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Investigating the neutrophil dependence of platelet recruitment to lungs inflamed by lipopolysaccharides

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Introduction

Bacterial lipopolysaccharides (LPS) cause an inflammatory reaction involving neutrophil recruitment to the lungs. Platelets can also be recruited to inflamed lungs, and in inflammatory settings both neutrophils and platelets release mediators that functionally alter each other. Previous work has shown that in mouse models, full pulmonary neutrophil recruitment responses following LPS inhalation are platelet dependent, but in this setting the dependence of platelet accumulation on neutrophils is unknown. We hypothesised that if platelet recruitment to lungs inflamed by LPS was dependent on neutrophils, the platelet response would be reduced by neutrophil depletion.

Methods

Balb/c mice (Female, 6-12 weeks) were treated with LPS (*E. coli*, O55:B5, 5mg/kg, intranasal) and lungs snap frozen 48 hours later. Recruitment of platelets and neutrophils to lungs was measured by quantifying the extent of CD42b platelet marker or neutrophil elastase immunostaining in respiratory portions of lung sections. Bronchoalveolar lavage was also taken from separate mice. These responses were then measured in control mice and mice depleted of neutrophils with anti-Ly6-G monoclonal antibody ([1A8], 25 mg/kg i.p., >80% depletion at 0h, >95% at +4h, +24h and +48h from LPS, $p<0.001$, n=12). Data are group means±standard error, with differences between groups detected using 2 or 3 way analysis of variance and Holm's p-value adjustment.

Results

LPS treatment caused platelet recruitment to lungs (PBS control vs. LPS + control IgG: 0.020±0.005 vs 0.37±0.06% of section CD42b+, $p<0.001$, n=6) with no detected effect of neutrophil depletion on the platelet response (LPS + control IgG vs. LPS + anti-Ly6-G: 0.37±0.06 vs. 0.30±0.09% of section CD42b+, n=6) whereas lung neutrophil accumulation was reduced (LPS + control IgG vs. LPS + anti-Ly6-G: 7.9±2.8 vs. 1.6±0.4% of section neutrophil elastase+, n=6, $p<0.01$). Platelet-derived CXCL4 (platelet factor-4) was detected by ELISA exclusively in bronchoalveolar lavage from LPS treated mice with no difference detected between neutrophil replete (71±6 pg/ml) and depleted mice (89±20 pg/ml, n=6), whereas LPS-induced migration of neutrophils into the bronchoalveolar space was reduced with neutrophil depletion (LPS + control IgG vs. LPS + anti-Ly6-G: 3.7±1.4 vs. $0.6\pm0.3\times10^6$ cells/ml, n=6, $p<0.05$).

Conclusions

Data presented here do not support the hypothesis that platelet responses to LPS depend on normal numbers of circulating neutrophils. Disease or host defence mechanisms involving platelets in similar inflammatory settings may not be influenced by solely targeting neutrophil responses, and the mechanisms and consequences of recruitment of activated platelets to inflamed lungs warrant further investigation.