Innate stimulus triggering preferential Th17 response in HLA-Cw6+ guttate psoriasis through skin-specific memory cells, epidermal cells and *Streptococcus pyogenes* interaction

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Introduction

Psoriasis has been considered to be triggered by innate stimuli, especially in guttate type, whose onset is often preceded by *Streptococcus pyogenes* (Sp) pharyngitis. Although Th17-cytokine immune response is clinically validated in psoriasis, there are no studies focusing on an Sp-specific Th17-induced response in these patients. In this study we have investigated how skin-homing CLA+ T cells together with lesional epidermal cells from guttate patients respond to Sp. Our results indicate that Sp induces a preferential Th17 response particularly for those cell cultures derived from HLA-Cw6+ guttate patients whose flares were associated to previous Sp pharyngitis.

Material and methods

The study included 14 guttate psoriatic patients and 6 healthy donors. Each participant underwent a blood extraction and skin punch biopsies. CLA+ and CLA- memory T cells were purified from blood samples through immunomagnetic separations, while epidermal cells (EPI) were obtained by chemical and mechanical treatment of skin punches. $5x10^4$ CLA+ or CLA- were seeded with $3x10^4$ autologous epidermal cells and activated by an extract of Sp (SE). Culture supernatants were collected after 5 days and cytokines were quantified by multiplex fluorescent bead-based immunoassay. For effector function evaluation, supernatants were used to stimulate *in vitro* cultured keratinocytes monolayers, followed by RNA extraction at 15h and psoriasis-associated gene expression analysis by quantitative real time PCR.

Results

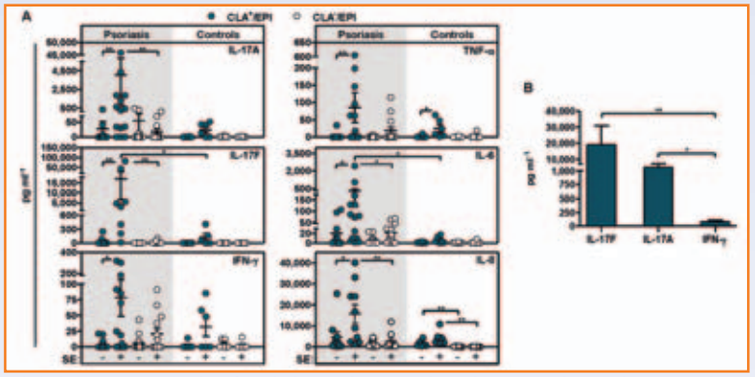

Figure 1. Sp induces the production of psoriasis-associated cytokines with a predominant Th17 response in cultures of CLA+ T cells and epidermal cells from guttate psoriatic patients. (a) Supernatants from CLA^+/CLA^- T cells cultured with autologous epidermal cells from 14 guttate psoriatic patients and 6 healthy controls, were collected at day 5 and IL-17A/F, IFN-γ, TNF-α, IL-6 and IL-8 were measured in culture conditions stimulated or not by SE. The cultures containing CLA^+ T cells and autologous epidermal cells (CLA^+/EPI) from psoriasis patients in the presence of SE displayed the highest activation. Each dot represents cytokine quantitation of one culture derived from individual psoriasis patient or control. **(b)** Mean comparison of T cell-derived cytokines revealed higher amounts of IL-17A and IL-17F than IFN-γ produced by SE-activation of psoriatic CLA^+/EPI cultures. Data is expressed as mean ± SEM.

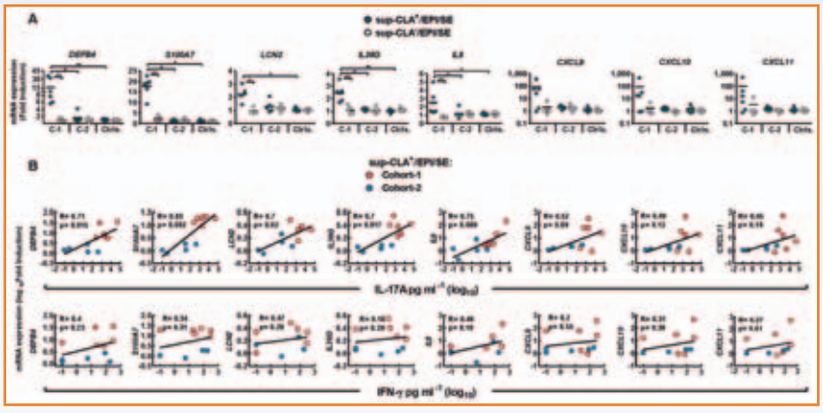

Figure 3. Supernatants of SE-stimulated CLA^+/EPI cultures from Cohort-1 guttate psoriatic patients induce a higher upregulation of psoriasis-associated genes in normal keratinocytes. (a) SE-activated CLA+/EPI or CLA-/EPI culture supernatants from Cohort-1 cocultures (n=6) is compared with Cohort-2 (n=5) and controls (n=5). Cohort-1-derived supernatants showed an increased effect when comparing their gene induction capacity with that elicited by Cohort-2, controls, or CLA^-/EPI/SE supernatants. **(b)** Correlation between IL-17A/IFN-γ of 5-days supernatants from SE-activated psoriatic CLA^+/EPI cocultures and the mRNA levels induced in keratinocytes. The amounts of IL-17A correlated with IL-17-related genes such as *DEFB4, S100A7, LCN2, IL36G* and *IL8*, while no significant correlations were obtained with IFN-γ. RNA from keratinocytes was extracted after 15h of supernatant-stimulation.

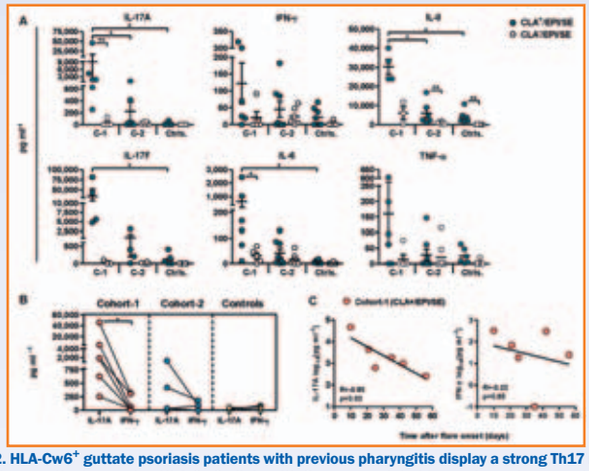

Figure 2. HLA-Cw6+ guttate psoriasis patients with previous pharyngitis display a strong Th17 response to SE. Those patients carrying HLA-Cw6 allele with flares associated to previous pharyngeal infection were grouped in Cohort-1 (C-1; n=6). The remaining patients not fulfilling any of these two criteria were grouped in the Cohort-2 (C-2; n=8). **(a)** SE-activated CLA^+/EPI cultures from Cohort-1 individuals showed higher cytokine production response than those from Cohort-2. Data is expressed as mean ±SEM. **(b)** Paired-test showed significant higher levels of IL-17A than IFN-γ within the same psoriasis patients from Cohort-1. **(c)** Interestingly, the amounts of IL-17A, but not IFN-γ, from Cohort-1 showed a significant inverse correlation with time elapsed since symptom onset, thus suggesting that Th17 circulating CLA^+ T cells would increase shortly after Sp infection and would progressively reduce. Each dot represents cytokine quantitation of one culture derived from one psoriasis patient or control.

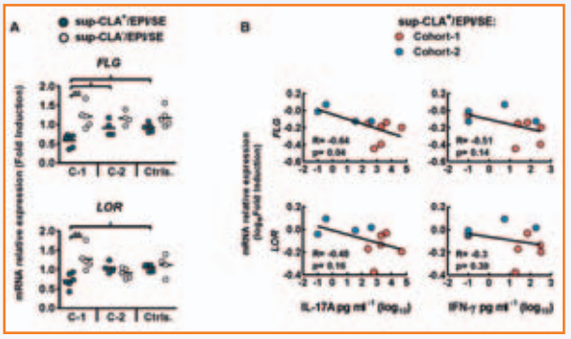

Figure 4. Skin barrier-associated filaggrin and loricrin are strongly downregulated in normal keratinocytes by supernatants from SE-stimulated psoriatic CLA^+/EPI cultures from Cohort-1. (a) SE-activated CLA^+/EPI coculture supernatants from Cohort-1 cocultures induced a reduction in mRNA expression of *FLG* and *LOR* in normal keratinocytes. **(b)** As it was observed for other IL-17A-associated upregulated genes, *FLG* relative expression levels in keratinocytes were also significantly inversely correlating with IL-17A-contents, but not with IFN-γ, of psoriatic CLA^+/EPI/SE cocultures supernatants, while LOR showed a non-significant trend.

Conclusion

These data suggest that those HLA-Cw6+ carriers presenting guttate psoriasis triggered by *S. pyogenes* throat infection develop a preferential Th17 response, which mainly induces an IL-17-associated altered gene expression profile in keratinocytes. This would be produced by Th17 circulating memory CLA^+ T cells that may increase shortly after streptococcal throat infection, which indicates that IL-17 might be a major effector immune factor in early events of the disease.

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