

The role of factor H binding protein (fHbp) in intracellular survival of *Neisseria meningitidis* after phagocytosis by human macrophages.

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Abstract

Project Rationale & Hypothesis: Factor H binding protein (fHbp) is a surface antigen of *N. meningitidis* which inhibits complement deposition on the bacterial surface and may also be protective against endogenous defenses. fHbp is a major component of new generation meningococcal vaccines.

Objectives: To determine whether fHbp influences meningococcal phagocytosis by primary human monocyte-derived macrophages (MDMs), and if fHbp enhances intracellular survival of *N. meningitidis* after phagocytosis by MDMs.

Methodology: *N. meningitidis* was co-incubated with 14 day-old MDMs at a range of multiplicities of infections, under opsonic and non-opsonic conditions. Bacterial association to the macrophage surface and internalisation were quantified by immunofluorescence. Intracellular survival of bacteria over 2 hours after phagocytosis was measured using a standard gentamicin protection assay. Uptake and survival of wild type organisms were compared with fHbp null mutants and a complemented strain.

Findings: fHbp significantly reduced internalisation of meningococci by MDMs opsonised by complement C6 deficient serum, but not under non-opsonic conditions. No difference was observed in intracellular survival of non-opsonised bacterial strains even when the MDMs were activated with Interferon- γ . We conclude that meningococcal fHbp inhibits opsonic uptake of *N. meningitidis* by macrophages, but has no effect on resistance to intracellular microbicidal mechanisms.

Introduction

- > *Neisseria meningitidis* infects the mucosal tissue within the nasopharynx.
- > Macrophages are the sentinel cell in the nasopharynx and a key component of the innate immune response.
- > *N. meningitidis* must invade the mucosal epithelium and survive in human serum to cause fulminant disease. Evoking a minimum host response is key to this, binding of factor H is just one method used by *N. meningitidis* to do this.

Factor H binding protein

- > Factor H is the main inhibitor of the alternative complement pathway. Factor H is a co-factor causing cleavage of C3b to the inactive C3bi. Factor H can cause irreversible dissociation of C3bBb (C3 convertase).
- > Factor H binding protein (fHbp) is a universally expressed, highly immunogenic, ~29kDa ligand for factor H, it is found as an outer membrane protein (OMP).
- > fHbp protects meningococci from complement-mediated death in human serum experiments, but has also been shown to protect meningococci from anti-microbial peptides *in vitro*.
- > Factor H binding protein is currently a vaccine candidate for serogroup B meningococcal disease, undergoing Phase III trials at the moment.



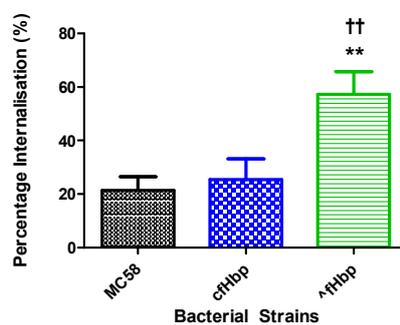
References

Cantini, F. et al. 2009, Journal of Biological Chemistry, vol 284, pp. 9022-9026.
 Seib, K.L. et al. 2009, Infection & Immunology, vol 77, pp. 292-299.
 Seib, K.L. et al. 2011, Infection & Immunology, vol 79, pp. 970-981.
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Results

Following opsonisation of *Neisseria meningitidis* and co-incubation of MDMs for 90 minutes there was a 3-fold greater uptake of MC58 Δ fHbp compared with wild type MC58 or complemented MC58.

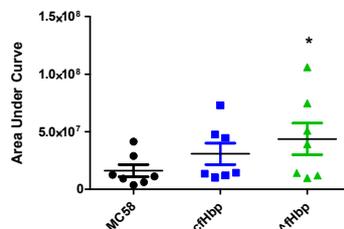
Percentage Internalisation of Opsonised Bacteria by MDMs at 90 minutes.



One way ANOVA analysis with Tukey's Multiple Comparison Test showed significance between sets of data. ** denotes significance ($p < 0.01$) between MC58 and MC58 Δ fHbp. †† denotes significance ($p < 0.01$) between MC58 cfHbp and MC58 Δ fHbp. Graph shows mean \pm SEM, n=7.

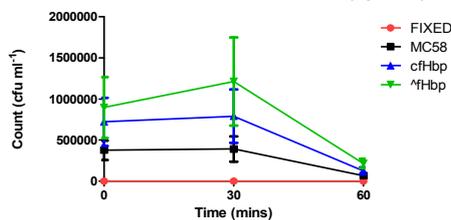
Intracellular viability (in absolute terms) of *Neisseria meningitidis*, measured by standard Gentamicin Protection Assay was enhanced in the Δ fHbp compared with wild type MC58.

Area Under Curve of Intracellular viable bacteria within MDMs (Opsonic)



One way ANOVA with Friedman test and Dunn's Multiple Comparison Test showed significance. * ($p < 0.05$) denotes significance between MC58 and MC58 Δ fHbp. Graph shows mean \pm SEM, n=7.

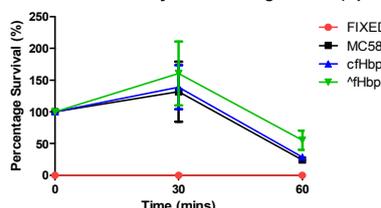
Intracellular Viable Bacteria within MDMs (Opsonic)



Graph shows actual viable counts for the intracellular bacteria (opsonised) following 90 minutes of infection and a 30 minute 'gentamicin pulse' (gentamicin 100 μ g/ml). MDMs were lysed using 2% Saponin for 10 minutes. Graph shows mean \pm SEM, n=7.

This 'increased viability' of MC58 Δ fHbp was entirely due to the enhanced uptake, as survival (measured as a percentage of viable counts at T=0) was equivalent for all strains.

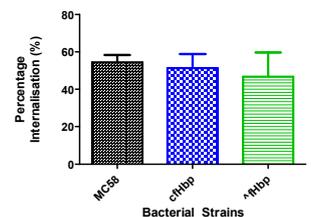
Intracellular Bacterial Viability as Percentage of T=0 (Opsonic)



Graph shows the intracellular survival of each bacteria (opsonised) strain when engulfed by MDMs. Graph shows mean \pm SEM, n=7.

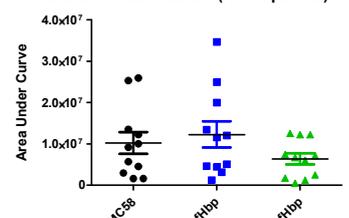
Under non-opsonic conditions it was expected that the wild type MC58 and MC58 Δ fHbp would be processed equivalently. This was confirmed by comparable uptake and equivalent intracellular viability and survival for all test strains.

Percentage Internalisation of Non-Opsonised Bacteria by MDMs at 90 minutes.



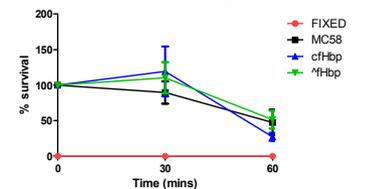
One way ANOVA analysis with Tukey's Multiple Comparison Test showed no significance between the sets of data. Graph shows mean \pm SEM, n=8.

Area Under Curve of Intracellular viable bacteria within MDMs (Non-Opsonic)



One way ANOVA analysis with Friedman test and Dunn's Multiple Comparison Test showed no significance between test groups. Graph shows mean \pm SEM, n=11.

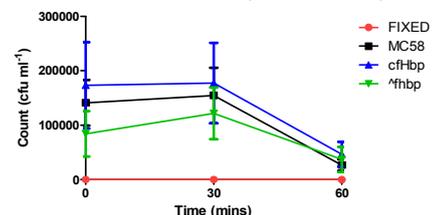
Intracellular Bacterial Viability as Percentage of T=0 (Non-Opsonic)



This graph shows the intracellular survival of each bacteria (non-opsonised) strain when engulfed by MDMs. Graph shows mean \pm SEM, n=12.

This pattern also occurs even when MDMs are pre-activated with IFN- γ .

Intracellular Viable Bacteria (Activated MDMs)



Graph shows actual viable counts for the intracellular bacteria. Graph shows mean \pm SEM, n=4.

Conclusions

- > Factor H binding protein retards phagocytic uptake of *Neisseria meningitidis* by human macrophages following opsonisation with complement-containing serum.
- > Factor H binding protein does not reduce phagocytic uptake of *Neisseria meningitidis* if bacteria are not opsonised.
- > The presence of factor H binding protein does not retard intracellular killing of *Neisseria meningitidis*.
- > Even when macrophages are activated by Interferon-gamma, therefore stressing the system with increased defensin production (in particular antimicrobial peptide LL-37), presence of fHbp does not appear to improve intracellular viability.
- > Factor H binding protein is key to the pathogenesis of *Neisseria meningitidis*, it is therefore important as a potential vaccine candidate.