Induction of cutaneous inflammation in normal Balb/c mice by human T cells

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Introduction

Current animal models to study the mechanisms of human T cell migration into cutaneous sites involve immunodeficient mice grafted with human skin (humanized mouse models), however those methods are expensive and time consuming. Most relevant human molecules involved in skin migration (CLA, VLA-4, LFA-1 and CCR10) crossreact with their mouse ligands. For this reason, we hypothesized whether it would be possible to develop a model where non-sensitizied normal Balb/c mice are injected i.v. with the human T cell line Hut-78 CLA$^+$ CCR10 transfected to induce ear inflammation.

Material and Methods

• Human CCR10 cloning, transfection and selection of CLA$^+$CCR10$^+$ and CLA$^-$CCR10$^+$ Hut-78 T-cell clones. Full length human CCR10 (AF215981) was amplified from cDNA of peripheral blood T cells using specific primers. Human CCR10 was cloned into the pCI-neo vector. Transfectans underwent selection in G418 and were further enriched/cloned from transmigrated cells in chemotaxis assays with mouse CTACK. Hut-78 CLA$^+$ cells CLA$^-$ T cells lines were generated as in (Santamaria-Babi et al, 1996).

• Hut-78 cell induced murine cutaneous inflammation. Male Balb/c (28-30 gr) were obtained from Harlam. 1% DNFB in acetone is applied topically on the right ear (10µl), acetone is applied on the left, and 3x10^5 Hut-78 T cells are injected intravenously in the tail vein in PBS 100µl. Net ear swelling in each mouse is calculated by weight subtraction between right and left ear (7mm diameter skin punch) after 24h of cells injection. A 7mm diameter skin punch was taken 24h after injection. Statistics were calculated by student´s T test. Data are presented as mean ± SD.

Results

As it is shown in **Figure 1**, simultaneous i.v. injection of Hut-78 CLA$^+$CCR10$^+$ cells together with topical 1% DNFB significantly induces higher neto ear weight than applying alone DNFB or Hut-78 CLA$^+$CCR10$^+$ T cells. DNFB-treated and Hut-78 CLA$^+$CCR10$^+$ T cells injected mice presented a net ear weight of 18,4 ± 4,7 mg. and DNFB-treated mice without human T cells of 7,3 ± 3,2 mg.

Hut-78 cells expressing both CLA and human CCR10 induced significantly the highest net weight in the ear (**Figure 2**), and skin inflammation depended on the number of i.v. administered cells and on the percentage of DNFB topically applied (**Figure 3A** and **3B**).

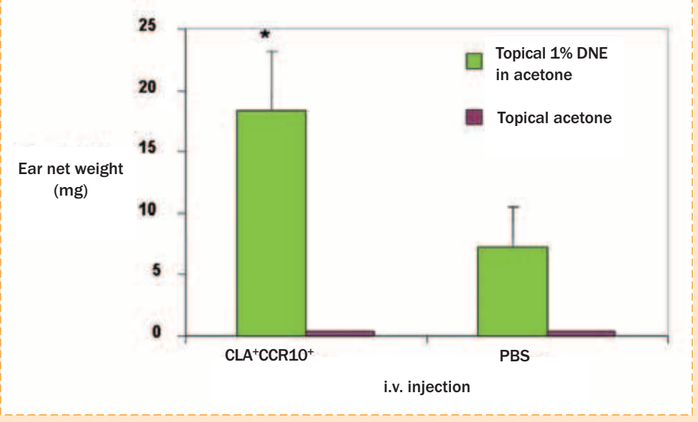
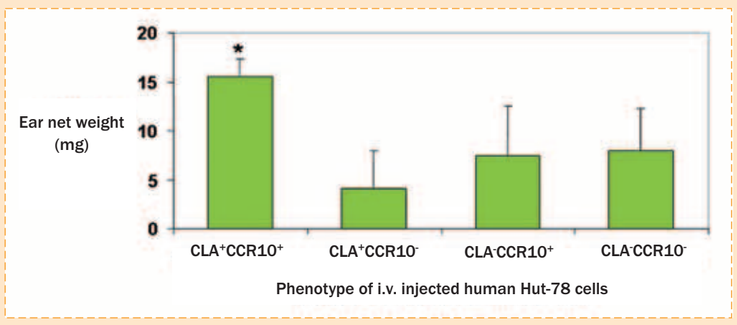


FIG. 1: Human Hut-78 CLA$^+$CCR10$^+$ human cells induce non-specific ear inflammation in non-sensitized Balb/c mice with topical DNFB (1%). Balb/c mice receiving i.v Hut-78 CLA$^+$CCR10$^+$ and 1% DNFB on the ear develop cutaneous inflammation. Results from 52 independent experiments with 3-5 mouse per group (*p<0,0001).

FIG. 2: Human Hut-78 expressing CLA and CCR10 induces higher inflammation than CLA$^+$CCR10, CLA$^-$CCR10$^+$ or CLA$^-$CCR10$^-$ cells. Balb/c mice received i.v. 4 different types of Hut-78 T cells. Results from 2 indepent experiments with 3 mouse per group (*p<0,05).



T cell tracking dye experiments with Hut-78 CLA$^+$CCR10$^+$ cells indicated the presence of fluorescence only in the inflamed right ear (55 positive cells per 60.000 total cutaneous cells), **Fig. 4A**. Histology clearly indicated signs of inflammation only in the right ears of animals injected with Hut-78 CLA$^+$CCR10$^+$ and topical DNFB (1%), **Fig 4B**. Finally, cutaneous inflammation induced by CLA$^+$ Hut-78 T cells could be inhibited by the previous *in vitro* incubation of cells with PTX or neuraminidase, **Fig. 5A** and **5B**.

FIG. 4A

	DNFB		DNFB & Hut-78 CLA$^+$CC10$^+$	
Injected cells	-		3x10^6	
Ear	Left	Rigth	Left	Right
Ear weight (mg)	15.1 ± 1.5	29.4 ± 1.5	14.8 ± 1.9	42.9 ± 1.9
Number of CFDA+ HECA-452+ cells in dermis	Not detected	Not detected	Not detected	55/60.000

FIG. 4B

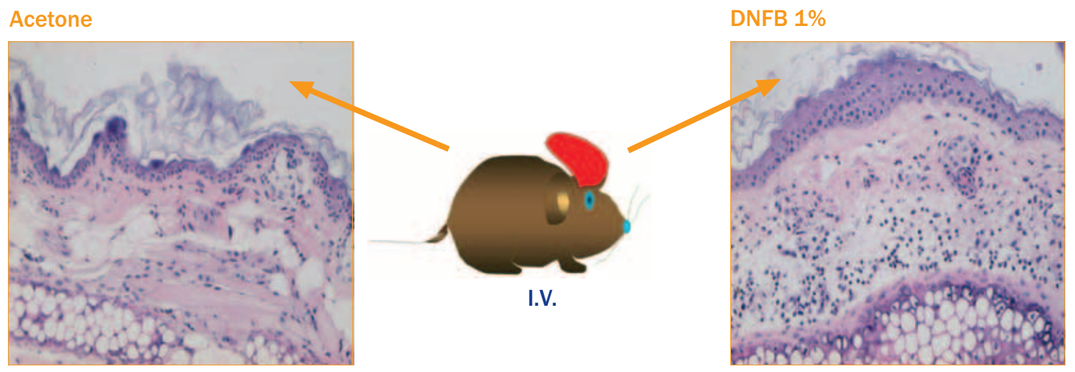


FIG. 4: Tracking dye experiment with CLA$^+$CCR10$^+$ Hut-78 cells and histology. Tracking dye experiments (Fig. 4A) from a representative experiment with 3 animals/group. 3x10^6 Hut-78 CLA$^+$CCR10$^+$ cells were stained with CFDA-SE dye and injected i.v. and ear samples were obtained at 6h hours after. Dermis was obtained after treating ears with dispase solution. In order to obtain a cell suspension, dermis were treated with an enzymatic cocktail of collagenase and DNase. Resulting cells were stained with HECA-452-PerCP. Histological pictures from mouse ears (**Fig. 4B**) correspond to a mice receiving topical 1% DNFB and CLA$^+$CCR10$^+$ Hut-78 cells i.v.

FIG. 3A

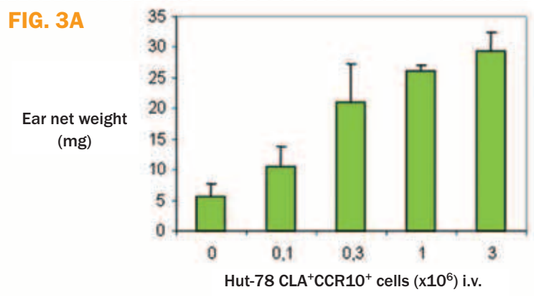


FIG. 3B

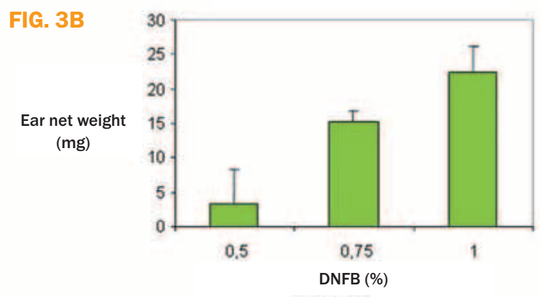


FIG. 3: CLA$^+$CCR10$^+$ Hut-78 cells induced cutaneous inflammation in Balb/c mouse depends on number of cells injected and percentage of DNFB applied. Balb/c mice received different amount of CLA$^+$CCR10$^+$ Hut-78 cells with DNFB 1%, **Fig. 3A**, or mice received 0.3x10^{-6} CLA$^+$CCR10$^+$ Hut-78 i.v. together with different percentage of DNFB, **Fig. 3B**. Results from 2 independent experiments with 3 mouse per group.

FIG. 5A

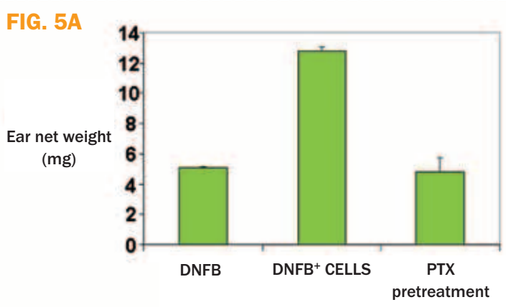


FIG. 5B

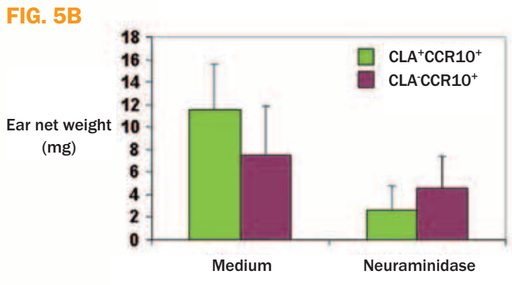


FIG. 5: *In vitro* treatment of Hut-78 CLA$^+$CCR10$^+$ T cells with Pertusis toxin (PTX) or Neuraminidase inhibit cutaneous inflammation. In the PTX experiment (**Fig. 5A**). Hut-78 CLA$^+$CCR10$^+$ T cells were incubated with 100 ng/ml of PTX for 2h at 37°C, washed and administered following standard procedure. Results are presented from a representative experiment with 3 animals/group. For the neuraminidase experiments (**Fig. 5B**), cells were incubated with 0.1U/ml of neuraminidase for 30 min at 25°C , washed and injected. The CLA antigen was eliminated by neuraminidase as it was assessed by flow cytometry.

Conclusions

Application of topical DNFB in non-sensitized Balb/c animals with circulating human Hut-78 CLA$^+$CCR10$^+$ provokes cutaneous inflammation. Such reaction is higher with cells expressing both CLA antigen and transfected human CCR10 and depends on number of i.v. injected cells, concentration of DNFB, G-protein couple receptors and a neuraminidase-sensitive carbohydrate structure.

Our data indicate that this novel animal model may be useful to study the mechanisms of human CLA$^+$ T cells migration to skin *in vivo*.

References

Santamaria Babi LF, Moser B, Perez Soler MT, Moser R, Loetscher P, Villiger B, Blaser K, Hauser C. The interleukin-8 receptor B and CXC chemokines can mediate transendothelial migration of human skin homing T cells. Eur J Immunol. 26(9):2056-61 (1996).