ABSTRACT

Those infected with Human Immunodeficiency Virus (HIV) classically progress to Acquired Immune Deficiency Syndrome (AIDS), an illness typified by an increase in opportunistic infections ranging from influenza to tuberculosis to several kinds of cancer. Since its identification in 1982, 24 million people worldwide have died as a result of AIDS. Not everyone infected with HIV progress to AIDS at the same rate, most falling into two groups: rapid progressors and long-term nonprogressors (LTNPs). These LTNPs are an exciting and important area of research on methods to inhibit progression to AIDS.

The murine AIDS (MAIDS) model used in this experiment consisted of the C57BL/6 mouse infected with murine leukemia virus (MuLV) to induce AIDS-like symptoms. The infection in MAIDS model mice typically mirrors human rapid progression in AIDS. These mice were compared to a BALB/c mice who, when infected with MuLV, more closely parallel a LTNP AIDS response.

Previous research has suggested a link between early expression of the immunosuppressant cytokine Interleukin-10 (IL-10) in MAIDS-susceptible C57BL/6 mice after three and seven days of infection with MuLV and the rapid progression AIDS response shown in these mice. C57BL/6 mice showed reduced levels of IL-10 and an accompanying high viral count after three and seven days. In this experiment, we hypothesized that, like in the previous research, BALB/c mice would exhibit low levels of IL-10 compared to C57BL/6 mice after both were infected with MuLV for 3.5 days. To investigate whether higher expression of inflammation typical of a successful antiviral response complements low expression of IL-10 and vice versa, the inflammatory cytokine Interferon-γ (IFN-γ) was hypothesized to have an inverse relationship with IL-10. This experiment imitated the methods of previous Stranford lab research, using the same animal models and Enzyme-Linked Immunoabsorbent Assays (ELISAs). BALB/c MAIDS-resistant mice were expected to express low levels of IL-10 and high levels of IFN-γ after 3.5 days of infection and C57BL/6 MAIDS-susceptible mice were expected to express high levels of IL-10 and low levels of IFN-γ after 3.5 days of infection. As in previous research, both mouse models showed similarly low expression levels of both IL-10 and IFN-γ before infection. Contrary to previous research and these hypotheses, however, the only significant difference was a rise in IL-10 concentrations between BALB/c pre-infection mice and day 3.5 post-infection mice. No significant differences between infection observation point or mouse strain were found in IFN-γ analysis. With a lower viral titer achieved this year than in previous research and adjustments to cytokine concentrations made for protein concentration in samples for the first time in this experiment, these results do not support the findings of previous research and may open an avenue of low-titer research in MuLV/MAIDS and eventually HIV/AIDS.

IFN-γ and IL-10 Regulation in the MuLV-Infected MAIDS Model

by

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INTRODUCTION

With its epidemic global spread in the late 1980s continuing through today and over 56,300 new cases a year in the United States alone, Human Immunodeficiency Virus (HIV) presents one of the foremost contemporary global health concerns (Hall et al., 2008). Those infected with HIV typically progress to Acquired Immune Deficiency Syndrome (AIDS), characterized by serious opportunistic infections, some more typically deadly like tuberculosis, and others that are usually more benign, like influenza, progressing to deadly conclusion (Hall et al., 2008). More than 30 years after its appearance in human populations, HIV remains a mysterious and deadly virus, and it is the goal of this experiment to contribute towards furthering scientific understanding of the modern scourge of humanity.

THE MAIDS MODEL

There are several animal models for AIDS, though for the most compact living quarters, relatively quick regeneration, and inexpensiveness, the murine AIDS (MAIDS) model fits laboratory purposes. In this mouse model, certain strains of mice are infected with the LP-BM5 strain of murine leukemia virus (MuLV) and develop AIDS-like symptoms, including immunodeficiency resulting in opportunistic infection and swollen lymph nodes and spleens (Jolicoeur, 1991). The Stranford lab uses two strains of mice as a comparison in

experimentation: The BALB/c mouse strain that is resistant to MAIDS by MuLV and the C57BL/6 mouse strain that is highly susceptible to developing MAIDS after MuLV infection (Pantaleo, 2001). Using these two strains of mice, the lab may compare the differences between a typical antiviral response and a MAIDS response against MuLV with the greater frame of solving the mysteries of HIV/AIDS in the future.

THE IMMUNE SYSTEM

Every day, the human body is barraged by countless infectious offensives seeking to infiltrate defensive barriers and feed on and replicate in the body. Fortunately, evolution has provided several physical and biochemical defenses to the human body to prevent infection. Barriers such as skin are physical walls between the external environment full of bacteria, fungi, and viruses looking for a new host in which to replicate. When the skin is broken, other physical barriers quickly fill in the wound. One of the most important is the platelet, an irregularly shaped cell fragment without a nucleus derived from megakaryocytes (Akira, Uematsu, & Takeuchi, 2006). Millions of platelets are in constant circulation in blood and are activated by tissue factor (TF) following blood vessel injury before coagulating and forming a stable fibrin clot, preventing any unwanted entrance by pathogens (any disease-producing agents) (Parham, 2009) (Dorland, 2007). Other factors preventing pathogen entrance are acidic environments in the vagina,

lysozymes that damage bacterial cell walls in tears, and mucus in the respiratory and gastrointestinal tracts that entangle and trap pathogens before crossing into blood and tissue (Parham, 2009). Pathogens, however, have also evolved ways to circumnavigate these barriers and still enter and infect the body. There are several cellular lines of defense against these pathogens, including innate immunity (immediate spontaneous response, non-specific to the pathogen) and adaptive immunity (delayed pathogen-specific response). These tactics are closely interwoven and provide an effective arsenal for defending the body against infection.

Cells involved in the immune system are all derived from hematopoietic stem cells in bone marrow (Morrison, Shah, & Anderson, 1997) (Figure 1). These stem cells form two lineages, the common lymphoid and common myeloid lineages (Romagnani, 1992). The common lymphoid lineage includes Natural Killer (NK) cells, T lymphocytes, and B lymphocytes, which eventually become plasma cells (Morrison et al., 1997). NK cells employ innate immune mechanisms while T and B lymphocytes are involved in the adaptive immune response. Common myeloid lineage includes the macrophage, neutrophil, basophil, megakaryocytes, eosinophil cells, all of which are involved in the innate immune response (Morrison et al., 1997). Besides NK cells, the common lymphoid lineage is mainly concerned with adaptive immunity.

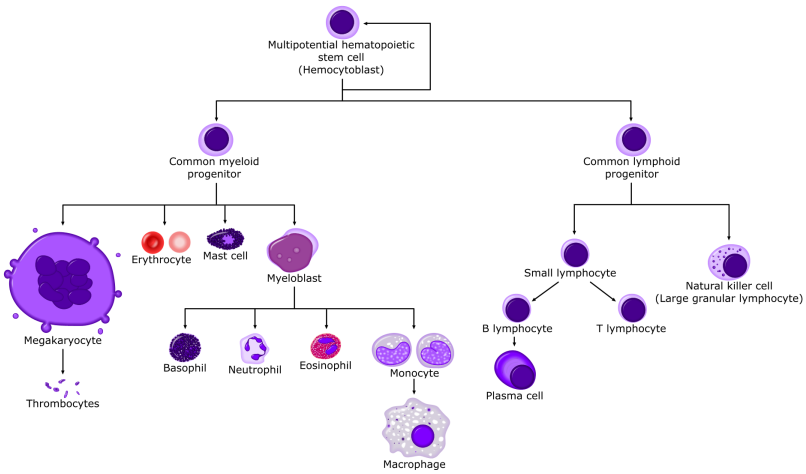

Figure 1. The developmental pathways for differentiating hematopoietic stem cells following the common lymphoid lineage or common myeloid lineage.

Source: National Library of Medicine, NIH. Link:

http://www.dentalarticles.com/visual/d/hematopoiesis.php

INNATE IMMUNITY

Once pathogens cross the physical and chemical barriers to the internal environment of the body, the immune system must correctly differentiate between self and non-self structures to mount a defensive (Parham, 2009). The innate, or non-specific, immune response is the fastest to respond, usually before the first twelve hours of infection (Parham, 2009). Pathogen-associated molecular patterns (PAMPs) trigger most of innate immunity after recognition by pattern recognition receptors (PRRs), which in turn will trigger adaptive, or specific, immunity (Akira et al., 2006). PAMPs include structures made solely by pathogens and not the host organism in question, like chitin in fungi or peptidoglycan in bacterial cell walls (Parham, 2009). PRRs can either be classified as signalling or endocytotic PRRs (Akira et al., 2006). Signaling PRRs include toll-like receptors (TLRs) on the outer membranes of monocytes, neutrophils, and macrophages, all of which culminate in the activation of the transcription factor nuclear factor kappaB, which in turn controls the transcription of a variety of inflammatory cytokines (Kawai & Akira, 2007), As seen in Figure 2, endocytotic PRRs are on the surfaces of phagocytotic cells like macrophages and promote the attachment of phagocytes to pathogens and endocytosis (Salomon & Robatzek, 2006).

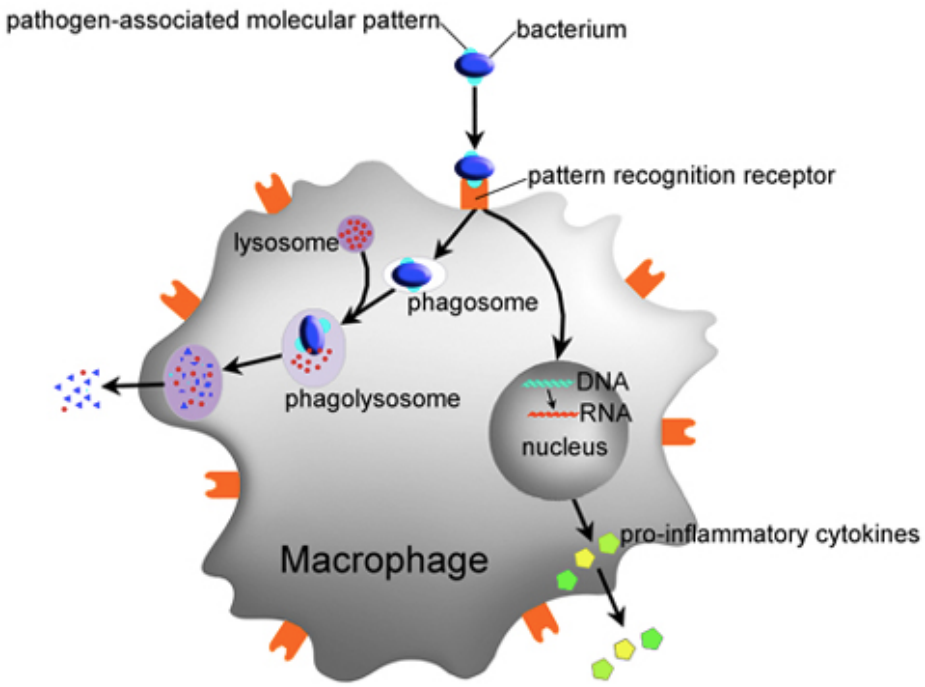

Figure 2. Macrophages and other APCs recognize PAMP s with their PRRs, signaling for endocytosis, pathogen destruction, and inflammatory cytokine expression. Source: Linus Pauling Institue, Oregon State University. Link: http://lpi.oregonstate.edu/infocenter/phagocytosis.html

Complement molecules always present in the blood can recognize these PAMPs and trigger a cascade leading to either the innate immune response eradicating the infection by 'complementing' the ability of phagocytotic cells to clear pathogen or further eliciting an adaptive immune response later in time (Akira et al., 2006). A C3 complement molecule will bind to various PAMPs and upon binding cleave into C3b, which stays bound to the pathogen, and C3a, which

acts as a chemotactic factor in the blood to lead phagocytotic cells that can consume and lyse the pathogen to the site of infection (Akira et al., 2006). C3b opsonizes the pathogen, covering it and creating a favorable environment for phagocytotic cell recognition(Akira et al., 2006). C5, similarly to C3, cleaves and leaves C5b bound to the pathogen while C5a acts as a chemotactic inflammatory paracrine to attract phagocytotic cells (Akira et al., 2006). Eventually complement molecules C6, C7, C8, and a number of C9 will bind and form the Membrane Attack Complex (MAC) that leads to pathogen cell lysis (Fleming & Tsokos, 2006). Complement is also active in the adaptive immune response discussed later in the introduction.

When C3a and C5a are cleaved and travel freely in the circulatory system, they are setting the stage for a larger recruitment of immune response molecules and cells at the site of infection. Inflammation is the priming of the infection site for an inundation of innate, followed by adaptive, immune response cells and molecules (Fleming & Tsokos, 2006). Symptoms of inflammation include vasodilatation, allowing more fluid and leukocytes into the site of infection, heat, and fluid and pus buildup. These complement molecules are also called anaphylotoxins and are able to trigger degranulation of innate immune cells like neutrophils, MAST cells, and NK cells (Fleming & Tsokos, 2006). Cells with cytotoxic degranulatory function hold relatively large amounts of cytotoxic molecules like perforin, which are meant to perforate the cell wall or plasma membrane of a pathogenic cell, that are released in directed exocytosis at the site

of infection upon recognizing anaphylotoxins (Betts et al., 2004). One of the key inflammatory molecules released by NK cells during an innate response can eventually trigger an adaptive response, and one of two key cytokines in this study, is Interferon-γ (IFN-γ), which can act as a chemokine to the inflamed region and triggers a strong adaptive response (Klein et al., 2006). Similarly, NK cells can release Tumor Necrosis Factor-α (TNF-α), which triggers an inflammatory cascade of interferons and signals between leukocytes, or interleukins(Klein et al., 2006).

While all interferons are antiviral agents, there exist the subtypes Type I and Type II interferons. Type I interferons all bind IFNα receptor on leukocytes and act in innate antiviral immunity (Trinchieri, 1997). IFN-γ is the only member of Type II interferon and is produced in NK cells and activated T cells during both innate and adaptive immune responses (Parham, 2009). The IFN-γ and TNF-α released by NK cells are also important in an adaptive immune response, explained in the next section. Neutrophils, the most abundant leukocyte in the human body, will also release proteins like serine proteases and defensins that break down microbial cell membranes and walls during inflammation (Akira et al., 2006). Neutrophils are the most common white blood cell present in pus and have a short life span. They will also phagoctyose relatively small opsonized pathogens, or pathogens encased by a layer of antibodies produced in the adaptive immune response explained in the next section, and digest them with the same cytotoxic proteins released from granules at the site of infection (Akira et al.,

2006). Leukocyte degranulation triggers further inflammation, attracting even more white blood cells, like macrophages, to fight infection. These first responders are not pathogen-specific, will only consume pathogen marked by complement or recognized by PRRs, and will have no memory of the pathogen for reference in future infection (Akira et al., 2006).

ADAPTIVE IMMUNITY

T and B-lymphocytes, both in the common lymphoid lineage, are the main responsive cells in an adaptive, pathogen-specific immune response. Primary lymphoid organs, where lymphocytes are first made and mature, include previously mentioned bone marrow, where hematopoietic stem cells differentiate into the common lineages, and the thymus, where only T cells mature (Parham, 2009). All other organs where lymphocytes are stored are called secondary lymphoid organs, and include lymph nodes, tonsils, Peyer's Patches, and the spleen (Parham, 2009).

These two types of lymphocytes both develop from the common lymphoid lineage of cells in the bone marrow and both are known for their pathogen-specific receptors. The specific parts of the pathogen the T and B cells may recognize with receptors are known as antigens (Ag) (Dorland, 2007). T and B cells specific for an invading pathogen's antigens will be selected for proliferation after exposure in a process called clonal selection, followed by clonal expansion

(Rajewsky, 1996). These two processes ensure that all lymphocytes selected by their specificity to an Ag are clones of one another (Rajewsky, 1996). The first time an Ag is recognized by adaptive immune cells is called the primary response and will start later and last longer than innate immunity (Parham, 2009). Unlike innate immunity which has no Ag-specific quality and therefore no memory of infection, many lymphocytes of adaptive immunity will persist and continue circulation in the blood, immediately recognizing and triggering an adaptive response upon future infection by the same pathogen (Kaech, Wherry, & Ahmed, 2002). These persisting Ag-specific lymphocytes are called memory B and T cells and are considered future protective immunity, the basis of secondary responses (Kaech et al., 2002). During a secondary response, memory lymphocytes can begin adaptive immunity immediately upon recognizing the same pathogen that triggered their previous activation (Kaech et al., 2002).

Lymphocytes can respond to many different categories of pathogen, including bacteria, viruses, fungi, and any number of parasites. Bacteria and viruses present two common pathogens for humans and lead to two different types of adaptive immune response due to their distinctive mechanisms of invasion and replication (Romagnani, 1992). Humoral immunity focuses on an antibody response from B cells to target bacteria and other pathogens in the interstitial fluid (Kuchroo et al., 1995). Viruses spend a short time in the humor but will quickly bind to a host cell membrane and inject genetic material, replication factors, and other viral proteins into the host cytoplasm; the adaptive immune response to

viruses therefore must center inside of the cells, called cell-mediated immunity (Parham, 2009). Each of these immune pathways uses different lymphocytes and soluble products to combat infection, including different T cell lines. These T cell lines include T helper 1 (Th1, involved in cell-mediated immunity), T helper 2 (Th2, involved in humoral immunity), T helper 17 (Th17), and Regulatory T (Treg, involved in immunosuppression) cells (Figure 3).

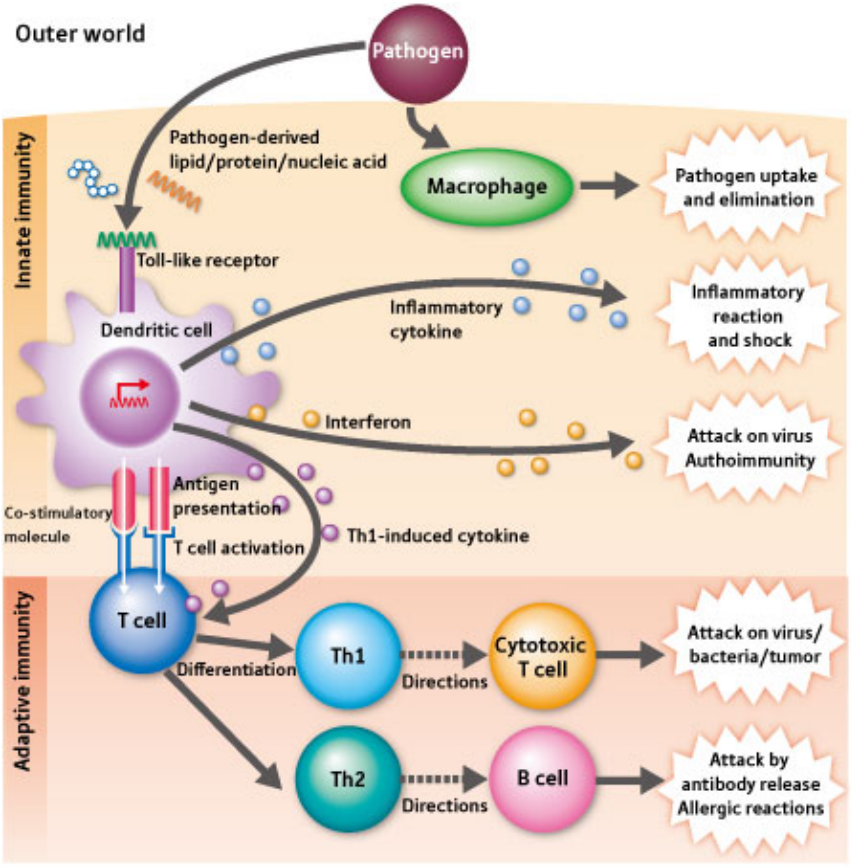

Figure 3. How innate and adaptive immune responses interact in response to infection by a pathogen. The upper half shows the innate response as a pathogen enters the body and leads to the lower half, illustrating the adaptive immune response. Source: Nature Publishing Group. Link: http://www.rikenresearch.riken.jp/en/sitemap/

CELL-MEDIATED IMMUNITY & THE TH1 PATHWAY

Cell-mediated immunity uses phagocytes and antigen-specific cytotoxicity to target cells affected by intracellular pathogens like viruses (Townsend & Bodmer, 1989). Several types of T lymphocytes coordinate cell-mediated immunity, including helper T cells, cytotoxic T cells, and T regulatory (Treg) cells (Romagnani, 1992). Each of these cells carries a T cell receptor (TCR) that binds to specific Ag's present on various pathogens (Romagnani, 1992). T cells are involved with both cell-mediated and humoral immunity, so these TCRs can bind to a wide variety of pathogens and not only those targeted by cell-mediated immunity (Parham, 2009). Helper T cells express a CD4 co-recepter and are therefore also called CD4+ T_H cells, while cytotoxic T cells express CD8 co-receptors and are called CD8+ T_C cells (Parham, 2009). As suggested in their name, the 'helper' or H in CD4+ T_H cells refer to the fact that these cells help other cells, like the B cells described later in 'Humoral Immunity,' perform their functions (Parham, 2009). The helper T cells most directly associated with a cell-mediated response are Th1 and Th17 cells, both proinflammatory (Steinman, 2007).

TCRs are unique in that their activation depends on specific interactions with antigen presenting cells (APCs) like dendritic cells, B cells, and macrophages. APCs phagocytose pathogen and present fragments of pathogen proteins on their surfaces in the binding cleft of their major histocompatability complex (MHC) molecules in what is called a peptide:MHC complex

(Romagnani, 1992). The two classes of MHC are I and II; MHC class I is present on most cell types and is recognized by CD8+ T_C cells in an antiviral response and MHC class II is on the surface of APCs, recognized by Th1 cells, CD4+ T_H cells involved in the cell-mediated response (Figure 4) (Romagnani, 1997). MHC is expressed on the plasma membranes of these cells and present short sections of digested intracellular proteins for potential recognition by TCRs(Romagnani, 1992). MHC class I presents intracellular proteins to the extracellular space, and only when presented by APCs are cytotoxic T cells activated (Townsend & Bodmer, 1989). Cytotoxic T cells carry granules of their namesake cytotoxic proteins that kill surrounding cells when released, so precise activation is key to preventing a hyper-immune response and hurting self-tissue (Townsend & Bodmer, 1989). Once activated in a primary lymphoid organ, a cytotoxic T cell can travel throughout the body searching for a non-APC cell presenting the same peptide:MHC class I complex, a sign that cell is infected, which causes the cytotoxic T cell to degranulate and kill the infected cell (Townsend & Bodmer, 1989). Helper T cells are involved in humoral immunity and eradicate pathogen directly, and will be discussed in the following section.

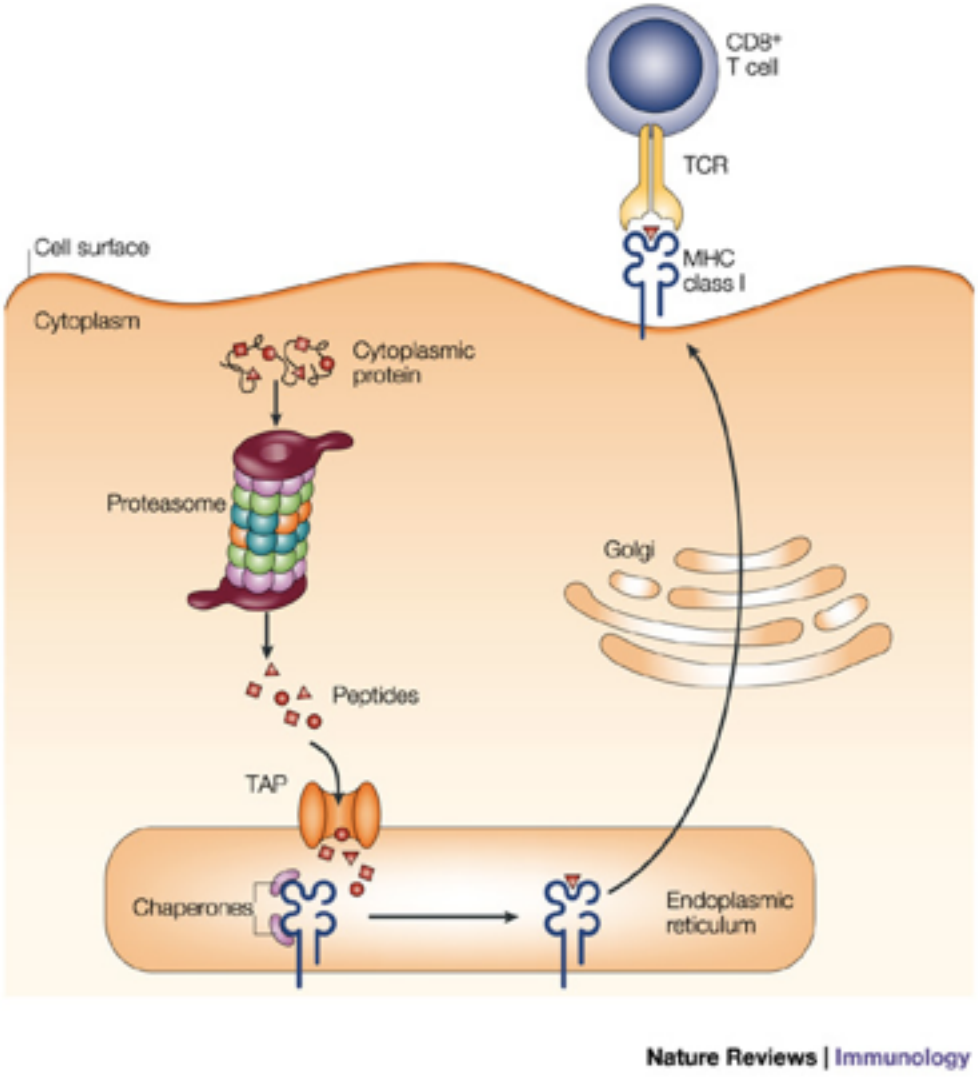

Figure 4. MHC Class I binds to specific sequences of peptides digested by any cell in the body and presents that small peptide on the cell surface where cytotoxic CD8+ T cells can recognize them. Source: Nature Reviews, Immunology.

Link: http://www.nature.com/nri/journal/v3/n12/fig_tab/nri1250_F1.html

INTERFERON-γ: INDICATOR OF THE TH1 PATHWAY

IFN-γ is one of the most well studied pro-inflammatory cytokines in the adaptive immune response, known as a strong antiviral cytokine. A dimer, and the only member of Type II interferons, it is mainly secreted by natural killer (NK) cells, some TH1 helper T cells, and cytotoxic T cells. NK cells work as an innate immune response against infection by recognizing infected cells with non-antigen specific surface receptors. NK cell cytotoxicity is inhibited by MHC Class I molecules on the surface of self cells, preventing random killing of healthy cells; they will, however, target self cells presenting low surface concentrations of MHC Class I molecules, sometimes a sign of viral infection (Wysocka et al., 1995). When activated, NK cells will turn on tumor necrosis factor-α (TNF-α) transcription, in turn activating IFN-γ production (Trinchieri, 1997). Secreted IFN-γ will help activate other innate responses in macrophages, dendritic cells, and neutrophils by binding to those cells' CD64 surface molecules (high affinity IFN-γ receptors) (Wysocka et al., 1995). Lymphocytes, however, do not express CD64 but will express IFN-γ once activated (Harris, Goodrich, Gerth, Peng, & Lund, 2005). B cells activated by Th1 cells can amplify a Th1 response by expressing IFN-γ, effectively amplifying the antiviral response overall with a B cell autocrine feedback loop of IFN-γ (Harris, Goodrich, Gerth, Peng, & Lund, 2005). This type of cell-mediated response directs T helper cells to differentiate into TH1 cells, in search of MHC Class II molecules presenting specific intracellular antigen (Kuchroo et al., 1995). TH1 cells will further secrete IFN-γ,

adding to the differentiation of B cells into plasma cells that will secrete antigen-specific antibodies to opsonize and neutralize virus in the bloodstream before infection of more cells (Kaech et al., 2002). Cytotoxic CD8+ T cells will also respond to high concentrations IFN-γ by actively inducing self cell MHC Class I surface presentation as a method of surveillance, killing cells that either do not present MHC Class I or those cells expressing peptide:MHC Class I complexes for which the CD8+ receptors are specific (Parham, 2009). In short, IFN-γ begins a cascade of cell-mediated immunity that builds by inducing even more IFN-γ secretion by other cells in response. High IFN-γ concentrations in the spleen and lymph nodes, where T cells gather and differentiate, indicates a strong TH1 pathway cell-mediated immune response, which favors virus eradication.

HUMORAL IMMUNITY & THE TH2, TH17 PATHWAYS

Humoral immunity acts against pathogens solely located in the extracellular space, like most bacteria and fungi. This type of immunity features B cells often with help from Th2 and Th17 cells, both CD4+ T_H cells (Peck & Mellins, 2010; Romagnani, 1997).

In a T cell- or thymus-independent humoral response, bacteria are marked by complement molecules C3b fragments by the time a B cell arrives to the site of inflammation. The B-cell co-receptor is a complex of three proteins that recognize and binds these fragments, beginning a signaling pathway that will eventually

allow the B cell to differentiate into an antibody (Ab) secreting plasma cell (Romagnani, 1997). The first of these proteins is CD21, which binds directly to C3b fragments; the second is CD19, which signals through the plasma membrane to begin response; the third is CD81 and has no known function (Romagnani, 1997).

In more common humoral responses, helper T cells are necessary for successful B cell activation in a thymus-dependent or Th2 response (Romagnani, 1997). Only APCs express MHC Class II to activate CD4+ T_H cells with the appropriate peptide:MHC complex and co-receptor signal (Romagnani, 1997). An activated helper T cell can then move into other parts of the body and into lymphoid organs to activate other effector cells like B cells and macrophages towards eliminating pathogen (Romagnani, 1997). Recognition of a peptide:MHC II complex and co-receptors between an APC and CD4+ T_H cell initiates a prolonged interaction where the T cell is activated, clonally selected, and then replicated in clonal expansion (Parham, 2009). In the presence of IL-4, the T cell will differentiate into a Th2 helper-T cell appropriate to aid in B cell activation through co-receptors like CD80 on activated B cells and CD28 on activated Th2 cell, or CD4+ T_H cell that activates B cells (Romagnani, 1992).

When a B cell enters a lymph node and encounters an activated Th2 cell with a TCR corresponding to a B cell's peptide:MHC II complex, they form a cognate pair and several receptor interactions help differentiate B cells into antigen-secreting plasma cells. These interactions include CD40 ligand to CD40,

and LFA-1 to ICAM-1 on Th2 cells and B cells respectively (Romagnani, 1997). The Th2 cell secretes IL-4 into the intermembrane space between the two cells, essential to B cell proliferation and differentiation (Romagnani, 1997).

In what is called the primary focus of clonal expansion, both activated Th2 cells and B cells will begin to proliferate over several days and B cells begin to secrete antigen-specific antibody into the bloodstream where it quickly reaches the site of infection (Romagnani, 1992). Upon beginning antibody secretion into the body, the B cells are known as plasma cells (Slifka & Ahmed, 1998). When antibodies begin binding to antigen on pathogens at the site of infection, the effector mechanisms used to destroy pathogen populations closely mirror those of innate immunity. Antibody may bind to a receptor key to bacterial replication and halt the life cycle (called neutralization), opsonize a pathogen and create a favorable environment for macrophage phagocytosis, or further activate the complement pathways (Harboe & Mollnes, 2008). Plasma cells that persist in the body long after an eradicated infection are called memory B cells and may quickly respond to future infections by the same pathogen (Slifka & Ahmed, 1998).

Th17 cells represent a subset of CD4+ helper T cells more recently reported than their Th1 and Th2 counterparts (Steinman, 2007). They secrete IL-17 at the site of infection as a proinflammatory method of neutrophil and macrophage recruitment (Steinman, 2007). While important in recognition of extracellular and intracellular pathogens alike, Th17 cells have been implicated in autoimmune

disease and transplant rejection if they recognize self-proteins (Afzali, Lombardi, Lechler, & Lord, 2007; Peck & Mellins, 2010).

STOPPING AN IMMUNE RESPONSE & THE TREG PATHWAY

During an immune response, cytokines like IFN-γ and TNF-α spread locally in the body and recruit both innate immune response cells like NK cells and macrophages and adaptive immune response cells like cytotoxic T cells to the site of infection and inflammation. When a pathogen is successfully eliminated, however, these cytokines are still in the bloodstream and are still triggering inflammation and cell recruitment. This can prolong inflammation to the point of destroying healthy tissue (Parham, 2009). This is where previously mentioned Treg cells, also carrying CD4+ receptors, inhibit inflammatory cytokines and phagocytotic APCs (Shevach, 2009). Tregs are identified by two proteins necessary for their function: transcription factor Foxp3 and outer membrane protein cytotoxic T lymphocyte antigen 4 (CTLA-4), also present on other T cells (Shevach, 2009). Tregs also express several anti-inflammatory cytokines like IL-10, TGF-β, and IL-4 (Shevach, 2009).

CTLA-4 has a structurally similar binding cleft to CD28 and also binds to CD80 on APCs, but CTLA-4 *downregulates* APC surface expression of necessary co-receptors for T cell activation like CD80 and CD86, the opposite function of CD28 (Shevach, 2009). By downregulating the expression of these co-receptors,

APCs are no longer able to activate either CD4+ T_H cells or CD8+ T_C cells, closing out both the cell-mediated and humoral immune responses (Shevach, 2009). Tregs that express a high level of CTLA-4 induce APCs to express the enzyme indoleamine 2,3-dioxygenase, abbreviated IDO (Shevach, 2009). Once expressed, IDO converts the amino acid tryptophan, key to T cell activity, into toxic kynurenine, inactivating T cells (Shevach, 2009).

Tregs also express an immunosuppressor called LAG-3 that binds to the MHC class II molecules on dendritic cells, preventing maturation and further T cell stimulation (Shevach, 2009). In order to suppress the activity of activated T cells, Tregs express CD39 on their outer membrane surfaces (Shevach, 2009). CD39 cleaves adenosine triphosphate (ATP), a common high energy molecule used in all cells, into just adenosine, downregulating inflammation. As a long-lasting immunosuppressing factor, Tregs can out-compete naïve T cells for APC interaction and activation. Located on the Treg cell surface, Neurophilin (Npr-1) is a competitor with effector cells for immature dendritic cell binding, preventing naïve T cells from connecting with dendritic cells in the first place (Figure 5) (Shevach, 2009).

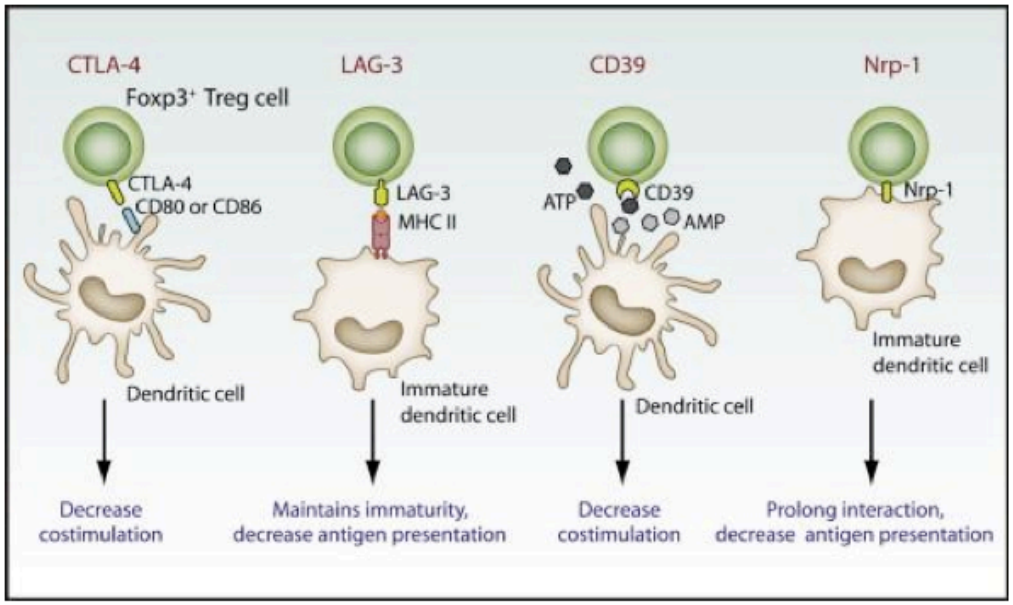

Figure 5. Four methods used by Tregs to suppress APC pro-inflammatory function. Source: Sevach, 2009 (see references).

Separately from the previously mentioned cell surface receptors, Tregs secrete various immunosuppressant cytokines, such as IL-10 and TGF-β, as a method to counter the inflammatory cytokines during an immune response (Chaudhry et al., 2011).

INTERLEUKIN-10: INDICATOR OF THE TREG PATHWAY

IL-10, on the other hand, is a cytokine that represents the anti-inflammatory side of the immune equation and can be secreted by a variety of cell types, most actively in Treg and TH2 cells. IL-10 aids in Treg differentiation and function while also inhibiting activation and effector function of other helper T cells, including inhibition of Th1 and Th17 cells' inflammatory functions (Chaudhry et al., 2011). With this inhibitory function, IL-10 serves as a cytokine that suppresses inflammatory immune responses (Figure 6). IL-10 is the best-studied inhibitor of IL-12 expression, a cytokine secreted by dendritic cells and macrophages that promotes pro-inflammatory IFN-γ production (Magram, et al., 1996). Through this interaction, IL-10 can inhibit up to 90% of cellular IFN-y production (Magram et al., 1996). It has also been suggested that IL-12 can trigger expression of low levels of IL-10 as a form of negative self-regulation (Meyaard, Hovencamp, Otto, & Miedema, 1996).

IL-10 can inhibit TNF-α production in macrophages previously activated by IFN-γ at both protein and mRNA-synthesis levels (Oswald, Wynn, Sher, & James, 1992). IL-10 induces endogenous production of TNF-α, a required co-stimulatory factor for macrophage activation, essentially diminishing the infection-killing abilities of phagocytotic cells, even if they've already been primed by IFN-γ (Oswald, Wynn, Sher, & James, 1992). Previous studies in the Stranford lab have shown elevated IL-10 levels in both MAIDS-resistant BALB/c and MAIDS-susceptible C57BL/6 mice in the first three days of infection compared to

infection-naïve mice, though C57B/6 mice have nearly triple the concentration of IL-10 as BALB/c mice in lymph nodes (Kwaa, 2012). Looking at known HIV/AIDS pathology, the initial interactions between virus and host cells are likely centered in the lymph nodes, so differences in early immune responses in these tissues may predict outcomes (Baveja & Rewari, 2004).

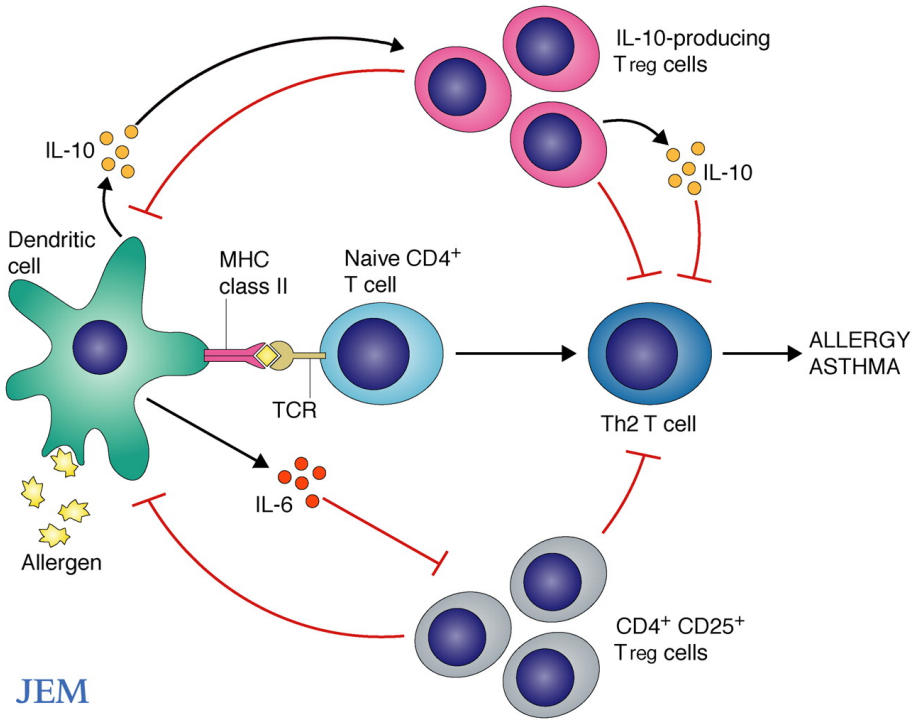

Figure 6. Inhibitory functionality using IL-10 between APCs (here pictured as the dendritic cell) and Tregs. This functionality works for pathogens other than only the allergens pictures. Source: Journal of Experimental Medicine. Link: http://jem.rupress.org/content/202/11/1459/F1.expansion.html

HIV/AIDS INFECTION & SYMPTOMS

HIV is a retrovirus that infects CD4+ T_H cells and uses virus-specific proteins such as reverse transcriptase and integrase to use host cell machinery to make copies of itself. A retrovirus has RNA as its genetic material and uses reverse transcriptase to transcribe the RNA into DNA and then incorporate its genome into the host genome for replication (Dorland, 2007). Symptoms of early infection include flu-like symptoms, though many infected with HIV will show little to no signs of initial infection. There are two types of HIV: HIV-1 and HIV-2 (Gandhi, Omobolaji T., Campbell-Yesufu, & Rajesh T., 2011). HIV-1 is the most common form of HIV and is known to progress to AIDS faster than HIV-2, which has a longer asymptomatic period (Gandhi,Omobolaji T.Campbell-Yesufu & Rajesh T., 2011; Walker,Rajesh T.Gandhi & Bruce D., 2002). Both infect CD4+ cells in the same manner, though HIV-1 appears to be more effective and more difficult to control both by the human immune system and medication with a higher viral count and more viral RNA, signs of greater viral proliferation in the host (Gandhi,Omobolaji T.Campbell-Yesufu & Rajesh T., 2011). Infection typically involves binding between gp120/41 on the viral envelope and a CD4 molecule on the T_H cell plasma membrane and a second chemokine interaction with either CCR5 or CXCR4 (Cicala, Arthos, & Fauci, 2011). HIV fuses to the host cell surface, relasing HIV RNA, reverse transcriptase, integrase, and other viral proteins into the host T cell (Cicala et al., 2011). Viral DNA is formed by reverse transcriptase in the host cytoplasm before integrase binds to the ends of

the viral DNA and enters the host nucleus. The viral DNA integrates into the host genome with the help of integrase and uses host transcription and translation machinery to replicate viral proteins. These new proteins and viral RNA are moved to the cell surface, are exocytosed as virions and infect other host T cells (Figure 7) (Cicala et al., 2011).

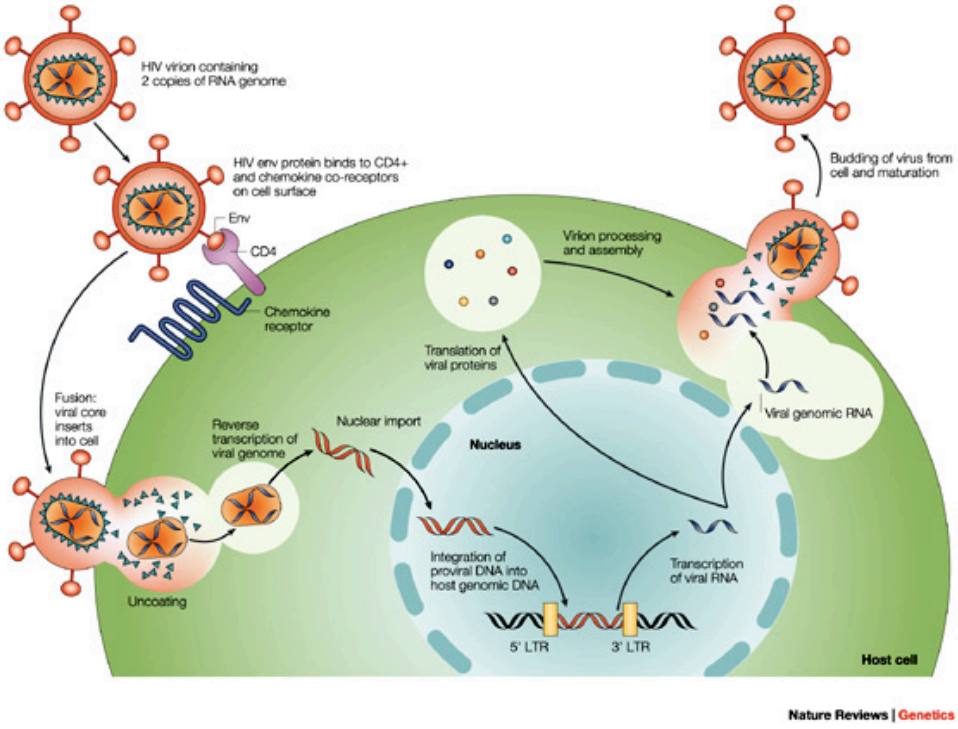

Figure 7. The HIV life cycle through host CD4+ T cells. HIV uses host machinery to transcribe and translate viral proteins to replicate and infect more CD4+ T cells, eventually leading to widespread CD4+ T cell death. Source: Nature Reviews, Genetics. Link: http://www.nature.com/nrg/journal/v5/n1/fig_tab/nrg1246_F1.html

This ‘hostile takeover’ of CD4+ T cell machinery transforms an immune response cell meant for immune protection into an HIV factory, leading to disabled immune responses. One sign of HIV infection progressing to AIDS is a low T cell count because the virus has advanced to T cell destruction. Upon the progression to AIDS and without a sufficient number of CD4+ T cells available, patients are susceptible to opportunistic infections like tuberculosis and meningitis, or more common ailments like influenza and rhinovirus, often with death as the outcome even with medications targeting and inhibiting specific proteins produced by HIV in its replication cycle, including integrase and proteases.

PREVIOUS RELATED RESEARCH IN THE STRANFORD LABORATORY

Previous honors research on the role of immunosuppressant interleukin-10 (IL-10) in the MAIDS model has suggested that early IL-10 expression in C57BL/6 susceptible mice within the first three days of infection with MuLV changes the course of the immune response from the typical antiviral cell-mediated response of low IL-10 expression and high interferon-γ (IFN-γ) expression observed in infection-resistant BALB/c mice, despite similar IL-10 levels observed in both strains before MuLV infection (Kwaa, 2012). The line of investigation is now directed at comparing the suggested rise in IL-10 levels in MAIDS-susceptible C57BL/6 mice compared to MAIDS-resistant BALB/c mice

to levels of pro-inflammatory IFN-γ, which would more strongly suggest a typical antiviral cell-mediated immune response than lower IL-10 expression alone.

GOALS OF THIS STUDY

This study aims to confirm previous research in the Stranford laboratory concerning high IL-10 expression as a representation of an anti-inflammatory response in MAIDS-susceptible C57BL/6 mice compared to MAIDS-resistant BALB/c mice in an early immune response against MuLV (Kwaa, 2012). Additionally, this study aims to look at the corresponding relationship between the mouse strains' IFN-γ expression as a representation of the inflammatory response, hypothesizing that IFN-γ concentrations will be higher in MAIDS-resistant BALB/c mice as an indicator of BALB/c mice entering a Th1 pathway. This study will test pooled axial, inguinal, and brachial lymph nodes pooled together as representative of the lymphatic system's T cell differentiating centers.

MATERIALS & METHODS

There were four major processes in this research: Sample preparation, Enzyme-Linked Immunosorbent Assays (ELISA), Bradford Protein Assay, Analysis of Variance (ANOVA) for the Bradford Protein Assay, and independent t tests for the ELISAs.

(1) Dissection and Sample Preparation

Four each of BALB/c and C57BL/6 mice (6 weeks old, female, Taconic Farms, Inc, Hudson, NY) were injected intraperitoneally with 1.0 mL of $3.2x10^3$ PFU/ml of LP-BM5 Murine Leukemia Virus (MuLV). Another four of each mouse strain were left untreated (naïve) and used as controls. At day 3.5, infected mice were sacrificed by CO_2 inhalation and dissected. Their axillary, brachial, and inguinal lymph nodes were isolated as a collective lymph node sample (LN). All samples were kept on ice. To increase the surface area on which 4° C cell lysis buffer (1X Protease Inhibitor Cocktail Cat #554779, BD Biosciences, San Jose, CA, and 0.5% Triton X-100 in PBS) works, these organs were cut into small pieces before being placed in the pooled LN tube for each mouse with 0.5 mL of lysis buffer. All samples were collected in 5 mL polystyrene round-bottom tubes. Uninfected control mice underwent the same procedure. These solutions were homogenized at room temperature for 90 seconds at ¾ speed with a Polytron PT-MR 3100 D (Cat #11090093, Kinematica AG, Lucerne, Switzerland) with a Polytron PT-DA Homogenizing Accessory for 0.1-2.0 ml (Cat #11030004, Kinematica AG, Lucerne, Switzerland). Samples were transferred to

microcentrifuge tubes and centrifuged at 12,000 rpm for 10 minutes at 4° C so that the pellets contained plasma membrane, connective tissue, and other dense cell parts, whereas the supernatant contained cytosolic components and interstitial fluid. The pellets were discarded and sample supernatant saved in 100 µl aliquots, immediately stored at -20° C.

(2) Determination of Sample IFN-γ and IL-10 Concentrations

To begin the two quantitative ELISAs for IFN-γ and IL-10, 96-well BD-Falcon plates were coated with 100 µl of the capture antibodies provided in each cytokine-specific BD OptEIA™ Mouse ELISA Set (Cat #551866 and #555252, respectively, BD Biosciences, San Jose, CA), diluted 1:180 in a 1.0 M phosphate buffer (pH 9.5) and incubated for 16 hours at 4° C. Each well was then washed 5 times with 300 µl of PBS, 0.05% Tween-20 (pH 7.0) wash buffer. For each wash in this experiment, the wells were filled with 300 µl of wash buffer, emptied with all remaining bubbles popped, and blotted before washing again. The plate was then blocked with 100 µl/well of assay diluent, consisting of PBS with 10% fetal bovine serum. After a one-hour incubation at room temperature, plates were washed 5 times, and standard and unknown samples plated in appropriately labeled wells. The cytokine standards were plated 100 µl/well in triplicate rows, A-G, from 2000 pg/ml by two fold serial dilution to 31.25 pg/ml, using the wells in row H as blanks filled with only assay diluent. LN supernatant samples were thawed on ice and then diluted with assay diluent to a 1:10 concentration and plated in triplicate with 100 µl per well. After a two-hour incubation at room

temperature, plates were washed 5 times. Cytokine-specific detection antibody and Streptavidin-Horseradish Protein (SAv-HRP), both provided by the BD ELISA kit, were diluted 1:250 with assay diluent and 100 µl of this mixture added to each well. After a one-hour incubation at room temperature, plates were washed 10 times and 100 µl/well of 1:1 substrate solution of tetramethylbenzidine (TMB) and hydrogen peroxide was added to each well. After a 30-minute incubation at room temperature, 50 µl of 1.0 M H_3PO_4 stop solution was added to each well to halt the color-changing chemical reaction. The plates were immediately read at 450 nm with a correction at 570 nm on a VersaMax™ Tunable Microplate Reader (Cat #97244, Molecular Devices, Sunnyvale, CA) with SoftMax® Pro 5.0 Software (Cat #0200-310, Molecular Devices, Sunnyvale, CA).

(3) Determination of Sample Protein Concentrations

The Coomassie (Bradford) Protein Assay Kit with BSA standard (Cat #23200, Thermo Scientific, Rockford, IL) was used on the same day as the ELISA procedure using an aliquot of the same thawed samples, to determine the concentrations of total protein in each sample. The results serve to correct ELISA cytokine concentration data for the amount of protein contained in each sample collected. In a BD-Falcon 96-well microplate, 5 µl of the BSA standard was plated in triplicate going down in concentration from rows A-H (2000, 1500, 1000, 750, 500, 250, 125, 25 mg/ml respectively). All dilutions were made with lysis buffer saved from the previous mouse dissection. 5 µl of each vortexed LN

supernatant sample was tested in triplicate in the remaining wells. A triplicate of lysis buffer alone served as the experimental blank. 250 µl of Coomassie Bradford reagent was added to each well. The plate was incubated for 10 minutes before being read at 595 nm on the VersaMax™ Tunable Microplate Reader with SoftMax Pro® 5 Software.

(4) Data Analysis

The existence of differences between observation points in protein concentration of samples was tested and the protein concentration measurements from the Bradford Assay were measured in mg/ml were then used to correct the ELISA cytokine concentration measurements for differences in total protein for each unknown sample. This was done by dividing the ELISA cytokine concentrations in pg/ml by the Bradford Assay total protein concentrations converted to mg/ml, providing data in pg of cytokine per mg of protein in each sample. An analysis of variances (ANOVA) was conducted using the Statistical Package for the Social Sciences (SPSS, version 19) on protein concentration data. The 2 x 2 factorial between subjects design had two different observation points (naïve [no MuLV infection] vs. Day 3.5 [post-MuLV infection]) and two different strains of mice (BALB/c [MAIDS-resistant] vs. C57BL/6 [MAIDS-susceptible]). It is important to note that observation point is not a within subjects factor since the mice were sacrificed at that observation point in order to obtain LN specimens. To meet the assumption of homogeneity of variance was tested using a Levene's test for equality of variances. When Levene's test was significant

($p<0.05$), variances were deemed unequal between the groups, outliers were examined and natural logarithms were then conducted in order to meet this critical assumption before proceeding with the full factorial ANOVA. A piori alpha was set at 0.05.

Planned pairwise comparisons using independent t tests were conducted to evaluate hypotheses.

RESULTS

Data was inspected for accuracy, descriptive statistics were calculated, and Box and Whisker plots were produced for each dependent variable (IL-10 and IFN-γ concentrations) by observation period and mouse strain. In addition, a preliminary assessment of protein concentration using analysis of variance (ANOVA) was conducted to ascertain the necessity of correcting the ELISA cytokine concentration for differences in total protein. Independent t-tests were used to elucidate relevant pairwise comparisons between observation points and mouse strains. Mouse 9, an infected day 3.5 BALB/c mouse, was confirmed as a statistical outlier. Thus all analyses were also performed with and without the outlier to ascertain the extent of which the outlier unduly influenced the findings.

It was concluded from the initial attempt to conduct ANOVA on the raw protein concentrations from the Bradford Assay, the assumption of homogeneity of variances was not met (Levene's test $p= 0.015$). Using the natural logarithm of protein concentrations resulted in ANOVA that met the assumption for homogeneity of variances (Levene's test $p=0.776$). There was no interaction between observation point and mouse strain ($p=0.780$) and no main effect of mouse strain ($p=0.206$). However, a significant main effect of observation points was detected ($p=0.003$). Mean protein concentration was significantly lower at the naïve observation point (mean=0.542, SD=0.257) as compared to day 3.5 (mean=1.497, SD=0.754), (Figure 8). Given the significant differences over

observation points, protein concentrations were used to correct cytokine concentrations in all following analyses as pg/mg of tissue.

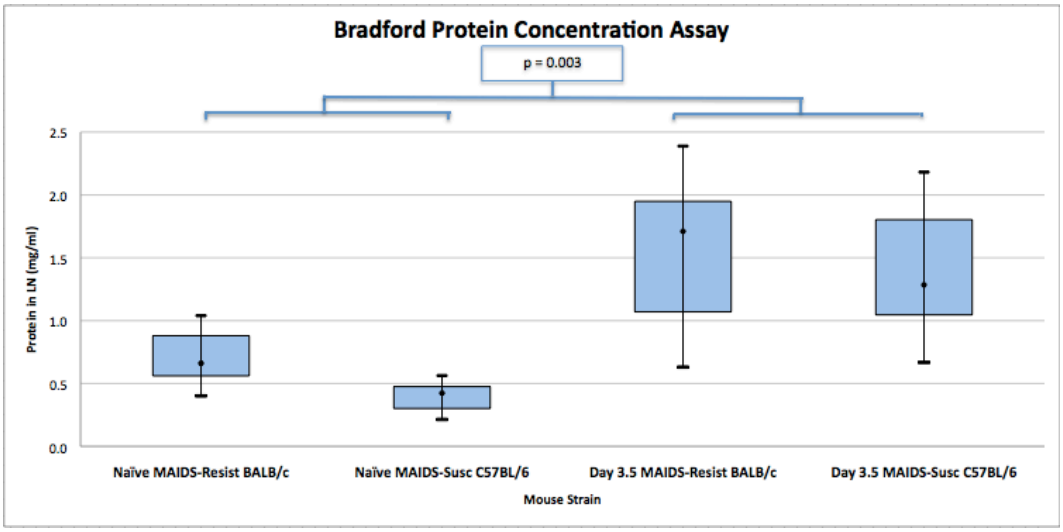

Figure 8. Protein concentrations by observation point and mouse strain as measured using a Bradford protein assay. Using the natural logarithm of protein concentration, naïve observations were significantly lower than Day 3.5 observations regardless of mouse strain.

Pairwise comparisons using independent t-tests were used to explore group differences of interest with respect to IL-10 concentrations in LN. There were no significant differences between mouse strains at the naïve observation point (p=0.646) or between mouse strains at the day 3.5 observation point (p=0.230) (Figure 9). There were also no significant differences (p=0.729) among the C57BL/6 mice comparing naïve and day 3.5 IL-10 concentrations. However, among the BALB/c mice, IL-10 concentrations were significantly lower (p=0.022) at the naïve observation point (mean=0.278, SD=0.839) as compared to the day 3.5 observation point (mean=-0.0352, SD=0.665). These t-tests were repeated without the outlier (Mouse 9 – infected day 3.5 BALB/c) and the conclusions were unchanged.

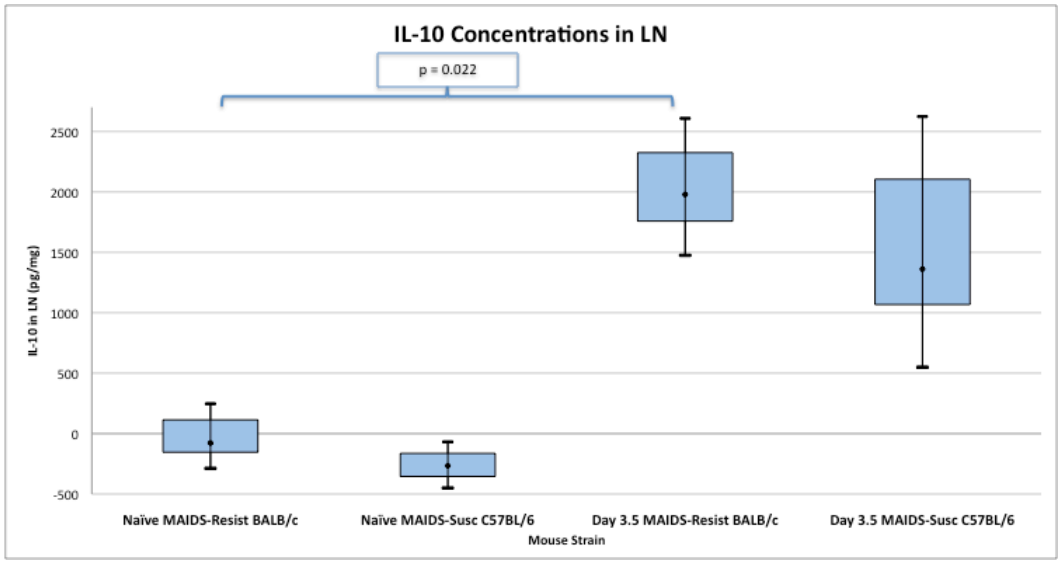

Figure 9. IL-10 concentrations corrected for total protein concentration in LN by observation point and mouse strain. Among BALB/c mice, significantly higher IL-10 concentrations were found at day 3.5 than at the naïve observation point.

Similar to IL-10 concentration analysis, pairwise comparisons using independent t-tests were used to explore group differences of interest with respect to IFN-γ concentrations in LN. There were no significant differences between mouse strains at the naïve observation point (p=0.892) or between mouse strains at the day 3.5 observation point (p=0.423) (Figure 11). There was also no significant difference (p=0.356) among the C57BL/6 mice comparing naïve and day 3.5 IFN-γ concentrations. Among the BALB/c mice, IFN-γ concentrations were also not significantly different (p=0.816) at the naïve observation point (mean=0.167, SD=0.873) as compared to the day 3.5 observation point (mean=-0.0373, SD=0.709). These t-tests were repeated without the outlier (Mouse 9 – infected day 3.5 BALB/c) but the conclusions reached were unchanged.

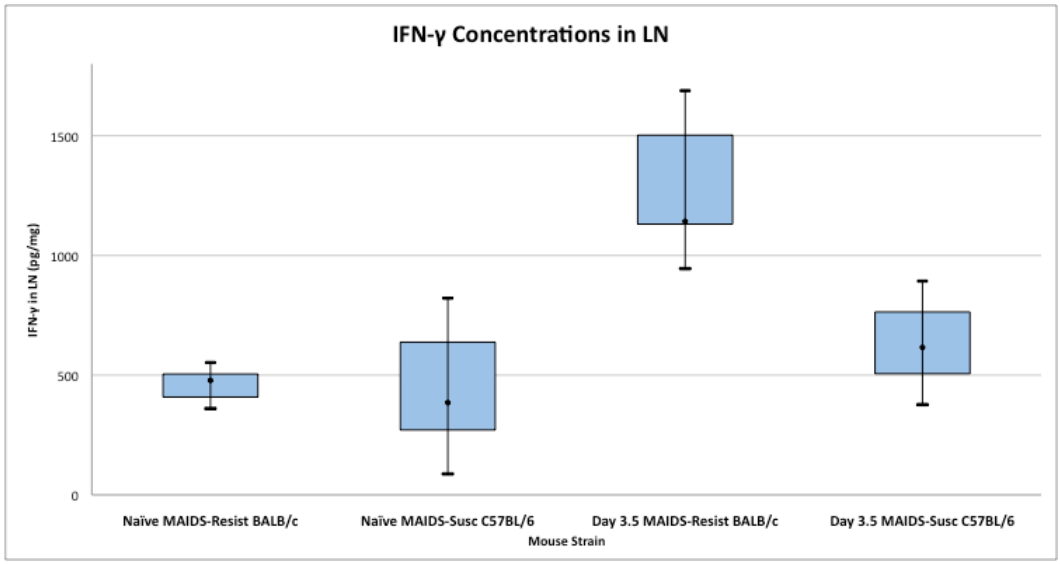

Figure 10. IFN-γ concentrations corrected for total protein concentrations by observation point and mouse strain. Using the natural logarithm of IFN-γ concentrations, no significant differences between observation points and mouse strains were found

DISCUSSION

The results from the Bradford Assay on total protein concentration in collected LN show significant differences between naïve pre-infection and infected day 3.5 groups for both MAIDS-resistant BALB/c and MAIDS-susceptible C57BL/6 mice (Figure 8). These significant differences confirmed the necessity of performing a Bradford Assay and using the resulting individual total protein concentrations to normalize cytokine concentrations measured in later ELISAs.

The cytokine quantification results from the IL-10 ELISA suggest that between naïve pre-infection and infected day 3.5 groups of both BALB/c and C57BL/6 mice, there are only significant differences in IL-10 expression between naïve and day 3.5 BALB/c mice (Figure 9). No significant differences in IFN-γ expression were found from IFN-γ ELISA results between naïve groups, between day 3.5 groups, between naïve and day 3.5 groups of the same mouse strains, or between mouse strains (Figure 10). It is worth noting that when the outlier (Mouse 9 – infected day 3.5 BALB/c) was removed from either the IL-10 or IFN-γ data set and independent t tests run again, the conclusions were unchanged.

There were, however, several variations in experimental design that may prevent this research from being an exact replicate of the Kwaa paper's that this research sought to confirm and could have impacted interpretation of these data. The first of these variations was the use of the Bradford Assay to normalize IL-10 or IFN-γ concentrations with the total protein concentration of each individual's

LN sample. This normalization was performed because of the following proposed logic: if two samples contained the same concentration of IL-10 or IFN-γ but sample A was twice the size with potentially twice the protein and number of cells of sample B, the T cells and APCs in sample A's LNs would have half of the contact with the cytokine in question. Therefore, IL-10 or IFN-γ in sample A's LNs would have half of the effect on T cells, APCs, and any other surrounding cells with the appropriate receptors. A more accurate and precise normalization would use protein concentrations from only the T cell centers of each lymph node used, as cytokine may not be evenly distributed throughout a lymph node and not reach differentiating T cells equally. This type of normalization may be useful in further study, but a total protein concentration of lymph nodes still leads to a more thorough analysis of cytokine concentration to size of area affected by that cytokine. If cytokine concentration data from Kwaa, 2012, could be normalized by the total protein concentrations of those particular samples, data would be more directly comparable.

An alternative hypothesis suggests the amount of cytokine by tissue mass is not the appropriate measure by which to compare MAIDS-resistant and MAIDS-susceptible mice, but that the sheer amount of cytokine in pg/ml will direct the immune response pathways. In any case, the non-protein concentration normalized cytokine levels calculated directly from the ELISAs still did not show the same pattern as previous research of IL-10 depression in BALB/c mice or a

rise in IL-10 expression in C57BL/6 mice and these results still do not confirm those of previous study.

Another, and possibly major, difference between previous research and this experiment is the variation between the viral titers used in these experiments. The MuLV used in Kwaa, 2012, came from the 5-20-2010 viral stock with a titer of 3.6×10^4 PFU/ml of LP-BM5 isolate of MuLV whereas this experiment used a different viral stock (2-3-2010) with a titer of 3.2×10^3 PFU/ml of the same viral isolate, both grown in the Stranford laboratory. Few other MAIDS studies reported in the literature used a titer lower than 10^4 PFU/ml of LP-BM5 MuLV or looked at an earlier time point than one week after infection for differences between strains (Green, Okazaki, Honjo, Cook, & Green, 2008). A difference of one degree of magnitude in viral titer has unknown effects on either BALB/c or C57BL/6 mouse strains, especially at the notably early observation point of 3.5 days. It may be possible that there is a threshold viral titer in MuLV at which immune responses begin to change their characterization or, in the extreme, overall pathway.

In humans, viral titers of HIV can be used to predict how long an HIV positive person has until developing AIDS, but the a difference in viral titer administered to mice makes before AIDS development is unexplored territory (Baveja & Rewari, 2004). While scientists would desire a viral titer of MuLV comparable in mice to the viral titer of HIV in humans normally found in AIDS, viral titers in mice and humans are difficult to compare, especially with the two

different retroviruses used. In fact, HIV and MuLV are measured in completely different units, virions/ml of blood and PFU/ml, respectively.

Possibly linked to a relatively low viral titer are the relatively low spectrophotometer readings of ELISA plates. While the standard curves for both IL-10 and IFN-γ ELISA kits from BD Biosciences produced R^2 values>0.900 and peak optical density (OD) readings were consistent, the concentrations measured were low across the board, except for the outlier (Mouse 9). In contrast, the Kwaa data show consistently low peak optical density readings that were just as consistent, but the concentrations measured were noticeably higher than those reported here (Kwaa, 2012). For example, Kwaa reported an average OD of 0.01 for Mouse 3, an infected day 3 BALB/c mouse, with a corresponding IL-10 concentration of 835.61 pg/ml against a standard with a peak OD of 0.068 for 2000 pg/ml of IL-10 and R^2=0.998 (Kwaa, 2012). In contrast, this experiment reported an average OD of 0.211 for Mouse 10, an infected day 3.5 BALB/c mouse, with a corresponding IL-10 concentration of 92.52 pg/ml against a standard with a peak OD of 1.6 for 2000 pg/ml of IL-10 and R^2=0.998. While these variable results of ELISAs exist, both sets of research report R^2 values of 0.998 for their respective standard curves, and thus the ELISA kit itself is an unlikely source of differences.

Besides differences in viral titers, other post-tissue removal techniques could lead to lower than expected cytokine readings. This includes the discrepancies in how an individual researcher completely homogenizes the

samples and effectively separated low-density cell materials (e.g. cytokines) from high-density cellular materials (e.g. fats, connective tissue), because failure to do this properly could result in the high-density pellet pulling in some low-density materials during centrifugation.

Variations in lysis buffer preparation and use could also play a role in how either IL-10 or IFN-γ successfully binds to the capture antibody in a sandwich ELISA. This includes variations such a temperature, age of the lysis buffer preparation, and the number of freeze/thaw cycles the lysis buffer has undergone. Other experimental variations can occur with centrifugation technique and accurately aliquoting sample and configuring samples so the centrifuge is properly balanced and works appropriately.

Another experimental difference between previous research and this experiment appears at observation point. Previous research used day 3 as the earliest infected observation point when this experiment used day 3.5 due to severe weather limiting access to the laboratory. If this extra 12 hours did affect results, it should only have served as more time for further differentiation on a Th1 or Treg pathway. This leads to the conclusion that observation point variation did not strongly affect results.

The lack of significant trends in IL-10 concentration between mouse strains fails to confirm previous results found in the Stranford laboratory, where IL-10 expression levels in naïve mice had no significant differences, but at day 3 BALB/c and C57BL/6 mouse strains showed significantly different expression

levels (Kwaa, 2012). The day 3 MAIDS-resistant BALB/c in the Kwaa paper showed depressed levels of IL-10, while day 3 MAIDS-susceptible C57BL/6 mice showed raised levels of IL-10 in the same LN pool collected here (Kwaa, 2012). These data were important because the MAIDS model had not previously been examined at such an early observation point because MAIDS usually develops after several weeks (Jolicoeur, 1991). Day 3, by comparison, is an extremely early observation point for measuring potential differentiation of the immune response between Th1 and Treg pathways, so by not confirming those previous results here, further replication attempts would be necessary to confidently report a difference in immune response at the early day 3 (or here, day 3.5) observation point.

A significant up-regulation of expression between IL-10 concentrations in naïve and day 3.5 BALB/c groups, but not in the corresponding C57BL/6 naïve and day 3.5 groups or in either mouse strain in IFN-γ, was still a noteworthy result because IL-10, as an indicator of the Treg response, is known as an immunosuppressant mainly after an inflammatory response is over and pathogen has been successfully eradicated (Chaudhry et al., 2011). Without a significant IFN-γ difference in the same naïve and infected day 3.5 individuals, and therefore no measured presence of a Th1 response, it would appear that the Treg response is beginning in day 3.5 BALB/c mice without any previous inflammatory immune response to suppress. This is the opposite response found in previous research

using a similar experimental design, where IL-10 levels were depressed in day 3 BALB/c mice (Kwaa, 2012).

In order to parse through these various explanations for disparate conclusions in such similar experiments, future studies could look at exactly how differing viral titers (e.g. 10^3, 10^4, 10^5, 10^6 PFU/ml) of LP-BM5 isolate of MuLV relate to IL-10 and IFN-γ concentrations in both BALB/c and C57BL/6 mouse strains at early time points, since there is no precedent for early infection behavior. It is possible that the MAIDS model does not take hold below a certain threshold of virus inoculums. This would have to be supported before these data can be used as evidence against previous research in the final interpretation of results, that there is actually no significant difference between the immune responses of either mouse strain at such an early time point and that other laboratories are correct in that the most interesting behavior occurs at the 3-6 week range in C57BL/6 mice that develop MAIDS.

If a low administered viral titer of LP-BM5 MuLV is the reason for nonsignificant differences between observation points and mouse strains on IL-10 and IFN-γ concentrations and low overall cytokine expression pattern in both MAIDS-resistant BALB/c and MAIDS-susceptible C57BL/6 mouse strains, then it is important that future studies take into account the need for high titers in order to achieve data appropriate to relate to MAIDS, and in discussion, human AIDS. However, it is also possible that there is no significant difference between mouse strains at the early day 3.5 time point of infection and that the increased variation

between individuals in each group mirrors varied adaptive immune response strengths in each individual mouse attempting to deal with MuLV. If this is indeed true in the MAIDS-susceptible C57BL/6 strain, it is conceivable that this mirrors the greatly varied early responses in HIV that confound many researchers today. However, there is also greatly more genetic variability between humans infected with HIV than between these two strains of laboratory mice infected with MuLV, so this direct comparison is likely not viable though it provides a new lens from which to look at early variation within these strains.

Even more interesting would be to infect many more C57BL/6 mice than the four in this experiment with the low viral titer and wait until one does not develop MAIDS. It is possible that a low viral titer acts as a vaccine in some mice, priming the immune system and resulting in a different immune response than developing MAIDS. Investigating why a C57BL/6 mouse may not develop MAIDS could give greater insight than comparing MAIDS-resistant BALB/c mice, though developing a MAIDS-resistant C57BL/6 mouse line would be appreciably more difficult, if possible. Assuming this MAIDS-resistant C57BL/6 mouse's resistance is genetic, data on this mouse's progeny's early infection period may give insight on how it successfully fought MuLV infection compared to the many C57BL/6 mice that would go on to develop MAIDS and eventually die. This type of experiment mirrors many Drosophila experiments where the odd variant out was picked out of the sea of thousands of other flies in order to understand its genetic mutations. This also presents limitations to this method of

further study, since mice cost much more than flies and take longer between generations. The amount of space necessary to house so many mice would be overwhelming to most labs attempting to study the MAIDS model.

In the end, this research failed to confirm previous results in the Stranford laboratory, though it was not an exact replication due to differences in viral titer and observation points. With these difficulties in mind, it is still remarkable that IL-10 concentrations significantly increased in only the BALB/c mice over observation points when the opposite, a depression in IL-10, was found in previous research. Proposed further study and an increased number of individuals tested, especially in the infected observation points, would help clarify and lend more confidence to these conflicting results and guide future inquiry in the Stranford laboratory.

Due to the hazardous qualities of HIV/AIDS research in humans or in the simian model, understanding the quirks and variations in the MAIDS model is key to further research pertaining to HIV/AIDS in many laboratories. Once the point of MuLV infection where immune response pathways differentiate between mouse strains, perhaps in the hypothesized Th1 and Treg dichotomy, experimentation on how to prevent MAIDS development in C57BL/6 mice at that point of infection can yield results that may present therapeutic targets useful to HIV/AIDS treatment. This is especially true if that prevention is focused on immune response control and not necessarily controlling HIV itself, the latter task proving difficult to all researchers today.

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