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IgE and FcεRI are highly expressed on innate cells in psoriasis

Running title: IgE involved in psoriasis

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Conflicts of interest: None declared.

What's already known about this topic?

Serum total IgE levels are elevated in psoriatic patients.

What does this study add?

The expression of IgE and FcεRI was particularly enhanced on innate cells in psoriatic lesions. The numbers of IgE+ and FcεRI+ cells in psoriatic lesions significantly decreased after treatment with ustekinumab.

Abstract

Background: Although elevated serum IgE levels have been reported in psoriasis, the role of IgE in psoriasis still needs to be clarified.

Objective: To analyze serum total IgE levels as well as the presence and distribution of IgE and FcεRI in psoriatic lesions, and investigate alteration of IgE and FcεRI after successful systemic treatment.

Methods: Total serum IgE levels were determined using ELISA. The expression and localization of IgE and FcεRI were investigated using immunohistochemistry and double-immunofluorescence.

Results: Elevated total serum IgE levels were found in 39% patients with psoriasis. The levels of total serum IgE were significantly higher in male patients compared to females. Furthermore, total serum IgE levels decreased after successful systemic treatment. A positive correlation between IgE+ and FcεRI + cells and a significant increase of these cells was found in psoriatic lesions as compared to normal skin. Interestingly, IgE+ and FcεRI + cells decreased significantly after successful therapy with ustekinumab. This article is protected by copyright. All rights reserved.

IgE and FcεRI were co-expressed on mast cells, epidermal Langerhans cells, dermal DCs, macrophages and a few neutrophils.

Conclusions: IgE might participate in the development of psoriasis by activating FcεRI-bearing cells.

Abbreviations

APCs; antigen presenting cells

IgE; Immunoglobulin E

DCs; dendritic cells

IDECs; inflammatory dendritic epidermal cells

FcεRI; Receptor I for the Fc region of immunoglobulin E

LS; lesional skin

N.elastase; neutrophil elastase

NKs; natural killer cells

NLS; non-lesional skin

Introduction

Psoriasis is a chronic relapsing inflammatory skin disease, characterized by interaction of keratinocytes with various innate and adaptive immune cells including distinct subtypes of dendritic cells (DCs), macrophages, neutrophils, mast cells and T cells including T helper (Th)1, Th17, and Th22 cells¹.

IgE plays a crucial role in innate and acquired immunity by binding to IgE receptors on immune cells. Two IgE receptors have been identified: the high-affinity Fc receptor (receptor I for the Fc region of IgE, designated FcεRI) and the low-affinity Fc receptor (FcεRII; also known as CD23). FcεRI is a tetrameric

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membrane protein complex consisting of 4 chains ($\alpha\beta\gamma_2$) and is constitutively expressed on mast cells and basophils. Its activation leads to cellular degranulation and release of various mediators (e.g. histamine, tryptase, leukotrienes), which play a major role in allergic diseases. In addition, Fc ϵ RI is also expressed as a trimeric complex ($\alpha\gamma_2$) on different DC subsets and macrophages. Activation of Fc ϵ RI on these cells has been reported to facilitate antigen presentation and promote cutaneous inflammation².

Previously, numerous studies have shown elevated serum total IgE levels in patients with psoriasis³⁻⁵. However, the expression of IgE and Fc ϵ RI in psoriatic skin and its modulation after successful systemic treatment has not yet been elucidated so far. In this study we therefore aimed to investigate the distribution and localization of IgE and Fc ϵ RI in psoriatic skin lesions. Moreover, we sought to analyze whether clinical improvement after successful systemic treatment may influence levels of serum total IgE and the *in situ* expression of IgE and Fc ϵ RI in psoriasis.

Materials and methods

Patients

Blood samples for IgE analysis were obtained from China (Huashan Hospital, Fudan University, Shanghai). Patient characteristics are shown in Table 1. 695 psoriatic patients (334 women, 361 men; mean age: 37 years, range: 7 to 82 years), and 200 healthy controls (106 women, 94 men; mean age: 39 years, range: 10 to 85 years) were enrolled. Skin samples were obtained from Switzerland (Inselspital, University Hospital, Bern). 20 psoriatic patients (3 women, 17 men; mean age: 49 years, range: 30 to 64 years;) without treatment, 7 psoriatic patients (2 women, 5 men; mean age: 52 years, range: 31 to 68 years;) treated with ustekinumab (Stelara®, Janssen Biotech Inc, Titusville, NJ) for 3 months, and 13 healthy

controls (6 women, 7 men; mean age: 49 years, range:18–68 years) were enrolled. Diagnosis of psoriasis was based on typical clinical and/or histopathological criteria. Patients with atopic dermatitis (negative personal and family history) were excluded from the study. The patients did not receive any local therapy at the skin biopsy site for at least 3 weeks prior to the investigation. Skin biopsy specimen were obtained from lesional and non-lesional skin. An additional skin biopsy specimen was obtained from lesional skin (adjacent to the initial lesional skin biopsy specimen) after successful treatment (PASI 75 response) with ustekinumab. Informed consent was obtained from all the participants. The study was approved by ethics Committees of the Huashan Hospital (for Chinese patients) and the Medical Ethics Committee of the Canton of Bern (for Swiss patients).

Detection of serum total and specific IgE

Serum total and allergen-specific IgE levels were determined at the Huashan Hospital using the ADVIA Centaur Sandwich-Immunoassay (Siemens Healthcare Diagnostics GmbH, Eschborn, Germany) and an immunoblotting assay (EUROIMMUN Medical Laboratory Diagnostics Stock Company, Lübeck, Germany), respectively. The normal range of the assay for serum total IgE was: 0 to 240 ng/ml. The detection limit of the assay for specific IgE was 0.35 kU/L. Specific IgE was classed from 0 to 5 (0:< 0.35; 1: 0.35-3.5; 2: 3.5-17.5, 3: 17.5-50; 4: 50-100; 5: > 100 kU/L).

Immunohistochemical staining of IgE and FcεRI

Immunostaining of skin tissues was performed as previously described⁶. All antibodies are listed in Table2. Briefly, cryostat tissue sections were air-dried, fixed in cold acetone for 10 min and subsequently

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rehydrated in Tris-buffered saline with 0.1% saponin. After blocking, the sections were incubated with the primary antibody, followed by a biotinylated secondary antibody and the streptavidin-biotin complex/alkaline phosphatase (K0376, DAKO Corp, Carpinteria, CA). All sections were developed using fuchsin substrate chromogen (K0625, DAKO Corp) and counterstained with hematoxylin. Substitution of the primary antibody with isotype-matched antibodies and omission of the primary antibody were used as negative controls.

Immunofluorescence double staining

Immunofluorescence double staining was performed as described elsewhere⁷. Briefly, tissue cryosections were incubated with rabbit anti-human IgE or one of the following mouse anti-human antibodies: anti-CD1a, anti-CD11c, anti-CD206, anti-CD207, anti-BDCA-2, anti-CD32, anti-neutrophil specific elastase and anti-mast-cell (MC) tryptase, followed by incubation with Alexa Fluor 594-conjugated goat anti-rabbit antibodies (Life Technologies, Paisley, United Kingdom) for IgE or with isotype-specific Alexa Fluor 594-conjugated goat anti-mouse antibodies (Life Technologies, Paisley, United Kingdom) for the remaining markers. Sections were then treated with mouse anti-FcεRI or rabbit anti-IgE, followed by incubation with fluorescein isothiocyanate-conjugated goat anti-mouse IgG2b or goat anti-rabbit IgG. Nuclear staining was performed with diamidinphenylindols (DAPI). The specificity of the reaction was confirmed by omitting the primary or secondary antibodies, as well as by using irrelevant isotype-matched antibodies as negative controls.

Evaluation of sections

The slides were analyzed with a Leitz Dialux 20EB microscope by two independent investigators. In each

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section immunohistochemical staining was assessed on 10-15 fields at $\times 400$ magnification. Cells displaying a strong cytoplasmic staining were considered positive. The positive stained cells were counted with a 0.063-mm^2 grid and the number of positive cells (mean $\pm$ SEM) per square millimeter was determined. Only positively stained cells with nucleus were included in the counting. Immunofluorescence double staining (n=3-6) was evaluated in a semi-quantitative manner using the following scale: 1: <25%, 2: 26-50%, 3: 51-75%, 4: 76-100% of positive cells.

Statistical analysis

Data analysis and graphs were performed on GraphPad Prism v.5 (GraphPad Software, San Diego, CA, USA). The data were expressed as mean $\pm$ SEM and if data was not normally distributed as median (25%; 75% quartiles). A p value of < 0.05 was interpreted as statistically significant. Statistical analysis was performed using either the Kruskal–Wallis nonparametric ANOVA test (with subsequent posthoc test: Mann-Whitney U test), the Wilcoxon matched-pairs signed rank test, the t-test or the chi-square test when appropriate. The Spearman's rank test was used to analyze the correlation between IgE+ and Fc ϵ RI + cells as well as between serum IgE levels and age or PASI, respectively.

Results

Serum total IgE levels are significantly elevated in patients with psoriasis vulgaris

As shown in Fig. 1 A elevated serum total IgE levels were found in 39% patients with psoriasis. The mean value in psoriatic patients (421.6 ± 23.80 ng/ml, n= 695) was significantly ($p < 0.0001$) higher than in normal controls (95.27 ± 4.24 ng/ml, n=200, Fig. 1 B). A significantly ($p < 0.05$) higher proportion of male patients (160/273, 58.6%) had elevated total serum IgE levels in comparison to females (113/273, 41.4%),

and the mean value in males (481.1 ± 35.15 ng/ml, $n=361$) was significantly ($p < 0.001$) higher than in females (357.2 ± 31.46 ng/ml, $n=334$, Fig. 1C). Furthermore, an over 5-fold increase in serum total IgE levels (> 1200 ng/ml) was detected in 12.7% (46/361) of the male patients, which was significantly ($p < 0.05$) higher in comparison to the female population (7%, 26/334). No statistically significant correlation was observed between elevated total serum IgE levels and age ($n=695$) or the psoriasis area severity index (PASI) ($n=92$).

Furthermore, specific IgE levels were analyzed in a subset of psoriatic patients with increased serum total IgE levels ($n=49$). Increased specific IgE levels to common aero- and food allergens (values ranged from 0.36-90 kU/L, Enzyme allergosorbent test classes (EAST) classes: 1-4) were detected in these patients. The type of allergens are shown in Fig. 1D. In addition, increased eosinophil counts ($> 4.5 \times 10^8$ /L) were detected in 11% of the patients with elevated serum total IgE levels.

IgE+ and FcεRI+ cells are significantly increased in psoriatic lesional skin and mainly expressed on mast cells, dendritic cells and macrophages

In order to investigate the protein expression and distribution of IgE and FcεRI in the skin we performed immunohistochemical analysis of lesional skin (LS) and non-lesional skin (NLS) from psoriatic patients (non-atopic without increased IgE levels) as well as of normal skin (NS) from controls. Representative stainings of IgE immunoreactivity in LS, NLS and NS are shown in Fig. 2A (upper panel). A marked enhancement of IgE immunoreactivity was particularly found in LS from psoriatic patients. IgE immunoreactivity was predominately observed in the perivascular or interstitial dermis and only on a few scattered cells in the epidermis. Quantification of positively stained cells is shown in Fig. 2B. The median number (25%; 75% quartiles) of IgE+ cells (230. 4 (166.4; 396.8), $n=13$) was significantly ($p < 0.001$)

increased in LS, as compared to NS (89.6 (0;166.4), n=12). No significant differences in numbers of IgE+ cells were observed between NLS (153.6 (12.8;230.4), n=9) and NS, as well as between LS and NLS.

Representative stainings of FcεRI immunoreactivity in LS, NLS and NS are shown in Fig. 2A (upper panel). As observed with IgE enhanced FcεRI immunoreactivity was particularly found in the dermis of LS from psoriatic patients. In addition, weak FcεRI immunoreactivity was also detected on some epidermal DCs. Quantification of positively stained cells is shown in Fig. 2C. The median number (25%; 75% quartiles) of FcεRI+ cells (537.6 (435.2, 716.8), n=20) in LS was significantly ($p < 0.001$ and $p < 0.0001$) higher in comparison to NLS (320.0 (192.0, 428.8), n=12) and NS (256.0 (166.4, 371.2), n=13), respectively. No significant difference in the number of FcεRI+ cells was observed between NLS and NS.

Next, the correlation between the increment of IgE+ and FcεRI+ cells in LS from psoriatic patients was analyzed. Representative immunofluorescence double-stainings for IgE and FcεRI are shown in Fig.3. The immunofluorescence staining of IgE and FcεRI was consistent with the initial immunohistochemical findings. Almost all IgE was located on FcεRI+ cells and a positive correlation was found between the number of IgE+ and FcεRI+ cells (n=13, $r=0.68$, $p < 0.05$).

In order to investigate the cellular identity of the IgE+ and FcεRI + cells in LS, we performed immunofluorescence double staining (n=3-6) with lineage markers of MCs (tryptase), various DCs (CD1a, CD11c, CD32, CD206, CD207, BDCA-2), macrophages (CD32, CD206) and neutrophils (neutrophil specific elastase). Representative stainings and semi-quantitative analysis of the percentage of each cell type expressing IgE (panel A) and FcεRI (panel B) are shown in Figs. 4 and 5. IgE and FcεRI were mainly located on tryptase+ mast cells, CD11c+ DCs and CD32+ DCs and macrophages and to a lower degree also on CD1a+, CD206+, CD207+, BDCA-2+ DCs or macrophages as well as a few neutrophils.

Total serum IgE levels and the number of IgE+ and FcεRI+ cells in psoriatic lesions decreased significantly with the improvement of disease

As shown in Fig.6 A, total serum IgE levels of 25 patients with improvement of psoriasis after conventional/non-biological treatment (acitretin or methotrexate) (862.6 ± 132.9 ng/ml) decreased significantly ($p < 0.0001$) in comparison to that before therapy (1144 ± 152.4 ng/ml). Moreover, as shown in Fig. 6 B and C the numbers of IgE+ cells ($n=7$, from 380.3 ± 74.20 to 212.1 ± 31.95) and FcεRI+ cells ($n=7$, from 727.8 ± 104.3 to 424.2 ± 69.97) were significantly ($p < 0.05$) reduced in LS after successful treatment ($> \text{PASI } 75$ response) with ustekinumab. In order to analysis which cells are reduced after treatment with ustekinumab, immunofluorescence double staining ($n=3$) was performed for the 3 cell populations (tryptase+, CD11c+ CD32+ cells) with the highest expression of FcεRI (stainings could not be performed for all markers due to limitation of samples). As shown in Fig. 7, a decrease of FcεRI bearing mast cells and particularly of CD32 and CD11c cells in skin lesions treated with ustekinumab was observed.

Discussion

This study demonstrates that 39% of Chinese patients with psoriasis have elevated serum total IgE levels. These results are consistent with some previous studies from China, Europe and the USA, which have reported increased serum total IgE levels in 42-46% of psoriatic patients³⁻⁵. Our results also demonstrate that mean serum IgE levels are significantly higher in male compared to females. Since smoking and alcohol consumption have been associated with elevated serum IgE levels^{8,9}, these factors may partially explain such differences between men and women. Previous studies have revealed contradictory results regarding serum IgE levels and disease severity³⁻⁵. In accordance with Ding and coworkers, we did not find any significant correlation between IgE levels and disease severity³. Interestingly, similar

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discrepancies have also been reported in atopic dermatitis¹⁰. IgE molecules preferentially bind to cells expressing IgE receptors (FcεRI and FcεRII) and serum IgE levels may not necessarily correlate with cell-bound IgE in the skin^{11,12}. Therefore, variation in the clearance of IgE molecules from the serum and its binding to cells expressing IgE receptors in the skin may partly explain the discordance between serum IgE levels and disease severity. In a subset of patients with psoriasis we could also detect increased specific IgE levels (EAST classes: 1-4) to common aero- and food allergens. Sensitization to common aeroallergens has also previously been reported in psoriasis patients from Europe, albeit their levels appear to be lower in psoriasis compared to atopic dermatitis¹³. Class-switching towards IgE in atopic individuals is mediated by Th-2 cytokine like IL-4, IL-13, which are down-regulated in psoriasis¹. Interestingly, some reports suggest that other cytokines like IL-17 and IL-21 may enhance IgE production^{14,15}. Since these cytokines are up-regulated in psoriasis, they may be involved in generating IgE in some psoriatic patients. Nevertheless, the precise regulatory mechanisms leading to enhanced production of IgE in psoriasis are still poorly understood and remain to be elucidated in future studies.

In order to better understand the involvement of IgE, we further investigated the immunohistochemical expression of IgE and FcεRI in psoriatic skin lesions. Our results show for the first time that IgE+ and FcεRI+ cells are significantly increased in psoriatic LS as compared to NS from controls. Co-localization analysis demonstrated that almost all IgE was located on FcεRI+ cells. These results are in line with previous findings in a pediatric population which have identified a positive correlation between the number of IgE+ and FcεRI+ cells¹¹. The enhanced expression of these molecules suggests that IgE/FcεRI interactions may participate in developing and maintaining inflammatory processes in psoriatic lesions. Whether initially high IgE levels initiate the inflammatory processes or increased numbers of pro-inflammatory cells secondarily lead to enhanced IgE production is currently not known. These mechanisms

may not be mutually exclusive. However, since IgE+ and FcεRI+ cells were not significantly enhanced in non-lesional psoriatic skin. we speculate that cytokines like IL-6, IL-17, IL-21 and possibly cellular interactions (CD40/CD40L) may secondarily enhance IgE production in psoriasis¹⁶.

In order to further determine the cellular identity of the IgE+ and FcεRI + cells, we analyzed the phenotypic characteristics of IgE/FcεRI-bearing cells. Our results demonstrate that IgE and FcεRI were co-expressed on various innate cell populations including mainly mast cells, some epidermal Langerhans cells (LCs), dermal DCs, macrophages and a few neutrophils. Increasing evidence suggests that innate immune cells are important in the pathogenesis of psoriasis¹. In particular, mast cells have been reported to be increased in skin lesions¹⁷. These cells are one of the main sources of IL-17, which plays a central role in the pathogenesis of psoriasis¹⁷⁻²⁰. In addition, mast cells can also release various other pro-inflammatory cytokines and mediators like IL-6, IL-8, IL-12, IL-22, IFN-γ and TNF-α, vascular endothelial growth factor, nitric oxide, which have all been implicated to participate in psoriatic inflammation¹⁷⁻²³. Mast cells may also directly interact with T cells inducing memory CD4+T to produce IFN-γ and IL-22²⁴. IL-22 promotes keratinocyte proliferation and is a crucial cytokine responsible for induction of acanthosis in psoriasis. Interestingly, a recent study also indicates that cellular interactions between mast cells and DCs modulate DCs to stimulate CD4+ T-cell proliferation and polarization towards Th1 and Th17 responses²⁵. Mast cells can be activated through different triggers and it remains to be elucidated in future studies how IgE/FcεRI interactions precisely influence their activation in psoriatic skin lesions.

Additionally to mast cells, DCs and macrophages are critically involved in psoriatic inflammation¹. Our study comprehensively demonstrates for the first time that IgE and FcεRI are expressed on different subsets of DCs including LCs (CD1a+, CD207+ cells), myeloid DCs (CD11c + cells), plasmacytoid DCs (BDCA-2+cells) and macrophages (CD32+, CD206+cells). LCs expressing FcεRI have been termed

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inflammatory dendritic epidermal cells (IDEC) and are known to produce pro-inflammatory cytokines like IL-12 and IL-18 upon ligation of FcεRI²⁶. LCs are also capable of inducing IL-17 and IL-22 in T cells and may thereby participate in promoting keratinocyte stimulation and up-regulation of antimicrobial peptides in psoriasis^{1,27,28}. IgE/FcεRI expression on different dermal DCs and macrophages may also participate in maintaining psoriatic inflammation, since stimulation of FcεRI on DCs and monocytes has been shown to promotes NF-κB signaling, prevent Fas/Fas-L-mediated apoptosis and release of proinflammatory mediators such as TNF-α, IL-6, IL-12^{29,30}. Interestingly, co-expression of IgE and FcεRI was also detected on some plasmacytoid DCs (pDCs), which has previously also been described in atopic dermatitis³¹. These cells may participate in initiating and promoting psoriatic inflammation through release of type 1 interferons, TNF-α, IL-6 and by promoting Th22 cells^{32,33}.

Another novel finding of this study is the detection of IgE and FcεRI on neutrophils. It has been previously observed, that IgE binding to FcεRI can enhance neutrophil survival³⁴ and result in release of IL-8³⁵. IL-8 is a known chemotactic factor for neutrophils, indicating that these mechanisms may further promote neutrophil recruitment and contribute to the self-amplifying loop of psoriatic inflammation.

Finally, our study demonstrates a significant reduction of IgE+ and FcεRI + cells after successful treatment with ustekinumab. Thereby, a decrease of FcεRI bearing mast cells and particularly of CD32 and CD11c cells in skin lesions treated with ustekinumab was observed. Ustekinumab is a human monoclonal antibody that binds to IL-12/23p40, which is mainly secreted by myeloid DCs and different macrophages (e.g. CD32+ macrophages)⁷. Treatment with ustekinumab particularly leads to down-regulation of the Th17 inflammatory pathway in psoriasis. Interestingly, IL-17 has recently been shown to promote IgE production in human B cells¹⁴. Up-regulation of IL-17 may partly explain the enhanced levels of IgE in psoriasis and furthermore, down-regulation of IL-17 after ustekinumab may be responsible for the reduced

number of IgE+ cells in our study.

Similar to psoriasis, skin lesions in patients with atopic dermatitis also harbor many FcεRI + and IgE+ cells, i.e. mast cells and also various DC subpopulations including myeloid and plasmacytoid DCs³¹. However, some quantitative differences such as an increased number of plasmacytoid DCs and particularly functional differences in the myeloid and plasmacytoid DCs have been reported in AD compared to psoriasis³⁶. In contrast to psoriasis, DCs in AD expressed chemokines like CCL17, 18 and 22, which are known to contribute to Th2 polarization³⁶. Furthermore, FcεRI+ plasmacytoid DCs have also been reported to produce IL-10 and less type 1 interferons in AD, which may further promote Th2 type immune responses³⁷. On the other hand, inducible nitric oxide synthase (INOS) and TNFα containing CD11c+DCs are enriched in psoriasis in comparison to AD³⁶. Thus, although both psoriasis and AD harbor many FcεRI + and IgE+ DCs, differences in their cytokine and chemokine production are likely to perpetuate distinct inflammatory pathways. It is also thought that FcεRI/ IgE+ DCs facilitate allergen and possibly also auto-antigen presentation, thereby contributing to inflammatory processes in AD³⁸. It is tempting to speculate that such mechanisms may also be involved in a subset of psoriatic patients, e.g. possibly in those with more pronounced itch or partly eczematous psoriasis.

In conclusion, we provide evidence of a role for IgE/FcεRI in psoriatic inflammation. The expression of IgE and FcεRI was particularly enhanced on innate cells in psoriatic lesions. Whether interruption of this pathway e.g. with anti-IgE-antibodies will lead to significant amelioration, remains to be elucidated in future studies.

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Figure Legends

Fig.1. Serum total and allergen specific IgE levels in psoriasis. (A) 39% of the patients with psoriasis had elevated serum total IgE levels. (B) The mean value of serum total IgE in psoriatic patients (421.6 ± 23.80 ng/ml, n= 695) was significantly higher than in normal controls (95.27 ± 4.24 ng/ml, n=200). (C) The mean value of serum total IgE was significantly higher in male (481.1 ± 35.15 ng/ml, n=361) compared to female patients (357.2 ± 31.46 ng/ml, n=334). (D) Specific IgE levels were analyzed in a subset of psoriatic patients with increased serum total IgE levels (n=49). The type of allergens and the percentage of patients with increased specific IgE are indicated.*** p< 0.0001, **p< 0.001

Fig. 2. The expression of IgE and FcεRI. (A) Representative staining of IgE and FcεRI. Scale bar, 50μm. (B) A significant increase in the number of IgE+ cells was observed in LS (n=13) from psoriatic patients, in comparison with NS (n=12). No significant difference was observed between LS and NS (n=9). (C) A

significant increase in the number of FcεRI+ cells was observed in LS(n=20), in comparison with NLS (n=12) and NS (n=13). Lines indicate median values (with 25%; 75% quartiles).*** p< 0.0001, **p< 0.001

Fig.3. The co-localization of IgE and FcεRI and its correlation in psoriatic skin. (A) Representative staining of IgE and FcεRI. Scale bar, 50μm. Two-colour immunofluorescence images were obtained for IgE (first panel of each row, red) and FcεRI (second panel of each row, green). The two images were then superimposed (third panel of each row). Double-positive cells are shown in yellow. Nucleus was stained with DAPI (4',6-diamidino-2-phenylindole). (B) The number of IgE+ cells was positively correlated with the number of FcεRI + cells (n=13, p = 0.05).

Fig. 4. Phenotype of cells expressing FcεRI in psoriatic skin lesions. Two-color immunofluorescence images were obtained for tryptase (a marker for mast cell), CD1a, CD206, CD207, CD11c, BDCA-2 (markers for DCs), CD32 (markers for macrophages), N.e elastase (a marker for neutrophils) (first panel of each row, red) and FcεRI (second panel of each row, green). The two images were the superimposed (third panel of each row). Double positive cells are shown in yellow. Scale bar, 10μm. Nucleus was stained with DAPI (4,6-diamidino-2-phenylindole).

Fig. 5. Cell populations expressing IgE and FcεRI in psoriasis. Staining was graded in terms of the percentage of double immunostained cells on a scale of 1-4. Cell types expressing IgE and FcεRI are shown on panel A and B, respectively.

Fig 6. Total serum IgE levels and expression of IgE and FcεRI in skin lesions after treatment. (A) Total serum IgE levels (n= 25) decreased significantly with improvement of psoriasis after conventional/non-biological treatment (acitretin or methotrexate). (B) Representative stainings of IgE and FcεRI before and after treatment with ustekinumab (n=7). Scale bar, 50μm. (C) The number of IgE+ and FcεRI+ cells in psoriatic lesions was significantly reduced after treatment with ustekinumab.*** p< 0.0001, * p<0.05

Fig.7. FcεRI bearing mast cells, dendritic cells and macrophages are reduced after treatment with ustekinumab. Representative stainings before and after treatment with ustekinumab and from normal controls are shown (n=3). Two-color immunofluorescence images were obtained for FcεRI (first panel of each row, red) and tryptase, CD11c and CD32 (second panel of each row, green). The two images were superimposed (third panel of each row). Double positive cells are shown in yellow. Scale bar, 50μm. Nucleus was stained with DAPI (4,6-diamidino-2-phenylindole).

Table 1. Patient characteristics

	Studies on serum totalIgE		Studies on skin biopsy specimens		
	Psoriasis	Normal controls	psoriasis	Psoriasis(treated with ustekinumab)	Normal controls
Subjects(n)	695	200	20	7	13
Mean age	37	39	49	52	49
(range)	(7-82)	(10-85)	(30-64)	(31-68)	(18-68)
Gender(m/f)	361/334	94/106	17/3	5/2	7/6
Mean PASIScore±SD	9.5 ±7.4*	not applicable	12.5 ±8.7	15.9±8.7	not applicable

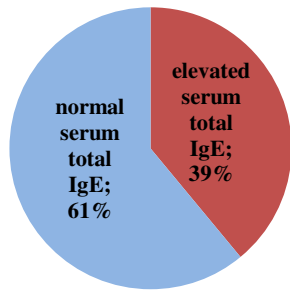
*available from n=180

Table2 antibodies used in this study

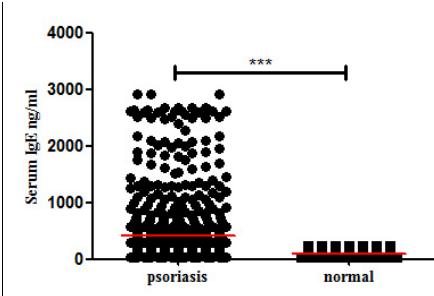
		Antigen	Format	Isotype	Clone	Product No.	Source
primary antibodies	rabbit anti-human	IgE		IgG		ab75673	Abcam, Cambridge, UK
	mouse anti-human	Fc epsilon RI		IgG2b	9E1	ab54411	Abcam, Cambridge, UK
	mouse anti-human	CD1a		IgG1	010	M3571	DAKO Corp, Carpinteria, CA
	mouse anti-human	CD11c		IgG1	KB90	M0732	DAKO Corp, Carpinteria, CA
	mouse anti-human	Tryptase		IgG1	AA1	M7052	DAKO Corp, Carpinteria, CA
	mouse anti-human	CD32		IgG1	KB61	M7190	DAKO Corp, Carpinteria, CA
	mouse anti-human	CD206		IgG1	19.2	555953	BD Bioscience, Franklin lakes, NJ
	mouse anti-human	CD207/langerin		IgG1	DCGM4	IM3449	immunotech, Marseilles, France
	mouse anti-human	BDCA-2		IgG1	AC144	130-090-690	MiltenyiBiotec GmbH
	mouse anti-human	Neutrophil Elastase		IgG1	NP57	M0752	DAKO Corp, Carpinteria, CA
second antibodies	rabbit anti-mouse immunoglobulin biotinylated					E0413	DAKO Corp, Carpinteria, CA
	swine anti-rabbit immunoglobulin biotinylated					E0431	DAKO Corp, Carpinteria, CA
	Goat anti-mouse IgG1		Alexa Fluor 594			A21125	Life Technologies, Paisley, United Kingdom
	Goat anti-mouse IgG2b		Fluoresceinisoithiocyanate			A90-109F	Bethy Laboratories Inc, Montgomery, TX
	Goat anti-rabbit IgG		Alexa Fluor 594			A11072	Life Technologies, Paisley, United Kingdom

Fig1
A

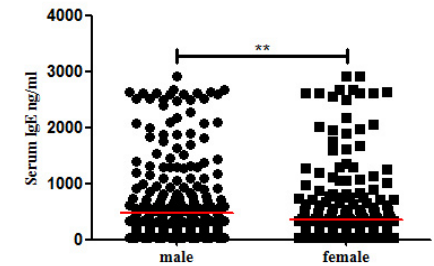
Figure 1. Serum total IgE levels in psoriasis patients.



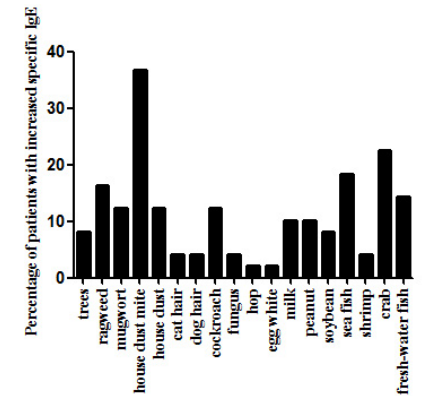
B



C



D1



D2

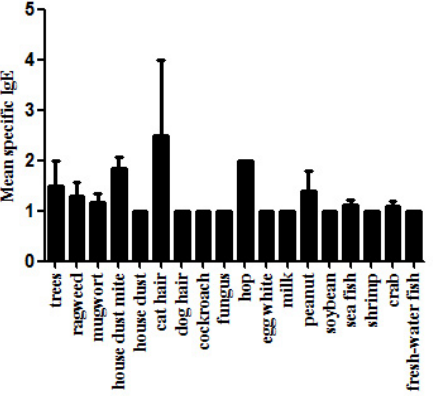
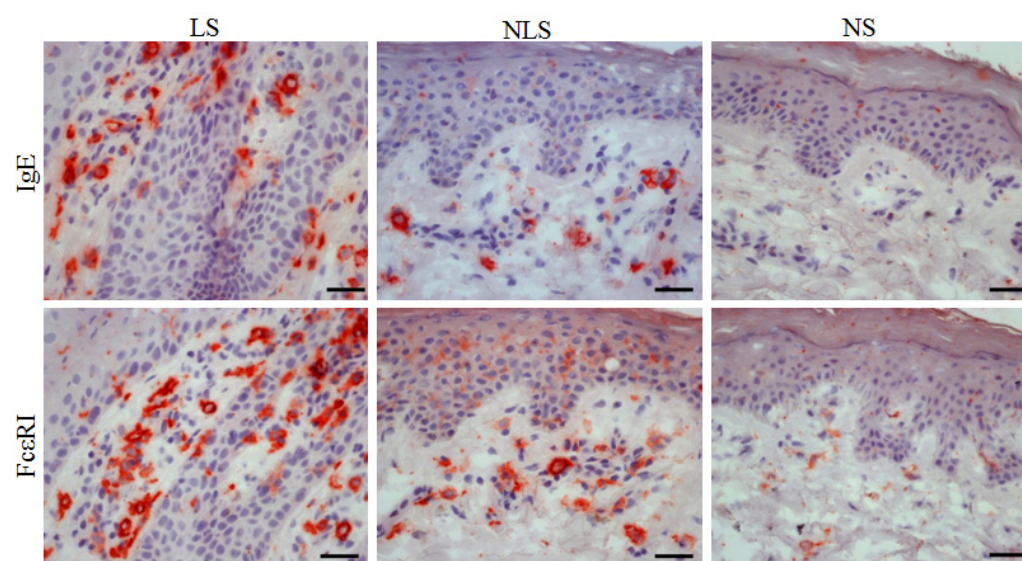
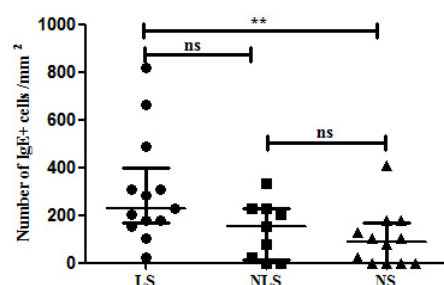


Fig2

A



B



C

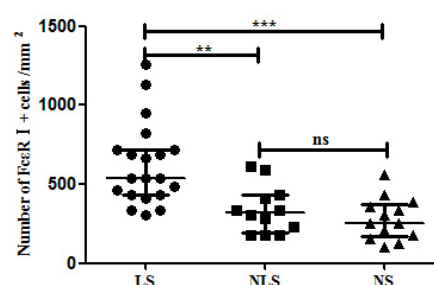


Fig 3

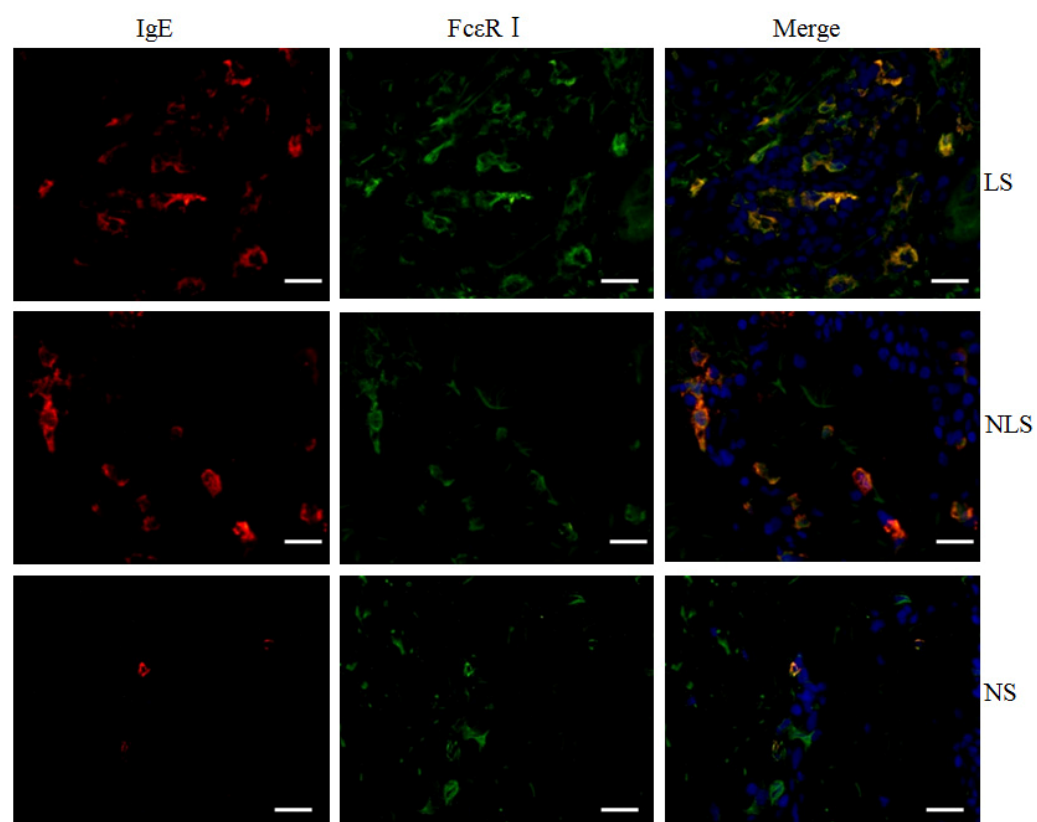


Fig 4

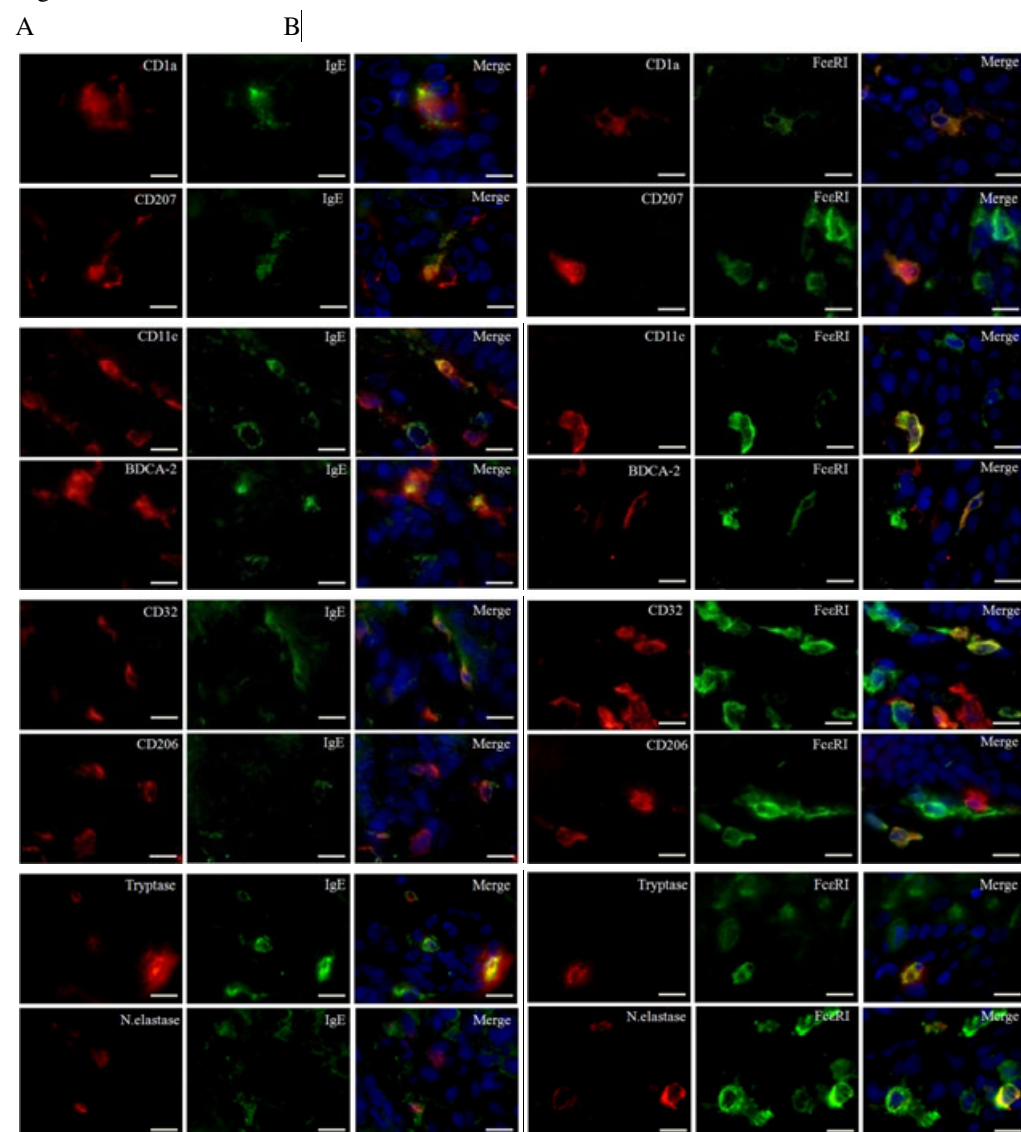


Fig 5

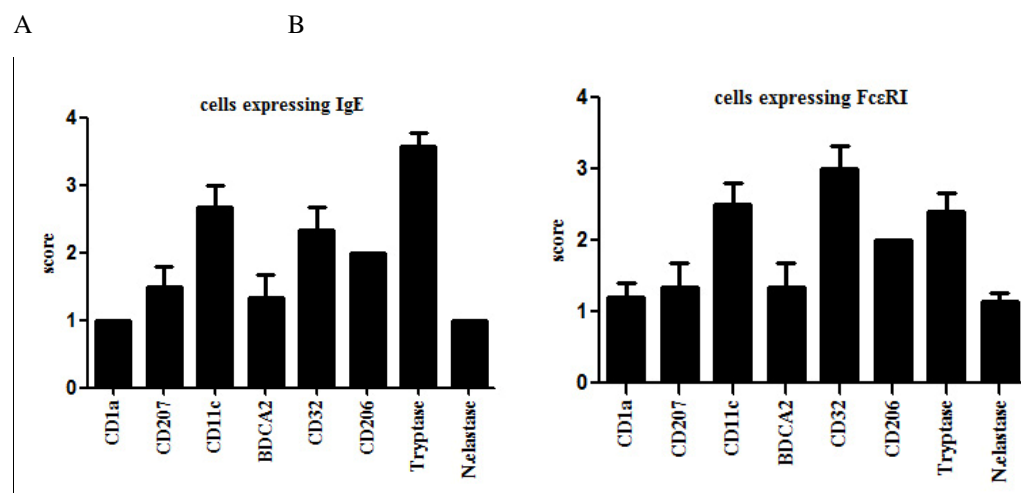
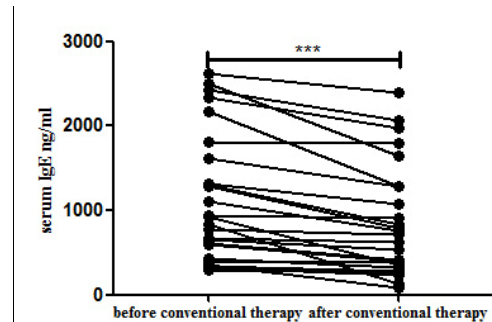
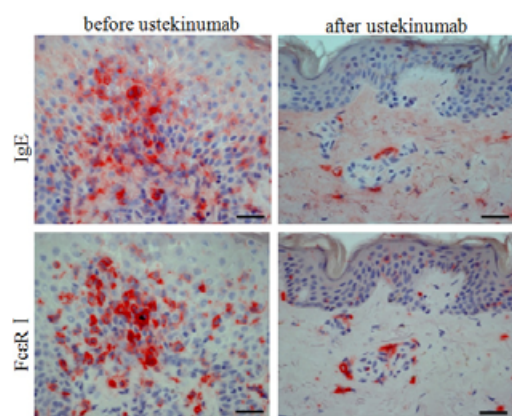


Fig 6

A



B



C

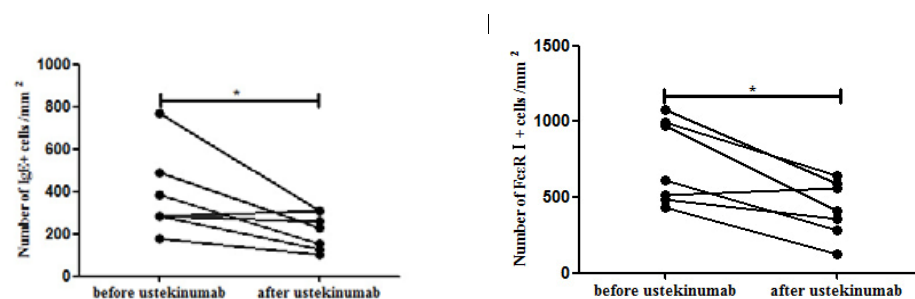


Fig7

