Age dependent role of microvascular endothelial and polymorphonuclear cells in LPS induced renal failure

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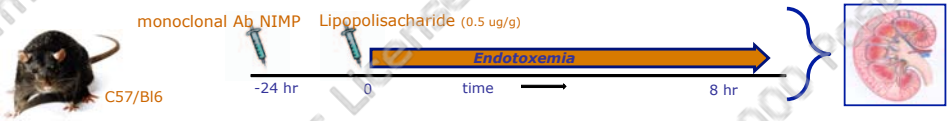
Introduction

The incidence of acute kidney injury (AKI) following severe sepsis is higher in elderly patients. We hypothesized that the microvascular endothelium is 'primed' by ageing and that sepsis represents a 'second hit' resulting in more severe microvascular complications in the elderly.

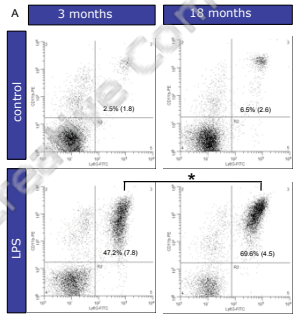


Materials & Methods

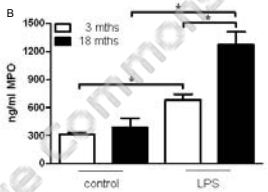
- 3 months and 18 months old female C57BL/6 mice were i.p. injected with 1,500 endotoxin units/gram body weight Lipopolysaccharide (LPS) and sacrificed after 8 hours.
- Neutrophil numbers in plasma were determined by flow cytometry and MPO ELISA.
- Quantitative (q)RT-PCR was used to analyze mRNA levels of P-selectin, E-selectin, VCAM-1, ICAM-1, Tie-2, and Angiopoietin (Ang)-1 and Ang-2. mRNA levels shown are relative to GAPDH as housekeeping gene and were determined by quantitative RT-PCR.
- In kidney tissue we assessed neutrophil influx and E-selectin protein expression.
- Neutrophils were depleted with the monoclonal Ab 24 hours prior to LPS challenge to study its consequences on kidney function. Plasma neutrophil gelatinase associated lipocalin (NGAL) levels were measured using ELISA in young and old mice.

Results

▪In aged mice the number of circulating PMNs after LPS challenge is higher than in young mice.

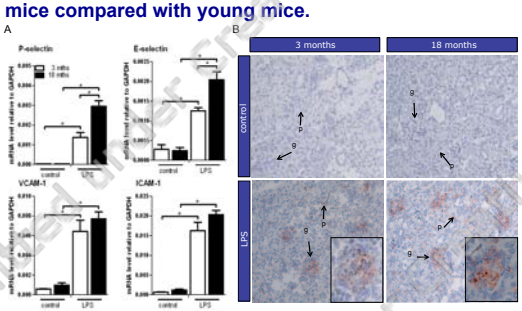


A. Flow cytometrical analysis of representative blood samples of a 3 months and 18 months old mouse with and without LPS injection. In each scatter plot, the right upper quadrant represents PMNs (Ly6G and CD11b positive). The relative increase in PMN count after LPS administration is lower (* p<0.5) in 3 months compared with aged mice. Value as % of PMN of total WBC count (± SD) of n=3.



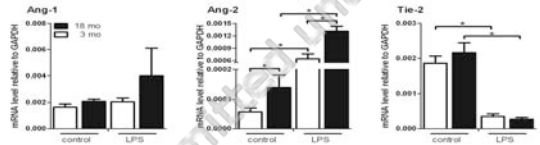
B. Quantification of MPO levels in plasma assessed by ELISA (mean [SD], N=8 per group; * P<0.05).

▪In the kidney, the expression of P- and E-selectin was more extensively induced by LPS in aged mice compared with young mice.



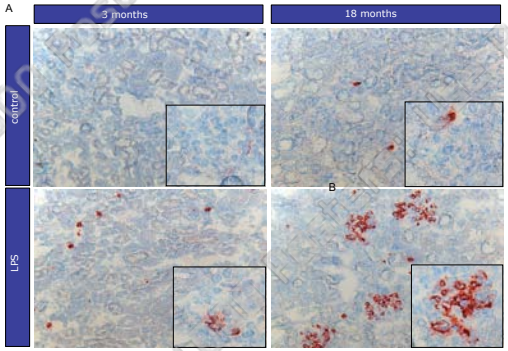
A. Expression of renal mRNA of P-selectin, E-selectin, VCAM-1, and ICAM-1 in 3 months old (white bars) and 18 months old (blackbars) mice subjected to LPS. (Mean [SD], N=6 per group; * P<0.05).
B. Frozen kidney tissue section were immunohistochemically stained for E-selectin (red), expression is visible in glomeruli (g) and peritubular (p) vasculature. Original magnification x200, Inserts: x400.

▪In aged mice Ang-2 mRNA levels in kidney were higher before and after LPS challenge.

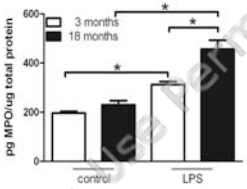


Ang-1, Ang-2 and Tie-2 in young, 3 months old (white bars) and elderly, 18 months old (blackbars) mice subjected to LPS. (Mean [SD], N=6 per group; * P<0.05).

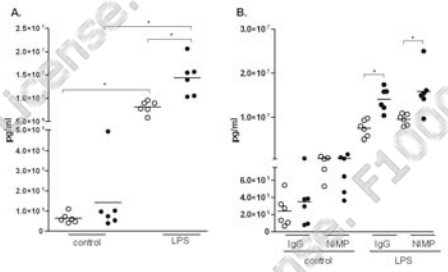
▪PMN infiltration in the kidney 8 hours after LPS injection is higher in aged mice.



A. Frozen kidney tissue sections were immunohistochemically stained for PMN (red) using Ly6G antibody. Original magnification x200, Inserts: x400.
B. Quantification of PMN infiltration in kidney tissue was performed using MPO ELISA. (Mean [SD], N=8 per group; * P<0.05).



▪After LPS challenge there was a significantly higher increase of NGAL concentration in plasma of older mice. PMN depletion is not protective.



A. NGAL was measured in plasma of 3 months old (white circles) and of 18 months old (black circles) mice.
B. Subset of mice were treated with anti-NIMP antibody 24 hours before LPS challenge to deplete polymorphonuclear neutrophils (PMN) or control IgG antibody that did not affect PMN count (B). Values are means +/- SD; *P ≤ 0.05 by ANOVA with post hoc comparison of all groups using Bonferroni correction.

Conclusion

- Ang-2 is increased in older mice which might cause priming of the endothelial cells.
- Endothelium responded by a more extensive increase in expression of P- an E-selectin in older mice and increased PMN influx.
- Loss of kidney function in aged mice after LPS challenge cannot be prevented by PMN depletion.