Self-defense against *Bacillus anthracis* toxins: Is P-selectin the key?

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Anthrax infections result from exposure to spores of the gram positive bacterium *Bacillus anthracis*. Anthrax spores are hardy and can persist in soil for years, posing an ongoing risk of infection to grazing animals. Natural infections in humans usually result from contact with infected animals or animal-derived products and subsequent spore acquisition through skin abrasions, ingestion, or inhalation. Cutaneous anthrax infections are the most common, but are still relatively rare and usually respond to medical care, while less common inhalational anthrax is the most frequently life-threatening. Prophylactic vaccination is available but is only approved in the US for groups at high risk of exposure, such as veterinarians, laboratory staff working with anthrax, and some members of the military. The US vaccine, anthrax vaccine absorbed (AVA; BioThrax®) consists of anthrax protective antigen (PA) adsorbed to aluminum hydroxide and is administered in 5 doses over 18 months with yearly boosters. Data from animal studies show that induction of toxin neutralizing antibodies to PA is the principal immune correlate of protection against anthrax.

However, it is likely that sufficient protective antibody responses will take at least 4 weeks to develop and patients will be at risk of disease in the interim given a median incubation period of 4 days between exposure and signs of symptoms. Therefore, administration of antimicrobials (ciprofloxacin or doxycycline) is recommended as complementary PEP to help prevent onset of disease following germination of spores to vegetative bacilli in the body (antimicrobials are inactive against non-germinated spores). Continuation on antimicrobials for 60 days is considered necessary as dormant spores can persist in the lungs for several weeks. While this PEP approach appears to have been effective in the aftermath of the 2001 anthrax exposure incident, a follow up study of the individuals who were prescribed antimicrobials revealed only 44% overall compliance with the complete 60 day antimicrobials regimen, raising the possibility that inadequate compliance may risk development of infections in potential future exposure incidents. Even with 100% patient regimen compliance and significant suppression of bacterial replication, cellular uptake and activity of anthrax toxins is not affected by antimicrobials.

Antibodies to heat, desiccation, and chemical disinfectants and the potential for widespread infections resulting from deliberate public exposure to spores has led to the employment of anthrax as a biological weapon. Most recently, in 2001, anthrax spores sent through the US postal service to recipients on the eastern seaboard were responsible for a total of 22 confirmed or suspected cases of cutaneous and inhalational anthrax, with 5 deaths due to inhalational infections. This incident and the potential for future bioterror attacks highlight the need for a comprehensive set of post-exposure prophylaxis (PEP) options. In cases of suspected exposure, the AVA vaccine can be used in the US under an Investigational New Drug protocol or Emergency Use Authorization in a 3 dose regimen over 1 month. But
PA binding to cell receptors, have been licensed by the US FDA in 2012 and 2016, respectively, for prevention and treatment of anthrax infections. With post-exposure vaccination, antimicrobials, and antitoxin antibodies, we now have a potent 3-pronged approach for anthrax PEP. However, to a large extent, PEP efficacy will be determined by how early treatment begins after exposure. When treatment is not initiated until after onset of significant symptoms, irreversible tissue injury may be too far developed for the patient to successfully respond to treatment. In this context, further knowledge of innate and natural defense mechanisms mounted by the body in response to established anthrax infections is urgently needed.

In this issue of Virulence, Sun and colleagues reveal a possible role for soluble P-selectin (sP-sel) in mitigating the pro-hemorrhagic effects of anthrax LT. The cell adhesion molecule P-selectin is expressed on surfaces of activated platelets and endothelial cells and is known to play a role in blood coagulation and thrombosis. Platelet P-sel, through interaction with its receptor PSGL-1, recruits tissue factor positive microparticles to developing thrombi where tissue factor initiates the coagulation cascade, leading to fibrin generation. sP-sel also has pro-coagulant effects through increasing numbers of circulating tissue factor expressing microparticles.

In the present work, Sun and colleagues used a model of anthrax pathology in which mice intravenously injected with LT develop key features of anthrax pathology such as hemorrhage and thrombocytopenia. Interestingly, despite this evidence of LT-induced impairment of coagulation, LT administration also increased levels of sP-sel in mouse plasma and increased circulating microparticles, the latter effect absent in P-sel or PSGL-1 knockout mice. A possible therapeutic effect for this LT-induced sP-sel was suggested by elevated mortality following LT challenge in either the P-sel or PSGL-1 deficient mice. To further investigate, the authors treated mice with P-sel-Fc prior to LT administration. P-sel-Fc treatment improved plasma clotting time in LT-administered mice, increased tissue factor positive microparticles in both control mice and mice administered LT, but not PSGL-1 deficient mice, and reduced other LT-induced pathology readouts. The pro-hemostatic effects of P-sel-Fc were, importantly, associated with significantly reduced mortality when mice were given a single P-sel-Fc treatment 4 hours before challenge with a lethal dose of LT or anthrax spores, with 83% and 23% survival, respectively. How LT induces elevated plasma sP-sel levels in this model remains to be determined.

Therapies mitigating anthrax-induced hemostatic and hemodynamic abnormalities would be an asset in anthrax PEP and a P-sel based approach could be attractive prospect in this respect. A similar sP-sel therapeutic approach has been suggested for hemophilia, for example, based on analogous findings in a mouse hemophilia model. However, a note of caution is in order as high levels of P-sel can lead to hypercoagulation disorders. Accordingly, the anti-P-sel antibody Inclacumab is currently in human testing for atherosclerotic cardiovascular diseases. Regarding potential sP-sel therapy for anthrax, additional studies would be informative. Given the findings of partial P-sel-Fc-mediated protection in anthrax spore challenged mice, it would be interesting to investigate the role of P-sel in non-human primates as the clinical course of anthrax infection and pathogenesis in these animals appears most relevant to humans. Whether sP-sel levels are elevated in the sera of anthrax patients is unknown, but it is an interesting question in light of a recent report that anthrax LT and ET do not directly inhibit human platelet function in vitro, a finding contradictory to previous animal model studies. In any case, the high human fatality rate for inhalational anthrax, up to 85–90%, suggests that natural elevation in sP-sel levels during infection is unlikely to be sufficient, in itself, as a protective mechanism. However, it is conceivable that this putative sP-sel self-rescue response may combine with other elements of the host’s innate responses to provide partial protection. For example, anthrax-infected macrophages have been reported to secrete adenosine triphosphate which is detected by other macrophages and induces IL-1β production and resistance to toxin-induced cell death. These and, possibly other innate and pathogenesis-mitigating responses may act together to effectively buy extra time, thus allowing vaccine, antimicrobial and anti-toxin PEP therapies to take effect.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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