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ORIGINAL ARTICLE

Production of Interleukin 8 by Circulating CLA+ T Cells With Skin Tropism in Patients With Psoriasis and in Healthy Controls

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KEYWORDS
Psoriasis;
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CLA;
Neutrophil;
IL-8

Abstract
Background: Psoriasis is an immune-mediated disease typically associated with cutaneous neutrophilic infiltration and Munro microabscesses. Interleukin (IL)-8 is one of the main neutrophil-attracting chemokines. Although keratinocytes have traditionally been considered to be the principal source of IL-8 in psoriasis, we present data that suggest that cutaneous lymphocyte associated antigen CLA+ T lymphocytes synthesize this cytokine.
Material and methods: Six patients with psoriasis and 6 healthy controls were studied. Immunomagnetic separation was used to isolate CLA+ and CLA- T lymphocytes and IL-8 and interferon (IFN)-γ production was quantified for each cell subpopulation using enzyme-linked immunosorbent assay. Finally, gene expression of IL-8 was analyzed by reverse transcriptase-polymerase chain reaction.
Results: CLA+ and CLA- T lymphocytes from patients with psoriasis and from controls showed a significantly increased production of IFN-γ when activated, whereas only activated CLA+ T lymphocytes (from patients and controls) synthesized IL-8. The higher level of expression of IL-8 and IFN-γ by CLA+ T lymphocytes in comparison to CLA- cells was confirmed.
Discussion: Previous studies have confirmed IL-8 production by T lymphocytes in inflammatory skin diseases with neutrophil-rich infiltrates, such as acute generalized exanthematous pustulosis, Behçet disease, and pustular psoriasis. We have confirmed the role of the subset of T lymphocytes with skin tropism (CLA+) in IL-8 production in nonpustular psoriasis.

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PALABRAS CLAVE
Psoriasis;
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Antígeno de linfocito cutáneo;
Neutrófilos;
IL-8

Producción preferente de IL-8 por linfocitos T CLA+ circulantes con tropismo cutáneo en pacientes con psoriasis y en sujetos sanos

Resumen

Introducción: La psoriasis es una enfermedad mediada inmunológicamente, de causa desconocida, que característicamente presenta infiltración cutánea por polimorfonucleares neutrófilos y microabscesos de Munro. La interleucina (IL)-8 es una de las principales quemocinas atrayentes de los polimorfonucleares neutrófilos. Aunque la producción de IL-8 en psoriasis se ha atribuido principalmente al queratinocito, presentamos datos que apoyan la producción de esta por los linfocitos T antígeno de linfocito cutáneo (CLA)+.
Material y métodos: Se incluyeron 6 pacientes con psoriasis y 6 sanos. Mediante técnicas de separación inmunomagnética se aislaron los linfocitos T CLA+ y CLA-. Se cuantificó la producción de IL-8 e IFNγ de cada subpoblación celular a través de un ELISA. Finalmente, se analizó su expresión génica mediante reacción en cadena de la polimerasa a tiempo real.
Resultados: Tanto los linfocitos T CLA+ como CLA-, de controles y pacientes con psoriasis, aumentaban significativamente la producción de IFNγ cuando eran activados, mientras que sólo los linfocitos T CLA+ activados, tanto de controles como de sujetos con psoriasis, producían IL-8. Se confirmó la expresión preferente de IL-8 e IFNγ en linfocitos T CLA+ respecto de CLA-.
Discusión: Estudios previos han demostrado la producción de IL-8 por linfocitos T en dermatosis inflamatorias ricas en neutrófilos, tales como la pustulosis exantemática aguda generalizada, la enfermedad de Behçet y la psoriasis pustulosa. Nosotros hemos confirmado el papel del subgrupo de linfocitos T con tropismo cutáneo (CLA+) en la producción de IL-8 en la psoriasis no pustulosa.

Introduction

Psoriasis is a chronic inflammatory skin disease with a major genetic component. It is characterized by abnormal keratinocyte growth and differentiation and multiple biochemical, immunologic, and vascular abnormalities.[1] Functional abnormalities in keratinocytes are believed to be triggered by immune system components such as T cells, dendritic cells, and various cytokines.[2] Polymorphonuclear neutrophil infiltration of the skin and Munro microabscesses are characteristic histologic findings in psoriasis, confirming that neutrophils have a role in the pathogenesis of this disease. 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.[3] The accumulation of neutrophils in the stratum corneum has been associated with the presence of highly inflamed, treatment-refractory psoriasis plaques.[3]

Neutrophil chemotaxis can be regulated by various chemokines, which can act synergistically. Examples are interleukin (IL) 8 (CXCL-8), C5a, MIP-1, MIP2, MIP-3, and GCP-2.[4] 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 in psoriasis. The cutaneous lymphocyte-associated antigen (CLA) is a marker for skin-homing memory T cells. The findings of several studies suggest that T cells migrate to the skin before psoriatic lesions begin to develop.[5-8] Recent studies of the role of circulating CLA+ T cells in acute phases of psoriasis have revealed a reduction in the number of circulating cells due to local infiltration.[9,10] In those studies, the extent of the reduction in circulating CLA+ T cells was correlated with disease severity.

The aim of this study was to investigate the possible interaction between CLA+ T cells and neutrophils in psoriasis by studying the production of IL-8 (a potent mediator of neutrophil chemotaxis) by circulating CLA+ memory T cells in patients with psoriasis and healthy control participants. Our results indicate that the activation of CLA+ T cells induces increased IL-8 production.

Material and Methods

The study included 6 patients with plaque psoriasis and 6 healthy individuals. Patients with erythroderma, pustular psoriasis, or arthritis were excluded. Blood samples were collected in all cases, and all the participants signed an informed consent form.

A confirmatory biopsy had been performed in all the patients prior to inclusion in the study. We assessed psoriasis area severity index scores, disease extent (body surface area affected), and clinical characteristics, including possible disease triggers such as streptococcal infection and stress. The 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 (cells with the ability to infiltrate the skin) were purified from peripheral blood lymphocytes obtained by Ficoll separation using 60 mL of blood. Three consecutive

immunomagnetic separations were then performed using antibodies and magnetic particle-conjugated antibodies, as per a previously described protocol.[11] The first two separations eliminated CD14+, CD19+, CD16+, and CD45RA+ lymphocytes, leaving a cell suspension containing just CD45RO+ memory T cells. The third procedure divided the sample into CLA+ and CLA- memory T-cell subpopulations.

Activation of CLA+/CLA–T Cells

The purified T cells were cultured in RPMI medium containing 10% fetal calf serum at a density of 5×10^5 cells/mL and activated with anti-CD3 and anti-CD28. After 48 hours, the supernatants were collected and frozen at -80ºC until needed to activate keratinocyte cultures. IL-8 and interferon (IFN)-γ were quantified by enzyme-linked immunosorbent assay (ELISA) (R&D Systems).

Analysis of IL-8 Gene Expression by Real-Time Polymerase Chain Reaction

RNA was extracted from CLA+ and CLA- T cells from both patients and control participants using a GenElute Mammalian kit (Sigma). Complementary DNA (cDNA) was then prepared by reverse transcription using 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).

The results were normalized to the expression levels of the housekeeping gene GAPDH using the formula $1.8^{-\Delta Ct} \times 10\,000$.

Results

Production of IL-8 and IFN-γ by Activated CLA+ and CLA–T Cells

Supernatants were analyzed by ELISA to determine the amounts of IFN-γ (positive control for activation) and IL-8 protein secreted. IFN-γ production was significantly increased in CLA+ and CLA- T cells activated with CD3/CD8 compared to inactivated cells from both patients and control participants (Figure 1), confirming the validity of the in vitro inactivation protocol. IL-8 production, in contrast, was detected only in activated CLA+ T cells, both in control participants and patients. The mean (SD) levels detected were 494 (254) pg/mL and 566.55 (464.414]) pg/mL, respectively.

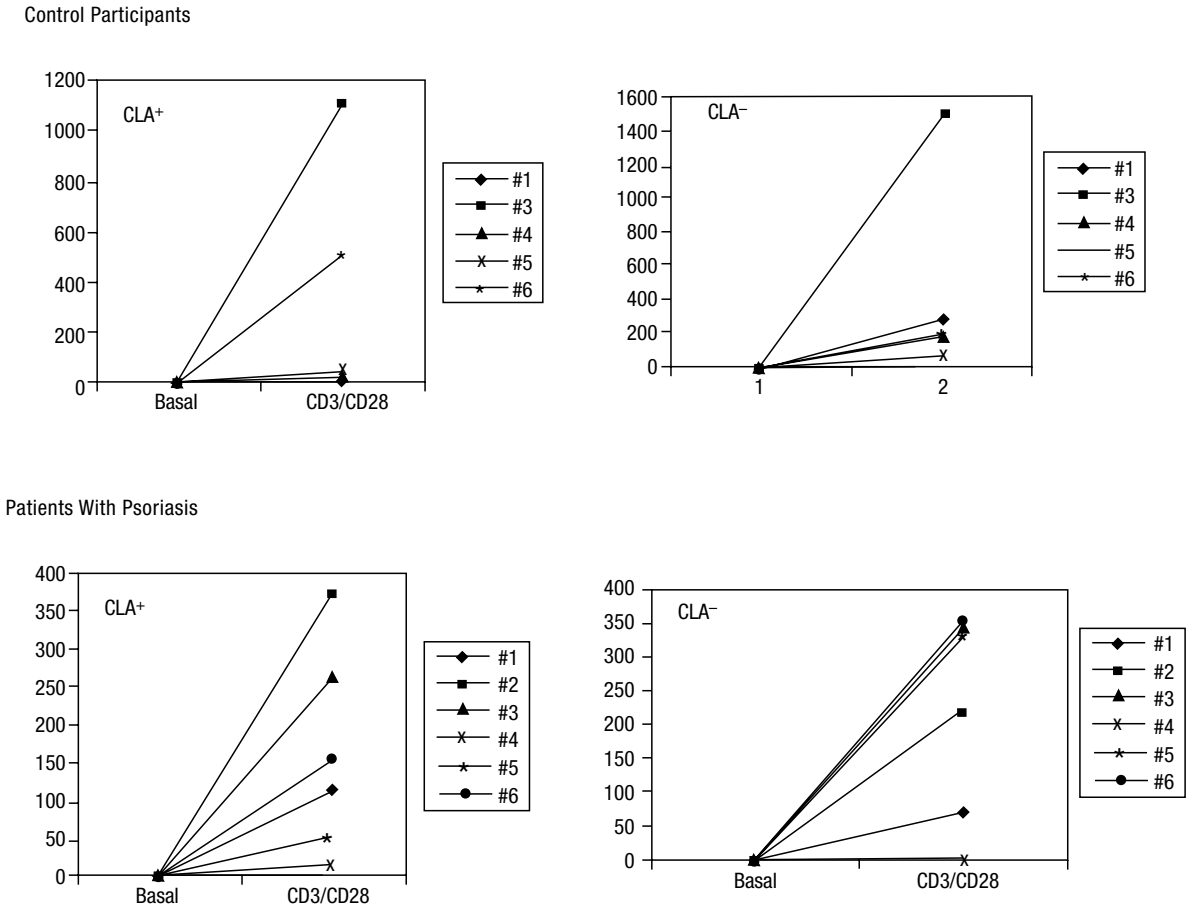

Figure 1 Production of interferon (IFN)-γ (pg/mL) at 48 hours. CLA indicates cutaneous lymphocyte-associated antigen.

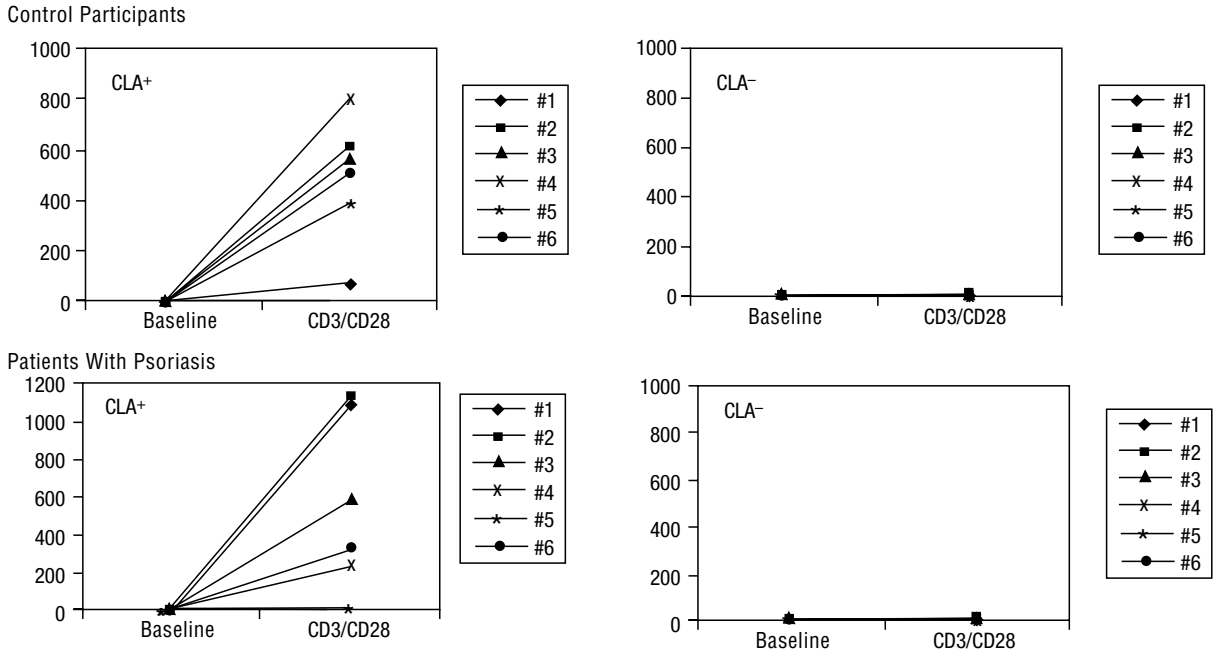

Figure 2 Production of interleukin (IL) 8 (pg/mL) by CLA^+ and CLA^- T cells (baseline and poststimulation levels with CD3/CD28) at 48 hours. CLA indicates cutaneous lymphocyte-associated antigen.

Real-time PCR Analysis of IL-8 and IFN-γ in CLA^+ and CLA^- T Cells

Real-time PCR analysis of CLA^+ and CLA^- T-cell expression in patients (n=2) showed increased expression of *IFN-γ* (96.51 [26]) and *IL-8* (29569.26 [8584]) in CLA^+ T cells compared to CLA^- T cells (7.71 [145] and 66.06 [31.64], respectively) (Figure 3).

Discussion

Our findings show, for the first time, that circulating skin-homing CLA^+ T cells produce considerable levels of IL-8 following activation in both patients with psoriasis and healthy individuals. The fact that we detected this cytokine in both patients and control participants supports the theory that this mechanism has a role in skin inflammation.

In bacterial and fungal infections, neutrophil recruitment is a rapid, T cell–independent process. Neutrophils tend to be among the first cells to be recruited to the infection site through various cytokines and chemokines. The pathophysiology of inflammatory diseases with infiltrates that are rich in neutrophils and in which sterile pustules may develop, however, is different. Neutrophil recruitment has traditionally been attributed to the production of IL-8 (CXCL8) by keratinocytes. Recent studies, however, have suggested that T cells, the first inflammatory cells to be recruited to the disease site, might also be involved in IL-8 production.[12-14]

The ability of T cells to produce IL-8 has been described in Behçet disease and pustular psoriasis,[15] with immunohistochemistry showing that infiltrating T cells (CD4+, CD8+), in addition to keratinocytes, produce IL-8

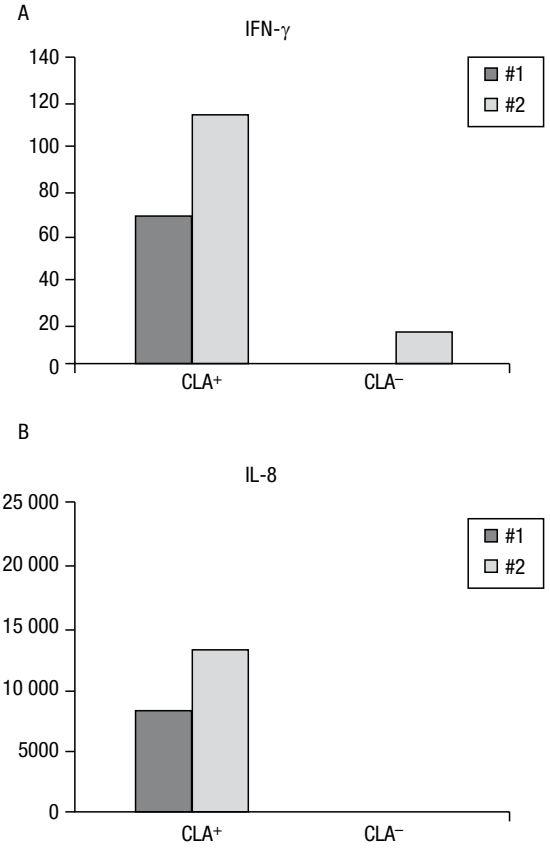

Figure 3 Expression levels of IFN-γ (A) and IL-8 (B) in CLA^+ and CLA^- T cells from patients with psoriasis. The results were normalized to GAPDH levels using the following formula: $1.8-\Delta Ct \times 10\,000$. IFN indicates interferon; CLA, cutaneous lymphocyte-associated antigen; IL, interleukin.

and CCR6. This T cell expression was not found in patients with atopic dermatitis. On analyzing supernatants following the in vitro activation of T cell clones derived from the skin of patients with Behçet disease and pustular psoriasis, the authors saw that the cells secreted both IL-8 and GM-CSF. The role of T cells in the recruitment of neutrophils in nonpustular psoriasis has not been analyzed previously. Our results confirm that T cells only produce IL-8 in psoriasis when stimulated. They also show a clear difference between CLA^+ and CLA^- T cells in terms of their ability to produce IL-8 in both psoriasis patients and healthy control participants. Furthermore, IL-8 production was associated with the ability of T cells to migrate to the skin. Indeed, an earlier study had already pointed to a relationship between CLA^+ T cell migration to the skin and both IL-8 and its receptor CXCR2, indicating a possible relationship between IL-8 and the pathophysiology of skin-homing T cells.[16]

Acute generalized exanthematous pustulosis (AGEP) is one of the diseases in which the role of T cells in the synthesis of IL-8 has been best characterized. It has been demonstrated that in addition to keratinocytes, drug-specific T cells (isolated in both the skin and peripheral blood of patients with AGEP) are capable of synthesizing IL-8 following stimulation.[13,14,17] On characterizing IL-8-expressing T cells in AGEP, Schaerli et al[17] observed that the supernatants from these cells were strongly chemotactic for neutrophils. Specifically, IL-8 was the main chemoattractant and appeared to act mainly through the CXCR1 and CXCR2 receptors. Schaerli et al also demonstrated that neutrophils treated with conditioned medium from IL-8-producing T cells showed a greater survival rate (with a 40% reduction in apoptosis). Because this subgroup of T cells had high CCR6 expression levels, the authors suggested that this chemokine receptor might play an important role in the recruitment of T cells in the initial phases of skin inflammation. Another study found high levels of CCR6 on the surface of CLA^+ T cells, an observation that supports our finding that IL-8-producing T cells appear to play a key role in skin homing.[18]

In conclusion, our data support the theory that IL-8 production by CLA^+ T cells plays an important role in chronic inflammatory skin diseases with sterile neutrophil infiltration such as psoriasis. The early migration of IL-8-producing CLA^+ T cells to the skin and their subsequent local activation would generate IL-8, which, in turn, would attract neutrophils. The IL-8 secreted by the T cells, 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.

Funding

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Conflicts of Interest

The authors declare no conflicts of interest.

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