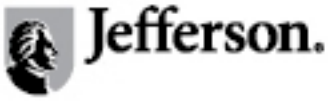

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Macrophages and neutrophils from humans and mice kill larval Strongyloides stercoralis during innate immunity

Sandra Bonne-Annee
Thomas Jefferson University, Sandra.Bonne-Annee@jefferson.edu

Laura A. Kerepesi
Thomas Jefferson University

Jessica A. Hess Ligas
Thomas Jefferson University, Jessica.Hess@jefferson.edu

David Abraham, PhD
Thomas Jefferson University, david.abraham@jefferson.edu

Macrophages and neutrophils from humans and mice kill larval *Strongyloides stercoralis* during innate immunity

Sandra Bonne-Année, Laura A. Kerepesi, Jessica A. Hess, David Abraham

Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA, USA, 19107

ABSTRACT

The parasitic nematode *Strongyloides stercoralis* (*Ss*) infects 30-100 million people worldwide, yet little is known about the immune response in humans. Previous studies on innate immunity to *Ss* in mice have demonstrated a role for eosinophils, neutrophils (PMN) and complement activation in the protective immune response. The goal of this study was to determine the role of macrophages (MΦ)) in innate immunity to *Ss* in humans and mice. When human MΦ or PMN were cultured independently, MΦ and PMN did not kill the larvae; however, larval killing did occur when both human MΦ and PMN were combined in vitro in the presence of complement. To examine the role of mouse MΦ in the immune response against *Ss*, bone marrow-derived MΦ were either: 1) cocultured with PMN and larvae in vitro or 2) placed in diffusion chambers with larvae and implanted subcutaneously into naïve mice. Larval killing only occurred in vitro if both MΦ and PMN were present. In addition, MΦ implanted in naïve mice killed the larvae within 7 days. To determine the phenotype of MΦ during the immune response to *Ss,* mice were infected subcutaneously with larvae and peritoneal exudates cells (PEC) were analyzed by flow cytometry to quantify classically activated MΦ (CAMΦ) and alternatively activated MΦ (AAMΦ). Analysis of PEC from mice with primary infections revealed an increase in AAMΦ in the peritoneal cavity at levels higher than in control mice. To determine if CAMΦ and/or AAMΦ functioned in killing the larvae, MΦ were stimulated in vitro with IL-4 to induce AAMΦ or IFN-γ/LPS to induce CAMΦ. AAMΦ, but not CAMΦ, killed the *Ss* larvae both in vitro and after 3 days within diffusion chambers in vivo. We conclude from these studies that both human and mouse MΦ, in conjunction with PMN, kill the parasitic nematode *Ss.* Furthermore, infection of mice with *Ss* results in the induction of AAMΦ which kill the parasite both in vitro and in vivo.

METHODS

Human

- Monocytes were obtained from donors primed with G-CSF to mobilize bone marrow-progenitors and stem cells.
- The monocytes were allowed to adhere to tissue culture plates for 2 hours and differentiated into macrophages after 7 days of culture with M-CSF.
- PMN were isolated from the blood of healthy donors by centrifugation using Lympholyte®-poly.

Mouse

- Bone marrow was isolated from naïve C57BL/6 mice and cultured for 5 days in conditioned media.
- MΦ were then left untreated or stimulated overnight with IL-4 to induce AAMΦ and IFN-γ/LPS to induce CAMΦ.
- PMN were isolated from the bone marrow of naïve C57BL/6 mice using Percoll® gradient centrifugation. The population was further enriched by negative selection using MACS® cell separation and monocyte depletion.

RESULTS

1.

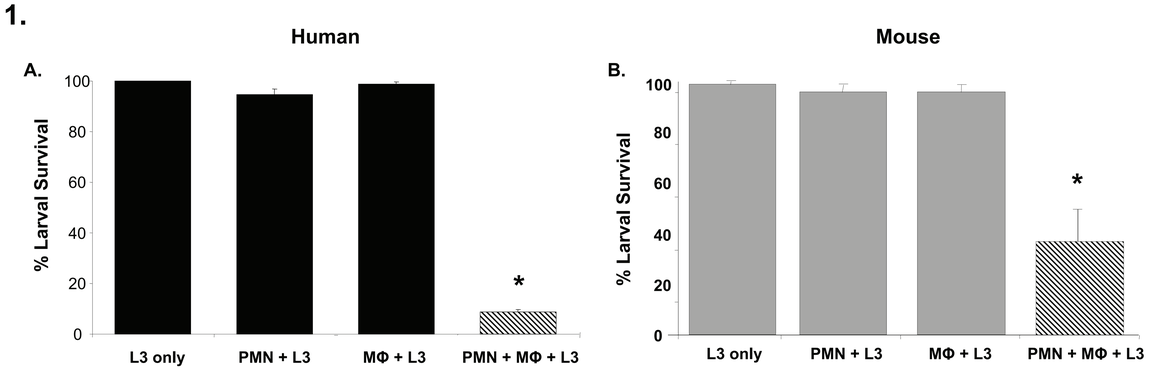

Figure1. MΦ and neutrophils are required for larval killing in vitro. A) Human MΦ and neutrophils were placed in 96-well plates with 50 L3 and autologous serum as a source of complement. Larval survival was assessed after 48 hours. **B)** MΦ and neutrophils isolated from C57BL/6 mice were placed in 96-well plates with 50 L3 for 22 hours. Normal mouse serum was added to all wells as a source of complement. Data are shown as mean ± SD *, p<0.05.

2.

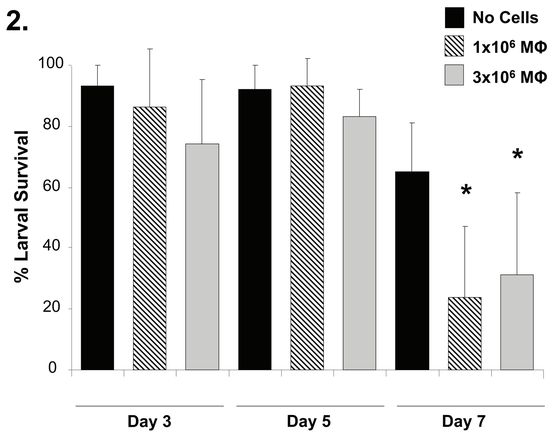

Figure 2. MΦ killing of larvae in vivo. MΦ were placed in cell impermeable diffusion chambers, with a membrane pore-size of 0.1µm, with 50 L3. The diffusion chambers were then implanted into naïve C57BL/6 mice for a period of 3, 5, 7 days. The viability of cells removed from diffusion chambers at each time point was greater than 96%. Data are shown as mean ± SD *, p<0.05.

3.

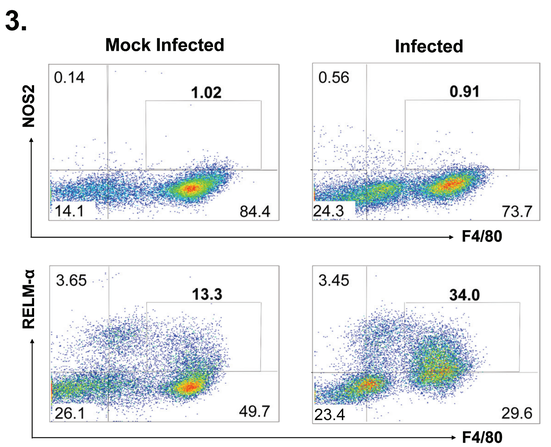

Figure 3. Analysis of MΦ subsets following infection in vivo. Mice were infected subcutaneously with 5,000 L3 or media. PEC were harvested 7 days post infection and stained for F4/80, CAMΦ marker NOS2, and AAMΦ marker RELM-α. Gate depicts the frequency of F4/80^{hi} double positive cells. All data are representative of two to five mice per group.

4.

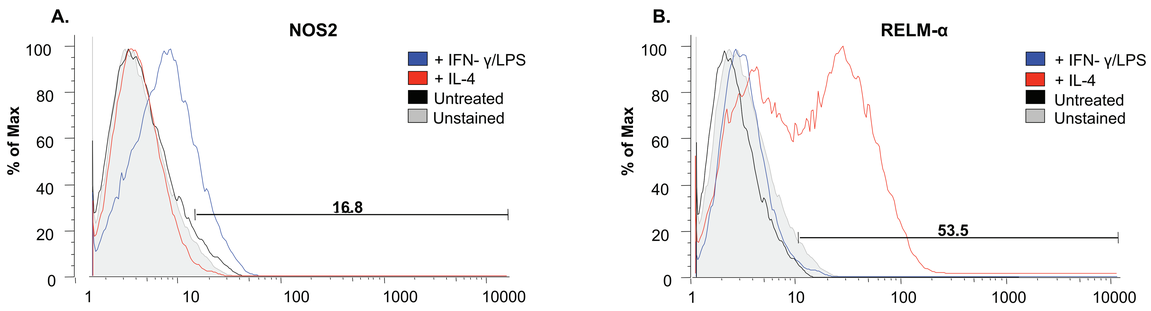

Figure 4. Induction of macrophage subsets in vitro. Bone marrow-derived macrophages were stimulated overnight with IL-4 to induce AAMΦ and IFN- γ/LPS to induce CAM Φ. Cells were then stained for flow cytometry using intracellular markers NOS2 for CAMΦ and RELM-α to confirm AAMΦ.

5.

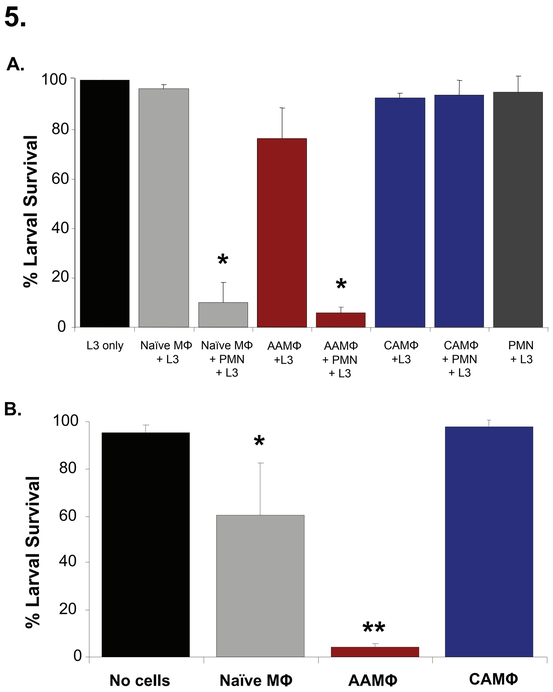

Figure 5. AAMΦ killing in vitro and in vivo. (**A**) MΦ were differentiated in vitro and placed in 96-well plates with PMN and larvae. Larval survival was assessed after 22 hours. MΦ recovered from wells had a viability greater than 95%. (**B**) MΦ subsets were placed into diffusion chambers containing larvae. The chambers were then implanted into naïve mice for 3 days. Cells recovered from chambers had a viability of greater than 95%. Data are shown as mean ± SD *, p< 0.05, **, p<0.05 when compared to naïve MΦ.

CONCLUSIONS

- **Both human MΦ and neutrophils are required to kill *S. stercoralis* larvae in vitro.**
- **Both mouse MΦ and neutrophils are required to kill *S. stercoralis* larvae in vitro.**
- **Infection with *S. stercoralis* larvae results in the induction of AAMΦ in mice.**
- **In mice, AAMΦ can kill *S. stercoralis* larvae during the innate immune response.**

ACKNOWLEDGEMENTS

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