Evaluation of immune response in preclinical settings

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 # 5383

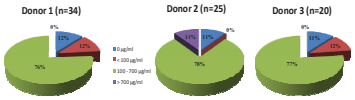
Introduction

Vaccines and other immunological modulators are highly promising approaches against cancer and many other diseases. They have the potential to activate the immune system to establish an active defense against pathological conditions (cancer cells, virus invasion, ...). In addition, antibodies, antibody fragments, and other biologics also can have a strong impact on the immune system which needs to be evaluated early on. In order to develop and accurately evaluate these immunology linked approaches, appropriate preclinical models with relevant immunological readouts are needed at different stages of therapy development. Ideally, methods should be available that allow predictive readouts in vitro, in vivo and ex vivo.

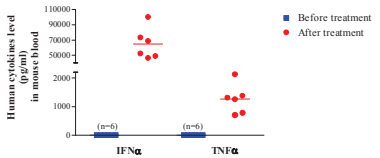
A comprehensive panel of tools was constructed and validated aimed at evaluating the modulation of the immune system by new therapies. In immunocompetent mice, immune cells were studied for their cell surface activation markers detection, induction of proliferative phenotype, antigen-specific T lymphocyte detection, secretion of soluble mediators such as cytokines, etc ... using FACS phenotyping, cytometric bead assay (CBA) or Luminex multiplex technologies and ELISPOT. In immunodeficient mice, the reconstitution of the mature human immune system with PBMC or naïve human immune system using hematopoietic stem cells were used to evaluate the modulation of those immune cells by therapies through human cytokine release and onset of graft versus host disease. Among other examples, characterization of antibody function (Fab and Fc mediated activities), quantification of the existence and function of antigen-specific T cell clones, phenotyping of immune cells for activation markers as well as multiplex assays to understand the cytokines network and differentiation of hematopoietic cells by colony formation unit will be described. All of these tools were analyzed in the context either of rodent syngenic models or humanized mouse models.

Readout in humanized mouse models

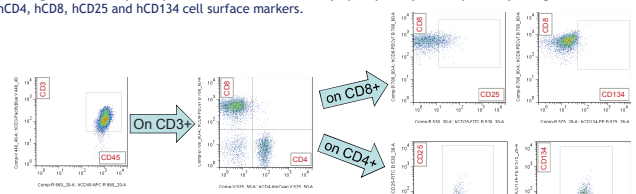
Irradiated SCID mice were engrafted with human PBMCs (three donors). Blood collection was performed 13 days post-engraftment to quantify total human IgG using ELISA assay. Human IgG level was used as a parameter to select humanized mice at the time of randomization.



Measurement of human cytokines level using Luminex analysis. Figure represents individual and mean level of IFNα and TNFα in plasma samples from NSG mice reconstituted with hematopoietic stem cells and receiving one injection of immunomodulator (IMM)12 weeks post-engraftment.



SCID mice were engrafted with human PBMCs after depletion of mouse immune system with whole-body irradiation and injection of mouse NK depleting antibody. Blood collection was performed 6 and 14 days post-engraftment to quantify total human activated T lymphocytes by flow cytometry using hCD45, hCD3, hCD4, hCD8, hCD25 and hCD134 cell surface markers.

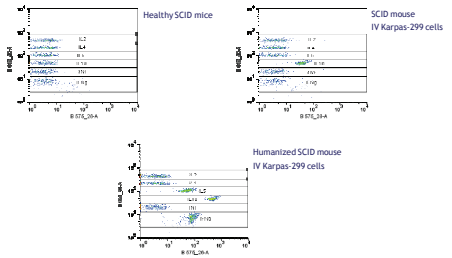


Graft *versus* host disease (GvHD) development was associated with the increased percentage of CD25+ and CD134+ cells among T cells as well as the decrease CD4+/CD8+ ratio.

Animals died within 2-3 weeks after engraftment with human PBMCs.

		hCD3+ (% of hCD45+)	hCD4+ (% of hCD3+)	hCD8+ (% of hCD3+)	ratio hCD4+/hCD8+	hCD25+ (% of hCD4+)	hCD134+ (% of hCD4+)	hCD25+ (% of hCD8+)	hCD134+ (% of hCD8+)
D6	mean	96.0	63.4	21.7	3.0	44.8	43.9	17.6	9.1
	SD	1.2	3.8	2.8	0.5	1.9	4.3	6.9	2.9
D14	mean	91.9	35.8	45.8	0.8	69.0	50.9	14.2	17.4
	SD	5.3	8.5	10.3	0.3	20.3	10.7	10.7	14.8

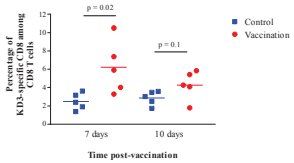
Adult NOD-SCID mice were IV injected with Karpas-299 human lymphoma cells and humanized with hPBMCs. Blood collection was performed to quantify release of human cytokines. Quantification was performed with CBA system using the following markers: hIL-2, hIL-4, hIL-5, hIL-10, hTNF and hIFNγ.



As known Karpas-299 tumor cell line expresses and secretes human IL10 cytokine. Human IL10 level was higher in mice engrafted with both tumor cells and hPBMC than in mice engrafted with only tumor cells. Moreover, secretion of human IL5 and IFNg was recorded only in mice engrafted with hPBMC.

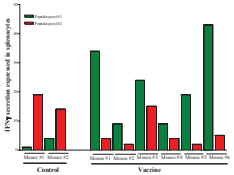
Readout in syngeneic models

Evaluation of specific T cell response to vaccination using FACS-Pentamer analysis. Figure represents individual and mean percentage of specific CD8+ cells in splenocyte samples from C57BL/6 mice receiving repeated injections of vaccine. Statistical analysis was performed using Bonferroni test.



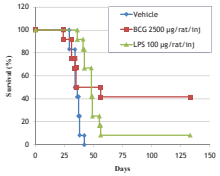
A significant level of specific CD8+ cells was detected in spleen from treated mice within 7-10 after vaccination.

Evaluation of T-cell mediated immune response against antigen-expressing syngeneic tumor grafted in mice treated with antigen-specific vaccine. Total splenocytes were restimulated with antigen-specific peptides divided in peptide-pool #1 and #2 and IFNγ secretion was monitored using ELISpot assay.



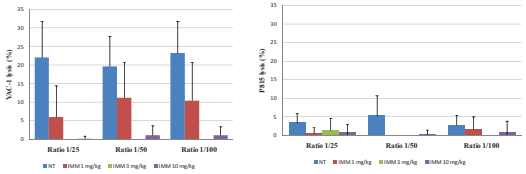
The results show that antigen-specific T cell mediated immune response could be detected in mice therapeutically vaccinated. Peptide-pool #2 was more potent than peptide-pool #1 at re-stimulating antigen-specific IFNγ producing cells.

Survival of BDIX rats bearing IP disseminated carcinomatosis of rat colon cancer after thrice weekly repeated IP injections of BCG at 2500 µg/rat/inj or LPS at 100 µg/rat/inj for 3 consecutive weeks starting at D10.



The median survival times of rats were increased by 34% and 37% for BCG (43 *vs* 35 days) and LPS (48 *vs* 35 days), respectively.

Ex vivo cytotoxic activity of mouse mononuclear spleen effector cells (mo-MSEC) against YAC-1 and P815 sensitive and resistant mouse target cells. The mo-MSEC were removed from mice after repeated daily injections of increasing doses of IMM.



IMM decreased significantly the natural cytotoxicity of mo-MSEC in a dose-dependant manner in both sensitive and resistant target cells.

Conclusions

- Novel therapeutic strategies are being developed that aim to implicate the immune system in the initiation, development and progression of tumors, by resetting or redirecting the immune effectors against tumor cells. These new strategies include vaccines, biologics, immunomodulators, antibodies...
- At the preclinical level, syngeneic rodent models are well described and allow evaluating various useful but rodent immunological readouts. However, there is a lack of relevance within these syngeneic models regarding human immune function as well as human tumor targets. Moreover, the therapeutic strategies are more and more human specific, increasing the needs for adapted preclinical models. Immunocompromised transgenic mouse strain humanized with human immune cells and bearing human tumors seem to be the appropriate preclinical tools to fill in the gap.
- Various examples of immunological readouts using either historical syngeneic models or newly established humanized mouse models are described here. The latter models are technically challenging as they require a P2 animal husbandry and ready access to human immune cells as well as human tumor samples. Our humanization was greatly improved and validated through numerous successful proof-of-concept studies and all logistical challenges were overcome (access to a P2 animal husbandry, approved biological resource center, strong network to collect fresh human samples).
- Nevertheless, there remain a number of biological challenges on which we are currently working to improve the use of a mouse host as a suitable environment for human immune cell function.
- In addition to their use in immune system modulating anti-cancer therapies, these humanized models also have a potential application in many other therapeutic areas such as autoimmune diseases, inflammation, anti-infectives... There is a huge interest of having a complete and functional human immune system within a mouse bearing a human tumor.