IL-21 production by streptococcal extract (SE) stimulated CLA+ T cells and epidermal cells in psoriasis: results from quantitative antibody arrays

Marta Ferran[1], Ana B Galvan[1], Catalina Rincon[2], Marc Sacrista[1], Ana M Giménez-Arnau[1], Antonio Celada[2], Ramon M Pujol[1], Luis F Santamaria-Babi[1,2]

1. Department of Dermatology. Hospital del Mar. Institut Municipal d'Investigació Mèdica (IMIM), Barcelona, Spain. 2. Biomedical Research Institute (IRB), Barcelona, Spain

Introduction

The clinical association between streptococcal infections and psoriasis is known for several decades. However the inflammatory mechanisms involved are poorly characterized. T cells are considered to represent a functional link between streptococcal tonsillitis and psoriatic inflammation. A direct evidence of the capacity of *Streptococcus* to induce hallmarks of psoriasis inflammatory response (Th1/Th17/Th22 inflammation and epidermal cell activation through the interaction of circulating CLA^+ T and epidermal cells) would support this concept. IL-21 is a cytokine present in psoriatic lesions that is produced by activated CLA^+CD4^+ T cells which induces epidermal hyperplasia, parakeratosis and T cell infiltration in the skin, in an IL-22-independent manner. However, it is not known whether IL-21 can be induced by a clinically relevant triggering factor of psoriasis. We have generated a coculture system comprising circulating CLA+/CLA- memory T cells and autologous epidermal cells to test streptococcal extract (SE) activation activity in patients with psoriasis and healthy individuals.

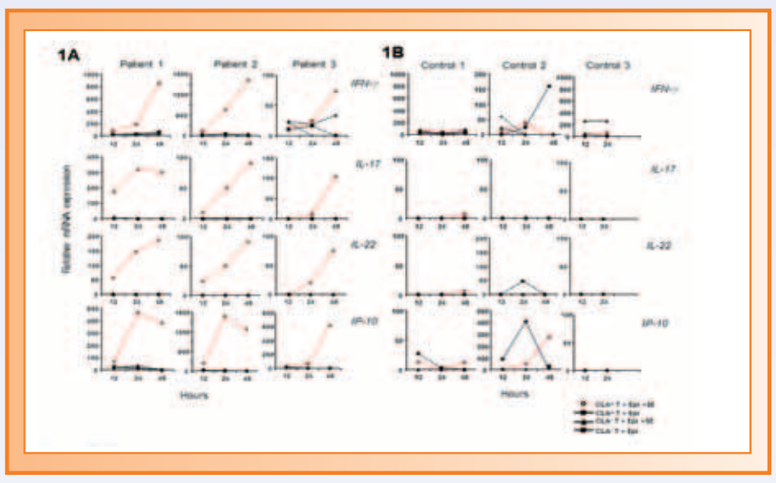
Material and methods

These results are part of a study with 15 non-treated moderate-to-severe psoriasis patients and 9 healthy controls that were enrolled in the study after giving written informed consent. CLA+/CLA- CD45R0+CD3+ were isolated by immunomagnetic separation from peripheral blood and epidermal cell suspensions were obtained from dispase/tryptase treatment of skin punch biopsies.

Results

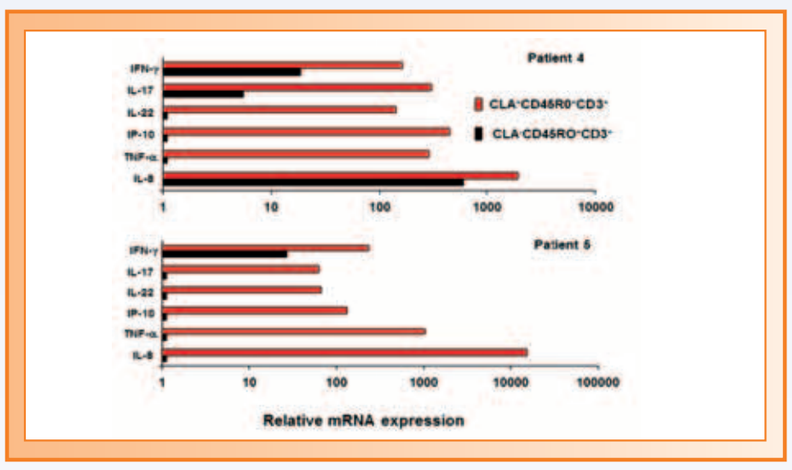
The results show for the first time that SE induces a strong activation of the autologous coculture of circulating CLA+ T cells together with epidermal cells in cells from psoriatic patients.

Figure 1. SE induces *IFN-γ*, *IL-17*, *IL-22* and *IP-10* gene expression only with psoriatic CLA^+ T cells and lesional autologous epidermal cells.



Time course experiment of gene expression analysis in the coculture. RNA was taken 12, 24 and 48h after activation with or without SE. Real time PCR was performed and gene expression was calculated. Increased values were calculated by subtracting normalized gene expression values in the culture for basal and SE-stimulated conditions.
Data presented from 3 psoriatic patients and 3 controls. Only in the coculture condition with CLA+ T cells and lesional autologous epidermal cell suspension, a time-dependent increase in gene expression for *IFN-γ*, *IL-17*, *IL-22* and *IP-10* is found.

Figure 2. StrepA extract increases psoriatic gene expression in non-lesional epidermal cells cocultured with CLA+ T cells from psoriasis patients.



CLA^+ T cells are present in the non-lesional marginal edge of psoriatic lesions before epidermal hyperplasia occurs, and are considered to be relevant elements in the early events of plaque psoriasis formation, thereby suggesting an initial involvement of skin-homing T cells in psoriatic lesion development. We therefore addressed whether SE induces psoriatic gene expression in cultures of non-lesional epidermal cells and autologous circulating CLA^+ T cells of psoriatic patients. The expression of *IFN-γ*, *IL-17*, *IL-22*, *IP-10*, *TNF-α* and *IL-8* was induced when non-lesional epidermal cells were cultured with CLA+ T cells and SE (Fig 2), whereas those genes were not detected in cells from healthy donors (data not shown). These results suggest that SE and CLA^+ T cells can induce some gene expression characteristic of the psoriatic lesion in epidermal cells obtained from non-lesional psoriatic skin.

Table I. Antibody array analysis of mediators produced during SE- induced CLA+ and epidermal cell activation in psoriasis

	Patient 6		Patient 7		Patient 8	
(pg/ml)[a]	CLA^+	CLA^-	CLA^+	CLA^-	CLA^+	CLA^-
IFN-γ	[illegible]	ND[b]	781	ND	[illegible]	ND
IL-17	[illegible]	ND	280	ND	1384	ND
IL-21	[illegible]	ND	[illegible]	ND	[illegible]	ND
IL-22	40	ND	ND	ND	2044	ND
IL-12p40	888	152	346	ND	129	ND
IL-12p70	116	ND	11	ND	38	ND
IL-23	ND	ND	ND	ND	ND	ND
IL-6	611	ND	36	ND	74	ND
TNF-α	87	ND	102	ND	140	ND
CXCL11	88	67	108	71	88	ND
CXCL10	1835	ND	281	ND	214	ND
CXCL9	[illegible]	567	[illegible]	100	[illegible]	ND
CXCL8	[illegible]	7828	[illegible]	4085	[illegible]	ND

a. Cytokine production after 5days activation of T cells and epidermal cells from three psoriatic patients
b. ND: Not detectable

To further characterize the production of mediators induced by the activation produced by SE in the culture of CLA^+ memory T cells and epidermal cells from psoriatic patients we performed a quantitative antibody array for thirteen cytokines (Table I) using 5 days supernatants of three psoriatic patients responding to SE. Cytokines produced by T-cells and epidermal psoriatic cells such as: IFN-γ, IL-17 and IL-21, IL-12p40, IL-12p70, IL-6, CXCL11, CXCL10, CXCL9, and CXCL8 were clearly increased by SE in the cultures of CLA+ T cells and epidermal cells in relation to CLA^-.

Conclusiones

The study shows that Streptococcal extract preferentially induces keratinocyte and CLA+ T cell (Th1, Th17, Th22) activation. IL-21 and other relevant mediators present in the psoriatic lesions can be induced by SE through circulating CLA+ memory T cells and epidermal cell interaction.

FUNDING The study was funded by FIS/ISCIII (Fondo de Investigación Sanitaria ISCIII) 2009.

Hospital del Mar

Parc de Salut MAR Barcelona