Streptococcal extract-induced activation of CLA+ T cells and epidermal cells coculture in psoriasis: gene and protein expression and in vivo epidermal hyperplasia induction

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Introduction

Psoriasis onset and exacerbations are associated with streptococcal throat infections. Up to now, it has not been clarified whether Streptococcal A (StrepA) extract can induce T-cell dependent keratinocyte activation, Th17/Th22 cytokine production and in vivo epidermal hyperplasia in psoriasis. We have generated a coculture system comprising circulating CLA+/CLA- memory T cells and autologous epidermal cells to test streptococcal extract activation activity in patients with psoriasis and healthy individuals.

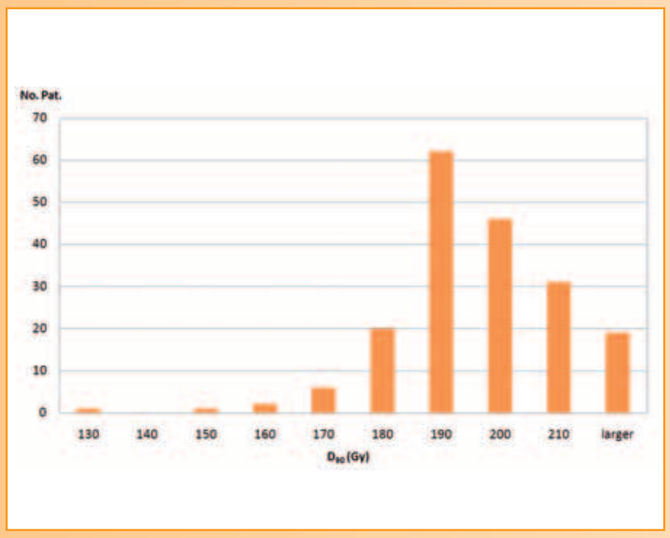
Material and methods

15 non-treated moderate-to-severe psoriasis patients and 9 healthy controls were enrolled in the study after giving written informed consent. A blood analysis and a skin biopsy were performed. CLA+/CLA- CD45RO+CD3+ were isolated by immunomagnetic separation from peripheral blood and epidermal cell suspensions were obtained from dispase/tryptase treatment of skin punch biopsies.

Results

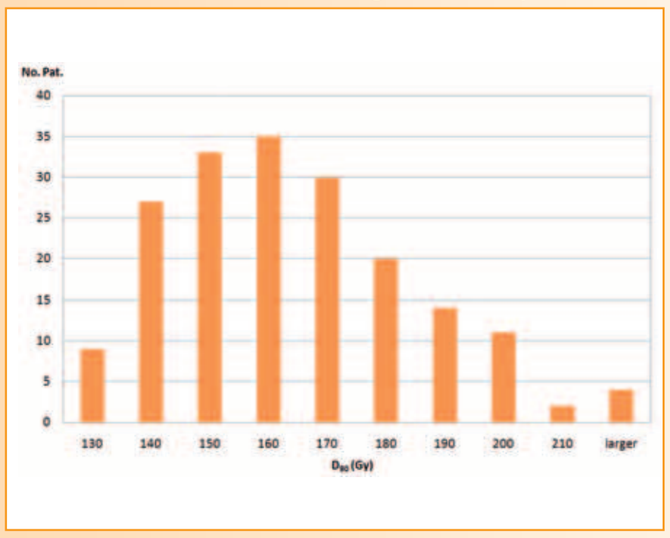
The results show for the first time that streptococcal extract induces a strong activation of the autologous coculture of circulating CLA+ T cells together with epidermal cells in cells from psoriatic patients.

FIGURE 1A. StrepA extract preferentially induces activation of cocultured CLA+ T cells and autologous lesional epidermal cells.



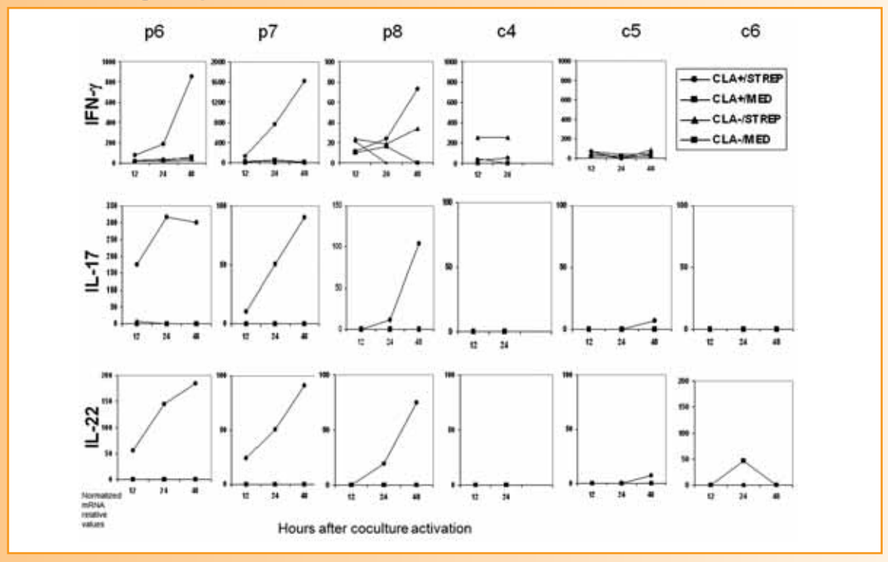
Coculture of freshly isolated circulating CLA+/CLA- CD45RO+CD3+ cells and autologous epidermal cells obtained from lesional psoriatic skin were incubated w/o Strep extract (photography was taken after 6 days of culture)
A: CLA+ T cells/epidermal cells + Strep extract.
B: CLA+ T cells/epidermal cells.
C: CLA- T cells/epidermal cells + Strep extract.
D: CLA- T cells/epidermal cells.

FIGURE 1B. StrepA induces IFN-γ and IL-17 production preferentially in the coculture of CLA+ T cells and autologous lesional epidermal cells.



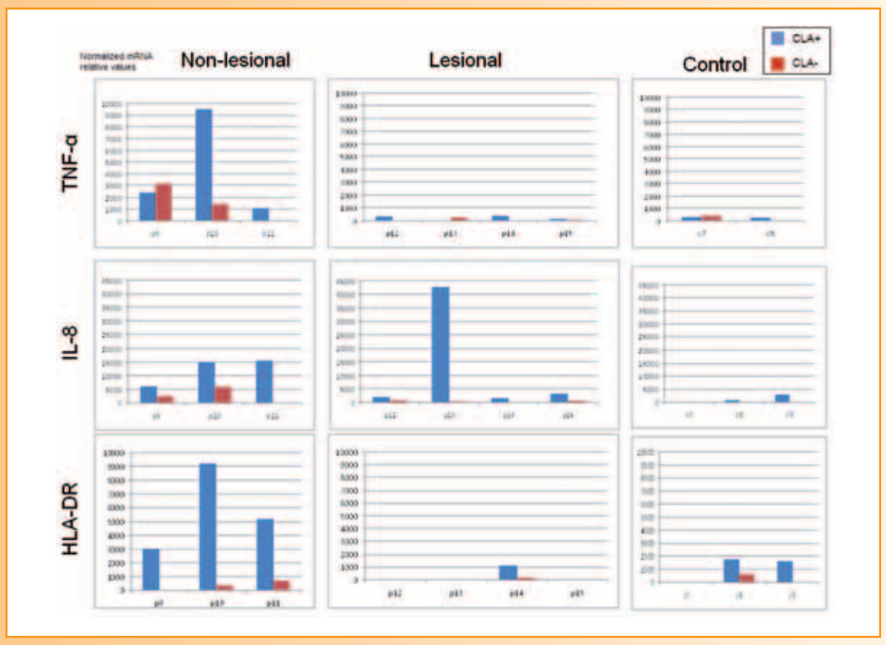
Supernatants from the coculture of T cells and autologous lesional epidermal cells (EPI) stimulated with Strep A extract or Staphylococcus aureus Enterotoxin B (SEB) were taken after 48h culture and IFN-γ an IL-17 were mesure by ELISA. Data are presented from 5 psoriatic patients (p) and 3 controls (c) as net cytokine production: (CLA+/StrepA or SEB/EPI)-(CLA+/EPI).
Only the coculture of CLA+ T cells and autologous lesional cells from psoriatic patients can induce IFN-γ and IL-17 production when stimulated with StrepA extract. Cocultures from controls do not respond to StrepA stimuli but they do respond to SEB.

FIGURE 2. StrepA induces IFN-γ, IL-17, IL-22 gene expression only with psoriatic CLA+ T cells and lesional autologous epidermal cells.



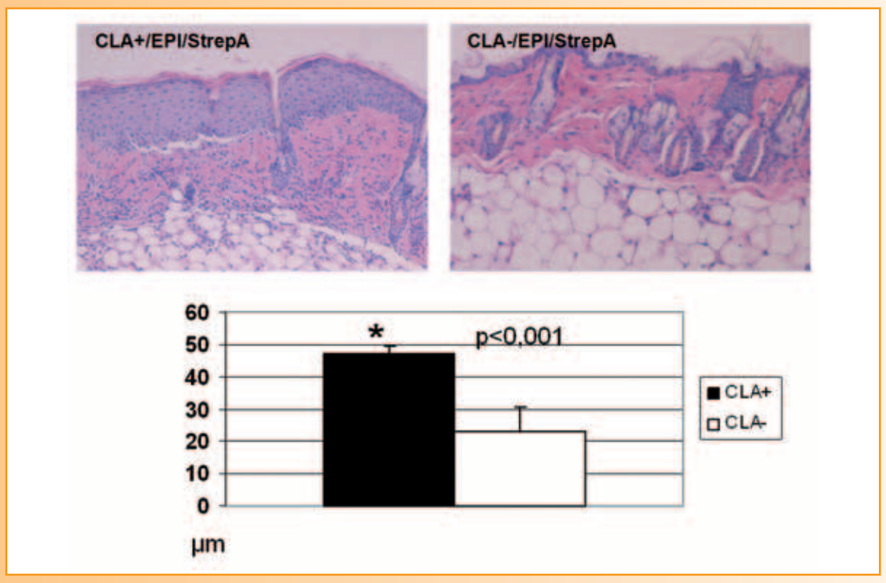
Time course experiment of gene expression analysis in the coculture. RNA was taken 12, 24 and 48h after activation with or without StrepA (MED=medium without StrepA extract). Real time PCR was performed and gene expression was calculated using the Δ-ΔCt method (using the mean cycle threshold value for GAPDH and the gene of interest for each sample). The equation 1.8 e(Ct_{GAPDH} -$Ct_{gene\ of\ interest}$) x 10^4 was used to obtain the normalized values. Data presented from 4 psoriatic patients and 3 controls. Only in the coculture condition with CLA+ T cells and lesional autologous epidermal cell suspension, a time-dependent increase in gene expression for IFN-γ, IL-7 and IL-22 is found.

FIGURE 3. StrepA induces TNF-α, IL-8 and HLA-DR gene expression in non-lesional epidermal cells cocultured with CLA+ T cells from psoriasis patients.



Gene expression in the coculture at 24h after activation was evaluated by RT-PCR and normalized to GAPDH. Data presented from 4 psoriatic patients and 3 controls.
Only CLA+ T cells form psoriatic patients plus StrepA induce expression in the coculture of non-lesional autologous epidermal cells.

FIGURE 4. Intradermal injection of supernatants from coculture of CLA+ T cells and epidermal cells activated with StrepA extract induces in vivo epidermal hyperplasia in Balb/c mouse.



6th day culture supernatant was injected intradermically in 4 Balb/c mice daily during 4 days and epidermal hyperplasia was evaluated at day 4 (results from 4 indepedent experiments). Histopathological figures (HEx20) and a comparative graphic show the differences of epidermal thickness in micrometers after supernatant injections.
Supernatants from CLA+T cells plus autologous epidermal cells activated with StrepA induce significantly higher epidermal hyperplasia compared to autologous CLA- T cells + epidermal cells incubated with StrepA.

Conclusions

The study shows that Streptococcal extract preferentially induces keratinocyte and CLA+ T cell (Th1, Th17, Th22) activation and in vivo epidermal hyperplasia. This ex vivo approach might contribute to clarify the role of streptococcal infections in early molecular mechanisms of psoriasis development.

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