

## ***In vitro* and *in vivo* determination of anti-TNF $\alpha$ activity in canine plasma from donors subject to preconditioning with endotoxin**

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**Background** Septic shock is characterized by cardiovascular and vasomotor failure that is induced by an uncontrolled cascade of inflammatory mediators such as TNF $\alpha$ , IL-1 $\beta$  and IL-6. In dogs, systemic bacterial infections, haemorrhage, trauma, gastric dilatation/volvulus and pancreatitis are the major causes of septic shock. While endotoxin is a recognized effector molecule that can initiate an inflammatory cascade, it has been reported that preconditioning with endotoxin can downregulate inflammatory cytokine responses to subsequent endotoxin challenge. This study reports the effect of endotoxin preconditioning on anti-TNF $\alpha$  activity present in plasma from canine donors.

**Materials and methods** Plasma from preconditioned (Caniplas®) and normal dogs (FFP) was provided blind to the study by a commercial supplier (Plasvacc Pty Ltd). *In vitro* anti-TNF $\alpha$  activity in canine donor plasma was determined by a L929 murine cell TNF $\alpha$  inhibition bioassay using recombinant murine TNF $\alpha$ . *In vivo* effects were tested by a rat subcutaneous skin pouch model. Rats were pretreated for 3 days with either Caniplas®, FFP, saline (2 ml/day, s.c.) or carprofen (5 mg/kg, s.c.) and inflammation was induced by injecting monosodium urate crystals into the pouch (5 mg/ml in 5 ml saline). Fluid was taken from pouches at specified intervals for cell count, TNF $\alpha$  and IL-6 analysis. Data analysis: normalized data were fitted to a four-parameter logistic curve. The fitted midpoints were compared statistically for datasets using an F-test and calculated fitted hill slopes.

**Results** In the rat skin pouch model, both Caniplas® and FFP reduced TNF $\alpha$  levels and Caniplas® was a more potent antagonist. The heightened anti-TNF $\alpha$  activity of Caniplas® compared with FFP was confirmed in the *in vitro* cell bioassay (Figure 1 - over). Neither Caniplas® nor FFP reduced inflammatory cell infiltration or the levels of IL-6.

**Conclusion** While we need to confirm the mechanism, we report that preconditioning with endotoxin does illicit specific anti-TNF $\alpha$  activity and that this observation has been confirmed in both *in vitro* testing and *in vivo* animal models. It is plausible that preconditioning animals with endotoxin induces an increase in the concentration of soluble TNF $\alpha$  receptors I and II in donor plasma, and that this is the probable source of TNF $\alpha$  antagonism. This report suggests that preconditioned plasma may be a beneficial treatment where inflammation causes increased expression of TNF $\alpha$ .

Figure 1

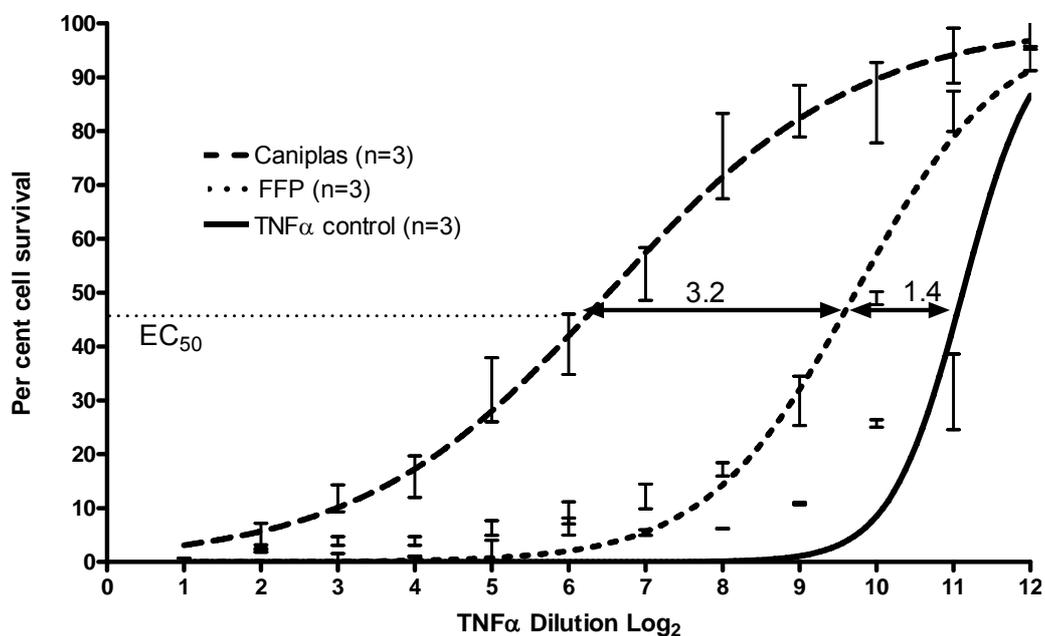


Figure 1 illustrates the difference in anti-TNF $\alpha$  activity between sera derived from pre-conditioned canine donors and untreated canine donors using an *in vitro* L929 cell bioassay. Different sera were mixed and incubated with serial dilutions (Log<sub>2</sub>) of recombinant mouse TNF $\alpha$  and then added to L929 cells. L929 cells are sensitive to TNF $\alpha$  and cell survival can be measured using crystal violet and absorbance at 630nm. Results are expressed as % cell survival versus dilution of mouse TNF $\alpha$  Log<sub>2</sub>. The difference in anti-TNF $\alpha$  activity between pre-conditioned and untreated sera at cell survival 50% (EC<sub>50</sub>) was significant ( $p < 0.0001$ ) using the F-test.

The electronic version of this abstract can be found at:

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