IL-15 and IL-23 synergize to trigger IL-17 response in CLA+ T cells in the presence of autologous epidermal cells through HLA class I and II molecules in psoriasis

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Introduction

IL-15 has emerged as a potential relevant target in psoriasis that could be important in the IL-17 response, together with other cytokines such as IL-23. Both IL-15 and IL-23 are constitutively expressed in psoriatic lesion. In addition, *IL15* gene is considered a susceptibility associated gene in psoriasis, together with other genes such as *IL23R* and *HLACW6*. However, IL-15 activity in humans has been poorly characterized. The purpose of this study was to evaluate the responses of psoriatic *ex vivo* cultures containing peripheral memory skin-homing CLA+, or CLA-, T cells, in presence of autologous lesional epidermal cells, under activation with IL-15 and IL-23.

Materials and methods

The study included 7 psoriatic patients and 3 healthy donors, who previously gave informed consent. Each participant underwent a blood extraction and 2 skin punch biopsies. Memory CLA+ and CLA- T cells were purified from blood samples through immunomagnetic separations, and epidermal cells (Epi) were obtained by chemical and mechanical treatment of skin punches. 1,6 x10⁴ CLA+ or CLA- were seeded with 1x10⁴ autologous epidermal cells and activated by 10 ng/mL of human recombinant IL-15 and/or IL-23. 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. After 5 days of culture, IL-17A and IFN-γ were measured by ELISA, and IL-17F by CBA fluorescent bead-based immunoassay. Data are represented by bar charts showing the mean ± SEM.

Results

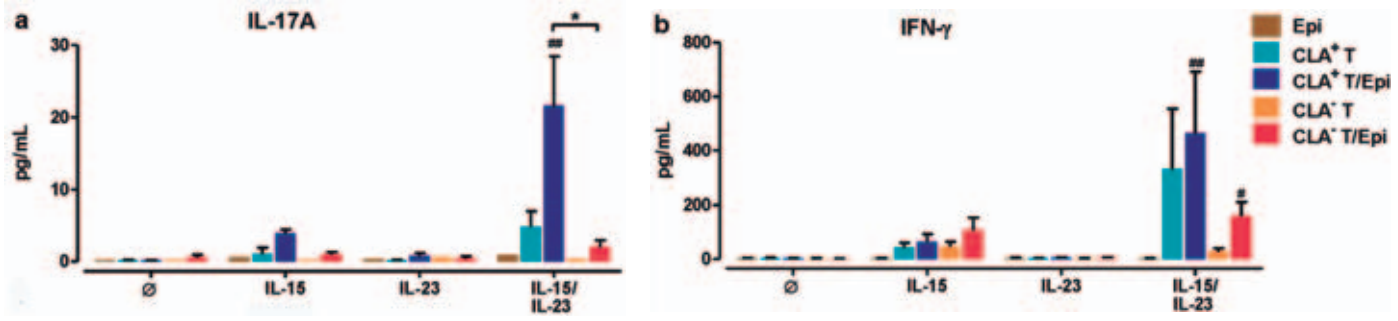

FIGURE 1. IL-23 synergize with IL-15 to produce IL-17A and IFN-γ. IL-17A production was especially enhanced by the presence of epidermal cells. Epidermal cells, CLA+T or CLA- T cells from psoriasis patients were cultured alone or in T/Epidermal cells coculture combinations, which were then activated by IL-15, IL-23, both, or were left untreated. Supernatants were collected at day 5 and IL-17A (n=3) (a) and IFN-γ (n=4) (b) were measured. One-way ANOVA and Dunnett's post-test were used to assess differences with basal untreated conditions (#: p<0.05; ##: p<0.01). Simple T-test was used to compare two different groups (*:p<0.05). Although levels of IFN-γ were higher than those of IL-17A, a more selective production of this latter was observed in CLA+ T/Epi cocultures, indicating a greater involvement of epidermal cells in the IL-15/IL-23 synergistic effect on IL-17A production.

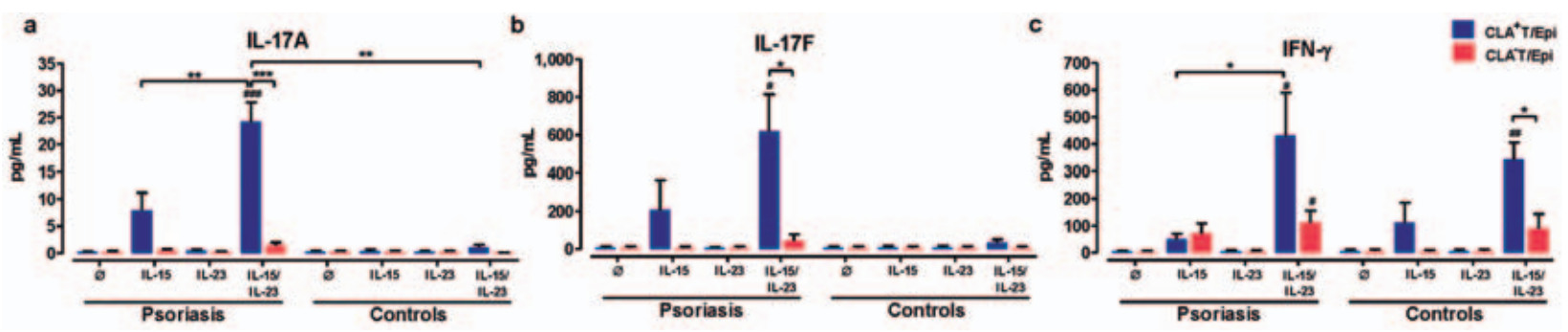

FIGURE 2. IL-17A and IL-17F synergistic production by CLA+ T cells and epidermal cells is induced in psoriasis by IL-15 and IL-23. Considering that the presence of epidermal cells was crucial for optimal cytokine production, we next sought to evaluate responses by psoriasis and healthy controls-derived cocultures. IL-17A (n=6) (a), IL-17F (n=4) (b) and IFN-γ (n=6) (c) were measured in 5-days supernatants. One-way ANOVA and Dunnett's post-test were used to assess differences with basal untreated conditions (#: p<0.05; ##: p<0.01; ###: p<0.001). Simple T-test was used to compare two different groups (*:p<0.05; **: p<0.01; ***:p<0.001). IL-17F was included in the analysis in order to gain sensitivity to the T17-response. IL-17A and IL-17F production by the synergistic effect of IL-15 and IL-23 was hardly found in healthy controls cocultures. Conversely, IFN-γ levels were similar in psoriasis and healthy controls.

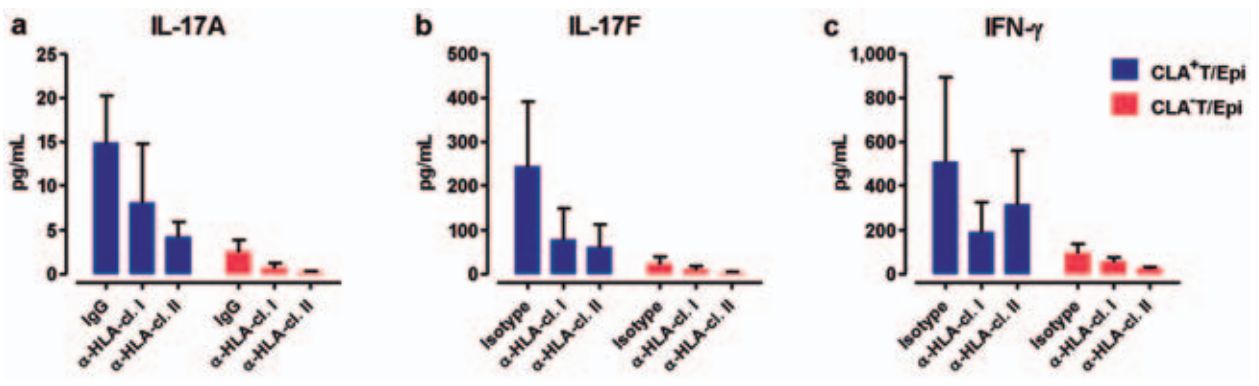

FIGURE 3. Production of IL-17A, IL-17F and IFN-γ induced by IL-15 and IL-23 is dependent on HLA-class I and II molecules. Blocking antibodies against HLA-A/B/C (class I) or HLA-DR (class II) were added to cocultures from psoriatic samples prior to activation with IL-15 and IL-23. After 5 days, supernatants were collected and IL-17A (n=4) (a), IL-17F (n=3) (b), and IFN-γ (n=4) (c) were quantified. Both antibodies reduced cytokine production (40-80%), although HLA-class II blockade showed a greater reduction on T17 products, IL-17A and IL-17F. This suggests an involvement of both, CD4+ and CD8+ T cells, and potentially through self-antigen presentation.

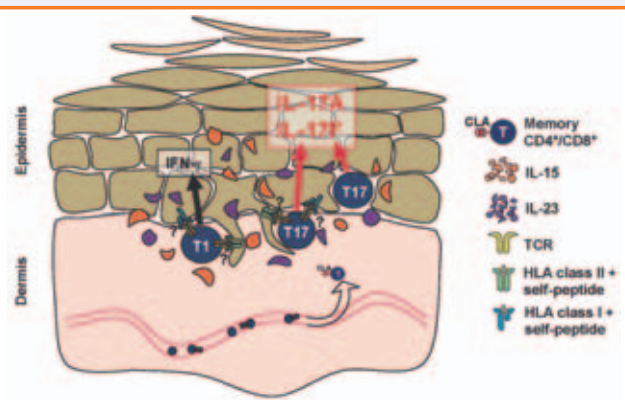

FIGURE 4. Schematic drawing. Natural sources of IL-15 and IL-23 in the skin include keratinocytes and langerhans cells. Both cytokines are present in the inflammatory milieu of psoriatic lesions. Then, circulatory CLA+ T cells enters into the skin and contact epidermal cells during skin inflammation. Our experimental approach reproduces this scenario in which skin-tropic CLA+T cells interact with epidermal cells, then IL-15 and IL-23 synergistically induce the production of IL-17A, IL-17F and IFN-γ possibly through self-antigen presentation since IL-15 is known to promote self-reactivity. However, the exact cellular and molecular mechanisms underlying the IL-15/IL-23 synergy have not yet been completely elucidated.

Remarks and conclusions

Here we report a novel synergistic effect of IL-15 and IL-23 in skin-homing CLA+ T cells that leads to IL-17A, IL-17F and IFN-γ in psoriasis. This effect was highly enhanced by the presence of autologous lesional epidermal cells in psoriatic *ex vivo* cocultures, especially for T17-derived cytokines such as IL-17A and IL-17F. This cytokine production would be triggered by self-antigen presentation in which CD4+ and CD8+ T cells might be involved, since it can be reduced by HLA-class I and II blockade. The fact that IFN-γ production was less enhanced by epidermal cells and that was similar in either psoriatic and healthy cocultures, unlike the IL-17A and IL-17F production, might suggest a differential role exerted by psoriatic lesional epidermal cells. In conclusion, these preliminary data reveal that mere infiltration of CLA+ T cells into the psoriatic lesion can trigger T17 products upon contact with IL-15, IL-23 and epidermal cells.

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