IgA plasma levels, but not IgG, against Streptococcus pyogenes identifies Anti-Streptolysin O negative chronic plaque psoriasis patients with increased specific CLA+ T cells IL17A and IL17F producers

Carmen de Jesús-Gil[1], Lidia Sans-de San Nicolas[1], Marta Ferran[2], Anca Chiriac[3], Antonio Celada[4], Ramon Pujol[2], Luis F Santamaria-Babi[1]

[1] Translational Immunology, Department of Cellular Biology, Physiology and Immunology, Faculty of Biology, Universitat de Barcelona, Spain.
[2] Department of Dermatology, Hospital del Mar, IMIM, Universitat Autònoma de Barcelona, Spain. [3] Department of Dermatophysiology, Apollonia University, Iasi, Romania.
[4] Macrophage Biology, Department of Cellular Biology, Physiology and Immunology, Faculty of Biology, Universitat de Barcelona, Spain.

Introduction

Streptococcus pyogenes tonsillar infection in early onset psoriasis patients can be associated with severe course of the disease and preferential biological therapy. Chronic plaque psoriasis patients have higher incidence of sore throat compared to controls. Although mucosal and cutaneous tissues are involved in psoriasis pathology, the interaction between their specific immune responses has not been deeply explored. The purpose of this study is identifying humoral immune response to *S. pyogenes* in psoriasis patients and address any connection with *in vitro* response in cocultures of CLA^+ T cells and epidermal cells after stimulation with *S. pyogenes* extract.

Material and Methods

The study included 27 non-treated psoriatic patients and 21 healthy controls, who previously gave informed consent. Each participant underwent a blood extraction and two skin punch biopsies. Homemade ELISA was developed to detect Streptococcus pyogenes specific IgA and IgG present in plasma. 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. $5x10^4$ CLA^+ or CLA^- T cells were cocultured with $3x10^4$ autologous epidermal cells and activated by 1μg/ml *Streptococcus pyogenes* extract (SE). After 5 days of culture, IL-17A, IL-17F, IFN-γ and IL-9 were measured by fluorescent bead-based immunoassay (FACs) or ELISA. Data are represented by scatter plots showing the mean (red bar) and 95% confidence interval (CI).

Results

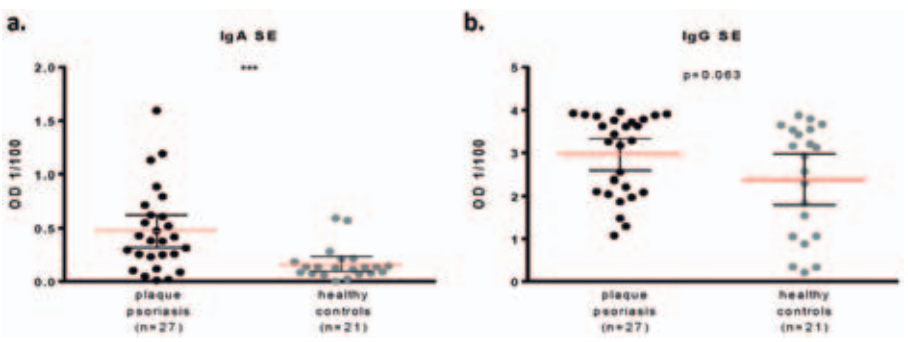

Figure 1. Plaque psoriasis patients show increased IgA levels against *S. pyogenes* compared to controls. ELISAs were performed with *Streptococcus pyogenes* extract (SE) as a substrate. Coated plates were incubated with diluted plasma from plaque psoriasis patients (n=27) and controls (n=15), and then with secondary antibodies against human IgA (a) and IgG (b). Optical density (OD) of plasma dilution 1/100, after background subtraction, is shown in vertical axis. In general, plaque psoriasis patients showed statistically significant increased levels of plasma anti-SE IgA but not IgG. Statistics lines are represented as medium with 95% confidence interval. Simple T-test was used to compare two different groups (*: p<0.05; **: p<0.01; ***: p<0.001).

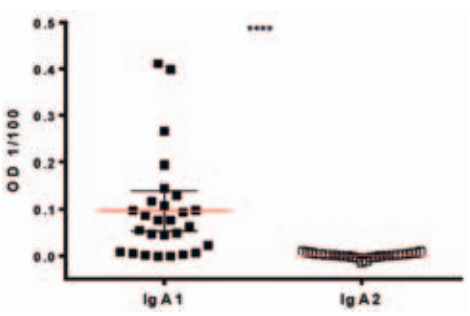

Figure 3. Anti-SE IgA response in psoriasis patients is associated to type 1 IgA. Specific IgA1 and IgA2 recognizing *S. pyogenes* extract (SE) were detected through ELISA in plasma collected from blood of plaque psoriasis patients (n=27). Optical density (OD) of 1/100 diluted plasma is reported. Psoriasis patients' IgA1 levels against SE proved to be higher than IgA2. Statistics lines are represented as medium with 95% confidence interval. Simple T-test was used to compare two different groups (*: p<0.05; **: p<0.01; ***: p<0.001).

Plaque psoriasis patients (n=27)	ASO	PASI	Length of disease (months)	Age of onset	HLA Cw6			Flare associated to streptococcal infection		
					Positive	Negative	UK	Yes	No	UK
Mean	99,95	15,13	34,14	34,14	40,74%	51,85%	7,41%	-	100%	-
SD	74,55	6,80	14,66	60,53	-	-	-	-	-	-

Table 1. Clinical features of plaque psoriasis patients' cohort. ASO: Anti-streptolysin O antibody titer, PASI: Psoriasis Area Severity Index, SD: standard deviation; UK: unknown.

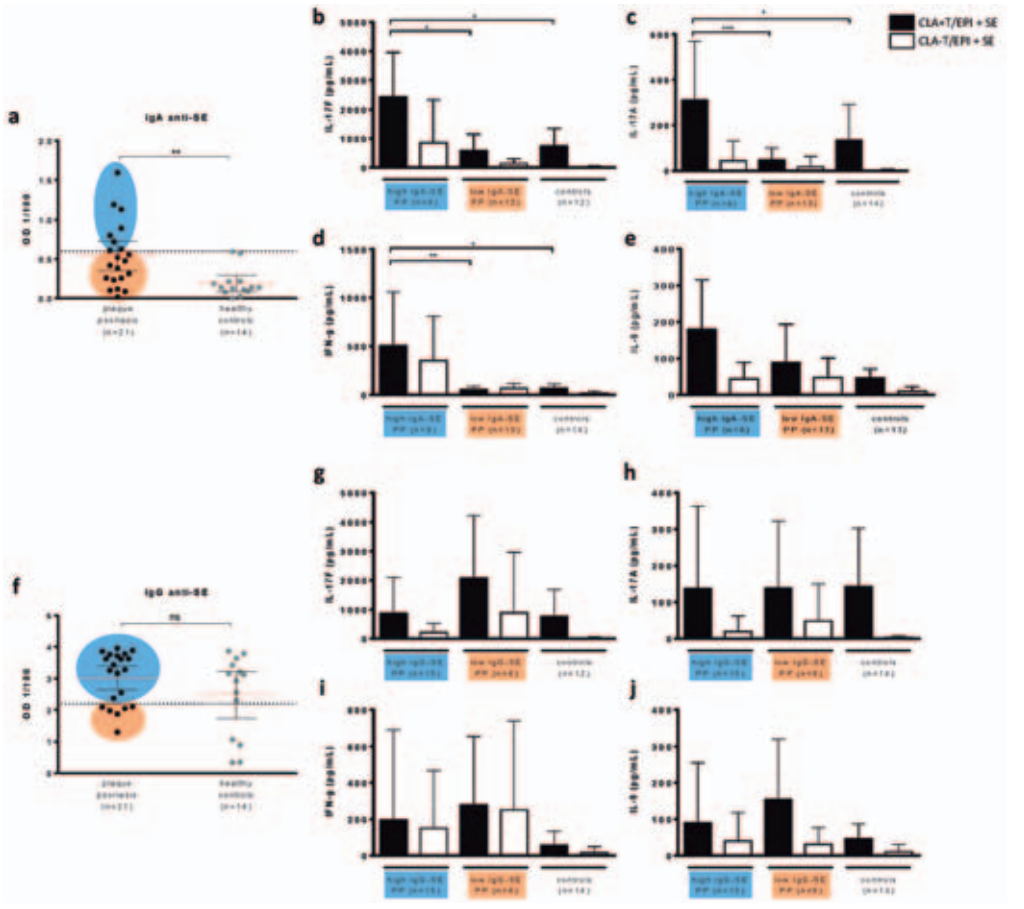

Figure 2. Plaque psoriasis patients with higher anti-SE IgA levels have stronger SE-dependent IL17 response *in vitro*. Cocultures of CLA^+ or CLA^- T cells with autologous epidermal cells were left untreated or stimulated with SE for 5 days. Afterwards, supernatants were collected and IL17F, IL17A, IFN-γ and IL9 concentrations were determined by cytometric bead assay or ELISA. According to their specific IgA (a) and IgG (f) anti-SE plasma levels, plaque psoriasis patients were classified in two groups: low or high. Importantly, PPP with high plasma IgA anti-SE levels have stronger SE dependent *in vitro* induction of IL17F (b), IL17A (c) by CLA^+ T cells, but not CLA^- T cells, when compared to plaque psoriasis patients with low IgA anti-SE or controls. SE-induced IFN-γ (d) response is also higher in patients with higher anti-SE IgA levels but similarly in CLA^+ and CLA^- T cell cultures. Finally, IL-9 levels (e) show similar tendency to IL17F and IL17A induction in CLA^+ T cells by patients with high IgA-SE. Interestingly, not association is observed between anti-SE IgG levels and the analyzed cytokines (g, h, i, j). Statistics lines are represented as mean with 95% confidence interval. Simple T-test was used to compare two different groups (*: p<0.05; **: p<0.01; ***: p<0.001).

Conclusions

The combined analysis of IgA and CLA^+ T cell response to the same antigen in psoriasis constitute a relevant tool to understand how microbial exposure in mucosa influence psoriasis trigger and development. Lilja M. et al had previously reported a direct correlation between secretory IgA-coated *Streptococcus pyogenes* and duration of tonsillitis. Our results suggest that microbe specific IgA could be considered a potential new biomarker to stratify chronic plaque psoriasis patients and a feasible tool to understand patients heterogeneity, which may shape their clinical course and response to treatments.

BIBLIOGRAPHY: Lilja M, Silvola J, Bye HM, Räisänen S, Stenfors LE. SIgA- and IgG-coated Streptococcus pyogenes on the tonsillar surfaces during acute tonsillitis. Acta Otolaryngol. 1999.

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