OUR
IMMUNO-ONCOLOGY OFFER

IMMUNODEFICIENT MODELS

BRGS A2DR2 mouse

- **Strain name:** C-*Rag2*^{tm1Fwa}-*Il2rg*^{tm1Cgn}-*Sirpα*^{NOD}-Tg HLA-A*02-HHD *Class I* and HLA-DRB1*15 *Class II*
- **Type:** Mutant inbred mouse, Mus musculus, GMO
- **Origin:** Institut Pasteur, France, 2022
- **Colour and related genotype:** Albino mouse
- Use of this strain is restricted to private sector users

PRESENTATION OF THE MODEL

The BRGS A2DR2 or Balb/c Rag2 γc Sirpα strain is a severely immunodeficient inbred (Balb/c background) strain model with 2 Knock Out (KO) genetic mutations and a NOD background gene: the *γc KO* (Interleukin 2 receptor gamma chain, *IL2rg*^{Tm1}) gene, the *Rag2 KO* (recombinase 2 activating gene) gene and the Sirpα (NOD Background) gene. This model also carries two transgenes expressing the HLA A2 and DR2 molecules.

The *Rag2*^{Tm1} mutation, commonly known as Rag2, is a KO mutation in the gene coding for the recombinase 2 enzyme, which plays a key role in the generation of T and B receptors in lymphocytes. This absence blocks the development of T and B lymphocytes and induces an immune deficiency. Mice homozygous for this mutation have a complete absence of T and B lymphocytes in the periphery.

The *IL2rg*^{Tm1} mutation called γc is a KO mutation of the gene encoding the γc chain common to (among others) interleukins (IL-2, IL-4, IL-7, IL-9 and IL-15). This gene is required for the differentiation and function of many hematopoietic cells with a complete impact on the development of Natural Killer (NK) cells.

The combination of these two mutations, *Rag2*^{Tm1}-*IL2rg*^{Tm1}, induces a severe immunodeficiency with absence of T, B and NK lymphocyte compartments.

The BRGS A2DR2 strain carries the Sirpα gene from the NOD background with a particular polymorphism. Expression of the Sirpα protein (NOD fund alleles) on the surface of bone marrow macrophages allows high-affinity binding to CD47 markers of human hematopoietic cells.

This binding induces a "don't eat me" signal that blocks murine macrophages and prevents phagocytosis of transplanted human cells.

This is a notable feature of the NOD background (transferred by backross to BRG Balb/c mutants) that gives it an advantage in human transplantation and xenotransplantation in general.

The BRGS A2DR2 strain carries transgenes expressing human HLA A2 and DR2 molecules (HLA-A*02-HHD *Class I* and HLA-DRB1*15 *Class II*).

BRGS A2DR2 differs from NXG (NOD-*Prkdc*^{scid}-*IL2rg*^{Tm1}/Rj) strains by the absence of the *Prkdc*^{scid} mutation, commonly referred to as "SCID" for Severe Combinated Immunodeficiency. The BRGS A2DR2 strain is thus more resistant to irradiation, injection of genotoxic products and stress, conferring a more stable and durable use to xenograft in general, and carry peripheral lymph nodes, allowing an optimisation of human immune system xenografts, by increasing the quality, quantity and functionality of the actors of immunity.

JANVIER LABS has licensed the BRGS A2DR2 strain from the Institut Pasteur. The use of this strain is restricted to private sector users.

The animals are bred to maintain both the genetic background and the mutations of interest in their homozygous forms.

The BRGS A2DR2 strain is bred in inbred mode and the phenotype is controlled in accordance with the JANVIER LABS GENETIC POLICY®.

Main application and research fields

Oncology
- Tumor implantation studies
- Studies on gene therapy
- Studies of cancer therapies
- Study on hematopoietic cancer cells
- Studies focused on breast cancer
- Humanized model for the evaluation of anticancer gene therapy

Immunology and immunotherapy

Human cell implantation in a murine model

Implantation of hematopoietic cells of human origin in a murine model

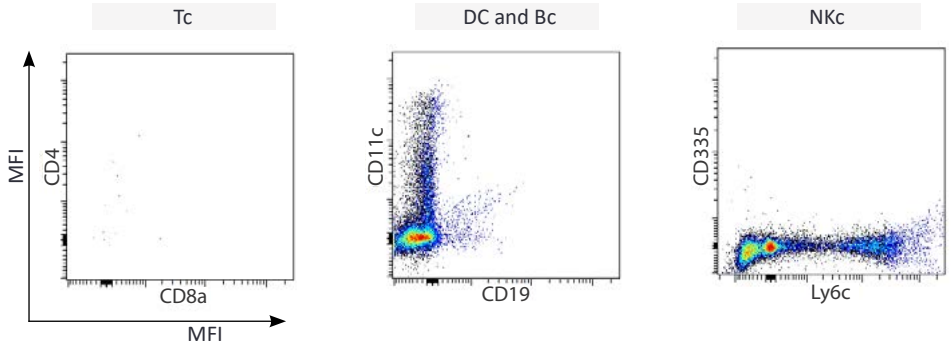
Transplants and grafts
- Human primary tumor xenografts of pulmonary origin
- A platform for the study of stem cells of epithelial origin
- Study the rejection of allograft after a pancreatic transplant against for type I diabetes

Infectious diseases
- Humanized models for the study of human-specific infectious diseases such as HIV, Epstein-Barr virus, malaria and dengue.

JANVIER LABS
Expertise in Rodent Research Models

IMMUNODEFICIENT MODELS

FLOW CYTOMETRY ANALYSIS, SPLEEN



Representative flow cytometry analysis confirming the absence of positive B cells (CD19), positive T cells (CD4 and CD8) and NK cells (CD335) in the peripheral blood of BRGS A2DR2 mice.

Fluorescence intensities (MFI) represent specific expressions of clusters of differentiations.

Clouds of points are obtained, each point representing a cell.

We can then determine the negative/single and positive/double positive cells in each population (defined by « Cluster of Differentiation ») , fixing or not the two antibodies carrying the fluorochromes.

PHENOTYPIC CHARACTERISATION

This model has been entirely characterized. The immunological and hematological parameters were characterized by Center of Immunophenomics (Ciphe, Marseille, France).

Background	Breeding	Coat	T Lymphocytes	B Lymphocytes	Leakiness	NK cells	Dendritic cells
BALB/c	Inbred	Albino	Absent	Absent	-	Absent	Dysfonctional
Macrophages	**Complement**	**Irradiation tolerance**	**Life span**	**Humoral immunity**	**Lymphoma outcome**	**Genes of interest**	
Normal	Normal	High	89 Wk.	Absent	Indefinite	*RAG2 IL2rg*	

ORIGIN/CREATION

Balb/c Rag2 γc Sirpα congenic animals were generated at the Institut Pasteur, and then backcrossed (speed congenic) to BALB/c(n) background for six generations.
The BRGS A2DR2 strain carries the transgenes expressing the human HLA molecules A2 and DR2.
HLA-A*02-HHD *class I* and HLA-DRB1*15 *class II* transgenic mice (B6 background), have been then backrossed (>10 generations) to the Balb/c Rag2 γc Sirpα strain (BRGS), to create BRGS A2DR2 Strain.

PHENOTYPICAL/PHYSIOLOGICAL OBSERVATIONS:

BRGS A2DR2 mice are viable, fertile, of normal size, and show no obvious physical or behavioral abnormalities. Histological examination of the lymphoid tissues reveals the absence of lymphoid cells and certain cystic structures in the thymus, an absence of follicles in the spleen, and markedly reduced cellularity of the lymph nodes.
BRGS A2DR2 mice are deficient in mature lymphocytes, serum Ig is not detectable.

These mice are resistant to the development of lymphomas even after treatment with sublethal radiation. These mutant mice have been shown to readily support the transplantation of human CD34+ hematopoietic stem cells and represent a superior long-lived model suitable for studies using xenotransplantation strategies.

REFERENCE/PUBLICATION

Accelerated thymopoiesis and improved T-cell responses in HLA-A2/-DR2 transgenic BRGS-based human immune system mice

Guillemette Masse-Ranson[1,2,3], Mathilde Duss´eaux[1,2], Oriane Fiquet[1,2], Sylvie Darche[1,2], Maud Boussand[1,2], Yan Li[1,2], Silvia Lopez-Lastra[1,2], Nicolas Legrand[4], Erwan Corcuff[4], Antoine Toubert[5,6], Mireille Centlivre[3], Timothee Bruel[3,7,8], Hergen Spits[9], Olivier Schwartz[3,7,8], Yves L´evy[3,10,11], Helene Strick-Marchand[1,2] and James P. Di Santo[1,2]

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IMMUNODEFICIENT MODELS

BRGS TSLP mouse

- **Strain name:** C-*Rag2*^{tm1Fwa}-*Il2*^{rgtm1Cgn}-*Sirpα*^{NOD}-Tg TSLP
- **Type:** Mutant inbred mouse, OGM
- **Origin:** Institut Pasteur, 2022
- **Colour and related genotype:** Albinos mouse
- **Use:** For private users only

PRESENTATION OF THE MODEL

The BRGS TSLP mouse model or Balb/c Rag2 γc Sirpα TSLP strain is a severely immunodeficient inbred (Balb/c background) strain model with 2 knock out (KO) genetic mutations and a NOD background gene: the γc KO gene (Interleukin 2 receptor gamma chain, *IL2rg*^{Tm1}), the Rag2 KO gene (recombinase 2 activating gene), and the Sirpα (NOD Background) gene. This model also carries a transgene expressing the molecule: thymic-stromal-cell-derived lymphopoietin.

The *Rag2*^{Tm1} mutation, commonly known as Rag2, is a KO mutation in the gene coding for the recombinase 2 enzyme, which plays a key role in the generation of T and B receptors in lymphocytes. This absence blocks the development of T and B lymphocytes and induces an immune deficiency. Mice wich are homozygous for this mutation show a complete absence of T and B lymphocytes in the periphery.

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The combination of these two mutations, *Rag2*^{Tm1}-*IL2rg*^{Tm1} , induces a severe immunodeficiency with absence of T, B and NK lymphocyte compartments.

The BRGS TSLP mouse model carries the Sirpα gene from the NOD background with a particular polymorphism. Expression of the Sirpα protein (NOD fund alleles) on the surface of bone marrow macrophages allows high-affinity binding to CD47 markers of human haematopoietic cells.

This binding induces a "don't eat me" signal that blocks murine macrophages and prevents phagocytosis of transplanted human cells.

This is a notable feature of the NOD background (transferred by backross to BRG Balb/c mutants) that gives it an advantage in human transplantation and xenografting in general.

The BRGS TSLP mouse model carries the TSLP transgene, thymic-stromal-cell-derived lymphopoietin, **to restore the development of secondary lymphoid nodes** absent in the so-called γc KO models (Interleukin 2 receptor gamma chain, *IL2rg*^{Tm1}) and whatever the genetic background. This absence of peripheral lymph nodes is related to the absence of lymphoid tissue inducer dependent on γc receptor mediated signals.

The BRGS TSLP mouse model differs from the NXG strains (NOD-*Prkdc*^{scid}-*IL2rg*^{Tm1} /Rj) by the absence of the *Prkdc*^{scid} mutation, commonly referred to as "SCID" for Severe Combinated Immunodeficiency. The BRGS T strain is thus more resistant to irradiation, injection of genotoxic products and stress, conferring a more stable and durable use to the xenograft in general, and carry peripheral lymph nodes, allowing an optimization of the xenografts of the human immune system, by increasing the quality, quantity and functionality of the actors of the immunity.

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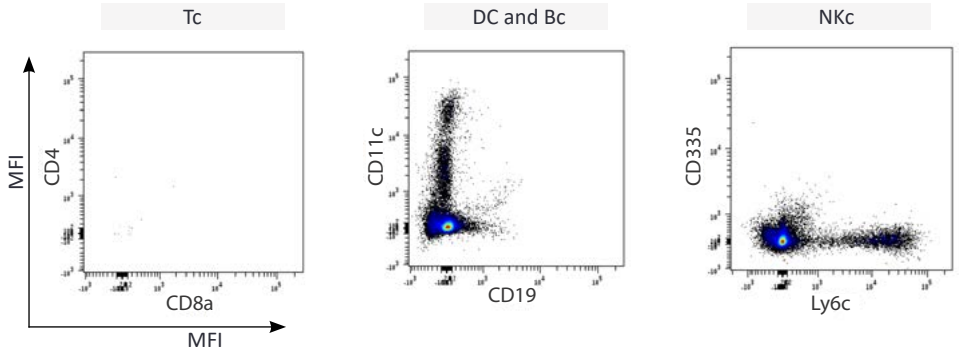

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Normal	Normal	High	89 Wk.	Absent	Indefinite	RAG2 IL2rg	

ORIGIN/CREATION

The BRGS TSLP model was generated at the Institut Pasteur.
The TSLP transgen expression is under the K14 control promoter, and previously created on the C57BL/6 genetic background.
TSLP transgenic mice were extensively back-crossed (> 10 generations) to the Balb/c *Rag2*$^{-/-}$-*Il2rg*$^{-/-}$-*Rag2 γc Sirpα* strain (BRGS) to create TSLP-transgenic BRSG (BRGST) hosts.

PHENOTYPICAL/PHYSIOLOGICAL OBSERVATIONS:

The BRGS TSLP mouse model are viable, fertile, of normal size, and show no obvious physical or behavioral abnormalities.
The BRGS TSLP mouse model levels were elevated in the serum of adult BRGS TSLP mice expressing K14 promoter-driven mouse TSLP compared with those in BRGS TSLP mice. Whereas gut NK cell and ILC (Innate Lymphoid Cells) subsets were severely depleted in *Il2rg*$^{-/-}$ BRGS TSLP mice compared with numbers in *Rag2*$^{-/-}$ mice, BRGS TSLP mice showed improved ILC development with no effect on NK cells, suggesting that TSLP complements IL-7 signaling pathways *in vivo*. Previous studies showed that TSLP overexpression can restore Lymphoid Tissue inducer (LTi) cell function in *Il2rg*$^{-/-}$ hosts.

This finding have been confirmed in BRGS TSLP mice, in which Lymph Node (LN) anlagen robustly developed throughout the body.
The few LN anlagens in BRGS TSLP mice were smaller than those in BRGS TSLP mice and visible only through dye staining.
In contrast, the defective Peyer's patches (PP) development secondary to Il2rg knockout was not rescued by TSLP overexpression.

BRGS TSLP mice are deficient in mature lymphocytes, and serum immunoglobulinsis not detectable. These mice are resistant to the development of lymphomas even after treatment with sublethal radiation. These mutant mice have been shown to readily support the transplantation of human CD34+ hematopoietic stem cells and represent a superior long-lived model suitable for studies using xenotransplantation strategies.

REFERENCE/PUBLICATION

A human immune system mouse model with robust lymph node development

*Yan Li[1,2], Guillemette Masse-Ranson[1,2,3], Zacarias Garcia[2,4], Timothée Bruel[3,5], Ayrin Kök[6,7], Helene Strick-Marchand[1,2], Gregory Jouvion[8], Nicolas Serafini[1,2], Ai Ing Lim[1,2], Mathilde Dusseaux[1,2], Thierry Hieu[6,7], Franck Bourgade[9], Antoine Toubert[10,11], Daniela Finke[12,13], Olivier Schwartz[3,5], Philippe Bousso[2,3,4], Hugo Mouquet[3,6,7] and James P. Di Santo[1,2]**

> https://www.researchgate.net/publication/326720721_A_human_immune_system_mouse_model_with_robust_lymph_node_development

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