Development of a fully human immune system in NOD/SCID/IL2Rγ (NSG) mice for oncology research purpose

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Abstract

Humanized mice are a promising translational research model for evaluation of pharmacological compounds efficacy and safety in Oncology. Their use has been enhanced by the development of new stocks of immunodeficient hosts, most notably mouse strain such as NOD-*scid*/*IL2R*γ *null* mice (NSG). As previously described (L. Schultz *et al.*, J. Immunol. 2005) the NSG mice could also be successfully humanized after engraftment of human hematopoietic stem cells (HSC). NSG mice have also been shown to be superior to other immunodeficient mice (BALB/c *nu/nu*, NOD/Shi-*scid*) for xenograft of tumour material. These models are particularly needed in preclinical development where there is no appropriate small animal model combining the human immune system and human tumour.

Material and Methods

Animals

One day-old newborn NSG mice were obtained from Charles River (L'Arbresle, France). Newborns with their mother were whole body irradiated (1.8 Gy, Co^{60}, Biomep SARL, France). Animal experiments were performed according to ethical guidelines of animal experimentation (1) and the English guidelines for welfare of animals in experimental neoplasia (2). All procedures with animals were submitted to the Animal Care and Use Committee of Pharmacy and Medicine University (Dijon).

Human umbilical cord sample and human stem cells transplantation

Freshly collected umbilical cord blood (UCB) samples from healthy volunteer donors were obtained from the Etablissement Français du Sang (EFS). The CD3+ (T cells) were depleted before purification of the mononuclear cells from UCB. The T cell depletion was evaluated by flow cytometry analysis.

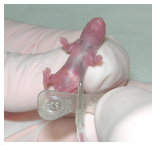
The purity and phenotyping of umbilical cord blood CD34+ HSCs were evaluated by flow cytometry analysis. Suspension of CD34+ cells was prepared at 6.10^5 CD34+ cells/ml in PBS. Whole body irradiated newborn mice were inoculated *via* the intracardiac route with 3.10^4 CD34+ cells as described by T. Pearson *et al.* (3)

FACS analysis

The human cells were quantified using expression of cell surface differentiation markers. Examination of hematopoietic chimerism was analysed on week 12 (W12) by detecting both mouse and human leukocytes On W16 by detecting human leukocytes: T and B lymphocytes, Monocytes, Macrophages or Granulocytes, natural killer and human platelets. Mouse central chimerism was analysed on W17 by detecting both mouse and human leukocytes in mice spleen, bone marrow and thymus.

Xenograft

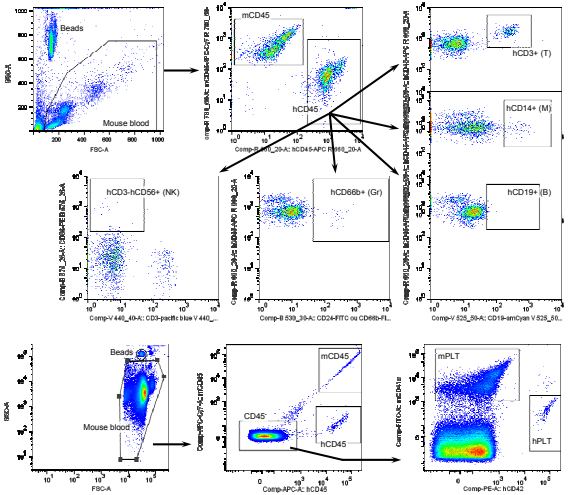
About twenty six weeks after humanization, the tumours were induced by injecting LoVo or BT-474 cells into the right flank or by injecting Ramos cells into the caudal vein of humanized NSG mice (D0). The viability and behaviour of mice were recorded every day, body weight and tumour volume of mice were recorded twice a week. All logistical issues of the study (collection, measurements, raw data, lethality, behavior and results of autopsy were managed using *Vivo* Manager software (Biosystemes, Dijon).



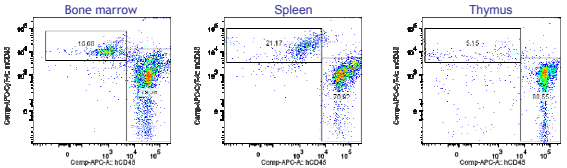
(1). Principe d'éthique de l'expérimentation animale. Directive n°86/609 CEE du 24 Nov. 1986, Décrêt n°87/848 du 19 Oct. 1987, Arrêté d'Application du 19 Avril 1988.
(2). WORKMAN P. et al., UKCCCR guideline. Br. J. Cancer, 77: 1-10, 1998.
(3). PEARSON T. et al., Current Protocols in Immunology, 15: 1-21, 2008.

Results

Peripheral blood chimerism W12, 7-color FACS analysis



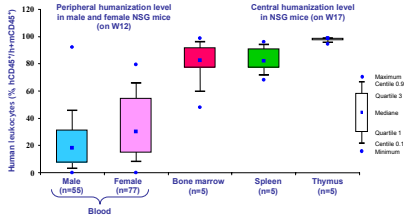
Central chimerism W17



Species	Antigens	Population	Fluorochrome
Mouse	mCD45	Leukocytes	APC-Cy7
	mCD41a	Platelets	FITC
Human	hCD45	Leukocytes	APC
	hCD3	Lymphocytes T	Pacific Blue™
	hCD19	Lymphocytes B	FITC
	hCD14	Monocytes, Macrophages, Granulocytes	PE-Cy7
	hCD56	NK cells	PE
	hCD42a	Platelets	PE

Results

Peripheral and central chimerism



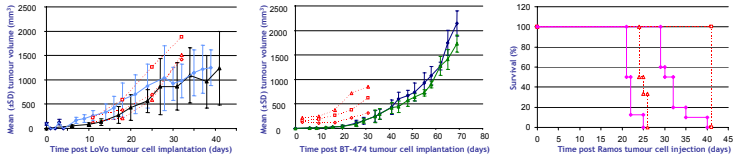
Peripheral blood human subpopulation

Human T lymphocyte (hCD3+), B lymphocyte (hCD19+), monocyte macrophages and granulocyte subset (hCD14+), natural killer (hCD56+), granulocytes (hCD66b) and platelets (hCD42a+) cells determined by blood FACS analysis 16 weeks after stem cells injection.

Blood subpopulation (gated on hCD45+)	Median (range, n=9)
hCD3+	52.9 (17.4 - 87.4)
hCD19+	5.4 (2.4 - 11.4)
hCD14+	23.7 (5.0 - 38.4)
hCD3-/hCD56+	2.6 (0.9 - 3.8)
hCD66b+	17.2 (3.7 - 29.7)

Platelet	Mean ± SD (% human vs total, n=12)
hCD42a+	2.7 ± 3.5

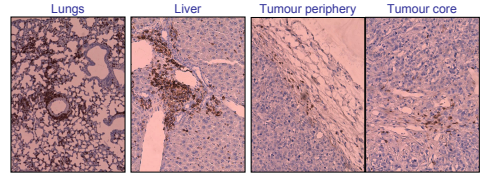
Tumor growth and survival curves of tumour-bearing humanized NSG mice



Chimerism analysis of tumour-bearing humanized NSG mice

Tumor model	Mouse sex	Peripheric chimerism (W12, %)	Xenograft (W27)	Mice death / termination	Chimerism (%) Blood	Chimerism (%) Bone Marrow	hCD3 (%) Blood
LoVo	M	20	W27	W31	11	30	99
	M	11			4	22	98
	M	18			8	60	93
	F	23					
	F	24	W16	NA	NA	NA	NA
	M	21					
BT-474	F	22	W25	W34	ND	ND	ND
	F	12			15	56	ND
	F	40			88	98	ND
Ramos	F	12	W28	W34	7	40	86
	F	16			24	71	92
	F	24		W30	ND	ND	ND
	F	32					
	M	15	W16	W20	NA	NA	NA
	F	22					

hCD45 IHC analysis of BT-474 tumour-bearing humanized NSG mice



Conclusions

- Twelve weeks after UCB HSCs injection, the peripheral blood contained about 30% of human leukocytes showing the normal proportion of different leucocytes sub-populations,
- Seventeen weeks after UCB HSCs injection, in bone marrow, spleen and thymus, human leucocytes has represented about 80%, 83% and 98% cells, respectively,
- Similar tumor growth curves were observed when comparing humanized NSG mice with non-humanized SWISS, NOD-SCID or SCID mice,
- This NSG mouse model opens new in vivo perspectives to study the complex relationships between the human immune system and human tumour cells under therapeutic treatments (antibody-dependent cell-mediated cytotoxicity with antibodies, antitumour vaccination strategies…).