Identifying the Molecular Filter Responsible for Phagocytosis and Phagocytosis + Inflammation

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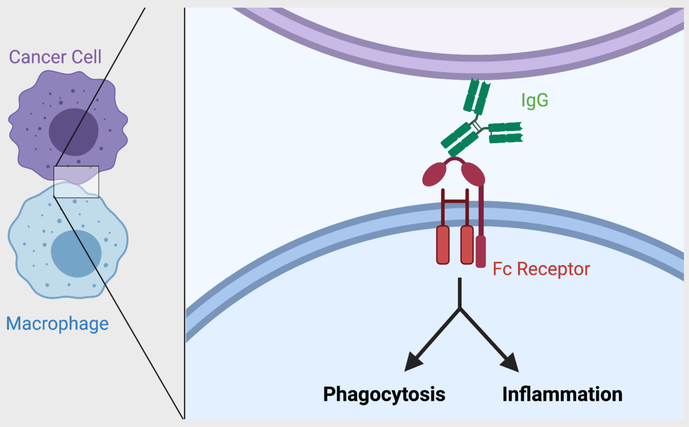
Abstract

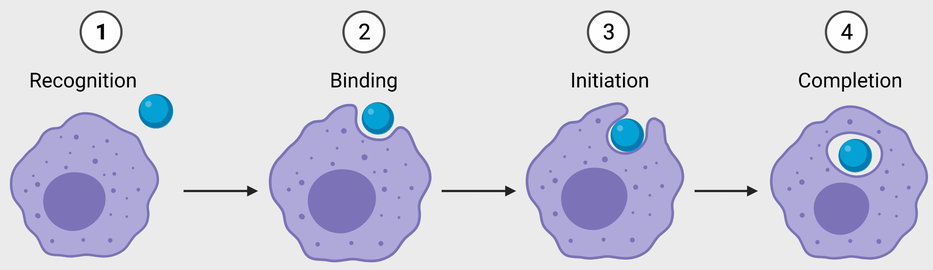
Previous immunotherapies used to target cancer have focused on T-cells, however, in the Morrissey lab we are taking advantage of macrophage's skilled ability of engulfing and eliminating foreign pathogens, including cancer cells through phagocytosis. Phagocytosis has seen to be triggered by the activation of the Fc Receptor by the "Eat me" signal IgG. The macrophage measures these signals from its environment and proceeds to make a decision. Previous research shows that at high levels of IgG a phagocytic event and inflammatory response is engaged and at low levels just phagocytosis happens. If we can find a protein that is recruited to the phagocytic cup at high levels and not at low levels of IgG, it could be the molecular fork in the road between these two responses. Once the filter has been identified, the mechanism can begin to be studied in order to eventually be able to precisely control when inflammation is triggered to increase the cancer killing efficacy of the immune system.

Background



- Macrophages are immune cells that measure "Eat me" and "Don't Eat me" signals throughout their environment
- The "Eat me" signal IgG is recognized by the Fc Receptor on the macrophage
- Previous work has shown High IgG levels induce Inflammation and Low IgG levels just trigger phagocytosis

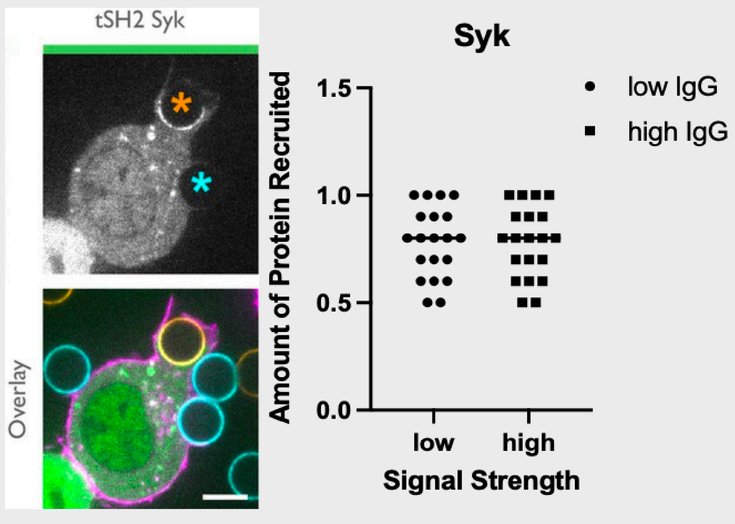
- No one knows how the macrophage makes this dose dependent decision between phagocytosis and phagocytosis + inflammation
- It is known how to trigger phagocytosis alone however, sometimes phagocytosis + inflammation occurs we want to be able to precisely control that response.



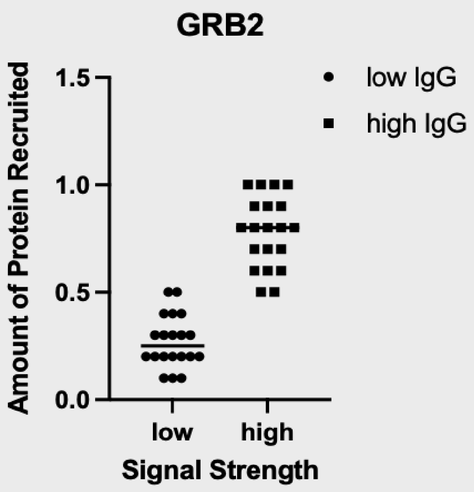
Process of Phagocytosis

Expected Results

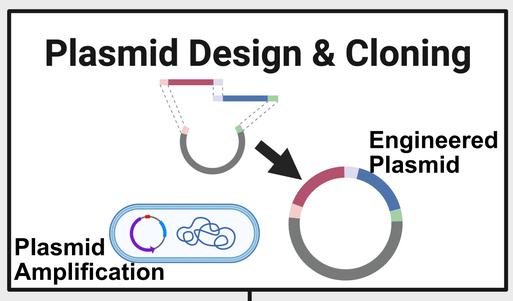
- Looking for protein recruitment to the phagocytic cup
- Highest recruitment is normalized to 1
- Each data point on the graph represents a single phagocytic event
- The brighter or more area covered by fluorescence indicates a positive result



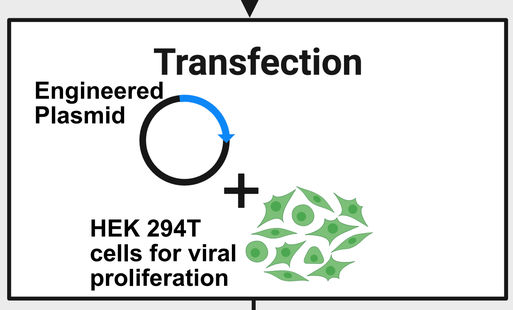
- Recruitment at High IgG and little to no recruitment at Low IgG is indicative of the molecular filter
- Indicates that this protein is needed for phagocytosis + inflammation and not just phagocytosis
- I hypothesize that GRB2 has the most potential for being this filter since it is a few proteins downstream of the Fc Receptor which is activated by IgG



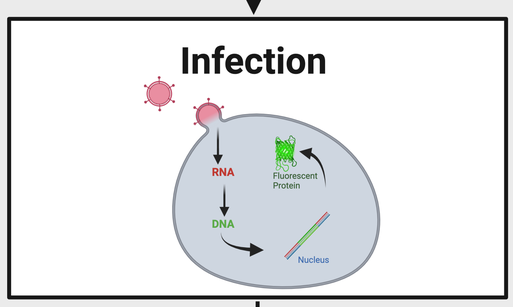
Experimental Design



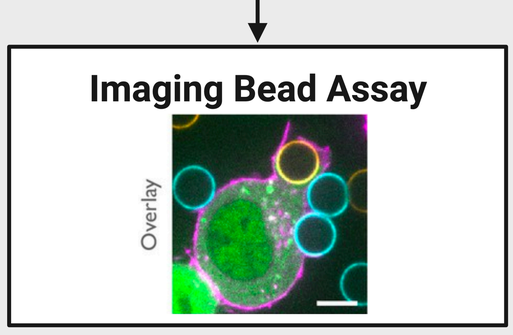
- Design plasmid fragments to have fluorescent tag on the gene of interest that codes for the protein we want to investigate.
- Assemble the plasmid into its circular form through a Gibson Assembly
- Amplify the plasmid through a bacterial transformation



- The plasmid is added with viral vectors into HEK 293T cells
- The cells will assemble the virus and the virus will exit the cells
- The virus now has our plasmid of interest inside it which it will use to infect the macrophages



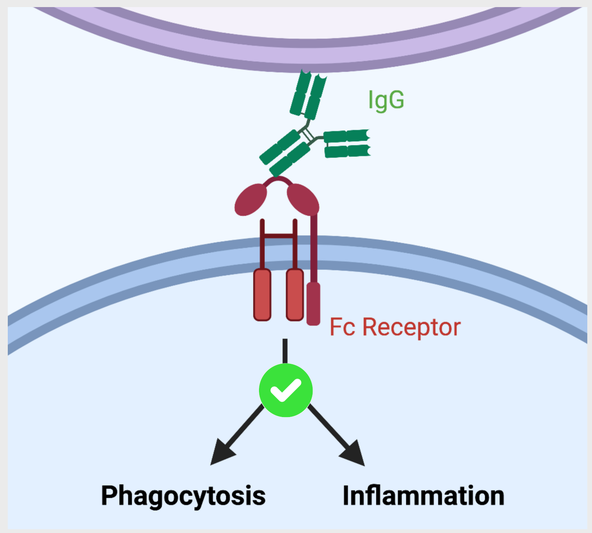
- The virus is capable of entering the macrophages and incorporating their viral DNA into the cells genome
- The cell's DNA replication machinery reads the incorporated DNA and produces the fluorescently tagged protein



- IgG coated beads are introduced to the infected macrophages
- A time lapse of the cells is taken to visualize the recruitment of the tagged protein

Conclusions

- Once we identify the protein that is the molecular filter, then we can begin to study the mechanism
- If we know the specific proteins needed and understand pathway we can engineer macrophages to trigger inflammation every time which would in turn increase cancer killing efficacy.



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NIH National Institutes of Health