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SELECTIVE REMOVAL OF INDUCIBLE NITRIC OXIDE SYNTHASE FROM PLASMA AS A THERAPY FOR SEPSIS, SEVERE SEPSIS AND SEPTIC SHOCK

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Purpose: The purpose of this research was to test our hypotheses (1) that the release of the intracellular protein inducible nitric oxide synthase (iNOS) into the circulation was partially responsible for the pathophysiology of sepsis and (2) that the selective removal of iNOS from the circulation might improve the outcome of septic patients. These hypotheses were based upon the findings made during our clinical trial for a new *in vitro* diagnostic test in which more than 1200 plasma samples obtained from ICU patients with sepsis or severe sepsis or at risk for developing sepsis were analyzed. Our analyses led to the discovery that iNOS was present only in the plasma of patients with sepsis and those who became septic within the next 48 hours, and iNOS was not found in normal controls or in non-septic ICU patients, such as trauma patients.

Methods: After developing and characterizing a panel of monoclonal antibodies (MAbs) specific for iNOS, the MAbs were used to develop very sensitive immunoassays that could detect and measure iNOS in plasma. In order to test our hypotheses, a mouse model of sepsis was developed that utilizes lipopolysaccharide (LPS) and iNOS isolated from induced DLD-1 cells to induce sepsis and that tests the ability of anti-iNOS MAbs to rescue the challenged mice from death by sepsis. Three chimeric mouse/human anti-iNOS MAbs were genetically engineered by incorporating a mouse anti-iNOS MAb binding domain into a human IgG1 backbone, and were tested for their ability to remove selectively iNOS from plasma when attached to an affinity support. These same anti-iNOS MAbs were also tested for their ability to neutralize *in vivo* the lethal effects of plasma iNOS.

Results: The chimeric mouse/human anti-iNOS MAbs were tested for their ability to protect challenged mice by physically removing iNOS from plasma with an anti-iNOS MAb loaded onto an affinity matrix. The physical removal of iNOS by treatment with an anti-iNOS MAb attached to resin was found to rescue challenged mice from death by sepsis, and the physical removal of iNOS was the more effective treatment as compared to the *in vivo* neutralization of iNOS. Up to 80% of the challenged mice that would otherwise have died from sepsis were rescued from death by the physical removal of iNOS using anti-iNOS MAbs bound to a solid support.

Conclusions: We concluded from these experiments (1) that plasma iNOS does contribute to the pathophysiology of sepsis, (2) that the physical removal of iNOS with the chimeric mouse/human anti-iNOS MAbs is protective and rescues the challenged mice from death by sepsis, and (3) that the physical removal of iNOS was the more effective treatment as compared to the *in vivo* neutralization of iNOS in plasma. Treatment with immobilized anti-iNOS MAb bonded to a solid support may prove to be an effective therapy for patients with sepsis, severe sepsis and septic shock.