Azithromycin suppresses effector functions of CD4+ T-cells via inhibition of mTOR signaling

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Background

- Antimicrobiell substances have secondary drug effects including immunomodulation

Question

- Do macrolides such as azithromycin (AZM) or clarithromycin (CLM) influence the activation of human CD4+ T-cells?

Material and Methods

- CD4+ T-cells were purified (>98.0%) from peripheral blood of 14 healthy individuals
- One hour prior to activation, T-cells were exposed to titrated concentration of AZM and CLM in therapeutic and supra-therapeutic concentrations.
- T-cells were activated using agonistic antiCD3/anti CD28 antibodies.
- Proliferation was measured by incorporation of 3H-Thymidine and dilution of fluorescence cell proliferation dyes.
- Cytokine secretion was measured using Luminex Mulitplex Technology.
- Apoptosis was assessed by Annexin V/ Propidium Iodide staining.
- Intracellular signaling was determined by immunoblotting and intra-cellular flow cytometry

Conclusion

- AZM (and CLM to a lesser extent) are **immunosupressive drugs**
- AZM partially **inhibits mTOR signaling**
- Relevance for patients?
- Clinical studies highly warranted

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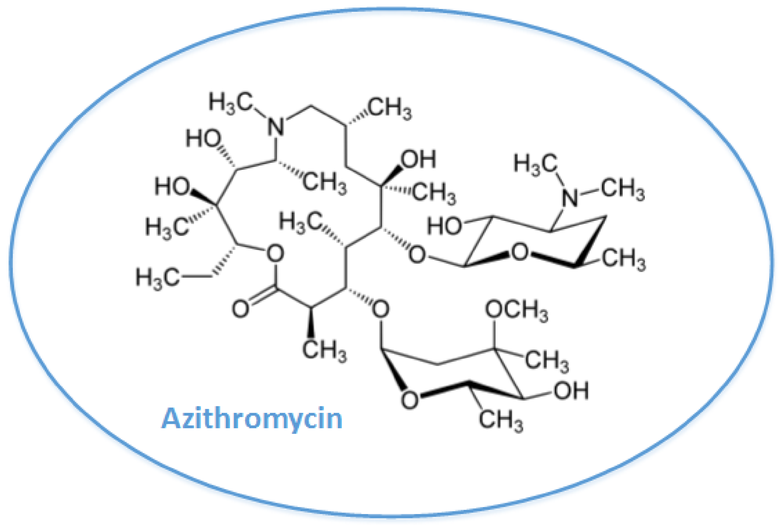
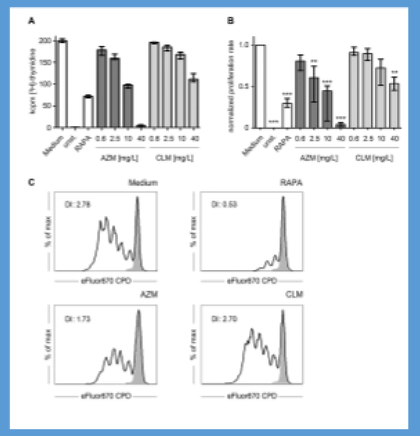
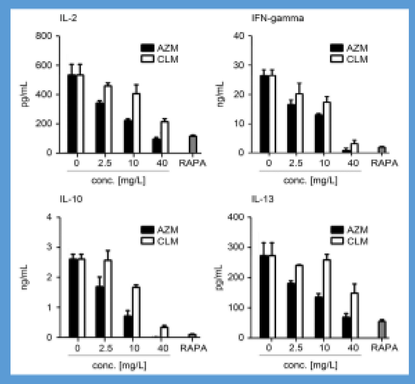




AZM suppresses activation of CD4+ T-cells

- Mean thymidine incorporation values of CD4+ T-cells
- Normalized proliferation values from ten donors
- Proliferation of eFluor670 CPD labeled CD4+ T-cells



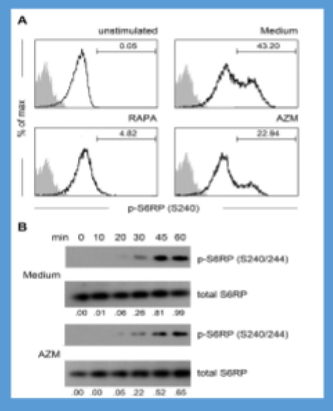
AZM suppresses cytokine production of CD4+ T-cells

Mean secretion values after

- 24 hours (IL-2)
- 72 hours (IFN-gamma, IL-10, IL-13)

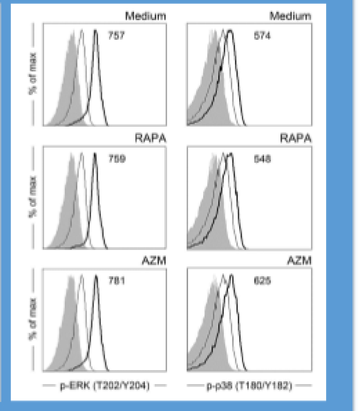
AZM inhibits phosphorylation of S6RP

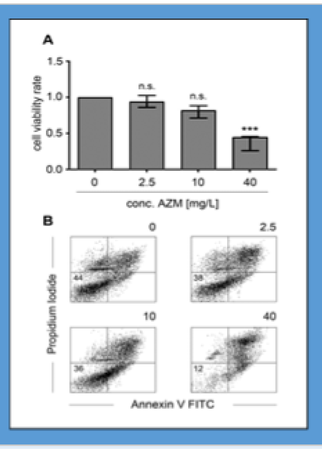
- A) Flow cytometry results using phosflow S6RP antibody
- B) Immunoblotting results of phospho-S6RP (upper lanes) and total S6RP (lower lanes); numbers indicate phospho-S6RP/total S6RP ratio



AZM does not interfere with MAP-kinase signaling

- Flow cytometry results for expression of phosphorylated ERK (left) or phosphorylated p38 (right) in activated T-cells





AZM does not compromise cell viability at therapeutical concentrations

- A) Mean normalized cell viability values from eight healthy individuals
- B) Dot plots depicting Annexin V (x-axis) and propidium iodide (y-axis) stainings from CD4+ T-cells from one representative individual

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