Streptococcus pyogenes activation of circulating CLA+ T cells in guttate and chronic plaque psoriasis patients: IL-9 production dependence on time of flare, PASI, ASO levels, and relationship with IL-17 response

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Introduction

Pharyngeal *Streptococcus pyogenes* infection can trigger both guttate and chronic plaque psoriasis types. At present, the immunological mechanisms behind such innate-induced adaptive immune response can be addressed by the *ex vivo* coculture of circulating memory CLA$^+$ T cells together with autologous epidermal cells activated by *Streptococcus pyogenes* extract (SE). In this study we have analyzed the response to SE in untreated guttate (n=16) and plaque psoriasis (n=12), and in healthy controls (n=10). Our results indicate that *ex vivo* CLA$^+$ T cell-dependent cytokine response in guttate patients is related to the clinical status of the patients such as the time elapsed since flare, PASI, and ASO level, with a peak at 1-2 months.

Materials 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. IL-9, HLA-A/B/C (class I) and HLA-DR (class II) neutralizing antibodies, or isotype IgG control, were added at day 0, at a final concentration of 10 µg/ml. Cytokines were measured by fluorescent bead-based multiplex assay or ELISA. SE was obtained from sonicated *Streptococcus pyogenes* isolated from throat swabs of patients with psoriasis and was used at a final concentration of 1 µg/ml.

Results

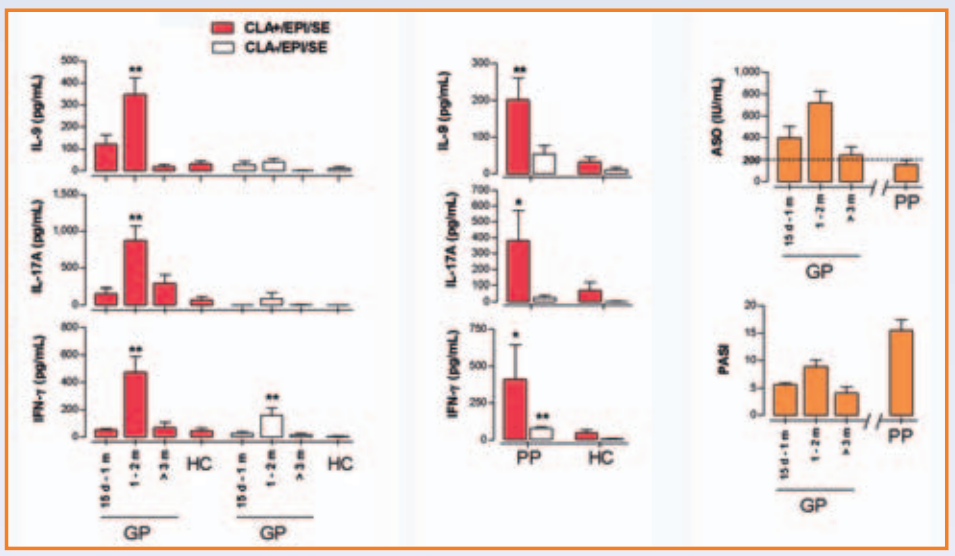

FIGURE 1. SE-INDUCED IL-9, IL-17A, and IFN-γ BY CLA+ MATCH WITH PASI AND ASO LEVELS 1-2 MONTHS AFTER STREP INFECTION

The *ex vivo* CLA$^+$ T cell-dependent cytokine response in guttate patients is related to the clinical status of the patients such as the time elapsed since flare, PASI, and ASO level, with a peak at 1-2 months of disease duration. Patients are shown in three separated groups according with length of disease onset: 15d-1m (n=3), 1-2 m (n=8) and >3m (n=5).GP: guttate psoriasis; PP: plaque psoriasis (n=12); HC: healthy controls (n=10); d: days; m: months. ASO: anti-streptolysin O. PASI: psoriasis area severity index. Significance is shown in relation to HC values.

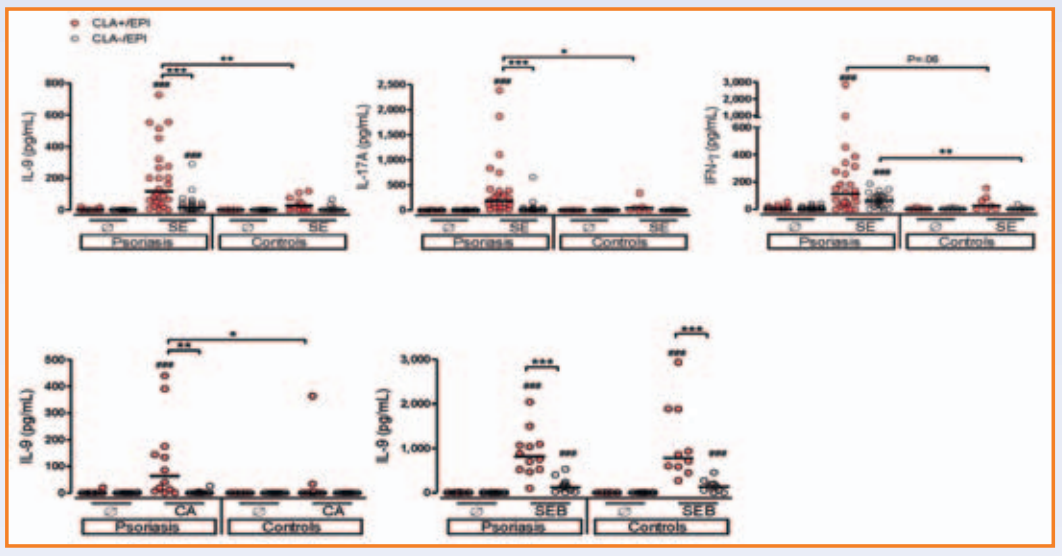

FIGURE 2. IL-9 IS INDUCED BY SE, TOGETHER WITH IL-17A AND IFN-γ, PREFERENTIALLY IN COCULTURE CONDITION OF PSORIATIC CLA+ T CELLS AND EPIDERMAL CELLS

IL-9 was preferentially induced in psoriatic-derived cocultures (n=27) containing CLA$^+$ T cells over non-skin homing CLA$^-$ T cells or healthy controls (n=10), as it was observed for IL-17A and IFN-γ responses. Other pathogens are likely to promote *ex vivo* activation of cocultures, such as *Candida albicans* (CA). Such psoriasis-associated selectivity was not due to poor response from controls, as activation with staphylococcal enterotoxin B (SEB) exerted the same IL-9 induction capacity in both groups of donors. (#): vs. respective unstimulated condition.

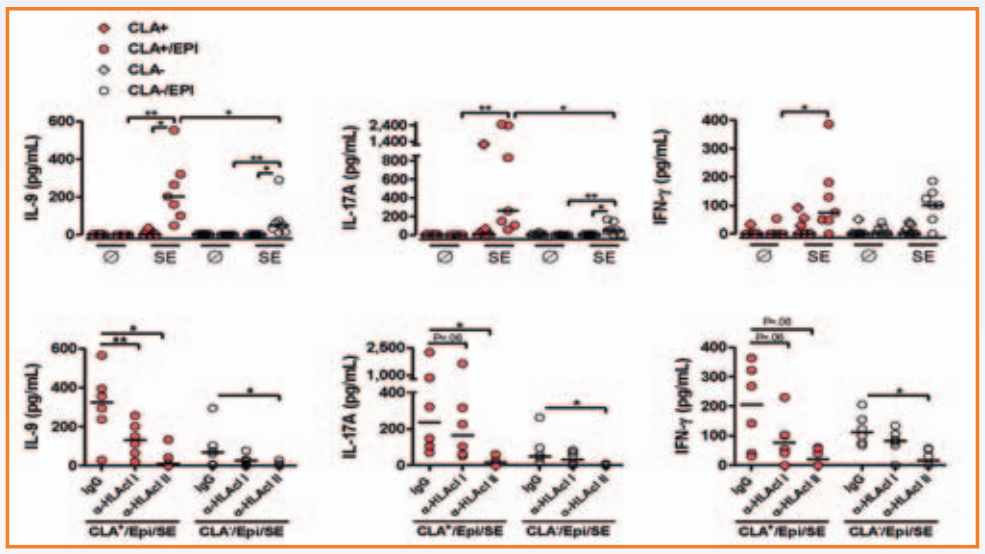

FIGURE 3. CLA-DEPENDENT IL-9 PRODUCTION DEPENDS ON THE PRESENCE OF EPIDERMAL CELLS AND MHC CLASS I AND CLASS II PRESENTATION

SE activity was minimal in T cells alone, but the presence of autologous lesional epidermal cells were actually responsible for production of IL-9, IL-17A and IFN-γ (n=7). Such response depended on the MHC class I (50%) and class II (90-100%) molecules (n=6).

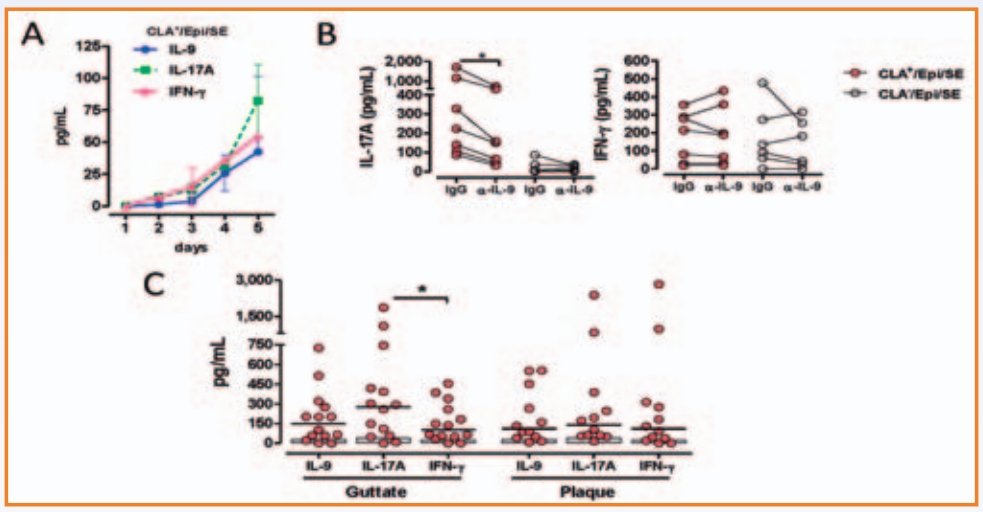

FIGURE 4. TIME COURSE OF IL-9 PRODUCTION, INFLUENCE OF IL-9 ON IL-17A PRODUCTION AND SIMILAR INDUCTION OF IL-9 BETWEEN GUTTATE AND CHRONIC PLAQUE PSORIASIS

(A) SE induces IL-9 production in parallel with IL-17A and IFN-γ (n=2-3).
(B) Neutralization of IL-9 activity by a specific monoclonal antibody reduced IL-17A production (50%), but no effect was observed in that of IFN-γ in CLA$^+$/EPI/SE cultures.
(C) Similar levels of IL-9 are induced in cocultures of CLA$^+$/EPI/SE from guttate (n=16) and chronic plaque psoriasis patients (n=12). Both types of psoriasis responded to SE in the *ex vivo* model and presented a mixed production of IL-9, IL-17A and IFN-γ. No significant difference was observed between guttate and plaque samples, and while the former showed a predominant Th17 profile, the latter showed no clear preference. Grey bars represent median cytokine production from healthy controls (n=10).

Conclusions

These results indicate that in psoriasis, the innate activation induced by SE leads to IL-9 production that supports IL-17A production. Such activation depends on CLA$^+$ T cell/epidermal cell interaction and HLA-mediated presentation. In guttate psoriasis the IL-9 production relates with clinical features of the patients. The induction of IL-9 by a clinically relevant trigger of psoriasis through skin-specific memory T cells, and its relationship with IL-17A production, supports the clinical translational relevance of these results for psoriasis.

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