Optimization of engraftment of human NK cells in NOD-SCID mice

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Background

- Successful engraftment of human (hu) peripheral blood mononuclear cells (PBMCs) into mice with severe combined immune deficiency (SCID) would enable evaluation of the efficacy of human IgG1 antibodies in ablating tumour growth by antibody-dependent cell cytotoxicity (ADCC).
- NK cells are a key effector population for ADCC elicited by human IgG1 antibodies and have been shown to play a major role in the ADCC activity mediated by hu PBMCs. NK cell interaction with human IgG1 molecules is mediated through binding to the FcγRIII receptor.
- The aim of this study was to establish optimal conditions for the engraftment of FcγRIII (CD16) positive hu NK cells in non-obese diabetic (NOD) SCID mice. The effect of whole body irradiation in combination with a murine NK cell-depleting antibody was examined and the engraftment monitored by determining the absolute number of hu NK cells in three organs/body compartments at two time-points.

Methods

- NOD-SCID mice were whole body irradiated (1.8 Gy) on day 0, treated with a mouse NK cell-depleting antibody (TMβ-1) 3 days later (day 3) and inoculated intraperitoneally (IP) with hu PBMCs the following day (day 4). A non-irradiated group also received antibody and PBMCs.
- Three healthy donors of PBMCs were selected based upon the percentage of CD45+CD3-CD56+CD16+ NK cells determined by flow cytometry. Cells from each donor were inoculated into both irradiated and non-irradiated groups.
- The engraftment of human NK cells in the peritoneal cavity, blood and spleen was analysed on both day 18 and 25 post-irradiation using 4-color flow cytometry (CD45, CD3, CD56 and CD16) with absolute cell numbers calculated per organ.

Group	Mice per group	Treatment	TMβ-1 Ab (dose, injection)	hu PBMC (donor number, injection)	Endpoint
1	6	Whole body irradiation (1.8 Gy)	34.8 mg/Kg, subcutaneous	D1, IP	Day 18
2				D2, IP	
3				D3, IP	
4				D1, IP	Day 25
5				D2, IP	
6				D3, IP	
7		Non-irradiated		D1, IP	Day 18
8				D2, IP	
9				D3, IP	
10				D1, IP	Day 25
11				D2, IP	
12				D3, IP	
Control	2	Whole body irradiation (1.8 Gy)		-	Day 18
	2	Non-irradiated			
	1	Whole body irradiation (1.8 Gy)			Day 25
	1	Non-irradiated			

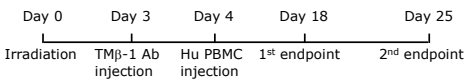

Figure 1 Study plan.

Description	Format
hu CD3	PerCP
hu CD16	FITC
hu CD56	PE
hu CD45	APC-Cy7

- PKH26 Reference Microbeads (Sigma) were also used to permit calculation of absolute cell counts.

Figure 2 4-color panel of Abs used for the analysis of hu NK cells by flow cytometry.

Donor	% of hu NK cells (CD45+CD3-CD56+CD16+)	Number of hu PBMC injected per mouse ($x10^6$)	Equivalent number of hu NK cells injected per mouse ($x10^6$)
D1	18.9	30	5.7
D2	10.2	30	3.1
D3	7.1	30	2.1

Figure 3 The three donors of PBMCs were selected based upon the percentage of NK cells.

Results

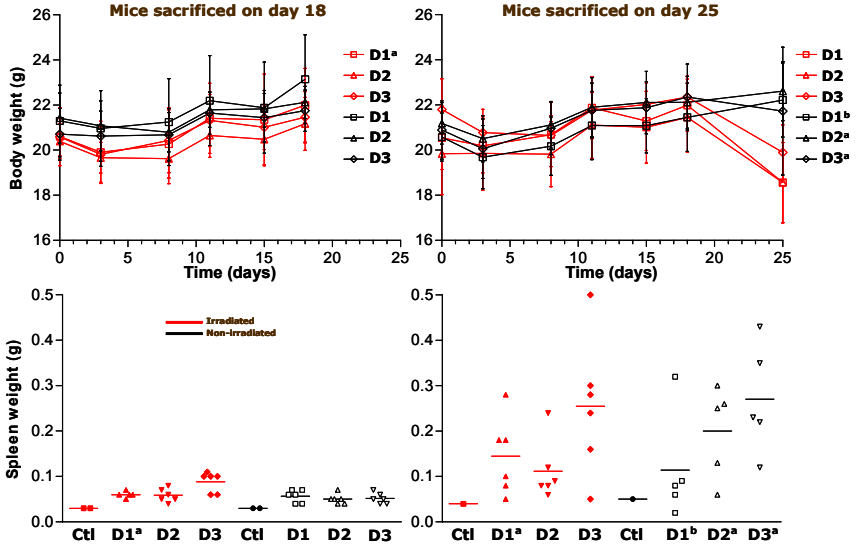

Figure 4 Body weight and spleen weight of irradiated (red) and non-irradiated (black) mice injected with hu PBMC from 3 different donors (D1, D2 and D3) and sacrificed at day 18 or day 25 after irradiation. Ctl: control mice that have not been injected with hu PBMC. [a]One dead mouse per group. [b]One mouse discarded per group.

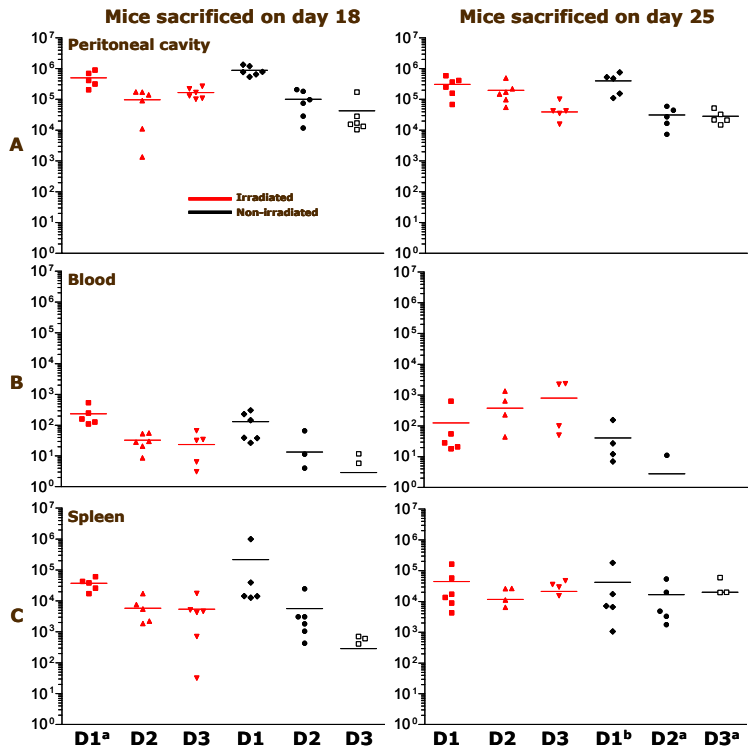

Figure 5 Absolute numbers of CD45+CD3-CD56+CD16+ hu NK cells recovered per peritoneal cavity (A), per 100µL blood (B) or per spleen (C) from irradiated (red) or non-irradiated (black) mice injected with hu PBMC from 3 different donors (D1, D2 and D3) and sacrificed at day 18 or day 25 after irradiation. [a]One dead mouse per group. [b]One mouse discarded per group.

Conclusions

- In the absence of irradiation, the numbers of NK cells from blood donors was a key factor for the engraftment: a trend between the number of NK cells injected at Day 4 and the number of NK cells recovered at Day 18/Day 25 was observed.
- Pre-treatment with irradiation resulted in higher numbers of NK cells recovered from peritoneum, blood and spleen, especially in mice injected with hu PBMC from donors with lower % of NK cells.
- The severe body weight loss in the irradiated group may indicate earlier development of graft-versus-host disease compared to the non-irradiated group.
- The increase in the weight of spleen may indicate accumulation of human cells at this site.