Radiolabeling of p-Bz-DOTA-CD-11c antibody with ^{88}Y: Conjugation, Labeling, Biodistribution studies

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Graft versus host desease, an alloimmune attack on host tissues mounted by donor T cells, is the most important toxicity of allogeneic bone marrow transplantation.
T cell responses are intitiated on antigen presenting cells. In allogeneic bone marrow transplantation (APC) the unusual situation arises in wich both host- and donor derived antigen-presenting cells are present. The suggestion was that depleting host APCs should abrogate graft versus host desease without the need for prolonged T cell-targeted immunosuppression.
Supporting a antibody-mediated APC depletion a radiolabelled anti-CD-11c antibody wich bind to all CD11c-expressing dendritic cell in both lymph nodes and spleen is discussed.
In this study we have evaluated the biodistribution of ^{88}Y-DOTA-anti-CD11c-antibody in black mice particularly to estimate accumulations in spleen and lymph nodes (1).

Ligand preparation:
p-NH_2-Bz-DOTA was synthesized by reduction of p-NO_2-Bz-DOTA with Pd/C and H_2 for 3 h in a MeOH/H_2O mixture. The amino derivative was then allowed to react with thiophosgene in 3 N HCl-solution.
This solution was extracted 4 times with $CHCl_3$, evaporated to dryness, dissolved in water and lyophilized.
Prior coupling the p-SCN-Bz-DOTA was dissolved in 1 ml 0,1 N HCl.

Conjugation procedure:
1,5 ml of a solution containing 1,94 mg/ ml CD-11c was purified 3 times with 0,15 M Na_2HPO_4 using microcentrifuge filters (MWCO 20000) and finally dissolved in 500 µl of this buffer.
2 ml 0.1 M $NaHCO_3$ and 5,6 mg p-SCN-Bz-DOTA in 280 µl 0,1.N HCL was added. After 3 hours this solution was washed 3 times with 0,1 M NH_4OAc buffer using the filters mentioned above.
Finally the conjugate was dissolved in 500 µl 0,1 M NH_4OAc.
A UV-spectrometric calibration (280 nm) gave a total yield of 1,547 mg DOTA-CD-11c.

Labeling procedure:
^{88}Y (500 µCi; 0,4 M HCl) was evaporated to dryness and dissolved in 0,5 ml 0,1 N HCl. The solution was purified on an ion-exchange resin. The ^{88}Y-fraction (2 M HCl) was evaporated to dryness and dissolved in 20 µl 0,01 N HCl.
The antibody solution and the yttrium solution were combined and stored at room temperature.
After 18 hours a quality control was performed (TLC Si60, 5x7,5 cm, 0.1 M Na_3Cit) indicating a quantitative yield (Fig1).

Mouse experiment:
Prior injection the final solution was adjusted to a volume of 3,5 ml using 0.9% NaCl.
150 µl of this solution (66 µg CD-11c; 15,5 µCi Y-88) were injected in each B6-mouse (8 weeks old). The animals were dissected after 24 h, 96 h and 1 week (168 h).

Each organ was weighted and measured 10 minutes using a auto-gamma counter. Couting yields were sufficient in all experiments. The resulting percent injected dose per gramme (%iD/g) for the organs of interest is shown in table 1.

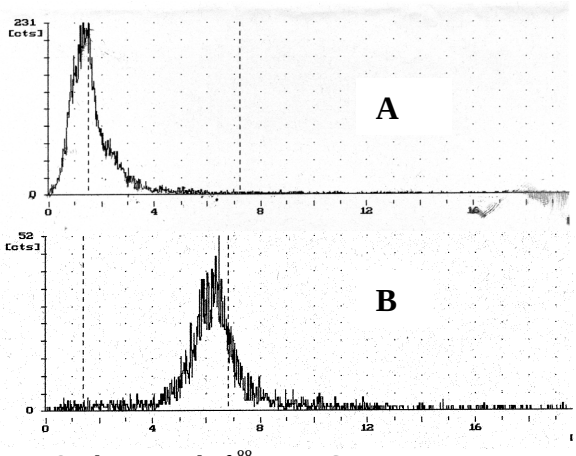

Fig. 1: Quality control of ^{88}Y-anti-CD11
A: Reaction mixture; B: free ^{88}Y

Table1: biodistribution data for ^{88}Y-DOTA-anti-CD11c in black B6 mice (N=3-5) (deviations in %)

time →	24 h		96 h		168 h	
organ ↓	%iD/g	+/-	%iD/g	+/-	%iD/g	+/-
blood	11,3	1,1	4,6	3,7	0,1	0,0
lung	6,5	0,2	5,1	1,3	1,6	0,3
spleen	36,4	2,5	70,9	11,8	30,9	4,4
skin	5,2	0,5	5,5	1,5	1,3	0,1
bone marrow	41,0	18,8	39,1	8,1	30,4	14,5
bone	5,5	0,7	8,1	2,1	6,9	0,9
lymph nodes	25,2	14,8	39,8	10,7	25,5	3,7
liver	15,0	1,5	16,0	4,9	13,3	2,4

Results:
Conjugation of the anti-CD11c-antibody was performed with high purity making a quantitative radiolabeling with ^{88}Y possible in less than 16 hours even at room temperature.
Because impurities of commercial available ^{88}Y-batches often prevent those high labeling yields, a purification by ion-exchange chromatography is recommended.
Considering the immunoreactivity of the antibody in respect to the labeling protocol, an accumulation of the labeled compound in organs with cells expressing CD-11c, i.e. lymph nodes, spleen and bone marrow, was expected.
As a proof of concept the biodistribution data indicate high accumulation of the labeled antibody in the mentioned organs.

(1) Schlomchik W.D., Couzens M.S., Tang C.B., McNiff J., Robert M.E., Liu J., Schlomchik M.J., Emerson S.G., Science 285 (1999) 411-415