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

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**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 naive mice. Larval killing only occurred in vitro if both MΦ and PMN were present. In addition, MΦ implanted in naive 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.

**RESULTS**

1. **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 Lymphoprep®-poly.

2. **Mouse**

   - Bone marrow was isolated from naive 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 naive C57BL/6 mice using Percoll® gradient centrifugation. The population was further enriched by negative selection using MACS® cell separation and monocyte depletion.

3. **In vitro**

   Culture with MΦ, PMN, L3 and complement

4. **In vivo**

   Diffusion Chamber 0.45 μm pore size membrane

5. **Naïve**

   Culture with MΦ, PMN, L3 and complement

**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 Lymphoprep®-poly.

**MOUSE**

- Bone marrow was isolated from naive 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 naive C57BL/6 mice using Percoll® gradient centrifugation. The population was further enriched by negative selection using MACS® cell separation and monocyte depletion.

**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.

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**Figure 1.** 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.

**Figure 2.** No Cells 1x10⁶ MΦ 3x10⁵ MΦ

**Figure 3.** 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 naive 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 naive MΦ.

**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 CAMΦ. Cells were then stained for flow cytometry using intracellular markers NOS2 for CAMΦ and RELM-α to confirm AAMΦ.

**Figure 5.** 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 RELM-α, CAMΦ marker NOS2, and AAMΦ marker RELM-α. Data depicts the frequency of RELM-α double positive cells. All data are representative of two to five mice per group.