

Wasp Venom Rush Immunotherapy Induces Transient Downregulation of B Cell Surface Molecule Expression

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Key Words

Antigen presentation · B cells · B cell phenotype ·
Rush immunotherapy · Wasp venom allergy

Abstract

Background: Little is known about the involvement of B cells in venom immunotherapy (VIT). To elucidate changes in the B cell phenotype during this process, we examined the expression of several surface molecules on peripheral B cells before and during VIT. **Methods:** 15 venom-allergic patients with a history of systemic reactions after a wasp sting and venom-specific skin test reactivity as well as serum IgE were investigated before VIT (day 1), 1 day after reaching a maintenance dose of 100 µg (day 6) during inpatient rush VIT, and again on day 26 during continued outpatient maintenance therapy. Changes in the serum levels of total IgE, allergen-specific IgE (sIgE) and sIgG4 were measured by ELISA. Expression of several surface molecules on double-labelled B cells was studied by flow cytometry of peripheral blood mononuclear cells. **Results:** Levels of total IgE, sIgE and sIgG4 showed a significant increase after 26 days of VIT. On day 6, cell surface expression of HLA-

II-DR, CD5, CD32 and CD54 was decreased in intensity and numbers of positive cells compared to day 1, while on day 26, expression of these molecules approached again baseline levels. Furthermore, a trend to decreased CD23 was noted on day 6. No changes were observed for CD40, CD86, CD95 and HLA-I-ABC. **Conclusion:** These data show that during initiation of rush VIT, B cell expression of surface molecules involved in T-B cell cooperation and antigen presentation is downmodulated. B cells may thus be additional direct or indirect targets of high-dose antigen therapy and contribute to the persistence of TH1 responses during maintenance VIT treatment.

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Introduction

Specific immunotherapy (IT) is the only currently available causal therapy for IgE-mediated allergies. The mechanisms responsible for the efficacy of IT are still not fully understood. So far, the main focus of research has been on changes of T helper cell (TH) cytokine patterns (decreased IL-4, IL-5 and IL-13 as well as an increased IL-10 production) [1–3]. Reduced mediator secretion

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from effector cells of allergic reactions, decreased leukocyte proliferation, lowered responsiveness of end organs and changes in immunoglobulin levels have been reported as well [4, 5]. The influence of IT on peripheral B cells has, however, received little attention, despite the obvious changes in B cell immunoglobulin production.

In order to analyze phenotypic changes of B cells during IT, we measured the expression of several surface molecules on B cells before and during rush venom IT (VIT). We examined molecules indicating the activation status, ability of antigen (Ag) presentation and T-B interaction of B cells. The following molecules were chosen: Fc receptors CD23 (FcRII) and CD32 (FcγRII), CD54 (ICAM-1) and CD86 which are costimulatory molecules for T cell activation, CD40 which plays a pivotal role during B-T cell interaction and isotype switching, CD95 (Fas) which mediates apoptosis, CD5 which is expressed on a subset of B cells (B1 cells), HLA-II-DR as key molecule for Ag presentation and HLA-I-ABC. CD23, CD54 and CD86 were also selected because of their involvement in IgE regulation [6–9]. Levels of total IgE, sIgE and sIgG4 were measured to gain functional data in parallel.

We show that rush VIT results in a transient downregulation of the expression of several B cell surface molecules, indicating systemic effects of VIT on B cell activation and thus implicating immunological changes induced during VIT at the B cell level.

Materials and Methods

Subjects and Rush VIT

Wasp venom-allergic patients (n = 15, 15–66 years of age, mean total IgE: 84.9 kU/l, mean specific IgE: 5.9 kU/l) were selected on the basis of a systemic allergic reaction after a wasp sting, a positive skin prick test and detection of specific IgE in the sera (CAP ≥ 2, Pharmacia). Therapy was carried out as rush VIT with ALK-lyophilized SQ, Scherax, Hamburg, Germany. Briefly, patients were hospitalized for 6 days; subcutaneous allergen injections were administered 5 times daily with increasing dosages starting with 0.001 µg allergen extract. All patients reached the maintenance dose of 100 µg allergen extract on day 5 with a cumulative dose of 335.21 µg allergen extract and were discharged on day 6. After the initiation phase, the first outpatient injection was administered 1 week later, the second after a further interval of 2 weeks, and all subsequent injections at monthly intervals.

Blood Sampling

Blood was obtained on the day of hospital admission (before VIT = day 1), 1 day after maintenance dose was reached (16 ± 2 h after the last injection) and on day 26 before the second outpatient allergen injection. Peripheral blood mononuclear cells (PBMC) were separated by Ficoll density gradient centrifugation.

Flow Cytometry

1 × 10⁶ cells were resuspended in staining buffer and then double-stained with 10 µl anti-CD19-FITC and 10 µl of one of the following monoclonal antibodies: anti-CD5-PE, anti-CD23-PE and anti-HLA-II-DR-PE (Biosource, Ratingen, Germany), anti-CD86-PE, anti-CD95-PE and anti-HLA-I-ABC-PE (Pharmingen, Hamburg, Germany), anti-CD32-PE, anti-CD40-PE and anti-CD54-PE (Immunotech, Krefeld, Germany). Antibodies with the respective isotype were used as a negative control (IgG1-FITC, IgG1-PE, IgG2a-PE all from Immunotech, and IgG2b-PE from Pharmingen). The percentage of double positive cells and mean fluorescence intensity (MFI) of the PE-labelled antibody were analyzed on a Coulter XL-Cytometer.

Total Serum IgE, Wasp Venom-Specific IgE and IgG4

Total IgE, wasp venom-specific IgE and wasp venom-specific IgG4 were determined by standard methods using the Pharmacia CAP-ELISA system (Pharmacia & Upjohn, Uppsala, Sweden).

Statistical Analysis

The Wilcoxon test for nonparametrical, paired data using SPSS software was carried out to analyze the statistical significance of the results.

Results

Significant Increase of Total IgE, sIgE and sIgG4 during VIT

The levels of total IgE (data not shown), wasp venom-specific IgE and sIgG4 showed a significant increase after 26 days of VIT (fig. 1a, b). This is in accordance with many previous studies which show significant increases in these parameters at comparable time points [4, 10, 11].

Transient Downregulation of Selected Molecules on Peripheral B Cells during VIT

CD5 is a molecule of unknown function that is constitutively expressed on a subset of B cells (B1 cells) [12] and found on all B cells after activation with anti-IgM or anti-IgG [13]. Both CD5 and CD32, a low affinity Fc receptor for IgG, seem to have inhibitory effects on B cell receptor (BCR) signal transduction [14, 15]. On day 6 of VIT, the percentages of both CD5- and CD32-expressing B cells and their MFI (data not shown) decreased and reached again baseline values on day 26 (fig. 2a, b).

For CD54, also known as ICAM-1, an adhesion molecule that is involved in IgE regulation via interaction with LFA-1 [9], a pattern very similar to that for CD5 and CD32, was observed with a reduction of the percentage of CD54-positive B cells (fig. 2c) and MFI on day 6 (data not shown).

HLA-II-DR plays an important role during Ag presentation and is upregulated after activation of B cells [6].

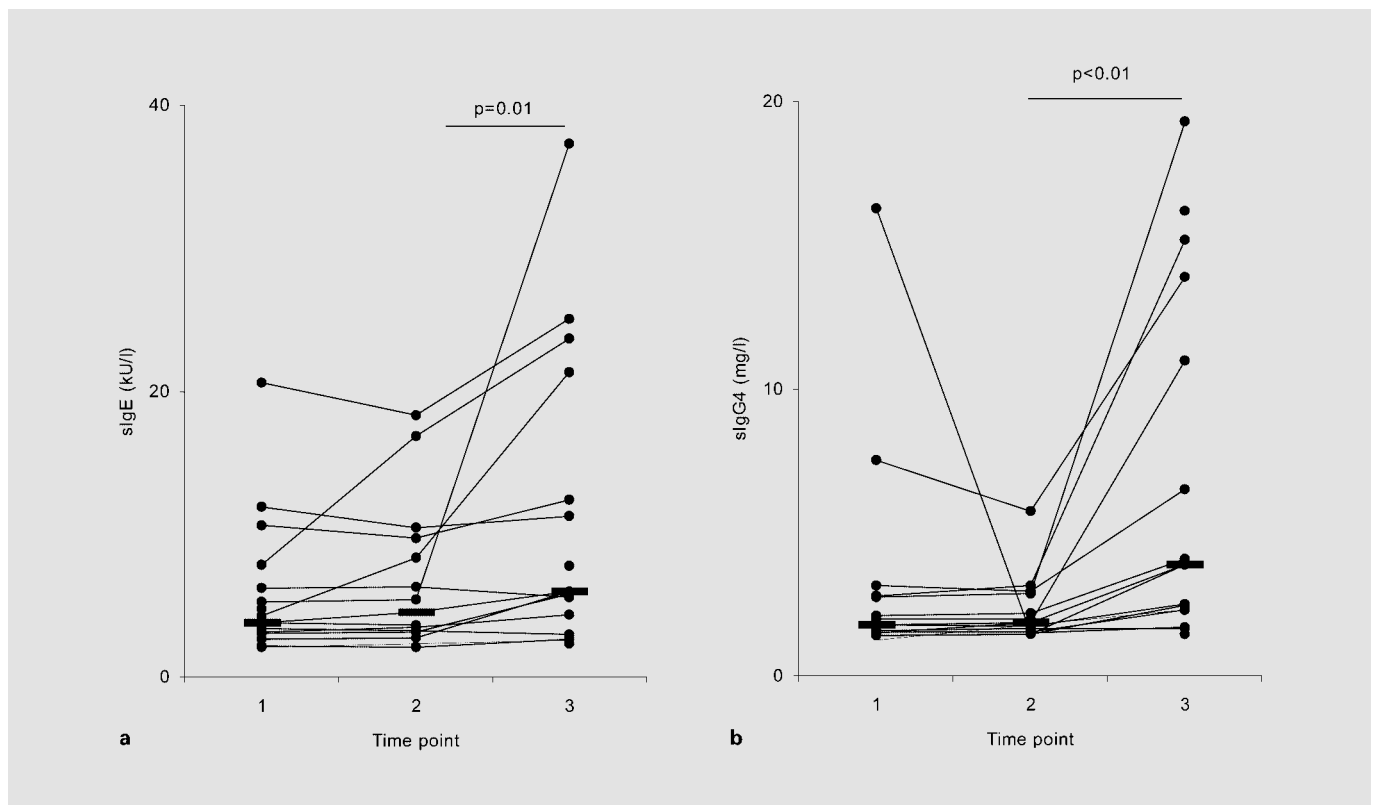


Fig. 1. Allergen-specific IgE (a) and IgG4 (b) before and during IT. Time point 1 = before IT; time point 2 = 1 day after reaching maintenance dose on the 6th day; time point 3 = before the 2nd outpatient allergen injection on day 26. The dots represent the level of a single patient, the horizontal bar represents the median.

Therefore HLA-II-DR was examined like the other molecules for the expression on CD19-positive B cells before and during VIT. At baseline, most B cells expressed HLA-II-DR on their surface; on day 6, however, there was a significant decrease of HLA-II-positive B cells (fig. 3). By day 26, median percentages reached again those found before initiation of VIT. There was also a significantly decreased MFI on day 6 compared to days 1 and 26 (data not shown). To illustrate the changes on the MFI figure 3 shows the HLA-II-DR expression of 1 patient before and during VIT.

The expression of CD23, the low affinity Fc receptor for IgE which participates in IgE regulation but has multiple additional immunological effects [16], was only measured in 10 patients. The expression showed a trend to a decreased percentage of positive B cells (fig. 2d) and MFI on day 6 (data not shown) but did not reach statistical significance due to the small number of analyzed patients.

Molecules Showing No Alterations of Expression during VIT

The expression of CD40, HLA-I-ABC, CD95 and CD86 did not change significantly during the course of VIT with regard to both the percentage of positive cells (table 1) and the MFI (data not shown). CD40, an important stimulatory B cell surface molecule, was expressed on almost all B cells, and the same was true for HLA-I-ABC. Expression of CD95, which is involved in apoptosis and is upregulated on activated lymphocytes, was noted on 28% of the B cells at baseline and increased during VIT, up to 37% on day 26. CD86, a costimulatory molecule also known as B7.2 and an activation marker for B cells, was detectable on only a minority of B cells before and during VIT.

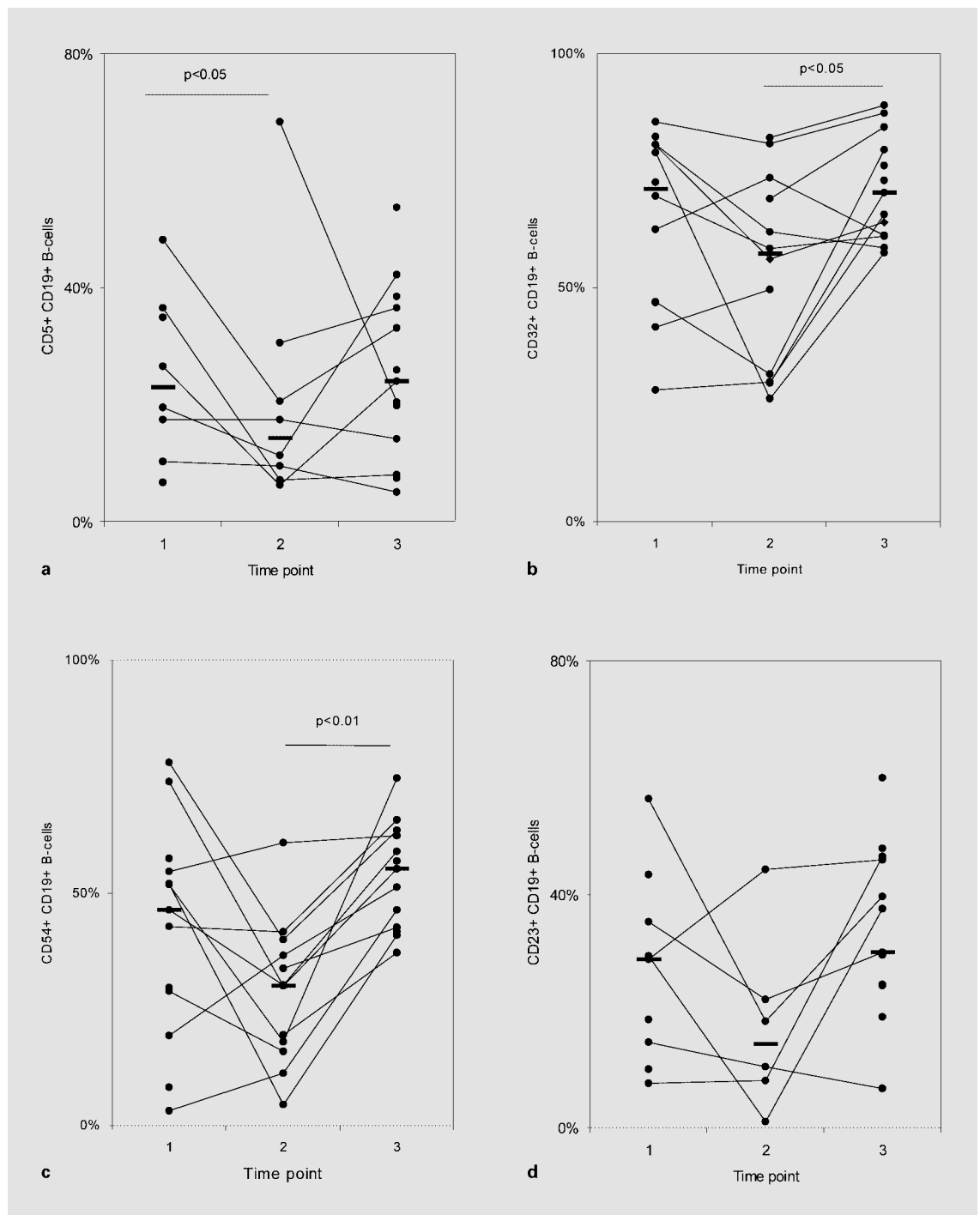


Fig. 2. Percentage of CD5 (a), CD32 (b), CD54 (c) and CD23 (d) expression on CD19+ B cells before and during IT. Time point 1 = before IT; time point 2 = 1 day after reaching maintenance dose on the 6th day; time point 3 = before the 2nd outpatient allergen injection on day 26. The dots represent the level of a single patient, the horizontal bar represents the median.

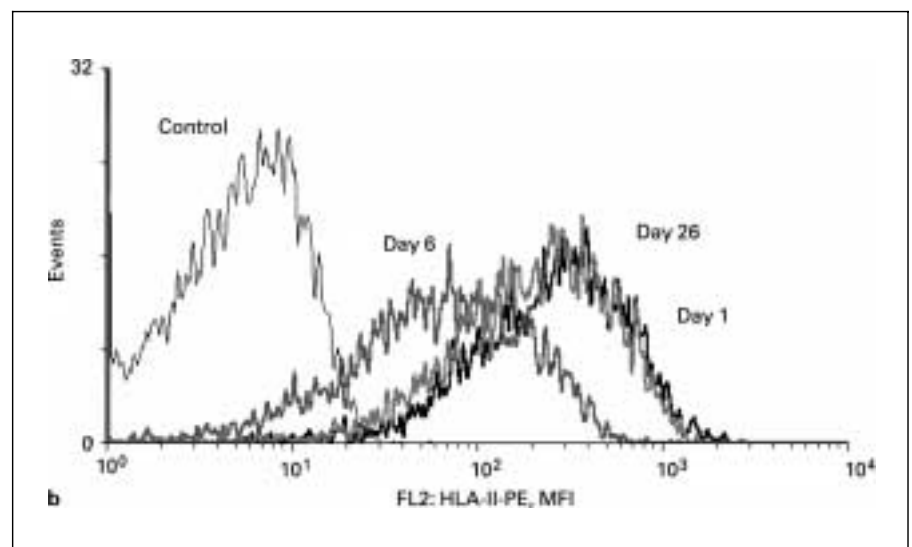
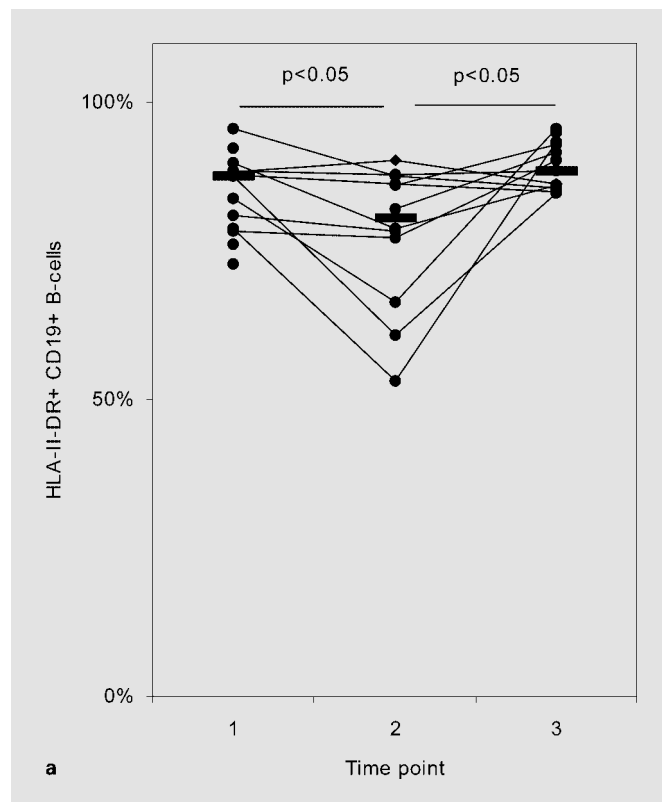


Fig. 3. HLA-II-DR expression on peripheral B cells before and during VIT. a Percentage of HLA-II-DR expression on CD19+ B cells before and during IT. Time point 1 = before IT; time point 2 = 1 day after reaching maintenance dose on the 6th day; time point 3 = before the 2nd outpatient allergen injection on day 26. The dots represent the level of a single patient, the horizontal bar represents the median. b HLA-II-DR expression of 1 patient before and during VIT.

Discussion

In this study, we have demonstrated a modulation of B cells during VIT, including a transient downregulation of CD32, CD5, CD54 and HLA-II-DR, and minor or no

changes regarding CD23, CD95, CD86, CD40 and HLA-I-ABC. Although the downregulation was not observed in the B cells from all patients, the findings were statistically significant. The decrease of the MFI during VIT indicates that the described observations are not due to a shift in B

Table 1. Median percentage of the expression of CD40, CD95, CD86 and HLA-ABC-I on CD19+ B cells during wasp VIT

	Day 1, %	Day 6, %	Day 26, %
CD40	95	95	96
CD86	7	5	7
CD95	28	31	37
HLA-ABC-I	99	98	99

Day 1 = before VIT; day 6 = 1 day after reaching maintenance dose; day 26 = before the 2nd outpatient allergen injection. The expression of these molecules did not change significantly during the course of the study.

cell populations, but that VIT modulates the B cell phenotype on a single cell level. We have also demonstrated changes in serum titers of total IgE, sIgE and sIgG4 with a significant increase after 4 weeks of VIT, which was also shown in previous studies [4, 10, 11]. Findings regarding CD32 expression are of interest since this low affinity Fc γ receptor (Fc γ RII) is an activation marker of B cells, and the present findings suggest a reduction of B cell activation during the initiation of VIT. CD32 is associated with the BCR and seems to negatively regulate BCR signalling [15]. Whether this decreased expression of CD32 contributes directly to the beneficial effect of VIT or whether the observed modulation is only a side phenomenon during VIT will need to be clarified in the future.

CD5, which was also transiently downregulated by VIT on peripheral B cells, is constitutively expressed only on B1 cells. CD5 also inhibits BCR signaling, but this seems to occur via mechanisms different than the BCR inhibition by Fc γ RIIB [14, 17]. While expression of CD5 on B2 cells (CD5-) is known to be induced through anti-Ig and not LPS stimulation [13], the role and function of CD5 in allergic disorders have so far not been well studied.

CD54 has been studied particularly well regarding its role in inflammatory processes where it mediates cell-specific adhesion, hence permitting the migration of activated leukocytes [18]. It is also involved in IgE-related processes by enhancing IL-4 and anti-CD40-induced IgE production by increasing germline transcripts [9]. In vitro, CD54 expression on B lymphocytes is strongly enhanced after stimulation with IL-4 and anti-CD40 antibody [6, 9]; therefore, the reduced IL-4 secretion during VIT might explain the described downregulation. How-

ever, the exact mechanisms by which CD54 expression is regulated during VIT remain to be clarified.

CD23 expression showed only a tendency to decrease during VIT in this study. This seems surprising in view of the numerous in vitro immunomodulatory effects of this low affinity Fc ϵ RII [16], but is probably due to small numbers. On B lymphocytes, its expression is induced by IL-4 and inhibited by IFN- γ . With regard to regulation of IgE synthesis, CD23-CD21 interactions lead to an increase of IL-4-induced IgE production [7], and soluble CD23 also enhances IL-4-induced IgE synthesis [16, 19]. In clinical studies, the seasonal increase in CD23 expression observed in atopic individuals with pollen allergies has been reported to be prevented by IT [20, 21]. In a further study, wasp venom-allergic patients have been reported to have not only reduced expression of CD23 on PBMC after 1 week of rush VIT, but also of IL-4R, CD40 and CD40L, while other surface molecules, e.g. HLA-DR, remained unchanged [3]. The differences to our findings may be related to the fact that expression of surface molecules on B cells was not specifically studied, so that modulation of cell populations other than B cells, e.g. monocytes, may have contributed to the findings. Interestingly we have observed in further in vitro studies that CD23 expression is downregulated in the presence of allergen in B cells but not monocytes (unpubl. data).

HLA-II is the key molecule for antigen-presenting cells (APC) and, besides Ig production, Ag presentation is an important B cell function. The transiently downregulated expression of HLA-II during VIT indicates a reduced ability of B cells to present Ag during this time period. It is worth noting that the type of the APC influences the immune response since monocytes enhance, for example, a TH1 response, whereas B cells enhance a TH2 response and IgE production [22, 23]. The fact that B cells are important APCs in situations with low Ag concentrations [24] is also interesting in view of our findings since the cumulative Ag dose is high during the initiation phase of VIT and would support APC other than B cells, and hence TH1. Reduced Ag presentation by B cell during VIT might, therefore, contribute to the shift from TH2 to a rather TH1-dominated reaction.

The changes described here in the surface molecule expression of B cells are not restricted to allergen-specific effects, as we measured all peripheral B cells and not only allergen-specific cells. Although most studies regarding the mechanisms of IT have described allergen-specific effects [2, 25], there is also evidence of allergen-nonspecific effects [3, 22, 26]. These unspecific effects can be partially explained by a reduced IL-4/IFN- γ ratio as

described in IT [2, 3]. For example, the expression of HLA-II, CD54 and also CD23 on peripheral B cells is regulated by IL-4 [6, 20] and their reduced expression during the early phase of VIT, as reported here, might be explained by the reduced IL-4 secretion observed during VIT. It remains, however, unclear why this downregulation occurs only transiently.

Besides the above-mentioned changes in cytokine secretion the described anergy of T cells induced by IT [27] might also have an influence on the changes in the B cell phenotype. Anergic T cells have a reduced CD40L expression [28]. Interestingly a reduced CD40L expression of T cells after initiation of VIT has been described [3]. The interaction of CD40L and CD40 is very important for B cell activation and enhances, for example, expression of costimulators such as CD54 and CD23 [6, 29]. T cell anergy might, therefore, contribute to the changes in the phenotype of B cells.

Another mechanism responsible for the observed modulation might be the high cumulative Ag dose (335.21 µg) during the 1st week of VIT. It is known that high Ag levels

preferably induce B cell anergy [30]. We, therefore, suppose that the supraoptimal Ag dose during the 1st week of VIT causes a transient reduced B cell activation.

Taken together, we have shown in this study that VIT modulates the phenotype of peripheral B cells during the high Ag dose initiation phase of therapy. Whether the observed phenotypical changes will provide any information of the clinical outcome of VIT will need to be analyzed in a prospective study, ideally proving clinical efficacy by sting provocation.

The mechanisms leading to the described findings are complex. Downregulation of HLA-II, CD54, CD5, CD32 and to a minor degree CD23 can be partially explained by changes of cytokine microenvironment, such as lowered IL-4 levels and thus indirect effects on B cells by IL-4-producing cells. Direct effects of Ag on B cells may, however, also contribute to the observed modulations. Whether the described effects are involved in the mechanisms of effective IT or whether they are just a side phenomenon will need to be clarified in further studies.

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