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Specific T cells targeting *Staphylococcus aureus* Fibronectin-binding protein 1 induce a type 2/type 1 inflammatory response in sensitized atopic dermatitis patients

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Abstract

Atopic dermatitis (AD) is one of the most common inflammatory skin diseases worldwide and *Staphylococcus aureus* colonization and secondary infections occur in the majority of AD patients. Allergic sensitizations against microbial antigens have been discussed as possible trigger factors of AD. Recently, we reported IgE-sensitization against Fibronectin-binding protein-1 (FBP1), an essential virulence component in *S. aureus*, in a subgroup of patients suffering from AD. To expand these findings by investigating delayed-type immune reactions, the objective of this study

was to detect and phenotypically characterize FBP1-specific T cells as possible trigger factors in AD.

Immunodominant T-cell epitopes were mapped by proliferation testing of patient-derived FBP1-specific T cell lines after stimulation with single 15mer peptides, which were derived from different functional domains of the FBP1-sequence. Major histocompatibility complex class II-tetramers carrying immunodominant epitopes successfully stained T helper cells in 8 out of 8 HLA-matched, IgE-sensitized AD patients. Cytokine profiling of multimer-sorted cells revealed that predominantly the type 2 cytokines IL-13 and IL-4 were secreted by these cells. In contrast, IL-17, the marker cytokine for response to extracellular pathogens, was scarcely detectable.

We thus demonstrate that FBP1 contains immunodominant peptides that induce a specific pro-inflammatory T helper cell response with increased Th2 levels that can drive an allergic inflammation in sensitized AD patients.

Keywords: Antigen-specific T cells, Atopic dermatitis, Fibronectin-binding protein-1, *Staphylococcus aureus*.

Introduction

Atopic dermatitis (AD) is a common inflammatory skin disease with a prevalence of 13% in children and up to 7% in adults (1, 2). A hallmark of AD is T cell infiltration into the skin, and T cells have been shown to be predominantly of a Th2 phenotype, while also Th1, Th17, and Th22 polarized T cells play important roles (3-5).

The most common complication of AD is secondary *Staphylococcus aureus* infection (6). Considering that *S. aureus* colonizes the lesional skin of nearly 90% of AD patients, knowledge on *S. aureus* factors that may trigger the disease represents an urgent need (7). With technologies such as the 16S ribosomal sequencing, Kong et al. showed a significant skin microbiome shift towards *S. aureus* dominance on lesional AD skin in AD pediatric patients that was restored after the inflammation was resolved (8). A positive correlation with disease severity has been suggested, and bacterial diversity diminishes with *S. aureus* prevalence (8, 9). Simpson et al. analyzed a cohort of 96 AD patients and found *S. aureus* skin colonization to be associated with

more severe clinical symptoms of AD, less type 2 inflammation, and weaker barrier disruption (10). The pathological role of *S. aureus* in exacerbating inflammation has been studied most intensively regarding toxins which act as superantigens (SAs) by causing non-specific activation of T cells and a subsequent inflammatory cytokine release (11-13). Many IgE-reactive SAs among bacterial enterotoxins have been described such as *Staphylococcal* enterotoxin A, B and E. However, IgE-sensitization also occurs to antigens without superantigen capacities. We identified earlier Fibronectin-binding protein-1 (FBP1) and other *S. aureus* antigens by binding to patients IgE followed by mass spectrometry (14, 15). FBP1 is a *S. aureus* MSCRAMM surface protein (acronym for "microbial surface components recognizing adhesive matrix molecules") and is essential in invasion and adhesion to epithelial cells. This is achieved by binding to fibronectin, an extracellular matrix protein present on host cells (16-18). *S. aureus* strains carrying a mutated FBP1 showed remarkably reduced colonization rate and decrease in mortality upon infected mice (19). Following presentation by antigen presenting cells, FBP1 triggers T cell proliferation in AD patients in incomparable levels to classical allergens, accompanied by a high release of pro-inflammatory cytokines (20). We showed earlier that PBMC (Peripheral Blood Mononuclear Cells) from sensitized AD patients released the cytokines IFN- γ , IL-6 and TNF- α in response to FBP1. These cytokines have been described in response to *S. aureus* (21) also by others and may contribute to a cytokine milieu that favors development of Th17 cells. Th17 cells have been shown to be present in the acute phase of AD whenever TGF- β and IL-1 are also present at the site of inflammation. Our *in vivo* experiments revealed that mice sensitized to FBP1 show a specific T cell proliferation and a mixed Th2/Th1 FBP1-specific T-cell phenotype, along with basophil degranulation (20).

To characterize FBP1-specific T cells in patients suffering from AD in a state-of-the-art manner, we identified FBP1 immunodominant epitopes in this study which led to the synthesis of MHC (major histocompatibility complex) class II tetramers.

Materials and methods

Study patients

Adult patients suffering from AD were consecutively recruited from the Hannover Medical School Department of Dermatology and Allergy's outpatient clinic. All patients recruited gave written

informed consent and this study was approved by the Hannover Medical School ethics committee (7565). The patients included were diagnosed with mild to very severe atopic dermatitis according to the Hanifin and Rajka Criteria for AD (22). Disease severity was assessed by SCORing Atopic Dermatitis (SCORAD). Investigators global assessment (IGA) was performed, ranging from 0 (clear), over 1 (mild), 2 (moderate) to 3 (severe). All patients recruited were not under systemic therapy. Patients selected for tetramer staining were HLA-typed for HLA-DRB1*15 via specific PCR primers as described below.

Proteins and Peptides

Recombinant FBP1 was produced as [His]₆-tagged fusion protein in *Escherichia coli* and purified by means of Ni²⁺ affinity chromatography, as described previously (20). LPS contamination was determined by LAL test (Limulus Amebocyte Lysate, Pyrochrome, East Falmouth, MA, USA) to be virtually absent (<5 EU/ml). Single peptides were synthesized at Peptides and Elephants, Hennigsdorf, Germany.

IgE sensitization testing

To identify patients with an IgE-sensitization to FBP1, a semi-quantitative ELISA was performed. Therefore, ELISA plates (Nunc maxisorb, 442404) were coated overnight with 5 µg/ml recombinant human FBP1 in PBS. Unspecific binding was inhibited by 1h incubation with 1% bovine serum albumin (BSA) in PBS. Patients' sera were applied undiluted in duplicates for 16h at 4°C. After washing, bound IgE was detected by 2µg/ml mouse-anti-human IgE (BectonDickinson, NJ, USA, clone G7-26) in PBS/1%BSA, followed by Streptavidin-horseradish peroxidase (Biotechne, MN, USA) in PBS/1%BSA and tetramethylbenzidine/hydrogen peroxide (substrate reagent pack, Biotechne, MN, USA). Patients were regarded positive if the observed value exceeded the mean of all samples measured by 3 standard deviations. Levels of Staphylococcal Enterotoxin B (SEB)-specific IgE were quantified by ImmunoCAP FEIA (ThermoFisher). Statistical test performed in Figure 1A was Mann-Whitney-Test.

Generation of T cell lines

Fresh human PBMC were isolated from the blood of 21 patients with a positive result in the FBP1-ELISA by density-gradient centrifugation and cultured in presence or absence of 2.5 µg/ml antigen at a density of 1×10^6 /ml in Iscove's medium supplemented with 4% human heat-inactivated AB serum, 2 mM glutamine, 50 mg/ml of gentamicin, 100 mg/ml penicillin/streptomycin, and nonessential amino acids (referred to as cell culture medium, CCM). After 7 days, rhIL-2 (10 U/ml) was added and after 14 days, cells were expanded with allogeneic, irradiated PBMC (55 Gy) as feeder cells in the presence of phytohemagglutinin (10 µg/ml) and rhIL-2 (10 U/ml).

Proliferation assay

The resulting cell lines were submitted to re-stimulation testing after three weeks. Therefore, autologous PBMC (7.5×10^4) were irradiated (55 Gy) and incubated with T cell lines (5×10^3) to present recombinant FBP1 (2.5 µg/ml) in a total volume of 200 µl cell culture medium. Experiments were performed in triplicates. After 5-7 days, ^3H -thymidine (18,500 Bq/well) was added to the culture and incubated overnight to measure incorporation using a beta counter (Microbeta2, PerkinElmer, Waltham, MA, USA). A stimulation index following FBP1 stimulation of greater than 1.5 was regarded as positive, and in those cell lines the proliferative capacity towards putative epitope peptides was assessed by ^3H -thymidine incorporation.

HLA-typing

HLA-DRB1 genotype of 22 FBP1-sensitized AD patients enrolled in the epitope mapping was assessed by PCR with sequence specific primers. To enlarge the subgroup of FBP1-sensitized AD patients that express the HLA type HLA-DRB1*15, further donors were screened for HLA-DRB1*15 in a targeted manner applying sequence specific primers following a published protocol (23).

Tetramer staining and cell sorting

Fresh PBMC were isolated from 8 FBP1-sensitized AD patients that express the HLA type HLA-DRB1*15 as mentioned above. Cells were cultured in the presence or absence of 10 µg/ml peptide 12, 16, and 17, respectively. As controls, 3 healthy donors expressing HLA-DR1*15 as well as 3 healthy donors not expressing HLA-DR1*15 were included. At days three, five, eight and eleven rhIL-2 (20 U/ml) was added to the culture. At day 14, the peptide-stimulated cells were stained for

1h at room temperature with MHC class II tetramers harboring peptide-epitopes 12, 16 or 17, respectively. MHC tetramers were supplied by the Tetramer Core Facility of the Benaroya Research Institute (Seattle, WA, USA). Following that, cells were stained by anti-CD4-allophycocyanin (Beckman Coulter, Brea, CA, USA), anti-CD14-brilliant violet 510 (Biolegend, San Diego, CA) and anti-CD19-brilliant violet 510 (Biolegend, San Diego, CA, USA). MHC tetramer⁺/CD4⁺/CD14⁻/CD19⁻ cells were sorted on a FACS Aria™ Fusion (Franklin Lakes, NJ, USA) in the Hannover Medical School Research Facility Cell Sorting. The sorted cells were stimulated using 10 µl of concanavalin A (Sigma-Aldrich, MO, USA) and after 2 days of incubation, the supernatants were collected. Of each donor, corresponding cells were cultured under the same conditions but in absence of peptides. These cultures served as controls in our experiments to subtract background cytokine levels after ConA stimulation.

Luminex multiplex assay

The resulting cell culture supernatants were analyzed by the Millipore hu Th17 13-Plex (Bio-Rad, CA, USA) assay, which was performed according to manufacturer's instructions to analyze IL-17F, IFN γ , IL-17A, IL-9, IL-2, IL-4, IL-5, IL-31, IL-10, IL-13, IL-21, IL-17E/IL-25, and TNF- α .

Results

In-silico prediction of immunodominant-FBP1 peptides

15-mer peptides were chosen by applying two T cell epitope prediction algorithms, SYFPEITHI (24) and the consensus algorithm of the immune epitope database (IEDB) (25), to the FBP1 sequence *S. aureus* (GeneBank HM245235, strain RN450). Relevant test values are depicted in Table 1. MHC binding to HLA-DRB1*01:01, DRB1*04:01, and DRB1*15:01 was taken into account since those occur with comparably high frequencies in the German population. FBP1 contains two known functional domains: The FbpA domain is conserved among pathogenic proteins that bind fibronectin to enter host cells, the DUF418 domain is of so far unknown function. Peptides 1-14 are located in the FbpA domain, peptides 15 and 16 in the DUF418 domain, and peptide 17 at the C-terminus (compare 20).

In vitro identification of immunodominant-FBP1 epitopes

Patients were included in the epitope mapping studies upon positive testing for FBP1-specific serum IgE by ELISA (n=21). No association between patient severity and response to FBP-1 was observed. Further information on those patients can be found in Table S1. Among testing a wider patient cohort we observed that IgE-sensitization frequency to FBP1 was not associated with age, disease severity as determined by SCORAD or total IgE levels (Figure 1A). IgE sensitization to SEB was detectable in several (33%) but not the majority of the FBP1-sensitized AD patient subgroup (Figure 1B), as reported for AD patients in general by Leung et al. 1993 (26).

T cell lines were generated from FBP1-sensitized patients' PBMCs in the presence of recombinant FBP1 according to an established protocol (27). After 21 days, proliferative capacity to recombinant FBP1 was tested by re-stimulation with autologous irradiated PBMCs as antigen presenting cells. T cell lines from 10 patients (out of 21 investigated patients) displayed distinct proliferation to recombinant FBP1 and were therefore considered as FBP1-specific. In these T cell lines, the proliferative capacity towards each of the seventeen FBP1 15mer peptides was measured by ³H-thymidine incorporation. Top proliferation indices were observed for the peptides 12, 16, and 17 (Figure 2). Since HLA-typing of reactive T cell lines revealed a relative high proportion of HLA-DRB1*15:01-positive donors and prediction algorithms displayed strong values regarding HLA-DRB1*15:01 (Table 1), this HLA was chosen for generating MHC class II tetramers.

Patients that displayed a T cell proliferation response towards FBP1 did not differ from those who did not in terms of SCORAD, total IgE, or IGA (Supplemental Figure 1).

Direct effects of *S. aureus* FBP1 on leukocytes

Allergens often possess intrinsic capacities to interact with the immune system, which push the immune response to Th2, for example due to homology to parasites (28). Furthermore, several allergens function as proteases, a mechanism shared with parasites, or act as adjuvants by activating the innate immune system (29, 30). To investigate whether FBP1 is capable of inducing immune responses by intrinsic properties, cytokine levels were measured in supernatants of patient-derived PBMC after 48h incubation with recombinant FBP1 with or without IL-2. In our set of experiments, there was a trend of a higher median of secreted IL-13 but also of IL-17, IL-22 and IFN- γ upon stimulation of the cells with FBP1 in combination with IL-2 (Supplemental Figure 2).

Presence of T cells specific for immunodominant FBP-1 epitopes in sensitized AD patients

Applying MHC-tetramers engineered on the basis of the three immunodominant epitopes, respective peptide-specific T cells were detected, quantified and sorted from the blood of 8 FBP1-sensitized HLA-DRB1*15:01 matched AD patients. Figure 3 shows staining of FBP1-specific CD4⁺ T cell lines generated in the presence or absence of the corresponding peptide. Without peptide stimulation prior to tetramer staining, only low frequencies of positive cells were observed that did often not exceed background levels. After peptide stimulation for three weeks, distinct populations of positive cells became detectable (frequencies within CD4⁺ cells: tetramer 12: 1.34%±1.55; tetramer 16: 0.44%±0.33; tetramer 17: 0.32%±0.28; n = 8 for all three tetramers). Detailed information on the responsiveness of each donor is given in Table S2.

FBP-1 peptide-specific T cells show a distinct pro-inflammatory profile

After sorting of the FBP-1 peptide-specific T cells from the 21 days T cell lines of the 8 FBP1-sensitized AD patients, we aimed to characterize the T cell polarization based on their cytokine expression by Luminex multiplex ELISA. Detected amounts of target cytokines in the cell culture supernatants of sorted and mitogen-activated peptide-specific T cells are shown in Figure 4. Interestingly, the overall cytokine profile resembled a type-2/type-1 response. The highest levels were reached by type-2 cytokines IL-13 and IL-4, while IL-17 was barely measurable. Further on, a considerable stimulation of IFN- γ could be observed. In healthy HLA-matched subjects we were not able to detect sufficient amounts of FBP1-specific T cells to perform similar measurements (data not shown).

Discussion

It is generally accepted that allergen-specific T cells are a major contributor to the allergic response in atopic patients, particularly in those suffering from late phase and delayed type allergic reactions such as AD, certain forms of asthma and chronic allergic rhinitis (31).

While individuals usually mount a type-1/type-3 immune response to antigens derived from microbial pathogens (32), patients suffering from atopic diseases also display hypersensitivity reactions towards bacterial antigens (33). In bronchial asthma, *S. aureus* SAs specific IgEs levels could be detected in serum and were associated with disease severity. It has been also demonstrated that *S. aureus* SAs specific IgE can predict the development of severe asthma with exacerbations over a long duration (34-36). In allergic rhinitis (AR), it has been demonstrated that

specific IgE to several *S. aureus* SAs including SEA, SEB and TSST-1 was detected in patients' serum contributing to the chronic disease course (37).

The type-2 inflammation bias is induced by *S. aureus* virulence factors, like enterotoxins, serine protease-like proteins, and FBP-1 as described here. Underlying mechanisms include the release of IL-33 from the epithelium, activation of Th2 cells and innate lymphoid cells with subsequent release of type-2 cytokines eventually leading to epithelial damage via mast cell degranulation, B-cell activation, and airway eosinophilia (38). Studies in mice suggest that this is of pathological relevance, since a pre-existing allergy led to diminished pathogen responses (39). Interestingly, a recent study showed that *S. aureus* proteins were among the most frequent underdiagnosed allergens proposing that the pathogenesis of non-allergic compared to allergic asthma is more similar than what was previously assumed (40). This would advocate for including *S. aureus* SA proteins among the standard allergens tested by allergists to improve patients' outcome.

State of the art in detecting allergen-specific T cells is the MHC tetramer staining technology. High sensitivity, epitope specificity and cell viability are believed to be the main advantages of this technology compared to other methods (41). The characterization of fine specificity and functions of FBP-1 specific T cells shown here for the first time confirms and extends our previous observations made on AD patients and a mouse model of FBP1 hypersensitivity, both depicting a type-1/type-2 response to this *S. aureus* protein. Type-1 cytokines have been described together with type-3 cytokines to represent the natural immune response to *S. aureus* in healthy donors (32), while also type-2 responses have been described to single proteins (32, 42). Besides type-2-responses, also type-1 cytokines have been reported in AD inflammation, which have been linked to disease chronicity (3, 43). The overweight of type-2 over type-1 cytokines observed here is of interest, since it may explain the frequent IgE-sensitizations to *S. aureus* and give an explanation on the findings that *S. aureus* even appears to precede AD (44) or allergy in general (42). Further, IgE sensitization to bacterial antigens may be caused through house dust mites who carry bacteria with them (45).

While in AD the predominant role of Th2-polarized T cells is well established, additional T cell subtypes such as Th17 and Th22 T cells have been described to play important roles (4, 5, 27). Since type-3 cytokines play key roles in fighting extracellular pathogens and *S. aureus* (32), it is a

current matter of debate if microbial allergens might contribute to the Th17 polarization in AD. Specific T cells targeting microbial allergens have been described, e.g. to *Malassezia* ssp., where IgE-levels to Mala s 13 correlate with disease severity (46, 47). We described earlier that Mala s 13 bears the potential to cross-react with a human homologous protein (thioredoxin), inducing an immune response containing IL-17 (48). In this context, it is of interest that induction of TNF- α and IL-6 by FBP-1 was reported before (14), a combination known to induce Th17 cells. However, IL-17 was only scarcely detectable, which might be explained by the overweight of type-1/type-2 cytokines. We show here that IgE and T cell sensitizations to allergens include bacterial antigens and contribute to the immune response driving the disease. Whether these sensitizations occur early in life or later as a consequence of an ongoing type-2 inflammation cannot be answered by this kind of study.

Targeting further pro-inflammatory mediators may be the solution for non-responders, since patients' allergen-specific T cells show a range of diverse phenotypes. A limitation that we encountered in our patient cohort was that different topical medication including steroids was not assessed in the study patients, in addition to that different experiments were performed with different number of study patients.

Taken together, we describe a type-2/type-1 response to the *S. aureus* antigen FBP1 in sensitized AD patients. While the type-2 immune response is well-known for its functions in parasite defense, it is most probably sub-optimal in controlling *S. aureus*. It may be hypothesized that by inducing a type-2 immune response via FBP1 in this subset of sensitized patients, *S. aureus* evades the immune system and in parallel aggravates ongoing inflammation, and thereby facilitates further self-growth and subsequent pathogenicity. Increased numbers of *S. aureus* on the skin lead in turn to an increased load of virulence factors that may further promote the skin inflammation combined with skin barrier defects. As a response, an increased suprabasal epidermal fibronectin deposition can be observed (49), which may lead to a vicious circle (Figure 5). Type-1 cytokines like IFN- γ are a substantial part of the immune response to *S. aureus* in healthy subjects (32), and appear to be outnumbered by type-2 responses in our measurements on FBP1. Targeted therapy that blocks type-2 immune responses has been shown recently to reduce *S. aureus* abundance and to increase bacterial diversity (50). It may thus be assumed that interfering with *S. aureus* type-2 mediated pathology is indeed beneficial for AD, and especially for FBP1-sensitized AD patients.

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Tables

Table 1. In-silico prediction of immunodominant-FBP1 peptides. Test values from SYFPEITHI and IEDB (consensus algorithm) are depicted for the HLA-types as indicated. Only relevant values are depicted.

No	position		sequence	DRB1*01:01		DRB1*04:01		DRB1*15:01	
				SYFPEITHI	IEDB	SYFPEITHI	IEDB	SYFPEITHI	IEDB
1	12	27	VESLQFLTGRVHKI	31	10.00	26	6.15	18	4.38
2	45	60	HQLLSIHPNFSRLQ	28	9.82	20	1.64	18	2.68

3	115	130	RTVILEIMGKHSNLI	31	15.82	20	7.96	18	1.18
4	126	141	SNLILVDENRKIEG			26	3.79		
5	138	153	IEGFKHLTPNTNHYR	25	6.34	22	0.99		
6	148	163	TNHYRTVMPGFNYEA			22	15.38		
7	169	184	INPYDITGAEVLKYI	30	16.56				
8	179	194	VLKYIDFNAGNIAKQ	26	30.98	22	7.96	18	9.93
9	198	213	PEGFSPLITNEIVSR	32	12.77	28	2.15		
10	212	227	RRQFMTSSTLPEAFD			16	1.06		
11	222	237	PEAFDEVMAETKLPP			22	16.71		
12	298	313	QQQLHKYQNKLAkli			20	12.1	28	1.76
13	324	339	EQLYGELITANIYRI	32	12.43	28	2.19		
14	354	369	EEVVIPLNPTKSPSA	32	19.54	20	2.83		
15	490	505	VVIFNDAPSDTTIKE			28	4.1		
16	522	537	PVDYTLIKNVHKPSG			28	0.96		
17	539	554	PGFVTYDNQKTLAT			22	2.11	26	8.28

Figure legends

Figure 1: IgE sensitization towards FBP1 in AD patients. A, Comparison between FBP1-sensitized (FBP1-IgE+, n=133) vs. non-sensitized (FBP1-IgE-, n=300) AD patients regarding disease severity (Scoring AD, SCORAD), total IgE level (kU/l) and age of subjects. p-values were calculated by Mann-Whitney-Test. B, SEB sensitization according to IgE levels (kU/l) in FBP1-sensitized AD patients.

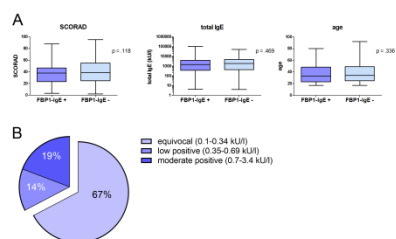
Figure 2: T helper cell epitope mapping of FBP1. Seventeen 15mer peptides of FBP1 that were predicted candidate binders by T cell epitope prediction algorithms were synthesized and tested for T cell proliferation by ³H thymidine intake in FBP1-reactive T cell lines derived from FBP1-IgE-sensitized AD patients. ns, non-stimulated control cells. Stimulation index (SI) is calculated as

the ratio between protein or peptide stimulated and unstimulated control cells. The three peptide epitopes displaying the highest proliferative capacity are depicted in red. Horizontal lines represent the median values.

Figure 3: MHC-II-tetramer staining of FBP1-specific T helper cells. A, MHC-II-tetramers harboring FBP1 peptide12 stained specifically a subgroup of CD4⁺ T cells in a 21-day T cell line (TCL peptide 12) grown from a sensitized DRB1*15:01 AD patient's PBMC in the presence of peptide12. Without stimulation, no distinct population is detectable (unstimulated). FMO, fluorescence minus one control lacking MHC-II-tetramer. B, exemplary MHC-II-tetramers staining of 3-week T cell lines generated from PBMC of DRB1*15:01 AD subjects in presence or absence of FBP1 peptide12, peptide16, and peptide17 followed by staining with their respective tetramer reagents.

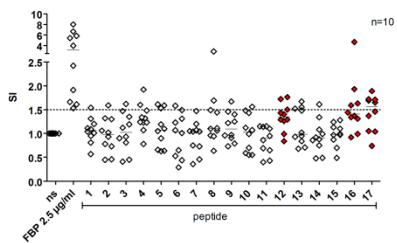
Figure 4: Cytokine expression capacities of FBP1-specific T cells derived from FBP1-sensitized AD patients. TCLs were grown in presence of respective peptide epitopes for 2 weeks. FBP1-specific T cells were sorted after MHC-tetramer staining from TCLs and activated with concanavalin A (ConA). Cytokine levels of corresponding cells cultured in absence of peptides before ConA activation were subtracted. Tetramer12 n=7; tetramer16 n=5; tetramer17 n=6.

Figure 5: The immune response to *S. aureus* FBP1 may facilitate *S. aureus* skin colonization in FBP1 sensitized AD patients. We hypothesize that the Th2-dominated and therefore bona fide suboptimal immune response to FBP1 leads to a facilitation of *S. aureus* adhesion and invasion of lesional AD skin epithelium. *S. aureus* virulence factors lead to inflammation that mediates increased expression of fibronectin by human keratinocytes, which again supports further *S. aureus* skin infection.



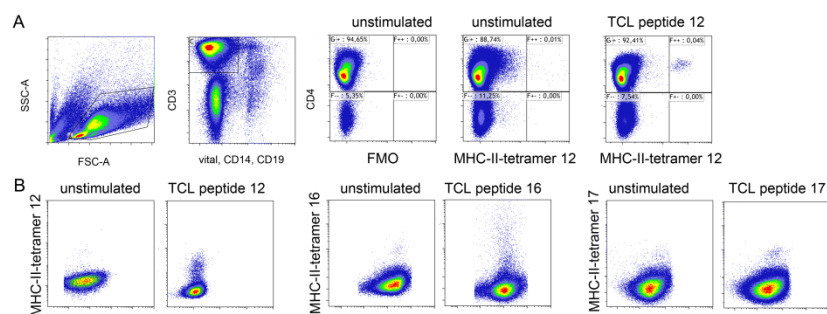
Farag et al. Fig.1

all_15120_f1.tif



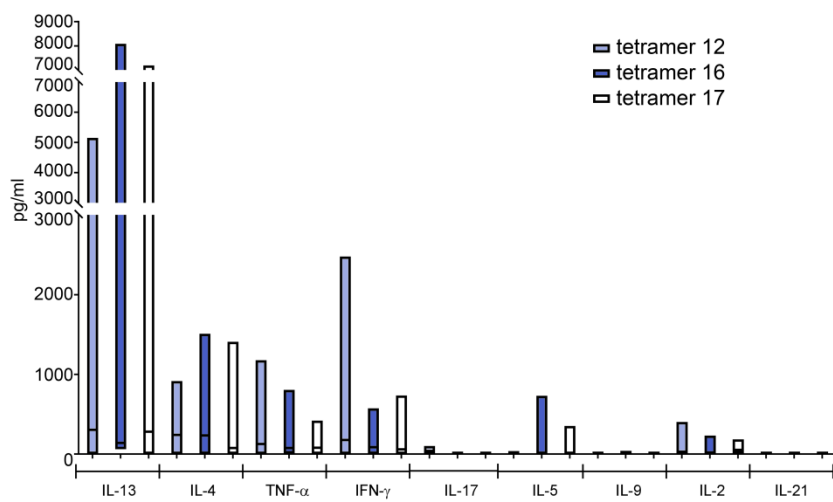
Farag et al. Fig.2

all_15120_f2.tif



Farag et al. Fig.3

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Farag et al. Fig 4

all_15120_f4.tif

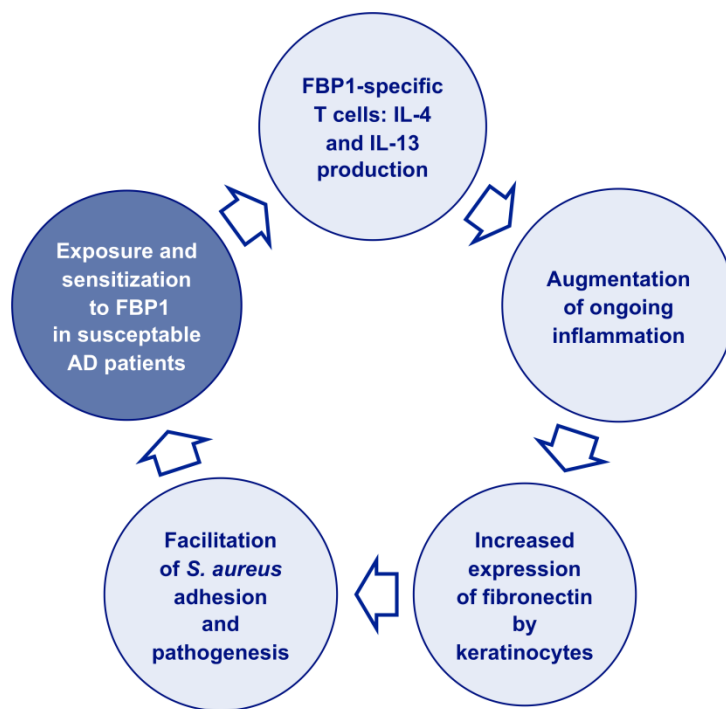
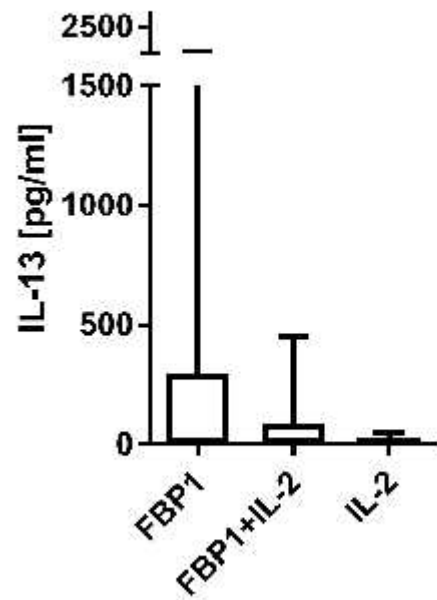
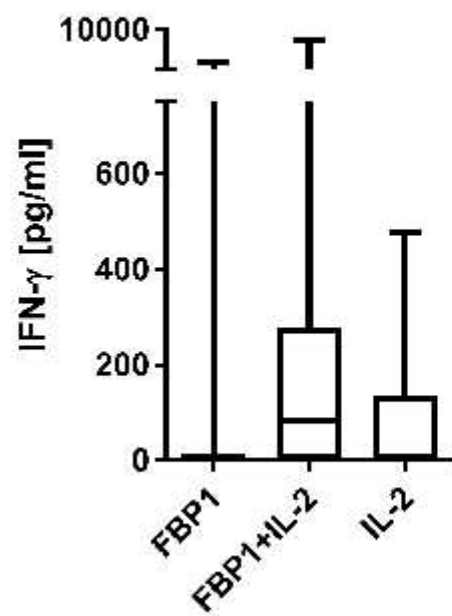
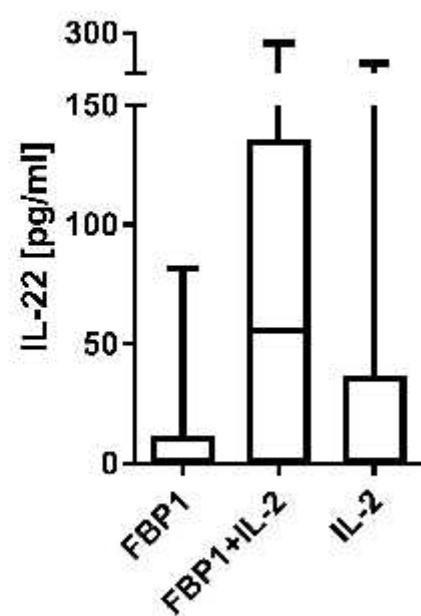
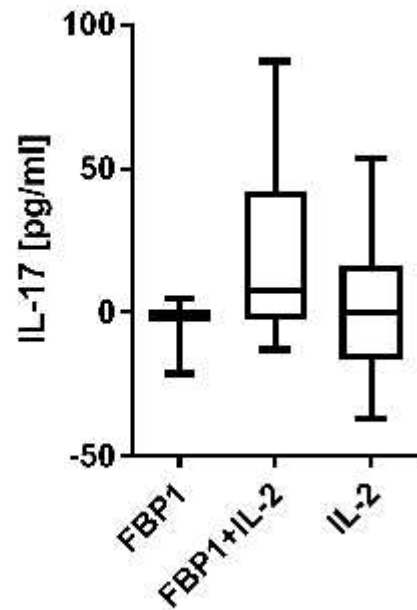


Figure 5_Farag et al.

all_15120_f5.tiff

IL-13**IFN- γ** **IL-22****IL-17**

Supplemental tables

Table S1. Patients 'characteristics and experiments performed.

#	age	IGA	SCORAD	total IgE	HLA-DRB1	epitope mapping	MHC-tetramer staining
1	42	3	74.5	7210	*03,*15	x	
2	73	2	18.6	102	*04	x	
3	44	3	77	16740	*08,*15	x	X
4	27	2	48	228	n.t.	x	
5	19	1	12	371	*01,*04	x	
6	47	1	17.5	2706	*01,*04	x	
7	28	2	41	215	*03,*12	x	
8	23	2	44.1	1315	*01,*15	x	X
9	42	n.a.	n.t.	38	n.t.	x	
10	34	2	41.3	12300	*03,*11	x	
11	35	2	33	445	*11,*15	x	
12	36	2	25.1	81	*01 or 02,*15	x	
13	45	3	48.8	708	*11,*12	x	
14	35	1	41.3	227	*08,*15	x	
15	29	3	68	4662	*07,*14	x	
16	50	2	26	1478	n.t.	x	
17	32	3	45	1450	n.t.	x	
18	37	2	43	5000	*13,*15	x	
19	49	n.a.	27.7	121	*03,*11	x	
20	27	2	44.8	142	*03,*15	x	
21	33	2	21	1042	*01,*03	x	
22	59	1	13	699	*07,*15		x
23	40	2	15	n.d.	*15 (PCR)		x
24	58	2	48.3	>5000	*15 (PCR)		x
25	28	1	15	n.d.	*15 (PCR)		x
26	56	1	23	>5000	*15 (PCR)		x
27	38	1	3	n.d.	*15 (PCR)		x

IGA, Investigator Global Assessment: 0, no symptoms; 1, mild; 2, moderate; 3, severe. SCORAD, Severity Scoring of Atopic Dermatitis. Total serum IgE levels (kU/l) were detected by ThermoFisher Phadia ImmunoCAP. The HLA-type is denoted "PCR" whenever HLA-DRB1*15 genotype was determined by a targeted PCR. n.a., not assessed.

Table S2. Evaluation of MHC-tetramer staining of T cell lines for each HLA-DRB1*15:01 donor and MHC-tetramer. Percentages of MHC-tetramer⁺ cells within the CD4⁺ T cell fraction are depicted. If only background levels were detectable, (-) is depicted. HLA-matched, healthy donors served as controls (ctrl.1-3). No signal was detectable when investigating HLA-mismatched cells (not shown).

	tetramer 12		tetramer 16		tetramer 17	
	ns	stim	ns	stim	ns	stim
3	0.64	1.42	0.64	0.40	0.64	0.41
8	-	2.14	0.47	0.51	-	0.28
22	0.63	1.15	0.83	1.10	0.80	1.92
23	0.32	0.36	0.38	0.58	0.32	0.54
24	-	4.77	-	-	-	-
25	-	0.12	-	-	-	0.19

26	0.95	0.62	0.43	0.52	0.42	0.47
27	-	0.14	-	-	-	-
ctrl 1	-	0.80	-	0.66	-	-
ctrl 2	-	-	0.85	-	-	-
ctrl 3	-	-	-	-	-	-

Supplemental figures (legends):

Supplemental Figure 1: Characteristics of FBP1-sensitized patients that displayed a proliferative response after FBP1 antigen stimulation of PBMC (FBP1 proliferation) compared to those who did not (no FBP1 proliferation). Disease severity (Scoring AD, SCORAD; IGA, Investigator Global Assessment: 0, no symptoms; 1, mild; 2, moderate; 3, severe) and total serum IgE levels (kU/l) are depicted.

Supplemental Figure 2: Cytokine levels in cell culture supernatants of freshly isolated PBMCs from FBP1-sensitized patients with AD stimulated for 48h with 2.5 µg/ml FBP1 in the presence or absence of IL-2. Cytokine levels of non-stimulated cells were subtracted. IL-13: n=11; IFN-γ, IL-22, IL-17: n=10.