Production and distribution of Exotoxin A in a dermal wound infected by

*Pseudomonas aeruginosa*

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Introduction

*Pseudomonas aeruginosa* is an opportunistic pathogen of animals and humans, particularly those with compromised immunity such as organ transplant recipients, cystic fibrosis patients, people with AIDS and the severely burned. Clinical strains often carry genes for multiple virulence factors. Collectively, these virulence factors assist the bacterium to attach, colonise and penetrate host tissue and destroy host cells. Exotoxin A (ETA) is a virulence factor that is expressed by approximately 80% of human clinical isolates. Systemic ETA kills cells in susceptible organs, including the liver and kidney, which can result in the death of the patient. Using a murine full thickness skin chamber model we investigated local production and systemic distribution of ETA in a dermal wound of infection.

Aims

To detect & measure ETA levels in wound exudate & distal organs of neutropenic & normal mice after a dermal wound was colonised by *P. aeruginosa*.

Material & Methods

All mice were implanted with the full thickness skin chamber that created a standardised full thickness wound through the dermis to the muscle fascia. The chamber provided protection from environmental contaminants whilst it contained wound fluid (exudate) and the injected bacteria. The screw cap allowed for repeat sampling from the wound. The mice were divided into two groups; one group received 200mg/kg cyclophosphamides to induce neutropenia. Each group received varying doses of an ETA-human clinical strain of *P. aeruginosa* delivered to the chamber. Blood, lung, spleen, liver & kidneys were harvested and homogenised. Bacteria were quantified by standard serial dilutions and colony counts on nutrient agar plates. ETA was quantified using a sandwich ELISA and standard curve developed for this purpose.

Results

Normal mice appeared unaffected by the wound infection. In contrast, neutropenic mice quickly became moribund necessitating euthanasia within 3-4 days.

*P. aeruginosa* in the wound exudate

The number of bacteria in the wound chamber exudate of both neutropenic mice and normal mice achieved a similar maximum level two to three days after inoculation and remained static independent of the initial inoculum concentration (Figure 3).

Exotoxin A in the wound exudate

The ETA concentration in wounds of neutropenic mice was approximately ten fold higher than levels achieved in the wounds of normal mice (Figure 2).

Systemic Exotoxin A and *P. aeruginosa*

No ETA or viable bacteria were detectable in the blood or peripheral organs of normal mice. However, in neutropenic mice viable bacteria were recovered from 93% (14/15) of spleen, and 33% (5/15) of blood samples. In all cases ETA was detected in the blood, or at least one organ, where viable bacteria were recovered from the spleen. Low levels (1-5ng/ml) of ETA was detected in 40% (6/15) of blood samples. ETA was most frequently detected in the liver (93%), and the highest concentration was found in the lung (566ng/g) (Table 1).

Conclusion

Bacterial levels in the wound chamber exudate were found to reach a maximum level independent of the number of bacteria in the inoculum and neutropenic state of the mice. In contrast, neutropenic mice had a ten-fold increase in ETA concentration in the wound chamber compared to normal mice. No systemic bacteria or ETA was detected in normal mice. However, neutropenia permitted bacteria to enter the circulation resulting in measurable ETA in the blood, or at least one susceptible organ, in all cases. These data suggest that ETA does not diffuse from the wound independent of bacteria entering the circulation; rather systemic toxicity is the result of ETA being generated by *P. aeruginosa* spreading systemically in immune-compromised patients.

References


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