Regulatory effects of KCa3.1 on Pro Inflammatory Macrophage Gene Expression

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Abstract

The intermediate conductance Ca^{2+}-activated K^+ channel (KCa3.1), encoded by KCNN4, is present in coronary smooth muscle cells as well as macrophages and has been shown to contribute to the development of atherosclerosis through its various signaling roles. Our objective was to study the effects of KCa3.1 on commonly expressed proinflammatory M1 macrophage genes using male apolipoprotein E knockout ($Apoe^{-/-}$) and $Apoe^{-/-}$/$KCNN4^{-/-}$ double knockout (DKO) mice. We hypothesized that knockout of KCa3.1 would inhibit macrophage shift to the M1 phenotype as evidenced by reduced inflammatory markers. Bone marrow derived macrophages (BMDM) were harvested from the femurs of male mice and cultured 7-10 days. BMDM cells were then treated with lipopolysaccharide (LPS, 100 ng/ml, proinflammatory stimulant), TRAM-34 (100 nM, selective KCa3.1 inhibitor), or LPS+TRAM-34 for 48 hours. BMDM cells were harvested, RNA isolated, and first-strand cDNA synthesized. KCNN4, IL-1β, and IL-6 mRNA expressions were quantified using Real-Time PCR (RT-PCR). KCNN4 expression was used to confirm knockout in DKO mice. IL-6 and IL-1β, which are highly expressed in proinflammatory macrophages, were used to indicate macrophage type after treatment. In all treatment groups relative KCNN4 expression was reduced (0.07 +/- 0.01) in DKO mice compared to $Apoe^{-/-}$ (1.00 +/- 0.17). With no LPS treatment there is very little expression of IL-6 or IL-1β in both groups. When treated with LPS, IL-6 expression increased 6.93 (+/- 0.38) fold in $Apoe^{-/-}$ mice and 6.07 (+/- 0.75) fold in DKO mice compared to control. With LPS treatment, IL-1β increased 175.73 (+/- 53.96) fold in $Apoe^{-/-}$ mice and 135.95 (+/- 49.12) fold in DKO mice. TRAM-34 did not inhibit the LPS-induced increase in IL-6 expression in either group (8.77 +/- 1.75 for $Apoe^{-/-}$ and 8.04 +/- 0.87 for DKO), nor did it affect IL-1β expression (283.49 +/- 65.02 for $Apoe^{-/-}$ and 199.00 +/- 54.10 for DKO). TRAM-34 alone was similar to control for IL-6 (0.81 +/- 0.32 for $Apoe^{-/-}$ and 0.98 +/- 0.8 for DKO) and IL-1β (1.16 +/- 0.43 for $Apoe^{-/-}$ and 0.46 +/- 0.17 for DKO). Based on these data we accepted the null hypothesis, neither genetic knockout of KCa3.1 nor inhibition by TRAM-34 prevented the LPS-induced macrophage shift to the M1 phenotype.

Hypothesis

Knockout of the KCa3.1 channel will inhibit macrophage shift to the M1 phenotype as evidenced by reduced inflammatory markers.

Data and Results

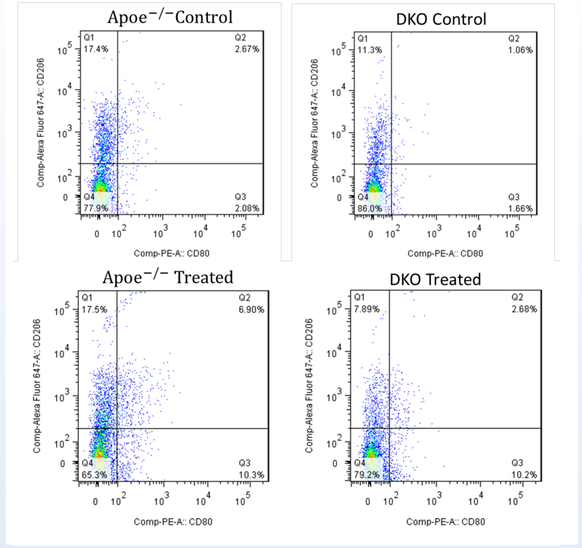

Figure 1. Flow Cytometry in $Apoe^{-/-}$ and DKO BMDM cells. Representative scatter plots of flow cytometry data. Isolated BMDM cells were cultured 7-10 days, then treated with INF-γ (10 ng/mL) and LPS (100ng/mL) or left untreated. After 48 hours BMDM cells were isolated and flow cytometry was performed. The gating strategy to identify M1 vs M0 cells was $CD45^+$, $CD11b^+$,$CD206^{+/-}$, $CD80^-$ (M0 cells) or $CD45^+$,$CD11b^+$,$CD206^{+/-}$,$CD80^+$ (M1 cells). The $Apoe^{-/-}$ cells had a fold change of 3.6 and the DKO cells 4.7, indicating that the DKO group had a slightly larger increase in M1 shift. ($p < 0.05$ n = 4). (Fold change determined by Q2 treated+Q3 treated/Q2 control+Q3 control.)

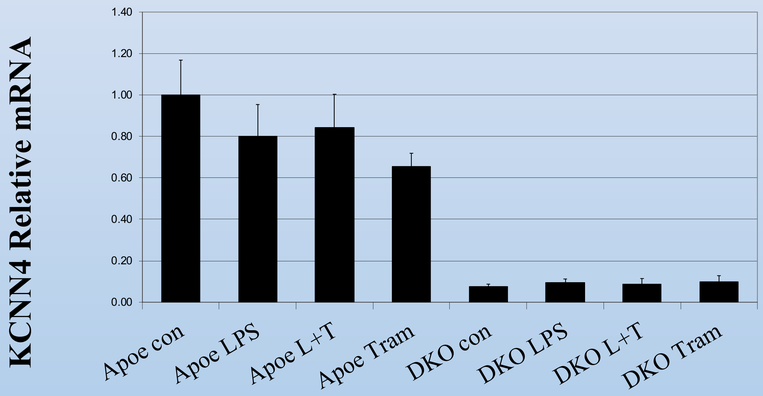

Figure 2. KCNN4 expression was reduced in DKO mice compared to $Apoe^{-/-}$. Relative mRNA expression of KCNN4 as determined by Real-Time PCR. KCNN4 is the gene that encodes for KCa3.1. BMDM cells were cultured for 7-10 days, then treated with lipopolysaccharide (LPS, 100 ng/ml, pro-inflammatory stimulant), TRAM-34 (100 nM, selective KCa3.1 inhibitor), or LPS+TRAM-34 (L+T) for 48 hours. Neither LPS nor TRAM-34 significantly altered expression of KCNN4. DKO mice had significantly reduced expression of KCNN4 compared to $Apoe^{-/-}$ mice ($p < 0.05$ n = 3-10 per group).

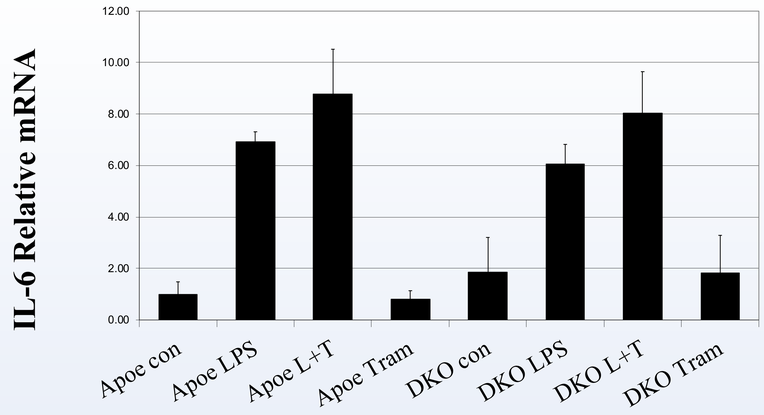

Figure 3. Relative IL-6 mRNA expression in $Apoe^{-/-}$ and DKO BMDM cells. IL-6 is a highly expressed marker in inflammatory M1 macrophages. LPS increased mRNA 6.03 +/- 0.38 fold in $Apoe^{-/-}$ and 6.07 +/- 0.75 in DKO (n=2-4 per group). TRAM-34 did not inhibit LPS-induced increases in either group, indicating that the absence of the KCa3.1 channel does not effect IL-6 macrophage expression.

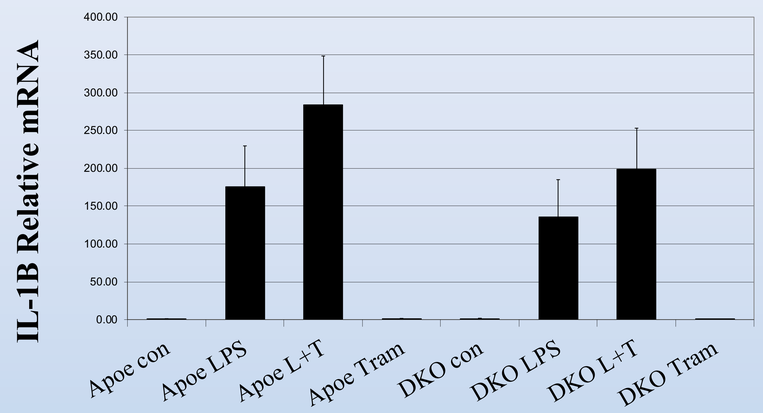

Figure 4. Relative IL-1β mRNA expression in $Apoe^{-/-}$ and DKO BMDM cells. IL-1β is a highly expressed marker in inflammatory M1 macrophages. A similar trend was seen in IL-1β expression as with IL-6 expression. With LPS stimulation, IL-1β expression increased 175.73 (+/- 53.96) fold in $Apoe^{-/-}$ mice and 135.95 (+/- 49.12) in DKO mice. There was no significant difference between $Apoe^{-/-}$ and DKO IL-1β expression. ($p<0.05$, n = 3-10 per group). LPS + TRAM-34 was not different from LPS alone.

Conclusion

Initial flow cytometry data demonstrated that knockout of KCa3.1 slightly upregulated M1 shift when defined as cell surface expression of CD80. However, neither knockout of the KCa3.1 channel, nor inhibition by TRAM-34, prevented LPS-induced increases in pro-inflammatory gene expression. Therefore, KCa3.1 does not appear to playing a major role in M1 inflammatory macrophage phenotypic shift.