charles river

RESEARCH MODELS

NOD CRISPR *Prkdc Il2r Gamma* (NCG) Triple-Immunodeficient Mouse Model

Nomenclature: NOD-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}/NjuCrl

Key Points:

- Tumors derived from LNCaP cells, a prostate cancer cell line, grow 2X in NCG versus NOD-SCID mice
- hPBMCs engraft with high efficiency in the NCG mouse, with 75% of all CD45^{+} cells of human origin by day 22
- Tumor-infiltrating lymphocytes (TIL) present in successfully engrafted PDX tumors in CD34^{+}-NCG mice
- GvHD response similar to other triple-immunodeficient mouse models

Summary

As researchers look for ways to optimize and translate studies from animals to humans, a push to "humanize" current models has occurred. This has been facilitated by the use of immunodeficient models that permit transplantation of foreign tissues with an attenuated host-versus-graft response. In light of this trend, Charles River Laboratories has acquired a mouse model that displays demonstrable improvements of foreign tissue transplantation and engraftment compared to previous generations of immunocompromised mice.

Uniquely created using CRISPR-Cas9 technology[1] to alter the *Prkdc* and *Il2rg* genes, this model is "triple-immunodeficient," more immunocompromised than commonly used immunodeficient mouse strains, including SCID and nude mice. *Prkdc* and *Il2rg* are part of the SCID (severe combined immunodeficiency) family of genes affecting the maturation and formation of T cells, B cells, NK cells, and, to a lesser degree, dendritic cells.[2,3] Disrupting the *Prkdc* gene, encoding for the catalytic subunit of the DNA-dependent protein kinase enzyme, reduces function required for V(D)J recombination necessary in propagating antibody diversity of maturing T and B cells. Disrupting *Il2rg*, encoding for the common gamma subunit found in IL-2 and multiple IL receptors (IL-4, IL-7, IL-9, IL-15, and IL-21), prevents immature lymphocytes (T, B, and NK cells) and other leukocytes from reaching maturation as the receptors are required to bind and induce cytokine-mediated signaling. The NCG mouse strain is similar to other triple-immunodeficient models capable of hosting xenograft cells, tissue and human immune system components, thus enabling studies of tumor biology and immuno-oncology, infectious disease, graft-versus-host disease (GvHD), hematopoiesis, and tissue transplant studies.

Development and Genetics of the NCG Model

The NOD-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}/NjuCrl was co-developed by Nanjing Biomedical Research Institute of Nanjing University and Nanjing Galaxy Biopharma in 2014 and transferred to Charles River (CRL) in 2016. This model was created by sequential CRISPR editing of the *Prkdc* and *Il2rg* loci in the NOD/Nju mouse, generating a mouse coisogenic to the NOD/Nju. The NOD/Nju carries a polymorphism in the *Sirpa* gene advantageous for human cell engraftment.[4] It is absent a hemolytic complement system, and displays reduced NK, dendritic and macrophage cell function.[5]

CRISPR/Cas9 technology was used to knock out both the *Prkdc* and *Il2rg* loci. The *Prkdc* gene was disrupted by a 52-base-pair deletion in exon 37 (starting at codon 1619). The *Il2rg* gene was disrupted by a 22-base-pair deletion in exon 3 (Codon 156). Note, the IL2RG protein produced is similar to other models that have a targeted disruption in exon 3 versus other models with a targeted disruption commencing within exon 7. NCG mice are homozygous for the *Prkdc* deletion, while, for the *Il2rg* deletion, female mice are homozygous and males hemizygous, as the *Il2rg* gene is carried on the X chromosome.

Preliminary Results: Performance of the NCG Model

Charles River Discovery Services and Nanjing Galaxy Biopharma carried out several studies to evaluate the performance of the NCG mouse model in widely used applications for triple-immunodeficient mice. This included assessing the model's ability to support difficult-to-develop tumor cell lines, humanization studies involving engraftment of human peripheral blood mononuclear cells (hPBMCs), an analysis of GvHD development following injection of PBMCs, as well as the ability to grow patient-derived tumors in humanized mice.

Human tumor cell engraftment using the NCG mouse

Charles River evaluated the performance of NCG mice for the growth of tumor cell lines that are difficult to establish in many other mouse strains. In particular, the human LNCaP prostate adenocarcinoma cell line was used to ascertain the ability of male NCG mice to grow solid tumors. Male NOD-SCID (CRL) mice were used for comparison purposes.

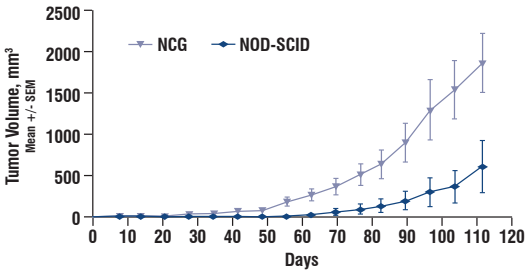

Figure 1. Tumor growth comparison among immunodeficient strains. NCG and NOD-SCID (CRL) mice were each implanted subcutaneously with 1x10^7 LNCaP tumor cells in 50% Matrigel® (Corning). Tumor volume measurements were taken twice weekly until the end of the study on day 112 post-implant. Tumors were measured with calipers in two dimensions and the tumor size was calculated using the formula:

$$\text{Tumor Volume (mm}^3\text{)} = \frac{w^2 \times l}{2}$$

where w = width and l = length, in mm, of a tumor. Values are in mean +/- SEM.

Conclusions

The results from this study indicate that the NCG triple-immunodeficient mouse model is more suitable for the growth of difficult tumor cell lines such as the human LNCaP prostate adenocarcinoma cell line than other immunodeficient strains such as the NOD-SCID mouse model.

Humanization studies using the NCG mouse model

Charles River evaluated NCG mice for the ability to engraft and develop a human immune system from hPBMCs.

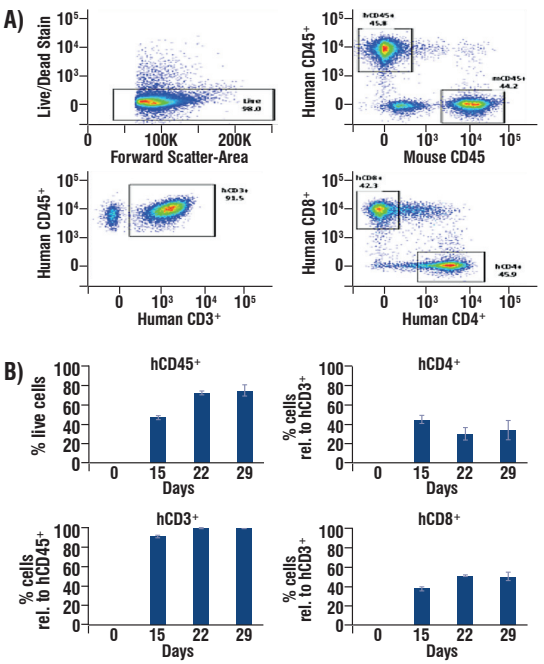

Figure 2. Immune profile of NCG mice humanized with human PBMCs. Panel **A)** represents the sequential gating strategy used to identify human T cells in peripheral blood of PBMC engrafted mice: singlets (not shown), live cells, hCD45$^+$, hCD3$^+$, hCD4$^+$/hCD8$^+$. Cell populations were quantified as a percentage of parent population gate. Panel **B)** Bar graphs display the engraftment kinetics of human PBMCs in the NCG triple-immunodeficient mouse model as determined by flow cytometry analysis. The leukocyte marker CD45$^+$ and T-cell markers CD3$^+$, CD4$^+$, and CD8$^+$ were evaluated from the day of engraftment until day 29 of the study. hCD3$^+$ values are relative to hCD45$^+$, while hCD4$^+$ and hCD8$^+$ values are relative to hCD3$^+$. Values are in mean +/- SEM.

Methods

On day 0, 8 female mice (7 to 10 weeks of age), were injected intravenously with 3 x 10^7 hPBMCs (Hemacare Bioresearch). Peripheral blood from engrafted animals was collected from each mouse on days 0 (n=8), 15 (n=6), 22 (n=4), 29 (n=4). The number of samples evaluated at each timepoint changed as animals had succumbed to the onset of graft-versus-host disease (GvHD) throughout the study. Immune cell populations were identified using flow cytometry, including: human (h)CD45$^+$ leukocyte markers, as well as T-cell markers hCD3$^+$, hCD4$^+$ hCD8$^+$ (hCD45$^+$CD3$^+$, hCD45$^+$CD3$^+$CD4$^+$, and hCD45$^+$CD3$^+$CD8$^+$ respectively).

Conclusion

NCG mice were successfully engrafted with human PBMCs. By Day 22, over 75% of circulating CD45$^+$ immune cells in the blood were human. The majority of these cells (99%) were of T-cell origin (CD3$^+$), consisting of CD4$^+$ or CD8$^+$ T cells. This is consistent with observations in other triple-immunodeficient mice.[6,7]

Patient-derived xenografts (PDX) engraftment in the NCG mouse

Nanjing Galaxy Biopharma conducted studies to evaluate the ability of the NCG mice to host patient-derived xenografts (PDX) humanized by engraftment of hematopoietic stem cells derived from cord blood (CD34$^+$ cells). A patient-derived lung tumor was implanted into humanized CD34$^+$-NCG mice and the tumor growth kinetics is shown below.

Methods

Newborn NCG mice were inoculated with 1 x 10^5 CD34$^+$ cells, purified and derived from human umbilical cord blood, via facial vein injection. LU1901, a patient-derived lung tumor tissue, was subsequently implanted subcutaneously. Tumor volume was assessed over a period of 21 days. Flow cytometry was used to evaluate the presence of human T cells (CD3$^+$, CD4$^+$, and CD8$^+$) from tumor-bearing CD34$^+$-NCG humanized mice.

Conclusions

PDX samples successfully engrafted in humanized NCG mice, displaying infiltration of human immune cells into the PDX tumor.

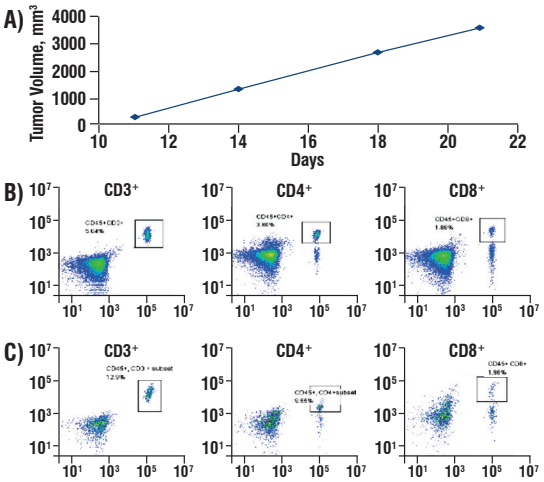

Figure 3. PDX growth kinetics in humanized CD34$^+$-NCG mice. A) Tumor growth kinetics of the PDX lung tumor in the NCG humanized mouse model. Values are in mean mm^3, +/- SEM. **B)** Circulating human T cells, post-engraftment of CD34$^+$ umbilical hematopoietic stem cells. CD3$^+$ (5.04%) CD4$^+$ (3.80%) CD8$^+$ (1.08%). **C)** Percent of tumor-infiltrating lymphocytes (TIL) obtained from tumors on day 21. CD3$^+$ (12.9%) CD4$^+$ (9.55%) CD8$^+$ (1.96%).

Assessment of GvHD response in the NCG mouse

Charles River evaluated the NCG mouse for xenogeneic GvHD response post-hPBMC injection. Triple-immunodeficient mice are an excellent model for GvHD as they display faster engraftment kinetics than standard immunodeficient mice without requiring (sublethal) total body irradiation.

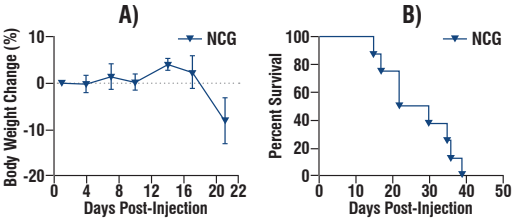

Figure 4. GvHD in the NCG mouse. NCG mice were engrafted with hPBMCs and the onset of GvHD was followed by tracking body weight changes. **A)** % change in body weight compared to starting weight (Day 1) shown up until 50% of the animals remained in the study (Day 21, n =6). Values are in mean +/- SEM. **B)** Kaplan-Meier analysis of NCG survival post-injection of hPBMCs. Median survival time = 26 days.

Methods

8 female mice aged from 7 to 10 weeks were injected intravenously with 3 x 10^7 hPBMCs. For this study, mice were euthanized when showing a body weight loss of > 30% or three consecutive measurements of > 25%; this timepoint was recorded as the survival time. GvHD was assessed by tracking body weight, weighing mice every 3 days, for 1.5 months. After 21 days, less than 50% remained in the study.

Conclusion

Development of GvHD occurred within 21 days of hPBMC engraftment as assessed by changes in body weight and clinical observations. The onset of GvHD varies with each hPBMC donor, which can be customized to the particular needs of each study. The results shown here demonstrate that the NCG mouse model is suitable for GvHD studies and is consistent with other triple-immunodeficient models.[8,9,10]

NCG Displays Excellent Engraftment of Human Tissue

The studies analyzing engraftment of human PBMCs, tumor growth, and GvHD reveal excellent performance of the NCG with similar results observed in other triple-immunodeficient mouse models.[6,7,8,9,10,11] NCG mice performed well in PDX experiments using tumor patient lung samples. Further studies are ongoing.

If you are interested in conducting an evaluation study, please contact askcharlesriver@crl.com or call 1.877.CRIVER1 (1.877.274.8371) for more information.

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