In vitro effect of canine hyperimmune sera on TNFa activity

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INTRODUCTION

Septic shock in dogs is caused by cardiovascular and vasomotor failure associated with an uncontrolled intrinsic release of inflammatory mediators [1–5]. The syndrome is characterized by cardiovascular dysfunction, vascular permeability alterations, pulmonary oedema and tissue hypoxia resulting from microthrombi which may culminate in disseminated intravascular coagulation and catastrophic multiple organ failure [6,7]. Systemic bacterial infection, particularly by Gram-negative enterobacteria, haemorrhagic trauma, gastric dilation/volvulus and pancreatitis are the major underlying causes leading to sepsis [8,9]. Because of haemodynamic instability and associated hypovolemia, fluid replacement therapy is generally applied to restore effective circulating volume. The use of fresh frozen plasma has been recommended in cases of coagulopathies as it has been recognized to assist restoration of haemodynamic stability [1,5,10,11]. There is increasing evidence that the drivers of the haemodynamic instability are inflammatory mediators (particularly TNFa) activated primarily by bacterial endotoxin [3,4,12,13].

MATERIALS AND METHODS

In this pilot study we examined the effect sera from a proprietary hyperimmune plasma product (Caniplas) on the activity of TNFain an in vitro L929 cell bioassay [12,14]. Two separate accessions of sera were provided. Initially, one hyperimmune preparation and one untreated serum sample were tested. On a separate occasion, three hyperimmune sera and one untreated sample were tested. All sera were supplied blind to the study. The effect of each serum preparation was tested against a dilution series of recombinant murine TNFa in a L929 cell bioassay[12,14]. The starting concentration of TNFa differed between the two accessions.

RESULTS

Figs. 1 (first accession) and 2 (second accession) illustrate that all sera tested exhibited antiTNFa activity in the in vitro bioassay.
Secondly, it appears that hyperimmune plasma may have enhanced activity over untreated sera.

DISCUSSION

In earlier work (unpublished data) we noted that equine hyperimmune and normal sera appeared to exhibit antiTNFa activity in an in vitro L929 cell bioassay. This pilot study was undertaken to determine if a similar effect could be found in canine sera.
There appears to be intrinsic antiTNFa activity by untreated canine serum, but this activity appears to be enhanced during the endotoxin vaccination regimen employed in preparing hyperimmune plasma. TNFa is rapidly degraded at the cellular level by neutrophil derived proteases [15] and there is increasing evidence that the cytokine is also modulated in the circulatory system by solubilized TNFa receptors [16]. Whilst this report is clearly preliminary and needs further confirmation, it does suggest that plasma (with hyperimmune plasma possibly having enhanced activity) may have a defined role in the modulation of TNFa activated inflammatory cascades characteristic of septic shock.

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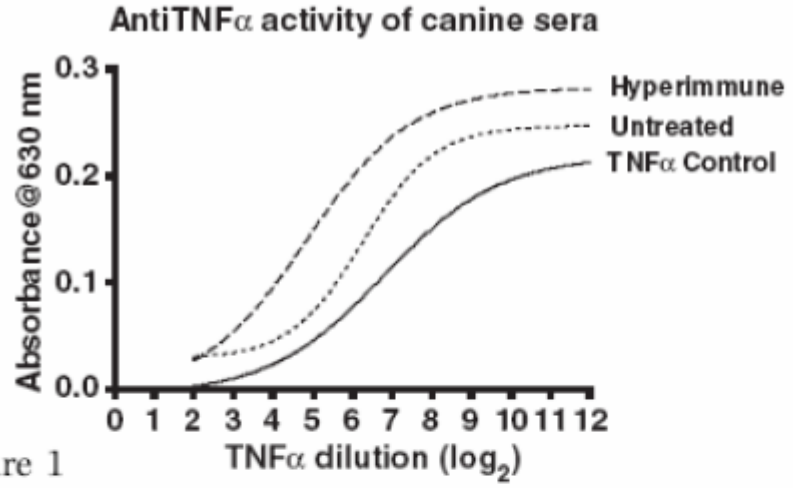

Figure 1

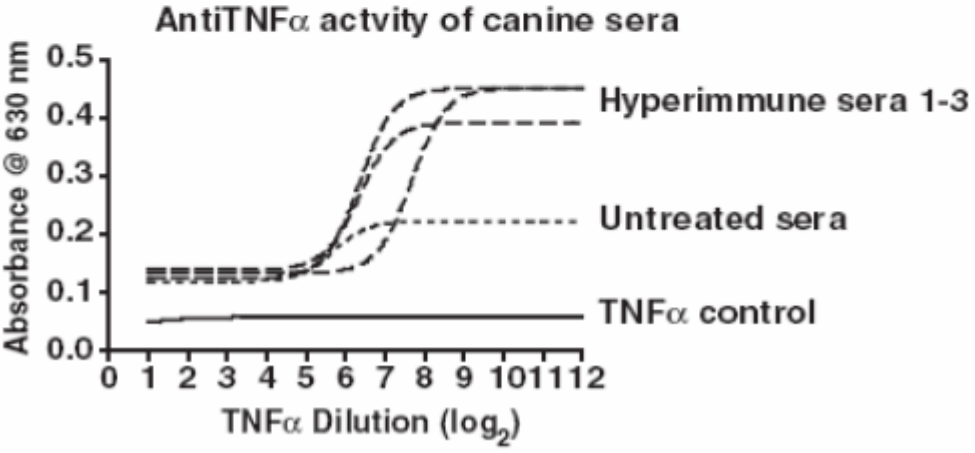

Figure 2