**BACKGROUND**

Septic shock is characterized by cardiovascular and vasomotor failure that is induced by an uncontrolled cascade of inflammatory mediators such as TNFα, IL-1β and IL-6. In dogs, systemic bacterial infections, haemorrhage, trauma, gastric distention/vomiting and pancreatitis are the major causes of septic shock. Whilst endotoxin is a recognized atherogenic molecule that can initiate an inflammatory cascade, it has been reported that preconditioning with endotoxin can down-regulate inflammatory cytokine responses to subsequent endotoxin challenge. This study reports the effect of endotoxin preconditioning on anti TNFα activity present in plasma from canine donors.

**MATERIALS**

Plasma from preconditioned (Caniplas®) and normal dogs (FFP) was provided blind to the study by a commercial supplier (Plasvacc Pty Ltd).

**METHODS**

*In vitro* anti TNFα activity in canine donor plasma was determined by a L929 murine cell TNFα inhibition bioassay using recombinant murine TNFα. *In vivo* effects were tested by a rat subcutaneous skin pouch model. Rats were pre-treated for 3 days with either Caniplas®, FFP native plasma (FFP), or recombinant (sTNFR1, α or μ) and inflammation induced by injecting monosodium urate crystals into the pouch (μg/ml in 1 ml saline). Fluid was taken from pouches at specified intervals for cell count. TNFα and IL-6 levels were determined by ELISA. Protein profiles of Caniplas® and FFP were determined by standard SDS-PAGE analysis. Examination of serum for soluble TNFα receptor 1 (sTNFR1) was performed by an immunofluorescence assay using a rabbit polyclonal anti-sTNFR1 antibody and a FITC conjugated goat anti rabbit antibody as the detection fluorochrome. Data analysis: Normalized cell survival % of maximal response (EC50) and 95% confidence intervals (CI).

**RESULTS**

In the rat skin pouch model, both Caniplas® and FFP reduced TNFα levels and Caniplas® was a more potent antagonist (data not shown). The heightened anti TNFα activity of Caniplas® compared to FFP was confirmed in the *in vitro* cell bioassay (Figure 1). Neither Caniplas® nor FFP reduced inflammatory cell infiltration or levels of IL-6. There was also possible evidence that the effector mechanism in Caniplas® may be increased levels of soluble TNFα receptor 1 (Figure 2). A difference in the protein profile between Caniplas® and FFP by SDS-PAGE analysis (Figure 3) was detected, although the nature and significance of this difference remains to be determined.

**CONCLUSION**

Whilst we remain to confirm the mechanism, we report that preconditioning with endotoxin does illicit specific anti TNFα 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 TNFα receptors I and II in donor plasma and that this is the likely source of TNFα antagonism. This report suggests that preconditioned plasma may be a beneficial treatment where inflammation causes increased expression of TNFα.