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CHE.496 Biological Systems Design

Project 1- Engineered Human Cells: SAY NO TO SEPSIS- A Review of the University of Ljubljana's iGEM Project

Goals:

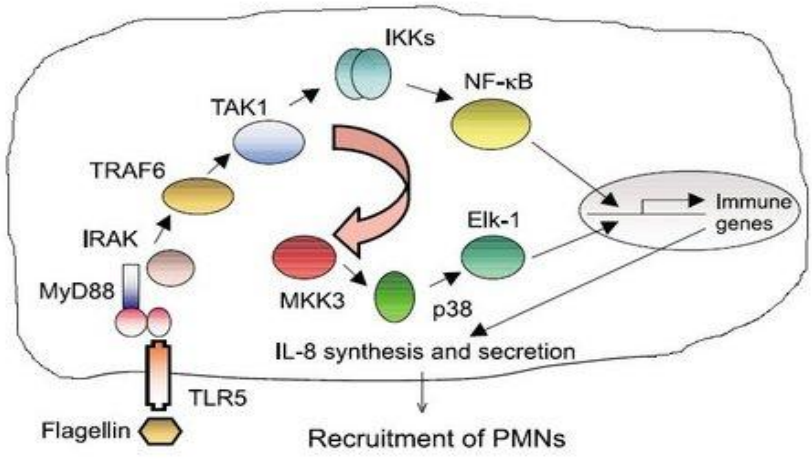
- Design a feedback pathway that, while retaining an effective pathway against infection, limits excessive cell stimulation and corresponding immune response

Background- Septic Shock

- Most common type of distributive shock
- High mortality rate ~40%
  - No novel advances since 1980s
- Associated mainly with gram-negative bacteremia
- Dysregulated release of chemokines (including cytokines)
- Additional injury due to endotoxins:
  - Coagulation cascade
  - Complement cascade
  - Vessel injury
  - Release of prostaglandins
- Eventually leads to multiple organ dysfunction syndrome (MODS)

Cellular Basis

- Toll-like receptors on surface of leukocytes
- Pathogen associated molecular patterns(PAMPs)
  - Lipopolysaccharides
  - Flagellin
  - Peptidoglycan



- Association of MyD88 (myeloid differentiation primary-response protein 88)
- MyD88- "adapter protein is involved in the signal transduction immediately after ligand-induced TLR oligomerization. This adapter protein is common to most TLRs before the signaling network branches into several phosphpryation cascades."

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Solution
- Inhibition via activation of dominant-negative adapter protein
- Decreased lifetime of adapter protein via rapid degradation tag (PEST sequence)

Results
- Construction of Biobricks
  - Promoter
  - Terminator
  - Protein coding sequences
    - Two inhibitory proteins of the signaling cascade (dnMyD88 and dnTRAF6)
    - Two reporters: *Renilla* luciferase
    - PEST sequence to decrease the lifetime of the inhibitor
- **Inhibition of cell signaling by a dnMyD88 feedback device**
  - ***Apparently* this shows a decreased response in the inhibited form**
- Decrease of protein lifetime via PEST sequence
  - Confirmed

Overall View
- Same principles of Biobricks can be used in eukaryotic cells
- Simplified model of the TLR signaling qualitatively captures most of the features of the natural system

Lessons for the VGEM Team
- Use citations
- Analysis- Give a more rigorous explanation of your reasoning behind accepting a hypothesis
- Defend your assumptions
- A model is critical to the understanding and development of your system