(1) Submission ID#1539434

Human transferrin and lactoferrin cooperatively support colonization by Neisseria meningitidis in the murine nasopharynx

---

Author(s)

Isaac Lee, MSc

Laboratory Technician

University of Toronto

Elissa Currie, PhD

Post-doctoral Fellow

University of Toronto

Muhamed-Kheir Taha, MD, PhD

Principal Investigator

Institut Pasteur

Anthony B. Schryvers, PhD/MD

Professor

University of Calgary

Scott Gray-Owen, PhD

Professor

University of Toronto

Background

Neisseria meningitidis (Nm) is a regular colonizer of the human nasopharynx, whose human-restricted nature makes in vivo studies of host-pathogen interaction difficult. Expression of human CEACAM1 (hCCM1) is required for Nm colonization of mouse nasal passages. Nm also has an exquisite specificity for the human forms of transferrin (hTf) and lactoferrin (hLf), being unable to utilize iron from mouse homologues of these proteins when infecting the murine nasopharynx.

Aim/Methods

To overcome this host restriction, we have used transgenic mice expressing various combinations of hCCM1, hTf and/or hLf to uncover the role of iron sources during Nm colonization.

Results

In vitro studies revealed that serum from hTf- or hLf-transgenic mice supported Nm growth, but serum from their wild type littermates did not. Notably, growth in the transgenic mice serum was comparable to that seen in human sera. Nasal infection of hCCM1 mice with iron-starved Nm resulted in no recoverable meningococci

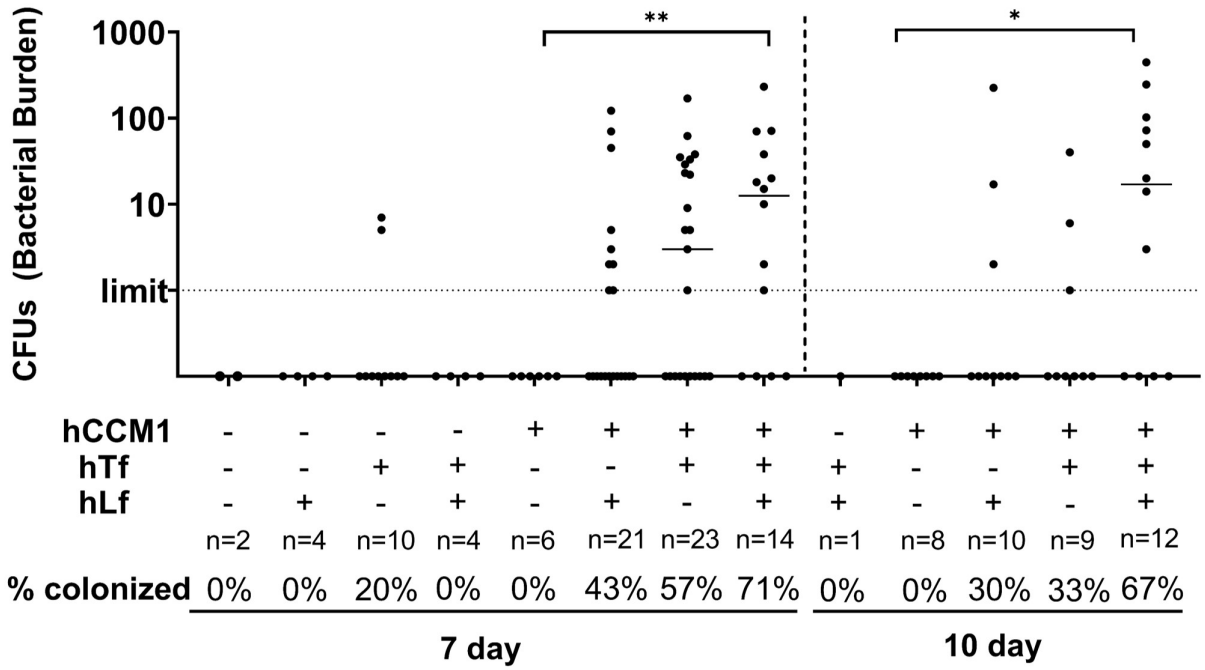
at 7 days post-infection. Mice co-expressing hTf and/or hLf along with hCCM1 allowed for Nm recovery at both 7 and 10 days post-infection. Notably, the expression of all 3 transgenes resulted in the highest median burden and colonization rates, suggesting that the two iron sources may have different contributions to Nm infection. The contribution of the two bacterial receptor systems was further explored by a competitive infection with a mixture of ΔtbpB and ΔlbpB strains of Nm in the triple transgenic (hCCM1/hTf/hLf) mice. At 3 days post-infection, 75% of mice were colonized by higher proportion of ΔlbpB Nm, suggesting that transferrin may be more useful during early colonization.

Conclusions
Collectively, this work establishes critical contributions of the meningococcal transferrin and lactoferrin receptors during nasal colonization and provides a novel in vivo model to better study Nm host-pathogen interaction at the mucosa.

Uploaded File(s)

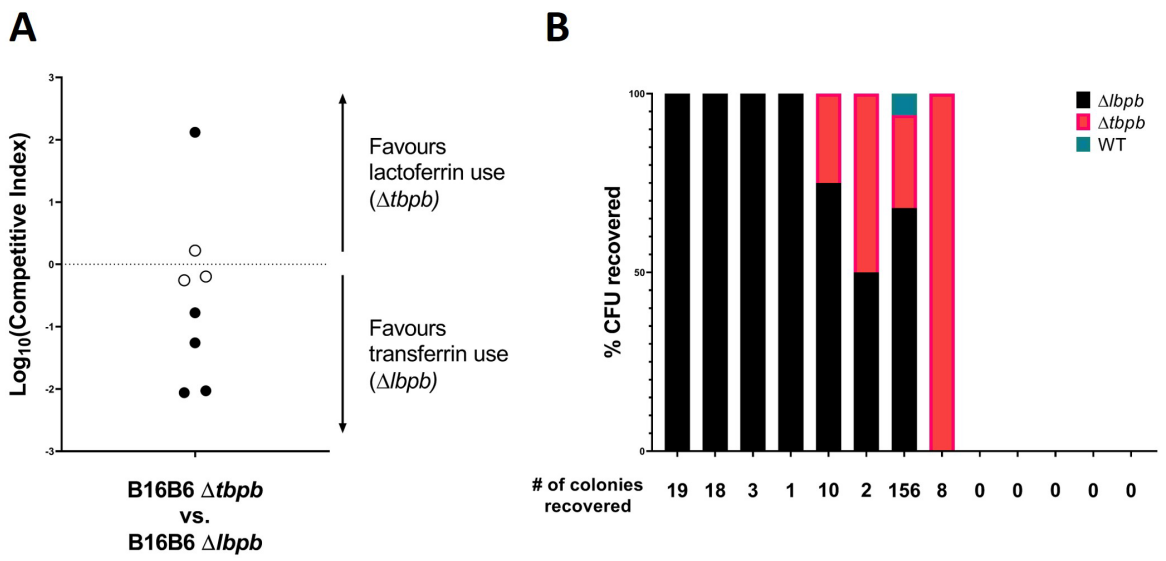
Supplemental Document Upload

Combined expression of hTf and hLf with hCEACAM1 enhances nasopharyngeal colonization rates at day 7 and 10 post-infection



Supplemental Figure 1. Combined expression of hTf and hLf with hCEACAM1 enhances nasopharyngeal colonization rates at day 7 and 10 post-infection. FvB mice were nasally infected with 10^6 CFU/mouse of *Nme* B16B6. Bacterial enumeration was done 7-and 10-days post-infection via nasal swabs. Data are pooled from 3 independent experiments. Limit represents limit of detection; bars represent group median. Kruskal-Wallis test with multiple comparisons. **p< .01, *p < .05. hCCM1, human CEACAM1; hTF, human transferrin; hLF, human lactoferrin.

Utilization of hTf may be more advantageous than hLf during early N. meningitidis colonization of the murine nasopharynx



Supplemental Figure 2. Utilization of hTf may be more advantageous than hLf during early *N. meningitidis* colonization of the murine nasopharynx. FvB hCEACAM1^{+} hTf^{+} hLf^{+} mice were nasally infected with a 1:1 mix of *Nme* B16B6 deficient in TbpB (Δ*tbpb*) or LbpB (Δ*lbpb*). Bacterial enumeration was done 5-days post-infection via nasal swabs. **A)** Competitive index of Δ*tbpb* vs. Δ*lbpb Nme*. **B)** Percentage of each mutant colonies recovered per mouse. Competitive index = (Δ*tbpb*:Δ*lbpb)/* (Δ*tbpb inoculum concentration/*Δ*lbpb inoculum concentration).* Log_{10}[Competitive index] > 0 = selective advantage for Δ*tbpb*; Log_{10}[Competitive index] > 0 = selective advantage for Δ*lbpb.* For the purpose of the calculation, value of 0.1 was used when no colonies were recovered for either mutant. Empty circles in **A)** indicate mice with mixed population of knockout strains recovered.