Last name, First name

**Microbiology (Lab based) Research Plan:

The Identification of Potential Memory Response against Re-infection with Babesia microti**

**Rationale**

Babesiosis is a tick-borne disease caused by the Apicomplexan parasite, Babesia microti (B. microti). More than 95% of cases occur in the Northeast, due to the increasing deer population. The disease is transmitted by a tick vector, known as *Ixodes Scapularis*, which passes the disease to humans through a blood meal. Babesiosis can also be transmitted through blood transfusions, which has become more common in the United States. This increase in blood transfusion cases is due to the asymptomatic nature of the disease, meaning it presented no symptoms in immunocompetent patients. Ticks initially obtained the infected sporozoites from a white-tailed deer or mouse. In previous years, there have been nearly 2,000 cases reported in the various parts of New York State, and this number continues to increase annually (CDC, 2012). Upon infection, the parasite enters the red blood cells and begins it replication phase, in which it continually multiplies within the red blood cells. In response to this replication, the immune system recruits inflammatory monocytes and specific cell types needed for the clearance of the parasite.

The mechanism implemented by the immune system to promote parasite clearance is unknown. This mechanism causes an abrupt decrease in the levels of parasitemia, as well as, killing of the parasites within the red blood cells. This unknown novel mechanism has caused a lack in efficient diagnosis and targeted treatment options; therefore, the number of Babesiosis-related deaths continue to increase, annually. Being able to understand and differentiate between the pathological and immunological aspects of Babesiosis will allow for the understanding into the differences which arise from patients who are immunocompromised compared to those who are not, and patients who are asymptotic in comparison to those who present symptoms. By understanding these differences, immunologically, it will allow for a targeted combating method for effectively diagnosing and treating Babesiosis in the long term. Previous studies have shown that there is a strong correlation in the recruitment of T cells and Th1 cytokine gamma interferon (IFN-y) for the control of B. microti, due to the functioning of humoral immunity, which acts as a defense mechanism against pathogens that try to invade the host immune system (Clawson, 2002; Mordue, 2017). This recruitment and activation of the innate immune response correlates to the importance of the cellular immunity because cell types, specifically those that function as cytotoxic cells, are necessary for the clearance of the protozoan.

In addition to the lack of knowledge surrounding the immunological and pathological aspects of Babesiosis, and how that influences the clearance of the parasite post-infection, the quantitative and qualitative findings about how re-challenged mice react to the reintroduction of Babesia microti into the immune system is unknown; ultimately coupled with how the memory cells work in tandem to influence the changes in the levels of parasitemia through the course of re-infection. In order to determine the establishment of a protective memory response, previous research was conducted in Apicomplexa parasites, and the development of a memory response was determined by IL-4 receptor expression on CD8+ T cells. The study showed that Il-4 receptors were necessary for the development of memory cells and protection against parasite challenge (Wipasa, 2010). Without an antigen-specific immune response, memory CD8+ T cells were not able to stabilize the parasite population in nonlymphoid organs. Therefore, without the presence of Il-4 receptors, CD8+ T cells are not able to be expressed, which leads to inability for the development of an antigen-specific CD8+ T cell response following immunization with the parasite (Morrot, 2005).

**Research Questions**

The gap in knowledge surrounding the pathological and immunological aspects of Babesiosis has led to the in depth questioning into the definitive mechanisms of the immune system, and how specific aspects such as B and T cells are crucial for the interaction between the host immune system and the parasite.

*Phase 1- Summer 2018:* Identification of novel mechanism used by the human immune system, in order to recruit specific cells and cytokines necessary for the clearance of the parasite following parasite replication on day 5, and the clearance of the parasite through parasite killing following day 7 post-infection.

1. What is the novel mechanism in the host immune system that causes the death of Babesia microti parasite following day 7 post-infection? Along with, how the levels of parasitemia changes through the course of the infection?
2. In the host blood and immune response, what are the specific cell types that are necessary for the clearance of the parasite, which correlate with parasite replication and parasite killing?

*Phase 2- Summer 2019*: Identify other critical immune cells and factors that are important for the regulation of the levels of parasitemia through the course of the infection (not such a big concept this year)

1. In using a re-challenged or vaccine model mice, what specific cell types are necessary for a protective memory response against reinfection with Babesia microti?
2. What aspects in the functionality of the immune system, does CD4+ T cells play in knockout mice, rather than in other mice strains?
3. Does immunodeficiency of B and T cells or immunocompetent wild type mice affect the regulation of the parasites at any crucial time stamp during the infection? (specifically, in SCID mice)

**Hypotheses**

1. It was hypothesized that if a mouse that was initially inoculated with Babesia microti was able to clear the parasite and retain a low level of parasitemia, then it was classified as protected against being re-challenged.
	1. This is due to the mouse genome containing the necessary cell types- such as an abundance of memory B cells and cytotoxic T cells- are required for the efficient clearance of the parasite.
2. It was hypothesized that if CD4+ T cells are depleted from the mice genome, and if SCID mice- B and T cells immune deficient mice- were used, then there will be no positive or negative regulation of the parasite.
	1. This lack of regulation and increased parasitemia would be due to the host’s immune system need for specific immune cells in the bloodstream for the clearance of the parasite.

**Expected Outcomes**

It is expected that when a mouse is initially infected with Babesia microti and is re-infected with the same parasite as a method of re-challenging, then the mouse will be protected against replication on day 1 through day 5 and dissemination following day 7 because of the functioning of the memory B cells in the immune system. These memory B cells will be able to more efficiently and effectively target and terminate the parasite before a spike of sharp increase in parasitemia as day 7, or peak parasitemia approaches. The effective targeting and termination of the parasite would be characterized by a parasitemia percentage of less than 35%. However, this process will not be able to be replicated in SCID mice who are immunodeficient of B and T cells, further validating their importance and role in the immune system’s inflammatory response and up-regulation as day 7 approaches. The up-regulation of primary immune cells would be due to the early recruitment process of specific inflammatory monocytes.

**Procedure**

*Role of Mentor*

* Inoculation of the various mice strains used throughout the course of the experiment. Mice strains include: Mu-MT mice strain without B cells, interferon gamma, CD4+ T cells, or CCR2 Knockout; as well as, wild type mice for the use of comparisons
	+ The involvement with direct use of mice would be performed by the qualified scientist in accordance with the Animal Care and Use Protocol, approved by the college (IACUC # 23-2-0718)
* Conducted sequential tail snips for the acquirement of infected red blood cells
* The mice used will be infected with Babesia microti by the transferring of 100ul of blood from infected mice on days 5-7 post-infection.
	+ Parasites would have to be serially passed in mice, as the parasites cannot be cultured outside of a mammalian host.
	+ On day 5,6, and 7 post-infection, 1 ml of blood will be collected from each of the 4 infected mice.
	+ 0.015% saponin will be used to lyse the red blood cells and liberate the parasites.
	+ The remaining pellet after centrifugation at 3000 rpm for 10 minutes contained parasite, peripheral blood mononuclear cells (PBMCs) and granulocytes.
* Provide student with blood for preparing blood smears for observations under the microscope

*Role of Student*

* Conducted parasitemia counts under the microscope for naive mice and do a comparison based on the re-challenged mice for day 1 through 5
* Analyze RNA-Sequencing data in order to continue working towards finding any other cell types that may be necessary for the clearance of the parasite following day 7 post-infection.
* Blood smears for each re-challenged mouse strain will be prepared before observing the parasitemia levels under the microscope
	+ A drop of the daily collected blood was used for the smearing of slide, all procedural aspects were conducted by qualified scientist
	+ Blood on the slide was smeared and stained using Giemsa stain, all of which was conducted by the qualified scientist
	+ Slides were then fixed in ice cold 100% methanol
* Parasite counts will be conducted using the Zeiss Primo Star Microscope under the Plan-ACHROMAT 100x/1.25 Oil objective lens, along with Immersol 518F, which will be applied to each slide to ensure optimal visibility
* The re-challenged mice will be observed beginning on day 1 through day 7 of the infection
* Each day will be recorded based on whether a parasite was seen in the red blood cells and how many would be seen in each field of vision
* The same process will be used to observe the wild type mice, knockout mice, SCID mice, and Mu-MT mice strains under the microscope or conduct any additional blood smears
* In order to look for any other crucial cell types necessary for the clearance of the parasite, the same procedure from last year will be used to analyze RNA-Sequencing Data

**Risk and Safety**

 Babesiosis is a blood borne pathogen that survives within red blood cells. Its natural mode of transmission is via tick bite. The primary risk factor is a skin injury allowing access into the bloodstream with Babesia microti contaminated sharp such as a needle contaminated with Babesia microti parasites. The risk of transmission is negligible even with an accidental spill or release if contaminated sharps are not generated. However, gloves must be worn during cleanup of spills to prevent inadvertent exposure through undetectable cuts in the skin. Undiluted vesphene will be applied directly to the spill and allowed 20 minutes to sit prior to clean up and discard of paper towels in a biohazard bag for offsite autoclaving. Volumes of liquid with viable parasites will never be greater than 50 mls (10 mls infected red blood cells plus 40 mls PBS). The biosafety cabinet will be decontaminated after use even in the absence of spills.

**Data Analysis**

 The analysis of the changing levels in parasitemia will be conducted through the calculation of mean averages and testing for statistical significance based on a p-value of 0.05% or lower. RNA Sequencing Data will be analyzed based on cross-references and as a method for validation with the data collected from the parasite counts. Specific genes and cell types will be sorted out based on an up-regulation from day 6 to day 7, and these up-regulated genes will be used to validate the importance of certain cell types in the clearance of the parasite; as well as, protection from reinfection.

**Vertebrate Animal Research**

Mice are a natural host species for Babesia microti. The parasite cannot currently be grown in red blood cell culture or any other culture conditions. This is a limitation to studies of Babesiosis. Currently the only way the parasite can be grown is in a mammalian host. Furthermore, the only way to study the disease babesiosis and the immune responses important during disease is through the use of an animal model like a mouse. There is no simpler tissue culture or non-mouse model that can substituted to recapitulate the disease since host mediators of protection and parasite mediators of pathology are entirely unknown. Without this research, the understanding of Babesiosis will not be understood, and differentiation from Lyme Disease can not be identified. Without this knowledge, the effects and mortality rates will be detrimental to any who may contract the disease; but more specifically, those who are immunocompromised.

The involvement with direct use of mice would be performed by the qualified scientist in accordance with the Animal Care and Use Protocol, approved by the college (IACUC # 23-2-0718). The mice used would be infected with Babesia microti- by the qualified scientist- by the transferring of 100ul of blood from infected mice on days 5-7 post-infection. Parasites would have to be serially passed in mice, as the parasites cannot be cultured outside of a mammalian host. On day 5,6, and 7 post-infection, 1 ml of blood would be collected from each of the 4 infected mice. 0.015% saponin would be used to lyse the red blood cells and liberate the parasites. The remaining pellet after centrifugation at 3000 rpm for 10 minutes contained parasite, peripheral blood mononuclear cells (PBMCs) and granulocytes.

**Potentially Hazardous Biological Agents Research**

The parasite is a BSL2 organism so any work with viable parasites would be performed in a biosafety level 2 biosafety cabinet in a BSL2 laboratory. The student will observe biosafety level 2 standards including wearing long pants, no open shoes, lab coat, gloves and safety glasses when working with viable parasites.

**Hazardous Chemicals, Activities and Devices**

Blood smears would be fixed and stained with Giemsa stain - which contains methanol, a flammable agent. Therefore, gloves will be worn when staining slides and no open flames will be used.

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