Characterization of CIEA NOG® mice reconstituted with human CD34+ stem cells (HSCs) or mature PBMCs

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Introduction

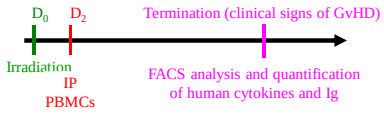
Human and rodent immune systems are different and results obtained in animal models are not always translated into human therapies. Furthermore, various human diseases don't have appropriate animal models. That is the reason why the development of humanized models was a huge need. Humanized mice can support studies in many areas of immunology, cancer…
The establishment and use of humanized animals require:
- Delivery of agreements and authorization from the Authorities for collection, storage and use of human tissues for scientific purposes,
- Access to a number of clinical centers through a Biological Resource Center to obtain human tissues in ethical and anonymity conditions and with associated clinical data, handling safety and traceability of samples,
- Approval of the research protocol by Ethical Committee on research animal care.

Therefore, mice reconstituted with human hematopoietic cells were developed directly to investigate the human immuno-hematopoietic system *in vivo*. To reach this goal, we are benefiting from the recent development and commercialization of new CIEA NOG® (NOD.Cg-PrkdcscidIl2rgtm1Sug/JicTac) knockout mice. As previously described (M. Ito *et al.*, Blood 2002), the NOG® mice have been shown to support human cell engraftment. We here describe the development of a human immune system in NOG® mice to study immune cell function.

Material and Methods: PBMCs

- Whole-body irradiation of female adult NOG® mice.
- IP injection of freshly prepared **PBMCs** (CD45+).
- Termination of mice when first clinical signs of Graft versus Host Disease (GvHD) appeared.
- Characterization of human leucocytes and activated T cells (CD45, CD8, CD4, CD25, CD134) by flow cytometry in blood and spleen.
- Calculation of human leucocytes and human T cells as absolute cell number and percentage.
- Quantification of human cytokines and human immunoglobulins using a multiplex assay system (Bio-Plex).

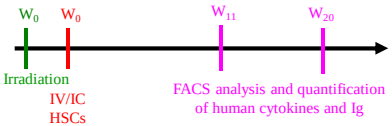
Study design for engraftment of mice with human PBMCs and evaluation of GvHD:



Material and Methods: HSCs

- Whole-body irradiation of adult/newborn NOG® mice.
- IV/IC injection of freshly prepared **CD3+ T cell-depleted HSCs** from umbilical cord blood (CD34+).
- Examination of hematopoietic chimerism in target tissues 11 and 20 week post-engraftment using flow cytometry.
- Calculation of human hematopoietic cells as absolute cell number and percentage.
- Quantification of human cytokines and human immunoglobulins using a multiplex assay system (Bio-Plex).

Study design for engraftment of mice with HSCs and evaluation of hematopoietic chimerism:

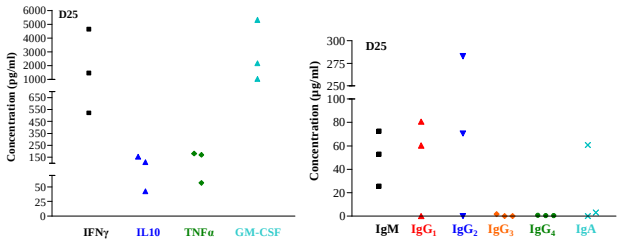


Results (PBMCs)

NOG® mice transplanted with $2x10^7$ PBMCs died within 23 ± 4 days (range: 18-28 days) after cell transfer. When NOG® mice received 10^7 PBMCs, the mice died between 48 ± 20 days (range D29 and D74).

The results evidenced the presence of human activated T lymphocytes in blood and spleen. The T human cells consisted mainly of CD3+ T lymphocytes (>90%) containing both CD4 and CD8 subsets (CD8+ cells at higher proportions than CD4+ cells).

D18		human Leucocytes	human T lymphocytes			Activated T lymphocytes				ratio CD4+/CD8+
		CD45+	CD3+	CD8+	CD4+	CD25+	CD134+	CD25+	CD134+	
		(% of live cells)	(% of CD45+)	(% of CD3+)		(% of CD8+)		(% of CD4+)		
Blood	mean	27.76	98.65	52.49	19.83	11.49	7.76	34.23	35.61	0.38
	SD	21.28	0.42	13.57	10.70	7.28	3.04	3.44	7.35	0.20
Spleen	mean	39.05	92.22	52.50	34.65	11.66	10.55	24.06	42.87	0.73
	SD	29.66	5.66	12.34	14.08	2.50	2.69	7.37	15.81	0.45

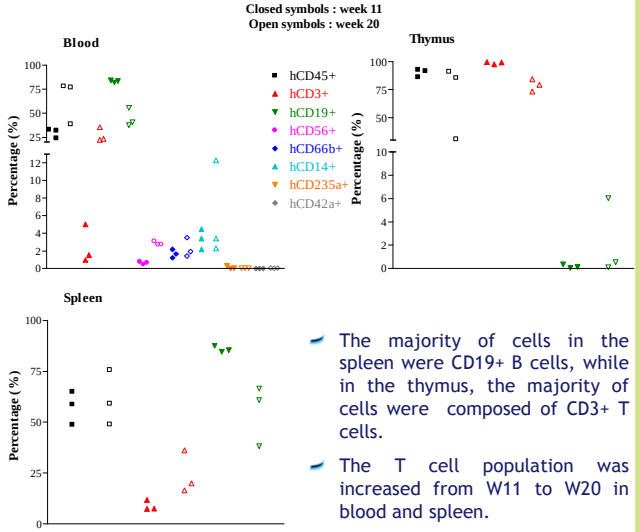


- Detection of IFN γ (T-helper 1) and IL10 (T-helper 2) as well as the cytokine GM-CSF and TNFα, involved in development of GvHD.
- Presence of plasma IgG and IgM suggesting that human B cells were fonctionnal for Ig secretion.

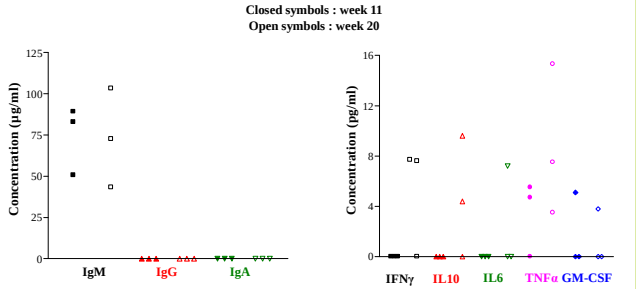
Conclusions and Perspectives

- PBMCs engrafted NOG® mice constitute a relevant model of GvHD associated with activation of T lymphocytes and cytokine release.
- HSCs engrafted NOG® mice leads to multi-lineage differentiation with presence of functional T-Helper (1-2) cells and activation of B lymphocytes.
- Functionality of human immune system could be completely demonstrated through antigen exposure and LPS response.
- Mice reconstituted with human HSCs are considered as a tool to investigate the human immune-hematopoietic system *in vivo*.

Results (HSCs)



- The majority of cells in the spleen were CD19+ B cells, while in the thymus, the majority of cells were composed of CD3+ T cells.
- The T cell population was increased from W11 to W20 in blood and spleen.
- Very low levels of CD45+ cells expressing markers of granulocytes, monocytes/macrophages and NK cells were detected and surprisingly circulating CD235a+ erythrocytes and CD42a+ platelets were not detected.



- High levels of plasma IgM were observed suggesting the activation of transferred naïve B cells.
- IFNγ (T-helper 1), IL10 (T-helper 2), inflammatory cytokine IL6, cytokine GM-CSF as well as TNFα were detected in humanized NOG® mice.