IMMUNOCOMPETENCE AND DECISION MAKING:

A NEUROSCIENCE AND LIFE HISTORY

THEORY PERSPECTIVE

by

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Submitted in partial fulfillment of the

requirements for Departmental Honors in

the Department of Neuroscience

Texas Christian University

Fort Worth, Texas

4 May 2015

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ABSTRACT

From an evolutionary perspective, the most fundamental task faced by all organisms is the successful utilization of resources—time, effort, energy, food—in the service of survival and reproduction. To help explain how organisms will allocate their energy, evolutionary biologists developed a theoretical framework called the Life History Theory. This model states that the decisions an individual makes during development typically form a predictive pattern of behaviors, often referred to as an individual's life history strategy. Individuals in a safe, resource abundant environment follow a slower life history strategy and tend to make more future-oriented, rational decisions. On the other hand, individuals in a dangerous, resource void environment follow a faster life history strategy and make more impulsive, present-focused decisions. In the past, many studies have been done to correlate external cues, such as childhood socioeconomic status, and life history strategies, but not much has been done to analyze how internal cues might drive life history strategies. This first-of-its-kind study sought to answer the question, "How does the reactivity of one's immune system influence one's desire for present- versus future-oriented outcomes?" In order to do this, we examined markers of immunological function and compared them to self-reported data. Although the results have not been completed, we confidently predict that individuals with less reactive immune systems will follow a faster life history strategy, making more impulsive, risky decisions, whereas those with more reactive immune systems will follow a slower life history strategy, making more rational, future-oriented decisions.

TABLE OF CONTENTS

Immunocompetence and Decision Making: A Neuroscience and Life History Theory Perspective

Introduction

Evolutionarily speaking, the most fundamental and important task presented to all organisms is maximizing one's fitness. Key predictors of an organism's long term fitness are the choices it makes in budgeting its limited energy between competing needs across the lifespan. Whether it be on reproduction, growth, food acquisition, or survival, energy must be spent wisely by an organism or death will surely follow. One question that has been researched and studied for decades is the question of what environmental factors predict how an organism will spend its energy. Life History Theory is a theoretical model based on evolutionary biology that is used to predict the tradeoffs than an organism will make in allocating its limited energy budget based on conditions in the organism's local ecology (Charnov, 1993; Kaplan & Gangestad, 2005; Roff, 1992; Stearns, 1992).

The decisions an individual makes during development typically form a predictive pattern of behaviors, often referred to as an individual's life history strategy (Del Giudice, Ellis, & Shirtcliff, 2011; Ellis et al., 2009). Life History Theory suggests that features of the environment, such as its harshness and unpredictability, that impact an organism's chances of survival are key predictors of which strategy that an organism will pursue. Organisms that live in an environment in which the future is uncertain and dangerous (e.g.. a songbird in a predator rich locale) typically pursue "fast strategies." A fast strategy is characterized by earlier reproduction, greater risk taking, more present-oriented decisions (Griskevicius, Tybur, et al., 2011). Because survival is uncertain in a tough environment, it is adaptive to allocate energy to present gains in order to achieve immediate rewards. When survivability is uncertain, investing energy toward

outcomes that provide immediate rewards – even if they come at the expense of larger, long-term pay-offs – is a favored course of action, because it decreases the likelihood that one will invest efforts in outcomes for which one will never reap the rewards. If the organism is unsure of its survival to the next day, then it is more beneficial to "spend" now while it still has a chance to gain the fitness benefits of reproducing now. On the other hand, organisms whose environments predict a high chance of long survivability are known as "slow strategists," This strategy is characterized by prolonged growth and delayed reproduction (Griskevicius et al., 2013; Griskevicius, Tybur, et al., 2011; Kaplan & Gangestad, 2005). Slower strategists have more time to invest in their own growth and prepare for reproduction, so they tend to make decisions that are more future oriented. Because there is no rush to reproduce quickly before dying, these organisms have the luxury of spending more of their energy on themselves and slowly accruing the larger, delayed rewards that will make for successful reproduction and competitive offspring in the future.

Although humans typically tend to favor a slower life history strategy than most organisms (e.g. guppies or frogs), there are still examples of people that favor faster versus slower strategies present in the research literature (Griskevicius, Tybur, et al., 2011; Kaplan & Gangestad, 2005). The former research finds that childhood socioeconomic status (SES) is a very influential external factor that drives life history strategies. Those who grew up in a poor, lower income household tend to follow a faster strategy throughout their life, regardless of their adult SES. Therefore, these individuals are seen maturing at a younger age, investing in offspring earlier, and making more present-minded decisions in order to maximize their chances of survival in an environment that is constantly changing and lacking in resources. On the other hand, those who grew up in a more affluent household follow a slower strategy, making more

delayed, future oriented decisions. The latter study finds that people living in a more violent, dangerous neighborhood tend to reproduce earlier than those living in a safer, more benign neighborhood. These individuals live in an environment in which the chances of survival are lower than those that are in a less violent neighborhood. Thus, they will invest in offspring at a younger age in order to maximize the chances of their genes being passed on to the next generation.

Essentially, existing research has found that external stressors (such as high rates of violent crime) play a major role in how an organism allocates its energy. External factors are those where an individual cannot minimize the threat by investing more somatic effort in oneself (Stearns, 2000). Though the link between extrinsic threat and pursuit of a faster life history strategy has been clearly linked in both the correlational and experimental literature (Griskevicius, Tybur, et al., 2011; Kaplan & Gangestad, 2005, Wilson & Daly; Simpson et al., Ellis et al 2009), not much research has been done to discover how internal, innate factors affect life history strategies.

The Threat of Disease

Throughout history, disease has posed a larger threat to survival than any anthropogenic cause. From 1918-1920, the Spanish Flu amassed a death toll of over 50 million, while affecting over 500 million (Johnson and Mueller, 2002). In the mid 1300's, the bubonic plaque, also known as the black death, has been estimated to have killed about 50% of the populations of Europe, Asia, and Africa (Gottfried, 2010). Even today, the World Health Organization estimates that greater than 15 million people die each year because of various diseases such as influenza, tuberculosis, and AIDS (WHO, 2004). It is clear that the treat of disease has made a

substantial impact on the history of mankind; because of this, biologists agree that disease has even played a critical role on evolution (Morens, Folkers, & Fauci, 2004).

Although the threat of disease has been seen throughout history, its severity can differ from person to person. If the individual invests enough energy, the disease can be avoided. If an individual has a stronger immune system, then the threat of disease is not as much of a threat to survival. Because of this, a person's internal ability tom fight disease is more of an intrinsic cue for those with good immune systems. Therefore, because there is no threat of death, those who have a stronger immune system will assume a slower life strategy—maturing slower, investing in development and growth first and reproduction later, and more future oriented decision making. Strong immune systems will suppress a threat to survival, making those with it more likely to assume a slower life strategy as there is no immediate risk of mortality, which allows the individual to make more delayed gratifying decisions.

On the other hand, weaker immune systems make the threat of disease more of an extrinsic threat; the threat is difficult to overcome and often uncontrollable. For those with weaker immune systems, the amount of energy that is devoted towards avoiding a possible disease threat is negligible because the individual will most likely acquire the disease regardless. Because of this, disease threat in the face of a weaker immune system is more of an extrinsic factor. A less reactive immune system will not be able to fend off the amount of disease that a stronger immune system can, which means that the threat of a disease is more likely to be associated with a threat of mortality. Thus, those who have weaker immune systems will tend to assume a faster life strategy. These individuals are more likely to make rasher, present-focused decisions, mature earlier, and reproduce faster. The lack of a strong immune system will allow

more dangerous disease to threaten survivability; because of this, the individual will spend its resources in order to elongate survival for as long as possible.

Past research at TCU has verified this assumption. This past study utilized the TCU SONA system in order to gather self-reported data to reveal that immune quality is in fact correlated to decision making. Students were asked questions on a four point Likert-like scale such as, "How often were you sick growing up?" and "I plan things carefully." These data proved that a weaker immune system is correlated to more impulsive, present-focused decisions and that stronger immune systems are related to more thought out, future-oriented decisions. The tables below show the data collected from the study, which reveal the correlation between self-reported immune strength and decision making.

Table 1. Pilot Study 1: Correlations between measures of impulsiveness and history of illness (higher scores correspond to more illness).

	Perceived Infectability
Difficulty Fighting Temptation	0.20*
Ability to Delay Gratification	-0.24**

*Note. * indicates significance at p < .05, while ** indicates significance at p < .01*

Table 2. Pilot Study 2: Correlations between measures of impulsiveness and history of illness (higher scores correspond to more illness).

	Overall Sickness History
Delay Discounting	0.29*
Barratt Impulsiveness Score	0.34**
Difficulty Fighting Temptation	0.31*
Ability to Delay Gratification	-0.37**

*Note. * indicates significance at p < .05, while ** indicates significance at p < .01*

From this study, further research needs to be done to measure markers of immunological function and compare these measurements with an individual's life history strategy. Our study seeks to find this correlation.

The Current Research

We are interested how an individual's internal state may affect how they respond to the threat posed by infectious diseases. Predominantly, we are interested in the types of decisions (and indirectly, energy allocation) that an individual makes in response to disease, based on their vulnerability to disease (as measured by immune system quality). Specifically, I examined risk perception and impulsivity as my dependent variables. This study looked both self-reported data and physiological markers in order to make a correlation between immune quality and decision making. The immunological markers examined are B-cells, T-cells and natural killer (NK) cells. These marks were obtained via a 50ml blood draw from each participant. In order to measure risk perception and impulsivity, self-reported data were gathered from the participants, which is then compared with the self-reported immune quality and immunological markers obtained from a blood draw.

To quantify immune strength, the peripheral blood mononuclear cells (PBMC) from each participant will be exposed to various mitogens to induce proliferation. According to the American Heritage Medical Dictionary, a mitogen is a substance that induces mitosis and cell transformation, especially lymphocyte transformation. Specifically, we used phytohaemaglutinin (PHA) and lipopolysaccharide (LPS), which activate T-cells and B-cells, respectively. PHA is a lectin found in plants (especially legumes) which binds to the sugars on glycosylated surface proteins, including the T-cell receptors (TCR), and thereby crosslinks them. Therefore, PHA crosslinks TCR and signal 1 (and possibly also signal 2 via crosslinking of co-stimulatory

molecules), which is required for T cell activation (Carlson, 2007). LPS is a mitogen from gram-negative bacteria (usually *E.*coli), which activates memory B-cells to differentiate into plasma cells (Zhang, et al., 1988). Guided by Promega protocol, proliferation of the PMBC's was measured at 24, 48, and 72 hour increments after being exposed to the mitogens in order to produce a growth curve over time. Using this information, we were able to obtain how reactive an individual's immune system was—the more PMBC proliferation, the stronger the immune system. Along with tracing proliferation of B-cells and T-cells, we also examined natural kill cell reactivity. In doing so, we exposed the natural killer cells to proliferating, malignant cancer cells. Specifically, K562 cells were used in this study in order to activate NK cells. NK cells essentially kill any cell that is foreign to the body. K562 is a non-adherent cell line obtained from a pleural effusion of a 53-year-old female with chronic myelogenous leukemia. K562 blasts are multipotential hematopoetic malignant cells that spontaneously differentiate into progenitors of erythrocytes, granulocytes and monocytes. This cell line is a perfect candidate for natural killer cell activation because it is low maintenance and can be proliferated throughout the study easily.

This is a novel study as it is the first of its kind to analyze markers of immunological function and correlate these data to decision making. Also, both kinesiology and psychology departments are involved. In this study we prompt the question, "How does the reactivity of one's immune system influence one's desire for present- versus future-oriented outcomes?" Using markers of immunological function, we hypothesize that those with weaker, less reactive immune systems will assume a faster life strategy and make more impulsive, present-focused decisions while those with stronger, more reactive immune systems will assume a slower life strategy and make more rational, future-oriented decisions.

Methods

Using the TCU SONA system, roughly 50 individuals were gathered to participate in a series of self-reports and a single 50-mL blood draw in exchange for 5 SONA credits. The first survey, Phoenix Ignition 1 was a simple diagnostic survey used to narrow the participant pool in order to allow only qualified participants to further engage in the study. A series of questions were asked, such as, "Are you taking birth control?" and "Are you taking any prescription medications? If so, which one(s)?" These are two sample questions that were used to eliminate participants using an external drug or contraceptive that can potentially disrupt the natural hormone cycle and immune system. For a complete list of prescription medications that were excluded from participation (Appendix A). Further questions were asked such as, "How often do you exercise a week?" and, for the women, a calendar was provided for the female participants to mark their average menstrual cycle. Only women who were not ovulating during a specific time were called back for further testing, as the immune system tends to lose reactivity when a woman is ovulating. Based on their responses, roughly 50 students were asked to return for a another survey and for a 50-mL blood draw.

Phoenix Ignition 2 was used to assess the types of decisions the individuals make, as well as demographic and personal history factors that may also impact decision making. In order to measure impulsivity and risky behavior, we utilized the Delayed Gratification Inventory (DGI), the Barratt Impulsivity Scale (BIS-II), and the Childhood Unpredictable Schema (CUS) (Hoerger, Quirk & Weed, 2011). Both the DGI and CUS are seven-point Likert-like scales, while the BIS-II is a four-point Likert-like scale. These three scales and inventories can be used to accurately quantify an individual's impulsivity and decision making practices.

As a part of this study, 50 mL of blood were drawn via an IV needle. Participants were asked to withdraw from any exercise or alcohol intake 48 hours before Phoenix Ignition 2 due to the fact that alcohol and exercise cause a boost in immune activity, which would give us unnatural results from our various assays. Immediately after the Phoenix Ignition 2 study, the blood was taken to the lab for experimentation in order to quantify the participant's immune quality. Prior to the blood collection, thrice weekly passages of the K562 cell line was performed in T-25 tissue culture flasks in order to ensure the leukemia cell proliferation. One day prior to the blood draw, the cell culture medium, Ficoll, sterile PBS, and mitogens for the blood draw were measured to assure sufficient quantities for the cell passages. After the blood draw, the blood was homogenized by inversion in heparinized tubes. The caps were then gently removed with sterile gauze. During the blood draw, the 4-mL tube of EDTA blood was drawn and then put on ice to be chilled and used as the blood plasma sample for future research. This EDTA blood is spun for 12 minutes at 1000 g and then stored at -80 °C. The 50 mL of collected blood was then mixed in a 1:1 ratio with PBS in 50-mL conical tubes. The PBS:blood mix was slowly added to Ficoll in order create a two layered liquid. The mixture was spun at 400 g for 20 minutes. Using a p1000 pipette, the white PBMC layer, a white halo looking stratum in the conical tube, was removed, without aspirating the Ficoll from the below the PBMC layer. An equal volume of HBSS, Hank's Balanced Salt Solution, was added to wash the cells. The mixture was spun at 4 °C at 100 g for 10 minutes. After spinning, the supernatant was directly poured into a container of NaOCl. The pellet was scraped to loosen. After this, 3 mL of HBSS to one tube was added, and then the liquid was transferred to a second tube to ensure that the sides of the first tube were washed with media in order to obtain the highest yield of PBMCs. The mixture was then spun again at 100 g for 10 minutes. The supernatant was poured off and the

pellet was again scraped to loosen. Two mL of full RPMI-1640 was added with 10% FBS to the cells. The cells are then counted to determine cell viability with trypan blue on the hemocytometer. The correct amount of medium was then added to the cells to be incubated at 37 °C in a 5.0% CO_2 environment. One hundred µL of cells were pipetted out into the wells of the plates to give a density of 2 $x10^5$ cells per well. Another 100 µL of one of three kinds of medium spiked with our two mitogens (PHA and LPS) was added to each well. Once the cells are in culture, the NK cell assay should be done after a 4 hour incubation period.

The proliferation and cytokine assay plates were done or stored (respectively) at 24, 48, and 72 hours after final plating. At each interval, the plasma of each well was removed in order to obtain cytokines of the cells for further research. CellTiter 96® AQueous One Solution Cell Proliferation Assay was used for the mitogen-induced proliferation assays, which is a colorimetric method for determine the number of viable cells in proliferation or cytotoxicity assays. The reagent for this assay contains a novel tetrazolium compound (MTS) and an electric coupling reagent (PES). The MTS tetrazolium compound was bioreduced by the cells' dehydrogenase enzymes into a colored formazan product that is soluble in tissue culture medium. The assays were performed by adding a small amount, 20 µL per well, of CellTiter 96® AQueous One Solution Reagent directly into the culture wells, which were then incubated for 2 hours and recorded for absorbance at 490 nm. The quantity of formazan product as measured by absorbance at 490 nm is directly proportional to the number of living cells in culture. This non-radioactive, fast, and convenient method is used to quantify the various amounts of immune cells in the participants' blood. Ninety-six-well plates suitable for tissue culture, repeating pipettes, and a 96-well plate reader were used for this portion of the experiment. The recorded data was plotted, using the corrected absorbance at 490 nm as the Y-axis versus the concentration of

growth factor as the X-axis. This protocol is in accordance to the Promega Corporation. This absorbance will allow us to properly quantify the immune quality in an individual's blood.

The NK cell assay utilized the *Cytotox 96 Non-Radioactive Cytotoxicity Assay*, which is manufactured by Promega, as well. This radioactive version measures the release of the radioactive isotope ^{51}Cr from cells that have been lysed by the NK cells (after 4 hr incubation of cell types together). Essentially, to begin the assay, the blood was spun down and again the PBMC layer was extracted and washed. One hundred μCi of ^{51}Cr was added for every 2 x 10^7 K562 cells total, which was calculated using a hemocytometer. The volume was adjusted to account for the decay of the isotope (see Appendix B). The radioactive chromium and K562 cells were then incubated for 1 hour; every 10 minutes during this incubation period, the tube was agitated. After the hour of incubation, the mixture was washed four times with HBSS and then re-suspended in full media, roughly 3 mL total. The cells were then counted and adjusted to 1 x 10^5 cells/ml for plating. Roughly 100 μL of adjusted cells were plated to give 10,000 cells/well. The PMBC's were then added to the K562/ ^{51}Cr mixture. In this, we desired 10,000 tumor cells total in all of the wells, adding them in a 50-μL volume. These tumor cells were added to varying numbers of PBMCs. The number of effector cells (PMBCs) was cut in half each time, as we moved down in terms of the Effector:Target (E:T) ratio (see Appendix C). Further, these cells were also added to 100 μL of plain medium for spontaneous release controls and to another 100 μL of 1% triton (in HBSS) for maximal release controls. These ought to be kept away from other wells on the plate. The plate was then centrifuged at 400g for 1 minute to bring the cells into good contact with each other. Then, the plate was incubated for 4 hours. After this, the plate was centrifuged again for 1 minute at 400 g. One hundred μL of supernatant was removed and placed into labeled 12 x 75 mm glass tubes. These samples were utilized for

counting in the gamma counter. The more radioactivity present in the supernatant, the more tumor cells lysed by the NK cells. Therefore, we can calculate the percent lysed by the participant's immune system in order to quantify its strength. The higher the percent lysed, the more reactive the immune system. The lower the percent lysed, the weaker the immune system.

Further, after completion of Phoenix Ignition 2, the participants will be asked to take a short, follow up study in exchange for 2 SONA credits or $25. No blood draws or other biological measures are involved in this follow up study, called Phoenix Ignition 3. The purpose of this survey was to look at how personal health and history factors associate with decision-making and various other traits that help determine life history strategies.

Predicted Results and Discussion

Because of mechanical malfunctions and human error, this study is still in progress. Therefore, our data has not yet been completed. However, we do have a strong prediction as to what the results will be. We believe that participants with less reactive immune systems (i.e. lower percentages of K562 cells lysed by the participants' NK cells and slower rates of PBMC proliferation when exposed to the mitogens) will tend to be more present-focused and make more impulsive, risky behaviors. We believe that this is evolutionarily advantageous because those who are faced with continuous threats to survival will be unsure about their chances of living to the next day; therefore, it is more advantageous to invest their energy in immediate rewards in order to quickly mature and to pass on their genes to the next generation while they still have a chance. On the other hand, we predict that those with a more reactive immune system will tend to make more future-oriented, delayed gratifying decisions as it is more advantageous to invest in hopes of acquiring a greater reward in the future. This is evolutionarily beneficial for an

organism because, when the threat of survival can be easily overcome, the organism does not have to fear death in the near future. Because of this, they ought to invest in the future, when rewards and outcomes may be more beneficial.

Evolutionarily speaking, the most fundamental task presented to every organism is maximizing one's energy. Life History Theory is used to predict how an organism will allocate its energy when faced with different stressors or threats. Those who are constantly presented with death and mortality will tend to make more present-focused, impulsive decisions; these organisms will mature and reproduce quickly in order to ensure that their genes are passed to the next generation. This lifestyle will not favor heavy parental investment because there is little advantage in investing in offspring that are most likely not going to survive. Because of this, these organisms will reproduce frequently in hopes that at least one of their offspring will survive into adulthood. Those who are presented with an abundance of resources in a safe, secure environment will tend to make more future-oriented decisions because it is more advantageous to invest in a greater reward in the future, knowing that they will live to reap these rewards. These organisms will mature slowly and reproduce later. This life history strategy favors parental investment because these organisms have more of a hope that their offspring will survive in the future.

Much research has been done in the past to look at how external cues drive the Life History Theory, while not much research has been done to analyze how internal cues can determine decision-making. This is a novel study because it correlates markers of immunological function and compares them with self-reported impulsivity and risk perception. Although this study has not been completed, we have a strong belief that our data will support our hypothesis.

If the data does not support the hypothesis, future research ought to analyze different markers of immunological function and compare them to self-reported decision making tendencies.

Appendices

Appendix A

1. **Steroid class drugs- The following drugs are EXCLUSIONARY for participation.**

Adalimumab- humira
Beclomethsone- aerobec, asmabec, beclazone, becloforte, beclomet, beclovent, beclodisk, becodisk, becotide, filair, qvar, rotahaler
Budesonide- entocort, pulmicort, symbicort
Certolizumab pegol- cimzia
Ciclesonide- alvesco
Dexamethasone- decadron
Etanercept- enbrel
Flunisolide- aerobid, flunitec, rhinalar
Fluticasone- flixotide, flovent, advair
Golimumab- simponi
Hydrocortisone- cortef
Infliximab- remicade
Methylprednisone- medrol
Mometasone- asmanex twisthaler, dulera, nasonex
Prednisolone- prelone
Prednisone- deltasone
Triamcinolone- azmacort

2. The following NSAID (nonsteroidal anti-inflammatory drugs) are OK to participate, as long as they don't take them within a day (24 HOURS) of the study

Aspirin (Anacin, Ascriptin, Bayer, Bufferin, Ecotrin, Excedrin) Choline and magnesium salicylates (CMT, Tricosal, Trilisate) Choline salicylate (Arthropan) Celecoxib (Celebrex) Diclofenac potassium (Cataflam) Diclofenac sodium (Voltaren, Voltaren XR) Diclofenac sodium with misoprostol (Arthrotec) Diflunisal (Dolobid) Etodolac (Lodine, Lodine XL) Fenoprofen calcium (Nalfon) Flurbiprofen (Ansaid) Ibuprofen (Advil, Motrin, Motrin IB, Nuprin) Indomethacin (Indocin, Indocin SR) Ketoprofen (Actron, Orudis, Orudis KT, Oruvail) Magnesium salicylate (Arthritab, Bayer Select, Doan's Pills, Magan, Mobidin, Mobogesic) Meclofenamate sodium (Meclomen) Mefenamic acid (Ponstel) Meloxicam (Mobic) Nabumetone (Relafen) Naproxen (Naprosyn, Naprelan*) Naproxen sodium (Aleve, Anaprox) Oxaprozin (Daypro) Piroxicam (Feldene) Rofecoxib (Vioxx) Salsalate (Amigesic,

Anaflex 750, Disalcid, Marthritic, Mono-Gesic, Salflex, Salsitab) Sodium salicylate (various generics) Sulindac (Clinoril) Tolmetin sodium (Tolectin) Valdecoxib (Bextra)

Appendix B

51-Cr decay factor table (1–29 days, days post-calibration; see ^{51}Cr decay sheet)

DAYS

	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
0	1.000	0.975	0.951	0.928	0.905	0.882	0.861	0.839	0.819	0.798
10	0.779	0.760	0.741	0.722	0.705	0.687	0.670	0.654	0.638	0.622
20	0.606	0.591	0.577	0.563	0.549	0.535	0.522	0.509	0.496	0.484

Appendix C

Effector:Target (E:T) ratio is as follows:

100:1 (100µl of PBMCs adjusted to 1 x 10^7 cells/mL)
50:1 (100µl of PBMCs adjusted to 5 x 10^6 cells/mL)
25:1 (100µl of PBMCs adjusted to 2.5 x 10^6 cells/mL)
12.5:1 (100µl of PBMCs adjusted to 1.25 x 10^6 cells/mL)

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