The GAS Serotype M2 Pilus Structure is Important for Both Cell Adhesion and Immune Evasion

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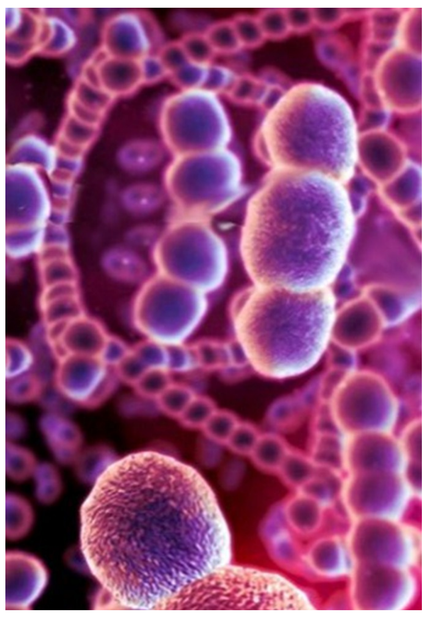
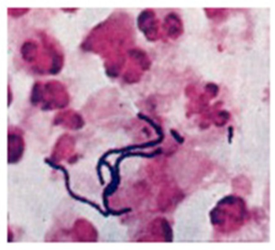
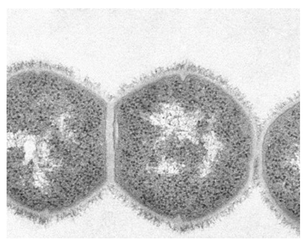
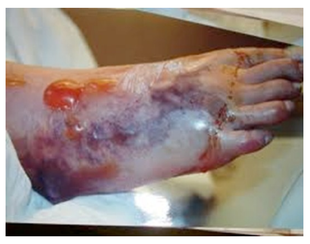
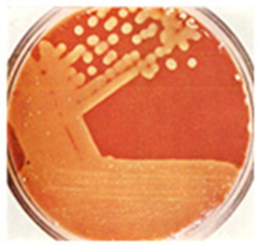


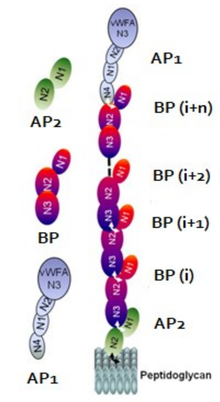








Streptococcus pyogenes (group A streptococcus, GAS) is an important human pathogen that frequently colonises the throat and skin. Pilus structures found recently in GAS have been shown to mediate adhesion to host cells and participate in biofilm formation. Pili are heteromeric structures consisting of multiple copies of backbone protein and 1 or 2 ancillary proteins. Among the nine types of pilus gene island identified thus far, the FCT-6 type pilus found in serotype M2 GAS strain shows low homology to the other better-studied types. Though the sequences of the two ancillary pilins are similar to those of the PI-1 pilus found in group B streptococcus (GBS), the similarity between the two backbone pilins is lower than 50%. To investigate the function of this unique pilus, we generated GAS pilus deletion mutants which were also modified to produce firefly luciferase for a strong bioluminescence signal. These bioluminescent GAS strains and recombinant proteins of individual pilins were used in various assays, to characterise the binding properties of each pilus subunit. Whole blood killing assay was also performed to address the importance of M2 pilus in bacterial survival.



MATERIALS & METHODOLOGY FRAMEWORK

Generation of bioluminescent wild-type and pilus deletion GAS strains

M2 GAS tip pilin (AP1)- or whole pilus operon-deletion mutants were generated by allelic replacement.

Firefly luciferase gene (*ffn*) was introduced into the bacteria by electroporation.

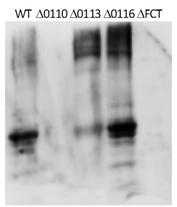
Bacterial adherence and invasion assays

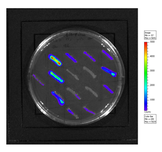
Monolayers of human epithelial cells were infected with bioluminescent GAS strains at an MOI of 10.

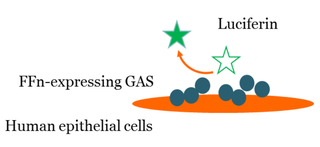
Adhered or Internalised bacteria were detected by bioluminescence signals.

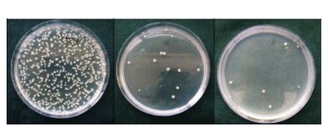
Whole blood killing assay

1000 cfu of late log phase GAS were added to 1 ml of heparinised human whole blood and incubated at 37°C for 2 h. Surviving cfu was quantified by plating serial dilutions.

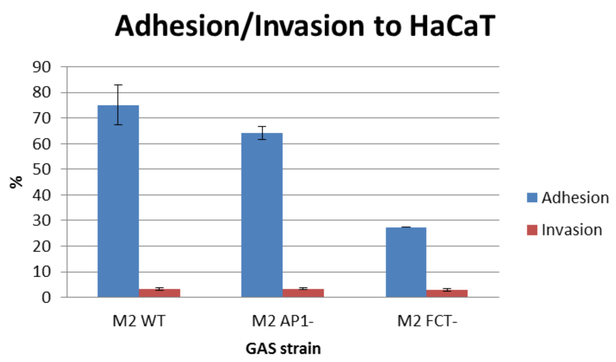


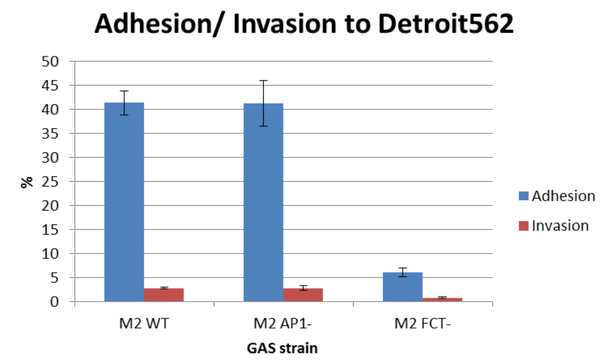






ADHERENCE OF M2 GAS TO EPITHELIAL CELLS

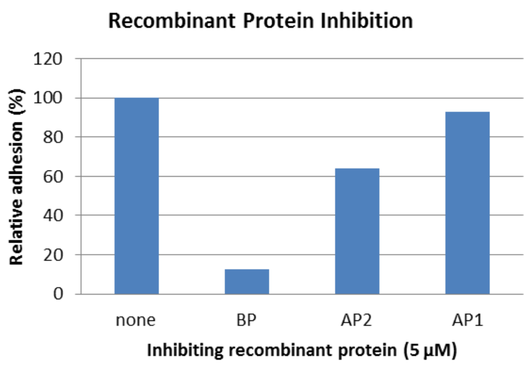


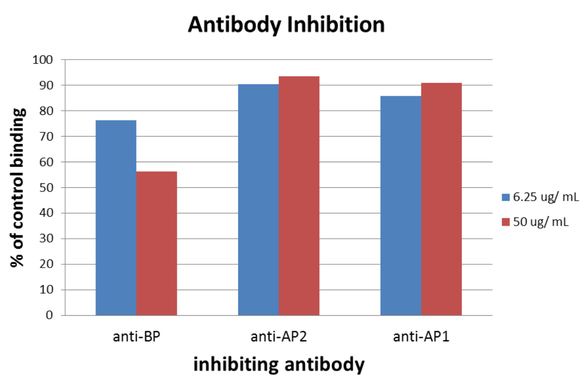


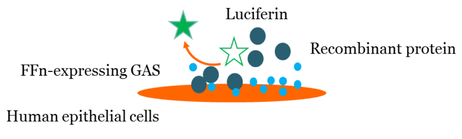
Adherence of wild type or pilus deletion M2 GAS strains to human epithelial cells. HaCaT (keratinocytes) or Detroit562 (pharyngeal epithelial cells) were infected with M2 strains at an MOI of 10 for 1 h.

Adhesion and invasion were quantified by measuring bioluminescence intensity, and shown as percentage of the bioluminescence intensity of initial inoculum. Deletion of tip pilin AP1 only partially reduced GAS adhesion or invasion to both cell lines tested, while whole pilus deletion showed a significant effect.

ROLES OF FCT-6 PILUS SUBUNITS IN ADHERENCE



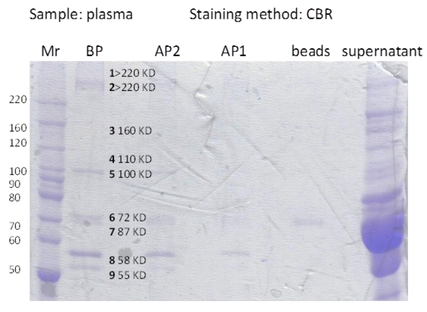


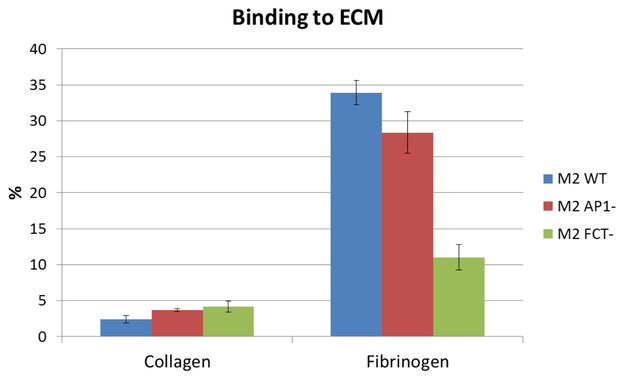


Binding specificity was determined by pre-incubating GAS with antibodies or recombinant proteins prior to infecting HaCaT cells.

Recombinant BP protein or specific antibody against BP showed the most significant effect on inhibiting adherence of M2 GAS to human epithelial cells.

IDENTIFICATION OF BINDING RECEPTORS

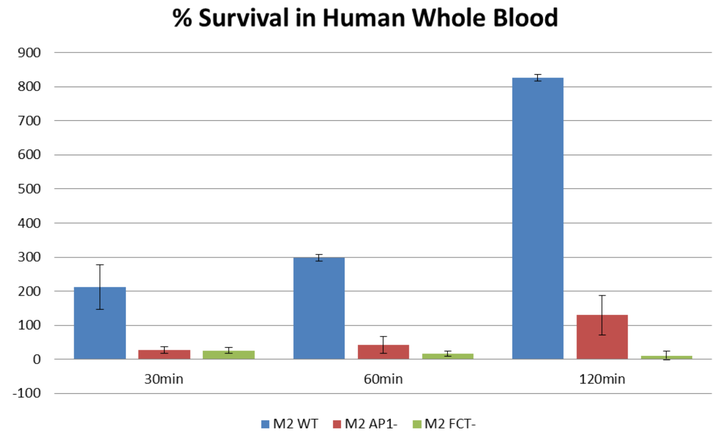




Beads coupled with BP, but not AP1 identified fibrinogen and fibronectin as binding partners of M2 pilus in pull-down assay using human plasma . Adherence of M2 GAS to fibrinogen was also confirmed by an ECM binding assay. Notably, deletion of AP1 only resulted in a moderate decrease in adherence, while deletion of the whole pilus conferred a marked effect.

No binding receptor was detected in pull-down assays with cell lysate (data not shown).

ROLE OF GAS M2 PILUS IN IMMUNE EVASION



The pilus is important for bacterial survival in whole human blood.

Deletion of AP1 significantly attenuated the growth of bacteria in whole human blood. M2 GAS was mostly killed after 2 h incubation without expression of pili, suggesting the pilus plays a protective role for bacterial survival.

CONCLUSION

- The M2 pilus is important for binding of GAS to HaCaT and Detroit562 cells. However, the backbone pilin (BP), but not the tip adhesin (AP1) seems to be responsible.
- Adhesion of BP to host cell lines is specific and can be blocked with recombinant proteins or antibodies.
- Pull-down assays identified 2 plasma proteins (fibrinogen and fibronectin) as binding partners for BP, but not for AP1. Adherence of fibrinogen to the pilus structure was confirmed in a binding assay.
- The M2 pilus, in particular the AP1 protein confers protection in whole human blood.

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