EFFECTOR FUNCTION OF CLA+ T LYMPHOCYTES ON INOS EXPRESSION IN AUTOLOGOUS KERATINOCTES IN PSORIASIS

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INTRODUCTION

CLA+ T cells represent a subset of effector memory T cells with cutaneous tropism which are involved in different cutaneous diseases mediated by T cells, including psoriasis[1]. It is known that skin infiltration by T lymphocytes is one of the first steps in the development of the psoriasis plaque, followed by epidermic hyperplasia[2]. Once they reach the skin, these lymphocytes get activated and develop effector functions on cutaneous resident cells. The aim of this study is to investigate the effect of CLA+ T lymphocytes on the keratinocyte gene expression in psoriasis, especially on inducible nitric oxide synthase (iNOS) expression.

MATERIAL AND METHODS

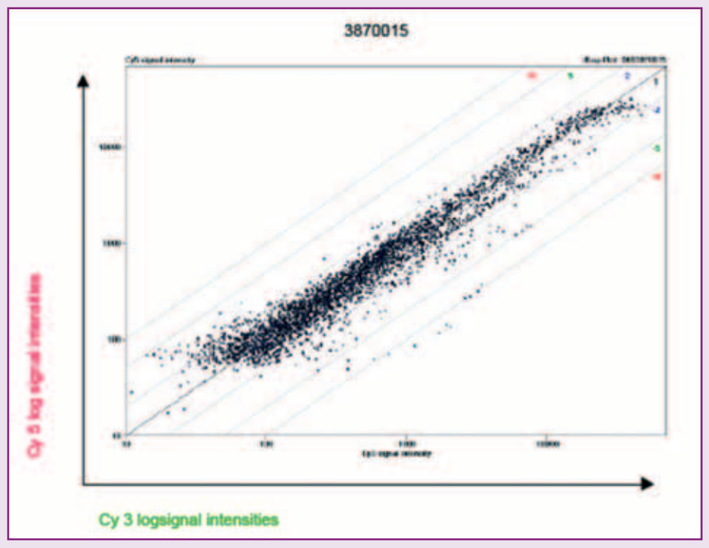
3 patients with plaque psoriasis and 2 healthy controls were included in the study, from whom we obtained a skin biopsy and a blood sample. A Ficoll gradient and immunomagnetic separations were performed to the blood in order to isolate different subpopulations of CLA+/- T cells, which were or not activated with anti-CD3/anti-CD28. Primary cultures of keratinocytes were developed from the cutaneous biopsies. Cultured keratinocytes were exposed to different activating conditions, including supernatant from CD3/CD28 activated CLA+ T cells. Initially, 18 genes related to psoriasis (Table 1) were selected to be analysed by RT-PCR and confirm the validity of the model.
From a expression gene array, a group of genes which were significantly higher induced by activated CLA+ T cells were identified in psoriatic keratinocytes, among which iNOS was present (Figure 1). mRNA expression level of iNOS was determined by quantitative real-time polymerase chain reaction in the different activating conditions tested in both psoriatic and healthy keratinocytes.

Table 1. Genes selected to study by RT-PCR in cultured keratinocytes under different conditions.

IL-7	TLR1
IL-8	TLR2
IP-10	TLR5
IL-15	IL-19
VEGF	IL-20
ICAM-1	CCL-27
TNF-α	Ki67
HLA-DR2	EGF
Psoriasin	ERB-B2

Figure 1. Results of gene array. Signal intensities of all spots. The signal intensities of each cDNA (represented by a dot) is shown in double logarithmic scale. Dashed diagonals define the areas of x-fold differential signal intensities.

X-axis: Cy3 signal intensity (keratinocytes incubated with supernatants of non-activated CLA+ T cells).
y-axis: Cy5 signal intensity. keratinocytes incubated with supernatants of activated CLA+ T cells).



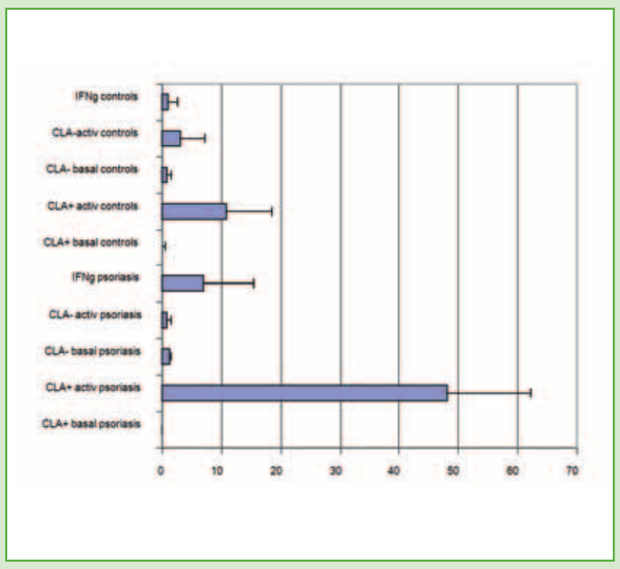
RESULTS

From 18 genes initially studied by RT-PCR, IP-10, HLA-DR2, ICAM-1 and IL19 are higher induced by CLA+ T cells, in comparison with CLA- T cells (Table 2).
iNOS was more upregulated in psoriatic keratinocytes stimulated by activated T cell supernatant (Figure 2).

Table 2. Gene expression in cultured keratinocytes induced by IFN-γ (activation control) o by supernatants of CLA+/- T cells activated with anti-CD3/anti-CD28. In psoriasis patients, a higher expression of IP-10, HLA-DR2 and ICAM-1 in keratinocytes incubated with CLA+ T cell supernatants was detected in comparison with healthy controls.

Genes	Psoriasis (n=3)			Healthy Controls (n=2)		
	IFNγ	CLA+	CLA-	IFNγ	CLA+	CLA-
IP-10	1646 ± 1281	**1253 ± 883**	447 ± 314	210 ± 74,9	722 ± 1011	79,7 ± 83,4
HLA-DR2	244 ± 166	**53,2 ± 23,6**	28,6 ± 17,1	144 ± 173	31,1 ± 21,8	7,7 ± 4,1
ICAM-1	46,2 ± 12,7	**63,0 ± 20,7**	18,7 ± 1,4	12,5 ± 4,7	14,2 ± 7,5	4,5 ± 3,3
IL-19	0,4 ± 0,06	**4,9 ± 1,9**	0,1 ± 0,08	0,5 ± 0,2	**6,0 ± 1,9**	0,9± 0,5

Figure 2. Confirmation by RT-PCR of iNOS mRNA expression in cultured keratinocytes under different conditions: psoriasis or healthy control keratinocytes incubated with IFN-γ, or with supernatant of activated (activ) or basal CLA+/- T lymphocytes. Results are presented as relative quantity (RQ).



DISCUSSION

Up to now the influence of T cells on keratinocytes has been studied by means of generation of T cell clones obtained from lymphocytes present in skin biopsies[3]. The drawbacks of this system are the difficulty in obtaining high quantities of lymphocytes and the need of artificial activations and antigen presenting cells, which can modify the lymphocyte phenotype. Our study manage to solve these problems, isolating CLA+ T cells from peripheral blood and activating them *ex vivo*. This model permit to characterize the effector function of T cells on keratinocytes. Our results suggest that T CLA+ lymphocytes induce a different profile of genetic expression on keratinocytes in comparison to CLA- T lymphocytes.
iNOS gene had previously been implicated in psoriasis[4,5], although it had not been associated with the effector activity of T lymphocytes. Since the effect of IFN-g on iNOS expression is significantly lower than activated T CLA+ lymphocyte supernatant effect, it can be suggested that other mediators produced by CLA+ T cells, apart from IFN-g, are able to induce its expression.
iNOS is an inducible nitric oxide (NO) sintetase. Although other 2 isoforms exist, both of them are expressed constitutively: endothelial and brain iNOS (eNOS and bNOS, respectively). In psoriasis, a higher expression of iNOS has been detected in lesional skin, in comparison with bNOS and eNOS. *In situ* hybridization and immunohistochemical studies found iNOS mRNA and protein on psoriatic keratinocytes and papillary dermis[6]. iNOS expression seemed to be induced by different cytokines, among which, IL-8[7].
iNOS produce NO, a free radical implicated in the pathogenesis of different inflammatory diseases, including psoriasis. Low levels of NO promote keratinocyte proliferation whereas high levels can arrest proliferation and initiate cellular differentiation[8]. Since in psoriasis there is a high keratinocyte proliferation, it seems that overexpressed iNOS might have a low activity, then a low effective antiproliferative action. It can be explained by arginase-1 activity (ARG-1), an enzyme which is also overexpressed in psoriatic keratinocytes and compete for a common substrate with iNOS, which is arginine, in order to produce L-ornitine as a precursor of polyamine synthesis[8].
On the contrary, some authors have described a high concentration of NO in psoriatic plaque or blood[4]. However, these studies analyse total production of NO, without specifying the type of cell which produced it. Apart from keratinocytes, other cells can synthesize NO from iNOS (dendritic cells) and other isoforms can produce NO, as well.
In conclusion, we have shown a model in which peripheral CLA+ T cells can activate psoriatic keratinocytes. Our study demonstrate that psoriatic keratinocytes exposed to supernatant of activated T cells exhibited the highest levels of iNOS mRNA expression, greater than the IFN-gamma condition. These results suggest that in psoriasis, some mediators produced by CLA+ T cells other than IFN-g might induce iNOS expression, a fact that links, again, acquired to innate immunitiy.

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