Comparison of TNF-α levels produced by macrophages treated with *Brucella abortus* and *Escherichia coli LPS*

Tajie Harris
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Abstract

Brucella abortus is a Gram-negative facultative intracellular pathogen. *B. abortus* evades the host immune system in order to enter host macrophages, replicate, and cause disease. Bacterial lipopolysaccharide (LPS) stimulates the innate immune response and induces the production of the inflammatory cytokines: TNF-α, IL-1, and IL-6. *B. abortus* LPS was isolated from a rough strain and used to treat RAW 264.7 macrophage-like cells. *E. coli* Re LPS was also added to RAW cells for comparison. Enzyme linked immunosorbent assays (ELISA) were performed to quantify the amount of TNF-α produced by the RAW cells treated with LPS. To induce similar TNF-α levels, 1,000 times more *B. abortus* LPS was required, compared to *E. coli* Re LPS. This illustrates that *B. abortus* LPS fails to induce a strong innate immune response and therefore is linked to the virulence of *B. abortus*.

Introduction

The members of the genus *Brucella* usually infect animal hosts. *B. abortus* is typically found in cows, but is a zoonosis and infects a wide range of hosts including humans. In cows, *B. abortus* causes the spontaneous abortion of fetuses. Humans develop brucellosis or undulant fever, which is characterized by an intermittent fever, arthritis, anorexia, fatigue, and headache. The disease can be contracted through wounds on the skin, if it is inhaled, or if it is ingested. Long-term infection leads to granulomatous lesions. Infection is hard to diagnose because of the localized infections throughout the body. Brucellosis can be treated with antibiotics. Due to the threat of biological warfare, a vaccine is being developed for an antibiotic resistant strain of *Brucella* (1).

In order to develop attenuated organisms to be used for a vaccine, the interactions between the bacteria and host need to be understood. For example, *B. abortus*'s virulence factors need to be determined.

It is known that *B. abortus* must gain entry to the host cells in order to replicate and cause infection. In order to do this, the bacteria must evade the innate immune system that functions early in an infection. The innate immune system recognizes lipopolysaccharide (LPS) molecules on the surface of Gram-negative bacteria. This leads to the production of cytokines that are necessary for an inflammatory response. These cytokines are TNF-α, IL-1, and IL-6 (4).

TNF-α is a cytokine that stimulates the immune system by binding receptors on certain cells. The cells survive, differentiate, or undergo apoptosis depending on the receptor that is bound and the signal that is transduced through the cell. Transcription factor NF-κB is activated, which controls the expression of genes that cause inflammation and regulate

the cell cycle. NF-κB also causes the production of anti-apoptotic molecules that prevent the destruction of tissue caused by TNF-α (5). TNF-α causes the apoptosis of cells infected with *B. abortus* (2). Therefore, inhibition of an inflammatory response allows the bacteria to survive in host cells. In addition, an inflammatory response would localize more immune cells to the site of infection and lead to a stronger response.

Since *B. abortus* evades the innate immune response, it was hypothesized that the LPS does not induce a strong inflammatory response. TNF-α production would serve as an indication of the strength of the response. *E. coli* LPS is known to trigger a vigorous response and serves as comparison. This paper reports the result of comparing the TNF-α produced by macrophage like cells in response to both types of LPS.

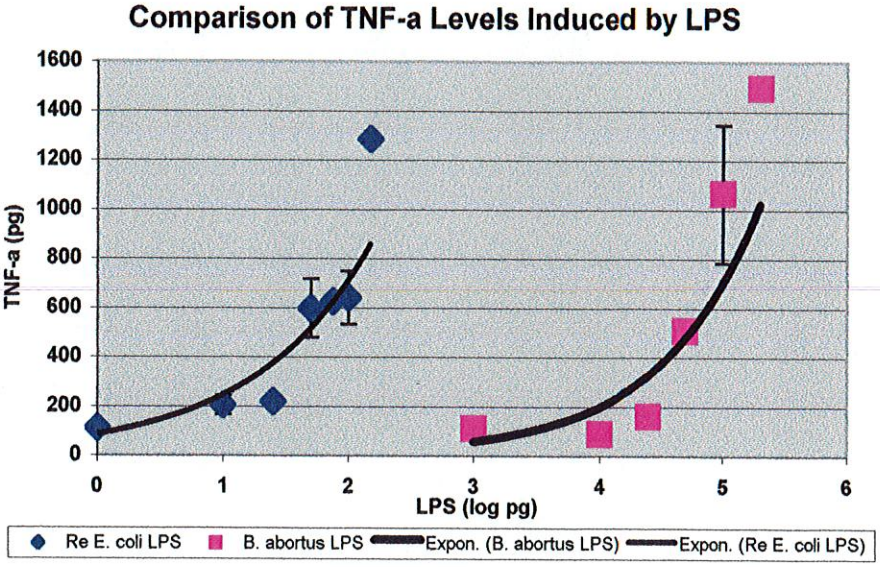
Materials and Methods

Cell Culture. RAW 264.7 murine cells were grown at 37° C with 5% CO_2. The cells were grown in RPMI 1640 supplemented with 10% fetal calf serum. Penicillin and streptomycin was added to the media to prevent bacterial contamination of the cells.

Treatment of cells with LPS. The RAW cells were scraped from the bottom of the flasks and 1 X 10^5 cells were plated to each well of a 12-well plate. Each well contained 1 ml of media. *B. abortus* LPS purified by Bruce Jarvis (University of Wisconsin, Madison) and *E. coli* Re LPS from company (city, state) were thawed and sonicated. The LPS was diluted with PBS. Appropriate concentrations of the LPS were added to each well with one left untreated. The cells incubated with LPS for 18 hours.

ELISA. A Quantikine® M Mouse TNF-α Immunoassay kit from R&D Systems (Minneapolis, MN) was used to perform the ELISA. A sample of media from the RAW cells treated with LPS was added to one well of the assay microplate. Known amounts of TNF-α and controls were also added to the plate. The plate had antibodies to TNF-α fixed to the bottom of each well. The samples, standards, and controls were allowed to incubate with the anti-TNF-α antibody for 2 hours. Then the wells were washed with wash buffer three times. Next, the TNF-α conjugate was added to each well and incubated for two hours. The wells were washed three times with wash buffer. Then, substrate solution was added for 30 minutes. Stop solution was added and the plate was tapped to mix the solutions. The plates were read in a plate reader at 450 nm. A standard curve was created from the known TNF-α amounts versus their O.D. reading. The amount of TNF-α produced in response to LPS was determined by interpolation from the standard curve.

Results



The results show that approximately 1,000 times more *B. abortus* LPS than *E. coli* Re LPS was needed in order to induce a similar TNF-α response. Data was collected from six separate experiments, the mean TNF-α level was calculated for each concentration of LPS, and the standard error was determined.

Discussion

The difference in *B. abortus'* ability to induce a TNF-α response compared to *E. coli*, is a strong indication that the LPS does not induce an inflammatory response and therefore allows *B. abortus* to evade the host immune system. TNF-α production during the inflammatory response would also cause the apoptosis of cells infected with *B. abortus.* Therefore, low TNF-α levels also ensure that the bacteria's host cell stays intact. In addition, LPS serves as a barrier to complement proteins, which lyse bacterial cells (3). For these reasons, LPS is an important virulence factor for *B. abortus.*

References

1. **Joklik, WK, HP Willet, and DB Amos, eds.** Brucella. Zinsser Microbiology, 18th ed. 1984. Appleton-Century-Crofts: Norwalk CT. 665-671.

2. **Gross, Antoine, Annie Terraza, Safia Ouahrani-Bettache, Jean-Pierre Liautard, and Jacques Dornand.** 2000. In Vitro *Brucella suis* Infection Prevents the Programmed Cell Death of Human Monocytic Cells. Infection and Immunity. **68**: 342-351.

3. **López-Urrutia, Luis, Andrés Alonso, Maria Luisa Nieto, Yolanda Bayón, Antonio Orduña, and Mariano Sanchez Crespo.** 2000. Lipopolysaccharides of *Brucella abortus* and *Brucella melitensis* Induce Nitric Oxide Synthesis in Rat Peritoneal Macrophages. Infection and Immunity. **68:** 1740-1745.

4. **Qursehi, Nilofer, Bruce W. Jarvis, and Kuni Takayama.** In: Brade, H., SM Opal, SN Vogel, and DC Morrison, eds. Endotoxin in Health and Disease: Nontoxic RsDPLA as a Potent Antagonist of Toxic Lipopolysaccharide.1999. Marcel Dekker, Inc.: New York. 687-698.

5. **Varghese, Jishy, Souvik Chattopadhaya, and Apurva Sarin.** 2001. Inhibition of p38 Kinase Reveals a TNF-α Mediated, Caspase-dependent, Apoptotic Death Pathway in a Human Myelomonocyte cell Line. Journal of Immunology. **11**: 6570-6577.

Glossary

- **Attenuate** – To decrease **virulence** of a pathogen and render it incapable of causing disease. Many vaccines are attenuated bacteria of viruses or bacteria that raise protective immunity without causing harmful infection.
- **Cell cycle** – The stages through which a cell passes from one cell division to the next.
- **Complement** – A group of serum proteins that participates in an enzymatic cascade, ultimately generating the cytolytic **membrane attack complex**.
- **Cytokine** – Any of numerous secreted, low-molecular-weight proteins that regulate the intensity and duration of the immune response by exerting a variety of effects on lymphocytes and other immune cells.
- **Enzyme-Linked Immunosorbent Assay (ELISA)** – An assay for quantifying either antibody or antigen by use of an enzyme-linked antibody and a substrate that forms a colored reaction product.
- **Facultative** - Capable of functioning under varying environmental conditions. Used of certain organisms, such as bacteria that can live with or without oxygen.
- **Genus** - A taxonomic category ranking below a family and above a species and generally consisting of a group of species exhibiting similar characteristics. In taxonomic nomenclature the genus name is used, either alone or followed by a Latin adjective or epithet, to form the name of a species.
- **Granuloma** – A tumor-like mass or nodule that arises because of a chronic **inflammatory response** and contains many activated **macrophages**, epithelioid cells, T_{DTH} cells, and multinucleated giant cells formed by the fusion of macrophages.
- **Innate immunity** – Nonspecific host defenses that exist prior to exposure to an antigen and involve anatomic, physiologic, endocytic and phagocytic, and inflammatory mechanisms.
- **Intracellular** - Occurring or situated within a cell or cells.
- **Interpolation** - To estimate a value of (a function or series) between two known values.
- **Lipopolysaccharide (LPS)** – An oligomer of lipid and carbohydrate that constitutes the endotoxin of gram-negative bacteria. LPS acts as a polyclonal activator of murine B cells, inducing their division and differentiation into antibody–producing plasma cells.
- **Lysis** - The dissolution or destruction of cells, such as blood cells or bacteria, as by the action of a specific lysin that disrupts the cell membrane.
- **Macrophage** – Mononuclear phagocytic leukocytes that play roles in adaptive and in innate immunity there are many types of macrophages; some are migratory whereas others are fixed in tissues.
- **Membrane attack complex** – The complex of **complement** components C5-C9, which is formed in the terminal steps of either the classical or the alternative complement pathway and mediates cell **lysis** by creating a membrane pore in the target cell.
- **Pathogen** – A disease-causing organism.
- **Programmed cell death** – An induced and ordered process in which the cell actively participates in bringing about its own death.
- **Rough strain** – A strain of bacteria that lacks the O-saccharide component of **LPS**.

- **Sonicate** - To expose a suspension of cells or microbes to the disruptive effect of the energy of high frequency sound waves.
- **Transcription factor** – Auxiliary protein (beyond the 8 to 14 distinct polypeptides that make up the polymerases) that binds to DNA and alter the transcription of nearby genes.
- **Virulence** – A measure of the infectious ability of a pathogen.
- **Zoonosis** - A disease of animals, such as rabies or psittacosis, that can be transmitted to humans.

Sources:

Karp, Gerald. 2002. Cell and Molecular Biology: Concepts and Experiments. John Wiley & Sons, Inc.: New York.

Goldsby, Richard A., Thomas J. Klindt, and Barbara A. Osborne. 2000. Kuby Immunology 4th Ed. W. H. Freeman and Company: New York.

Dictonary.com. April 12, 2002. http://www.dictionary.com