THE EFFECT OF EQUINE HYPERIMMUNE SERA ON TNFα ACTIVITY IN A L929 CELL BIOASSAY

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Adult equine endotoxaemia (AEE) remains a major cause of equine morbidity and mortality. The syndrome is common in horses presenting with acute abdominal disorders such as intestinal strangulation and severe colitis[1]. Death may result from an endotoxin (also known as lipopolysaccharide or LPS) induced, cytokine mediated, hyperinflammatory cascade that can lead to hypovolemic shock, coagulopathy and catastrophic multiple organ failure[2]. Endotoxin is a lipid A moiety located in the outer membrane of Gram negative bacteria such as intestinal *Escherichia coli.* The toxin may gain access to the systemic circulation through loss of gut mucosal integrity induced by events such as severe colitis, either by translocation of live bacteria or endotoxin leakage from the gut lumen[3]. Endotoxin is then rapidly bound to an LPS binding protein (LPB) in serum which potentiates its presentation to antigen presenting cells such as macrophages. Activated macrophages secrete inflammatory cytokines- predominantly TNFα and IL1β which initiate the uncontrolled cascade leading to a potential for the catastrophic pathology[4].

Because of haemodynamic instability and associated hypovolemia, fluid replacement therapy is generally applied to restore effective circulating volume[5]. The use of fresh frozen plasma has been recommended in cases of coagulopathies as it has been recognized to assist restoration of haemodynamic stability[6].

In this pilot study we examined the effect of sera from a proprietary hyperimmune plasma product (Equiplas) on the activity of TNFα in an *in vitro* L929 cell bioassay[7]. In brief, we report observations from 2 accessions of sera. Accession 1 describes the antiTNFα activity of 3 hyperimmune sera and an untreated serum sample that were provided blind to the study. Accession 2 reports a comparison of antiTNFα activity found in 3 paired hyperimmune sera collected following a multiple endotoxin vaccination regimen.

Figure 1
AntiTNFα activity of Equine sera

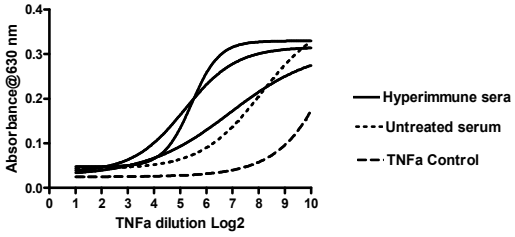


Figure 2.
Effect of Donor A 1st serum sample on TNFα activity

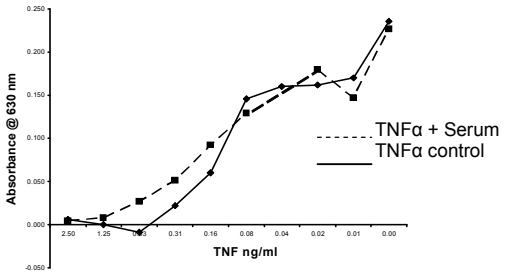
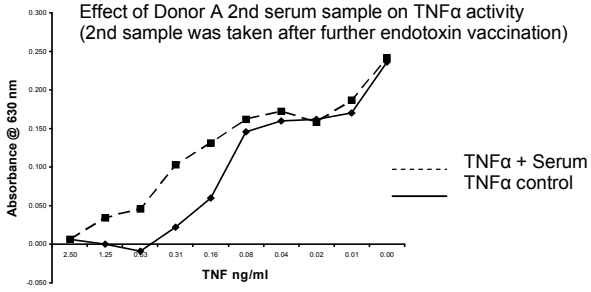


Figure 3.
Effect of Donor A 2nd serum sample on TNFα activity
(2nd sample was taken after further endotoxin vaccination)



We report here observations which we acknowledge require further work for statistical validation. **Figure1** suggests that there is intrinsic antiTNFα activity in all equine sera (Accession 1), including the untreated equine serum. It also appears that the proprietary endotoxin vaccination regimen employed in the preparation of hyperimmune plasma (Equiplas) enhances the antiTNFα activity of serum, as all 3 hyperimmune samples appear to exhibit greater activity than the untreated serum (although one specimen of hyperimmune serum appears to be only marginally enhanced). **Figures 2 & 3** are representative of results for a donor subjected to a 2nd regimen of endotoxin vaccination. The results suggest that in some cases there may be further enrichment of antiTNFα bioactivity **(Figure 3).** However, only 2 of the 3 donors subjected to further vaccination (Accession 2) demonstrated such enhancement. If these results are indicative of what occurs, then it may not be too surprising to find that there is an intrinsic biological limit to the titre achievable for an active factor. Whilst it is known that TNFα is rapidly degraded at the cellular level by neutrophil derived proteases, there is increasing evidence that the cytokine is also modulated in the systemic circulation by solubilized TNFα receptors (sTNFR)[8]. The sTNFRs appear to be solubilised from parent cell membranes by proteases in the presence of excess TNFα and may be a host means for moderating the cytokine's systemic influences[8,9]. Whilst our observations are preliminary and require confirmation, it does seem plausible that the endotoxin vaccination regimen used in the preparation of the hyperimmune plasma may similarly stimulate the systemic release of sTNFRs and so account, at least in part, for the apparent antiTNFα activity. Clearly, further work needs to be undertaken to confirm our observations and to determine the nature of the antiTNFα activity.

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ROY, M.F. (2004) *Vet Clin North Am Equine Pract*. 20(1):41-61
SOUTHWOOD, L.L. (2004) *Vet Clin North Am Equine Pract*. 20(1):167-197
TOMLINSON, J.E. & BLIKSLAGER, A.T. (2004) *AJVR* 65:1377-1383
TINKER, M.K et al., (1994) Equine Vet J 29:448-453
LUCUS, C.E. et al., (1995) *Am J Surgery* 171:399-404
HELSTERN, P. & HAUBELT, H. (2002) *Thrombosis Research* 107:S19-S22
WADHWA, M. *et al*., (1995) In *Cytokines: a practical approach* (Ed FR Balkwill) IRL Press, Oxford pp 357-391
ADERKA, D et al., (1998) *J Clin Invest*. 101:650-659
ALEXANDER, W & HILTON, D (2004) *Ann Rev Immunol* 22:503-529