Cell and Tissue Transplantation and Therapy

ORIGINAL RESEARCH

The Immune Response in Inbred and Outbred Strains of Mice before and after Bone Marrow Transplantation

Christopher L. Shultz, Michael Badowski and David T. Harris

Department Immunobiology, University of Arizona, Tucson, AZ. Corresponding author email: davidh@email.arizona.edu

Abstract: Understanding the immune response is critical to evaluate the response to infection and vaccines, particularly in bone marrow transplant (BMT) recipients who may exhibit an altered immune system. Such studies have relied upon inbred mouse models due to genetic consistency and experimental reproducibility. Recent studies suggest that inbred mice vary substantially from outbred counterparts in terms of immune responses. These experiments quantified differences in immune responses of inbred and outbred mice before and after BMT. Inbred and outbred adult mice were lethally irradiated and syngeneic bone marrow transplants performed. 6–9 weeks after engraftment, the mice were immunized with allogeneic cells. Immune cell changes were analyzed by flow cytometry. Immunization resulted in significant leukocyte increases in all groups. B cells only varied for transplanted inbred mice. Outbred mice had significantly greater baseline T cells due to increased CD4+ T cells. CD8+ T cell numbers were comparable between the strains and groups. Interestingly, in outbred mice both CD4 and CD8 T cells responded equally while in inbred mice CD8 T cells were predominant. Outbred mice had peak responses later and more prolonged than inbred mice. Thus, inbred mice may not be an accurate model for testing immune responses in humans, especially after BMT.

Keywords: BMT, outbred mice, immune kinetics

Cell & Tissue Transplantation & Therapy 2013:5 9–18

doi: 10.4137/CTTT.S10938

This article is available from http://www.la-press.com.

Introduction

Stem cell transplantation, as exemplified by bone marrow transplants (BMT) and cord blood transplants (CBT), is commonly used to treat a variety of malignant and genetic hematological diseases.[1,2] Previous studies have demonstrated that immune dysfunctions occur in the transplant recipients post-transplantation,[3–5] but rarely have investigators analyzed how the immune system responds to re-immunization after stable hematological engraftment.[6–9] In that the immune system in BMT recipients is generally destroyed and replaced by the transplanted stem cells, with all immune memory being lost, it would not be unexpected that patients exhibit immune dysfunction. As patients are required to repeat their immunizations (eg, measles, mumps, rubella) in order to regain immune protection against common infectious organisms, such changes could be detrimental.

A recent study by Avetisyan et al[9] examined the immune responses of 18 control and 14 allogeneic stem cell transplant patients to challenge with seasonal influenza vaccines. Four weeks post-vaccination all patients demonstrated significant immune responses to influenza, but only 29% of the transplant patients demonstrated protective levels of anti-influenza antibody. Furthermore, the immune responses of the control patients were observed to be 10–20-fold higher than that of the transplant patients, even for those patients more than 6 months post-transplant. No data concerning the kinetics of the immune response to antigenic challenge were presented. A previous study by this group[8] had reported similar results in a smaller cohort of transplant patients. Similar results have also been reported by Engelhard et al[6] and Pauksen et al.[7] Thus, it appears that the immune response of stem cell transplant patients generally does not return to an entirely normal state, even at times long after hematopoietic engraftment and supposed immune homeostasis has been achieved.

The purpose of this study was to analyze the immune response to a vigorous antigenic challenge in a murine bone marrow transplantation model. Historically, inbred mouse models have been utilized for such studies as their limited genetic variability removes much of the inter-subject variability from the experimental results, making results more reproducible and data interpretation simpler. However, recent studies have suggested that such genetically identical mice may not serve as an accurate model for the human condition (which are generally outbred) in terms of their immune responses.[10–12] To evaluate the utility of the inbred mouse model, outbred mice were also analyzed in these experiments. Both inbred and outbred mice were lethally irradiated and syngeneic BMT (the optimal situation) was performed. After stable hematopoietic engraftment, responses to immunization were measured by changes in B cells and T cells (CD4 and CD8). Total white blood cells (WBC) were also measured over a one month period following vaccination. Non-transplanted and transplanted inbred and outbred mice were compared for similarities and differences. It was observed that significant differences between inbred and outbred mice existed in terms of the kinetics of the immune response as assessed by changes in total CD4+ and CD8+ T cells, but generally not B cells, which was evident before and after BMT. Therefore, use of inbred mouse strains, particularly following stem cell transplant, may not be representative of outcomes observed in human patients.

Materials and Methods

Mice

All mice were obtained from Jackson Laboratories (Bar Harbor, ME) and used according to an IACUC approved protocol. All care and handling of mice was in accordance with the AAALAC guidelines.

Bone marrow transplantation (BMT)

C57BL/6 (B6, inbred) and Swiss-Webster (ND4, outbred) mice (6–8 weeks of age) were irradiated (850+/– 50 rads) and 4 hours later were injected with 20×10^6 mononuclear cells (MNC) harvested from the femoral bone marrow of respective syngeneic mice.[9] This dose of radiation was lethal for both strains of mice if stem cell transplantation did not occur. Thus, mice were kept in sterile microisolator cages throughout the duration of engraftment and immunological challenge, and were provided with sterile food and water. Mice were analyzed for T-cells, B-cells and neutrophils by flow cytometric (FACS) analysis starting at 6–8 weeks post transplantation. Once the presence of normal levels of T-cells and B-cells in the peripheral blood indicated successful engraftment, the immunological challenges were initiated (generally at d100 post-transplant or later). A total of 6 mice

(in 2 experiments) were used for each of the strains in each of the conditions. Transplanted mice are indicated by the Tx designation (ie, B6-Tx and ND4-Tx).

Immunization

Spleens were harvested from B6 and ND4 donor mice, homogenized, and resuspended as single cell suspensions in phosphate-buffered saline (PBS). MNC were isolated by Lymphocyte M centrifugation from the splenocyte suspension as described previously.[9] A total of 40×10^6 splenocyte MNC were injected subcutaneously into either B6 or ND4 mice (whether transplanted or not) as a vigorous antigenic challenge by both allogeneic MHC class I and class II antigens (ie, B6 cells injected into ND4 mice, and vice-versa). In the BMT mice, the immunizations did not occur until a minimum of 6–9 weeks post engraftment. Mice were analyzed for various immunological parameters prior to challenge as well as up to 33 days post-immunization. All analyses for all groups were performed at the same time to minimize variability.

FACS analysis

Approximately 20 µL of peripheral blood was collected from the cheek pouch and lysed in ACK lysis buffer for 5 minutes at 37 °C to remove red blood cells. Cells were then resuspended in 200 µL of PBS-5% FBS. All F_c receptors were blocked using 10 µL (0.01 mg/mL) of mouse IgG for 5 minutes to reduce spurious antibody binding. Cell suspensions were then stained using the following antibody-flurochrome conjugates (all at 0.01 mg/mL): CD3-Horizon v450 and CD19 PerCP-Cy 5.5 (BD Biosciences), CD4-FITC, CD8-PE-Cy7 and Gr-1-PE. Cells were analyzed by FACS for samples collected at –3, 3, 6, 8, 11, 14, 18, 21, 27 and 33 days after immunization. All cell counts were obtained with the use of a Hemavet Analyzer (Drew Scientific, Dallas, TX). Each mouse of each strain under each treatment condition was sequentially analyzed over the course of 33 days.

Results

Kinetics of the immune response following transplantation and immunization

Inbred C57Bl/6 (B6) and outbred Swiss-Webster (ND4) mice were transplanted with syngeneic bone marrow (to mimic an autologous transplant without the complications of rejection or graft-versus-host disease), allowed to stably engraft for a minimum of 6–9 weeks, and then challenged with MHC class I and II allogeneic spleen cells. Immune responses as determined by overall changes in specific immune subpopulations were assessed by FACS, and compared to non-transplanted control mice of the same strain. Assessments were made prior to immunization and again every several days afterwards for approximately 1 month. As shown in Figure 1, inbred B6 mice displayed higher pre-immunization WBC levels after BMT but outbred ND4 mice generally exhibited higher levels of peripheral blood white blood cells (neutrophils + lymphocytes) than B6 mice, regardless of transplant condition and time after challenge. The B6 groups demonstrated peak responses to antigen challenge at days 8–10 (typical of a primary immune response) which eventually returned to baseline by day 28. ND4 groups however, had peak responses at days 10–18, being more prolonged in the response but also returning to baseline levels by day 28. Interestingly, the inbred mice demonstrated higher baseline levels of WBC (cells/mL) after transplant, as compared to non-transplanted mice, while WBC levels in outbred mice remained unchanged. Inbred mice displayed a diminished magnitude of WBC response after transplant (2.5-fold pre- and 1.3-fold post-transplant), while the magnitude of the outbred mouse response was more consistent (2.2-fold pre- and 2.4-fold post-transplant).

Further analyses of the observed WBC changes during the immune response were performed and are shown in Figure 2 for the changes over time observed in total lymphocyte counts. Analysis of total lymphocyte counts (including both T and B cells) revealed striking changes over time. Transplanted B6 mice displayed higher levels of total lymphocytes at d3 (baseline) compared to control B6 mice (by almost 3-fold) which was maintained with time after immunization. Both groups of ND4 mice were initially equivalent at day 3 (baseline) for total lymphocyte counts but displayed elongated immune responses (peak responses from days 10–18) as compared to B6 mice. Further, the magnitude of the immune response was diminished in transplanted B6 mice (1.2-fold vs. 1.8-fold), while the immune responses were similar for the transplanted ND4 mice as compared to control ND4 mice (2-fold vs. 2.2-fold).

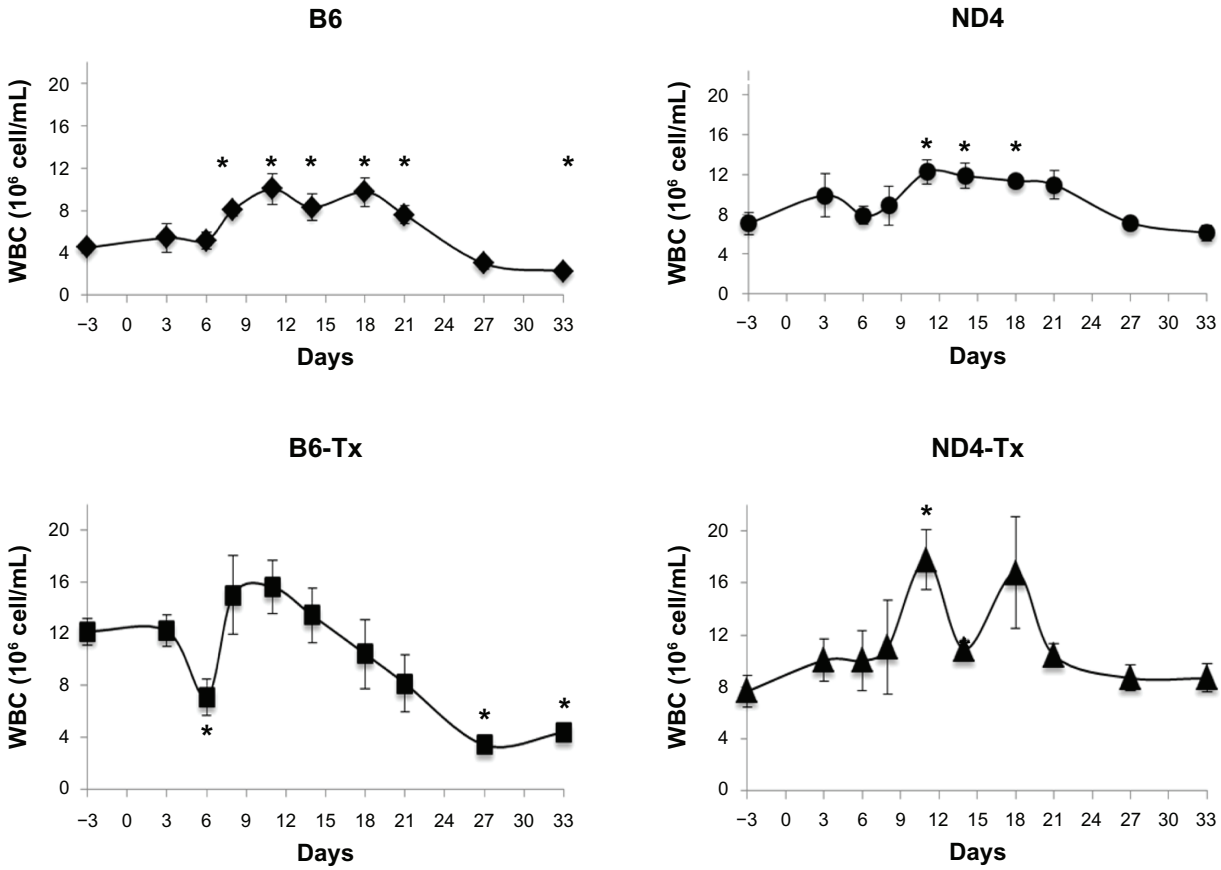

Figure 1. Changes in total WBC following transplantation and immunization.
Notes: Inbred (B6) and outbred (ND4) mice were subjected to syngeneic bone marrow transplants as described. Non-transplanted B6 and ND4 mice served as controls. After stable engraftment all animals were immunized and followed for up to 33 days post vaccination. At the indicated times blood was drawn from each mouse and analyzed for total white blood cell counts. A total of 6 mice were used for each group in each strain. Data is presented as the mean of the 6 measurements plus/minus the standard error of the mean. Transplanted mice are indicated by the Tx designation (ie, B6-Tx and ND4-Tx). *indicates $P < 0.05$ significance level compared to day-3 timepoint as determined by an unpaired Student's *t*-test.

As shown in Figure 3, baseline B cells counts were similar in both strains of mice. However, transplanted inbred B6 mice demonstrated higher baseline levels of B cells than any of the other mouse groups or strains, while transplanted ND4 mice similar exhibited B cell numbers before and after transplant. Upon antigenic challenge B6-Tx mice displayed weak B cell expansion between days 8–21, similar to that seen with control B6 mice (albeit the control mice had B cell levels 2-fold or lower in numbers). Control and transplanted ND4 mice showed similar small increases in B cell numbers after challenge. Thus, it appeared that B cell responses were impaired in both inbred and outbred mice as a result of transplant, and that B cell numbers were significantly altered in transplanted B6 mice.

Examination of the T cell responses to allogeneic MHC class I and class II immunization (Fig. 4) revealed that the outbred ND4 mice possessed higher baseline T cell numbers than did the inbred B6 mice (by approximately 2-fold). Both control and transplanted B6 mice rapidly expanded total T cell numbers (by 3-fold or greater) which peaked on days 8–9 and returned to baseline by day 27. Outbred ND4 mice displayed different kinetics in T cell responses in that both control and transplanted ND4 mice displayed peak T cell changes on day 12 which remained elevated even at day 33. Further, the magnitude of the ND4 responses (approximately 2-fold) was smaller than those of the B6 mice.

When total T cells were analyzed for CD4 and CD8 subsets, additional differences were found. Outbred ND4 mice had greater numbers of total CD4+ T cells (5-fold higher) than inbred B6 mice, and this immune cell subset comprised 80%–90% of the total T cells in the peripheral blood (Fig. 5). In response to immunization, both strains of mice responded with increased CD4+ T cell numbers

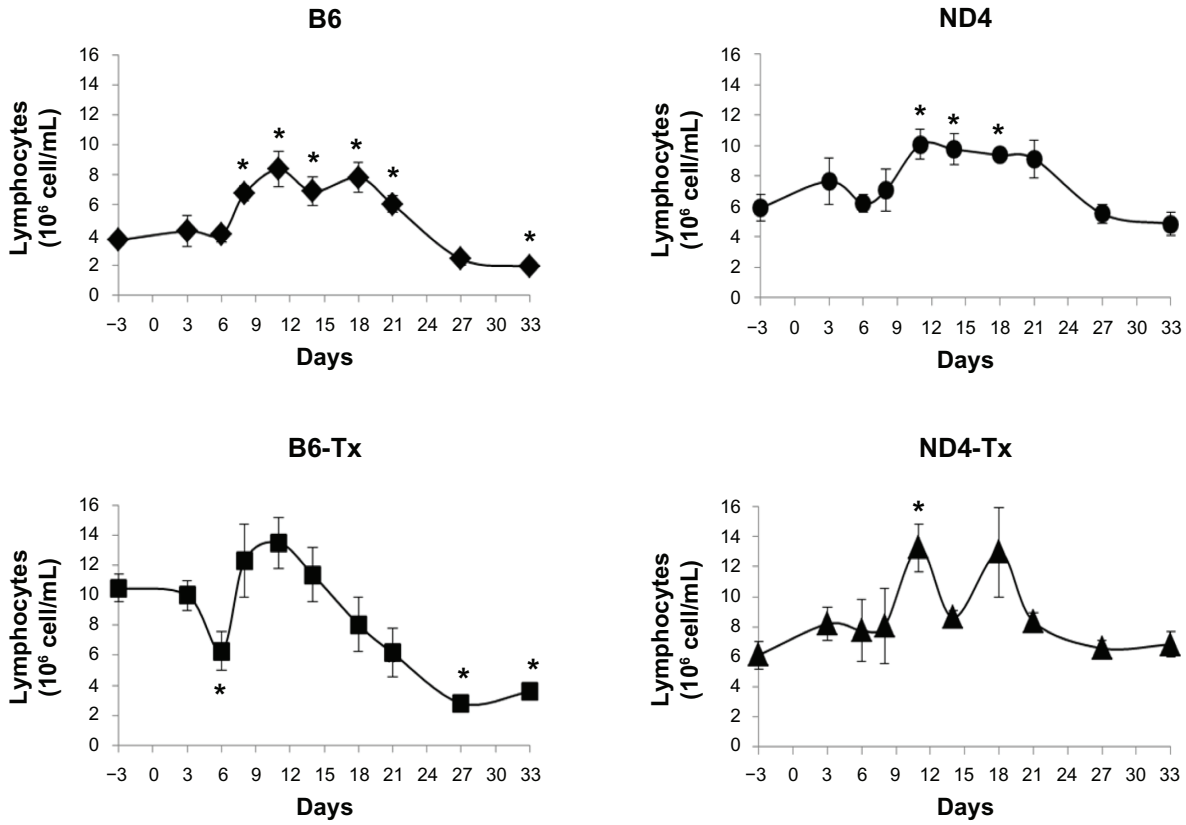

Figure 2. Changes in total lymphocytes following transplantation and immunization.
Notes: Inbred (B6) and outbred (ND4) mice were subjected to syngeneic bone marrow transplants as described. Non-transplanted B6 and ND4 mice served as controls. After stable engraftment all animals were immunized and followed for up to 33 days post vaccination. At the indicated times blood was drawn from each mouse and analyzed for total lymphocyte counts. A total of 6 mice were used for each group in each strain. Data is presented as the mean of the 6 measurements plus/minus the standard error of the mean. Transplanted mice are indicated by the Tx designation (ie, B6-Tx and ND4-Tx). *indicates $P < 0.05$ significance level compared to day-3 timepoint as determined by an unpaired Student's *t*-test.

although the percentages of total T cells remained fairly constant. Both groups of B6 mice displayed peak responses of approximately 3-fold over baseline levels. Peak responses of the outbred mice were smaller (approximately 2-fold). Control inbred B6 mice exhibited peak responses at day 8–9 as expected, and again at day 21, which was not observed in the transplanted B6 mice. Both groups of ND4 mice had CD4+ T cells which peaked around days 8–10 although the transplanted ND4 mice had more prolonged responses than that seen with the control mice.

Analysis of the CD8+ T cell fraction demonstrated that both strains of mice at baseline times had comparable absolute numbers of CD8+ T cells, which meant that inbred B6 mice had a greater percentage of total T cells expressing the CD8 molecule (35%–40% vs. 15%) than did outbred ND4 mice (Fig. 6). B6 responses to immunization (5–7-fold) peaked around day 8 for both controls and transplanted mice, while ND4 responses peaked (3–6-fold) later and were more prolonged. Transplanted ND4 mice displayed smaller responses than control ND4 mice. The percentage of CD8+ T cells increased from 35% to 60% in both groups of B6 mice, while the percentage of CD8+ T cells did not significantly change in the outbred ND4 mice. Thus, for the outbred mice the magnitude of the CD4+ T cell response was greater in terms of absolute numbers than for inbred B6 mice whose CD4+ and CD8+ T cell numbers were comparable at the time of peak responses. Thus, transplantation seemed to affect CD8+ T cell responses the least in the B6 mice, while similar responses were muted in the outbred mice. Interestingly, the CD4 to CD8 ratios were significantly different for the 2 strains of mice, albeit the ratios did not change greatly after transplant. Inbred mice demonstrated a 60/40 ratio before transplant and a 65/35 ratio afterwards. Outbred mice however, showed an 80/20 ratio and an 85/15 ratio before and after transplant, respectively.

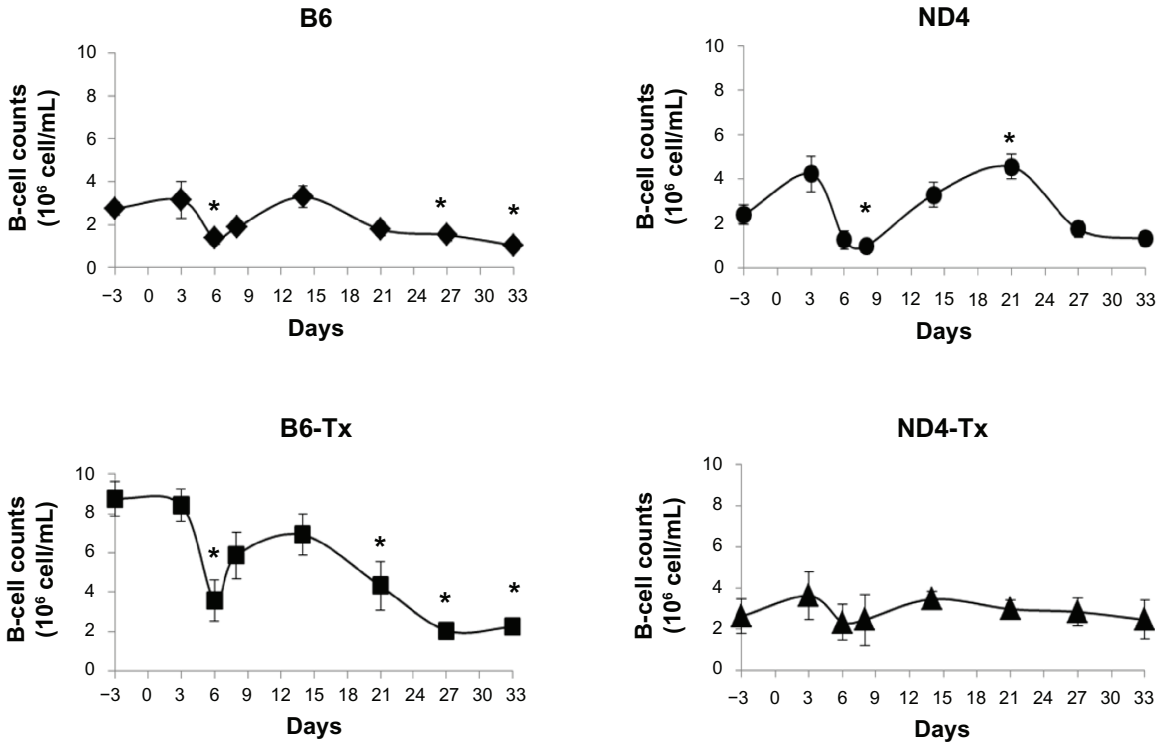

Figure 3. Changes in B cell numbers following transplantation and immunization.
Notes: Inbred (B6) and outbred (ND4) mice were subjected to syngeneic bone marrow transplants as described. Non-transplanted B6 and ND4 mice served as controls. After stable engraftment all animals were immunized and followed for up to 33 days post vaccination. At the indicated times blood was drawn from each mouse and analyzed for B cell counts by flow cytometry. A total of 6 mice were used for each group in each strain. Data is presented as the mean of the 6 measurements plus/minus the standard error of the mean. Transplanted mice are indicated by the Tx designation (ie, B6-Tx and ND4-Tx). *indicates $P < 0.05$ significance level compared to day-3 timepoint as determined by an unpaired Student's *t*-test.

Discussion

In this study we assessed the immune response to a MHC (class I and II) tissue mismatch in bone marrow transplanted and control mice using both an inbred (B6) and outbred (ND4) mouse models. We wished to determine how immune responses were altered following stem cell transplantation, and whether commonly used inbred strains of mice were good models for the human condition. We chose to perform syngeneic bone marrow transplants as this setting would represent a best case scenario where reconstitution should occur rapidly and immune responses should not be hindered by variables of rejection or graft versus host disease. In order to assess all aspects of the immune response (B cells, helper and cytotoxic T cells), we chose to immunize the mice with allogeneic class I and class II disparate murine spleen cells as this approach induces a vigorous immune response. Splenic cells were chosen as this tissue contains numerous antigen presenting cells (macrophages, dendritic cells and B cells) that display both MHC class I and II antigens.

Prior to immunization, absolute peripheral blood leukocyte numbers were found to be higher in transplanted B6 mice than in control B6 mice, while in the outbred model both transplanted and control mice had equal numbers of leukocytes. This initial observation suggested that not only might there be differences between transplanted and control mice in their immune responses, but that inbred and outbred mouse strains might also display different immune responses. The observed differences in leukocyte numbers prior to immunization may be a characteristic of the homeostatic immune system that is unique to and different between these groups of mice. Unfortunately, few such studies have been reported, with even fewer studies examining the similarity or dissimilarity of the immune response between these strains before and after stem cell transplantation.

A recent study[11] examined the CD8+ T cell response to bacterial and viral infections in inbred Balb.c and B6 and compared it to similar responses in outbred Swiss-Webster mice. While the investigators observed coordinated immune responses in the inbred

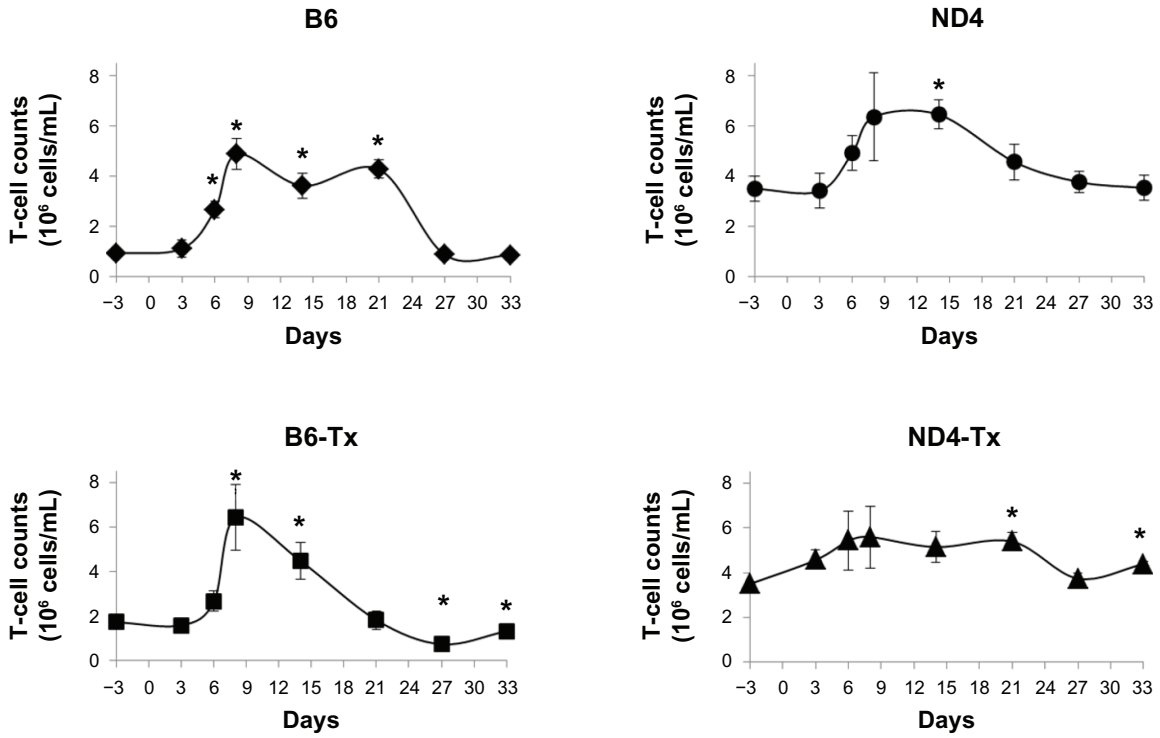

Figure 4. Changes in T cell counts following transplantation and immunization.
Notes: Inbred (B6) and outbred (ND4) mice were subjected to syngeneic bone marrow transplants as described. Non-transplanted B6 and ND4 mice served as controls. After stable engraftment all animals were immunized and followed for up to 33 days post vaccination. At the indicated times blood was drawn from each mouse and analyzed for total CD3+ T cell counts by flow cytometry. A total of 6 mice were used for each group in each strain. Data is presented as the mean of the 6 measurements plus/minus the standard error of the mean. Transplanted mice are indicated by the Tx designation (ie, B6-Tx and ND4-Tx). *indicates $P < 0.05$ significance level compared to day-3 timepoint as determined by an unpaired Student's *t*-test.

strains of mice, there were significant differences found with the outbred mice in terms of the kinetics and magnitude of the immune responses. The authors concluded that inbred mice might be limited in their use as preclinical human models. Similar results were reported by Rettinger et al[12] who compared CBA mice to Swiss-Webster mice in terms of the kinetics of the immune response to a protozoan infection.

As anticipated, we observed that the immune response to challenge with alloantigens was more consistent in the inbred groups of mice when compared to the outbred mouse groups. The typical textbook primary immune response peaks between 7 to 10 days post-challenge.[13] This type of immune response was observed with both control and BMT B6 mice. Although there were differences in terms of the magnitude of the responses, the kinetics of the immune responses was almost identical. However, the outbred ND4 mice differed from the respective B6 groups of mice. That is, not only were there differences in the magnitude of the response between the BMT and control ND4 mice, but the kinetics of the responses were quite different with the transplanted mice. In general, outbred ND4 mice were delayed in peak responses to antigenic challenge as compared to B6 mice, and peak responses were more prolonged as compared to B6 mice. Very interestingly, outbred mice had much higher absolute numbers of T cells than inbred mice, with a much skewed CD4/CD8 ratio found in the peripheral blood. B6 mice had a 60/40 ratio of CD4/CD8 T cells while ND4 mice had an 80% CD4/20% CD8 T cell ratio. The reason for this difference, as well as any consequences, is unclear. In addition, upon antigenic challenge outbred ND4 mice produce a predominant CD4+ T cell response while inbred B6 mice display a predominant CD8+ T cell response. Interestingly, transplanted B6 mice appeared to have higher fold increases in CD8 T-cells after immunization compared to the ND4 counterparts, although both strains exhibited suppressed T cell responses in general after stem cell transplant. Again, the reason for such observations and any consequences is not known, although it may indicate a homeostatic CD8 T cell imbalance in these mice.

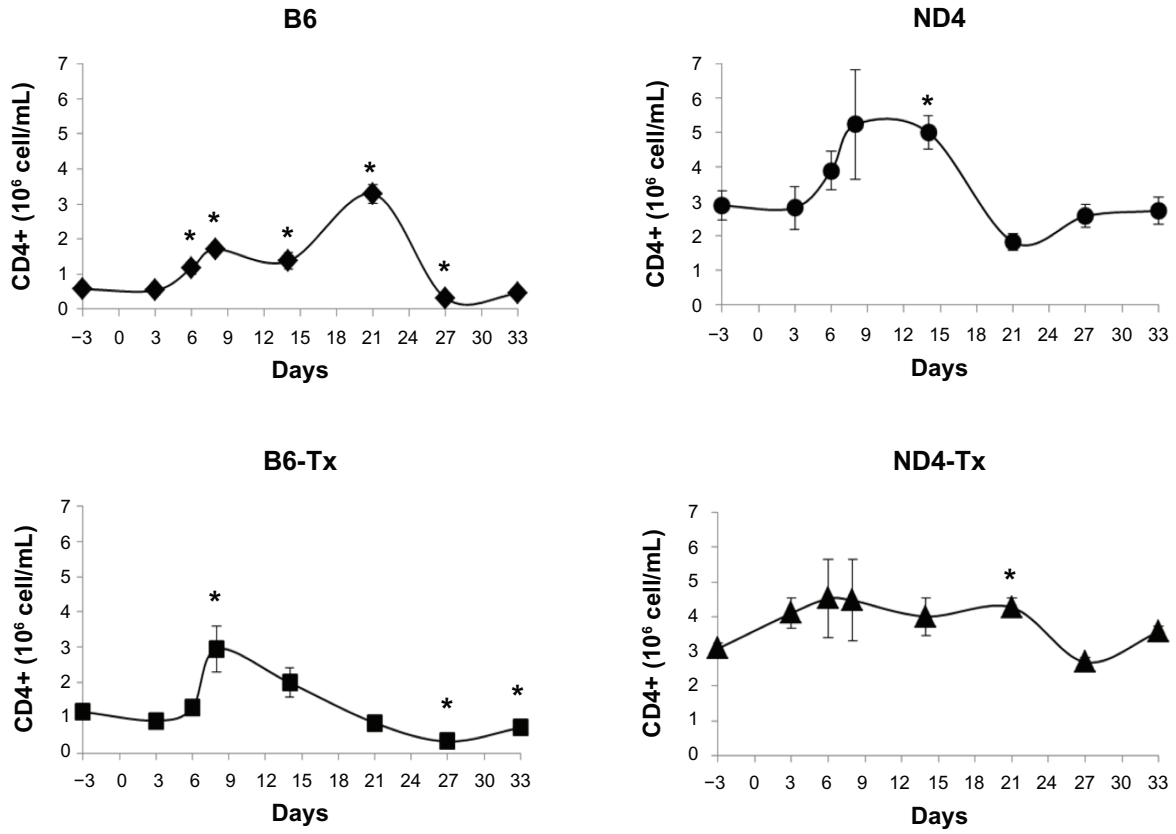

Figure 5. Changes in CD4+ T cell counts following transplantation and immunization.
Notes: Inbred (B6) and outbred (ND4) mice were subjected to syngeneic bone marrow transplants as described. Non-transplanted B6 and ND4 mice served as controls. After stable engraftment all animals were immunized and followed for up to 33 days post vaccination. At the indicated times blood was drawn from each mouse and analyzed for total CD4+ T cell counts by flow cytometry. A total of 6 mice were used for each group in each strain. Data is presented as the mean of the 6 measurements plus/minus the standard error of the mean. Transplanted mice are indicated by the Tx designation (ie, B6-Tx and ND4-Tx). *indicates $P < 0.05$ significance level compared to day-3 timepoint as determined by an unpaired Student's *t*-test.

Further experiments may benefit from utilizing live infectious pathogens to characterize the kinetics of the immune response between these groups of mice, determining antigen-specific T cell responses through the use of tetramers, and from determination of functional T cell responses via detection of secreted cytokines and/or survival outcomes. However, we do not imagine that anything other than the magnitude of the responses will be different from what has been reported herein for total B and T cells (and T cell subsets).

Interestingly, B cell responses were greater in the outbred strain of mice when compared to the inbred strain. These responses were muted in both strains of animals after transplant, particularly in the inbred B6 mice. B cells are known to recover soon after stem cell transplant,[14] but require reacquisition of T helper cell function in order to respond normally.[14] This finding may explain why outbred ND4 mice display better B cell responses sooner after transplant. It is not, however, evident why B6 mice are poor B cell responders to alloantigen challenge. Close examination of the data seems to indicate that the B6 mice actually experienced a decline in B cell numbers over time. However, we believe this finding is due to a margination of B cells from the peripheral blood into the tissues, rather than a loss (or death) of total B cells. Conservatively, we have termed this response a weak expansion to immunization.

In conclusion, it appears that the kinetics of the immune responses observed in outbred mice better model those of a healthy human subject.[15] Although only murine responses were analyzed in the current study, published literature suggests similar results are to be expected within the human population. The difference in magnitude of the immune responses between inbred and outbred mice, as well as the individually responding immune subsets, should deter future studies from modeling immune responses in an inbred mouse model, as the responses appear to

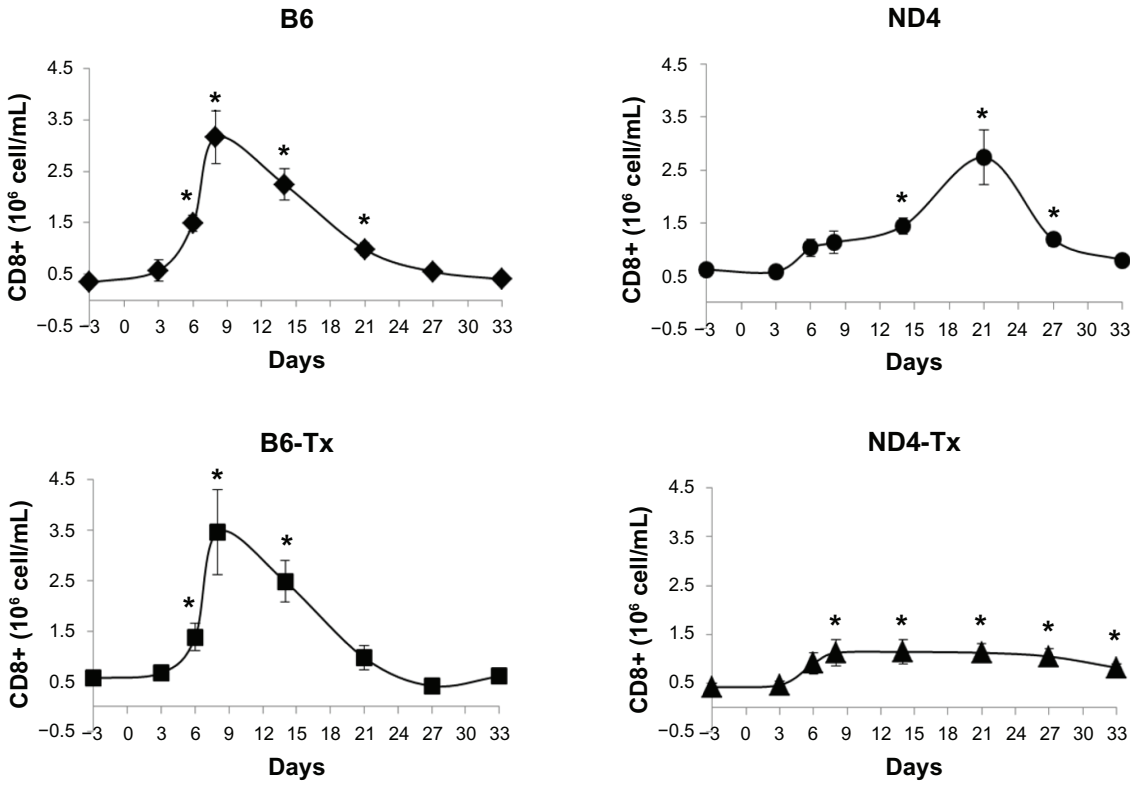

Figure 6. Changes in CD8+ T cell counts following transplantation and immunization.
Notes: Inbred (B6) and outbred (ND4) mice were subjected to syngeneic bone marrow transplants as described. Non-transplanted B6 and ND4 mice served as controls. After stable engraftment all animals were immunized and followed for up to 33 days post vaccination. At the indicated times blood was drawn from each mouse and analyzed for CD8+ T cell counts by flow cytometry. A total of 6 mice were used for each group in each strain. Data is presented as the mean of the 6 measurements plus/minus the standard error of the mean. Transplanted mice are indicated by the Tx designation (ie, B6-Tx and ND4-Tx). *indicates $P < 0.05$ significance level compared to day-3 timepoint as determined by an unpaired Student's *t*-test .

be significantly different. When stem cell transplant patients are considered, it appears that the inbred mouse model may be even less characteristic of the human setting. It appears that even long after successful transplant (even in the absence of GVHD) there continue to be immune cell homeostatic issues that are particularly acute in the inbred B6 strain. Thus, although there are many reasons for using genetically inbred mouse strains in research, pre-clinical modeling of the immune response seems most analogous to humans in the outbred mouse model, particularly with respect to stem cell transplant patients. A potential caveat in these experiments is that we did not challenge the different groups of mice with a third party allogeneic splenocyte population. Although we do not have reason to believe that dissimilar immune responses would be observed, this caution must be noted.

However, differences have been reported in the literature between inbred strains of mice with regard to lymphocyte subset proportions,[16] although these differences were only significant when comparing normal strains of inbred mice to those exhibiting autoimmune tendencies (eg, B6 mice versus SLJ and NOD mice). Only small differences between normal strains of mice (such as B6, C57BR, Balb/c, and B6D2) were noted. However, as we have not examined multiple inbred strains of mice and made comparisons with multiple outbred strains of mice, even though reports in the literature are consistent with our conclusions, we must note this caveat.

Acknowledgements

We would like to acknowledge the expert technical assistance of the Nikolich laboratory regarding use of the HemaVet apparatus and of Paula Campbell for assistance with the flow cytometric analyses.

Conflict of Interest

Each of the authors declares that they have no conflict of interests, financial or otherwise, in relation to the work presented in this manuscript.

Author Contributions

Conceived and designed the experiments: DTH. Analysed the data: CLS, MB. Wrote the first draft of the manuscript: DTH. Contributed to the writing of the manuscript: CLS, DTH. Agree with manuscript results and conclusions: CLS, MD, STH. Jointly developed the structure and arguments for the paper: CLS, DTH. Made critical revisions and approved final version: CLS, MD, DTH. All authors reviewed and approved of the final manuscript.

Funding

Author(s) disclose no funding sources.

Competing Interests

None of the authors have a financial interest with any company that might be concerned with the topic of this work.

Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

References

1. Hows JM. Histocompatible unrelated donors for bone marrow transplantation. *Bone Marrow Transplant*. 1987;1(3):259–63.
2. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med*. 1998;339(22):1565–677.
3. Fujimaki K, Maruta A, Yoshida M, et al. Immune reconstitution assessed during five years after allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 2001;27(12):1275–81.
4. Steingrimsdottir H, Gruber A, Bjorkholm M, Svensson A, Hansson M. Immune reconstitution after autologous hematopoietic stem cell transplantation in relation to underlying disease, type of high-dose therapy and infectious complications. *Haematologica*. 2000;85(8):832–8.
5. Berger M, Figari O, Bruno B, et al. Lymphocyte subsets recovery following allogeneic bone marrow transplantation (BMT): CD4+ cell count and transplant-related mortality. *Bone Marrow Transplant*. 2008;41(1):55–62.
6. Engelhard D, Nagler A, Hardan I, et al. Antibody response to a two-dose regimen of influenza vaccine in allogeneic T cell-depleted and autologous BMT recipients. *Bone Marrow Transplant*. 1993;11(1):1–5.
7. Pauksen K, Linde A, Hammarstrom V, et al. Granulocyte-macrophage colony stimulating factor as immunomodulating factor together with influenza vaccination in stem cell transplant patients. *Clin Infect Dis*. 2000; 30(2):342–8.
8. Avetisyan G, Ragnavolgyi E, Toth GT, Hassan M, Ljungman P. Cell mediated immune responses to influenza vaccination in healthy volunteers and allogeneic stem cell transplant recipients. *Bone Marrow Transplant*. 2005;36(5):411–5.
9. Avetisyan G, Aschan J, Hassan M, Ljungman P. Evaluation of immune responses to seasonal influenza vaccination in healthy volunteers and in patients after stem cell transplantation. *Transplantation*. 2008;86(2): 257–63.
10. Blom-Potar MC, Chamond N, Cosson A, et al. Trypanosoma vivax Infections: Pushing Ahead with Mouse Models for the Study of Nagana. II. Immunobiological Dysfunctions. *PLoS Negl Trop Dis*. 2010;4(8):e793.
11. Rai D, Pham NL, Harty JT, Badovinac VP. Tracking the total CD8 T cell response to infection reveals substantial discordance in magnitude and kinetics between inbred and outbred hosts. *J Immunol*. 2009;183(12):7672–81.
12. Rettingner C, Leclipteux T, de Meerschman F, Focanti C, Losson B. Survival, immune response and tissue cyst production in outbred (Swiss white) and inbred (CBA/Ca) strains of mice experimentally infected with Neospora caninum tachyzoites. *Vet Res*. 2004;35(2):225–32.
13. Haribhai D, Lin W, Relland LM, Truong N, Williams, CB, Chatila TA. Regulatory T Cells Dynamically Control the Primary Immune Response to Foreign Antigen. *J Immunol*. 2007;178(5):2961–72.
14. Atkinson K. Reconstruction of the haemopoietic and immune systems after marrow transplantation. *Bone Marrow Transplant*. 1990;5(4):209–26.
15. Huo A, Bissett SL, Giemza R, Beddows S, Oeser C, Lewis DJM. Systemic and Mucosal Immune Responses to Sublingual or Intramuscular Human Papilloma Virus Antigens in Healthy Female Volunteers. *PLOS One*. 2012;7(3):e33736.
16. Chen J, Harrison DE. Quantitative train loci regulating relative lymphocyte proportions in mouse peripheral blood. *Blood*. 2002;99(2):561–6.