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**Omalizumab response correlates with reduced IFN- γ -, IL-10- and IL-31-secreting cells
in chronic spontaneous urticaria**

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Author contribution:

MMR wrote the manuscript, performed the experiments, and analyzed the data. CM and WP designed the study and revised the manuscript. LH and JP contributed to the acquisition of clinical data and revised the manuscript. All authors finally approved the manuscript.

ABSTRACT

Background: The anti-IgE antibody omalizumab has been approved for the treatment of chronic spontaneous urticaria (CSU) in patients insufficiently responding to antihistamines.

However, its mode of action in CSU is not clearly understood.

Objectives: The aim of this study was to get a better insight in the immune mechanisms involved in clinical improvement of CSU patients treated with omalizumab.

Methods: CSU patients (n=15) were followed for five months after initiation of omalizumab treatment. Clinical symptoms were assessed by UCT and CU-Q2oL. Cell-bound IgE was quantified on both FcεRI- and FcεRII-cell populations in peripheral blood. In addition, IgE and IgG as well as their receptors were measured on basophils and basophil activation was assessed with different concentrations of anti-FcεRI and fMLP. Furthermore, the frequencies of different T cell subsets secreting IL-5, IL-10, IL-31 or IFN-γ were analyzed by ELISpot assay.

Results: Seven patients showed a full, five a partial and three no clinical response to omalizumab. Cell-bound IgE was reduced on FcεRI-bearing cells, but not on FcεRII-expressing cells. Likewise, the expression of FcεRI declined. Basophil activation increased upon FcεRI-stimulation while their sensitivity was not affected. Both basophil and T cell frequencies remained unchanged. However, when comparing the individual response to omalizumab treatment with distinct T cell subsets, a significant correlation was found between improved UCT and decreased frequencies of IL-10-, IL-31- and IFN-γ-secreting T cells.

Conclusions: We here show that besides addressing IgE-dependent immune mechanisms, omalizumab treatment of CSU patients has effects on distinct T cell subsets, which correlate with clinical improvement.

Abbreviations:

AUC:	area under the curve
CSU:	chronic spontaneous urticaria
CU-Q _{2o} L:	chronic urticaria quality of life score 2
fMLP:	N-formyl-methionyl-leucyl-phenylalanine
IFN:	interferon
IL:	interleukin
MFI:	mean fluorescence intensity
PBMC:	peripheral blood mononuclear cells
UCT:	urticaria control test

INTRODUCTION

Patients with chronic spontaneous urticaria (CSU) suffer from recurrent wheals and pruritus induced by the release of inflammatory mediators from cutaneous mast cells. How mast cells are activated in CSU is still the focus of intense research. In approximately one third of CSU patients, IgG autoantibodies against IgE or its high affinity receptor, FcεRI, are found^{1, 2}, which are linked to disease severity³. Furthermore, IgE antibodies directed against different autoantigens can be detected^{4, 5}. Besides mast cells, basophils leaving the peripheral blood compartment and migrating into the skin might be of pathological relevance. The degree of peripheral blood basopenia, one of the earliest and most consistently reported findings in CSU^{6, 7}, correlates with the severity of clinical symptoms and is associated with autoreactivity^{8, 9}. Moreover, in a subgroup of CSU patients, basophils are non-responsive to stimulation of the FcεRI^{10, 11}. An important question is if other immune cells besides mast cells and basophils might play an important role in the pathogenesis of CSU. In this regard, FcεRIα-specific T cells have lately been described in peripheral blood of CSU patients¹².

First line treatment of CSU is antihistamines blocking the binding of histamine released by mast cells and basophils to the histamine receptor 1. However, only about 60% of CSU patients benefit from this treatment¹³. For patients that do not sufficiently respond to antihistamines the monoclonal anti-IgE antibody omalizumab was approved, which has been shown efficacious in up to 80% in several studies¹⁴⁻¹⁶. While its clinical efficacy is well established, the immunological mode of action of omalizumab in CSU is not that well

understood. So far, it has been demonstrated that omalizumab reduces free serum IgE¹⁷, thereby leading to a reduction of cell-bound IgE and downregulation of cell surface FcεRI expression¹⁸⁻²¹. Furthermore, basophil releasability upon IgE-mediated activation, which is disturbed in a subgroup of CSU patients^{9, 22}, recovers during omalizumab therapy²³. In addition, individual immune alterations might contribute to a differentiated therapeutic^{20, 24-26}. For example, recent studies indicate that the presence of autoantibodies could lead to a delayed treatment response^{20, 27} and low total serum IgE levels might even be an indicator of treatment failure²⁸.

To better understand the immunological effects of omalizumab in CSU and shed more light on the pathogenesis of this disease, we conducted an experimental study investigating different immune parameters in CSU patients before initiating and during therapy with omalizumab. An additional point of interest was to identify potential biomarkers indicating a therapeutic response to omalizumab.

METHODS

Patients

A total of 15 patients (12 females/ 3 males) with CSU non-sufficiently controlled by antihistamines were treated with 300mg omalizumab subcutaneously every four weeks and followed for up to 20 weeks. All patients suffered from CSU for more than 8 weeks. Chronic as well as acute infections were ruled out as a possible trigger by history and routine blood analysis. None of the patients had received systemic corticosteroids within the last four weeks or was previously treated with omalizumab. During the study period, patients were allowed to take non-sedating antihistamines. Peripheral blood was drawn for further analysis before the first (V1), second (V2), third (V3), fourth (V4) and sixth (V5) injection of omalizumab. Two of the patients discontinued the study after V4 (patient B1 and B14; Table 1) due to non-treatment related reasons. Clinical symptoms were recorded by the urticaria control test (UCT)²⁹ and the German version of the CU quality of life score (CU-Q_{2oL})³⁰. According to the grading system of the UCT^{27,29}, patients with well-controlled CSU at the final visit of the study ($UCT \geq 12$) were defined as full-responder. CSU patients who improved in a clinically relevant range of minimum 3 UCT points according to Ohanyan et al.³¹ were classified as partial-responder. Patients that did not experience clinically relevant improvement were graded as non-responder to omalizumab treatment. All patients provided written informed consent before inclusion in the trial. The study was approved by the ethics committee of the medical faculty of the Philipps-Universität Marburg.

A detailed description of methods is provided in this article's Supporting Information.

RESULTS

CSU patients show a differentiated clinical response to anti-IgE treatment

Patients with CSU were treated for up to five months by monthly subcutaneous injections of the monoclonal anti-IgE antibody omalizumab and analyzed before as well as after one, two, three and five months of treatment (Fig. 1a). Clinical evaluation by UCT and CU-Q2oL revealed a significant improvement of symptoms in most patients already after one month of treatment (Fig. 1b, c; $p < 0.05$). Within the group of omalizumab responders, seven patients showed full and six-five partial response to omalizumab according to our grading system at the final visit. However, ~~two~~ three patients did not improve until the last visit in comparison to pre-treatment values (Table 1, patients B3 and B4, indicated in bold). Notably, two of these three patients were less severely affected when starting omalizumab.

All patients showed an 1.2- to 11-fold increase of total serum IgE as determined by using two independent measure systems indicating the well-known development of IgE-omalizumab complexes (Fig. 1d, e; $p < 0.05$), which results in enhanced serum IgE concentrations due to an extended half-life of complexed IgE^{32, 33}. It has been speculated that serum IgE measured by ADVIA Centaur would quantify free IgE in an indirect fashion due to competing epitopes for the detection antibodies and omalizumab³⁴. However, we found a strong correlation of

total IgE measured by ADVIA Centaur and ImmunoCAP ($r = -0.9700$, $p < 0.0001$, data not shown).

Depletion of cell-bound IgE

To evaluate the amount of IgE bound to cell surface receptors, IgE was stained on FcεRI- and FcεRII-bearing cells, in order to obtain a relative measure for total cell-bound IgE on different cell populations. This staining strategy is based on the size and granularity as well as the distribution of either FcεRI or FcεRII on different cell populations. By this means, we were able to quantify IgE on FcεRI+ (basophils, monocytes/dendritic cells) and FcεRII+ cells (B cells, eosinophils/neutrophils). Here, a significant reduction was detected on FcεRI-bearing cells like basophils (Fig. 2a; $p < 0.001$), monocytes and dendritic cells (Fig. 2c; $p < 0.001$), but not on FcεRII-expressing cells like eosinophils and B lymphocytes (Fig. 2b, d).

Reduction of IgE and FcεRI on basophils

Basophils have gained interest in the pathophysiology of CSU. Not only as a peripheral blood surrogate of cutaneous mast cells, but also because basopenia is a common feature in CSU³⁵. Several studies have described diminished basophil-bound IgE and decreased expression of its high affinity receptor, FcεRI, during omalizumab treatment^{18, 36}. Likewise, patients in this

study showed a reduced MFI of cell surface IgE and FcεRI expression after one month lasting for the whole treatment period compared to pre-treatment levels (Fig. 3a, b; $p < 0.001$). To investigate these parameters in more details, we specifically focused on their presence on basophils. Notably, we detected declining numbers of IgE+ basophils (Fig. 3c; $p < 0.05$) but not of FcεRI+ basophils (Fig. 3d). Thus, while surface IgE and FcεRI is diminished by omalizumab, more than 60% of basophils still have IgE bound to their surface and 90% continue to express FcεRI.

Since basophils also exhibit FcγRII, which is able to inhibit IgE-mediated basophil activation³³³⁷, its expression and the presence of IgG bound by the receptor was determined on basophils. However, no significant changes were detected during omalizumab treatment (Fig. 3e, f).

Basophil reactivity increases upon omalizumab treatment

Since increased peripheral blood numbers¹¹ and sensitivity⁷ of basophils have been linked to a more moderate course of CSU, these parameters were also closely investigated in the present study. However, quantifying basophil frequency in peripheral blood by flow cytometry, we could not detect a rise of basophil numbers during omalizumab treatment when investigating the whole cohort although a trend became apparent at V2 ($p = 0.08$; data not shown). Notably, all patients showing pre-treatment basopenia ($\leq 0.1\%$ of peripheral

blood cells, $n = 5$) responded to treatment (either fully ($n = 3$) or partially ($n = 2$); table 1, indicated in light grey) and in the majority of these (4/5) the number of basophils normalized. Interestingly, one of the patients who did not experience a benefit by omalizumab developed basopenia during the study (Table 1, B4).

To investigate their reactivity to FcεRI-mediated activation, basophils were stimulated with different concentrations of anti-FcεRIα. Thereby three basophil phenotypes could be differentiated as recently published⁹: firstly, reactive to anti-FcεRIα (CU-R, 60%), secondly, non-reactive to anti-FcεRIα (CU-NR, 7%) and thirdly non-reactive with basopenia and already pre-activated basophils (CU-NR_{B0}, 33%). Upon omalizumab therapy, this distribution shifted towards a more reactive phenotype (CU-R: 67%, CU-NR: 13%, CU-NR_{B0}, 20%, Table 1). In addition, the median dose response curves showed an enhanced frequency of activated, CD63+ basophils compared to pre-treatment numbers (Fig. 4a). Increased basophil activation became even more obvious by calculating the AUC (Fig. 4b; $p < 0.05$). In contrast to the AUC, which is a measure that combines reactivity and sensitivity of basophil activation, the EC₅₀, resembling the concentration giving half-maximal activation, displays only sensitivity³⁴³⁸. Here, the EC₅₀ of FcεRI-mediated activation remained unchanged during omalizumab treatment (Figure 4c). In addition, non-specific stimulation by fMLP also resulted in increased basophil activation albeit not as distinct and only at some time points (Fig. 4d; $p < 0.05$). In summary, basophil reactivity to anti-FcεRIα increases, without affecting the sensitivity.

Omalizumab therapy leads to T cellular alterations

Since previous studies showed elevated T cellular cytokine concentrations like IL-10³⁹ and IL-31⁴⁰ in untreated CSU patients and a decrease of IL-31 serum concentrations during omalizumab treatment⁴¹, the question arose if the frequency of T cells secreting these cytokines would be altered by omalizumab therapy. In addition, the number of IFN- γ - and IL-5-secreting cells as marker cytokines for T_H1 and T_H2 cells was assessed, respectively. Within the whole cohort of treated patients, the investigated T cell frequencies did not change statistically (Fig. 5a-d). However, there was a trend to reduced frequencies of IFN- γ -, IL-10- and IL-31-secreting cells in the first visits during omalizumab treatment.

Therapeutic efficacy correlates with decreased numbers of IL-10- and IL-31-secreting cells

To identify potential biomarkers for clinical outcome of omalizumab therapy, the investigated immune parameters were correlated with treatment response at the final visit (Δ V1-V5). Correlating the alterations in IL-10+, IL-31+ and IFN- γ + cells with the improvement in UCT, an inverse relationship was discovered (Table 2; IL-10: $r = -0.6052$, $p < 0.05$; IL-31: $r = -0.6066$, $p < 0.05$; IFN- γ : $r = -0.5645$, $p < 0.05$) (Fig. 5e-g). Therapeutic response recorded by CU-Q2oL did not significantly correlate with changes in these cellular subtypes (data not

shown). Furthermore, immune pre-treatment parameters were correlated with improvement in UCT at the end of the observation period, looking for predictive markers for treatment success, but no significant relation was seen (Suppl. Table E1). To identify markers of early response clinical improvement at V2 was correlated with immune changes at this time point, however no significant correlation was obtained either. Still the best although not statistically significant association for early response was noticed for total serum IgE (Suppl. Table E2).

DISCUSSION

In 2014, the monoclonal humanized anti-IgE antibody omalizumab was approved for the treatment of antihistamine-resistant CSU in the European Union and in the US. Several double-blind placebo-controlled^{14, 24} and real-life studies⁴² have proven its clinical efficacy in CSU. However, the mechanisms by which omalizumab leads to improvement in CSU are still under investigation. To shed more light on its mode of action, we analyzed different immune parameters in CSU patients treated for 20 weeks with omalizumab and correlated them with clinical efficacy. Interestingly, besides its known effects on IgE-mediated immune mechanisms, omalizumab affected distinct T cell populations with clinical improvement primarily associated with decreased frequencies of IL-10-, IL-31- and IFN- γ -secreting cells.

Several studies have investigated the impact of omalizumab therapy on IgE in the serum as well as fixed on cells, reporting that free IgE is cleared from the circulation¹⁷ and

subsequently also lost from the cell surface^{18, 43} by an indirect fashion since omalizumab cannot target receptor bound IgE^{44, 45}. Thus, pathogenic IgE autoantibodies directed for example against thyroid peroxidase⁴ or IL-24⁵ should be depleted. Since IgE itself might also serve as an autoantigen^{3, 46}, removal of IgE antibodies complexed with omalizumab should also have an impact on CSU symptoms. In addition to IgE, the expression of its high affinity receptor FcεRI is downregulated on peripheral blood basophils, as shown here and by others^{36, 47}. Basophil-bound IgE declined already after the first omalizumab application, accompanied by a strong reduction of FcεRI expression. Of note, a very recent study reported that reduced surface density of FcεRI is not only seen on basophils, but also on skin cells¹⁸.

Different patterns of basophil activation to anti-IgE or allergen stimulation during omalizumab therapy have been described, showing in peanut-allergic patients reduced^{44,48} and in CSU patients unaltered sensitivity¹⁹, the latter finding similar to our results determined by calculating the EC₅₀. On the other hand and in line with a previous report^{45,19} basophil reactivity rather increased.

We and others¹¹ have recently demonstrated that CSU patients can be classified into three distinct immunological endotypes based on their basophil reactivity to FcεRI stimulation, namely responders, non-responders and a basopenic group with the least disease control⁹. However, following the frequency of basophils during omalizumab therapy, their number did not rise significantly, which is in line with a previous report by Metz et al.¹⁸ but in contrast to another recently published study⁴⁹. Nevertheless, all of the basopenic patients included

responded at least partially to omalizumab treatment, with the majority displaying a substantial recovery of their basophil number. It is assumed that basophils of CSU patients migrate to the affected skin, because they are more abundant in cutaneous lesions of CSU patients than in healthy control skin⁵⁰. Moreover, they are recruited to the skin after autologous serum injection in patients with autoreactive CSU^{48,51}. Hence, it could be hypothesized that in basopenic patients the response to omalizumab treatment is partly due to diminished basophil migration to or redistribution from the skin resulting in normalization of basophil numbers in peripheral blood.

A major finding of our study is the association of reduced T lymphocyte frequencies, namely cells secreting IL-10, IL-31 and IFN- γ , with the improvement of clinical symptoms during omalizumab therapy. Evidence for an involvement of T cells in CSU stems from examinations showing enhanced frequencies of CD4⁺ and CD8⁺ cells in CSU patients compared to healthy control skin^{48,51} and increased cutaneous mRNA expression of IFN- γ , IL-4 and IL-5⁵². Beyond that, in peripheral blood of CSU patients T cell cytokines like IL-31⁴⁰, IL-17 and IL-33⁵³ are elevated and IFN- γ -secreting, autoreactive T lymphocytes targeting Fc ϵ RI α can be found¹². For this reason, we investigated the influence of omalizumab on different T cell subsets. While the frequencies of IL-5-, IL-10-, IL-31- and IFN- γ -secreting cells remained unaltered in the whole cohort of studied patients, treatment response correlated with decreased numbers of IL-10-, IL-31- and IFN- γ -secreting T lymphocytes. Thus, omalizumab is not only affecting IgE-mediated immune mechanisms and

immediate type effector cells but in addition seems to modulate inflammatory T cell responses. This is also suggested by reduced serum levels of IL-31 in CSU patients⁴⁰ and diminished frequencies of T cells in asthmatics^{51,54} treated with omalizumab. So far, the role of T cells in CSU remains obscure; however, the clinical efficacy of the T cell suppressant drug cyclosporine further affirms the importance of targeting T cells in CSU^{55, 56}. Considering CSU an autoimmune disease^{5, 12}, it is evident that autoantigen-specific T cells are of importance triggering B lymphocytes to become autoantibody-secreting plasma cells⁵⁷,⁵⁸. During omalizumab treatment, FcεRI is reduced on dendritic cells and monocytes as antigen-presenting cells^{59, 60}. While IgE fixation on this receptor is crucial for efficient FcεR-mediated antigen presentation to T cells^{61, 62}, we here show that IgE is rather diminished on these cells. Thus, omalizumab might indirectly reduce the percentage of inflammatory T cells by preventing binding of IgE to the FcεR on antigen-presenting cells and thereby prohibit their stimulation of T cells.

One limitation of this trial is the relatively low number of participants. Our investigation was setup as a pilot study covering a variety of different cellular and molecular parameters, thereby uncovering potential immunological candidates of pathophysiologic relevance targeted by omalizumab. Furthermore, we cannot exclude that we might have missed very early immune effects initiated by omalizumab days or even hours after its first application.

In summary, while we were able to confirm important influences of omalizumab on its primary target, IgE, and IgE-dependent parameters like FcεRI expression and basophil

releasability, we also could show that omalizumab has an impact on inflammatory T cell activity, as observed by a significant correlation between clinical treatment response and reduced numbers of IL-10-, IL-31- and IFN- γ -secreting cells. Our results thus point to an extended spectrum of immune alterations induced by anti-IgE antibodies, opening potential new perspectives for their therapeutic implementation.

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FIGURE LEGENDS

Fig. 1: Patients with CSU were treated with monthly subcutaneous omalizumab injections (indicated by arrows) for five months. Blood was drawn before (V1), after one (V2), two (V3), three (V4) and five months (V5) of omalizumab therapy (a). CSU-related symptoms were quantified on the questionnaires UCT (b) and CU-Q2oL (c). Total IgE measured by ADVIA centaur (d) as well as by ImmunoCAP (e) were compared before and during omalizumab treatment. Statistical significant differences *** $p < 0.001$.

Fig. 2: The amount of cell-bound IgE in relation to the frequency in the peripheral blood was quantified on basophils (a), B cells (b), monocytes/ dendritic cells (c) and eosinophils (d) by flow cytometry. Statistical significant differences *** $p < 0.001$.

Fig. 3: The mean fluorescence intensity (MFI) of IgE (a) and FcεRI (b) was quantified on the surface of basophils by flow cytometry. In addition, the percentage of IgE+ (c) and FcεRI+ (d) as well as IgG+ (e) and FcγRII+ (f) basophils of CSU patient before and during omalizumab was determined. Statistical significant differences * $p < 0.05$, *** $p < 0.001$.

Fig. 4: Basophils of patients with CSU were stimulated with different concentrations of anti-FcεRIα and median dose response curves were compared before and during the course of

omalizumab therapy (**a**). The area under the curve of the individual dose response curves (**b**) and the respective doses giving half-maximal activation, the EC₅₀ were calculated (**c**). Some of the CSU patients' basophils could not be activated by anti-FcεRIα. Their EC₅₀ was arbitrarily set to 0.001. As an unspecific control stimulus, fMLP was used to activate basophils in an FcεRI-independent way (**d**). Statistical significant differences *p<0.05, **p<0.01.

Fig. 5: Frequencies of IFN-γ- (**a**), IL-5- (**b**), IL-10- (**c**) and IL-31-secreting cells (**d**) of CSU patients before and during omalizumab treatment were quantified by ELISpot assay after stimulation with CD3/ CD28 beads. The treatment efficacy was correlated with the change in IL-10+ (**e**), IL-31+ (**f**) and IFN-γ+ spots (**g**) at the end of the therapy with omalizumab. Open circles depict patient that did not respond to omalizumab treatment.

Table 1: Patient characteristics

Patient number	Sex	Age [years]	CSU since [months]	Basophil frequency		Basophil phenotype		UCT	
				V1	V5	V1	V5	V1	V5
1	f	22	10	0.02	0.15	CU-NRB0	CU-NR	5	16
2	f	49	17	0.09	0.26	CU-NRB0	CU-R	3	16
3	f	21	7	0.1	0.08	CU-NRB0	CU-NRB0	7	16
4	m	21	6	0.01	0.03	CU-NRB0	CU-NRB0	6	10
5	f	36	4	0.05	0.16*	CU-NRB0	CU-NR*	1	7*
6	m	42	6	0.69	1.05	CU-R	CU-R	3	16
7	f	18	13	0.61	0.55	CU-R	CU-R	1	13
8	f	66	3	0.71	0.64	CU-R	CU-R	6	13
9	f	28	44	0.32	0.18*	CU-R	CU-R*	4	12*
10	f	34	137	0.16	0.33	CU-R	CU-R	2	11
11	f	55	6	0.44	0.49	CU-R	CU-R	5	10
12	m	52	119	0.62	0.29	CU-R	CU-R	4	10
13	f	39	137	0.39	0.39	CU-NR	CU-R	6	7
14	f	23	69	0.22	0.54	CU-R	CU-R	11	9
15	f	24	26	0.31	0.03	CU-R	CU-NRB0	11	11

*indicates when values of V4 were displayed, because V5 was not available; patients in bold were non-responsive to omalizumab treatment; patients marked with grey background were basopenic at V1; CU-R: basophils responsive to FcεRI-stimulation, .CU-NR: basophils nonresponsive to FcεRI-stimulation, CU-NRB0: basopenia with basophils non-responsive to FcεRI-stimulation

Table 2: Correlation clinical efficacy

Correlation with UCT ΔV1-V5	Spearman r-values	Spearman p-values ↓	Benjamini- Hochberg significance
IL-31+ cells	-0,6066	0,0174	significant
IL-10+ cells	-0,6052	0,0180	significant
IFN-γ+ cells	-0,5645	0,0292	significant
IL-5+ cells	-0,5081	0,0526	not significant
FcγRII on basophils	0,4208	0,1191	not significant
IgE on B cells	-0,4135	0,1213	not significant
IgE on basophils	0,3366	0,2186	not significant
ex vivo activation	-0,3151	0,2452	not significant
IgE on eosinophils	-0,2954	0,2768	not significant
basophils	0,2742	0,3192	not significant
FcεRI on basophils	0,2739	0,3206	not significant
fMLP activation	0,2650	0,3371	not significant
anti-FcεRIα AUC	0,2363	0,3934	not significant
IgE on basophils	0,1325	0,6356	not significant
serum IgE (ImmunoCAP)	-0,1092	0,6873	not significant
IgE on monocytes	-0,08423	0,7533	not significant
anti-FcεRIα EC ₅₀	0,05031	0,8592	not significant
serum IgE (ADVIA)	-0,01970	0,936	not significant
IgG on basophils	0,01170	0,9678	not significant

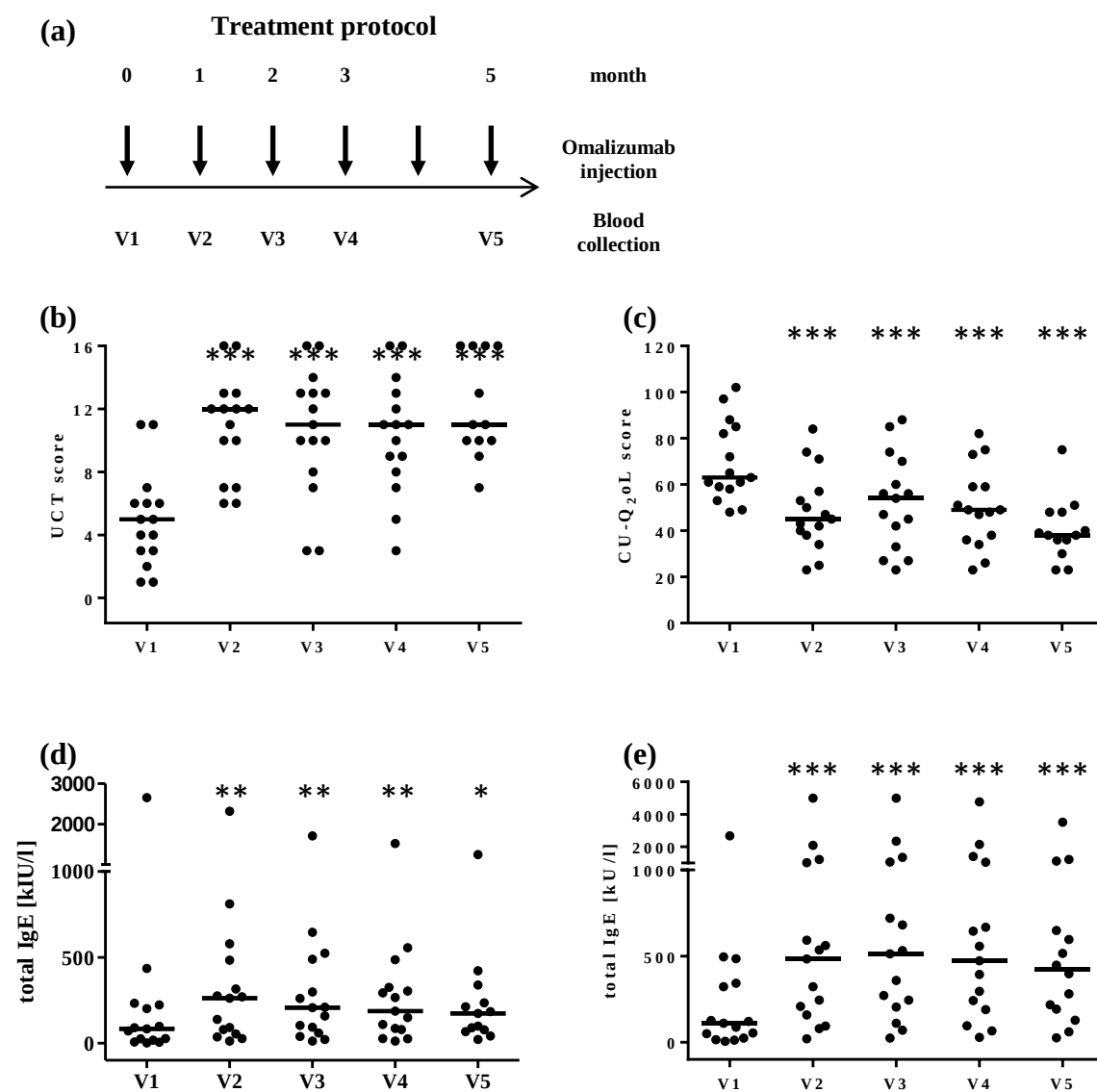


Figure 1

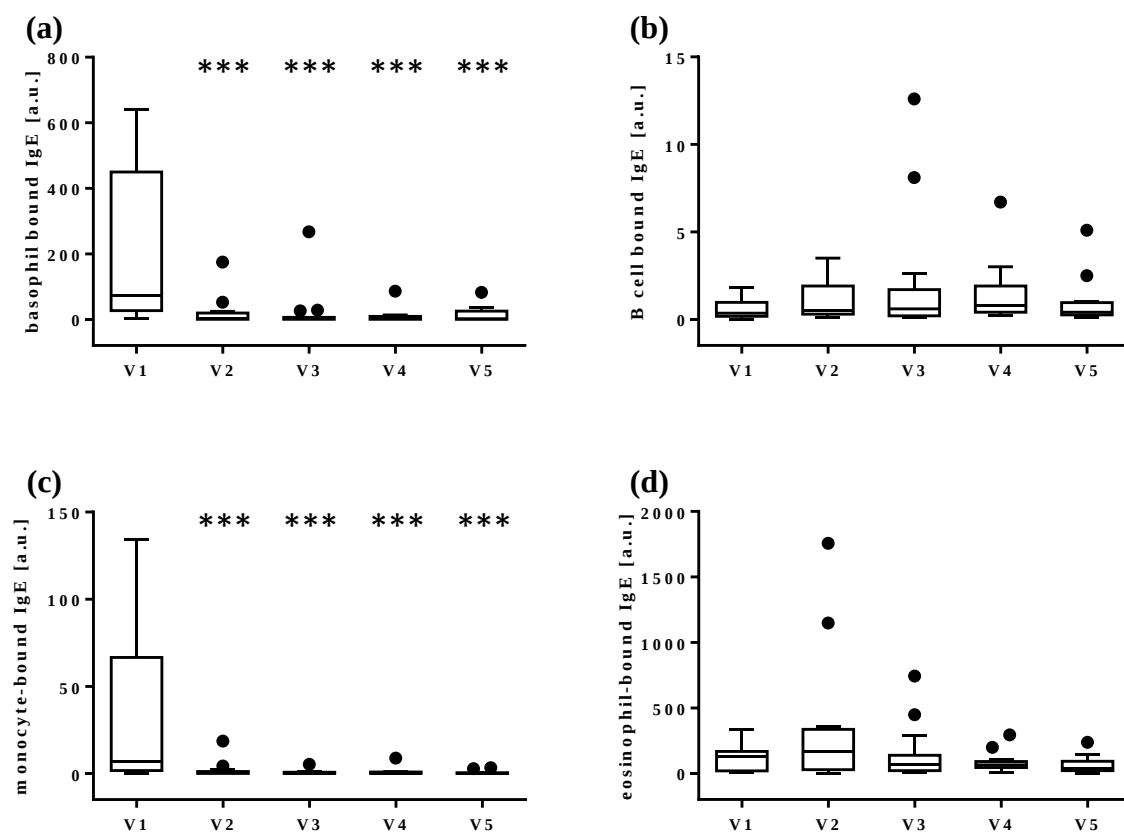


Figure 2

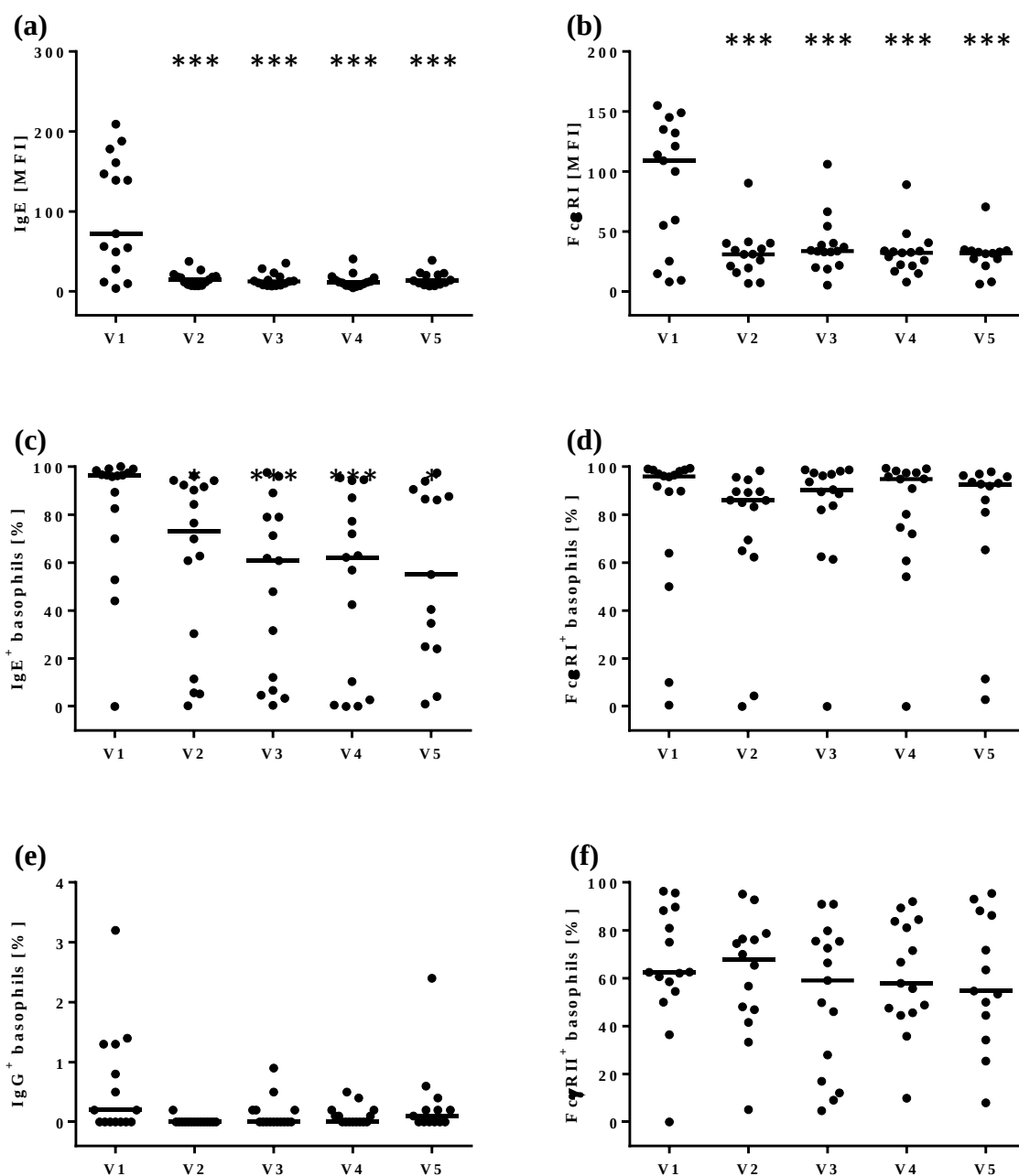


Figure 3

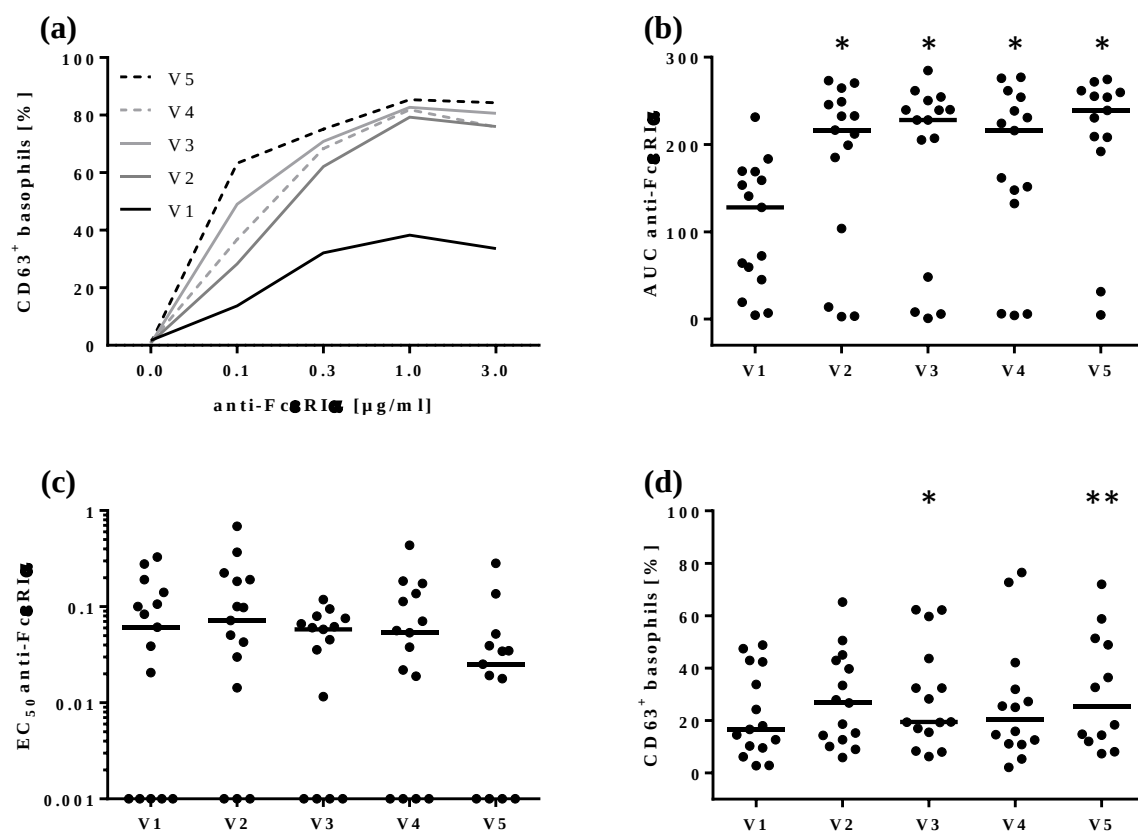


Figure 4

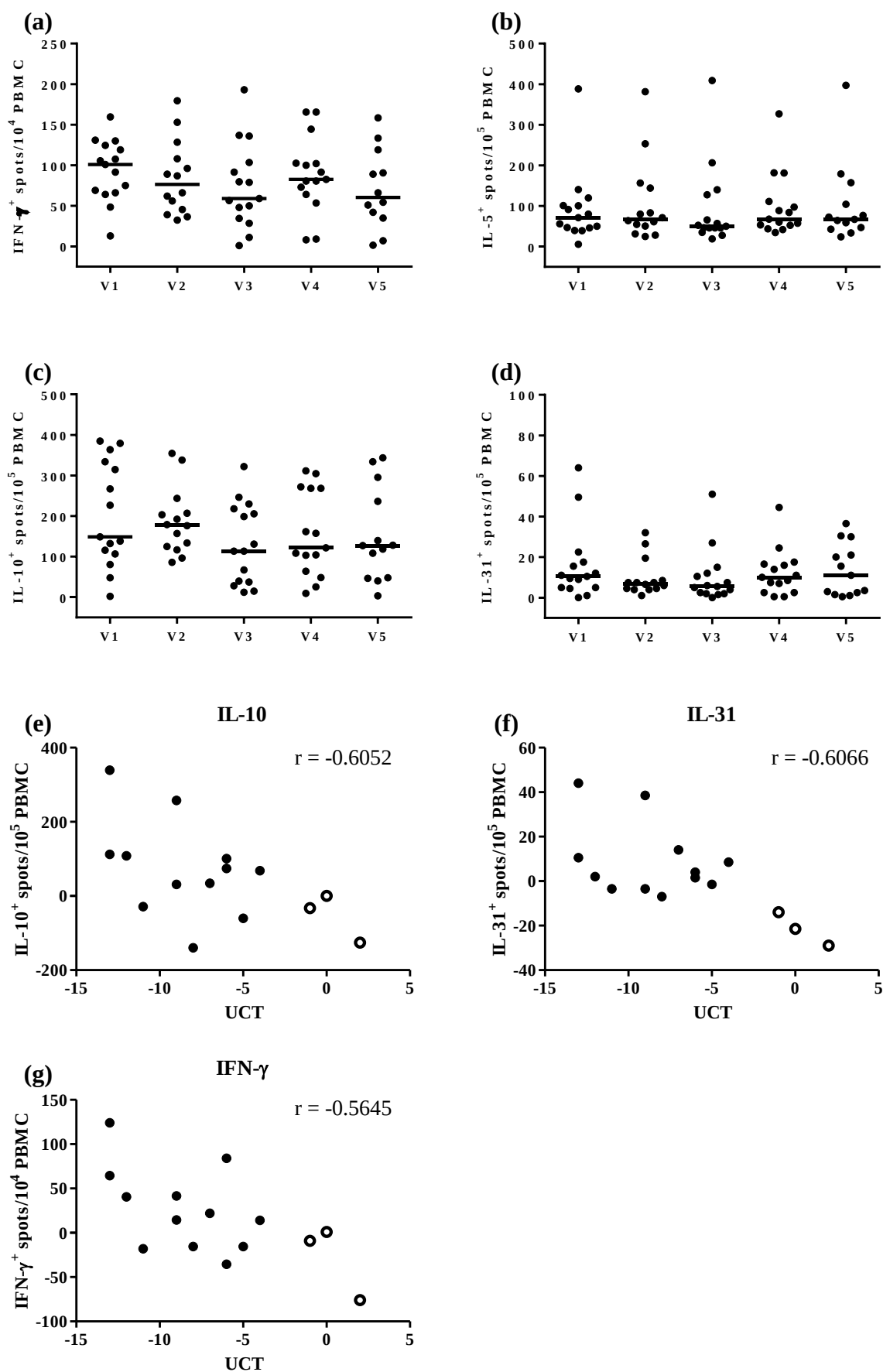
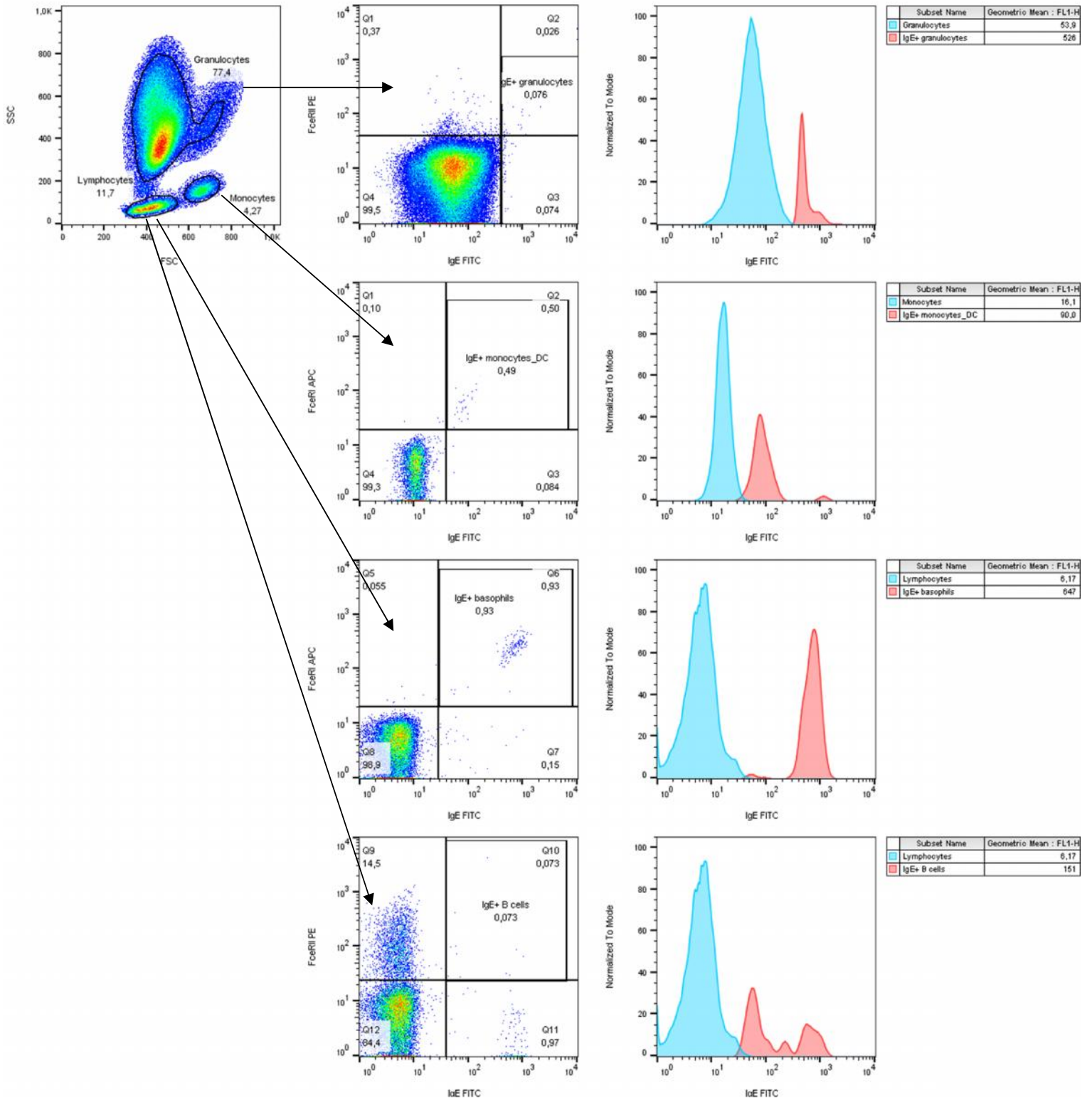


Figure 5



Suppl. Figure 1

1 **METHODS**

2 **Measurement of total serum IgE**

3 Serum concentrations of total IgE were measured by ImmunoCAP and ADVIA Centaur
4 according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, USA;
5 Siemens, Munich, Germany).

7 **Quantification of cell-bound IgE, IgG and their receptors**

8 The relative amount of cell-bound IgE and its respective receptors were assessed on different
9 peripheral blood cells as described by Liang and Ganley-Leal (1). Briefly, peripheral blood
10 was depleted of erythrocytes by incubation with lysis buffer and the remaining cells were
11 stained with anti-IgE-FITC (eBioscience, San Diego, USA), anti-FcεRII-PE (BD Biosciences,
12 San Jose, USA) and anti-FcεRI-APC (eBioscience, San Diego, USA) for 20 min at 4°C
13 followed by flow cytometric analysis. To differentiate IgE-bearing cell populations, the
14 expression of FcεRI and FcεRII as well as the size and granularity were used for gating of
15 peripheral blood cells (Suppl. Fig. 1). Thereby, FSC and SSC low cells (lymphocyte gate)
16 contained two major cell populations carrying IgE on their surface, FcεRII⁺ B cells and
17 FcεRI⁺ basophils. Monocytes/dendritic cells have a higher FSC value and are characterized
18 by a high FcεRI expression, whereas eosinophils/neutrophils are larger and more granular
19 than lymphocytes and express mostly FcεRII. By these criteria, cells were gated and their

frequency within the peripheral blood was determined. The mean fluorescence intensity (MFI) of IgE was determined on the four different cell populations and the MFI of the isotype control was subtracted. Resulting values were multiplied with the respective frequency of the peripheral blood cell populations. In addition, IgE, IgG and their respective receptors (FcεRI, FcγRII) were quantified on the surface of basophils as recently described (2).

Basophil activation test

For basophil analysis, peripheral blood was sampled in ethylenediaminetetraacetic acid-containing blood collection tubes and processed within 2 h after venepuncture. To measure dose-dependent basophil activation, cells were stimulated with anti-FcεRIα (0.1, 0.3, 1 and 3 µg/ml) and N-formyl-methionyl-leucyl-phenylalanine (fMLP) as non-specific positive control in IL-3-containing buffer using Flow CAST (Bühlmann, Schönenbuch, Switzerland) according to the manufacturer's instructions. Basophils were gated as CCR3⁺ SSC^{low} cells and CD63⁺ basophils were regarded as activated. Based on the anti-FcεRIα dose-response curve, the area under the curve (AUC) was estimated by applying the trapezoidal rule:

$$AUC_{0-x_n} = \frac{1}{2} \sum_{i=1}^{n-1} (x_{i+1} - x_i)(y_i + y_{i+1})$$

The concentration giving a half-maximal activation of basophils, EC₅₀, was determined by fitting a non-linear curve. AUC was used as a measure for basophil releasability and EC₅₀ as a measure for basophil sensitivity to FcεRI-mediated stimulation. In some patients, basophils

did not show activation after stimulation with different concentrations of anti-FcεRIα. Thus, EC₅₀ could not be calculated in these patients and was arbitrarily set to 0.001 in the respective figure.

ELISpot analysis

Peripheral blood mononuclear cells (PBMC) were isolated from citrate-phosphate-dextrose adenine-containing blood by density gradient centrifugation as previously described (3) and stored in liquid nitrogen until further use. For ELISpot analysis, PBMC were thawed and cultured with or without CD3/CD28 Dynabeads (Thermo Fisher Scientific, Waltham, USA) in RPMI-1640 (Capricorn Scientific, Ebsdorfergrund, Germany), supplemented with 10% pooled human serum, 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mmol/l L-glutamine (all from PAA Laboratories, Pasching, Austria) in a humidified atmosphere with 37°C and 5% CO₂, for 24 h at a density of 10⁶ PBMC/ml in 48 well plates. ELISpot plates coated with anti-interleukin (IL)-5-, -10-, -31- or interferon (IFN)-γ-capture antibodies were used to culture the resuspended cells in duplicates for further 20 h at a density of 10⁵ (IL-5, IL-10, IL-31) or 10⁴ (IFN-γ) PBMC/well. Single cell spots were detected according to the manufacturer's instruction (for IL-31 R&D Systems, Minneapolis, USA; for all others BD Biosciences, San Jose, USA) and counted automatically (A.EL.VIS, Hannover, Germany).

57 **Statistics**

58 Comparison of multiple paired samples was performed by the non-parametric Skillings-Mack
59 test (4) (χ^2 distribution; degrees of freedom = 4, B = 1000), which is an equivalent to the
60 Friedman test in a balanced incomplete block design. When rank sums differed significantly
61 between time points, post-hoc analysis with Wilcoxon-signed-rank test was applied.
62 Differences were considered statistically significant at p values < 0.05. Correlations between
63 treatment efficacy and immunological parameters were calculated with Spearman's rank
64 correlation test. To adjust the false discovery rate for multiple comparisons the Benjamini-
65 Hochberg procedure (false discovery rate = 0.15) was utilized. Statistical analysis was
66 evaluated with RStudio© and GraphPad Prism software (GraphPad Software, La Jolla, USA).

67

68 **Supplementary Figure Legends**

69 **Supplementary Figure 1:** Gating strategy for cell-bound IgE staining with exemplary gating
70 of IgE⁺ granulocytes, monocytes, basophils and B cells in relation to isotype control.

71 **Supplementary Table E1:** Predictive values

Correlation with UCT V1	Spearman r-values	Spearman p-values ↓	Benjamini- Hochberg significance
IL-31+ cells	-0,4888	0,0634	not significant
IL-10+ cells	-0,3635	0,1778	not significant
IgE on eosinophils	-0,3062	0,2812	not significant
FcγRII on basophils	-0,2131	0,4349	not significant
anti-FcεRIα AUC	0,2124	0,4432	not significant
IgE on B cells	-0,2058	0,4451	not significant
IgE on basophils	0,2070	0,4743	not significant
IgE on monocytes	-0,1806	0,5287	not significant
anti-FcεRIα EC ₅₀	-0,1532	0,5289	not significant
serum IgE (ImmunoCAP)	0,1737	0,533	not significant
IgG on basophils	-0,0936	0,5902	not significant
IL-5+ cells	-0,1307	0,631	not significant
serum IgE (ADVIA)	0,1146	0,6825	not significant
IgE on basophils	0,0986	0,7243	not significant
IFN-γ+ cells	0,0842	0,7648	not significant
FcεRI on basophils	0,0734	0,7945	not significant
basophil	-0,0483	0,8546	not significant
ex vivo activation	0,0448	0,8734	not significant
fMLP activation	0,0036	0,9923	not significant

Correlation with UCT ΔV1-V2	Spearman r-values	Spearman p-values ↓	Benjamini- Hochberg significance
serum IgE (ADVIA)	-0,5018	0,0528	not significant
IgE on eosinophils	-0,4856	0,0617	not significant
IgE on B cells	-0,4029	0,1227	not significant
basophils	0,3933	0,1466	not significant
anti-FcεRI AUC	-0,2924	0,27	not significant
IL-10+ cells	-0,2509	0,3435	not significant
IL-5+ cells	-0,2450	0,3521	not significant
serum IgE (ImmunoCAP)	-0,2437	0,3574	not significant
FcεRI on basophils	0,2473	0,3715	not significant
IgE on basophils	0,2004	0,4712	not significant
fMLP activation	0,1950	0,4835	not significant
IgE on basophils	0,1932	0,4876	not significant
IL-31+ cells	-0,1825	0,4883	not significant
anti-FcεRI EC ₅₀	-0,1412	0,5784	not significant
ex vivo activation	-0,1173	0,6471	not significant
IgE on monocytes	-0,0931	0,7076	not significant
IgG on basophils	0,0915	0,7434	not significant
IFN-γ+ cells	0,0325	0,9083	not significant
FcγRII on basophils	-0,0217	0,9098	not significant

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