Establishment and Optimization of the NSG Mouse Model for Antibody Development

Pitcher, Jennifer L.[1]; Harman, Ben[2]; Strake, Brandy[2]; Nungesser, Donna[1]; Emmell, Eva[1]

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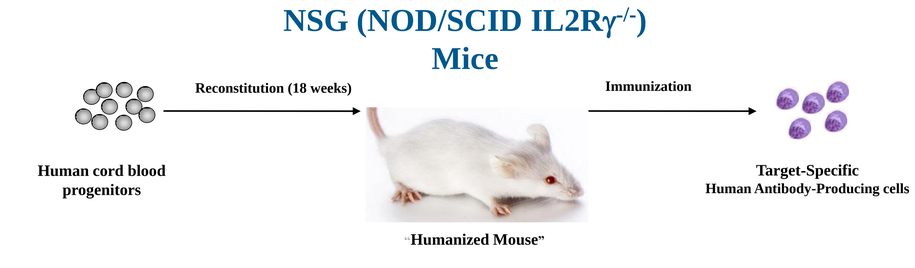
Abstract

The NSG or NOD SCID IL-2 rγ<-/->mouse (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ 005557) is a strain of genetically–deficient mice that do not produce a functional immune system. Due to genetic defects, NSG mice are excellent hosts for the propagation of human blood cells *in vivo*. Transfer of human hematopoietic progenitors leads to successful engraftment of human peripheral B & T cells, although reconstitution is somewhat variable and time-consuming, taking up to 18 weeks for the appearance of mature T cells in the peripheral blood. During our early experiments, human CD34+ stem cell reconstituted mice who had undergone whole body irradiation at 250cGy exhibited many complications compromising their health requiring daily monitoring and frequent supportive care. Our facility has worked diligently over the last 2 years to successfully remedy many of these health issues. In optimization studies we have found that lowering the irradiation dose and adding high fat feed prior to transfer of human cells resulted in optimal multi-lineage reconstitution of human peripheral cells, better general health and resulted in prolonged survival. These improvements helped support further development of this model as a system for the study of human hematopoiesis and human immune responses.

Introduction

The NSG mouse or NOD/SCID gamma mouse (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) is a strain of genetically –deficient mice that lack mature T cells, NK, and B cells and therefore serve as an excellent host for the generation of human blood cells from hematopoietic stem cells (HSCs) following low dose whole body irradiation. Circulating human B & T cells can be detected in the peripheral blood of these mice at 18 weeks post-reconstitution, providing a potential platform for the generation of human antigen-specific antibodies following immunization with human proteins. Although, this strain generally handles the preconditioning regimen and reconstitution well, reconstitution efficiency shows variability within each cohort of mice. Moreover, complications such as hair loss, weight loss, anemia, dietary issues, irradiation sensitivity, and malocclusion are frequently seen in some mice. Therefore, this animal model requires careful monitoring by both scientists and animal care staff to maintain overall health and assess suitability of individual animals for experiments. In an effort to improve the overall health of reconstituted mice and reduce variability, we performed studies investigating the effect of different preconditioning and dietary regimens on survival and human reconstitution. These studies allowed us to refine the model and improve the overall health and lifespan of the reconstituted mice, as well as reducing the variability of human engraftment. We hope our findings will reduce the number of animal that we use in our studies by increasing the numbers of mice available for study and decreasing the requirement for large group sizes.



Methods and Results for Irradiation Dose Response and Diet Studies

For the irradiation & dose response study, forty-two (42) female NSG mice at 8 weeks of age acclimated for 14 days prior to this experiment. Mice were housed in filter top plastic cages and supplied with autoclaved food (NIH-31 6% fat) and filtered/acidified water. On Day 0, animals were stratified by weight into 6 groups. Once grouped, 5 of the 7 mice per group underwent whole body irradiation (0, 50, 100, 150, 200, and 250 cGy) to deplete any murine hematopoietic cells. On Day 1, the same mice within each group received a single IV injection of 2x10e5 human CD34+ cells. The 2 remaining untreated mice in each group served as naïve controls. All groups were weighed and scored (Table 1) at Day 0 and once weekly for 20 weeks. Animals scoring 2 or higher were removed from the study and humanely euthanized. Once weight loss was observed in any groups, highly nutritional supportive care (ClearH20 DietGel 76A) was added to the cage three times weekly throughout the remainder of the study. Whole blood samples of 10-15ul were collected via tail snip from mice at Day -1 and at Weeks 2, 8, 12, 16, and 20. Whole blood was collected into heparinized tubes and transferred into 96 well round bottom plate containing 190ul of RPMI media containing 10% FBS. Samples were analyzed by flow cytometry for cell population counts. For the diet study, fourteen (14) female NSG mice were grouped by weight into 2 groups. Group 1 was fed our facility's normal rodent chow (Lab Diet/Laboratory Autoclavable Rodent Diet 5010) ad lib. Group 2 was fed a higher fat rodent chow (NIH 31 Modified Open Formula Mouse/Rat Seterilizable Diet containing 6% fat) ad lib. Acclimation, housing, water, supportive care, and identification were as previously described. On Day 0, 5 animals per group animals received whole body irradiation at 250cGy. This exposure is what had been previously demonstrated in literature. On Day 1, the same 5 mice were given a single IV injection of 2x10e5 human CD 34+ cells. The two additional mice per cage remained untreated and served as naïve controls. All mice were then weighed, scored, and monitored for malocclusions and recorded once weekly for 20 weeks.

Table 1.

Normal Alert and Reactive		0
Ruffed Hair coat Decreased activity Ocular Discharge		1
Hunched posture Moderate Hypothermia or hyperthermia Labored breathing during prodding	1-2 degrees above or below baseline	2
Labored breathing during rest Ataxia Tremor Hypothermia or hyperthermia	3-5 degrees above or below baseline	3
Loss of ability to ambulate with gentle prodding Unconsciousness		4
Death		5

Fig 1.

Effect of Diet on Body Weight and Survival

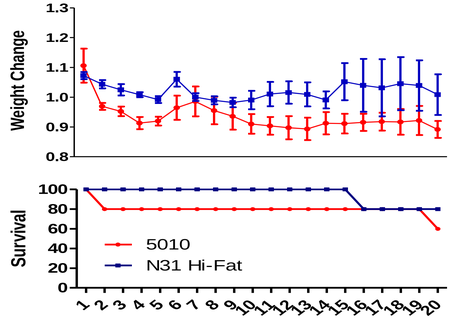
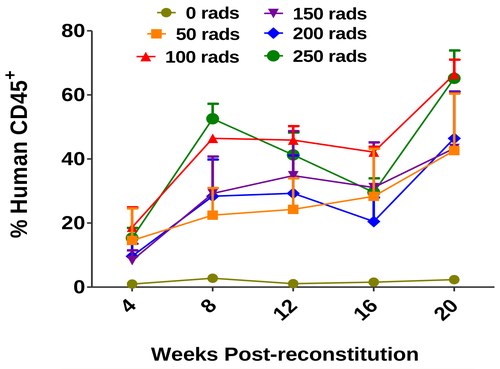


Fig 2.

Human Engraftment in Peripheral Blood



Conclusion

To date, we have successfully reconstituted 18 Cohorts each consisting of 60 mice. Cohorts 1-11 were irradiated at 250cGy, at which time we noticed over 50% of the mice with each cohort developing malocclusions, and having to remove 18-20% of the mice from study prior to the 18 week time point (time of full reconstitution) due to rapid weight loss, malocclusions, blindness, and in some cases hair loss. The irradiation dose response study (Fig.2) concluded that we could not only get the same level of engraftment with less irradiation, but that we could also eliminate the majority of the health issues we were observing. Cohorts 12-18 were irradiated at 100cGy, with zero malocclusions and zero blindness. The diet study results (Fig.1) shows us that with a higher fat rodent feed, in comparison to our normal facility feed, reduced the amount of weight loss over the 20 week period thus eliminating the amount of animals having to be removed from study due to ethical guidelines. The ultimate goals of these experiments were to a) reduce or eliminate the number of animals that were unable to be used in future cohorts b) having the mice maintain a healthy weight throughout the study time course of both reconstitution and any immunization protocol thereafter , and c) reduce or eliminate the occurrences of blindness and malocclusions due to cGy exposure. By reducing the irradiation and changing over to a higher fat diet and higher nutritional supportive care, we have been able to successfully establish this animal model for antibody development in our facility.

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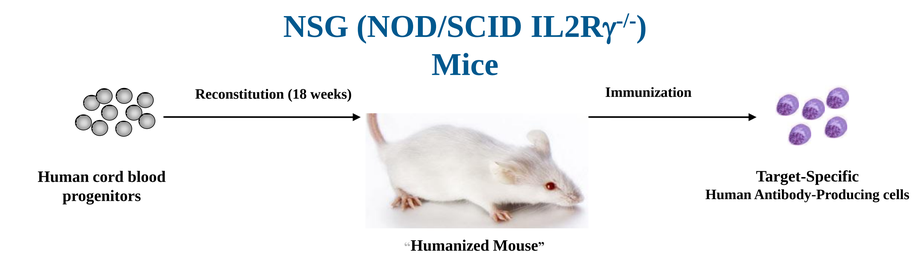
Introduction

The NSG mouse or NOD/SCID gamma mouse (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) is a strain of genetically –deficient mice that lack mature T cells, NK, and B cells and therefore serve as an excellent host for the generation of human blood cells from hematopoietic stem cells (HSCs) following low dose whole body irradiation. Circulating human B & T cells can be detected in the peripheral blood of these mice at 18 weeks post-reconstitution, providing a potential platform for the generation of human antigen-specific antibodies following immunization with human proteins. Although, this strain generally handles the preconditioning regimen and reconstitution well, reconstitution efficiency shows variability within each cohort of mice. Moreover, complications such as hair loss, weight loss, anemia, dietary issues, irradiation sensitivity, and malocclusion are frequently seen in some mice. Therefore, this animal model requires careful monitoring by both scientists and animal care staff to maintain overall health and assess suitability of individual animals for experiments. In an effort to improve the overall health of reconstituted mice and reduce variability, we performed studies investigating the effect of different preconditioning and dietary regimens on survival and human reconstitution. These studies allowed us to refine the model and improve the overall health and lifespan of the reconstituted mice, as well as reducing the variability of human engraftment. We hope our findings will reduce the number of animal that we use in our studies by increasing the numbers of mice available for study and decreasing the requirement for large group sizes.



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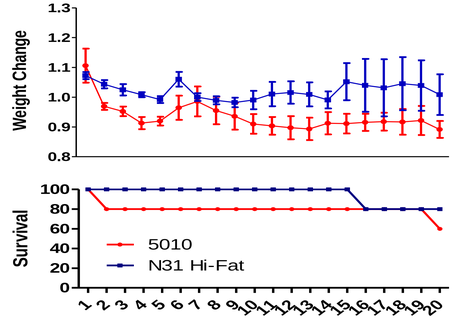

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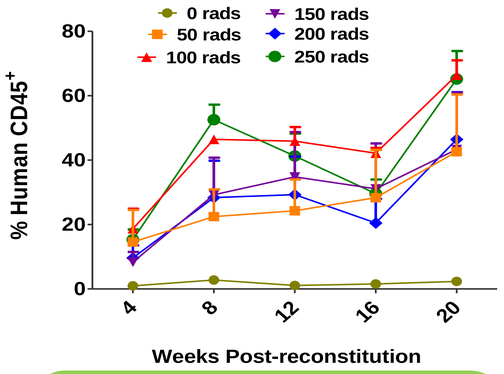

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