COMBINATION ANTI-CYTOKINE IMMUNOTHERAPY IN AN OVINE MODEL OF GRAM-NEGATIVE SEPTIC SHOCK

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by

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SUMMARY

This thesis examines the role of anti-cytokine monoclonal antibodies (mAb) as adjunctive treatment in an animal model of septic shock. In particular, the role of combination immunotherapies directed against more than one cytokine is investigated.

The study aims were to: 1) develop a lethal hyperdynamic ovine model of gram-negative septic shock which reproduces the pathophysiologic derangements observed in human septic shock; 2) evaluate the efficacy of a mAb directed against (ovine) interleukin-1β (IL-1β), a pivotal mediator in the pathogenesis of septic shock, to ameliorate the pathophysiologic derangements and reduce mortality in a lethal hyperdynamic ovine model of gram-negative septic shock; and 3) test the hypothesis that a combination of anti-cytokine immunotherapeutic strategies directed against two pivotal mediators in the pathogenesis of septic shock, namely IL-1β and tumour necrosis factor-α (TNF-α), is superior to monotherapy against either cytokine alone.

The first results chapter (Chapter 3) describes the development of the ovine model of septic shock. The intravenous (iv.) dose of live E.coli required to produce 100% mortality (LD₁₀₀ dose) in awake, non-resuscitated sheep was determined to be 2 – 3 x 10⁹ live organisms/ml. The administration of basic supportive therapies (fluids, antibiotics) following the iv. LD₁₀₀ infusion of live E.coli in awake sheep was associated with the development of a reproducible hyperdynamic model of septic shock. Multiple pathophysiological derangements were demonstrated including myocardial depression (decreased stroke volume and left ventricular stroke work indices), progressive metabolic acidosis (decreased arterial and mixed venous pH and increased plasma lactate levels), increased plasma endotoxin and immunoreactive cytokine (IL-1β, TNF-α) levels and multiple organ injury. Mortality was 100% and survival time ranged from 8.4 hours to 15.0 hours.
Chapter 4 examines the efficacy of a neutralising mAb directed against sheep IL-1β (3.41) to:
(1) inhibit the live bacterial-induced pathophysiological derangements and; (2) improve survival, in the established ovine model of gram-negative septic shock. The prophylactic iv. infusion of anti-ovine IL-1β mAb 3.41 one hour prior to an iv. LD_{100} live E.coli infusion was associated with an ameliorated hypodynamic response, a sustained increase in systemic mean arterial pressure and attenuated metabolic acidosis (increased arterial and mixed venous pH and decreased plasma lactate levels) relative to control animals not receiving anti-ovine IL-1β mAb 3.41. However, the beneficial effects were incomplete. In particular, myocardial depression, multiple organ injury and elevated circulating cytokine levels (TNF-α) were not prevented. In addition, survival 24 hours post-infectious challenge was not significantly improved following the administration of anti-ovine IL-1β mAb 3.41.

The therapeutic effects of dual cytokine blockade employing a combination of mAb directed against ovine IL-1β (3.41) and TNF-α (6.09) are investigated in Chapter 5. Compared with anti-ovine TNF-α mAb 6.09 alone, prophylactic iv. combination anti-ovine IL-1β and TNF-α immunotherapy in sheep receiving an iv. LD_{100} infusion of live E.coli afforded no additional beneficial effects with respect to the observed amelioration of hypotension, metabolic acidosis and leucopaenia. Similarly, both monotherapy with anti-ovine TNF-α mAb 6.09 and combination anti-ovine IL-1β 3.41 and anti-ovine TNF-α mAb 6.09 therapy were associated with a progressive improvement in cardiac performance (increased cardiac index) over the 24 hour study period. However, divergent effects on mixed venous oxygen were demonstrated. With regard to organ dysfunction, neither monotherapy with anti-ovine TNF-α mAb 6.09 or combination anti-ovine IL-1β 3.41 and anti-ovine TNF-α mAb 6.09 therapy prevented multiple organ injury and survival was not significantly different between the two treatment groups.
In conclusion, there were significant beneficial effects of mAb targeting either IL-1β or TNF-α. However, an incremental improvement with dual cytokine blockade was not demonstrated in the current model of ovine gram-negative septic shock induced by a lethal infusion of live E.coli.