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In-vitro Stimulation of CD4+ Lymphocytes Following Renal Ischemia/Reperfusion Injury

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Abstract

Th17 cells have been implicated in pathogenesis of immune-mediated diseases like arthritis and allergic asthma. Activated Th17 cells attract neutrophils through chemokine and cytokine secretion. Previous studies in our laboratory have implicated a role of Th17 cells in Acute Kidney Injury (AKI). When AKI rats were place on high salt diet to hasten Chronic Kidney Disease (CKD) the number of Th17 cells was significantly increased. Interestingly, there was an increase in circulating Th17 cells in CKD rats. Because angiotensin II is known to influence lymphocyte activity, we treated post ischemic rats with angiotensin receptor blocker, losartan, along with high salt diet. As expected, the induction of Th17 cells was reduced in rats treated with losartan as compared to untreated rats (Mehrotra et al., Kidney Int., 2015).

To further explore the Th-17 activation mechanism, renal CD4+ cells were isolated from post ischemic rats and stimulated in-vitro with varying conditions. These conditions focused on the effect of adenosine on IL-17 response using RT-PCR. Additionally, inhibitors of adenosine were added to test their IL-17 response in conjunction with adenosine. Specifically, an inhibitor for the Orai1 calcium channel. Furthermore, adenosine agonists were tested with to determine their effect on IL-17 response in the in-vitro stimulation plate. To compare to positive controls that stimulated IL-17 responses, angiotensin II and salt, as well as a cell stimulation cocktail was added to the in-vitro plate for comparison. A negative control containing only DMSO was also used for comparison.

Background

•Acute Kidney Injury (AKI) secondary to ischemia represents a major clinical problem associated with significant mortality. AKI is becoming widely recognized as a risk factor for the development of Chronic Kidney Disease (CKD).

•In previous work from the laboratory, rats were subjected to unilateral I/R injury or sham surgery. CD4+ cells were collected and stimulated in differing concentrations of adenosine. The 25 mM concentration consistently exhibited the most IL-17 response. Additional experiments focused on the 25 mM adenosine response in addition to different adenosine agonists.

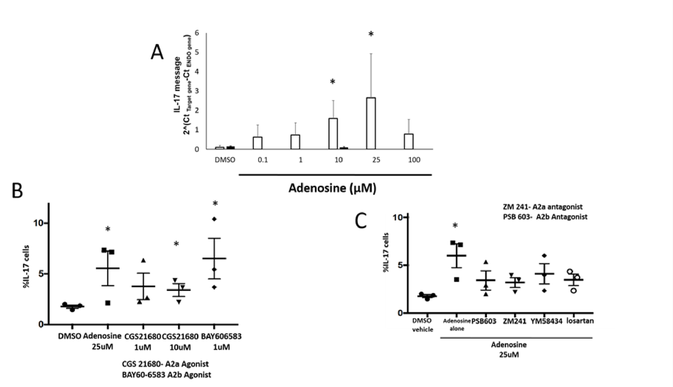


Figure 1 Evaluation of adenosine and adenosine receptors on the potential to stimulate IL-17 expression in CD4+ lymphocytes primed by AKI. (A) This graph shows that adenosine in a potential stimulator of IL-17 activity in CD4+ lymphocytes. Shown is a dose response of lymphocytes isolated from kidneys of rats following 7 days of recovery from AKI (open bar) or from sham rats. Cells were stimulated overnight in culture with increasing concentrations of adenosine resulting in an increase in IL-17A mRNA by real time PCR. Data represent mean of 4-5 different rats. No response was observed in cells from sham rats. (B) We focused initially on A2 receptors. Panel B shows responses of AKI primed lymphocytes in culture relative to A2A and A2B agonists. Note both agonists shows a stimulatory response indicating a potential role for A2 receptors. (C) This panel indicates effects of various antagonists on stimulation secondary to adenosine (25mM). Initial concentrations of antagonists were 10 mM. These results suggest that A2 receptors may signal cooperatively with Ang II/AT1 receptors and may utilize the store-operated Ca2+ channel pathway.

- In previous work from the laboratory, rats were subjected to unilateral I/R injury or sham surgery. CD4+ cells were collected and stimulated in elevated ang II and salt, along with other differing inhibitors (Figure 1). These data show a large Il-17 response in injured rats with both salt and ang II and differing inhibitors with the responses.

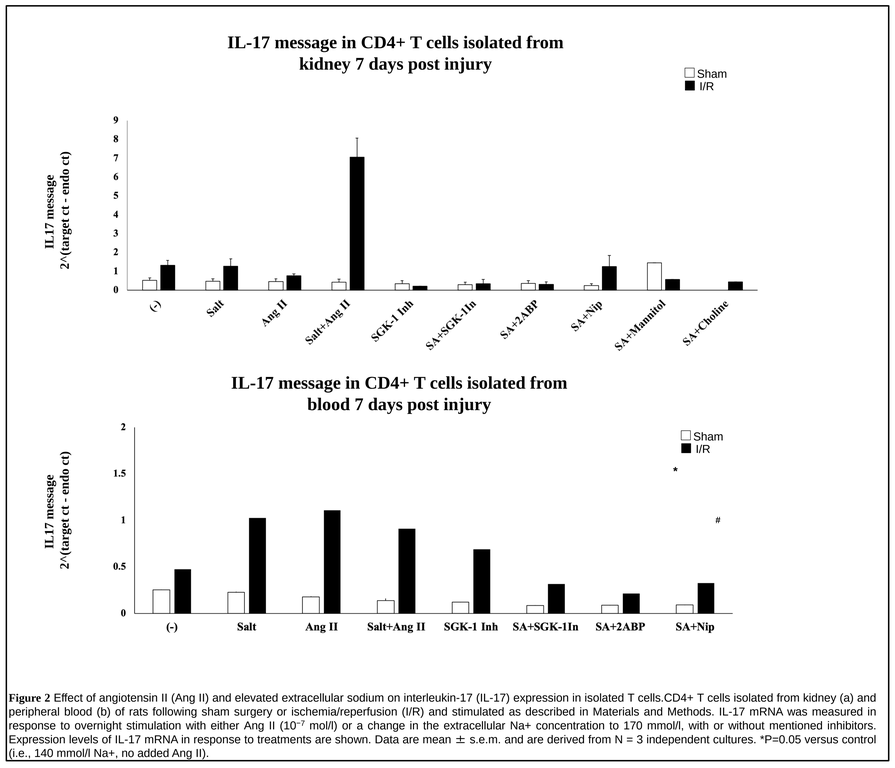
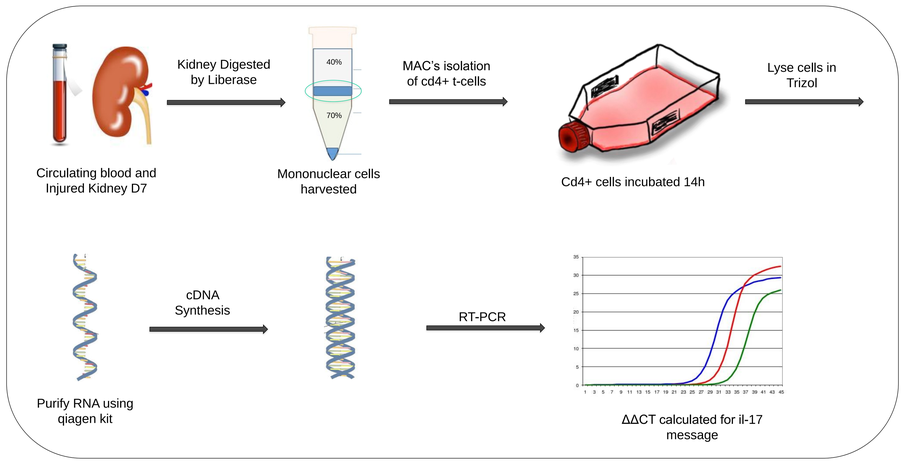


Figure 2 Effect of angiotensin II (Ang II) and elevated extracellular sodium on interleukin-17 (IL-17) expression in isolated T cells.CD4+ T cells isolated from kidney (a) and peripheral blood (b) of rats following sham surgery or ischemia/reperfusion (I/R) and stimulated as described in Materials and Methods. IL-17 mRNA was measured in response to overnight stimulation with either Ang II (10^{-7} mol/l) or a change in the extracellular Na+ concentration to 170 mmol/l, with or without mentioned inhibitors. Expression levels of IL-17 mRNA in response to treatments are shown. Data are mean ± s.e.m. and are derived from N = 3 independent cultures. *P=0.05 versus control (i.e., 140 mmol/l Na+, no added Ang II).

Procedure



Rats were subjected to bilateral I/R injury or sham surgery and allowed to recover for 7 days. The rats were sacrificed at 7 days post surgery and the kidney harvested. CD4+ cells were isolated from blood and kidney were incubated 12-14 hours with various in-vitro stimulation conditions.

Results

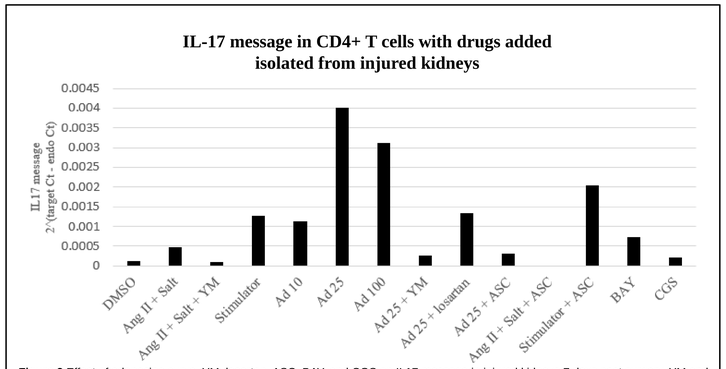


Figure 3 Effect of adenosine curve, YM, losartan, ASC, BAY, and CGS on IL17 message in injured kidneys 7 days post surgery. YM and losartan showed inhibiting effects on IL17 message compared to the control adenosine. ASC also showed inhibiting effects, especially in comparison to the positive control (Ang II + Salt). When ASC was added to the Stimulator, an inverse effect was shown. CGS and BAY are adenosine agonists. BAY displayed a higher level of inducing effect than CGS. Data is shown in IL17 mRNA response measured by RT-PCR.

- Rats were injured from ischemia/reperfusion surgery and their kidneys were taken out seven days post-surgery. The CD4+ T Cells were isolated according to the procedure previously described. Once RNA was extracted, cDNA was used to measure IL17 message. The negative control in this experiment was created by only adding DMSO to the well. The positive control is the well with Ang II and Salt added.

- According to the results, the adenosine curve shows that a 25 M concentration of adenosine will cause the highest IL17 response. When inhibitors were added to the 25 M adenosine cocktail, the IL17 message was attenuated significantly. IL17 agonists, BAY and CGS, showed a significant IL17 response compared to the negative control

Conclusion

•Increasing adenosine concentration to 25 M showed the most drastic effect on Il-17 regulation from AKI CD4+ T cells isolated from the kidney. This was consistent with previous research on adenosine curves in CD4+ T Cells from AKI rats. Other reagents show significant inhibition effect in the kidney CD4+ T Cells.

•YM displayed a significant effect on IL17 response inhibition. This effect was larger than losartan's inhibitive effect. ASC also showed a significant inhibition of IL17 response in the positive control, but a lesser effect in adenosine 25 M well BAY and ASC show an inducing effect on IL17 response. Due to a low sample size, more research needs to be conducted to ensure the trends are consistent and accurate.

References

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