Animal models to inform clinical research: vitamin A supplementation combats invasive non-typhoidal *Salmonella* infection

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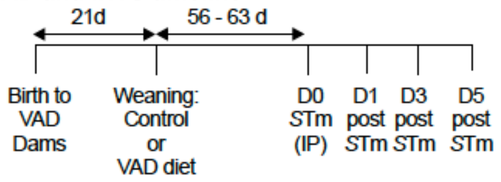
Introduction

There is an epidemic of invasive non-typhoidal *Salmonella* (iNTS) infection, with 3.4 million cases and approximately 680,000 deaths occurring annually worldwide (1). Children in sub-Saharan Africa are particularly vulnerable, with a case fatality of 20-25% (1,2). An important risk factor of iNTS infection is malnutrition (2). In 2009, the WHO estimated that 190 million preschool age children were vitamin A deficient (VAD) (3). Animal models of infectious disease are critical for elucidating immunological mechanisms underlying susceptibility and informing next steps in clinical research. Using a mouse model of vitamin A deficiency, our laboratory has found that VAD mice are more susceptible to developing iNTS. Importantly, vitamin A supplementation (VAS) improves control of infection. VAS is not new to global health; it is efficacious prevention in reducing incidence of diarrhea in children from 6 months to 5 years of age (4). Our studies are novel in that we are investigating vitamin A as a treatment for iNTS bloodstream infection, which often presents as a febrile systemic illness without diarrhea. The purpose of our animal model of vitamin A deficiency and iNTS is to inform clinical research and contribute to the improvement of human health outcomes globally.

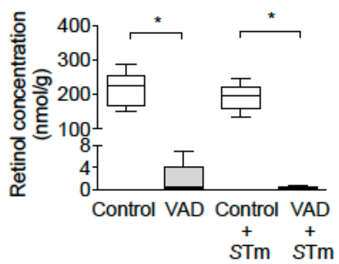
Mouse model of vitamin A deficiency and invasive non-typhoidal *Salmonella*

A. VAD and iNTS timeline



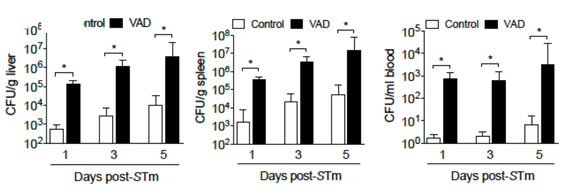
A. Generation of VAD mice and experimental design of systemic *S.* Typhimurium (STm) colonization.

B. Hepatic retinol is decreased in VAD mice



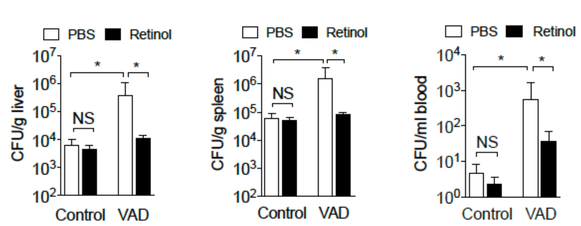
B. Hepatic retinol concentration in control and VAD mice in uninfected mice and mice 3 days after infection with STm. Experiment was done with 6-12 mice per group. Statistical significance was determined on log-transformed values using a one-way analysis of variance (ANOVA) with a post-hoc Tukey test.

C. *Salmonella* burden is increased at systemic sites in VAD mice



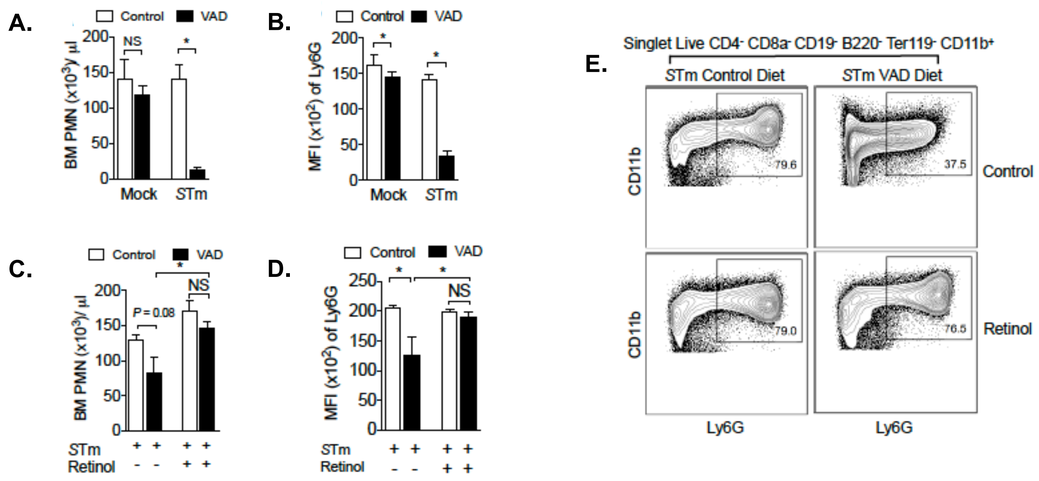
C. STm colonization at systemic sites 1, 3, and 5 days after infection of control and VAD mice. Bars represent mean + SEM of 4-9 mice per group. Statistical significance was determined on log-transformed values using an unpaired Student's *t* test with significance at P < 0.05.

D. Retinol treatment leads to improved control of *Salmonella* burden at systemic sites in VAD mice



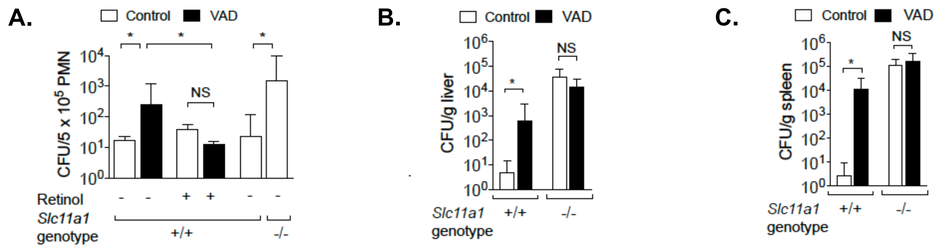
D. STm colonization at systemic sites 3 days after infection in control and VAD mice treated with either PBS or retinyl palmitate (600 IU delivered IG) at 7 days and 3 days prior to infection. Bars represent the mean + SEM of 6-16 mice per group. Statistical significance was determined on log-transformed values using a one-way analysis of variance (ANOVA) with a post-hoc Tukey test.

Granulopoiesis is compromised during invasive non-typhoidal *Salmonella* infection and vitamin A deficiency



A. Number of bone marrow neutrophils (singlet live CD4$^-$CD8a$^-$CD19$^-$B220$^-$Ter119$^-$CD11b$^+$Ly6G$^+$) 1 day after STm infection or mock infection. Data represent the mean + SEM of 5-6 mice per group. Statistical significance was determined on log-transformed values using an unpaired Student's *t* test with significance at P < 0.05.
B. Median fluorescent intensity (MFI) of Ly6G 1 day after STm infection or mock infection. Data represent the mean + SEM of 5-6 mice per group. Statistical significance was determined on log-transformed values using an unpaired Student's *t* test with significance at P < 0.05.
C. Number of bone marrow neutrophils 3 days after STm infection with or without retinyl palmitate treatment (600 IU delivered IG) administered 7 days and 3 days prior to infection in VAD and control mice.
D. Median fluorescent intensity (MFI) of Ly6G 3 days after STm infection with or without retinyl palmitate treatment (600 IU delivered IG) administered 7 days and 3 days prior to infection in VAD and control mice.
E. Representative flow cytometry plots from male mice for bone marrow neutrophil frequency (singlet live CD4$^-$CD8a$^-$CD19$^-$B220$^-$Ter119$^-$CD11b$^+$Ly6G$^+$).

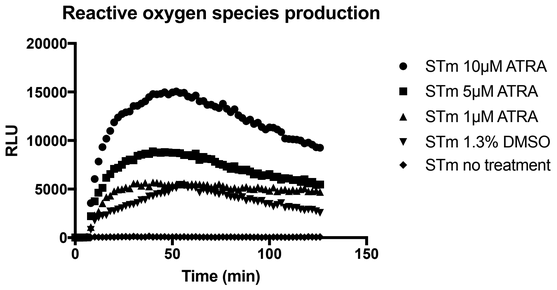
Vitamin A deficiency reduces *Slc11a1*-mediated neutrophil bactericidal activity



A. STm burden in splenic neutrophils 3 days after infection in control and VAD *Slc11a1*$^{+/+}$ mice treated with PBS or retinyl palmitate (600 IU delivered IG) at 7 days and 3 days prior to infection. Control mice analyzed include both *Slc11a1*$^{+/+}$ and *Slc11a1*$^{-/-}$. Data represent the mean + SEM 3-4 mice per group. Statistical significance was determined on log-transformed values using a one-way analysis of variance (ANOVA) with a post-hoc Sidak test.
B. STm colonization of the liver and spleen (**C**) 3 days after oral infection from control and VAD *Slc11a1*$^{+/+}$ and *Slc11a1*$^{-/-}$ mice. Bars represent mean + SEM of 6-8 mice per group. Statistical significance was determined on log-transformed values using an unpaired Student's *t* test with P<0.05.

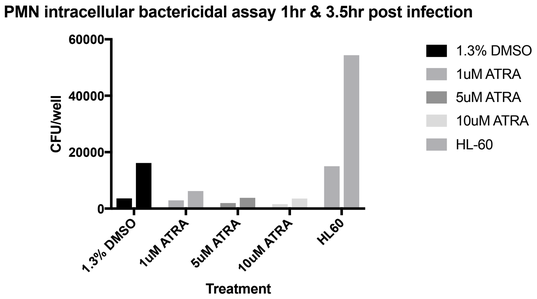
Reactive oxygen species production and intracellular bactericidal activity of neutrophils in cell culture is dependent on vitamin A concentration

A. Reactive oxygen species production



A. Pilot assay for reactive oxygen species production. HL-60 cells were treated with 1.3% DMSO or a titration of all-trans retinoic acid (ATRA) for 5 days to differentiate into neutrophils. 5E4 cells/well of a luminol-treated cell suspension was seeded per well in a 96-well plate and read for basal luminescence. Bacteria opsonized with 20% normal human serum were added per well at an MOI of 10:1. A control of 20% normal human serum only was used. Kinetic luminescence was read for 2 hours and recorded as relative light units (RLU).

B. PMN intracellular bactericidal assay 1hr & 3.5hr post infection



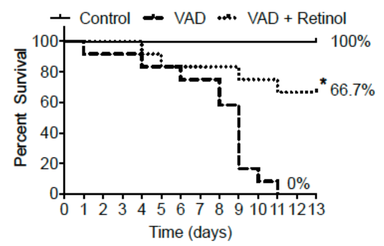
B. Pilot assay for intracellular bactericidal activity. HL-60 cells were treated with 1.3% DMSO or a titration of all-trans retinoic acid (ATRA) for 5 days to differentiate into neutrophils. Undifferentiated HL-60 cells were used as a control. 5E5 neutrophils were seeded per well in a 24-well plate and opsonized STm was added for an MOI of 10:1. After 30 minutes allowed for internalization, gentamicin treatment was applied to remove any viable extracellular bacteria. CFUs were plated at 1 hour and 3.5 hours after infection.

Conclusions

- Vitamin A deficiency in mice can be used as a model of how malnutrition compromises the immune response to iNTS infection.
- Molecular mechanism:
  - Emergency granulopoiesis is impaired during iNTS infection in VAD mice.
  - Neutrophils recruited to systemic sites during iNTS infection in mice have compromised control of the infection and is dependent on *Slc11a1*.
  - Reactive oxygen species formation is dependent on vitamin A concentration in cell culture.
  - Intracellular bactericidal capacity is dependent on vitamin A concentration in cell culture.
- Clinical application:
  - Vitamin A supplementation rescues the immunologic phenotype of iNTS infection in VAD mice.

Future directions

Vitamin A supplementation increases survival in VAD mice with iNTS



Percent survival of mice treated with two consecutive doses of either PBS or retinyl palmitate (600 IU delivered IG) starting one day after STm infection. Data represent percent survival of 12 mice per group from 3 experiments. Statistical significance was determined using a Log-rank (Mantel-Cox) test.

- Clinical application: Assess the combination of vitamin A supplementation and antibiotic treatment in combating multidrug-resistant STm in the mouse model.
- Molecular mechanism: Investigate the role of *Slc11a1* in formation of reactive oxygen species, intracellular killing function, and maturation state of neutrophils during iNTS and vitamin A deficiency utilizing both mouse models and cell culture.

References

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