Kinetics of the Immune Response in Inbred and Outbred Mice Before and After Bone Marrow Transplantation

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A Thesis Submitted in Partial Fulfillment of the Bachelors of Science degree with Honors in Biochemistry

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May 2011

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Table of Contents

Abstract pg. 4
Introduction pg. 6
Materials and Methods pg. 9
Results pg. 11
Discussion pg. 18
References pg. 21
Acknowledgments pg. 23
Figures pg. 24
Abstract
Studying immune system kinetics plays a valuable role in understanding immune system function and is critical in evaluating the efficacy of vaccine candidates, particularly in bone marrow transplant recipients who are suspect of having an altered immune system. Historically, studies of immune kinetics and vaccination efficacy have relied upon inbred mouse models due to their genetic consistency and reproducibility in experimentation. However, recent studies suggest that these inbred mouse models vary substantially from their outbred counterparts in terms of immune response. The purpose of these experiments was to quantify differences in the immune response of inbred and outbred mice before and after bone marrow transplant (BMT). C57BL/6 (inbred) and ND4 (outbred) adult mice were lethally irradiated and given syngeneic bone marrow transplants at 5-10 weeks of age. C57BL/6 and ND4 mice were then immunized with ND4 or C57BL/6 tissue mismatched splenocytes at 6-9 weeks post engraftment. Peripheral blood was collected from the cheek pouch and analyzed using flow cytometry and blood smears at day -2, 3, 5, 7, 10, 14, 18, 21, and 25 relative to immunization. T-cells were indentified by CD3 expression while B-cells were identified by CD19 and B220 expression. T-cell/B-cell ratios revealed that both BMT and non BMT ND4 mice displayed a substantially elevated T-cell response in contrast to BMT and non BMT C57BL/6 mice. No significant differences in T-cell/B-cell kinetics were observed between BMT and non-BMT ND4 mice, though on average the ratio did appear slightly lower, indicating a possible minor deficiency of T-cell responses in the BMT group. Interestingly, BMT C57BL/6 immune responses continuously cycled over the
duration of the experiment. NK 1.1 (NK cell marker), GR-1 (granulocyte marker), and Mac-1 (macrophage marker) were observed in low numbers in the periphery as expected. These data suggest that BMT and non BMT C57BL/6 mice were similar in their immune kinetics, while the cycling of the BMT C57BL/6 response may be a result of homeostatic factors triggered by transplantation. Furthermore, immune kinetics in BMT and non BMT ND4 mice were more variable, likely due to their increased genetic diversity as compared to B6 mice. These data illustrate significant differences between inbred and outbred immune responses, particularly after BMT, suggesting that inbred mice may not serve as an accurate model for testing immune responses and vaccination efficacy in humans.
Introduction

Cancer has been a prevalent disease in human existence, dating back to 1600 BC when the ancient Egyptians first recorded the presence of cancer on papyrus documents (1). Human kind has made several leaps and bounds in treatment since the earliest discoveries of cancer, though our treatments are still widely limited to surgery, radiation, chemotherapy, or some combination of the three. Bone marrow transplantation (BMT) is one such treatment that utilizes a combination of radiation and stem cell therapy, and has become a common procedure for treating certain cancers such as leukemia and lymphoma (2). BMT transplant may utilize either one’s own stem cells (autologous BMT) or stem cells from a donor (allogeneic BMT).

The use of BMT in the treatment of cancer begins with the irradiation or chemotherapy of the patient in order to destroy the cancer, which in turn destroys the dividing stem cells within the bone marrow. As bone marrow derived stem cells give rise to cells of the blood and the immune system (leukocytes), untreated irradiated patients are left with a weak to non-existent immune system, unable to combat infection. The solution to this issue is the transplantation of bone marrow from themselves or donor. This procedure alone is wrought with inherent flaws, such as graft vs. host disease (GvHD), where the donor’s bone marrow (the graft) will actually react immunological to the host (the recipient patient) (3), thus requiring genetically similar donors and recipients. Survival after BMT is reported to range from 25 to 62%, dependent upon the cancer, age, and gender of the patient (4). Previous studies have quantified the immune deficiencies post irradiation and transplantation (5,6,7), but have come short in determining how the immune
system would respond to immunization post engraftment. As the immune system in BMT recipients has been virtually wiped out and replaced, all immune memory is lost, requiring patients to repeat their immunizations (measles, mumps, rubella, etc.). The reactivity of the “de novo” immune system to immunization is often valuable.

The purpose of this study was to quantify the immune response to immunization in a bone marrow transplanted mouse model. Historically, inbred mouse models have been preferred for their identical genomes, limiting the genetic variability in an experiment. However, recent studies have suggested that these genetically identical mice may not serve as an accurate model for humans (which are outbred) in terms of immunology (8). To evaluate the veracity of the inbred mouse model, outbred mice were also included in this experiment. Inbred and outbred mice were irradiated and subject to BMT. Their responses to immunization, measured by T-cell/B-cell ratios and leukocyte concentrations, were measured over one month and compared to the responses of the non-BMT inbred and outbred mice.

**Summary of Immunological Response to Pathogens**

The first step in preventing infection is mediated by innate immunity. Innate immunity is the non-specific system by which a mammal’s immune system utilizes physical barriers, such as skin and mucus membranes, in addition to cellular responses from non-specific immune cells such as phagocytes. However, innate immunity is non-specific, and therefore cannot “remember” the original pathogen
that it was exposed to in order to mount a more effective response upon secondary exposure. In order to better respond to a secondary exposure, mammals have developed adaptive immunity.

The key players in adaptive immunity are the lymphocytes, which can be divided into various subpopulations based upon their function. The two main lymphocyte groups are the T-cells and the B-cells. The T-cells can be further divided into two populations: T-helper cells and T-cytotoxic cells. The T-helper cells can be considered the “heart” of the immune system, as it is involved in nearly every aspect of the adaptive immune response. The T-helper cell’s role is to recognize pathogen and mobilize the rest of the immune system. The T-helper cell uses a specific surface protein called CD4, which binds to a surface protein called MHC II on antigen presenting cells (a.k.a dendritic cells). MHC antigens are universal markers on every mammalian leukocyte (white blood cell) and somatic cell, specific to a single organism, meaning that nearly every human has a unique MHC. Upon binding to MHC on antigen presenting cells (APC), the T-helper cell will rapidly divide and send a variety of chemical signals (cytokines) that activate B-cells to make antibodies specific to the presented pathogen, and other lymphocytes to begin attacking the pathogen. In addition to immune mobilization, memory T and B cells specific to the pathogen are also preserved in order to mount a faster immune response upon future exposure to pathogen (17).
Materials and Methods

Bone Marrow Transplantation (BMT)

C57BL/6 (B6) (inbred) mice were irradiated (800-900 rads) and treated that same day with 20x10^6 mononuclear cells (MNC) harvested from the bone marrow of the femurs of a sacrificed B6 syngeneic mouse. MNC were counted using a hemacytometer as described previously (9). Swiss-Webster ND4 (outbred) mice were similarly treated. Mice were kept in sterile microisolator cages through the duration of engraftment. Mice were tested for T-cells and B-cells using FACS analysis 6 weeks post transplantation. The presence of T-cells and B-cells in the peripheral blood indicated successful engraftment. All care and handling of mice was in accordance with the Institutional Animal Care and Use Committee (IACUC).

Immunization

The spleen of a sacrificed B6 mouse was harvested, homogenized, and suspended in PBS. MNC were counted from the splenocyte suspension using a hemacytometer as described previously (9). 40x10^6 splenocyte MNC were injected in to each of the ND4 bone marrow transplanted (BMT) mice and non-bone marrow transplanted control (non-BMT) mice subcutaneously between 6 and 9 weeks post engraftment. The spleen of a sacrificed ND4 mouse was harvested, homogenized, and suspended in PBS. 40x10^6 MNC were counted and injected in to each of the B6 bone marrow transplanted (BMT) mice and non-bone marrow transplanted control (non-BMT) mice subcutaneously between 6 and 9 weeks post engraftment.
**Blood Analysis**

Approximately 20uL of peripheral blood (PB) was collected from the cheek pouch and lysed in ACK lysis buffer for 5 minutes at 37°C. Cells were then resuspended in 200uL of 5% FBS/PBS. Fc receptors were blocked using 10uL (.01mg/ml) of mouse IgG for 5 minutes to reduce spurious antibody binding. Cell suspensions were then stained using the following antibody-flurochromes conjugates (all at .01mg/ml): CD3-Pacific Blue and CD19 PerCP-Cy 5.5 (BD Biosciences). Cells were analyzed using flow cytometry (FACS) at -2, 3, 5, 7, 10, 14, 18, 21, and 25 days relative to immunization. T-Cells were identified by CD3 expression while B-Cells were identified by CD19 and B220 expression.

5uL of PB was collected at -2, 3, 5, 7, 10, 14, 18, 21, and 25 days relative to infection and smeared on a glass slide and were stained with giemsa fixative. 5 photos were taken of each slide (Figure 1). Red blood cells were counted using CellProfiler 2.0 (10) while leukocytes were counted manually.
Results

*Pre-immunization Leukocyte Concentrations in inbred and outbred, bone marrow transplanted and non-bone marrow transplanted mice*

Comparison of pre-immune leukocyte populations between inbred and outbred mice is crucial to understanding how immunological studies in inbred mice may be interpreted to their outbred counterparts. Peripheral blood (PB) was collected from the cheek pouch of B6 non-BMT (n=4), B6 BMT (n=5), ND4 non-BMT (n=4), and ND4 BMT (n=4) mice and smeared/stained 2 days prior to immunization with mismatched splenocytes. Using CellProfiler 2.0, leukocytes and red blood cells (RBC) were counted. Paired sample analysis revealed significantly higher percentage of leukocytes (WBC) in the peripheral blood (PB) of the inbred transplant group when compared to the inbred control group pre-immunization (Figure 2) (p < .05). In contrast, the outbred BMT mice leukocyte population only comprised .06% of PB while the outbred non-BMT control leukocyte population comprised .18% of PB (p < .05).

*Leukocyte Kinetics of Inbred Mice Post Immunization*

Quantification of the immune response in terms of the leukocyte population post vaccination is useful in determining how an organism might fight infection. The pathways by which the immune system becomes activated are also known to vary by immunogen (11). Previous studies have characterized the immune response in terms of specific T-cell populations and various immunogens, and have revealed peaks of the response within 8 to 12 days of vaccination (11,12). Therefore, it was necessary to quantify global changes in leukocyte activity both in BMT and non-BMT
inbred mice. Analysis of blood smears for B6 non BMT mice revealed, on average, 
three phases of oscillation between .07 and .15% of PB, and equal peaks at days 3, 
10 and 21 (Figure 3). Analysis of B6 BMT mice on average reveal a primary peak at
day 10 and secondary, lower peak at day 21. (Figure 3). However, when examining 
individual mice (Figure 5, Figure 6) within the B6 BMT and B6 non-BMT groups, we 
see that each group contains one mouse that responds differently than the others 
(outliers noted with black data points). The mouse designated B6cntl044R (noted 
with black circles) had an early response peaking at day 3 that was higher in 
magnitude by .05% of the average. Interestingly, the outlier in the B6 BMT group, 
mouse B6tx042R, (noted with black squares) peaked early at day 3 with a 
magnitude five times that of the other mice in its group. Mouse B6tx042R also 
experienced a secondary peak at day 14 lower than its first peak yet still higher than 
that of its counterparts. This data suggest that there may be some component other 
than genetic factors to the immunological response, and that this unknown factor 
may be the result of development and behavior.

*Leukocyte Kinetics of Outbred Mice Post Immunization*

Studying immune kinetics within an inbred model in theory limits variation 
between organisms and yields higher reproducibility of results. However, immune 
kinetic results of inbred mice may not be consistent with their outbred (8) 
counterparts, making it necessary to study the immune system in both an inbred 
and outbred model. To measure the immune kinetics of BMT and non-BMT outbred 
mice, BMT and non-BMT ND4 mice were vaccinated with a splenocyte tissue 
mismatch. Their blood was analyzed for total leukocytes populations using blood
smears as described previously. This experiment was repeated on outbred mice that had received syngeneic bone marrow transplants. In the non-BMT outbred mice, three of the four mice peaked in leukocyte concentration between 10 and 14 days, while one mouse ND4cntl941R had no change in leukocyte concentrations for the duration of the experiment (Figure 7). Similarly, three of the four BMT outbred mice peaked between 10 and 14 days, while one mouse ND4tx940R peaked early at day 3 (Figure 8). Interestingly, when comparing the fold changes in leukocyte concentrations (Figure 9), BMT outbred mice appear to have 5, 7, and 12 fold increases at their relative peaks while non-BMT outbred appear to have at the highest a 1.5 fold increase, suggesting that the BMT outbred mice have a more dramatic response to tissue mismatched antigen. However, the greater fold increase in leukocytes may not mean that the BMT ND4 mice have a stronger immune system, as both respond (Figure 7, Figure 8) to the antigen. This ramped immune response may be the result of disrupted homeostatic factors caused by bone marrow transplantation.

**Differences of Leukocyte Concentrations Between Inbred and Outbred Mice**

Historically, studies of immune kinetics have relied upon inbred mouse models due to their attractiveness in reproducibility of results. However, recent studies have suggested that the knowledge gained from these studies may not translate to the understanding of the outbred (human) immune system (13). To compare these two models, non-BMT ND4 (outbred) and non-BMT B6 (inbred) mice were immunized with tissue mismatched splenocytes. Their blood was analyzed for total leukocytes populations using blood smears as described previously. This experiment was
repeated in BMT ND4 and BMT B6 mice. Comparison of non-BMT B6 and non-BMT ND4 mice revealed a significantly higher leukocyte concentration in ND4 mice pre-immunization (p<.05). However, in post immunization measures no significant differences in response were found in terms of leukocyte concentration. Analysis of BMT B6 and BMT ND4 mice revealed no significant differences in leukocyte concentration, suggesting that both transplant groups have a similar response to tissue mismatched antigen in terms of the leukocyte kinetics and magnitude.

**T/B-Cell Ratios of Inbred Mice post-Vaccination**

Comparison of T-cell (CD3+) and B- cell (CD19+) counts are useful indicators of the immune response, and are frequently utilized as measures of bone marrow engraftment after transplantation (5,6). T-cell and B-cell ratios can also be useful determinants of how the immune system is responding to various immunogens, where a high T-cell/B-cell ratio is characteristic of a T-cell immune response to challenge (14). Blood was withdrawn from BMT and non-BMT B6 mice at -2, 3, 5, 7, 10, 14, 18, 21, and 25 days relative to immunization and analyzed using flow cytometry (FACS). T-cell/B-cell ratios were determined using FACS analysis of CD3+/CD19+ events (FACS gating Figure 9). FACS analysis revealed an oscillation of lymphocytes in the BMT B6 which peaked at days 5, 10, and 18, with the highest peak occurring on day 10 (Figure 11). This oscillation was also observed in the leukocyte concentrations of the BMT B6 group, suggesting that the two measures may be inherently related. Analysis of non-BMT B6 mice revealed an increase of lymphocytes peaking at day 18, 8 days later than the BMT B6 group (Figure 11). In both BMT and non-BMT, B6 mice were observed to have a drop in lymphocytes
between days 5 and 7, preceding the peak response. Immune responses on days 10, 14, 18, and 21 were all significantly different (p<.05). Upon closer examination of individual mice, it was found that one BMT B6 mouse in particular, mouse B6tx042R (noted with black squares), peaked 70% higher in T-cell/B-cell ratios than any other mouse in its group on day 5 (Figure 12), indicating that this mouse seemed to have a unique immune response in terms of leukocyte counts and T-cell/B-cell ratios. These data further suggest that there may be additional homeostatic factors that are not genetically dependent.

**T/B-Cell Ratios of Outbred Mice post-Vaccination**

Comparison of T-cell/B-cell ratios in BMT and non-BMT inbred mice provided insight into possible differences in immune responses, though an outbred model may be a more accurate model of how the human immune system would respond before and after BMT. In this experiment, BMT and non-BMT ND4 mice (outbred) were immunized and analyzed at -2, 3, 5, 7, 10, 14, 18, 21, and 25 days relative to immunization using FACS as described previously. Analysis of T-cell/B-cell ratios revealed a peak at day 7 in the non-BMT ND4 though upon closer examination of individual mice (Figure 13) 3 of the 4 non-BMT mice peaked at day 7 with a secondary minor peak between days 14 and 18, while the other mouse presented a weaker peak at day 10 followed by a stronger peak at day 18. This variation in both time and magnitude of the response are not unexpected in these genetically outbred mice. Analysis of the BMT ND4 mice revealed 2 to 3 fold increases in T-cell/B-cell ratios, peaking between days 3 and 7 (Figure 14). However, comparison of BMT and
non-BMT ND4 mice revealed no significant differences in the immune response (Figure 14).

**T/B-cell Ratios in Outbred and Inbred, BMT and non-BMT mice**

As discussed previously, there were no substantial differences in the magnitude of response when comparing BMT and non-BMT mice by strain (B6 or ND4). FACS analysis of T-cell/B-cell ratios revealed significant differences (p<.05) between non-BMT B6 and non-BMT kinetics (figure-14). No real differences in peak days appeared, though the magnitudes of the responses varied dramatically. The T-cell/B-cell ratio in non-BMT B6 mice was consistently between 0 and 1, indicating a near equal quantity of T and B cells responding. However, the non-BMT T-cell/B-cell ratio ranged from 2 to 6.5, indicating a consistent surplus of T-cells. This same T-cell response was also observed when comparing BMT B6 with BMT ND4 mice (p<.05) (Figure 16), though the BMT ND4 mice varied much more than their non-BMT counterparts in the magnitude of the T-cell/B-cell ratios.

**Fold Changes in Fraction of T-cell and B-cell Populations Relative to pre-Vaccination**

Tracking fraction changes in T-cell and B-cells through the course of the immune response in B6 non-BMT mice revealed significant (p<.05 on day 3, 7, 10, 14, 18, 21) increases in T-cell fraction of lymphocytes relative to pre-immunization when compared ND4 non-BMT mice (Figure 17). Comparison of T-cell fractions between B6 BMT and ND4 BMT mice revealed significant increases (p<.05 on day 3 and 10) in B6 BMT (Figure 18). Interestingly, T-cell fractions between B6 BMT and B6 non-BMT mice revealed a lower T-cell fraction that peaked later in B6 BMT (Figure 18,
Figure 19). Comparison of B-cells in B6 BMT and B6 non-BMT showed similar downward trends with minima between day 14 and 18, though no significant differences were found (Figure 20). Similarly, comparison of B-cell fraction of lymphocytes between ND4 BMT and ND4 non-BMT revealed downward trends with no significant differences.
Discussion

In this study, we assessed the immune response to a MHC (I and II) splenocyte tissue mismatch in BMT and non-BMT mice using both an inbred and outbred mouse model. Prior to immunization, the leukocyte percentage of PB was higher in BMT than non-BMT mice in the inbred model. The opposite effect was found in the outbred model, with non-BMT mice having a higher leukocyte percentage than BMT mice. This initial observation suggested that the BMT inbred mice may actually have a different immune response than their non-BMT equivalents; whereas the BMT outbred mice may actually present an even different immune response relative to non-BMT controls. However, no statistical differences in leukocyte concentration were observed between BMT and non-BMT within the inbred and outbred strains. The observed differences in leukocyte concentration prior to immunization may be a characteristic of the immune system in a resting phase that is unique to both inbred and outbred, BMT and non-BMT mice. Unfortunately, few studies have been conducted on non-induced immune kinetics. More long-term studies on the natural cycling and variation of adaptive immunity prior to immunization may shed light on this issue.

As expected, the immune response was more consistent in the inbred group compared to the outbred groups. However, the oscillation of leukocytes in the immune response of BMT B6 mice was unexpected (Figure 6). The “typical” immune response peaks between 10 to 14 days (15); our BMT B6 mice oscillated in magnitude of response over the 25 days of the experiment. This oscillation was also
observed in the T-cell/B-cell ratio of B6 BMT mice (Figure 12), though the oscillations were out of phase. This “abnormal” reaction in B6 BMT mice may be due to some deficiency in the regulatory mechanisms of the immune response, possibly triggered by the transplantation. These data suggest that this “abnormality” in immune response may also arise in human BMT patients, though no studies have been conducted on human BMT pathogen induced kinetics to confirm this.

Interestingly, a much stronger and earlier peak in leukocytes was observed in one of the four inbred BMT mice (Figure 6). This irregularity was also seen in the same mouse’s T-cell/B-cell ratio (Figure 12). It is worth noting that this mouse received the same BMT donor as the other B6 BMT mice in the group, strongly suggesting that there are other non-genetic factors regulating the immune response.

T-cell/B-cell ratios revealed that both BMT and non BMT ND4 mice were substantially elevated in T-cell responses in contrast to BMT and non BMT B6 mice (Figure 15, Figure 16). No significant differences in T-cell/B-cell kinetics were observed between BMT and non-BMT ND4 mice, though on average the ratio did appear slightly lower, indicating a possible lower T-cell response in the BMT group (Figure 14). As expected, outbred T-cell/B-cell ratios were much more variable than the inbred counterparts, both in BMT and non-BMT groups, though all mice peaked in T-cell/B-cell ratios between days 5 and 7. The outbred pre-immunization T-cell/B-cell ratios seemed to better model those of a healthy human subject, which tend to have twice as many T-cells than B-cells in their PB (16). The difference in magnitude of T-cell/B-cell ratio between inbred and outbred mice may deter future
experiments from modeling immune responses in an inbred mouse model, as the lymphocyte activities appear to be significantly different.

Interestingly, both B6 BMT and B6 non-BMT appeared to have consistently higher fold increases of T-cell fractions of lymphocytes compared to the ND4 counterparts relative to pre-immunization. This observed increase in T-cell fraction in B6 mice may be compensatory of the B6 mice’s low T-cell/B-cell ratios, enabling B6 mice to mount a T-cell response despite their significantly low T-cell/B-cell ratios when compared to inbred ND4 mice. Unfortunately, total lymphocyte concentrations in the PB were not measured, thus making global increases or decreases of T-cell and B-cell populations incapable of determining.

Future studies will aim to more thoroughly quantify the immune response by measuring individual CD4 and CD8 T-cell subpopulations. Further experiments may also benefit from utilizing other pathogens in order to characterize the kinetic differences of immune response. Total lymphocyte counts should also be performed using complete blood count (CBC) techniques in order provide a global hematological picture of the immune response.
References


Acknowledgements

First and foremost, I thank my loving wife for her continued support and faith in my career as a scientist and future physician. I also thank Dr. David Harris and the Undergraduate Biology Research Program for the opportunity to actively participate in scientific research. And finally I thank Dr. Michael Badowski for showing me what relentless passion for family and science look like.
Figures

**Figure 1.** Photo of a blood smear slide. Light pink circles are red blood cells. Leukocytes are stained dark purple.

**Figure 2.** Pre-Immunization Leukocyte (WBC) percentage of total peripheral blood (PB) cells in B6 (inbred) and ND4 (outbred) mice. Error bars are 1 SD. (*p < .05)
Figure 3. Percentage of leukocytes in peripheral blood (PB) in inbred B6 BMT (n=5) and inbred B6 non-BMT (n=4) controls reveal an oscillation in response in the transplanted mice. Error bars are 1 SD.

Figure 4. Percentage of leukocytes in peripheral blood (PB) in outbred ND4 BMT (n=4) and outbred ND4 non-BMT (n=4) reveal on average a late peak in the transplanted outbred mice. Error bars are 1 SD.
Figure 5. B6 non-BMT mouse % leukocyte in PB. 2 B6 non-BMT mice (shown in purple and blue lines) indicate a strong increase in leukocytes at day 21, significantly later than the other non-BMT mice in the group. 1 B6 non-BMT mouse (shown in black circles) also appeared to peak earlier and stronger than its counter parts. Each line represents 1 mouse.

Figure 6. B6 BMT % leukocyte in PB. B6 BMT mice reveal a unique oscillation of their immune response, while 1 B6 BMT mouse (shown with black squares) peaked earlier and stronger in response despite being genetically identical to those in its group. Each line represents 1 mouse.
Figure 7. ND4 (outbred) non-BMT percentage of leukocytes in PB reveal high variation between mice. 1 mouse (shown in red) appears to not have responded to the tissue mismatch antigen, while the other 3 peak between 10 and 14 days.

Figure 8. ND4 BMT % Leukocyte of PB reveal immune response in all 4 mice, though mouse 940R (shown in red) appears to peak early at day 3 and then again at day 14, while the other 3 mice peak as expected between days 10 and 14.
**Figure 9.** ND4 (outbred) fold increase in % leukocytes of PB illustrates a dramatic increase in leukocytes in BMT ND4 mice relative to non-BMT control mice. This increase may be the result of failed regulatory mechanisms or due to transplantation.

**Figure 10.** Gating strategy of leukocyte analysis using flow cytometry (FACS). Gating of lymphocytes is shown in A with all events in P1. CD3+ cells (T-cells) are gated in P4 as a percent of P1 (shown in 9-B). CD19+ (B-cells) are gated in P6 as a percent parent of P1.
Figure 11. B6 (inbred) T-cell/B-cell ratios in BMT (n=5) and non-BMT (n=4) mice, revealing a late peak in non-BMT mice and a unique oscillation in response of BMT mice. Error bars are 1 SD.

Figure 12. Inbred T-cell/B-cell ratios of individual mice. One BMT B6 mouse in particular (show with black squares) had a unique increase earlier and higher magnitude than its other BMT counterparts.
**Figure 13.** Non-BMT ND4 (outbred) T-cell/B-cell ratios indicate peaking of response at day 7 in three of four control mice, while mouse 941L peaked later at day 10 with a lower magnitude. A primary and lesser secondary response is seen in all four mice.

**Figure 14.** BMT and non-BMT ND4 (outbred) T-cell/B-cell ratios reveal no statistical difference in immune response, though non-BMT control mice tend higher in ratio.
Figure 15. Comparison of non-BMT T/B-cell ratios indicates a surplus of T-cells significantly (p<.05) higher in ND4 (outbred) mice than that of B6 (inbred) mice.

Figure 16. Comparison of BMT T/B-cell ratios also indicates a surplus of T-cells significantly (p<.05) higher in ND4 (outbred) mice than that of B6 (inbred) mice.
**Figure 17.** Fraction of T-cells in lymphocyte population relative to pre-immunization revealed higher increase in T-cell fraction in inbred B6 non-BMT mice (n=4) when compared to outbred ND4 non-BMT mice (n=4). Error bars are 1 SD. (p<.05 at day 3, 7, 10, 14, 18 and 21).

**Figure 18.** Fraction of T-cells in lymphocyte population relative to pre-immunization revealed oscillation in T-cell fraction in inbred B6 BMT mice (n=5) when compared to the low fraction changes observed outbred ND4 BMT mice (n=4). Error bars are 1 SD. (p<.05 at day 3 and 10).
Figure 19. Fractional changes of B-cell populations of lymphocytes in B6 BMT (n=5) and B6 non-BMT (n=4) relative to pre-immunization revealed minima at day 14 and 18. Error bars are 1 SD.

Figure 20. Fractional changes of B-cell populations of lymphocytes in ND4 BMT (n=4) and non-BMT (n=4) relative to pre-immunization reveal no significant differences in B-cell fraction, though both populations tend to decrease to day 18. Error bars are 1 SD.