Adaptive Immune Response in Equine Pigeon Fever

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Abstract

Corynebacterium pseudotuberculosis is a gram-positive intracellular anaerobe responsible for the development of abscesses within its hosts.[1] Commonly called “Pigeon fever”, a successful vaccine has yet to be developed for the nitrate positive *equi* biovar infecting horses. We believe examining the adaptive immune response, particularly the role of T helper cells in disease manifestation, will help us to better understand how this infection functions and how it can be targeted with a vaccine. In similar intracellular infections, it is often the hosts with Th1 dominant responses that develop mild forms while those with Th2 dominant responses have more severe infections. The relationship between the T_H1 dominant response and reduced pigeon fever severity suggests that a T_H1 dominant response is advantageous in *C. Pseudotuberculosis* infection.

Introduction

The United States equine population is experiencing a rapid increase in cases of an infection commonly called “Pigeon fever”.[1] The proliferation of this infection has not only seen an increase in quantity, but an expansion geographically as well.[1] Pigeon fever is the result of a gram-positive bacterium known as *Corynebacterium Pseudotuberculosis.*[2]

Pigeon fever manifests in 3 common forms occurring at varying frequencies. External abscesses dictate ~91% of cases and are rarely fatal.[3] Internal abscesses account for ~8% of cases and are defined by growths in the liver, spleen, kidney, and/or respiratory tract.[3] Due to the vitality of the organs affected by internal abscesses, a higher mortality rate of ~40% is observed in cases of internal infection.[3] In fewer than 1% of cases, pigeon fever occurs in the lymphatic system of the legs and is known as ulcerative lymphangitis.[3] A pathogenic agent of pigeon fever is the extracellular exotoxin phospholipase D (PLD).[2] A sphingomyelinase, PLD can alter cell membrane integrity increasing vascular permeability and thus aiding the dissemination of the infection.[2]

An essential component of fighting such infections is the activation of the adaptive immune response, composed of lymphocytes called B cells (responsible for antibody production) and T cells. Cytotoxic T cells (T_C) kill infected host cells; T helper cells (T_H) are involved in modulating the response of immune cells. There are many types of T helper cells; this study focuses on T_H1, T_H2, and T_H17. T_H1 dominant responses result in the activation of macrophages and cytotoxic T-cells; cell types that are important in fighting intracellular pathogens . T_H2 dominant responses result in recruitment of B-cells and antibody production. T_H17 cells increase immune cell recruitment and stimulate T_H1 cells. Investigating the role of T helper cells and their subtypes may reveal a pattern in why some hosts of *C. Pseudotuberculosis* develop mild external abscesses while others develop severe internal infections.

Cytokine Regulation of T-Cell Subgroups

Name:	Type:	Response Subtype:
Beta-GUS	Housekeeping Gene	N/A
GATA	Transcription Factor	T_H2
tBet	Transcription Factor	T_H1
TNFa	Cytokine	T_H1
IFNg	Cytokine	T_H1
IL-10	Cytokine	T_{reg}
IL-12	Cytokine	T_H1
IL-17A	Cytokine	T_H17
IL-2	Cytokine	T_{reg}
IL-4	Cytokine	T_H2
IL-1b	Cytokine	T_H17

Figure 1: Factors Measured and Their Effect on T cell differentiation. Each cytokine or transcription factors aids in the differentiation of the T cell response to a particular subtype.

Helper T Cell Differentiation

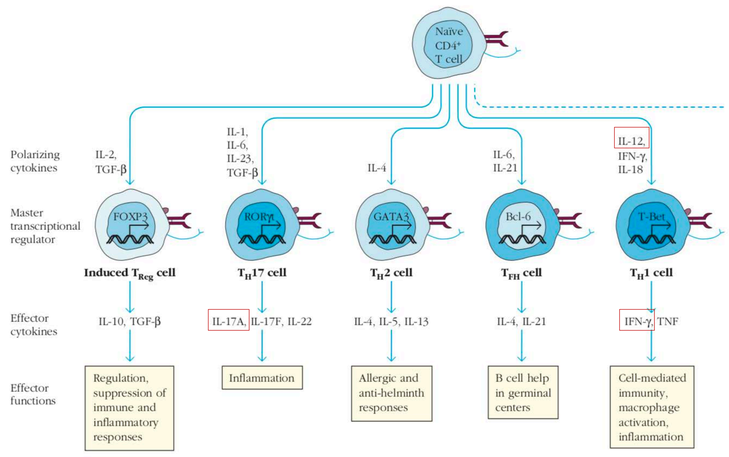
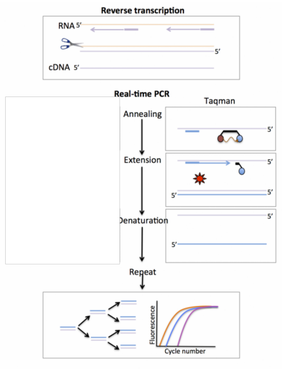


Figure 2: Helper T Cell Subset Differentiation. Polarizing cytokines cause helper T cell differentiation. Effector cytokines create the effects of the differentiated cells. IFN-γ and IL-12 are polarizing cytokines resulting in T_H1 differentiation. IFN-γ is also an effector cytokine for T_H1 while IL-17A is an effector cytokine for T_H17.

RT-PCR Experiment Design

Horse blood samples → **Stimulate white blood cells to induce cytokine Production** →



RT: Starting from a non-sequence specific primer (oligo-dT or random sequence combinations) reverse transcriptase enzyme makes a complementary DNA strand mRNA as a template. The RNA template is then degraded

QPCR: cDNA is bound by sequence specific probe labeled with a linked dye and quencher. DNA polymerase extension of sequence specific primer causes degradation of the probe, separating the dye and quencher molecules and allowing fluorescence of the dye

IFN-γ Relative Quantity

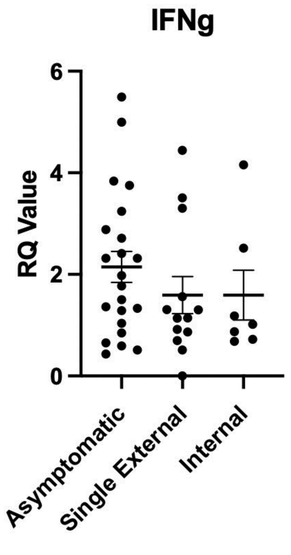


Figure 3: Relative Quantity of IFN-γ in asymptomatic, externally infected, and internally infected horses. IFN-γ quantity decreases as the severity of the infection increases. Internally infected horses show the lowest quantity of IFN-γ . Asymptomatic horses show the highest levels of IFN-γ.

IL-17A Relative Quantity

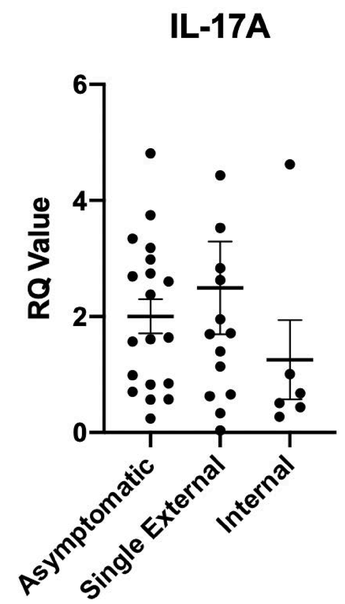


Figure 4: Relative Quantity of IL-17A in asymptomatic, externally infected, and internally infected horses. IL-17A levels appear the lowest in internally infected horses and highest in externally infected horses.

Conclusion

- IL-17A levels appear the lowest in internally infected horses suggesting they have a less dominant T_H17 response than asymptomatic and externally infected horses.
- IFN-γ levels suggest that asymptomatic horses have a strong T_H1 response compared to internally and externally infected horses.
- The difference between the means of differently infected horses for both IL-17A and IFN-γ were not statistically significant
- We cannot conclude that IFN-γ and IL-17A levels are higher in horses with milder cases of pigeon fever
- We cannot conclude that IFN-γ and IL-17A levels are lower in horses with more severe cases of pigeon fever.

Future Directions

- Gathering more samples particularly from internally infected horses will allow a greater chance to achieve statistical significance in trends
- Obtaining horse samples locally would allow cell stimulation without a freeze/thaw cycle, increasing the number of viable cells.
  - More cells would allow collection at multiple time points after stimulation, allowing for better results for genes that are expressed at different rates and levels.

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