Effects of Vaccination Routes on Generating Innate Immune Memory

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Abstract

Traditionally vaccines rely on adaptive immunity, by activating the immune system with an attenuated pathogen or pathogen subunit to elicit a heightened response at subsequent exposures. Thus, adaptive immune responses provide memory-based immunity. However, recent work with Mycobacterium tuberculosis and other pathogens has identified a role for "trained" macrophages in reducing bacterial burdens and improving disease outcomes. Here we studied the potential role of trained monocytes in immune responses to Francisella tularensis, an intracellular bacterium that replicates within mammalian macrophages. We used an in vitro culture approach that serves as a functional correlate to predicts vaccine-induced protection. We vaccinated mice using Francisella tularensis Live Vaccine Strain (LVS). We infected murine macrophages from naïve mice, from mice vaccinated intradermally, or from mice vaccinated intravenously with LVS. LVS-infected macrophages were then cultured alone, or co-cultured with naïve splenocytes, splenocytes from mice vaccinated intradermally, or splenocytes from mice vaccinated intravenously. In co-cultures, immune (but not naïve) splenocytes reduced intramacrophage bacterial replication. We observed no differences in control of intramacrophage bacterial replication when comparing co-cultures with naïve macrophages or macrophages from intradermally vaccinated mice. However, in co-culture experiments carried out using cells from intravenously vaccinated mice, we observed subtle differences in control of growth. LVS-immune immune splenocytes controlled bacterial replication in primed macrophages better than in naïve macrophages. Similarly, nitric oxide and IFN-g production in the corresponding supernatants were greater when using primed macrophages. Nonetheless, in the context of this in vitro co-culture assay, the data do not support substantial development of trained monocytes in either intradermally or intravenously mice vaccinated with LVS.

Introduction

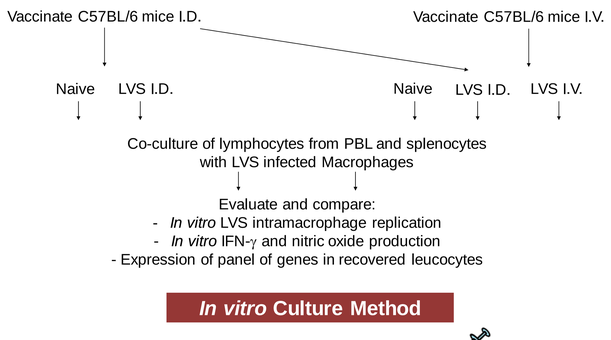
Previous studies indicated that:

- Along with the generation of memory T and B lymphocytes, vaccination may stimulate durable innate immune responses by myeloid cells such as monocytes and macrophages, dubbed "trained" immunity.
- Bacillus Calmette-Guérin (BCG) vaccination enhances production of myeloid cell-derived proinflammatory cytokines in response to secondary infections in some circumstances, particularly when BCG is administered intravenously. In addition to traditional adaptive immunity, this "trained" immunity from vaccination is associated with accelerated clearance of *Mycobacterium tuberculosis*.
- Immune responses to intracellular bacterium *Francisella tularensis* and the Live Vaccine Strain (LVS) of *F. tularensis* are very similar to those against *M. tuberculosis*. We have used this BSL-2 rodent model to study the nature of T cell-mediated protective immunity to intracellular bacteria in general.
- We have used an *in vitro* co-culture system to study mechanisms by which immune T cells control the replication of *Francisella* or *M. tuberculosis* within macrophages. This approach provides a means to readily evaluate the functions of T cells and macrophages from vaccinated animals *in vitro*.

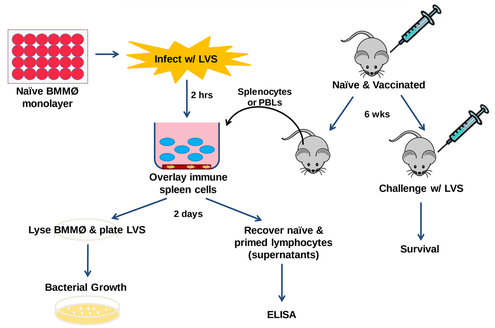
Therefore, the objective of this study is to compare intramacrophage bacterial control by LVS-immune lymphocytes in bone marrow-derived macrophages from naïve and LVS-vaccinated mice.

Methods

Experimental design



In vitro Culture Method

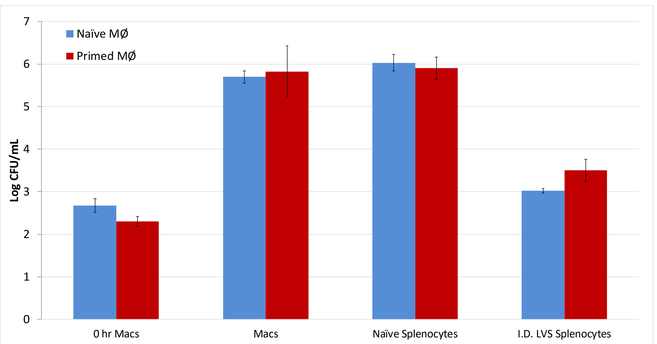


Synopsis: *Francisella tularensis* (LVS) vaccinated mice do not show evidence of trained monocytes in response to vaccination.

Results

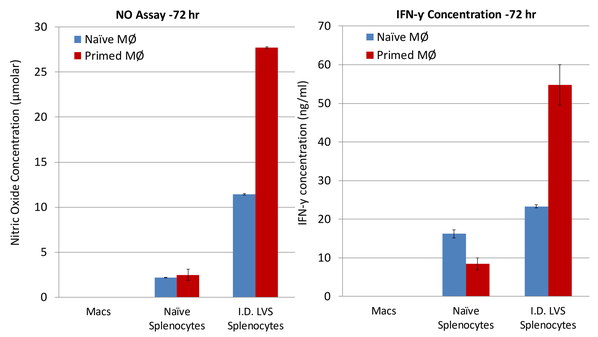
I.D. Vaccination

Fig 1. Control of *in vitro* bacterial replication is comparable when using bone marrow derived macrophages from naïve mice or mice vaccinated i.d. with LVS



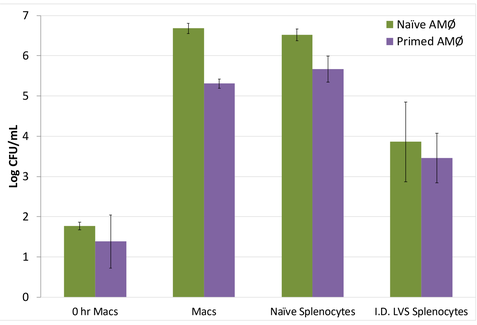
Mice were vaccinated i.d. with 10^4 LVS. Eight weeks later, bone marrow-derived macrophages were prepared in 24 well plates from naïve or vaccinated mice. Confluent macrophages were infected with LVS, and then naïve or immune splenocytes were overlaid on infected macrophages. Three days later, supernatants were collected (see Fig. 2), and macrophages then lysed and plated on agar to count recovered bacteria. Numbers of bacterial colony forming units (CFU) +/ SD from triplicate wells of the indicated combinations from one representative experiment of 3 are shown.

Fig 2. Nitric oxide and IFN-γ production are enhanced when using bone marrow derived macrophages from mice vaccinated i.d. with LVS



Supernatants collected from co-cultures described in Figure 1 were assessed for levels of nitric oxide by Griess reaction (left panel) or for IFN-γ by capture ELISA (right panel). NO levels in µmole +/ SD from triplicate wells of the indicated combinations from one representative experiment are shown (left panel). IFN-γ levels in ng/ml +/ SD from triplicate wells of the indicated combinations from one representative experiment are shown (right panel).

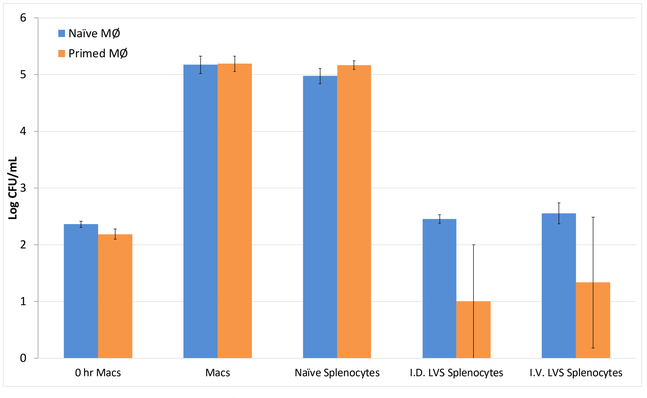
Fig 3. Control of *in vitro* bacterial replication is comparable when using alveolar macrophages from naïve mice or mice vaccinated i.d. with LVS



Mice were vaccinated i.d. with 10^4 LVS. Eight weeks later, alveolar macrophages were prepared in 96 well plates from naïve or vaccinated mice. Confluent macrophages were infected with LVS, and then naïve or immune splenocytes were overlaid on infected macrophages. Three days later macrophages were lysed and plated on agar to count recovered bacteria. Numbers of bacterial colony forming units (CFU) +/ SD from triplicate wells of the indicated combinations from one representative experiment are shown.

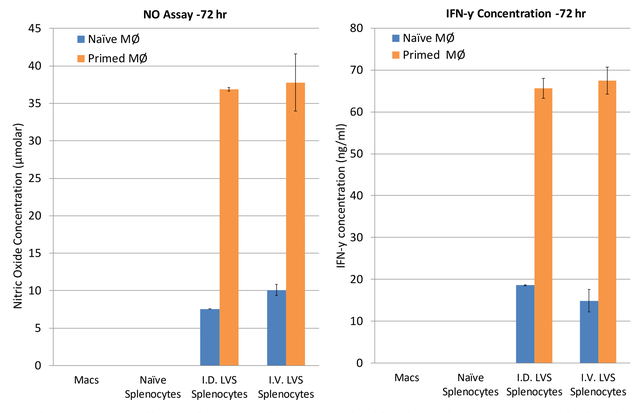
I.V. Vaccination

Fig 4. Control of *in vitro* bacterial replication is comparable when using bone marrow derived macrophages from naïve mice or mice vaccinated i.v. with LVS



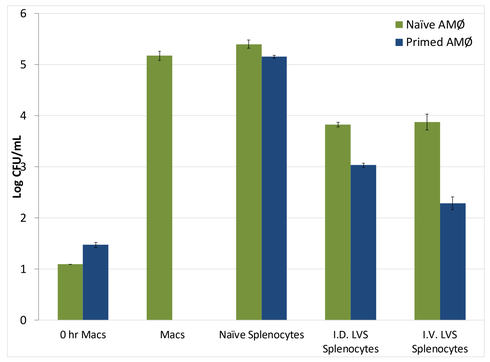
Mice were vaccinated i.v. with 10^2 LVS. Eight weeks later, bone marrow-derived macrophages were prepared in 24 well plates from naïve or vaccinated mice. Confluent macrophages were infected with LVS, and then naïve or immune splenocytes were overlaid on infected macrophages. Three days later, supernatants were collected (see Fig. 5), and macrophages then lysed and plated on agar to count recovered bacteria. Numbers of bacterial colony forming units (CFU) +/ SD from triplicate wells of the indicated combinations from one representative experiment of 5 are shown.

Fig 5. Nitric oxide and IFN-γ production are enhanced when using bone marrow derived macrophages from mice vaccinated i.v. with LVS



Supernatants collected from co-cultures described in Figure 3 were assessed for levels of nitric oxide by Griess reaction (left panel) or for IFN-γ by capture ELISA (right panel). NO levels in µmole +/ SD from triplicate wells of the indicated combinations from one representative experiment are shown (left panel). IFN-γ levels in ng/ml +/ SD from triplicate wells of the indicated combinations from one representative experiment are shown (right panel).

Fig 6. Control of *in vitro* bacterial replication is comparable when using alveolar macrophages from naïve mice or mice vaccinated i.v. with LVS



Mice were vaccinated i.v. with 10^2 LVS. Eight weeks later, alveolar macrophages were prepared in 96 well plates from naïve or vaccinated mice. Confluent macrophages were infected with LVS, and then naïve or immune splenocytes were overlaid on infected macrophages. Three days later macrophages were lysed and plated on agar to count recovered bacteria. Numbers of bacterial colony forming units (CFU) +/ SD from triplicate wells of the indicated combinations from one representative experiment are shown.

Conclusions

- Lymphocyte-mediated control of *in vitro* bacterial replication is comparable when using monocytes derived from the bone marrow or the lungs of naïve mice or mice vaccinated with LVS by either the i.d. or i.v. routes.
- Therefore, in the context of our *in vitro* co-culture system, the data to date does not support substantial development of trained monocytes in bone marrow and lungs of LVS-vaccinated mice.
- Future studies can still evaluate the role of innate immune responses through *in vivo* approaches. For example, we can vaccinate animals with LVS and challenge them with non-specific pathogens to assess the development of "trained" myeloid cells.
- We could also look at the immune response to a secondary, nonspecific infection in KO mice for a necessary component of trained monocytes, such as hypoxia-inducible factor 1-alpha (HIF1-α).
- Such studies will contribute to determining whether next generation *Francisella* vaccines should be directed to eliciting these additional innate immune responses.