Generation of Rag1 and Il2rg immunodeficient rats and application to humanization studies

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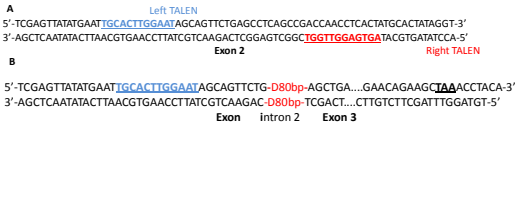
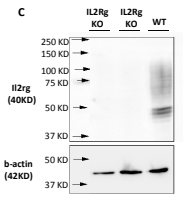
Modeling the immune response and rational immune approaches in vivo have relied heavily on studies performed in immunodeficient mouse models. These mouse models are ground-breaking platforms to humanize different tissues and organs and to evaluate compounds to treat a variety of human diseases, from cancer and infectious diseases to allergies, inflammation and GVHD. Rats are ideally suited to perform experiments in which larger size is needed and are still a small animal model suitable for rodent facilities.

We describe here the generation of Il2rg-deficient rats and their crossing with previously described Rag1-deficient rats, generating for the first time double-mutant Rat Rag1 and Il2rG KO (RRG) animals.

Generation of immunodeficient rats with *Rag1* and *Il2rg* gene deletions

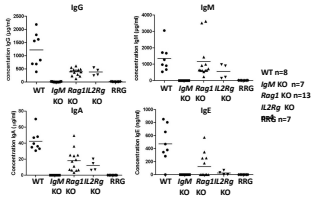
Generation of Il2rg-deficient rats using TALENs.





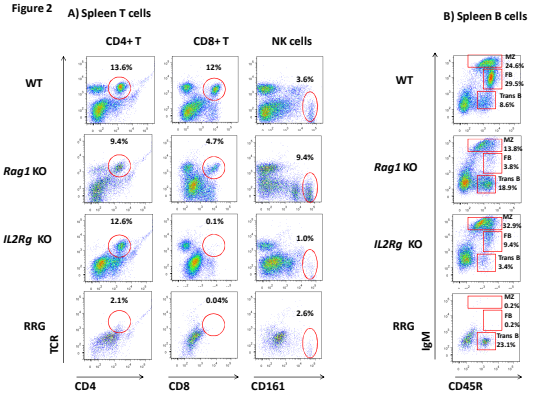
TALENs recognizing rat *Il2rg* sequences were generated and used for microinjection into rat zygotes. **A)** *Il2rg* DNA sequences recognized by the pair of TALENs used in the generation of *Il2rg*-deficient rats. **B)** *Il2rg*-mutated sequence (80 bp deletion) of the rat line used in this study. Intron 2 sequence only depicted for the first nucleotides. Premature stop codon in exon 3 underlined and in bold. **C)** Western blot analyses of two males of the Il2rg-deficient rat line used in this study and a WT animal as control using an anti-Il2rg antibody and anti-b actin as loading control.

Serum immunoglobulin isotypes.



Serum was drawn from adult rats and the amount of different immunoglobulin isotypes quantified by ELISA. *IgM*-mutated rats (Menoret et al., 2010) were used as control for the absence of all immunoglobulin isotypes.

Phenotype of lymphocyte populations.



Spleen cells from adult males were analyzed for lymphocyte populations. **A)** T (TCR$^+$) CD4$^+$ and CD8$^+$ cells as well as NK cells (CD161^{bright} TCR$^-$) were analyzed using MAbs and cytofluorimetry. **B)** B cells were analyzed and different differentiation stages defined using anti-IgM and anti-IgD MAbs. Representative experiment from n=3 to 7 for each rat line.

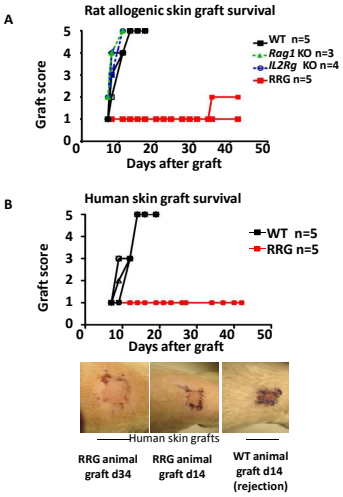
Absolute numbers of immune cells in different lymphoid compartments of Rag1 KO, Il2rg KO and RRG rats.

Organ	WT	Rag1 KO	Il2rg KO	RRG
Thymus				
Total cells number x10^8	10±1,8	10.9±1.3	0.05±0.04^d	0.00045±0.0002^d
TCR$^+$ cells	2.0±0.8	0.3±0.05^b	0.02±0.02^b	0.00009±0.0001^b
Cervical+axilar lymph nodes				
Total cells number x10^6	24.0±6,7	5.4±2.1^b	0.2±0.3^c	0.7±0.7^d
Spleen				
Total cells number x10^6	533.6±146.8	37.6±13^d	124.5±57.2^d	8.3±10.2^d
IgM$^+$ B cells	193.7±48.2	4.8±4.4^d	54.5±21.4^d	0.2±0.5^d
TCR$^+$ CD4$^+$	75.7±34.4	3.5±2.6^d	17±7.3^b	0.2±0.2^d
TCR$^+$ CD8$^+$	55.5±20.8	2.0±0.8^d	0.3±0.1^d	0.1±0.3^d
CD161^{high} CD3$^-$ (NK)	16.7±4.7	7.4±1.6^c	3.5±2.6^d	0.3±0.1^d
CD172a	158±18.2	19±2.7^c	101.3±8.9^a	9.6±4.0^d
Bone marrow				
Total cells number x 10^6	132.5±28.5	40.1±3.3^c	67.5±16.4^b	17.3±7.6^d
CD45R$^+$ IgM$^+$ B cells	23.0±0.8	2±0.1^c	1.7±1.0^b	0.4±0.3^d
CD45R$^+$ IgM$^-$ B cells	23.6±5.5	15.6±0.7^c	10.2±2.3^c	6.5±6^d
TCR$^+$ cells	1.0±0.8	0.6±0.2	0.6±0.3	0.5±1.3

WT (n=4) for thymus, cervical+axillaris lymph nodes and bone marrow and **(n=7)** for spleen; **Rag1KO (n=5)** for thymus, cervical+axillaris lymph nodes and bone marrow and **(n=8)** for spleen; **Il2Rg KO (n=3)** for thymus, cervical+axillaris lymph nodes and bone marrow and **(n=5)** for spleen. **RRG (n=7)** for thymus, cervical+axillaris lymph nodes and bone marrow and **(n=11)** for spleen. **a** P<0.05, **b** P<0.005, **c** P<0.0002 and **d**<0.0001 Mann-Whitney test.

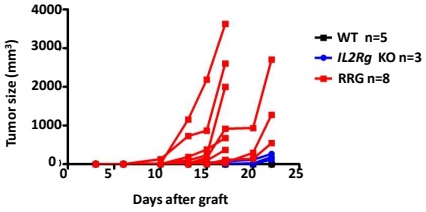
Human tissue grafting models

Skin graft survival.



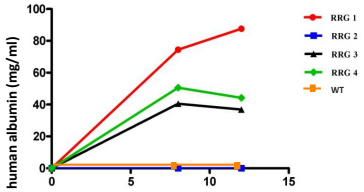
Skin from allogeneic or human origin were grafted into different rat lines and rejection scored by visual inspection. **A)** Major histocompatibility complex (MHC) mismatch skin from Lewis.1A rats (RT1^a MHC haplotype) was grafted into different the rat lines (WT, *Rag1*-deficient, ^{Il2rg}-deficient and RRG) all of SD origin (predominantly RT1^u MHC haplotype). **B upper).** Human skin was grafted into the different rat lines (WT, *Il2rg*-deficient and RRG). Graft score for A and B: 1: normal; 2: dry skin and <25% necrosis inside the graft; 3: dry skin and 25-75% necrosis; 4: dry skin and > 75% necrosis; 5: 100% necrosis. **B below).** Human skin transplanted into WT rats showed grade 5 rejection at day 14 after transplantation (left), human skin transplanted into RRG animals show normal grade 1 at day 14 (middle) and 34 (right) after transplantation.

Human tumor graft growth.



Human A2780 cells were embedded in Matrigel and implanted subcutaneously into WT (n=6), *IL2rg*-deficient (n=3) and RRG (n=7) animals. Tumor growth was quantified and expressed as mm^3. Each curve represents a single animal.

Liver humanization



8 weeks old rats received two doses of Retrorsine at 30mg/Kg 15 days apart by intra peritoneal injection. Four weeks after the second injection, a two-thirds partial hepatectomy was performed. Immediately after, 2.106 freshly-thawed human primary hepatocytes (Biopredic International, St Gregoire, France) were injected into the portal vein. **A)** Follow-up of human albumin production in the sera of individual RRG and WT animals is indicated at the indicated time points.

CONCLUSION :

Rag1 and Il2rg KO rats were crossed to obtain double KO RRG rats with a more pronounced T, B and NK immunodeficient phenotype that each single KO. This newly RRG rats can be use as a valuable model in several domains, such as stem cells-based medicine, transplantation research and for testing immunogenic molecules in preclinical tests

Publication of RRG animals: *Transplantation*, 2018, in press.

RRG animals are available upon request. ianegon@nantes.inserm.fr or severine.menoret@univ-nantes.fr