PRODUCTION OF IL-8 BY CIRCULANT CLA+ T CELL LYMPHOCYTES WITH SKIN TROPISM IN PSORIASIS AND HEALTHY CONTROLS

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BACKGROUND

Psoriasis is an immune-mediated disease typically associated with cutaneous neutrophilic infiltration and Munro microabscesses. It has been postulated that in addition to influencing keratinocyte growth and differentiation, neutrophils in the epidermis might also trigger T-cell activation by inducing cell-surface expression of HLA-DR[1]. The accumulation of neutrophils in the stratum corneum has been associated with the presence of highly inflamed, treatment-refractory psoriasis plaques[1]. Neutrophil chemotaxis can be regulated by various chemokines, which can act synergistically, such as interleukin (IL)-8 (CXCL-8), C5a, MIP-1, MIP2, MIP-3, and GCP-2[2]. In psoriasis, IL-8 is produced following activation of cells resident in the skin, in particular keratinocytes. The interaction between neutrophils and T cells that migrate to the skin, however, has not been widely studied.

OBJECTIVES

The aim of this study was to investigate the possible interaction between skin-homing memory T cells (CLA+ T) cells and neutrophils in psoriasis by studying the production of IL-8 by circulating CLA+ memory T cells in patients with psoriasis and healthy control participants.

METHODS

The study included 6 patients with plaque psoriasis and 6 healthy individuals. Blood samples were collected after a minimum period of 6 weeks without treatment of any kind.

Purification and activation of Circulating Peripheral Blood CLA+ T cells: CLA+ T cells were purified from peripheral blood lymphocytes obtained by Ficoll followed by three consecutive immunomagnetic separations (Macs) in order to obtain CLA+ and CLA– memory T-cell subpopulations. The purified T cells were activated with anti-CD3 and anti-CD28, collecting the supernatants 48 hours later.

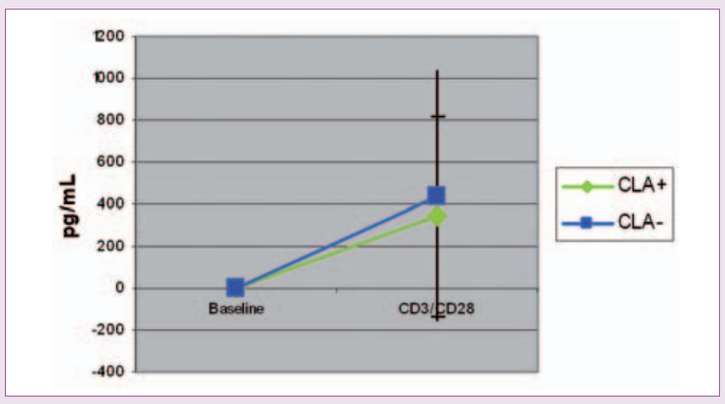
Enzyme-linked immunosorbent assay (ELISA): An ELISA (R&D Systems) was performed on the collected supernatants in order to quantify interferon (IFN)-g (positive control for activation) and IL-8.

Analysis of IL-8 Gene Expression by Real-Time Polymerase Chain Reaction (RT-PCR): RNA was extracted from patient activated CLA+ and CLA– T cells using the GenElute Mammalian kit (Sigma). Complementary DNA (cDNA) was prepared with the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) and analyzed by real-time polymerase chain reaction (PCR) with ABI7900HT (Applied Biosystems). The data were processed using SDS software (version 1.0) (Applied Biosystems).

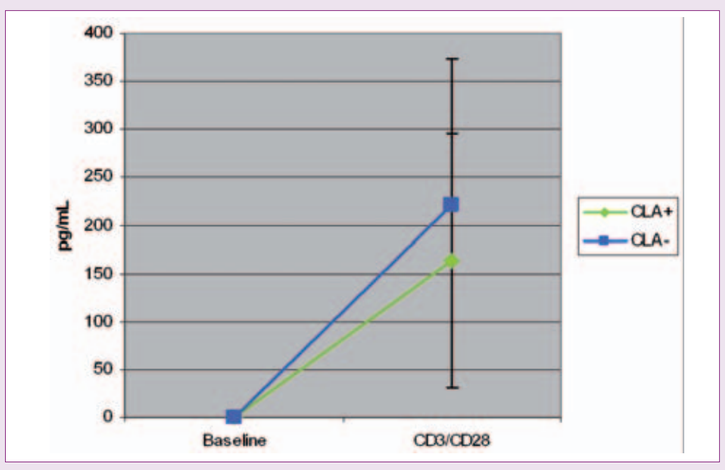
RESULTS

Production of IL-8 and IFN-g by Activated CLA+ and CLA– T Cells: IFN-g production was significantly increased in CLA+ and CLA– T cells activated compared to inactivated cells from both patients and control participants, confirming the validity of the in vitro activation protocol. IL-8 production, in contrast, was detected only in activated CLA+ T cells, both in control participants and patients.

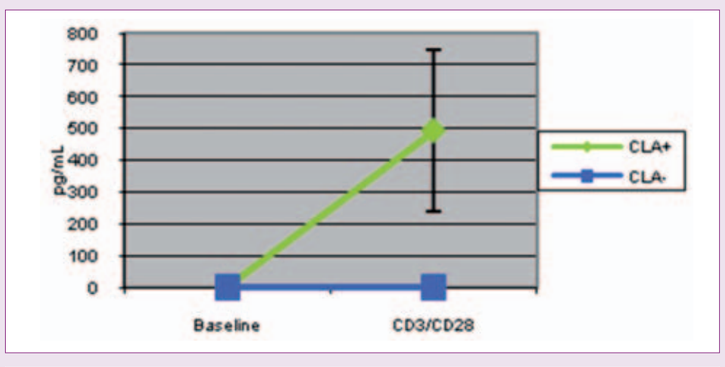
Production of IFNg in control participants



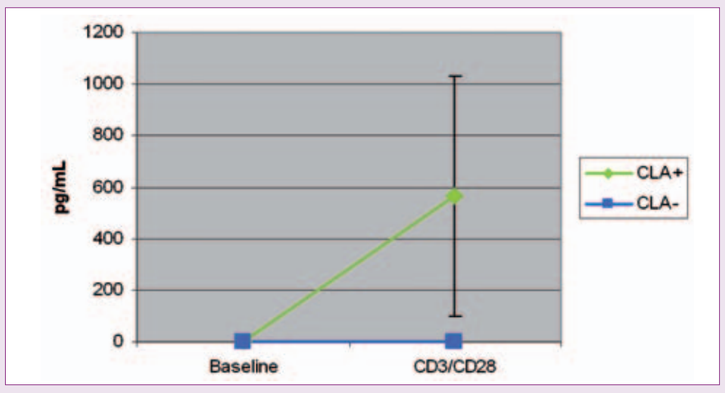
Production of IFNg in psoriatic patients



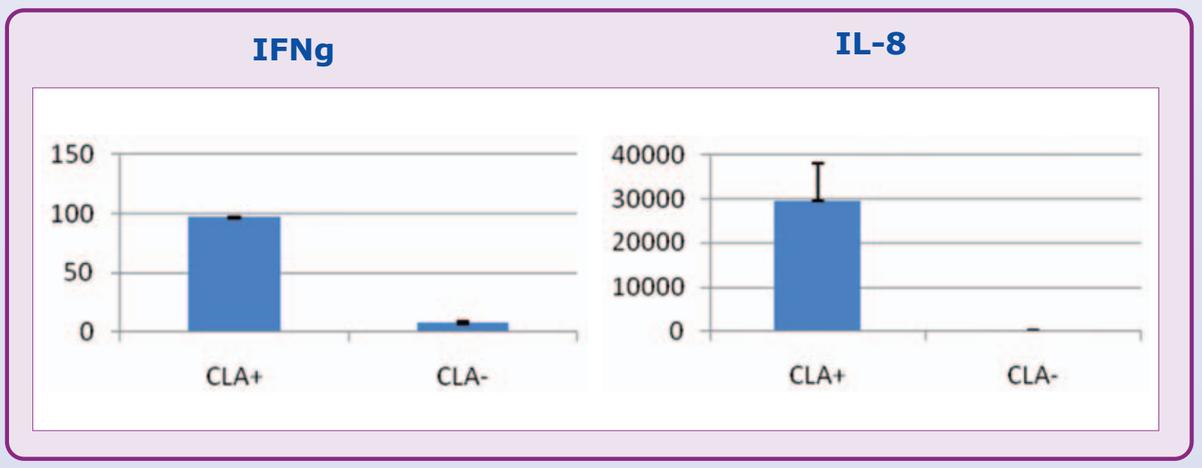
Production of IL-8 in control participants



Production of IL-8 in psoriatic patients



Real-time PCR Analysis of IL-8 and IFN-g in CLA+ and CLA– T Cells: Real-time PCR analysis of activated CLA+ and CLA– T-cell expression in patients (n=2) showed increased expression of *IFN*-g (96.51±26) and *IL-8* (29569.26 ±8584]) in CLA+ T cells compared to CLA– T cells (7.71±1.45 and 66.06 ±31.64, respectively).



The results are normalized to the expression levels of the housekeeping gene GAPDH using the formula $1.8^{-\Delta Ct \times 10000}$.

CONCLUSION

Circulating skin-homing CLA+ T cells produce considerable levels of IL-8 following activation in both patients with psoriasis and healthy individuals when stimulated. In addition, RT-PCR analysis confirms that in psoriatic patients, CLA+ T cells significantly express more IL-8 than CLA- T cell subset. IL-8 production by circulating CLA+ T cell memory subset relates T cell migration into inflamed skin in chronic inflammatory skin diseases with sterile neutrophil infiltration in psoriasis. This process could be involved in the recruitment and survival of polymorphonuclear neutrophils in nonpustular psoriasis[3-5].

The early migration of IL-8- producing CLA+ T cells to the skin and their subsequent local activation would generate IL-8 which, together with other cytokines/chemokines derived from other cells resident in the skin such as keratinocytes, would give rise to the recruitment and survival of polymorphonuclear neutrophils in nonpustular psoriasis.

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