JANVIER LABS

HUMANIZED MICE:
NEXT GENERATION OF *IN VIVO* MODELS

2024

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IMMUNODEFICIENT MODELS BY JANVIER LABS

Janvier Labs has an extensive array of immunodeficient models featuring diverse characteristics and genetic backgrounds. These models can accommodate all your xenograft requirements, including tumors, immune cells, organs, and beyond.

+	Background	Breeding	Coat	T Lymphocytes	B Lymphocytes	Leakiness	NK cells	Dendritic cells	Macrophages	Complement	Irradiation tolerance	Life span	Humoral immunity	Phagocytic tolerance	Mutations of interest
NXG	NOD	Inbred	Albino	Absent	Absent	-	Absent	Dysfun.	Dysfun.	-	Low	89 Wk.	Absent	High	*Sirpa^{NOD} Prkdc^{scid} Il2rg^{tm1}*
NRG	NOD	Inbred	Albino	Absent	Absent	-	Absent	Dysfun.	Normal	-	High	89 Wk.	Absent	High	*Sirpa^{NOD} Rag2^{tm1} Il2rg^{tm1}*
NRG Nude	NOD	Inbred	Albino	Absent	Absent	-	Absent	Dysfun.	Normal	-	High	54 Wk.	Absent	High	*Sirpa^{NOD} Rag2^{tm1} Il2rg^{tm1} Foxn1^{nu}*
BRGS A2DR2	BALB/c	Inbred	Albino	Absent	Absent	-	Absent	Dysfun.	Normal	Normal	High	89 Wk.	Absent	High	*Sirpa^{NOD} Rag2^{tm1} Il2rg^{tm1}*
BRGS TSLP	BALB/c	Inbred	Albino	Absent	Absent	-	Absent	Dysfun.	Normal	Normal	High	89 Wk.	Absent	High	*Sirpa^{NOD} Rag2^{tm1} Il2rg^{tm1}*
B6N Rag2γc sirpa Nude	C57BL/ 6NRj	Inbred	Nude	Absent	Absent	-	Absent	Dysfun.	Normal	Normal	High	54 Wk.	Absent	High	*Sirpa^{NOD} Rag2^{tm1} Il2rg^{tm1} Foxn1^{nu}*
B6N Rag2γc sirpa	C57BL/ 6NRj	Inbred	Black	Absent	Absent	-	Absent	Dysfun.	Normal	Normal	High	89 Wk.	Absent	Low	*Rag2^{tm1} Il2rg^{tm1}*
B6N Rag2γc	C57BL/ 6NRj	Inbred	Black	Absent	Absent	-	Absent	Dysfun.	Normal	Normal	High	Min 54 Wk.	Absent	Low	*Rag2^{tm1} Il2rg^{tm1}*
NOD Scid	NOD	Inbred	Albino	Absent	Absent	Medium	Dysfon.	Low	Dysfun.	-	Low	36 Wk.	Absent	High	*Sirpa^{NOD} Prkdc^{scid}*
B6N Rag2 Nude	C57BL/ 6NRj	Inbred	Nude	Absent	Absent	-	Normal	Dysfun.	Normal	Normal	High	54 Wk.	Absent	Low	*Rag2^{tm1} Foxn1^{nu}*
CB17 Scid Nude	CB17	Inbred	Nude	Absent	Absent	-	Low	Dysfun.	Dysfun.	Normal	Low	36 Wk.	Absent	Low	*Prkdc^{scid} Foxn1^{nu}*
B6N Rag2	C57BL/ 6NRj	Inbred	Black	Absent	Absent	-	Normal	Dysfun.	Normal	Normal	High	Min 54 Wk.	Absent	Low	*Rag2^{tm1}*
B6N γc	C57BL/ 6NRj	Inbred	Black	Low	Low	-	Absent	Low	Normal	Normal	High	Min 54 Wk.	Absent	Low	*Il2rg^{tm1}*
CB17 Scid	CB17	Inbred	Albino	Absent	Absent	High	Low	Low	Dysfun.	Normal	Low	36 Wk.	Absent	Low	*Prkdc^{scid}*
B6N Nude	C57BL/ 6NRj	Inbred	Nude	Absent	Normal	-	Normal	Normal	Normal	Normal	High	Min 54 Wk.	Normal	Low	*Foxn1^{nu}*
BALBc/ Nude	BALB/c	Inbred	Nude	Absent	Normal	-	Normal	Normal	Normal	Normal	High	Min 54 Wk.	Normal	Low	*Foxn1^{nu}*
NMRI Nude	NMRI	Outbred	Nude	Absent	Normal	-	Normal	Normal	Normal	Normal	High	Min 54 Wk.	Normal	Low	*Foxn1^{nu}*
ATHYMIC Nude	BALB/c	Outbred	Nude	Absent	Normal	-	Normal	Normal	Normal	Normal	High	Min 54 Wk.	Normal	Low	*Foxn1^{nu}*

WHAT ARE HUMANIZED IMMUNE SYSTEM MICE?

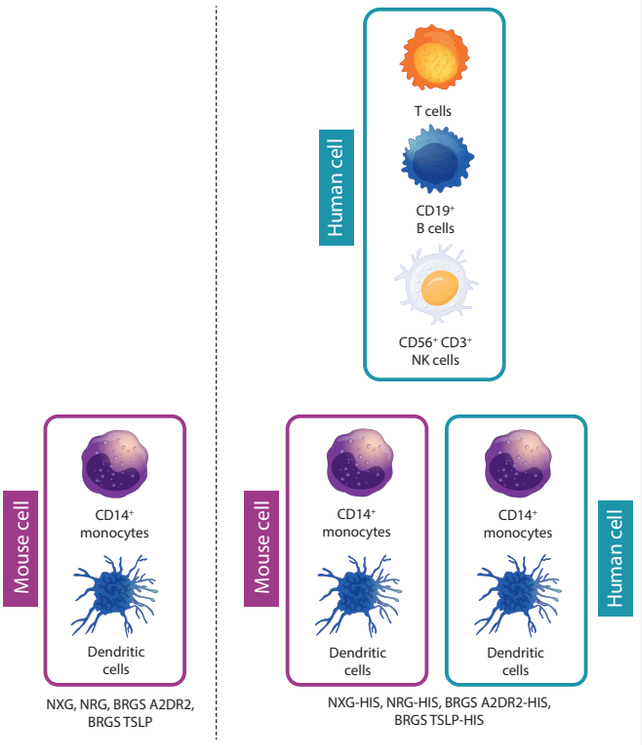
As the demand for advanced therapies to address unmet medical needs like cancer and autoimmune diseases continues to rise, there is an increasing necessity for enhanced preclinical models with greater predictive capability. While *in vitro* studies utilizing human cells or organoids are valuable, animal models offer a more comprehensive understanding of complex biological processes in mammals.

Humanized immune system (HIS) mice involve the engraftment of human immune cells into immunodeficient mice, resulting in various types of humanized immune systems depending on the engrafted cell type.

Janvier Labs offers diverse HIS models generated by engrafting cord blood CD34+ hematopoietic stem and progenitor cells.

Humanized CD34+ mice represent the pinnacle of mouse models as surrogate systems for human research. Their applications span across immunology, oncology, infectious diseases, safety studies and beyond. This innovative approach serves to bridge the gap between *in vitro* experimentation and clinical trials, furnishing a platform for more precise and translational investigations.

Compared lymphoid and myeloid cells presence in standard vs. HIS models



WHY SHOULD YOU CHOOSE JANVIER LABS' HIS MODELS?

JANVIER LABS combined its expertise together with the emergence of new technologies to develop new high-value models for scientists involved into target validation and efficacy studies, safety evaluation of new innovative therapies.

Benefit from the proximity of our expert to guide you in the choice of your research models.

1. A ready-to-use mouse with a guaranteed humanization rate
2. Quality and stability of the grafted immune system
3. Extended study window with the ability to lead 3- to 4-months preclinical studies
4. Opportunity to perform tumor co-transplant
5. Models produced in Europe and distributed by specialized JANVIER LABS carriers
6. SOPF status to facilitate the entry in your animal facilities
7. Mainly females which offer a guarantee of quality and stability
8. No license acquisition required for the use of mice

HIS MODELS BY JANVIER LABS

Janvier Labs prides itself in having one of the most robust HIS mice production platforms available on the market.

THE HUMANIZATION PROCESS:

The process of humanization involves injecting cord blood-derived human CD34+ cells into mice after inducing myeloablation (irradiation or chemoablation). This technique allows for the development of a functional and multi-lineage human immune system within the murine host. The use of cord blood as a source of hematopoietic stem cells offers advantages such as accessibility, ethical considerations, and the potential for diverse immune cell populations.

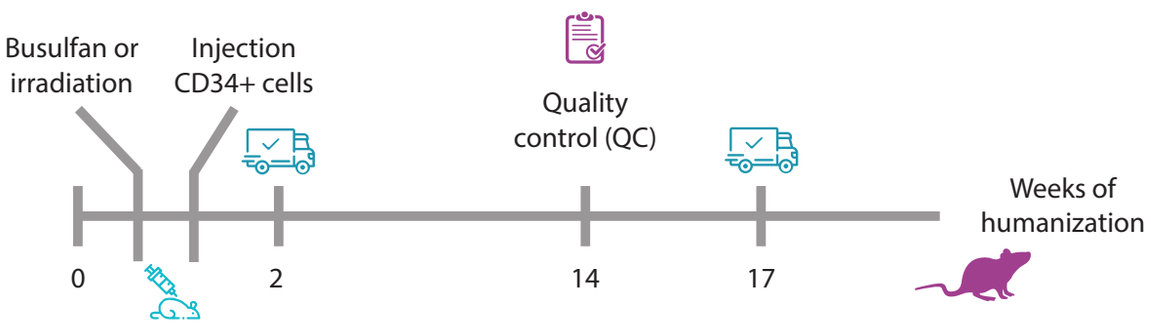
THE QUALITY CONTROL:

Quality control allows to measure the relative humanization rate of humanized mice in the peripheral blood. The standardization of quality control is performed by flow cytometry of the different human populations: hematopoietic, lymphoid and myeloid. Each HIS is tested and analyzed before sending and use. HIS mice are produced with SOPF status. Each batch provided is supplied with a list showing the animal repartition according to the different CD34+ donors. Quality control is typically performed 14-15 weeks post engraftment and allows to measure the relative humanization rate of humanized mice in the peripheral blood.

THE MODELS:

Janvier Labs offers 4 different models of HIS mice to its customers with different areas of application, perfect to accommodate your needs along your research process.

MODELS	NXG-HIS	NRG-HIS	BRGS A2DR2-HIS	BRGS TSLP-HIS
AVAILABILITY	Off-the-shelf	On-demand		
DELIVERY	- Starting 2 weeks post-engraftment without FACS QC - 17 weeks post-engraftment with FACS QC			
KEY FEATURES	- Most versatile model - Highest degree of hu CD45+ cells engraftment - Sensitive to irradiation	- Similar to the NXG-HIS model - Very resistant to genotoxic agents	- Expresses HLA class I and II - Enhances T-cell population development - Human-like TCR repertoire	- Overexpressed mouse TSLP - Full development of secondary lymphoid tissues - Model of atopic dermatitis



THE SERVICES:

The scientific team at Janvier Labs is always available to assist you in optimizing the utilization of your HIS mice. Reach out to us to explore how we can contribute to the success of your experiments.

- Typically, our mice, along with a comprehensive report on human system engraftment and sanitary status, are shipped to customers around week 16 post-engraftment. But we can offer to liberate the animals sooner to accommodate your needs.
- Engraftment of CD34+ cells carrying a specific HLA from a donor can be a valuable tool for your research. We can routinely offer engraftment with HLA-A*02:01 donors but can accommodate additional requests.
- If specific percentages of the lymphoid and myeloid compartments are desired to tailor the use of Janvier Labs' HIS mice to your research needs, we can selectively choose animals that best suit your requirements.

GENETICALLY ENGINEERED MODELS (GEM)

NATURAL IMMUNO-DEFICIENT

WILD TYPE

NATURAL MUTANTS

MICE
Mutant inbred

NXG-HIS Mouse

Strain name: NOD-*Prkdc*^{scid}-*Il2rg*^{tm1}/Rj

Type: Inbred transgenic mouse, GEMM

Origin: Janvier Labs, in 2021

Colour and related genotype: Albino mouse

Grafted human cells: CD34 from blood cord

Treatment: Busulfan or irradiation

Presentation of the model

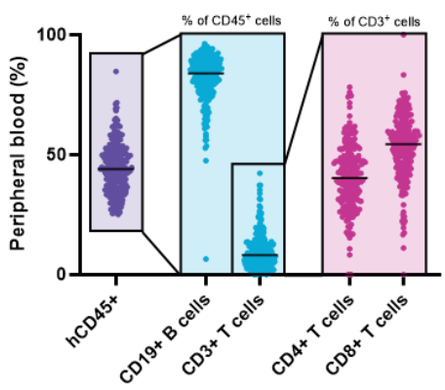
NXG-HIS mice are your best ally for all your research by relying on one of the best immunodeficient models and an optimized protocol allowing for the optimal development of multi-lineage human immune cells.

The NXG mouse model:
The NXG mouse is an inbred strain model on the NOD genetic background, sharing similarities with other strains like NSG, NcG, NOG. It carries two crucial mutations: *Prkdc*^{scid,} known as "SCID," which inhibits T and B cell development, resulting in their absence; and *Il2rg*^{tm1}, a knockout of the interleukin-2 receptor subunit gamma gene, essential for various immune cells, causing severe immunodeficiency with the absence of T, B, and NK lymphocytic cells. Additionally, the NXG strain expresses the NOD variant of the *Sirpa* gene, promoting reduced phagocytosis of transplanted human cells due to cross-recognition with CD47 ligands on human cells.

All these factors contribute to establishing the NXG strain as one of the best performing models in the context of humanizing the immune system.

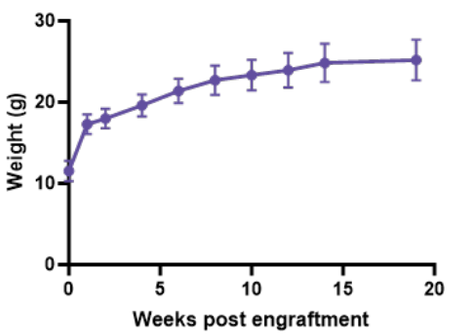
Peripheral blood and weight

Flow cytometry analysis of human cells 14 weeks after engraftment



Relative percentage of human CD45+ cells, T-cells and B-cells

Growth curve



GENETICALLY ENGINEERED MODELS (GEM)

NATURAL IMMUNO-DEFICIENT

WILD TYPE

NATURAL MUTANTS

MICE
Mutant inbred

NRG-HIS Mouse

Strain name: NOD-*Rag2*^{tm1}-*Il2rg*^{tm1}/Rj

Type: Inbred transgenic mouse, GEMM

Origin: Janvier Labs, in 2023

Colour and related genotype: Albino mouse

Grafted human cells: CD34 from blood cord

Treatment: Irradiation

Presentation of the model

NRG-HIS mice are your best ally for all your research by relying on one of the best immunodeficient models and an optimized protocol allowing for the optimal development of multi-lineage human immune cells. They are an attractive model for immune system immunization, especially in the frame of research related to radiotherapy or chemotherapy.

The NRG mouse model:

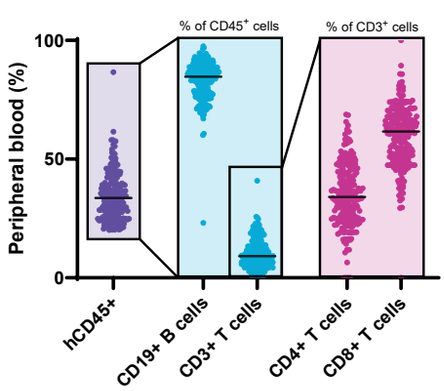
The NRG or NOD Rag2 γc strain is a highly immunodeficient inbred model characterized by knockout mutations in the *Il2rg* and *Rag2* genes. The *Rag2*^{tm1} mutation disrupts recombinase activity crucial for B and T cell receptor formation, resulting in a total absence of T and B lymphocytes. The *Il2rg*^{tm1} mutation knocks out the gamma c chain, essential for the proper functioning of various immune cells, including NK cells, leading to severe immunodeficiency. The combination of *Rag2*^{tm1} and *Il2rg*^{tm1} mutations induces the absence of T, B, and NK cells.

The NRG strain also expresses the NOD variant of the *Sirpa* gene, reducing phagocytosis of transplanted human cells. In comparison to the NXG strain, the NRG strain is more resistant to irradiation, genotoxic products, and stress due to the replacement of the *Prkdc*^{scid} mutation with the *Rag2* knockout, enhancing its durability and stability for xenograft purposes.

NRG-HIS mice stand as one of the most robust model of immune system humanized mice while displaying a great resistance to genotoxic agents

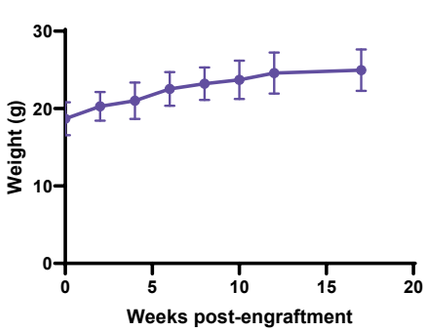
Peripheral blood and weight

Flow cytometry analysis of human cells 14 weeks after engraftment



Relative percentage of human CD45+ cells, T-cells and B-cells

Growth curve



GENETICALLY ENGINEERED MODELS (GEM)

NATURAL IMMUNO-DEFICIENT

WILD TYPE

NATURAL MUTANTS

MICE
Mutant inbred

BRGS A2DR2-HIS Mouse

Strain name: C-*Rag2*^{tm1}-*Il2rg*^{tm1}-*Sirpa*^{NOD}-Tg(HLA-A*02- HHD)-Tg(HLA-DR2)/Rj

Type: Inbred transgenic mouse, GEMM

Origin: Pasteur institute, 2022

Colour and related genotype: Albino mouse

Grafted human cells: CD34 from blood cord

Treatment: Irradiation

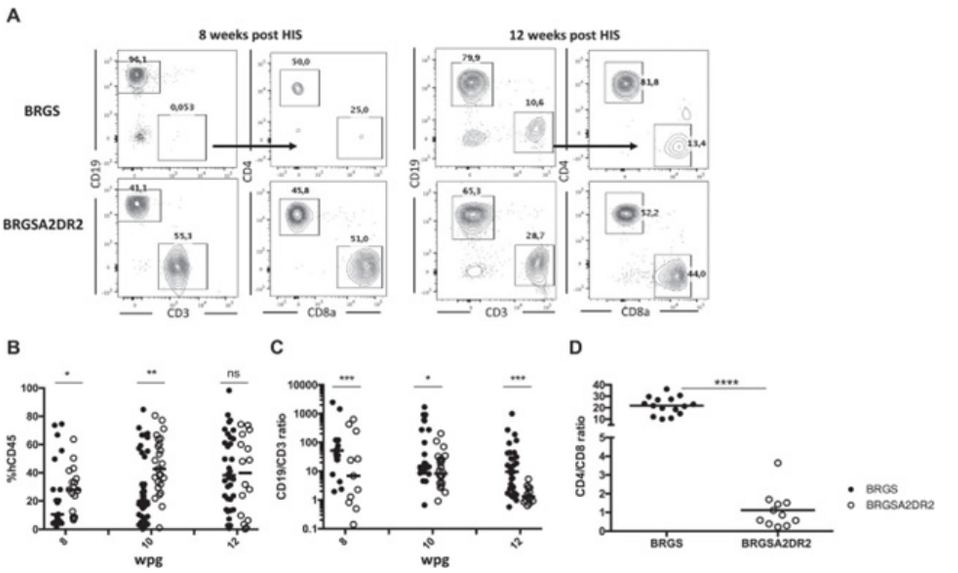
Presentation of the model

BRGS A2DR2-HIS mice go one step further in comparison to other humanized models by providing accelerated thymopoiesis as well as improved T-cell responses. These mice stand as a versatile model, resistant to genotoxic agents and perfect for any applications related to T-cell therapies (TCR redirected T-cells, vaccines…).

The BRGS A2DR2 mouse model:

The BRGS A2DR2 strain is a highly immunodeficient inbred model characterized by knockout mutations in the *Il2rg* and *Rag2* genes, with a NOD background gene. The *Rag2* knockout inhibits the formation of B and T cell receptors, leading to the absence of T and B lymphocytes. The *Il2rg* knockout affects the gamma c chain crucial for immune cell differentiation, resulting in the absence of T, B, and NK cells. The combination of these mutations induces severe immunodeficiency. Additionally, the strain carries the NOD variant of the *Sirpa* gene, reducing phagocytosis of transplanted human cells. Finally, this strain expresses human HLA-A*02 and HLA-DR2 transgenes.

The presence of HLA-A*02 and HLA-DR2 transgenes allows for a more rapid emergence of T cells in the hosts' circulation, indicating a potentially more efficient development of human T cells in the mouse thymus following the engraftment of human CD34+ stem cells.
The acceleration of CD4+ and CD8+ T cell development in BRGS A2DR2-HIS mice leads to a more balanced composition of B and T cell compartments in peripheral lymphoid organs. The presence of human HLA transgenes enhances both B- and T-cell functions, resulting in elevated levels of class switched Ig, increased percentages of polyfunctional T cells, and clear indications of antigen-specific T-cell responses post-immunization.



Kinetics of human cell reconstitution in the blood of BRGS versus BRGSA 2DR2-HIS mice. (A) Representative flow cytometry immune-phenotypic analysis of hCD45+ blood cells at 8 weeks and 12 weeks post graft (wpg) in humanized BRGS versus BRGS HLA-A*02-HHD class I and HLA-DRB1*15 class II transgenic mice (BRGS A2DR2-HIS) reconstituted with HLA-A*02+ HLA-DRB1*15+ HSCs. (B) Analysis of hCD45+ cells using the hCD45% × 100/ (hCD45% + mCD45%) formula (B) or CD19/CD3 ratio (C) over time (wpg) in BRGS (full circle) and BRGS A2DR2-HIS (open circle). (D) Comparison of the CD4/CD8 ratios in both models at 12 wpg. Each dot represents one mouse and data are representative from at least five independent experiments (14–34 mice in each group).

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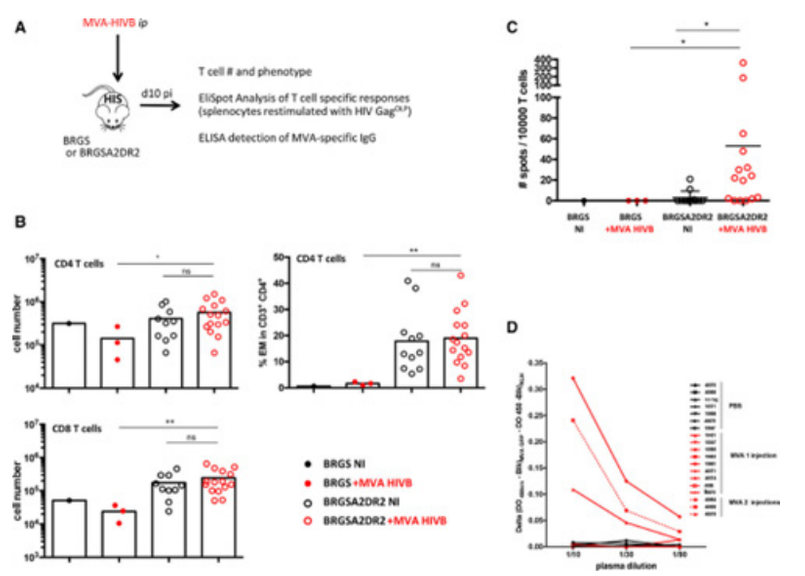

Antigen-specific HIV responses in BRGS versus BRGS A2DR2 mice. (A) Schematic overview of the MVA-HIVB immunization of HIS mice between 12 and 16 wpg. (B) Splenic CD4+ and CD8+ T cells numbers in nonimmunized (black symbols) and immunized (red symbols) from BRGS (full symbols) and BRGS A2DR2 (open symbols) HIS mice. (C) ELISPOT IFN-γ analysis of splenocytes from BRGS (full circles) and BRGS A2DR2 (open circles) reconstituted with HLA-A*02+ HLA-DRB1*15+ HSCs 10 days after immunization with MVA-HIVGPN viral particles (red symbols). (D) MVA vector-specific IgG response in the plasma determined by ELISA from MVA-HIVB immunized and nonimmunized BRGS A2DR2-HIS mice. Non-immunized BRGS and BRGS A2DR2 animals were also analyzed as controls (black circles). Each dot represents one mouse. Representative data of three independent experiments with one (BRGS NI) to seven mice analyzed per group.

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GENETICALLY ENGINEERED MODELS (GEM)

NATURAL IMMUNO-DEFICIENT

WILD TYPE

NATURAL MUTANTS

MICE
Mutant inbred

BRGS TSLP-HIS Mouse

Strain name: C-*Rag2*^{tm1}-*Il2rg*^{tm1}-*Sirpa*^{NOD}-Tg(*Tslp*)/Rj

Type: Mutant inbred mouse

Origin: Institut Pasteur, France, 2022

Colour and related genotype: Albino mouse

Use of this strain: Is restricted to private sector users

Grafted human cells: CD34 from blood cord

Treatment: Irradiation

Presentation of the model

BRGS TSLP-HIS mice provides additional features in comparison to standard models by displaying a complete development of secondary lymphoid tissues (SLT) as well as consisting in the best model up to date for atopic dermatitis.

The BRGS TSLP mouse model:

The BRGS TSLP strain is a highly immunodeficient inbred model characterized by two knockout mutations in the *Il2rg* and *Rag2* genes, resulting in a lack of T, B, and NK cells. The *Rag2* knockout hinders VDJ gene recombination necessary for B and T cell receptor formation, while the *Il2rg* knockout affects the gamma c chain, essential for immune cell differentiation and function. This strain also carries the NOD variant of the *Sirpa* gene, reducing phagocytosis of transplanted human cells. Compared to the NXG strain, it replaces the *Prkdc*^{scid} mutation with *Rag2* knockout, making it more resistant to various stressors and improving its stability for xenograft purposes. Additionally, it overexpresses murine thymic-stromal-cell-derived lymphopoietin (*Tslp*).

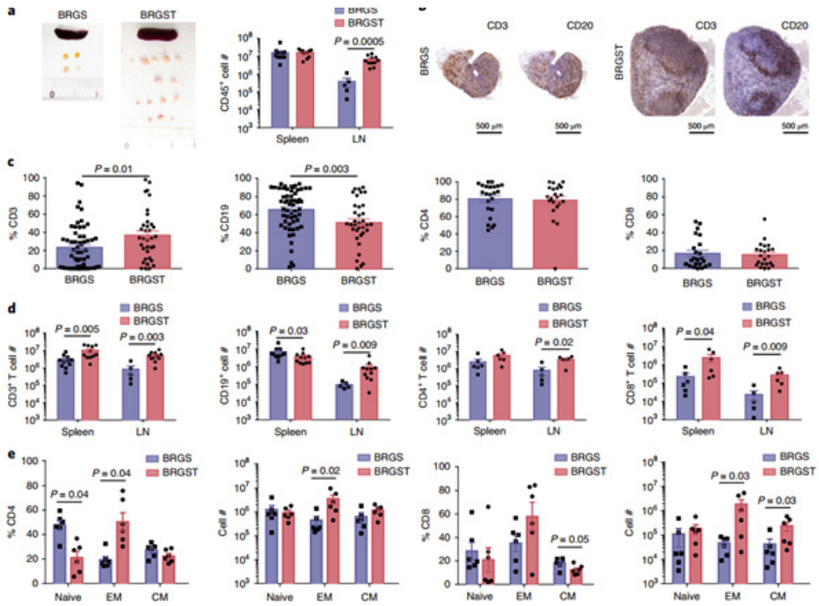
The overexpression of the *Tslp* gene leads to the formation of a complete range of lymph nodes (LNs) containing segregated human B and T cells. In comparison to other immune system humanized mice, BRGST HIS mice exhibit a larger thymus, increased presence of mature B cells, and a higher number of IL-21-producing follicular helper T (T_{FH}) cells, resulting in augmented antigen-specific responses. In essence, BRGS TSLP-HIS mice accurately represent the impact of SLT development on human immune responses and offer a valuable model for studying and manipulating immune regulation.

A model for human atopic dermatitis:

Atopic dermatitis is a chronic inflammatory skin condition characterized by dry, itchy, and inflamed patches of skin. It commonly affects individuals with a family history of allergies, asthma, or eczema, and can flare up due to triggers such as allergens, irritants, or stress. It is a prevalent condition, affecting approximately 15-20% of children and 1-3% of adults worldwide. This disease places not only heavy financial costs on patients and society, but also tremendous negative impact on the quality of life for patients and their family.

While non humanized BRGS TLSP do not show signs of AD development, BRGS TSLP-HIS mice universally demonstrate clinical, histological, immunological and skin commensal changes resembling human AD. Multiple human hematopoietic lineages associated with human AD were identified in BRGS TSLP-HIS mice, such as skin infiltrating Th2 and Th22 cells, IgE secreting B cells and hFcεRIα expressing mast cells and basophils. Similar to clinical findings, AD progression and skin homing chemokine receptor expression on CD4 T cells were highly correlated.

Together, BRGS TSLP-HIS constitutes a novel humanized model, showcasing enhanced immune system development that mirrors the impact of SLT development on human immune responses. This model offers a platform for visualizing and investigating immune regulatory mechanisms. Moreover, these mice serve as the first spontaneous human AD model and represent an optimal preclinical model for evaluating novel therapeutic interventions.



Characterization of human immune cell reconstitution in BRGS TSLP-HIS mice.
a, Representative organs (left; scale bars in centimeters) and absolute numbers of human CD45+ cells (right) in the spleens and LNs of BRGS and BRGS TSLP HIS mice (n = 11 mice per group). All visible LNs in BRGS or BRGS TSLP-HIS mice were pooled. b, Representative immunohistochemistry images of LN sections from BRGS (left) and BRGS TSLP (right) HIS mice. Scale bars, 500 µm.
c, Percentages of human T cells and B cells (n = 60 (BRGS) or 35 (BRGS TSLP) mice) and CD4+ and CD8+ T cells (n = 23 (BRGS) or 22 (BRGS TSLP) mice) among human hematopoietic cells (hCD45+mCD45– (h, human; m, mouse)) in the blood of the indicated mice. d, Absolute numbers of human T and B cells (n = 11 mice per group) and CD4+ and CD8+ T cells (n = 6 mice per group) in the spleen and LNs of the indicated groups of mice. All visible LNs in BRGS or BRGS TSLP HIS mice were pooled. e, Percentages and absolute numbers of human naive and memory cell subsets, identified by expression of CCR7 and CD45RA among hCD45+CD3+CD4+ cells and hCD45+CD3+CD8+ cells in the spleen of the indicated groups (n = 6 mice per group). EM, effector memory; CM, central memory. a,c–e, Data are shown as mean ± s.e.m.; P values determined by two-tailed Mann–Whitney U test. Each black square or circle represents an individual mouse.
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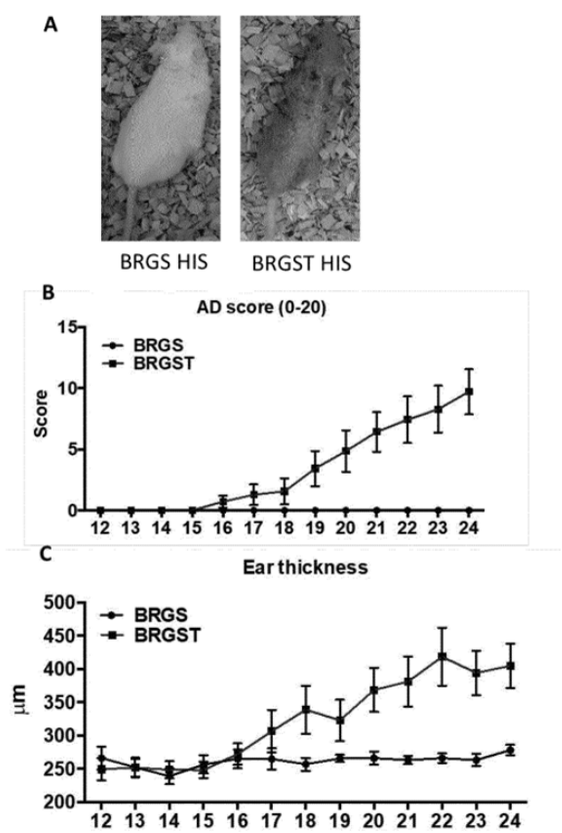

Characterization of the development of AD in BRGST TSLP-HIS mice. a, Representative photographs of BRGS and BRGS TSLP-HIS mice 25 weeks post CD34+ cells engraftment. b, AD scores evolution post CD34+ cells engraftment. The scoring system based on a system scoring the hair density and coverage, the presence of erosions, crusts and scales from 4 regions of the body on a scale of 0 to 4. c, Ear thickness evolution post CD34+ cells engraftment.

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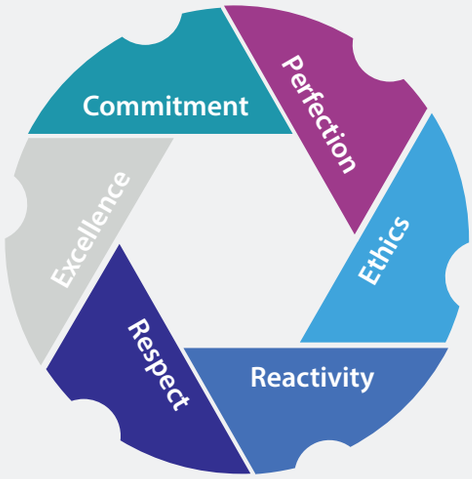

YOUR SATISFACTION: OUR PRIORITY

OUR COMMITMENTS: ADAPTABILITY AND REACTIVITY

- Flexibility in order-taking
- Tailor-made response to each of your requirements
- Personalised accompaniment for your projects
- A scientific follow-up specialised in rodents breeding
- Sales assistance on hand and ready to help
- Local sales representative for your specific needs
- Team of veterinary surgeons and experts to advise you
- An ISO 9001 certified company for all our activities

MY NOTES

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