Malaria parasite infection dampens the immune response to *Salmonella* Typhimurium: a role for macrophage polarization

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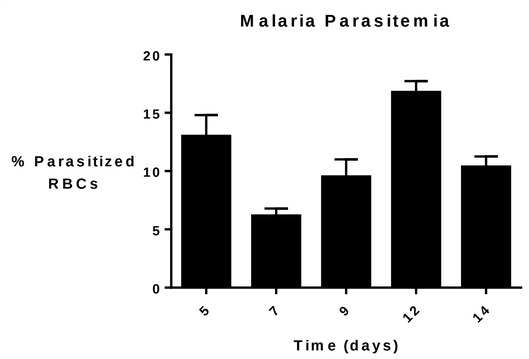
Introduction

Malaria is an important risk factor of disseminated non-typhoidal *Salmonella* (NTS), which has a case fatality of 20-25% in children in sub-Saharan Africa.[1] Multiple co-infection mouse studies have begun to elucidate molecular mechanisms behind this impaired immune response to NTS during malaria parasite infection. For instance, Lokken et al. (2014) found that IL-10 produced during malaria infection likely suppresses the ability of macrophages to control bacterial infection.[2] Macrophage activation can be seen on a spectrum, from more pro-inflammatory macrophages (M1) that have enhanced killing of intracellular microorganisms to more anti-inflammatory macrophages (M2) that dampen the immune response.[3] We hypothesize that malaria infection polarizes the macrophage playing field from M1 to M2, weakening the immunological response to subsequent NTS infection. Elucidating molecular mechanisms during co-infection can reveal important targets for therapeutic intervention.

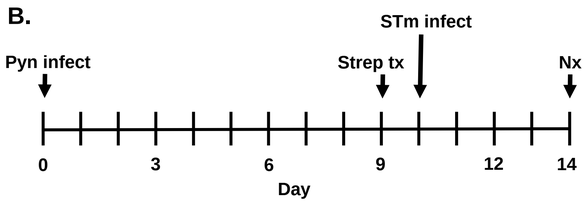
Animal Model in C57BL/6 mice

A.



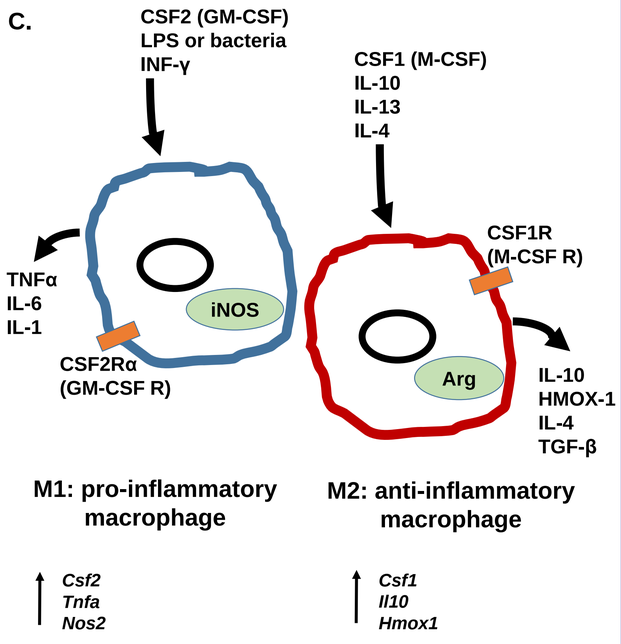
A. Parasitemia in *P. yoelii*-infected mice. Time is in days after malaria infection. C57BL/6 mice were inoculated via intraperitoneal injection with *P. yoelii*-infected RBCs. Control mice received the same amount of blood from uninfected mice. Data represent the mean ± SEM of 4 mice.

B.



B. Experimental set-up. Eight-week old mice were parasite-infected (n=4) or mock-infected (n=4) via intraperitoneal injection. All mice were treated via oral gavage with streptomycin on day 9 followed by oral gavage of 10^8 CFU of a *Salmonella* Typhimurium (STm) overnight culture on day 10. Necrospy was performed on day 14, and RNA was isolated from liver CD11b+ cells. RNA was normalized to 40 ng/uL, reverse transcribed to cDNA, and analyzed by quantitative real-time PCR.

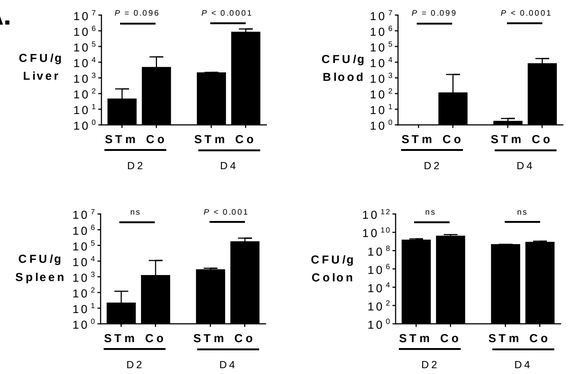
C.



C. Macrophage polarization schematic and hypothesized transcript changes.[4]

Malaria parasite infection alters the inflammatory cytokine environment to promote *Salmonella* outgrowth

A.

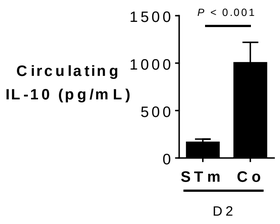


A. STm outgrowth in liver, spleen, blood, and colon contents on days 12 and 14 after malaria parasite infection and day 2 and 4 after STm inoculation. Data are represented as means and SEM (n=3-4). Statistical significance was determined using an unpaired Student's *t* test.

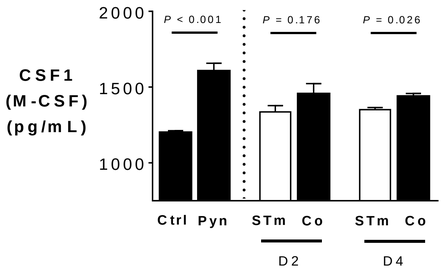
B. ELISA results. Levels of circulating IL-10 (pg/mL) 12 days after parasite or mock infection and 2 days after STm infection (n=5). Data are represented as means and SEM. Statistical significance was determined using an unpaired Student's *t* test.

C. ELISA results. Levels of CSF1 (M-CSF) (pg/mL) 10 days after malaria parasite infection (Pyn) compared to mock-infected control (Ctrl) (n=3-4). D2 represents 12 days after malaria parasite infection (Co) or mock infection (STm) and 2 days after STm infection (n=4). D4 represents 14 days after malaria parasite infection (Co) or mock infection (STm) and 4 days after STm infection (n=3). Data are represented as means and SEM. Statistical significance was determined using an unpaired Student's *t* test.

B.

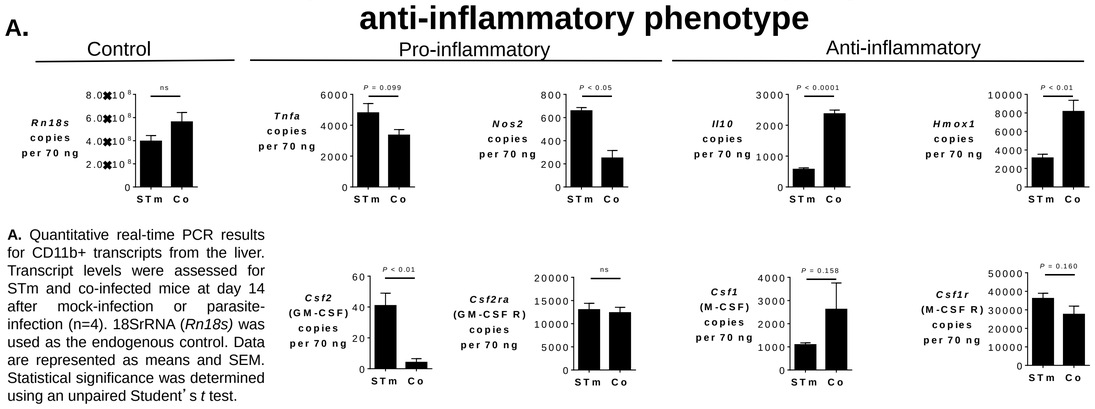


C.



Malaria parasite infection shifts macrophages to an anti-inflammatory phenotype

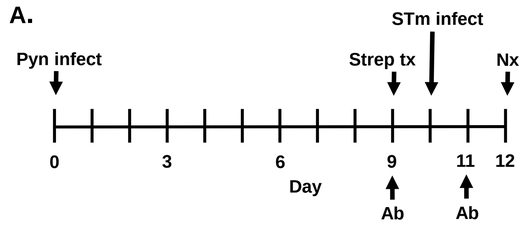
A.



A. Quantitative real-time PCR results for CD11b+ transcripts from the liver. Transcript levels were assessed for STm and co-infected mice at day 14 after mock-infection or parasite-infection (n=4). 18SrRNA (*Rn18s)* was used as the endogenous control. Data are represented as means and SEM. Statistical significance was determined using an unpaired Student's *t* test.

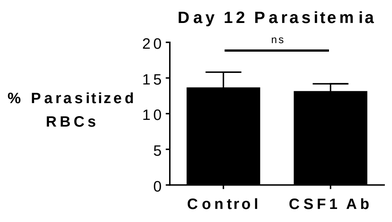
CSF1 (M-CSF) depletion leads to increased pro-inflammatory cytokines and mixed infection control of *Salmonella* outgrowth

A.



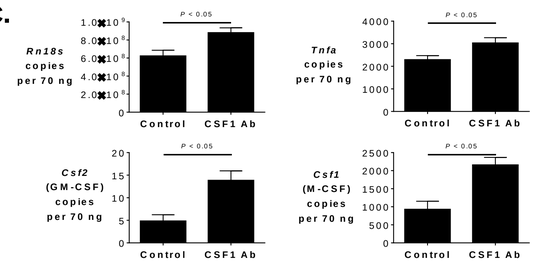
A. Experimental set-up. Eight-week old mice were parasite-infected via intraperitoneal injection. All mice were treated via oral gavage with streptomycin on day 9 followed by oral gavage of 10^8 CFU of a STm overnight culture. Antibody treatments of either anti-CSF1 (n=4) or control IgG (n=4) were given on days 9 and 11 in the quantity of 0.5 mg per mouse, intraperitoneally.

B.



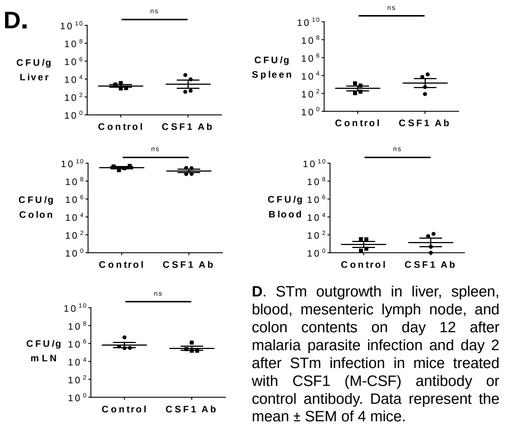
B. Parasitemia in *P. yoelii*-infected mice at day 12 post-infection. C57BL/6 mice were inoculated via intraperitoneal injection with *P. yoelii*-infected RBCs. Data represent the mean ± SEM of 4 mice.

C.



C. Quantitative real-time PCR results for CD11b+ transcripts from the liver. Transcript levels were assessed for co-infected animals treated with anti-CSF1 (n=4) or a control IgG (n=4). Endogenous control used for *Tnfa, Csf2, and Csf1* is represented by the *Rn18s* graph. Data are represented as means and SEM. Statistical significance was determined using an unpaired Student's *t* test.

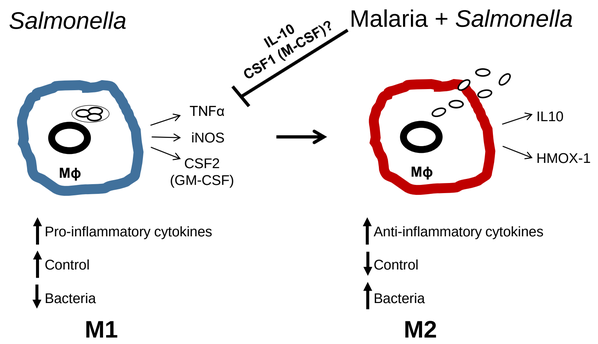
D.



D. STm outgrowth in liver, spleen, blood, mesenteric lymph node, and colon contents on day 12 after malaria parasite infection and day 2 after STm infection in mice treated with CSF1 (M-CSF) antibody or control antibody. Data represent the mean ± SEM of 4 mice.

Conclusions

- Malaria parasite infection leads to outgrowth of STm at systemic sites, suggesting that malaria dampens the immune response to STm.
- Malaria parasite infection leads to increased circulating IL-10 and CSF1, anti-inflammatory cytokines.
- Malaria parasite infection shifts macrophages to an anti-inflammatory phenotype.



- *Csf1* transcripts are unchanged by malaria, suggesting that the increase in circulating CSF1 does not come from CD11b+ cells in the liver.
- *Csf1r* and *Csf2ra* transcripts are unchanged by malaria, suggesting that CD11b+ cells in the liver do not significantly change receptor transcription for CSF1 and CSF2.
- Depletion of CSF1 leads to:
  - increased transcript levels of *Tnfa,* suggesting a pro-inflammatory shift in the absence of CSF1.
  - increased transcript levels of *Csf2,* suggesting a pro-inflammatory shift in the absence of CSF1.
  - increased transcript levels of *Csf1,* suggesting compensation.
  - mixed control of infection at systemic sites, suggesting that increased CSF1 is not the main driver of the anti-inflammatory shift during malaria at days 9-12.

Future directions

- Assess protein levels of CSF1, CSF1R, CSF2, CSF2Rα and see if they correlate with transcript findings.
- Add back CSF2 during co-infection and see if it restores control of the STm infection.
- Deplete CSF1 earlier on during malaria infection and see if there is a time-dependent shift in pro-inflammatory and anti-inflammatory cytokines that leads to better control of STm infection.

References

1) Feasey, N. A., et al. (2012). "Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa." Lancet 379(9835): 2489-2499.
2) Lokken, K. L., et al. (2014). "Malaria parasite infection compromises control of concurrent systemic non-typhoidal Salmonella infection via IL-10-mediated alteration of myeloid cell function." PLoS Pathog 10(5): e1004049.
3) Mosser, D. M. and J. P. Edwards (2008). "Exploring the full spectrum of macrophage activation." Nat Rev Immunol 8(12): 958-969.
4) Bosurgi, L., et al. (2011). "Macrophages in Injured Skeletal Muscle: A Perpetuum Mobile Causing and Limiting Fibrosis, Prompting or Restricting Resolution and Regeneration." Front Immunol 2: 62.

Acknowledgements

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