In vivo model to study migration of circulating human Sezary cells Hut-78 ($CLA^+CD4^+CCR10^+$) T cells into mouse skin

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Introduction

Cutaneous T-cell lymphoma originates from a clonal proliferation of $CD4^+CLA^+$ memory T-cells. Little is known about the factors that drive a mature T-cell clone to accumulate in the skin. At the moment there is a need of *in vivo* models to study the migration of those tumor cells into skin.

Material and methods

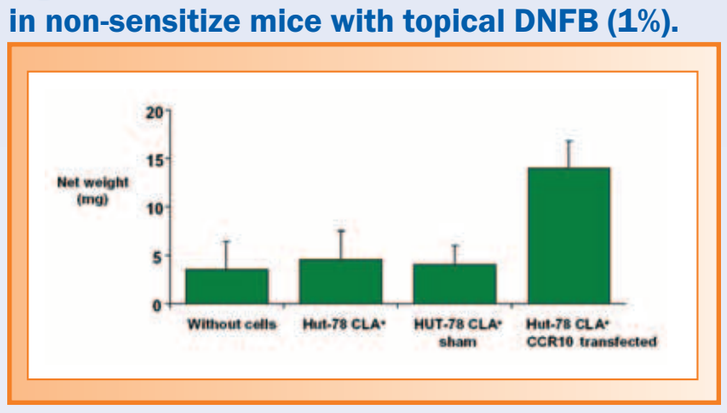
The Hut-78 is a human Sezary syndrome cell line expressing low levels of CLA and poor chemotaxis to CCR10 ligands. The line was enriched for cells line stably expressing the CLA antigen and transfected with the human CCR10.

Selection of Hut-78 CLA^+ cells and CCR10 transfection: CLA^+Hut-78 cells were generated by sorting for CLA^+ T cells in the Hut-78 cell line. Full length human CCR10 (AF215981) was amplified from cDNA of peripheral blood T cell using specific primers. Human CCR10 was cloned into the pCI-neo vector. CLA^+ Hut-78 cells transfectants underwent selection in G418 and were further enriched from transmigrated cells in chemotaxis assays with mouse CTACK/CCL27.
Hut-78 CLA^+CCR10^+ induced cutaneous inflammation: Female Balb/ mouse (28-30gr) were obtained from Harlam. 1% DNFB in acetone is applied topically on the right ear (10 μl), acetone is applied on the left and Hut-78CLA^+CCR10^+ are injected i.v. in the tail vein in 200 μl PBS. Net ear swelling in each mouse is calculated by weigth subtraction between right and left ear (7mm diameter skin punch) after 24h of cell injection. Statistics were calculated by student's T test. Data are presented as mean ± sd.

Results

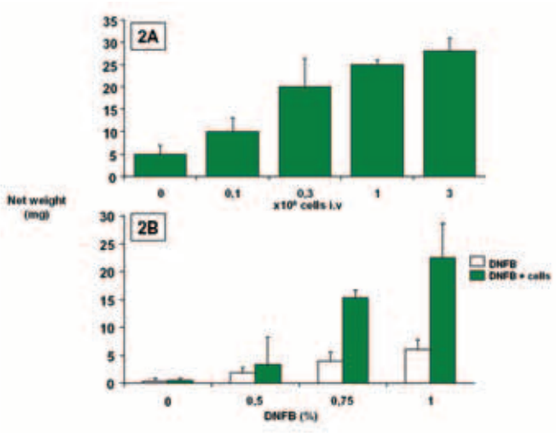
Simultaneous i.v. injection of Hut-78 CLA^+CCR10^+ cells together with topical DNFB 1% induces higher net ear weight than injecting sham-transfected CLA+T cells or parental Hut-78 cell line (**Figure 1**). Ear inflammation depended on the number of Hut-78 CLA^+ $CCR10^+$ injected, concentration of DNFB applied (**Figure 2**), G-protein coupled receptor (Pertusis Toxin), CLA (neuraminidase) and CCL27 (**Figure 3**). Fluorescence dye-labelled cells (Hut-78 CLA+ CCR10+ or peripheral $CD4^+$ $CD45RO^+$ cells from a Sezary Syndrome patient) could be detected by in vivo imaging (IVIS) on right ear 24h after injection. One month after a single injection of cells clear infiltration of abnormal cells, extensive angiogenesis and epidermal hyperplasia was more pronounced on right ear (DNFB) than in left ear (acetone), **Figures 4** and **5**.

Figure 1. Human Hut-78 CLA^+CCR10^+ cells induce non-specific ear inflammation in non-sensitize mice with topical DNFB (1%).



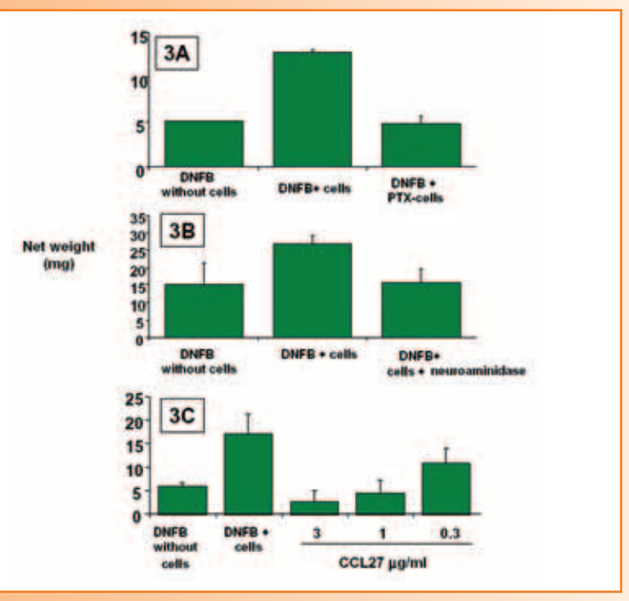
Mice were injected with $3x10^5$ cells i.v. and ear punches were taken after 24h. Four independent experiments with animals/ condition (p=0.02)

Figure 2. Hut-78 CLA^+CCR10^+ cells induced non-specific ear inflammation in non-sensitize mice by DNFB depending on cell number and DNFB concentration



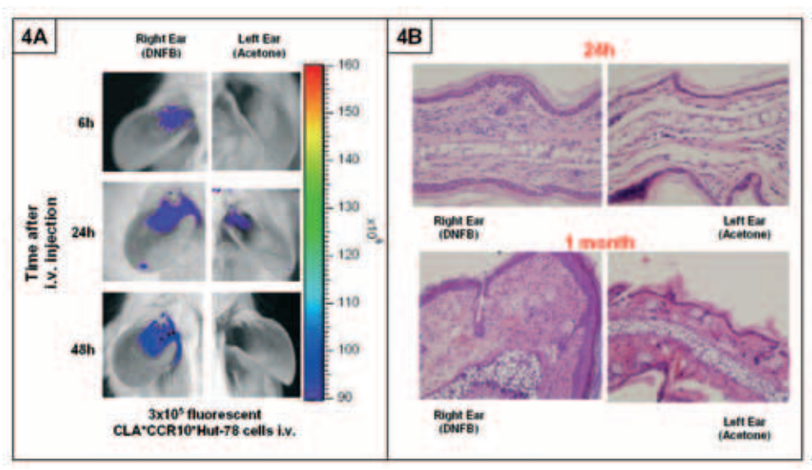
Balb/c mice received different amounts of Hut-78 CLA^+CCR10^+ cells with 1% DNFB (**Fig 2A**), or were injected with $3x10^5$ cells i.v. together with different concentration of DNFB (**Fig 2B**). Results from two independent experiments using 3 mice/condition.

Figure 3. In vitro treatment of Hut-78 CLA^+CCR10^+ cells with pertusis toxin PTX), neuraminidase or CCL27, inhibit cutaneous inflammation



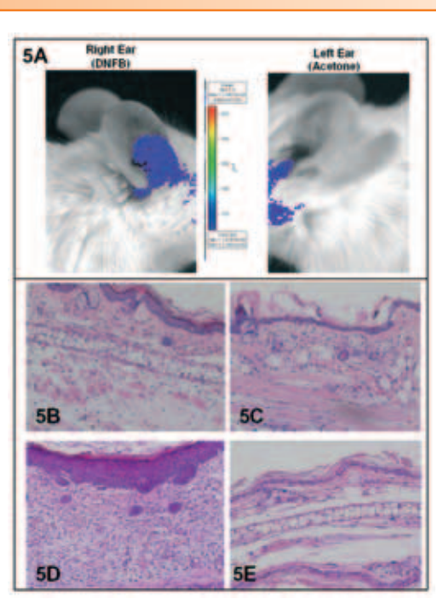
Cells were preincubated with 100 ng/ml of PTX for 2h (**Fig 3A**), 0.1U/ml of neuraminidase for 30 min (**Fig 3B**), or with different concentration of mouse CCL27 for 15 at 37 °C (**Fig 3C**). Washed cells were injected and DNFB was applied

Figure 4. In vivo imaging (IVIS) and histology after iv injection of Hut-78 CLA^+CCR10^+



Cells fluorescently dyed can be detected in right ear at different times after iv. Injection, **Fig 4A**. Histologies performed 24h, and one month after single injection of $3x10^5$ cells and a single DNFB application, **Fig 4B**

Figure 5. In vivo imaging (IVIS) and histology after iv injection of circulating memory T cells from a Sezary Syndrome patient



Cells fluorescently dyed can be detected in right ear at different times after iv injection. Histologies performed 24h (5B, 1% DNFB; 5C acetone) and one month (5D, 1% DNFB; 5E, acetone) after single injection of $3x10^5$ cells and a single DNFB application.

Conclusions

Our data suggest that this novel animal model may be useful to study the molecular mechanisms of migration to skin, and survival of the human HUT-78, CD4+ memory cells from Sezary Syndrome patients *in vivo*.

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