cold 5% dextrose, passed through an 18-gauge needle to break up solids, and stored in aliquots at -80°C. For injection, the cecal slurry was thawed rapidly at 37°C and then equilibrated to room temperature. Groups of 8 mice each received intraperitoneal (IP) injections at doses ranging from 0.5 gm/kg to 4 gm/kg. Animals were monitored every hour and deaths recorded. The results are depicted in the figure at right. Survival was dose dependent.



Applications & Endpoints

Noble's Non-Surgical Model of Sepsis and Inflammation can be used in a variety of different types of studies including development of antibiotics, antithrombotics, immune modifiers, and diagnostics with a variety of endpoints.

Cytokines	The effects of therapeutics on the time course of induction of a wide variety of cytokines and chemokines can be studied.
Disseminated Intravascular Coagulopathy	DIC can be monitored by following Fibrinogen Degradation Products (FgnDP) using an ELISA assay with a goat anti-mouse fibrinogen antibody conjugated to horseradish peroxidase.
White cell and Platelet levels	Change in levels and compositions of white cells and platelets can be monitored during the progression of symptoms.
Bacteremia	Blood and organs can be harvested and the numbers of bacteria quantitated.
Survival	The effects of therapeutic interventions on survival can be monitored. Mice in extremis are humanely euthanized.

Advantages

This Noble Life Sciences model provides a convenient, effective animal model for compound screening. The model is less cumbersome than cecal ligation and puncture; surgery is not required, the dose can be titrated and endpoints can be studied either in a non-lethal setting or by measuring survival.

Poly-microbial	Unlike LPS models, Noble's Inflammation/Sepsis Model is a polymicrobial infection model that activates multiple Pattern Recognition Receptors.
Non-surgical	Unlike cecal ligation and puncture, Noble's Inflammation/Sepsis Model does not require surgery resulting in a highly reproducible response.
Use for Screening	Noble's Inflammation/Sepsis Model can be used to screen a large number of mice since it doesn't require surgery enabling large comparative studies to be undertaken.
Titratable	Noble's Inflammation/Sepsis Model is titratable and can be used to look at inflammatory mediators in a non-lethal mode or to look at survival after polymicrobial sepsis.
Coagulation	Noble's Inflammation/Sepsis Model can be used to look at disseminated intravascular coagulation by priming with LPS.