**Pseudomonas aeruginosa; progression from dermal infection to septicaemia.**

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**Introduction**

Pseudomonas aeruginosa infection of a dermal wound can cause life threatening septicaemia, especially in humans and animals with compromised immunity (eg AIDS, organ recipients, burns patients). Most clinical strains produce multiple virulence factors (VF) to facilitate the colonisation, destruction and penetration of the host. ETA and elastase digest collagen, elastin and disrupt cell-to-cell junctions, and we are investigating how *P. aeruginosa* utilises these two of its significant virulence factors to penetrate the circulatory system of the host.

**Previous results**

In previous experiments we have found:

- The level of ETA in wound exudates are about 8 X times higher in mice treated with cyclophosphamide (CP) compared to normal mice (Figure 1), even though both groups had the same bacterial load in the chamber.
- CP reduced the concentration of leukocytes in wound exudates (Figure 2). These mice, with reduced leukocytes and increased ETA levels, developed septicaemia.

**Hypothesis & aims**

Septicaemia is due to the increased levels of VFs in the wound exudate, particularly ETA & elastase, and this makes the tissue in the wound more permeable to penetration by *P. aeruginosa*. Our aims are to compare the levels of elastase in normal and CP (immunosuppressed) treated mice, and to investigate the effect of purified *P. aeruginosa* elastase on the permeability of host tissue in a dermal wound using a fluorescently labelled 40 KDa marker.

**Materials & Methods**

**Purification of elastase**

*Pseudomonas aeruginosa* elastase was purified from a culture of strain PABO. The culture volume was reduced, buffer exchanged in Sephadex G-50, loaded on a DEAE sepharose column and eluted with increasing NaCl concentration. Elastase fractions were identified using the elastin congo red assay (Figure 3). The enzymatic activity of the purified product was quantified using the DQ elastin assay (Invitrogen).

**Mouse experiments**

Mice were implanted with a full thickness skin chamber on day 0. (Figure 4). On day 1 and day 6, mouse skin chambers were either given 1 unit of elastase in phosphate buffered saline (PBS) or PBS. Following, an overnight in vivo incubation, the chambers were evacuated by pipette and 24g of a 40KDa fluorescently labelled dextran (Invitrogen) was aliquoted to each skin chamber. 4d samples were taken from each skin chamber over the following 6 hrs, diluted in PBS and the fluorescence was quantified using a fluorimeter.

**Results**

- The concentration of ETA & elastase within skin chambers of CP treated mice was about six to eight times higher than in skin chambers of normal mice infected with similar colony forming units of *P. aeruginosa* (Figure 1 & 5).
- The CP treatment reduced the leukocyte concentration, and an increase in VF concentration in the chamber was observed (Figure 2). It is likely that the increased VF concentration was caused by the reduced number of leukocytes in the chamber.
- Elastase treatment resulted in vascular permeability indicated by a decrease in dextran marker concentration (Figure 6), an increase in exudate volume in the chamber (Figure 7), and increased the influx of RBCs.
- Collectively, these results support our hypothesis that the increased concentration of VFs is responsible for the progression of *P. aeruginosa* from dermal infection to septicaemia.

**Conclusions**

- The concentration of ETA & elastase within skin chambers of CP treated mice was about six to eight times higher than in skin chambers of normal mice infected with similar colony forming units of *P. aeruginosa* (Figure 1 & 5).
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**References**


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