Streptococcus-induced activation of circulating CLA+ T and epidermal cells in HLA-Cw6+ patients: a predominant Th17 response in guttate psoriasis patients and Th1/Th17/Th9 in chronic plaque responders

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Introduction

Guttate psoriasis triggered by *Streptococcus pyogenes* (Strep) throat infection in HLA-Cw6+ patients is clinically well defined, but the immunological mechanisms involved, as well as the effector functions of CLA+ T cells on epidermal cells, are poorly characterized. Our previous work showed that circulating skin-homing CLA+ T cells cocultured together with autologous cutaneous epidermal cells and activated by an extract of *Streptococcus pyogenes* (SE), result in an *ex vivo* Th17/Th1 immune response and epidermal cell activation, only in psoriasis. We have used this model to characterize the strep-specific immune response and effector function on keratinocytes using clinical material obtained from guttate, acute and plaque psoriasis patients (n=21).

Material and Methods

Purified circulating CLA+/CLA- memory T cells and autologous epidermal cells were cocultured under the presence or not of SE, and supernatants were collected at day 5. Cytokines were measured by fluorescent bead-based multiplex assay or ELISA. For *in vitro* studies, normal human keratinocytes (NHKs) were incubated with 1/10 diluted conditioned coculture supernatant. Keratinocytes mRNA was isolated after 15h stimulation and cDNA expression was measured by qPCR.

Results

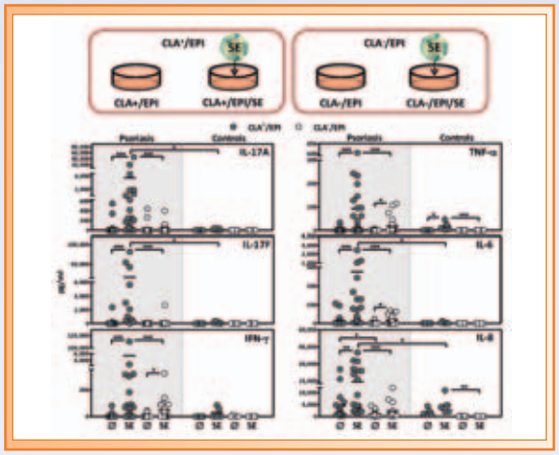

Figure 1. SE INDUCES IL-17A, IFN-γ, TNF-α, IL-6 AND IL-8 PRODUCTION PREFERENTIALLY IN CLA+ T CELL COCULTURES

Activation of cocultures of circulating CLA+ memory T cells (CLA+) with autologous epidermal cells (EPI) through a streptococcal extract (SE) resulted in the production of proinflammatory cytokines such as IL-17A, IFN-γ, TNF-α, IL-6 and IL-8, which are important mediators in psoriatic lesions (Psoriasis n=21; Controls n=5).

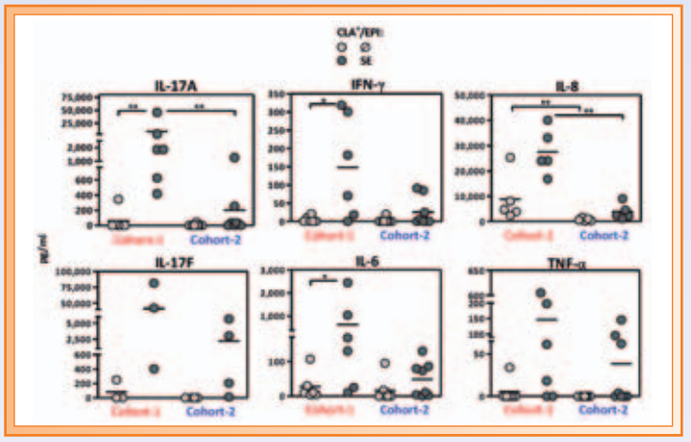

Figure 2. GUTTATE TYPE HLA-CW6 POSITIVE PSORIASIS PATIENTS WITH PREVIOUS PHARYNGITIS DISPLAY A STRONGER RESPONSE TO SE WITH HIGHER PRODUCTION OF IL-17A/F

Patients with acute onset of psoriasis were classified into two cohorts: those with guttate psoriasis type, HLA-Cw6 allele carriers and with flare associated to previous pharyngeal infection were grouped in Cohort-1 (n=5). The remaining patients not satisfying any of these three parameters were grouped in the Cohort-2 (n=9). SE-activated cocultures of CLA+ T cells with autologous lesional epidermal cells in Cohort-1 showed higher cytokine production response than Cohort-2, with predominating Th17 response (IL-17A/F).

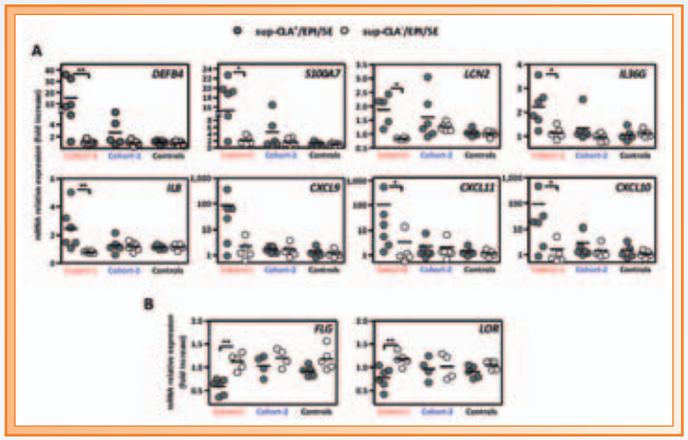

Figure 3. KERATINOCYTES STIMULATED WITH COHORT-1 ENRICHED SUPERNATANTS SHOW AN INCREASED INDUCTION OF PSORIATIC ASSOCIATED GENES AND A DECREASED EXPRESSION OF SKIN BARRIER GENES

(A) According to the substantial differences observed in cytokine production between the two cohorts previously described, we wondered if upregulated psoriasis-associated genes examined in keratinocytes cultures were differentially expressed. Effector function of Cohort-1 coculture supernatants (n=5-6) showed a more marked increase when comparing their gene induction capacity with that elicited by Cohort-2 supernatants (n=4-5). (B) Gene expression of proteins involved in the proper formation of the cornified envelope, such as loricrin and filaggrin, which has been described to be reduced in psoriasis lesions, was downregulated in keratinocytes exposed to Cohort-1 supernatants.

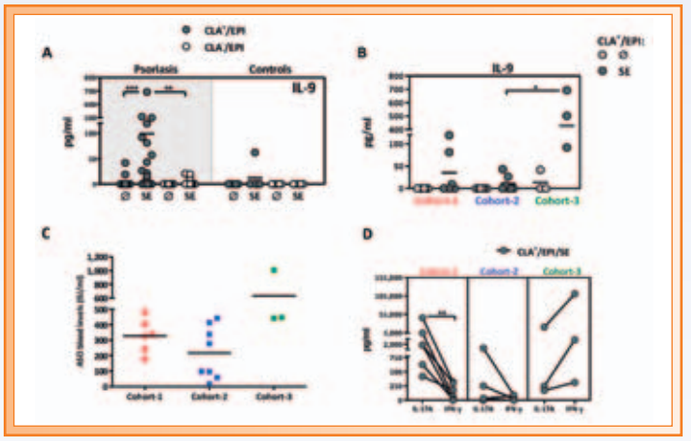

Figure 4. CHRONIC PLAQUE PSORIASIS PATIENTS, WITHOUT PREVIOUS GUTTATE PSORIASIS AND THROAT INFLAMMATION, RESPOND DIFFERENTLY TO STREPTOCOCCUS, SHOWING A HIGHER PRODUCTION OF IL-9 AND PREDOMINANT TH1 PROFILE IN OUR *EX VIVO* MODEL OF PSORIASIS

(A) IL-9 production was detected in supernatants of SE-activated psoriatic cocultures of CLA+ cells with autologous lesional epidermal cells. (B) Chronic plaque psoriasis patients (Cohort-3; n=3) derived cocultures that responded strongly to SE with a higher production of IL-9 compared to Cohort-1 and 2 patients, were associated with high levels of anti-streptolysin O in blood despite their psoriasis was not apparently triggered by a throat infection (C). (D) SE-activated cocultures displayed preferential Th17 response type, as IL-17A production compared to IFN-γ was higher, which is more evident in Cohort-1. Interestingly, psoriatic plaque patients from Cohort-3 exhibited a stronger Th1 response.

Conclusion

This model proposes an innate-induced preferential Th17 immune response in type I psoriasis patients, typically suffering acute stages of the disease, which might help us to understand the mechanisms behind this characteristic clinical presentation. Furthermore, Strep induces a different adaptive immune response in the model (Th17/Th1/Th9) in chronic plaque psoriasis patients without previous guttate episode nor recurrent throat infections but elevated levels of anti-streptolysin O, showing a Th1 predominating profile and high production of IL-9.

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