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Specific IgA and CLA+ T-cell IL-17 response to *Streptococcus pyogenes* in psoriasis

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ABBREVIATIONS

ASO: anti-streptolysin O antibody

CLA: cutaneous lymphocyte antigen

EOP: early onset psoriasis

GAS: group A streptococci

GP: guttate psoriasis

PP: plaque psoriasis

SE: *Streptococcus pyogenes* extract

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ABSTRACT

Streptococcus pyogenes tonsillar infection is well-known to trigger and exacerbate psoriasis lesions in both guttate and plaque forms of the disease. Although mucosal and cutaneous tissues are closely involved in psoriasis pathology, the interaction between their specific immune responses has not been deeply explored. This work aims to address and characterize the presence of humoral responses against *Streptococcus pyogenes* in psoriasis patients and its putative association with cytokine responses detected *in vitro* in our psoriasis ex vivo model, based on the coculture of CLA+/- T cells with autologous epidermal cells. Psoriasis patients presented increased IgA response to *S. pyogenes* when compared to control subjects. Surprisingly, in plaque psoriasis patients, despite being negative for anti-streptolysin O antibody titer, IgA plasma levels against *S. pyogenes* correlated with CLA+ T cell dependent IL-17F response *in vitro*. Not association is observed for IgG levels in plaque psoriasis. Similar association is observed for IgA anti-SE and IL-17A in guttate psoriasis patients. We propose *S. pyogenes* specific IgA as a potential new perspective for better understanding the role of *S. pyogenes* in psoriasis development.

KEY WORDS

Plaque psoriasis, guttate psoriasis, IgA, IgG, *Streptococcus pyogenes*, CLA, IL-17

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INTRODUCTION

Psoriasis is a chronic inflammatory T cell-mediated skin disease, consequence of a combination of genetic and environmental factors (Hawkes et al. 2017). The role of microorganisms in psoriasis development has been discussed for years, and several bacteria, fungi and virus have been associated with the either the onset or new flares of the disease (Fry et al. 2013). *Streptococcus pyogenes* tonsillar infection is the strongest environmental factor linked to trigger and/or to exacerbate psoriasis skin lesions, not only in guttate form of the disease (Norrlind 1955), but also in chronic plaques (Cohen Tervaert and Esseveld 1970; Thorleifsdottir et al. 2017; Wardrop et al. 1998). In fact, tonsillectomy has proved to ameliorate psoriatic symptoms in patients, even leading to disease clearance in some cases (Thorleifsdottir et al. 2017). Patients with early onset of psoriasis (EOP) are more likely to develop flares after an upper respiratory tract infection, to have a family history psoriasis and to present higher disease severity (Theodorakopoulou et al. 2016). These EOP patients tend to require systemic treatments, being more likely to receive biologics and suggesting that streptococcal throat infection somehow conditions psoriasis development and the clinical management of the disease. However, the exact pathogenetic links between *Streptococcus pyogenes* tonsillar infection and psoriasis are not fully elucidated. Streptococci are able to be internalized and to survive into tonsillar cells as intracellular structures. This immune evasion mechanism allows *S. pyogenes* to remain within the organism, giving rise to a reservoir of persistent putative pathogenic antigens (Österlund et al. 1997). The well-established association between *S. pyogenes* throat sore and psoriasis has led to the hypothesis that cutaneous lesions are mediated by T cells originated in tonsils that later migrate to the skin, where they activate and secrete pro-inflammatory cytokines (Valdimarsson et al. 2009). This hypothesis is further supported by the demonstration of identical *TCRVB* gene rearrangements in cutaneous and tonsillar T cells isolated from the same patient (Diluvio et al. 2006).

There is a subset of memory T cells whose activity is confined to cutaneous tissue, which are identified by the expression of the cutaneous lymphocyte-associated antigen (CLA). During cutaneous inflammation, these skin homing T cells are able to recirculate between skin lesions and blood and are considered peripheral biomarkers of human T-cell mediated cutaneous diseases (Ferran et al. 2013).

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Interestingly, psoriasis patients have increased levels of CLA+ T cells expressing IL-23 receptor in blood and tonsils (Sigurdardottir et al. 2013). Variations in the number and phenotype of circulating CLA+ T cells are closely related to improved clinical outcome of psoriasis patients (Thorleifsdottir et al. 2012). Our group has established a psoriasis *ex vivo* model based on the coculture of circulant CLA+ or CLA- T cells and autologous epidermal cells, isolated from lesional psoriasis patients' skin biopsies, which are then activated by a *S. pyogenes* extract, leading to specific IL-17 response in CLA+ but not CLA- T cells cultures (Ferran et al. 2013; Ruiz-Romeu et al. 2018; Ruiz-Romeu et al. 2016).

As considered a T-cell mediated disease, the study of the cellular immune response in psoriasis has been widely approached. Nevertheless, few results addressing the humoral immune response against *Streptococcus pyogenes* in psoriasis patients have been undertaken. It has been reported that plaque psoriasis patients had increased plasma IgG levels recognizing *S. pyogenes* heat shock proteins (Pérez-Lorenzo et al. 2003), specifically the 60KDa protein named rHSP60Sp compared to guttate psoriasis patients and controls (Cancino-Díaz et al. 2004), which was associated with higher PASI and ASO titter. More recently, non-treated chronic plaque psoriasis patients showed increased blood levels of IgG against secreted *S. pyogenes* proteins, but not cellular components compared to control subjects (El-Rachkidy et al. 2007). Notably, palatine tonsils are key components of the mucosal immune system at the oropharyngeal tract and are commonly infected by streptococci in psoriasis. Although mucosal and cutaneous tissues are closely involved in psoriasis pathology, the interaction between their specific immune responses has not been deeply explored. The role of IgA as the major antibody participating in humoral mucosal immunity may be of great interest in psoriasis, despite presence of B cells is not required in psoriasis pathogenesis (Thomas et al. 2019). Lately, Thomas J et al showed increased IgA blood levels in plaque psoriasis patients, that positively correlated with IgA producing plasma cells (CD19+ CD24- CD38+ CD138+) and Psoriasis Area Severity Index (PASI). However, less is known about the presence of specific IgA against *S. pyogenes* in those patients. This work aims to address and characterize the presence of mucosal immune response against *Streptococcus pyogenes*

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in psoriasis patients and its putative association with the IL-17A, IL-17F, IFN-γ and IL-9 cytokine response detected *in vitro* in our psoriasis model.

RESULTS

Psoriasis patients show elevated anti-SE IgA compared to control subjects

To study the psoriasis-specific humoral immune response against a relevant microbial trigger of the disease such as *Streptococcus pyogenes*, plasma from psoriasis (n=62), psoriatic arthritis (n=13), atopic dermatitis (n=17) patients, and healthy controls (n=21) were collected and analyzed by ELISA against the microorganism extract. Psoriasis patients have proved to have higher immunoglobulins (Ig) A levels against *S. pyogenes* extract (SE) when compared to atopic dermatitis and controls individuals, but similar to psoriatic arthritis (Figure 1a). However, differences in anti-SE IgG plasma levels are not detected within psoriasis, psoriatic arthritis, atopic dermatitis and healthy controls subjects, (Figure 1c). Interestingly, according to their subtype of disease, plaque (n=34) and guttate (n=28) psoriasis patients show increased anti-SE IgA both relative to atopic dermatitis and control subjects (Figure 1b, d). Nonetheless, neither plaque nor guttate psoriasis showed higher anti-SE IgG plasma levels compared to control subjects (Figure 1d). Of important note, anti-SE IgG was detected in plasma from many control subjects at similar levels to those in psoriasis patients, whereas anti-SE IgA was hardly detectable in controls.

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Increased anti-SE IgA levels in ASO negative plaque psoriasis

Anti-streptolysin O antibody (ASO) titer is an easy and frequently used tool to assess recent group A *Streptococcus* infection. Considering that 71,43% of guttate psoriasis flares in our first cohort were associated to *S. pyogenes* infection and they present elevated ASO titer (Table 1), increased anti-SE IgG plasma levels were expected in these patients (n=28). Surprisingly, higher anti-SE IgA levels compared to control subjects were observed too. However, although our cohort of plaque psoriasis patients (n=34) showed negative ASO titer (<200U/ml) and their flares were not associated to clinical signs of *S. pyogenes* infection (Table 1), we were able to detect statistically significant increased anti-SE IgA levels in plasma compared to controls too. Besides, plaque psoriasis patients showed a more severe disease according to Psoriasis Area and Severity Index (PASI) (mean value of 14,58 (±6,55

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SD) versus 7,10 (±3,14) in guttate psoriasis, p value <0,0001), longer clinical evolution (26,69 (±62,73) months versus 2,71 (±7,12), p value <0,01), slightly later onset of the disease and less association to the presence of HLA-Cw6 allele (Table 1) when compared to individuals with guttate psoriasis. Altogether, our results point out that some plaque psoriasis patients have been exposed to *S. pyogenes,* despite having negative clinical signs of recent infection, and that elevated anti-SE IgA levels may be a sign of chronic immune response against *S. pyogenes*.

Psoriasis patients anti-SE IgA plasma levels, but not IgG, correlate with CLA+ dependent-IL-17 response *in vitro*

Next, we wanted to see whether anti-SE IgA or IgG plasma levels were associated to *in vitro* response to *S. pyogenes* (SE) activation in cocultures of CLA+/- T cells together with autologous epidermal cells. For that purpose, cytokines such as IL-17A, IL-17F, IFN-γ and IL-9 were quantified in culture supernatant from some plaque psoriasis (PP, n=27), guttate psoriasis (GP, n=26) and healthy control (n=14) subjects. Interestingly, a significant direct correlation (r = 0,501; p value = 0,0056) is established between IgA anti-SE plasma levels and CLA+ T cells dependent IL-17F response to *S. pyogenes* in plaque psoriasis patients (Figure 2a). This association is not observed for CLA- T cells IL-17F response. A direct correlation is also observed between IgA anti-SE and IFN-γ induction in both CLA+ and CLA- T cells cultures (Figure 2c) for plaque form of the disease. However, there is not clear association between IgA anti-SE plasma levels and IL-17A and IL-9 responses *in vitro* for plaque psoriasis patients (Figure 2b, d). Regarding guttate psoriasis patients we observed distinct patterns of association between IgA anti-SE and cytokines induced in culture. On the one hand, there is no association with neither IL-17F nor IFN-γ (Figure 2e, g), as observed for plaque psoriasis. On the contrary, there is clear direct correlation between IgA anti-SE and CLA+ T cells dependent IL-17A induction (r = 0,586; p value = 0,0017), but not in CLA- T cells (Figure 2f). And also, a direct correlation with IL-9 levels for both CLA+ and CLA- T cells (Figure 2h). Notably, no correlation was found with IgG anti-SE plasma levels (Supplementary Table 1). Altogether, plasma levels of anti-SE IgA –but not IgG– from plaque psoriasis patients revealed *in vitro* IL-17 response of CLA+ T cell and epidermal cell cocultures stimulated with *S. pyogenes* extract.

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Anti-SE IgA response in psoriasis patients is associated to type 1 IgA

Having proved the presence of IgA recognizing *S. pyogenes* in plasma from psoriasis patients, even in those with non-associated sore throat infection, we seek to explore the putative source of mucosal immune response against these microorganisms by analyzing IgA subtypes. Plasma from psoriasis patients was then analyzed by a similar ELISA but using anti-human IgA1 or IgA2 as secondary antibodies. We observed that psoriasis patients showed higher anti-SE IgA1 than IgA2 subtype in plasma (Figure 3a). Although optical density values detected were lower than for IgA, positive and negative controls proved that antibodies worked in each ELISA (Figure S1). When looking at the distribution of specific IgA1 and IgA2 against *S. pyogenes* between the different forms of the disease, we observe that both plaque (Figure 3b) and guttate psoriasis (Figure 3c) patients have slightly increased levels of anti-SE IgA1 compared to IgA2.

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DISCUSSION

Streptococcus pyogenes infection can influence psoriasis development and evolution (De Jesús-Gil et al. 2018; Norrlind 1955). Our results show that IgA against *S. pyogenes* is present in plasma from plaque and guttate psoriasis patients and its levels are directly associated to CLA+ T cell dependent IL-17 response in our *ex vivo* model of the disease, that correlates with the clinic (Ruiz-Romeu et al. 2018).

We found that our cohort of plaque psoriasis patients, with no history of streptococcal mediated tonsillitis and negative ASO titer, had developed humoral response against *S. pyogenes*. Despite what other clinical parameters may indicate, anti-SE IgA levels found in plaque psoriasis patients' plasma proved that these patients have been exposed to this microorganism. Although B cells are not indispensable for psoriasis development, as proved by a case of full psoriasis vulgaris phenotype in a patient with common variable immunodeficiency (CVID) (Thomas et al. 2019), the presence of anti-SE IgA response may condition disease development or progression. More surprisingly, in those plaque psoriasis patients values for IgA-SE correlated with CLA+ T cells mediated IL-17F response *in vitro*. However, higher but not significantly increased anti-SE IgG blood levels were detected in plaque psoriasis patients compared to control subjects, even though previous works showed increased blood levels of IgG recognizing secreted *S. pyogenes* proteins (Cancino-Díaz et al. 2004; Pérez-Lorenzo et al. 2003), probably due to the different source of antigen used to determine IgG against *S. pyogenes*. Increased levels of IgA anti-SE were also detected in guttate psoriasis patients when compared to healthy individuals, but these were directly correlated with IL-17A secretion by CLA+ T cells *in vitro* after *S. pyogenes* stimulation.

Although the association between IL-17 and IgA in psoriasis has not been previously reported to our knowledge, the link between IL-17 and immunoglobulins had been previously described in animal models. IL-17 deficient (IL-$17^{-/-}$) mice showed impaired immunoglobulin response in allergic (Nakae et al. 2002) and autoimmune (Nakae et al. 2003) disease models. Despite molecular mechanisms underlying this effect remain to be fully understood, *in vitro* studies suggest that IL-17 may be indirectly involved in antibodies production by enhancing B cell activators by other cells (Shibui et al.

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2012) and that IL-17A/IL-17RA axis modulates B cell migration within the germinal centers (Ferretti et al. 2016). Most of the evidence about the association between Th17 and IgA *in vivo* derives from studies of the intestinal immunity, where Th17 cells have proved to be relevant for IgA isotype switch and secretion (Cao et al. 2012; Hirota et al. 2013). Interestingly, Christensen *et al* have recently demonstrated how parentally primed Th17 cells induce antigen specific IgA in lungs from immunized mice (Christensen et al. 2017), confirming the link between Th17 cells and IgA responses in the airways too. Regarding the published data, we hypothesize that long term exposure to *S. pyogenes* activated Th17 cells could induce IgA synthesis and secretion in tonsils. However, proving the molecular mechanism behind remain a limitation of this study.

Tonsils from psoriasis patients are more frequently infected by group A *Streptococcus* (GAS) than those from control subjects, and it often precedes the appearance of psoriatic lesions in skin. Recently, the development of GAS extra- and intracellular biofilms has been reported in tonsillectomy specimen from psoriasis, supporting the microbial role in the pathogenesis of the disease (Allen et al. 2018). Biofilm formation within the tonsils may be responsible for the recurrent relapses over time, since antistreptococcal agents are not able to penetrate them, and may explain why not all patients present upraised ASO titers, leading to a lack of *S. pyogenes* infection serum markers (Kim et al. 2010). Given the relevant role of palatine tonsils in *S. pyogenes* infection in psoriasis and as they are key components of the mucosal immune system, we believed the study of IgA against this microorganism in patients would be of great interest. Of note, analyzing presence of *S. pyogenes* in the tonsils from our cohort of patients and compare the results with those of IgA anti-SE in plasma remain a limitation of our study. Our results postulate anti-SE IgA blood levels as a new parameter of exposition to *S. pyogenes*, which is also present in patients with negative ASO titers, whose disease is nowadays considered to be independent to *S. pyogenes* infection.

Previous published data from out group showed CLA+ T cell dependent Th17 responses after *S. pyogenes* activation *in vitro* in psoriasis patients (De Jesús-Gil et al. 2018; Ruiz-Romeu et al. 2016). Surprisingly, we found a direct correlation between IgA anti-SE and IL-17F -for plaque psoriasis- or IL-17A -for guttate psoriasis- responses *in vitro* when CLA+ T cells were cocultured with autologous

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epidermal cells and activated with *S. pyogenes* extract. Considering that IgA anti-SE was detected in plasma from patients with no history of streptococcal throat infection and negative ASO titter, we thought that these patients could have been exposed to this microorganism in an alternative manner. For that reason, type 1 and type 2 IgA against *S. pyogenes* were analyzed. Of note, although IgA1 is the most common subtype present in circulation, the fact that IgA response was type 1 -and type 2 was completely absent- may indicate that the origin of this humoral immune response took place in the upper respiratory tract (Pakkanen et al. 2010). During disease development, increased levels of CD4+ and CD8+ CLA+ T cells expressing IL-23 receptor are found in blood and tonsils (Sigurdardottir et al. 2013). Coexistence of *S. pyogenes* and CLA+ T cells in tonsils lead to the hypothesis that cutaneous lesions are mediated by T cells initially originated in tonsils that then migrate to the skin, where the pro-inflammatory environment induces their activation and therefore secretion of IL-17 cytokines (Valdimarsson et al. 2009). Our findings support this linkage between mucosal tissue -palatine tonsils-, microbe -*S. pyogenes*- and skin immune response -CLA+ T cells-.

. Although mean values of IgA anti-SE OD are similar in plaque and guttate patients, the number of patients whose values are over this mean are higher in plaque guttate patients. This uneven distribution may reflect the differences between these two types of disease. On the one hand, guttate psoriasis is an acute form, closely related to *S. pyogenes* upper respiratory tract infection and generally associated with better prognosis; however, some patients evolve to chronic plaque form of the disease. Few long-term follow-up studies have attempted to identify guttate psoriasis patients that are likely to progress to chronic forms of the disease according to clinical and laboratory data, but not clear parameters have been established to date (Ko et al. 2010; Martin et al. 1996; Pfingstler et al. 2016). We hypothesize that presence of elevated anti-SE IgA levels in guttate psoriasis patients could help tracking progression towards plaque form of the disease. However, long-term follow-up studies are required to confirm this idea. On the other hand, plaque psoriasis flares can also be exacerbated by *S. pyogenes* infection (Cohen Tervaert and Esseveld 1970; Wardrop et al. 1998) and tonsillectomy has been proved to be an effective treatment for the cutaneous lesions in some patients (Thorleifsdottir et al. 2017). Anti-Streptolysin O (ASO) antibody titer is an easy and frequently tool used to asses group A

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Streptococcus infection, which begin to increase around one week and peak three to six weeks after infection (Gerber et al. 2009). Nonetheless, the course of plaque psoriasis disease can take several months or even years, leading to negative ASO titers that may cover the presence and relevance of *S. pyogenes* infection in those patients. Indeed, previous studies reported that secretory IgA coated *S. pyogenes* increased in chronic tonsillitis, whereas IgG coated pathogens levels increased in acute forms and remained equal despite disease duration (Lilja et al. 1999). These findings support our observation of anti-SE IgA in psoriasis patients.

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MATERIAL AND METHODS

Patients

This study was performed with human samples and in accordance with the Declaration of Helsinki. A total of 62 non-treated psoriasis patients and 21 healthy individuals were enrolled. All participants contributed voluntarily and provided written informed consent and human material collection has been approved by the Comité Ético de Investigación Clínica (CEIC) from the Parc Salut Mar (Hospital del Mar, Barcelona). Psoriatic samples were from patients with guttate and plaque lesions, without any age or sex restriction. Patients that received any systemic treatment for the last 6 weeks were excluded in order not to obtain underestimated cellular activation. Psoriasis patients and healthy subjects underwent two skin biopsies, which were punched in lesional skin in psoriatic patients, and a blood extraction. Additionally, plasma samples from psoriatic arthritis (n=13) and atopic dermatitis (n=17) were analyzed as examples of other chronic inflammatory diseases.

ELISA against *Streptococcus pyogenes*

S. pyogenes extract (SE) was as previously described by Baker BS et al (BAKER et al. 1991). Briefly, group A beta-haemolytic streptococcus was isolated from throat swabs of psoriasis patients and cultured in liquid Tood-Hewitt medium for 24h at 37ºC, followed by four washes with PBS. Finally, bacteria were adjusted to 1mg protein/ml, sonicated and maintained in sterile conditions. SE was diluted in coating buffer (50mM $NaHCO_3$ in miliQ H_2O, pH=9.6) to a final protein concentration of 5ug/ml and incubated for 3 hours at 37ºC. The same extract was used for all described experiments. Wells were then washed five times with PBS, blocked with 5% skimmed milk powder in PBS overnight at 4ºC, and then washed again five times in PBS-Tween 0,05%. Plasma samples were diluted 100-fold in PBS-1% skimmed milk, added to coated wells and incubated 2 hours at 37ºC. Wells were again washed five times with PBS-T and incubated with alkaline phosphatase labelled goat anti-human IgA, IgA1, IgA2 or IgG (SIGMA-Aldrich, St Louis, Mo) diluted 1:4000 in PBS-1% skimmed milk for 90 min at 37ºC. Following further five washes with PBS-T 0,05%, p-nitrophenyl phosphate substrate (SIGMA-Aldrich) was incubated for 30 minutes at room temperature and finally

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enzymatic reaction was stopped by adding NaOH 3M solution. Plates were read within the next 30 minutes at 405 nm and 570 nm for background signal extraction, according to suppliers' instructions. The titer of reactive IgA, IgA1, IgA2 or IgG was taken as the OD_{405nm} after background signal (OD_{570nm}) and negative control well signal, incubated with only PBS-1% skimmed milk, subtraction. Positive control wells were coated with human IgA/G isotype controls (Invitrogen), incubated with PBS as primary antibody and the corresponding secondary antibody before substrate addition.

Circulating memory T cell and epidermal cell isolation

Peripheral blood mononuclear cells were isolated by Ficoll gradient (GE Healthcare, Princeton, NJ) and, after subsequent immunomagnetic separations (Miltenyi Biotech, Bergisch Gladbach, Germany) memory $CD45RA^-$ CLA^+ and CLA^- T cells were purified as previously described(Santamaria Babi et al. 1995). Punch skin biopsies (4-6 mm) were incubated overnight in dispase (Corning, Bedford, Mass) at 4ºC, then epidermal sheet was peeled off from the dermis. The epidermis was cut in smaller pieces and incubated in trypsin solution (Biological Industries, Kibbutz Beit Haemek, Israel) for 15 minutes at 37ºC. Equal volume of RPMI media (SIGMA-Aldrich, St Louis, Mo) containing 10% of FBS (Gibco, Grand Island, NY) was added to inhibit trypsin action. Epidermal tissue was then mechanically disaggregated by gently up and down pipetting. Epidermal cell suspension (Epi) was transferred to fresh media [RPMI, 10% FBS, 1% penicillin-streptomycin (SIGMA-Aldrich)], and the remaining tissue leftovers were discarded.

Cultures and pathogen activation

Ex vivo cocultures consisted of $5x10^4$ CLA^+ or CLA^- T cells plated together with $3x10^4$ autologous epidermal cells (CLA^+/Epi or CLA^-/Epi, respectively), in 96-well flat-bottom plates (SIGMA-Aldrich, St Louis, Mo), in the culture media described above. Cocultures were left untreated or activated with *S. pyogenes* extract (SE) at 1ug/ml final well concentration. After 5 days of culture, supernatants were collected and kept frozen at -20ºC for later cytokine quantification.

Cytokine quantification

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Multiplex fluorescent bead-based immunoassays were used to measure IL-17A and IFN-γ (Diaclone SAS, Besançon, France) and IL-17F (BD Bioscience, Franklin Lakes, NJ) concentration in collected culture supernatants. IL-9 concentration was quantified by using pre-coated ELISA kits (Biolegend, San Diego, CA).

Statistical Analysis

Data are generally represented as the mean and 95% confidence interval (CI). Differences between two groups were analyzed by the Mann-Whitney test. Differences were considered significant at a P value of less than 0.05 and represented by symbols as follows: (*): $P<0.05$; (**): P<;0.01; and (***): $P<0.001$.

DATA AVAILABILITY

No datasets were generated or analyzed during the current study.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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FIGURE AND TABLE LEGENDS

Figure 1. Psoriasis patients show increased plasma IgA levels against *S. pyogenes* extract. Specific immunoglobulins A and G recognizing *S. pyogenes* extract (SE) were detected through ELISA in plasma collected from blood of psoriasis (n=62), psoriatic arthritis (n=13) and atopic dermatitis (n=17) patients and healthy controls (n=21). Optical density (OD) of 1/100 diluted plasma is reported. Psoriasis patients' IgA **(a)** and IgG **(c)** levels against SE are shown. According to their subtype of disease **(b, d)**, guttate and plaque psoriasis show differential anti-SE immunoglobulin profile. Statistics lines are represented as mean with 95% confidence interval. Mann-Whitney test was used to compare two different groups (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$). PsO: psoriasis; PsA: psoriatic arthritis; AD: atopic dermatitis; HC: healthy controls; p-PsO: plaque psoriasis; g-PsO: guttate psoriasis.

Figure 2. Psoriasis patients anti-SE IgA plasma levels correlate with CLA+ dependent-IL-17 response *in vitro.* IL-17F (a, e), IL-17A (b, f), IFN-γ (c, g) and IL-9 (d, h) concentration were measured in culture supernatants after 5 days of *S. pyogenes* stimulation and correlated to levels of anti-SE IgA in plaque (n=27) and guttate (n=26) psoriasis. Statistics lines are represented as linear regression and Pearson r values are indicated. P values are represented as *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

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 psoriasis patients (n=62). Optical density (OD) of 1/100 diluted plasma is reported. Psoriasis patients' IgA1 levels against SE proved to be higher than IgA2 (**a**). This preferential anti-SE IgA1 over IgA2 response is maintained for plaque (**b**) and guttate (**c**) psoriasis patients. Statistics lines are represented as mean with 95% confidence interval. Mann-Whitney test was used to compare two different groups (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$).

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<u>Table 1. Clinical features of guttate and plaque psoriasis patients.</u> Mean values (± standard deviation) or total number (percentages) are shown. Mann-Whitney test was used to compare numerical variables (*: p<0.05; **: p<0.01; ***: p<0.001). ASO: Anti-streptolysin O antibody titer, PASI: Psoriasis Area Severity Index, NA: not assigned.

		Plaque psoriasis (n=34)	Guttate psoriasis (n=28)	P value
ASO		99,36 (±67,74)	442,3 (±260,9)	< 0,001
PASI		14,58 (±6,55)	7,10 (±3,14)	< 0,001
Length of disease (months)		26,69 (±62,73)	2,71 (±7,12)	0,0084
Age of onset		37,46 (±14,33)	27,19 (±11,98)	0,0316
HLA Cw6 n (%)	**Positive**	11 (32,35%)	22 (78,57%)	NA
	Negative	14 (41,18%)	2 (7,14%)	NA
	Unknown	9 (26,47%)	4 (14,19%)	NA
Flare associated to Streptococcal infection n (%)	**Yes**	-	20 (71,43%)	NA
	No	34 (100%)	3 (10,71%)	NA
	Unknown	-	5 (17,86%)	NA

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Cytokine	Culture condition	Statistic	Plaque psoriasis (n=29)		Guttate psoriasis (n=26)		Healthy controls (n=14)	
			IgA-SE	IgG-SE	IgA-SE	IgG-SE	IgA-SE	IgG-SE
IL-17F	CLA+/EPI/SE	Pearson r	0,5011	0,0234	0,3154	0,2742	-0,308	0,0004
		P value	0,0056	0,9018	0,1165	0,1753	0,3288	0,9990
	CLA-/EPI/SE	Pearson r	0,2908	-0,0759	0,3663	0,2449	0,4681	0,1487
		P value	0,1259	0,6953	0,0657	0,2279	0,1248	0,0221
IL-17A	CLA+/EPI/SE	Pearson r	0,1894	0,189	0,5859	0,359	-0,0667	0,2251
		P value	0,3251	0,367	0,0017	0,071	0,8209	0,4392
	CLA-/EPI/SE	Pearson r	0,1874	-0,087	0,3732	0,208	0,2114	0,1944
		P value	0,3293	0,655	0,0604	0,308	0,4681	0,5054
IFN-γ	CLA+/EPI/SE	Pearson r	0,4643	0,005	0,1603	0,116	-0,0346	-0,1731
		P value	0,0112	0,718	0,4340	0,089	0,9066	0,5540
	CLA-/EPI/SE	Pearson r	0,4149	0,005	0,1739	0,070	0,4917	0,0757
		P value	0,0252	0,703	0,3956	0,191	0,0742	0,7970
IL-9	CLA+/EPI/SE	Pearson r	0,2787	-0,018	0,5650	0,4242	-0,0571	-0,0366
		P value	0,1432	0,9247	0,0026	0,0308	0,8464	0,9012
	CLA-/EPI/SE	Pearson r	0,1250	0,1637	0,4338	0,3760	0,1359	0,2818
		P value	0,5182	0,3961	0,0268	0,0584	0,6431	0,3291

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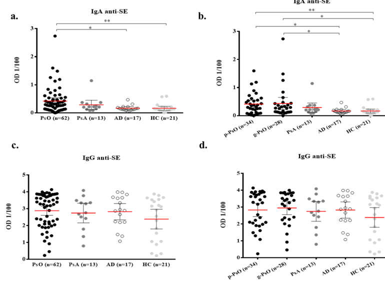

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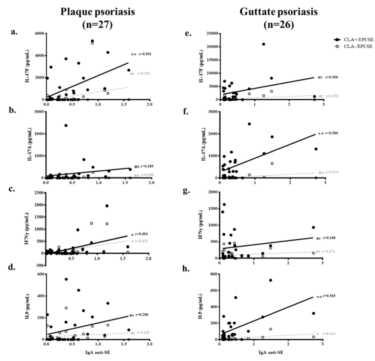

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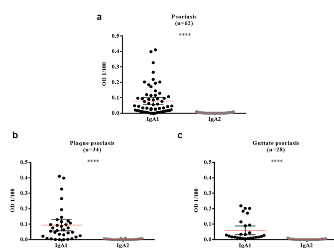

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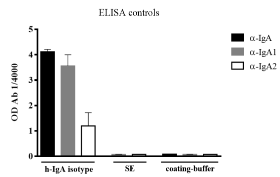

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SUPPLEMENTARY MATERIAL

Table S1. Correlation between IL-17F, IL-17A, IFN-γ and IL-9 responses *in vitro* and plasma immunoglobulins against *S. pyogenes* from plaque and guttate psoriasis patients and healthy controls. Pearson r and p values are indicated for each culture condition.

Figure S1. Positive and negative controls in our handmade ELISA. Plates were coated with human IgA isotype as positive control, then incubated with PBS-1% milk and finally with secondary antibodies targeting IgA, IgA1 and IgA2. For negative controls, plates were coated with *S. pyogenes* extract or only coating-buffer, then incubated with PBS-1% milk and finally with enzyme-conjugated secondary antibodies.

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