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13. ABSTRACT (Maximum 200 words)

Biological warfare agents cause lethality by activating systemic inflammation. After infection, cells of the innate immune system, including macrophages release soluble factors like TNF, HMGB1 in addition to other proinflammatory cytokines that cause shock and widespread tissue injury. We discovered that high levels of HMGB1 are produced in humans and animals with infection. We have developed and tested monoclonal antibodies that specifically neutralize HMGB1 bioactivity in vitro. With DARPA support we have discovered that inhibition of HMGB1 activity attenuates lethality in live infection models. In addition to HMGB1, we have extended our studies in understanding the role of an acetylcholine receptor subunit, known as alpha-7. Alpha-7 receptor agonists like acetylcholine and nicotine, inhibit HMGB1 release by macrophages, thus controlling the innate immune response. We have proved the effectiveness of anti-inflammatory agents, designed to activate the alpha-7 subunit and modulate the lethal immune response. Our studies have accomplished many of the project's goals by developing medical countermeasures that target either HMGB1 or alpha-7, to protect against biological warfare agents.

14. SUBJECT TERMS
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Final Progress Report

Statement of the problem studied

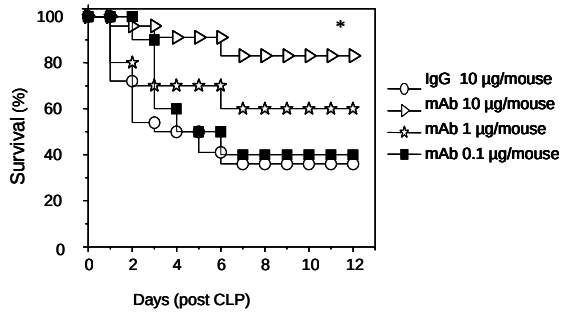
Biological warfare agents cause lethality by activating systemic inflammation. After infection, cells of the innate immune system, including macrophages release soluble factors like TNF, HMGB1 in addition to other proinflammatory cytokines that cause shock and widespread tissue injury. We originally identified a cytokine role for HMGB1, a protein known previously as a transcription factor, and have since focused our efforts on studying the role of this mediator in the pathogenesis of severe sepsis. We discovered that high levels of HMGB1 are produced in humans and animals with sepsis. Administration of HMGB1 to experimental animals causes lethal organ damage. We have developed and tested monoclonal antibodies that specifically neutralize HMGB1 bioactivity in vitro. With DARPA support we have discovered that inhibition of HMGB1 activity attenuates lethality in live animals with infection.

In addition to HMGB1, we have extended our studies in understanding the role of an acetylcholine receptor subunit, known as alpha-7. Alpha-7 receptor is expressed on the macrophages and plays an important role in the innate immune system by controlling activation of macrophages. Alpha-7 receptor agonists like acetylcholine and nicotine, inhibit HMGB1 release by macrophages, thus controlling the innate immune response. With DARPA support, we have proved the effectiveness of anti-inflammatory agents designed to activate the alpha-7 subunit and modulate the lethal immune response.

Summary of the most important results

Anti-HMGB1 antibody protects against lethality in established murine sepsis. To determine whether inhibiting HMGB1 attenuates the lethality of sepsis, we subjected mice to severe sepsis using cecal ligation and puncture (CLP) technique. CLP is a widely used method for inducing sepsis conditions in experimental animals.

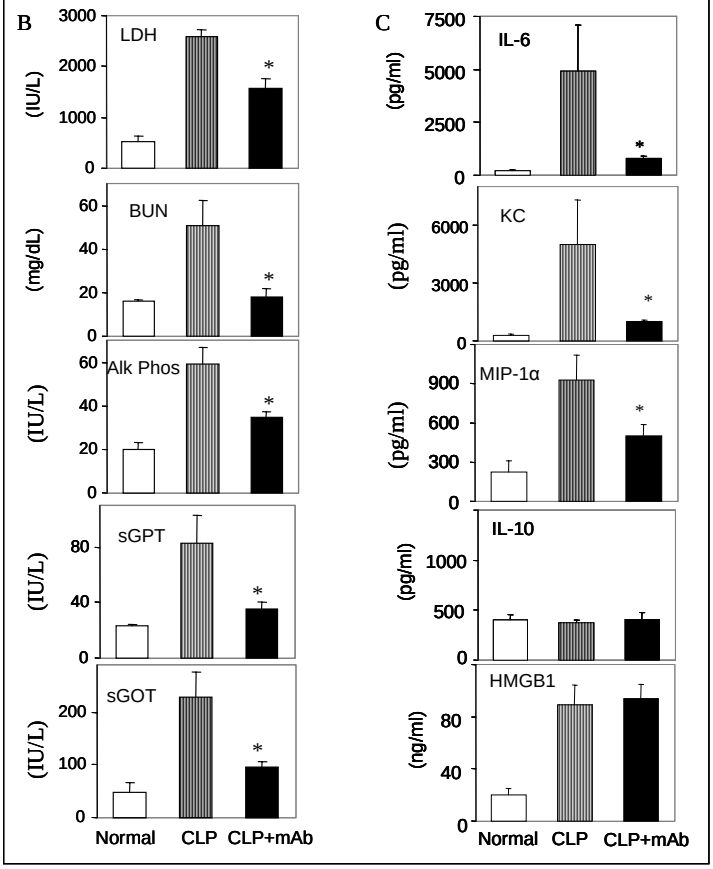
Figure 1 . Anti-HMGB1 antibody improves survival in sepsis



Administration of neutralizing anti-HMGB1 antibodies to animals with severe sepsis was protective, converting lethality rates from 70% to 20% (Figure 1). Interestingly, passive immunization with a single dose of anti-HMGB1 antibodies was protective even when the treatment was started 24hrs after the onset of sepsis, a time frame that is consistent with HMGB1 release kinetics. Dose response and kinetic experiments revealed that the protective effect of the neutralizing antibody is dose-dependent.

Anti-HMGB1 antibodies reduce serum levels of enzymes and cytokines. Onset of sepsis leads to increased serum levels of enzymes and cytokines. As shown in Figure 2, serum levels of lactate dehydrogenase (LDH), blood urea nitrogen (BUN), alkaline phosphatase, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminae (SGOT) were all elevated in severe sepsis. Treatment with neutralizing anti-HMGB1 antibody significantly reduced the serum levels of these enzymes, indicating that the treatment reduces tissue injury. Anti-HMGB1 antibody also significantly reduced serum levels of inflammatory cytokines and chemokines including IL-6, KC and MIP-1α. Together, these experimental data suggest that targeting HMGB1 activity inhibits systemic inflammatory responses and improves survival in live infection models.

Figure 2. Anti-HMGB1 antibody reduces serum enzymes and cytokines

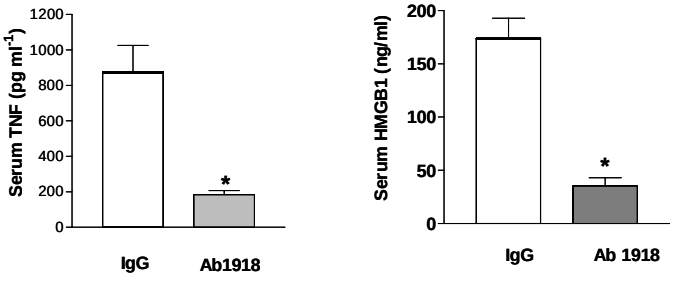


Alpha-7 agonists are protective in experimental models of sepsis. We have synthesized a lead alpha-7 agonist CAP 201-0068 that inhibits HMGB1 release by macrophages in vitro. To determine if activation of acetylcholine receptor subunit, alpha-7, improves survival, we subjected mice to severe sepsis either by CLP or by lethal endotoxin challenge. Administration of alpha-7 agonists, to mice with established severe sepsis, improves survival significantly, converting

lethality rates from 80% to 10%. A dose-dependent protective effect of Alpha-7 agonist was observed indicating the specificity of the agonist effect.

Anti-alpha-7 antibody protects against lethality in established murine sepsis. We have developed agonist antibodies against the alpha-7 receptor, in addition to chemically synthesized reagents. Our lead anti-alpha-7 antibody Ab1918, inhibits TNF and HMGB1 release by macrophages in vitro. In addition, administration of the antibody to animals with established sepsis resulted in decrease of serum TNF and HMGB1 levels (Figure 3) and improved survival significantly (Figure 4).

Figure 3. Treatment with anti-alpha-7 antibody decreases serum TNF and HMGB1 levels in experimental sepsis.



Taken together, these studies strongly suggest that reagents that will target either HMGB1 or alpha-7, leading to attenuation of the lethal responses will be effective as countermeasures to biological warfare.

Studies of anti-HMGB1 antibody treatment in Yersinia pestis infection. The next critical step in the development of anti-HMGB1 and CAP 201-0068/ anti-alpha-7 as countermeasures to biological warfare is to test effectiveness in animal models of such threat agents. To begin to understand the efficacy of these treatments, we conducted studies in the animal model of plague (*Yersinia pestis*) in collaboration with Dr. Rick Lyons (University of New Mexico). Mice were challenged with *Yersinia pestis* infection and were treated with anti-HMGB1 antibody 24 hr post-infection. In our preliminary studies, all the animals appeared very sick and treatment with anti-HMGB1 antibody did not improve survival. These studies are still inconclusive as there are several factors that need to be addressed in detail, such as time of antibody administration and dose of antibody administration. However, it should be possible to address these queries in the future.

Figure 4. Anti-Alpha-7 antibody improves survival in sepsis.