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Fc-competent cell depleting agents assessment in FcγR humanized and BRGSF-HIS models

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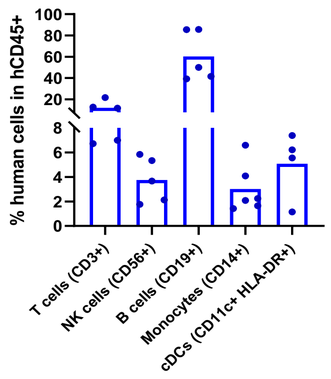
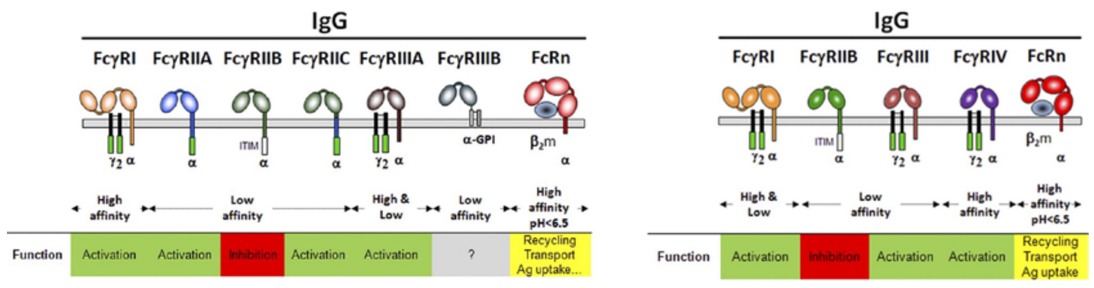
Background: Depletion of undesirable immune cells has shown to be a promising approach to treat cancer. B-cell depleting antibodies such as rituximab are often used to treat patients with lymphoma and was shown to improve patients' survival. Cell depletion can be triggered by antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC). ADCP and ADCC activities are mediated by Fcγ receptors (FcγR) expressed on immune cells such as natural killer (NK) and macrophages. Translatable assessment of cell-depleting agents in preclinical models requires the presence of functional immune cells and expression of FcγR receptors. Importantly, FcγR expression in mouse and human are distinct, thus increasing the complexity of depleting-antibodies assessment studies in preclinical models. Herein, we described two preclinical humanized models for assessment of cell-depleting agents.

1. BRGSF-HIS model features

- BRGSF (Balb/C Rag2$^{-/-}$ IL2Rγ$^{-/-}$, SIRPα^{NOD} and Flt3$^{-/-}$):
  - Highly immunodeficient with reduced murine myeloid cells
  - Normal life span (no anemia, no weight loss, normal fur texture & integrity, normal activity & posture)
- Upon hCD34^{+} cell injection:
  - Lymphoid and myeloid compartment development
  - Boost by hFlt3L enhances human myeloid cell development and accumulation



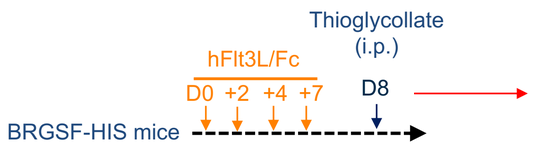
1. Humanized FcγR model features



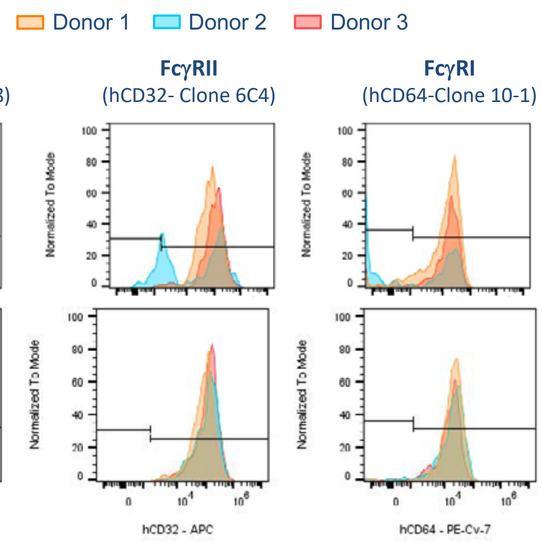
From: Properties of mouse and human IgG receptors and their contribution to disease models. P. Bruhns, Blood, 2012

- FcγR are different in mouse and human
- FcγR feature distinct activities and expression pattern
- No expression of mouse FcγR in FcγR-humanized mice
- FcγR humanized mouse model expresses human FcγR with a "human-like" distribution

2. FcγR expression in Flt3L-boosted BRGSF-HIS mice

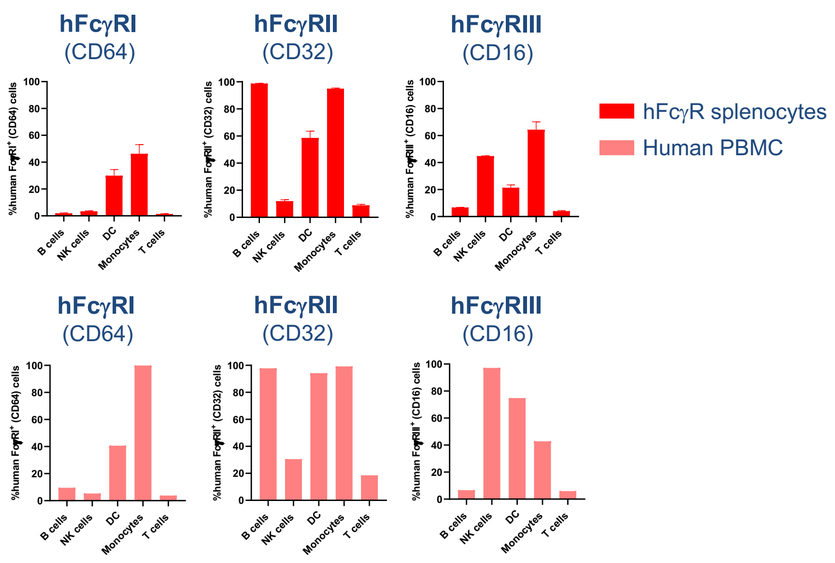


Analysis of FcγR expression on:
- Thioglycollate-elicited peritoneal macrophages incubated for 3 days with M-CSF
- Bone marrow-derived macrophages



- FcγRI, II and III are expressed on peritoneal macrophages and BMDM

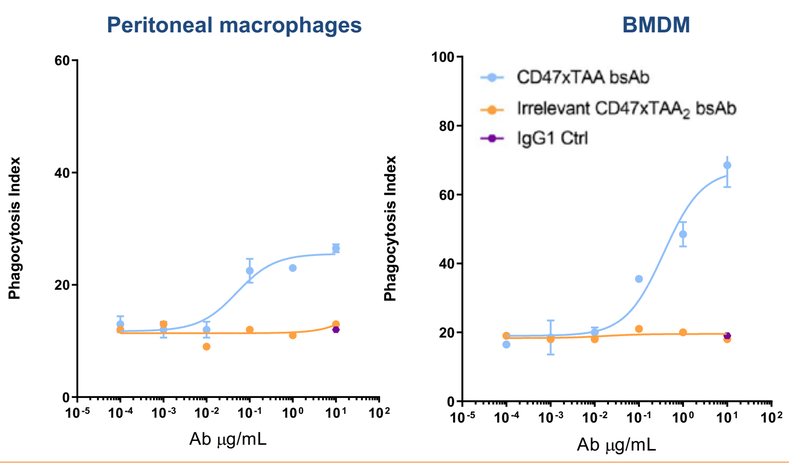
2. Similar expression of FcγR in hFcγR mice and human PBMC



- In hFcγR mice and human PBMC, the FcγR are expressed on the same populations

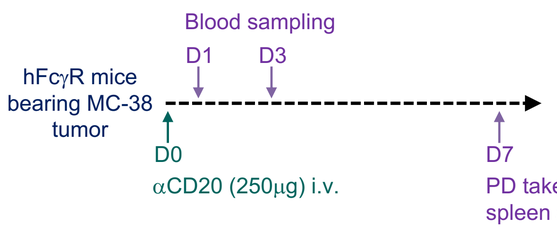
3. *Ex vivo* ADCP induction using CD47-TAA BsAb

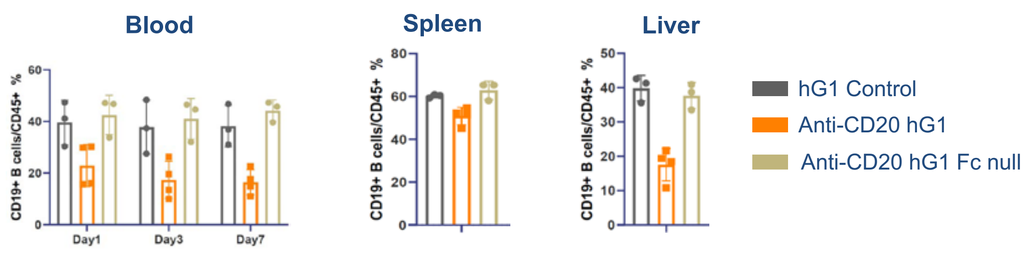
- BRGSF-HIS mice were boosted with Flt3L and thioglycolate-elicited peritoneal macrophages and bone marrow-derived macrophages were incubated with CD47-TAA BsAb and TAA-expressing MKN45 cells for 2.5h
- Phagocytic index was determined by fluorescence *(average number of target cells engulfed by 100 macrophages)*



- Human macrophages phagocyte target cells when incubated with anti CD47-TAA bsAb

3. Anti-CD20 induced depletion of B cells in hFcγR mice

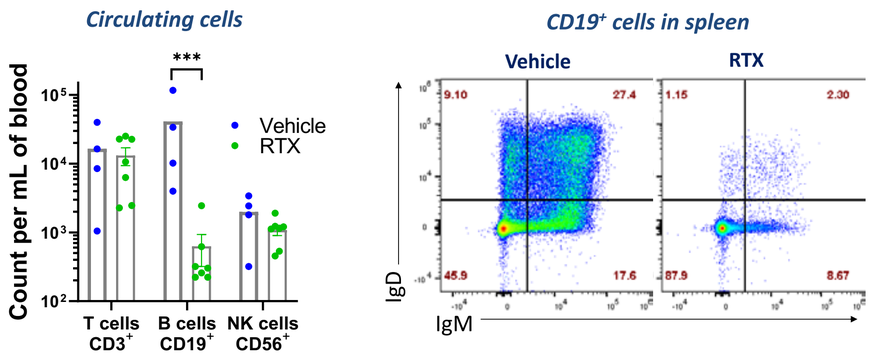




- B-cell depletion induced by anti-CD20 antibody is dependent on Fc effector function

4. FcγR-mediated function in BRGSF-HIS mice

- Rituximab (RTX): anti-CD20 mAb (IgG1) which binds via FcγR (mostly FcγRIIa and FcγRIIIa) and triggers CDC, ADCC and ADCP dependent mechanisms
  (Teige *et al.*, Front. Immunol., 2019)
- B-cell depletion was assessed in Flt3L-boosted BRGSF-HIS mice 48h post injection with RTX 30mg/kg (i.v)



- RTX induces circulating mature B-cell depletion, suggesting FcγR-mediated monocytes and NK cell cytotoxicity

4. Next generation of the FcγR humanized model

- **FcγR/hIgG1**: to enable tolerability to human IgG1 therapeutic antibodies through expression of human IgG1
- **FcγR/hSA/hFcRn**: to improve PK translatability of therapeutics with enhanced half-life through binding to human serum albumin or human FcRn
- **FcγR/CD71 (TFRC)**: better biodistribution of therapeutics to the brain by enabling drug shuttling through the blood–brain barrier
- Intercrossing with different immune checkpoint humanized models: assessment of bispecific or combo therapies with competent Fc, targeting immune checkpoints

Conclusion: Antibody-mediated depletion of immune cells is achieved in the two humanized models described here, confirming their value to assess efficacy of depleting agents. Although the fully functional immune system and the expression of humanized FcγR on stromal cells is an advantage of the hFcγR model, assessment of mouse biology is a drawback. BRGSF-HIS mice could be used to assess depleting agents in the presence of human immune cells.

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