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Letter to the Editor

Langerhans cells are predominant high affinity immunoglobulin E receptor bearing cells in the epidermis of bullous pemphigoid skin[☆]



Bullous pemphigoid (BP) is driven by IgG autoantibodies against hemidesmosomal protein non-collagenous 16A(NC16a) part of BP180 [1]. However, the number of observations suggests the supportive role of IgE [2,3]. Total serum IgE is commonly elevated in BP and specific IgE to BP180 NC16a antigen correlates with disease activity [3]. IgE deposits can be found in the basal membrane zone (BMZ) [4]. High affinity IgE receptor I (FcεRI) is expressed on mast cells and eosinophils in the dermis of BP skin [5]. FcεRI can be expressed on other cell types that are located in the epidermis such as CD207+CD1a+ Langerhans cells (LC) and CD207-CD1a+ inflammatory dendritic epidermal cells (IDEC) as in atopic dermatitis (AD). We wanted to confirm the presence of FcεRI bearing cells in epidermis of BP skin and that there is FcεRI on LC in BP. We also wanted to evaluate if the presence of epidermal FcεRI+ cells is related to the total serum IgE and disease activity.

Biopsies from peri-lesional and lesional (urticarial/erythematous) skin of 21 patients with BP, lesional AD and healthy controls were double stained for FcεRI and: LC (CD207), myeloid epidermal DC (CD1a) and eosinophils (major basophilic protein – MBP).

We have found FcεRI+ cells within the epidermis of BP patients (Fig. 1). Microscopic quantitative analysis [6] revealed the mean density of epidermal FcεRI+ cells of 77.2 and 95.12 per mm² in BP peri-lesional and BP lesional skin respectively and 49.64 cells/mm² for healthy control and 168.82 cells/mm² for AD. There was a statistical difference among these four groups (p = 0.0001). When a post hoc analysis was performed a significant difference between healthy control and lesional BP skin was found but not between healthy control and peri-lesional BP skin. The majority of FcεRI+ cells were CD207+ LC with dendritic morphology in both lesional and peri-lesional skin. A proportion of CD207- cells could be found within epidermal FcεRI+ cells in all studied groups and differed significantly among them (p = 0.0001; Fig. 1f). The density of FcεRI+CD207+ LC differed significantly among all four groups (Fig. 1g). However there were no significant differences, except the one between healthy control and AD lesional skin, when post hoc analysis was performed for pairs separately. The density of non LC FcεRI+CD207- cells differed significantly among four groups and was significantly higher in lesional BP skin than in peri-lesional BP and healthy control (Fig. 1h).

Significant correlation was found between total serum IgE and total epidermal FcεRI+ cells in peri-lesional skin but not in lesional one (Fig. 2a and b).

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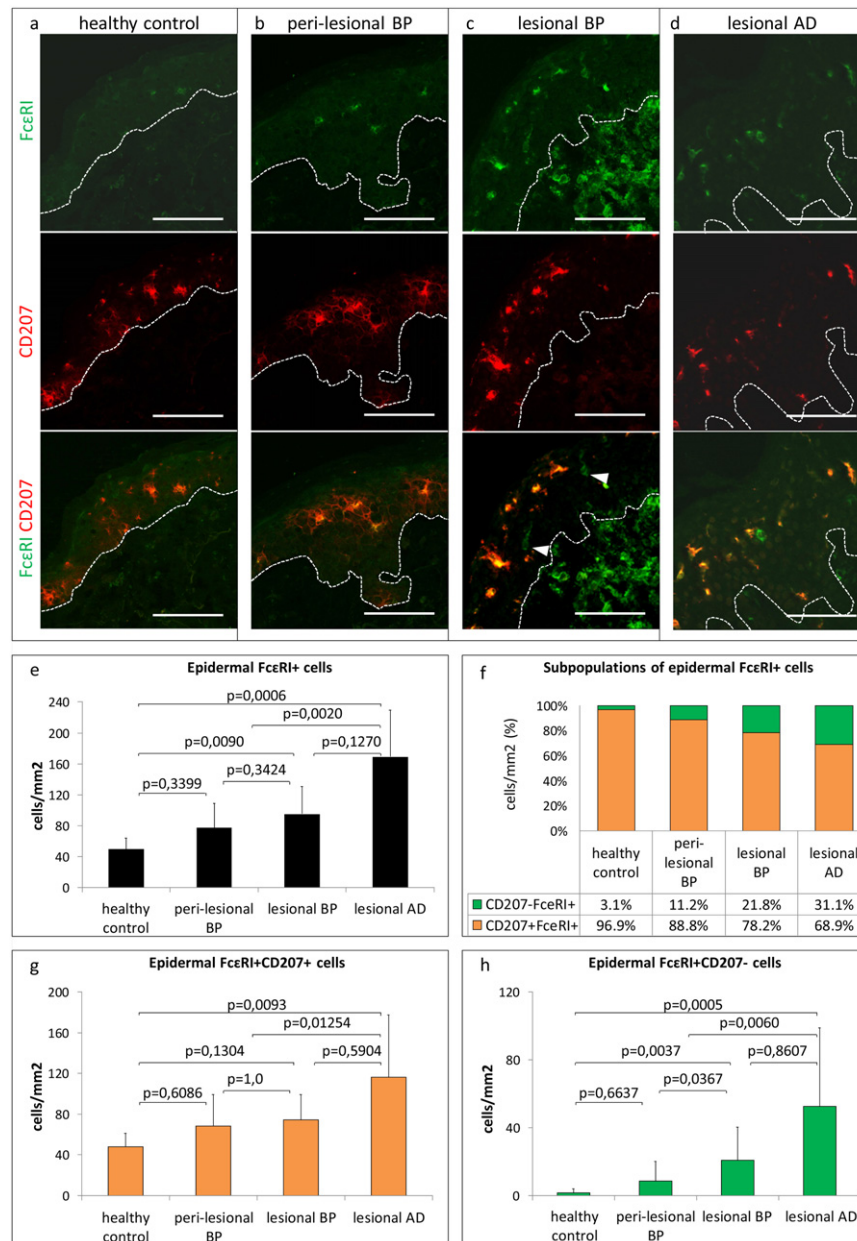


Fig. 1. Comparison of the expression of high affinity IgE receptor (FcεRI) on epidermal cells in healthy control (a; representative for 7), peri-lesional (b) and lesional (c) bullous pemphigoid (BP; representative for 21), and in atopic dermatitis skin (AD) (d, representative for 5). FcεRI is mostly expressed on epidermal CD207+ cells with dendritic morphology as shown on the merge pictures. Two populations of FcεRI+ are visualized as yellow–orange FcεRI+ CD207+ Langerhans cells (LC) and green FcεRI+ CD207- non-LC on the picture of BP lesional and AD skin (c and d). Some of the FcεRI+ CD207- non-LC have clear dendritic morphology (c, white arrows). A dense infiltrate of FcεRI+CD207- cells is seen in the dermal compartment adjacent to basal membrane zone (white dotted line) in BP lesional skin. White bar – 100 μm; (e) The comparison of the number of FcεRI+ cells in the epidermis of healthy control patients (n = 7), peri-lesional and lesional BP (n = 21) as well as atopic dermatitis (n = 5) revealed significant differences between all four groups (Kruskal-Wallis test: $p < 0.0001$). The density of epidermal FcεRI+ cells differed significantly between lesional BP and healthy skin, between AD and healthy and AD and peri-lesional skin in post hoc analysis (Dunn's test; $p = 0.0009$; $p = 0.0006$ and $p = 0.002$ respectively). (f) Percentages of two populations of FcεRI+ cells differed significantly among four studied groups (χ^2 test: $p = 0.0001$) and CD207+ cells were predominant population in each group (orange portion of bars). (g–h) Peri-lesional and lesional BP skin, healthy and atopic dermatitis (AD) control skin differed significantly in terms of density of epidermal FcεRI+ CD207+ LC and FcεRI+ CD207- non-LC (Kruskal-Wallis test: $p = 0.0118$ and $p = 0.0001$ respectively). However no significant differences between the density of BP lesional and peri-lesional FcεRI+ CD207+ LC ($p = 1.0$) and no differences between pairs of BP peri-lesional and healthy control skin as well as between pairs of BP lesional and healthy control was found in post hoc analysis (Dunn's tests; $p = 0.6086$ and $p = 0.1304$) (g). The same analysis for the density of epidermal FcεRI+ CD207- non-LC revealed significant differences between lesional BP skin and peri-lesional ($p = 0.0367$) and between lesional BP and healthy skin ($p = 0.0037$) (h).

We were not able to confirm significant correlation of disease severity (measured with BPDAl) with total FcεRI+, and subpopulations of LC CD207+ and non-LC CD207- FcεRI+ cells (data not shown). However when values counting for urticaria/erythema and erosions/blisters scores were extracted from 'total' BPDAl, a

significant correlation was found between lesional FcεRI+CD207- cells and urticaria/erythema activity score (Fig. 2d).

We have confirmed previous observations that blood eosinophilia correlates with disease activity in untreated BP patients in our study and have found a positive correlation eosinophilia and BPDAl

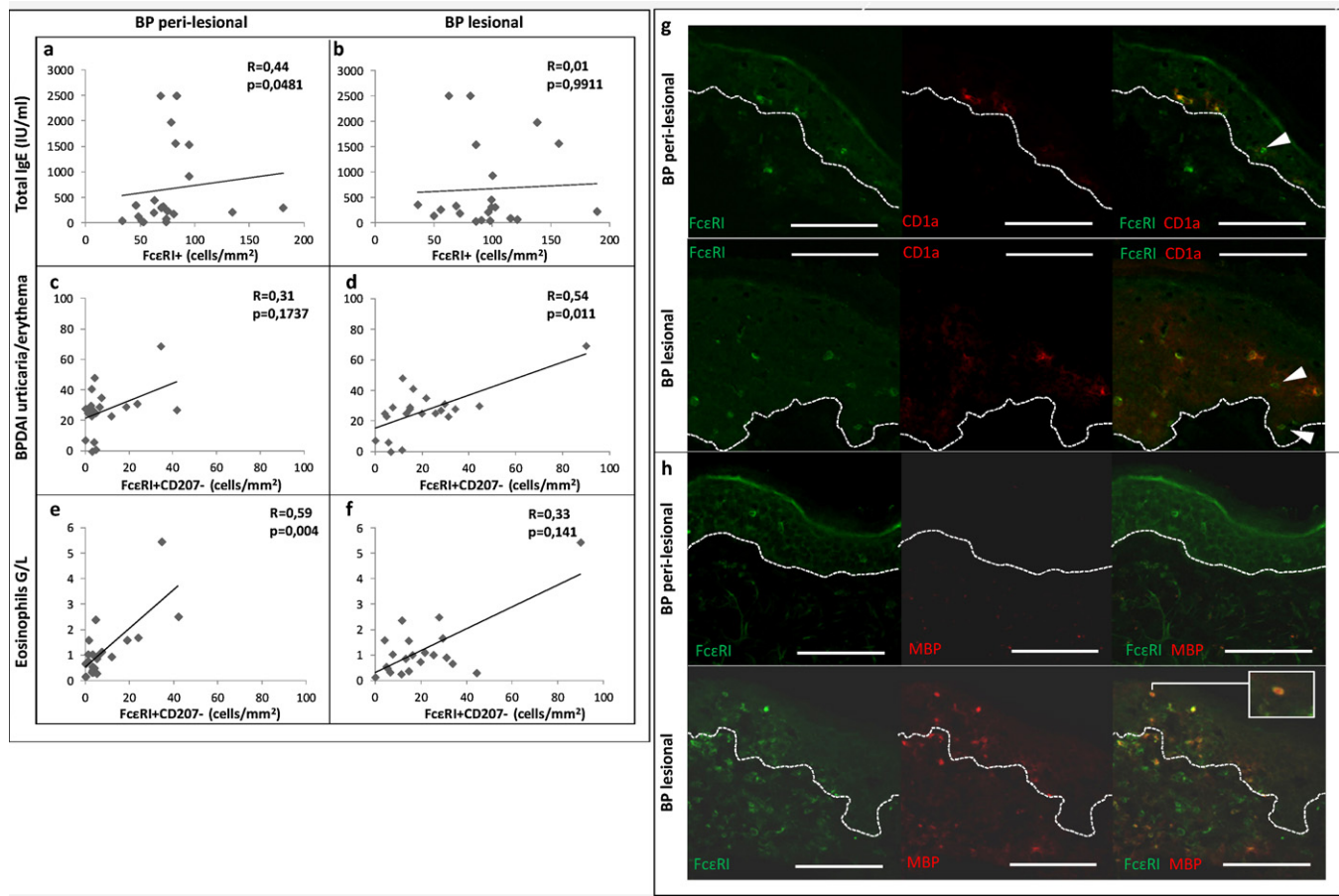


Fig. 2. (a,b) Significant positive correlation of total IgE serum concentration (IU/ml) with the density of epidermal FcεRI+ (cells/mm²) in peri-lesional bullous pemphigoid (BP) skin ($R=0,44$; $p<0,048$). There is no such correlation for lesional BP skin ($R=0,01$; $p=0,9911$; $n=21$). (c,d) Significant positive correlation was found between urticaria/erythema component of BP disease area index (BPDAL) and density of epidermal FcεRI+ CD207- cells in lesional ($R=0,54$; $p=0,0115$) but not in peri-lesional skin ($R=0,31$; $p=0,1737$; $n=21$). (e,f) Peripheral blood eosinophilia significantly positively correlates with the density of epidermal FcεRI+ CD207- cells (cells/mm²) in peri-lesional BP skin ($R=0,59$; $p=0,004$) but not in lesional one ($R=0,33$; $p=0,141$; $n=21$). All correlations (a–f) were calculated according to Pearson and/or Spearman test. All p values $<0,05$ were considered to be statistically significant. (g) Staining for FcεRI (green) and epidermal myeloid dendritic cells (CD1a+; red) of peri-lesional and lesional BP skin ($n=6$). Most of epidermal FcεRI+ cells were double positive (yellow) CD1a+ myeloid DC in both peri-lesional and lesional BP skin. FcεRI+CD1a- cells were only occasionally seen in epidermis in peri-lesional skin but more commonly in lesional one (white arrows on merge picture). White bar – 100 μm; White dotted line – basal membrane. (h) Staining for FcεRI (green) and eosinophils marker – major basic protein (MBP; red) of peri-lesional and lesional BP skin (representative for 1 out of 6). Presence of double positive round FcεRI+MBP+ eosinophils (yellow) can be seen focally only in the epidermis of lesional skin. These cells have clear non-dendritic morphology as shown on magnified part of merge picture. Usually such cells are present in the areas of dense FcεRI+ positive infiltrate in the adjacent dermis. This infiltrate is composed from MBP+ eosinophils and from MBP- cells. White bar – 100 μm; White dotted line – basal membrane.

and for urticaria/erythema part of BPDAL but not for erosions/blisters part (data not shown). We have found a positive correlation of eosinophilia with peri-lesional FcεRI+CD207- cells but not with lesional ones (Fig. 2e and f).

FcεRI+CD1a- cells were present in lesional BP epidermis and only occasionally in the peri-lesional one (Fig. 2g). Majority of FcεRI+ cells was CD1a+ as expected after CD207/FcεRI staining. Round FcεRI+MBP+ eosinophils were present only in lesional epidermis (Fig. 2h).

The role of FcεRI+ LC and non LC epidermal cells in the pathogenesis of BP remains unknown. It is possible that, being in close proximity to IgE attached to BMZ, they are somehow participants in the disease process. FcεRI+LC maintain tolerance and prime immune responses in allergy. Crosslinking FcεRI on LC enhances T-cell activation and activated LC produce chemokines that attract precursors of DC into epidermis [7]. We have shown that some epidermal FcεRI+ that are non-LC have dendritic morphology (Fig. 1c). This population could represent inflammatory dendritic epidermal cells (IDEC), as it was shown for AD. The third cell population of FcεRI+ cells was demonstrated by us only in lesional BP epidermis, which were oval non-dendritic FcεRI+MBP+

eosinophils. Eosinophilic epidermal infiltrate was already described in BP. However, no data on the presence of FcεRI on their surface was available until now. These eosinophils can be responsible for the formation of erythematous lesions. This is supported by our finding of the correlation of the lesional FcεRI+CD207- cells with erythema/urticaria part of BPDAL.

An open question remains about the nature of FcεRI+CD207- cells in peri-lesional skin. They are not high in number when compared to lesional skin (8,65 cells/mm² vs. 20,71 cells/mm²) and consist only 11.2% of all FcεRI+ epidermal cells (Fig. 1f). The number of these cells correlates with eosinophilia. We were not able to find FcεRI+MBP+ eosinophils in peri-lesional epidermis (Fig. 2h). We can speculate that FcεRI+ CD207- population could be responsible for the production of cytokines and chemokines for eosinophils and thus promote eosinophilia. There were several reports on the presence of chemokines attracting eosinophils in blister fluids and BP skin [8,9]. We speculate that FcεRI+ eosinophils could be attracted into epidermis by FcεRI+ non-LC myeloid DC (IDEC) or their CD1a- precursors [7]. We cannot exclude that other FcεRI+ cells e.g. basophils or plasmacytoid DC, are homing to epidermis in BP.

We have found a correlation of serum IgE and epidermal FcεRI+ cells in peri-lesional skin, but not in lesional one. It was previously reported that expression of FcεRI+ on eosinophils does not correlate with serum IgE [10]. This finding can explain our results, as we have identified eosinophils as common FcεRI+ cells in lesional BP epidermis, but not in peri-lesional areas.

Epidermal FcεRI+ cells found in our study are the natural target for newly proposed BP treatment with omalizumab. Reducing circulating IgE could influence the number of FcεRI+ cells in peri-lesional skin. Simultaneously it could impact the function of FcεRI+ LC and non-LC DC as well as FcεRI+ eosinophils.

Conflict of interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jdermsci.2016.09.012>.

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Letter to the Editor

First Japanese case of congenital generalized hypertrichosis with a copy number variation on chromosome 17q24



To the Editor

Hypertrichosis is defined as excessive hair growth compared with individuals of the same age, race and sex [1]. Congenital generalized hypertrichosis (CGH) is a rare syndrome characterized by universal hair overgrowth which is not caused by androgen stimulation [1,2]. In addition to hypertrichosis, patients with CGH can have other associated abnormalities, such as cardiomyopathy and gingival hyperplasia [1,2]. CGH can show X-linked recessive (OMIM 307150), autosomal dominant or autosomal recessive (OMIM 135400) inheritance trait, and recent advances in molecular genetics have gradually disclosed the pathogenesis of CGH [3–6]. Of these findings, it has been reported that autosomal dominant form of CGH with or without gingival hyperplasia is caused by copy number variations (CNVs) on chromosome (Ch) 17q24.2–q24.3 [4]. Interestingly, *SOX9* gene, which is known to play a role in

maintenance of hair follicle (HF) stem cells [7,8], is located close to the region of the CNVs, and it has been demonstrated that a CNV on Ch17q24.2–q24.3 resulted in reduced expression of the *SOX9*-mRNA, most likely due to a position effect [6]. Furthermore, autosomal recessive form of CGH has been shown to be caused by a loss-of-function mutation in *ABCA5* gene encoding a member of the ABC transporter [6]. Importantly, the *ABCA5* gene resides on Ch17q24.3, where the CNVs occurred in patients with autosomal dominant CGH [4]. Besides these findings, however, the molecular basis of CGH in Japanese population had remained totally unknown. In this study, we analyzed a Japanese female patient with CGH and successfully identified a CNV on Ch17q24. We further investigated how the CNV affected expression of *SOX9* and *ABCA5* proteins in the patient's HFs. The detailed Materials and Methods are shown elsewhere (Supplementary information online).

The patient was a 6 month-old girl of Japanese origin. Both of her parents, as well as her elder sister, were unaffected. There were no consanguinities between the parents. She has shown obvious hypertrichosis on the entire body surface since her birth (Fig. 1A, B). In addition, she has had gingival hyperplasia (Fig. 1A), while neither mental retardation nor cardiomyopathy was evident. She was referred to National Center for Child Health and Development Hospital due to her hair symptoms. Based on the clinical features, we diagnosed her as having CGH with gingival hyperplasia.

Supplementary material: Reagents

Reagent	Company
FITC conjugated mouse anti-human FcεRI (clone AER-37)	Biolegend, USA
PE conjugated mouse anti-human CD207 antibody (clone DCGM4)	Beckman Culture, USA
PE conjugated mouse anti-human CD1a antibody (clone HI149)	eBioscience, USA
mouse anti human major basophilic protein (MBP) antibody (clone BMK13)	Chemicon, USA
rabbit anti-mouse IgG Cy-5 (AP160S)	Chemicon, USA