

Understanding the Innate Immune Response to Group A Streptococcus Pili

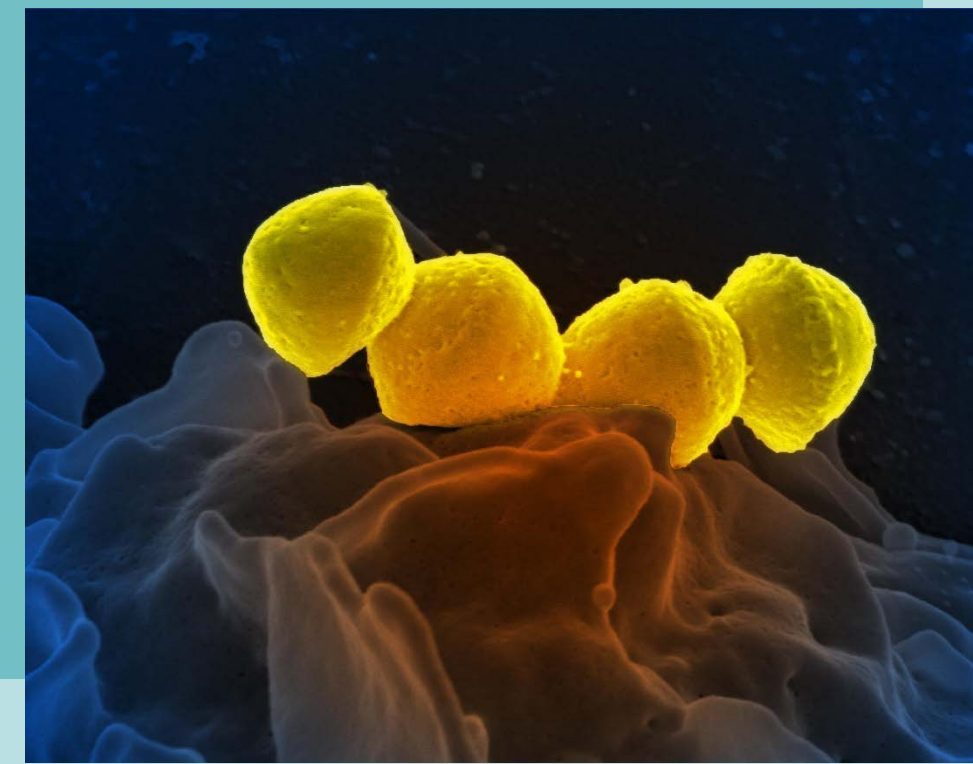


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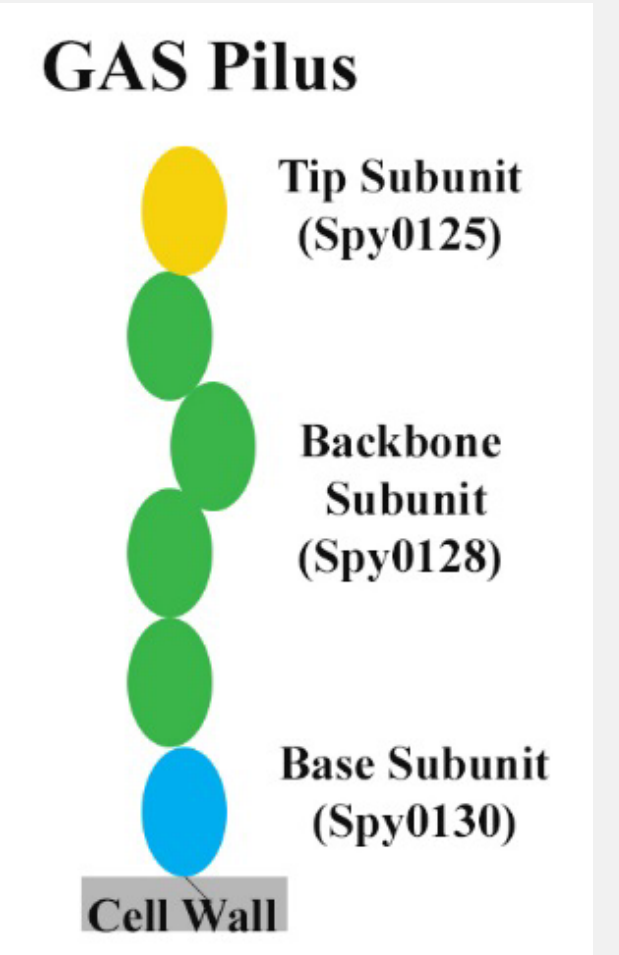


Introduction

Streptococcus pyogenes, or Group A Streptococcus (GAS), is a bacterial pathogen exclusive to humans. It is associated with a wide range of diseases, from pharyngitis to necrotising fasciitis and immune sequelae such as rheumatic heart disease. The pilus of GAS is a promising candidate for a vaccine against GAS infections.

The pilus (pl. pili) is a long, flexible, hair-like structure anchored to the cell surface. It is a virulence factor key in the initiation of infection and colonisation, due to its function of adhering to the surface of host cells.

Pili based vaccine has the potential to offer broader strain coverage and has been shown to be able to stimulate the production of protective antibodies. However, the innate immune response involved is yet to be defined.

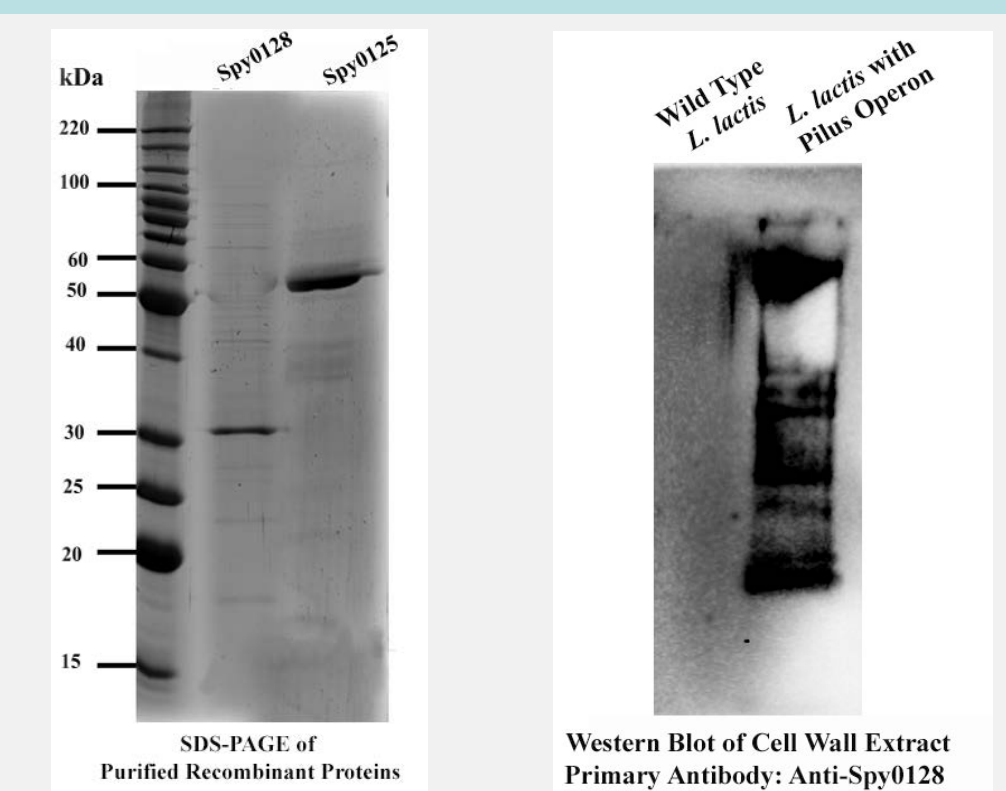


Aim

The aim of this project is to investigate the GAS pili's immunomodulation capacity by measuring cellular responses to recombinant proteins of pilus subunits, and to fully assembled pili on the surface of a *L. lactis* gain of function strain.

Methods and Results

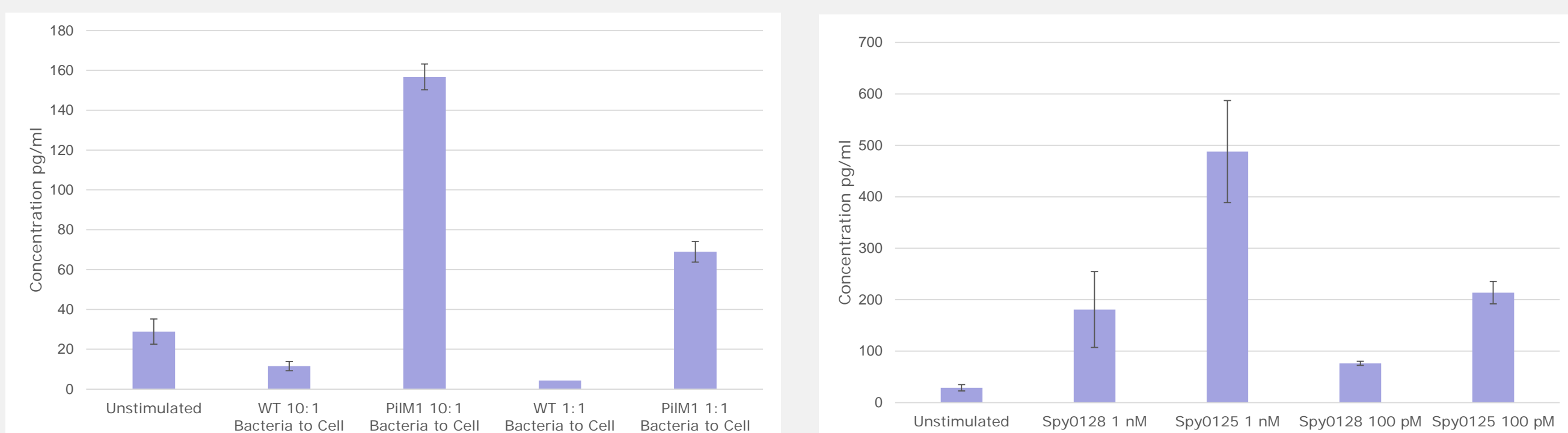
- The pilus backbone subunit (Spy0128) and tip subunit (Spy0125) were produced in *E. coli* as soluble, 6×histidine tagged recombinant proteins → → The proteins were then purified using immobilised metal affinity chromatography.
- Surface expression of the GAS pili were achieved by electroporation of a plasmid harboring the FCT-2 pilus operon, driven by a strong constitutive promoter, into *L. lactis*. Pilus expression was confirmed by a western blot of the cell wall protein extract.



Pili Mediated Secretion of Pro-Inflammatory Cytokines

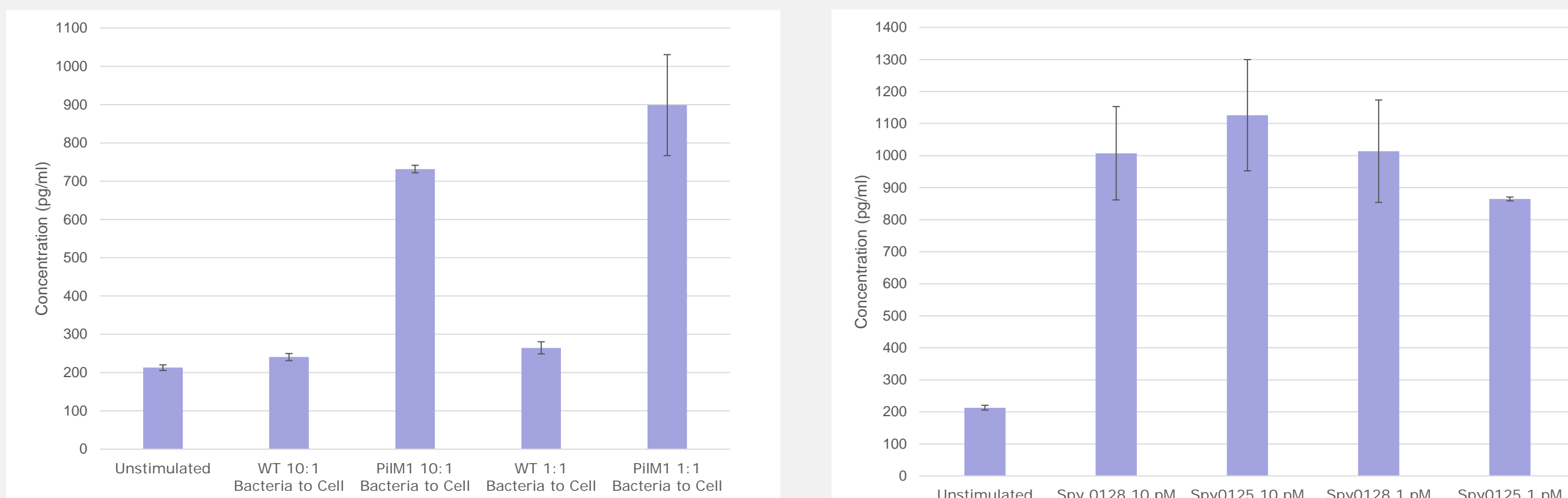
- TNF-α and IL-8 production in response to pilus subunits and whole pili expressed on *L. lactis* was measured using THP-1 cells, a human monocytic cell line.
- Cells were incubated for 20 hours with either tip subunit (Spy0125), backbone subunit (Spy0128), *L. lactis* expressing pili (PiIM1) or wild type *L. lactis* (WT).
- The supernatant was collected and the cytokine levels were quantified by ELISA.

TNF-α Secretion



- TNF-α secretion was highest in cells incubated with PiIM1.
- The tip subunit (Spy0125) appeared to be the greatest contributor to this response.

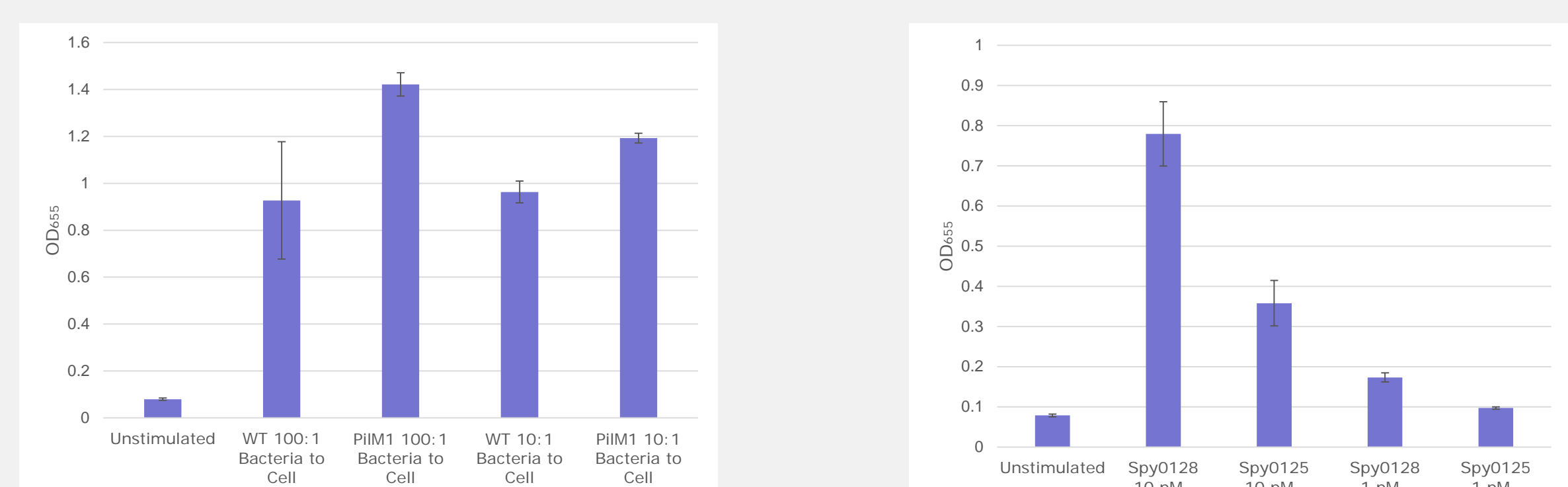
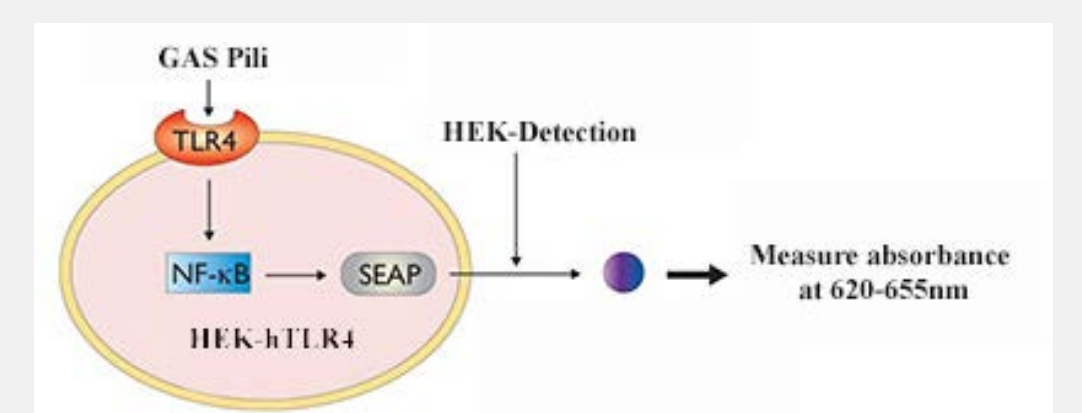
IL-8 Secretion



- IL-8 secretion was highest in cells incubated with PiIM1.
- High concentrations of IL-8 was secreted from both cells incubated with backbone subunits (Spy0128) and cells incubated with tip subunits (Spy0125).

Interaction between Pili and Toll Like Receptors-(TLRs)

- The interaction between pili and the TLRs, the most prominent pattern recognition receptor (PRR) for GAS, will be determined using HEK293 reporter cell lines expressing hTLR-2, hTLR-4 or hTLR-5 individually.
- In a preliminary experiment HEK293-hTLR2 cells were incubated for 9 hours with tip subunit (Spy0125), backbone subunit (Spy0128), pili expressing *L. lactis* (PiIM1) or wild type *L. lactis* (WT).



- PiIM1 activated TLR2 on the HEK293-hTLR2 cells.
- Both subunits appeared to be contributing but the backbone subunit (Spy0128) stimulated greater activation.

Conclusion

- Whole GAS pili and pilus subunits stimulate the innate immune system to release pro-inflammatory cytokines.
- The tip subunit in particular stimulates strong TNF-α release.
- Pili and pilus subunits induce a very strong IL-8 response.

- The pilus stimulates cytokine release via interaction with TLR2
- Both subunits activate TLR2 but activation levels do not correspond to the pattern of cytokine release, suggesting other receptors or pathways may also be involved.

These results indicate that GAS pili are able to induce innate immunity. This strengthens the pili's potential as a vaccine candidate as the innate immune system has the function of enhancing the adaptive immune response required to produce protective antibodies.

Further investigation is needed to fully characterise which branches of the innate immune system the pili specifically stimulate.

References

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