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DESPITE NFkB ACTIVATION IN CIRCULATING MONOCYTES FOLLOWING CARDIAC SURGERY WITH CARDIOPULMONARY BYPASS (CPB) THE NET PLASMA BIOACTIVITY IS ANTI-INFLAMMATORY

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Introduction. Mechanism and clinical significance of CPB-induced systemic inflammatory response is the subject of intense debate. In particular, the relationship between leukocyte activation and pro- and anti-inflammatory cytokine response remains unclear. Since NFkB is a transcription factor controlling the expression of a multitude of inflammatory genes as well a major target of many inflammatory stimuli, we monitored NFkB activity in monocytes of patients undergoing cardiac surgery utilising CPB.

Methods. Blood samples were collected from patients before and various time periods after the initiation of CPB. Cytospins were prepared from isolated mononuclear cells and nuclear translocation of NFkB was monitored by immunocytochemistry as evidence of NFkB activation. Plasma cytokines and inhibitory factors were monitored by ELISA. Net inflammatory activity of the plasma was assessed using an in vitro bioassay based on the ability of plasma to influence the expression of E-selectin, an NFkB dependant adhesion molecule on cultured pulmonary artery endothelial cells (PAEC) using flow cytometry.

Results. NFkB activation significantly increased in monocytes following CPB ($38.4 \pm 6.9\%$ vs $20.4 \pm 3.5\%$, $p=0.047$, $n=6$). IL-1 was undetectable in all plasma whereas TNF was detectable in only one patient out of post CPB (36.7pg/ml). In addition, there was no

evidence of net inflammatory activity in either the pre and post CPB plasma capable of inducing E-selectin expression on PAEC (3.7 ± 0.5 and $3.3 \pm 0.4\%$ of positive cells, respectively, vs $3.2 \pm 0.4\%$). In contrast, TNF (0.1ng/ml) and IL-1 (0.02ng/ml) readily induced E-selectin expression (51.4 ± 9.3 and $50.4 \pm 12.9\%$ positive cells). These effects of cytokines were inhibited by co-treatment with post CPB plasma ($71 \pm 6\%$ of control and $57 \pm 12\%$ of control, respectively, $p<0.05$, $n=13$) but not with pre CPB plasma.

Pre CPB plasma concentrations of soluble TNF Receptor (sTNFR) 1 ($859.9 \pm 117.1\text{pg/ml}$) and IL-1 receptor antagonist (IL-1RA) ($917.3 \pm 44.7\text{pg/ml}$) were significantly increased 2 hours after the initiation of CPB ($1460.9 \pm 180.1\text{pg/ml}$ and $2886.5 \pm 1202.7\text{pg/ml}$, respectively, $P<0.05$, $n=13$) with peak levels observed at 5 hours post CPB (1634.5 ± 270.8 and $22191.3 \pm 763.5\text{pg/ml}$, respectively). Pre CPB Plasma levels of sTNFR2 ($2156.3 \pm 209.3\text{pg/ml}$) and soluble IL-1 Receptor2 ($24.4 \pm 3.2\text{ng/ml}$) were not significantly altered ($2825.5 \pm 371.4\text{pg/ml}$ and $13.7 \pm 11.9\text{ng/ml}$, respectively, $n=13$).

There was a statistically significant correlation between plasma sTNFR1 and IL-1RA levels and the ability of plasma to inhibit TNF ($r^2 = 0.423$, $p=0.016$) and IL-1 ($r^2 = 0.423$, 0.364 , $p=0.029$)-induced E-selectin expression, respectively.

Summary And Conclusion. These data demonstrate that although cardiac surgery utilising CPB appears to activate monocytes, as judged by the activation of the inflammatory transcription factor NFkB, this neither parallels with significant increase in plasma levels of monocyte-derived pro-inflammatory cytokines nor results in increased proinflammatory activity of the plasma capable of eliciting pro-inflammatory intracellular signaling. Contrary, the predominant response appears to be a rise of anti-inflammatory cytokines producing a net anti-inflammatory activity capable of modulating TNF and IL-1-induced signaling.