



Suppression of allergen-specific B lymphocytes by chimeric protein-engineered antibodies

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ABSTRACT

House dust mites *Dermatophagoides pteronyssinus* (Dpt) are among the most frequent causes of allergy symptoms in Europe. Der p 1 is one of the major allergenic compounds of Dpt and the pathological Der p 1-specific B cells play a key role as producers of allergen-binding antibodies. The selective elimination of these cells by artificial protein molecules which inhibit the production of Dpt-recognizing IgE antibodies is a perspective therapeutic goal of allergy.

A protein engineered chimeric molecule has been constructed, which binds Der p 1-specific B cells via their BCR and suppresses selectively the production of anti-Der p 1 antibodies via CR1. The synthetic peptide Der p 1 p52-71 and an anti-CD35 monoclonal antibody were used for the construction of Der p 1 chimera. The functional effects of engineered antibodies were analyzed in vitro using PBMCs from allergy patients.

Significant inhibition of allergen-specific proliferation and reduction of Der p 1-IgE antibody production were observed after treatment of PBMCs from allergic patients with Der p 1-peptide chimera. Culturing of these PBMCs in the presence of the chimeric molecule increased the percentage of apoptotic (Annexin V-positive) B lymphocytes, but not T lymphocytes.

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Introduction

Hypersensitivity to house dust mite (HDM) allergens is the most common allergic response affecting up to 20% of the population in the industrialized world. It is also one of the main asthma-causing factors with more than 50% of all asthmatics worldwide being sensitized to HDM-borne aeroallergens (Zock et al. 2000; Gaffin and Phipatanakul 2009). Different approaches have been used to

investigate the mechanisms of human allergic reaction, characterized by immune inflammation and high levels of IgE production. The allergic reaction is triggered by the cross-linking of a polyvalent allergen molecule to IgE antibodies bound by high-affinity IgE receptors (FcεRI) on the surface of effector cells (Basophils and Mastocytes). The following intracellular signal transduction results in activation and release of molecules mediating allergic inflammation.

Based on the mechanism of allergic reaction epitope-specific therapeutic strategies can be developed (Taketomi et al. 2006). Several attempts for immune response modulation and inhibition of IgE production have been made using modified allergen epitopes (Yang et al. 1993; Ewan 1990). Different methods of chemical modification such as polymerization, denaturation and conjugation of allergens with a variety of carriers have been tested (Dreborg and Akerblom 1990; Ball et al. 1999; Doull et al. 1997). Such modifications may reduce the number of allergen applications necessary for efficient immunotherapy or, enhance the induction of tolerance (Petrunov et al. 1996).

House dust mites *Dermatophagoides pteronyssinus* (Dpt) are the most important indoor allergens (Charbonnier et al. 2003; Wan et al. 1999; Kalsheker et al. 1996; Pestel et al. 1994; Duez et al. 1996, 2000). One of the major allergenic molecules of Dpt is

Abbreviations: Dpt, *Dermatophagoides pteronyssinus*; CR1, complement receptor 1; PBMCs, peripheral blood mononuclear cells; HDM, house dust mite; dsDNA, double stranded DNA; scFv, single chain Fv; SLE, systemic lupus erythematosus; BCR, B cell receptor; FITC, fluorescein isothiocyanate; PE, phycoerythrin; ELISA, enzyme-linked immunosorbent analysis; ELISpot, enzyme-linked immunosorbent spot; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; FACS, fluorescence-activated cell sorting; FCS, fetal calf serum; mAb, monoclonal antibody; SCID, severe combined immunodeficiency; IFN-γ, interferon gamma; SIT, specific immunotherapy; SD, standard deviation; EDC, 1-ethyl-3-(3'-dimethylaminopropyl) NBT, nitro blue tetrazolium; BCIP, 5-bromo-4-chloro-3-indolyl phosphate.

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the glycoprotein Der p 1, and some potential epitopes have been predicted by bioinformatic methods. Using a three-dimensional model, Der p 1 (222-amino acids based protein) has been mapped, four potential B cell epitopes have been selected and their IgE-dependent biological activities demonstrated (Greene and Thomas 1992; Jeannin et al. 1992; Duez et al. 2001). One of these epitopes (peptide p52–71) was used as a model epitope in the present study.

The pathological Der p 1-specific B cells in allergic patients trigger the disease development as producers of sensitizing IgE antibodies. Therefore, the selective elimination or suppression of anti-Der p 1 B-lymphocytes is a reasonable therapeutic purpose. Erdei et al., have shown that the complement receptor type 1 (CR1, CD35) provides potent inhibitory signals to human B lymphocytes (Jozsi et al. 2002). We have previously shown that the targeting of an epitope to the available epitope-specific B cells may have a strong positive or negative effect on their response in murine or humanized models. This targeting can be achieved by a chimeric molecule containing both B and T cell epitopes, and a whole molecule or scFv fragment from an antibody specific to an activating or inhibitory cell surface receptor. Such chimeras were produced either by genetic or by protein engineering (Baiu et al. 1999; Prechl et al. 1999; Ivanovska et al. 2006; Tchorbanov et al. 2007; Mihaylova et al. 2007; Gesheva et al. 2012).

We have constructed a chimeric molecule able to cross-link cell surface immunoglobulin with the inhibitory complement receptor type 1 (CD35) on DNA-specific B cells from SLE patients with the aim of silencing them selectively. This engineered molecule consisted of B-cell DNA-mimotope peptide DWEYSVWLSN coupled to a monoclonal anti-human CR1 antibody. The ability of the DNA-like chimera to bind human B cell CD35 molecule was demonstrated by flow cytometry (Voynova et al. 2008). Also, we found by ELISpot analysis that the DNA-like peptide chimera induced a dose-dependent suppression of anti-dsDNA IgG antibody-secreting plasma cells when PBMCs from lupus patients were cultured in the presence of the chimera (Kerekov et al. 2011a,b).

SCID mice reconstituted with PBMCs from SLE patients showed presence of auto-antibodies against dsDNA as well as human immunoglobulin deposition in the renal glomeruli. Treatment of the transferred SCID mice with anti-human DNA-like chimera prevented the appearance of anti-dsDNA antibodies and proteinuria, observed in PBS-injected animals.

Based on these results, we hypothesized that it may be possible to suppress selectively the production of anti-Der p 1 IgG and IgE antibodies by a chimeric protein molecule, containing a monoclonal antibody specific for the human inhibitory receptor CR1, coupled to the p52–71 peptide from the major Dpt allergen, Der p 1. This protein-engineered molecule would bind selectively to Allergen-peptide-associated B-cells by recognizing their Der p 1-specific BCR, and deliver a strong inhibitory signal via the surface CR1 receptors (Fig. 1).

The aim of the present study was to specify the effects of the above-described chimeric molecules on anti-Der p 1 B-lymphocytes from allergic patients.

Materials and methods

Monoclonal antibodies

Purified mouse 3D9 IgG1 monoclonal antibody (mAb) specific to human complement receptor type I (CD35) was prepared as described (Voynova et al. 2008). Briefly, the mouse 3D9 hybridoma producing IgG1 antibody specific to human complement receptor type I (CD35) was adapted to grow in serum-free CHO medium (Gibco, Gaithersburg, MD). Antibodies from the supernatant were

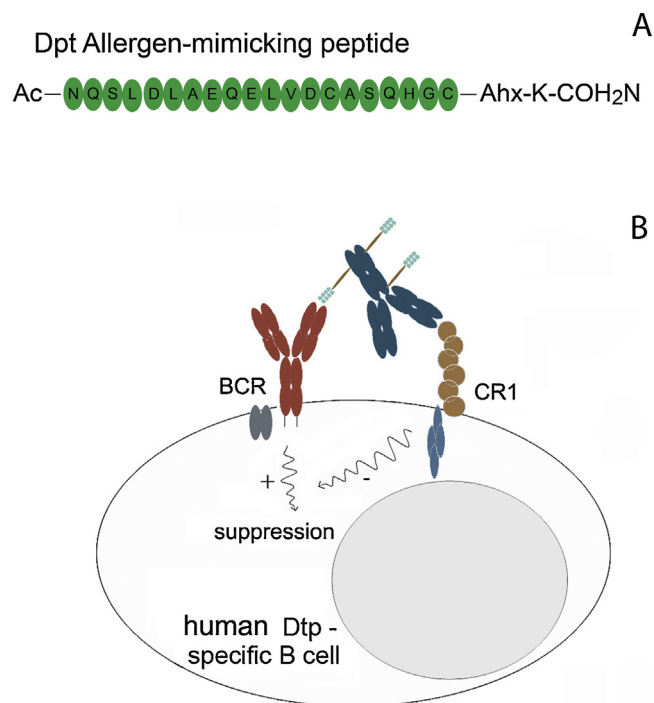


Fig. 1. Receptor co-crosslinking by a protein engineered chimeric molecule. (A) Dpt allergen-mimicking peptide. (B) Schematic view of receptor co-crosslinking.

obtained by 50% ammonium sulfate precipitation and subsequent purification on Protein G column.

FITC-conjugated anti-human CD35 and anti-human CD19-PE (Pharmingen BD, San Diego, CA) as well as FITC-conjugated anti-mouse IgG (Sigma–Aldrich, Taufkirchen, Germany) were used for FACS experiments.

Construction of chimeric molecules containing allergen epitope and inhibitory cell surface receptor-binding antibody for targeting the allergen-specific B lymphocytes

For the construction of chimeric molecules we used two synthetic peptides and an antibody to human CR1. The peptide p52–71 (Ac-NQSLDLAEQELVDCASQHGCAhx-K-NH₂) from Der p 1 and an irrelevant peptide (Ac-DEACLQCGSEHDQAVQNLLS-Ahx-K-NH₂), containing the same amino acids in a shuffled order were purchased from Caslo Laboratory (Lyngby, Denmark).

The chimeric molecules: an Allergen-peptide chimera, consisting of the Der p 1 peptide and the anti-human CR1 mAb 3D9, and an irrelevant peptide chimera, comprising the irrelevant peptide conjugated to the 3D9 mAb were constructed using the classical EDC (1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide-HCl), (Fluka AG, Buchs, Switzerland) cross-linking technique (Voynova et al. 2008; Bauminger and Wilchek 1980). Briefly, during the peptide synthesis an Ahx linker with lysine was introduced to the peptides C-end. The antibody and peptide were mixed at a 20-fold molar excess of the peptide and 60-fold molar excess of carbodiimide. The reaction mixture was stirred overnight at 4 °C, dialyzed against PBS and concentrated by ultrafiltration.

Allergic patients and healthy blood donors

The study comprised 12 recently diagnosed untreated patients with bronchial asthma and/or allergic rhinitis, sensitive to Dpt (female to male ratio 5:7; mean age (20–46)). Inclusion criteria were: allergy to HDM first diagnosed at least 2 years before the study by a skin allergy test and/or basophil degranulation flow

cytometry test, and presence of high titers of allergen specific IgE antibodies as demonstrated by ELISA. The control group consisted of six age- and sex-matched healthy donors. Permission from local Ethical Committee and Informed consent were obtained in all cases.

PBMCs from patients and controls were isolated by standard Ficoll-Paque technique (Amersham Bioscience, Sweden) and cultured in RPMI-1640 (Gibco, Gaithersburg, MD) supplemented with 10% FCS, 4 mM L-glutamine, 50 μ M 2-mercaptoethanol and antibiotics in 5% CO₂ at 37 °C.

Flow cytometry analysis

The specificity of constructed chimeras was tested on the CR1-expressing human leukemic cell line LAMA-84 (DSMZ: ACC 168) (Champelovier et al. 2004) as well as on human PBMCs. Briefly, LAMA-84 cells (1×10^6 cells/ml) were double-washed with PBS and incubated with the Allergen-peptide chimera, or with the Irrelevant chimera, or with unconjugated 3D9 MoAb (1 μ g/ 10^6 cells), or with PBS alone for 30 min at 4 °C, followed by two washes. A second incubation with FITC-conjugated anti-mouse IgG for 30 min at 4 °C was performed.

In a separate experiment PBMCs from allergic patients were incubated with the Allergen-peptide chimera, or with the Irrelevant chimera, or with unconjugated 3D9 MoAb (1 μ g/ 10^6 cells), or with PBS alone for 30 min at 4 °C, followed by two washes. A second incubation with FITC-conjugated anti-human CR1 or FITC-conjugated anti-mouse IgG antibody combined with anti-human CD19-PE for 30 min at 4 °C was performed.

Ten thousand cells were analyzed from each sample with a BD LSR II flow cytometer using the Diva 6.1.1 software (BD Biosciences, San Jose, CA).

ELISA for Der p 1 peptide detection

96-well Maxisorp immunoplates (Nunc, Roskilde, Denmark) were coated with Allergen-peptide chimera or with Irrelevant chimera (0.5 mg/ml in PBS) overnight at 4 °C. The plates were washed and blocked with 1.0% BSA for 2 h at room temperature, followed by two washes. Pre-diluted (1:20) sera from allergic patients or healthy donors were incubated for 1 h at room temperature. The binding of the human anti-Der p 1 IgG and IgE antibodies was detected by subsequent incubation with an anti-human IgG conjugated with alkaline phosphatase or biotin-conjugated anti-human IgE for 1 h, followed by incubation with streptavidin-conjugated alkaline phosphatase (all from Sigma). The plates were washed again and phosphatase substrate (104, Sigma) was added. Optical density was measured at 405 nm.

PAGE and Western blotting

Both constructed chimeras were subjected to SDS-PAGE and Western blot analysis. SDS-PAGE was performed using 10% gels and a MiniProtein II system (BioRad, Richmond, CA) in the presence of 0.1% SDS. After the electrophoresis, the proteins were transferred to a nitrocellulose membrane (0.45 μ m, from Sartorius, Germany) using a MiniTrans Blot device (from BioRad) in a buffer containing 48 mM Tris and 110 mM glycine in the presence of 20% (v/v) methanol. The membranes were blocked overnight in a TBS buffer, containing 0.4% Tween 20, cut into strips and each slot was incubated for 1 h at room temperature with diluted sera from allergic patients or healthy donors. After washing, the membranes were incubated in an optimal dilution of an anti-human IgG conjugated with alkaline phosphatase or biotin-conjugated anti-human IgE for 1 h, followed by incubation with streptavidin-conjugated alkaline

phosphatase. The reaction was revealed using the nitro blue tetrazolium/bromo-chloro-indolyl-phosphate substrates (Sigma).

ELISpot assay for counting Dpt-specific IgE antibody-secreting cells

The effects of chimeric molecules on the number of IgE anti-Dpt antibody producing plasma cells was assessed by ELISpot. PBMCs from clinically relevant allergic patients and healthy non-allergic volunteers were isolated by Ficoll-Paque gradient centrifugation of heparinized blood and were cultured (2×10^6 /ml) in complete RPMI-1640 medium (see above). The cells were stimulated with standard Dpt allergen preparation (from BulBio, Sofia, Bulgaria) and cultured for 96 h in the presence of increasing concentrations (ranging from 40 ng/ml to 1000 ng/ml) of the Allergen-peptide chimera, or of Irrelevant peptide chimera. Cells incubated with 10 μ g/ml lipopolysaccharide (LPS) (from *Escherichia coli*, Sigma, L-2630) or with medium alone were used as positive and negative control respectively.

ELISpot 96-well plates (Millipore, Bedford, MA, USA) were activated with 35% ethanol, coated with 40 μ g/ml standard Dpt allergen preparation for 60 min at room temperature, washed with PBS and blocked with 1% BSA in PBS. The pre-cultured cells were transferred to ELISpot plates with the Dpt allergen-coated membranes and were further cultured for 4 h in a humidified 5% CO₂ atmosphere at 37 °C. Next, the plates were washed, incubated with a biotin-conjugated anti-human IgE for 1 h, followed by incubation with streptavidin-conjugated alkaline phosphatase and developed by NBT-BCIP substrate. The number of spots corresponding to anti-Der p 1 IgE antibodies producing cells was counted by C.T.L. Immunospot S5 Versa Analyzer (Bonn, Germany).

Detection of apoptosis

Peripheral blood mononuclear cells from untreated allergic patients and healthy volunteers were cultured for 4 days (2×10^6 cells/ml in complete RPMI-1640 medium) as described above. The cells were washed, stained with PE/Cy5-conjugated anti-human CD19 or anti-human CD3 antibodies and the apoptosis of gated B (CD19+) or T (CD3+) cells was measured by flow cytometry using the Annexin V-FITC apoptosis detection Kit I (Pharmingen BD).

Statistical analysis

Values in the figures correspond to mean $\pm$ SD. The unpaired Student *t*-test was used to determine differences between each two groups. A value of *p* < 0.05 was considered as statistically significant.

Results

Construction of protein chimeric molecules

The protein chimeras were constructed by coupling copies of the two synthetic peptides to the 3D9 monoclonal anti-human CR1 antibody. The Allergen-peptide chimera contained the Der p 1 peptides p52–71 (Ac-NQSLDLAEQELVDCASQHGCAhx-K-NH₂); the Irrelevant peptide chimera contained an irrelevant peptides (Ac-DEACLQCGSEHDHQAQVQNLLSAhx-K-NH₂), comprising the same amino acids in a shuffled order, both coupled to the anti-CR1 antibody. According to mass-spectral analysis data 14–16 peptides were bound to each single immunoglobulin molecule (Mihaylova et al. 2009).

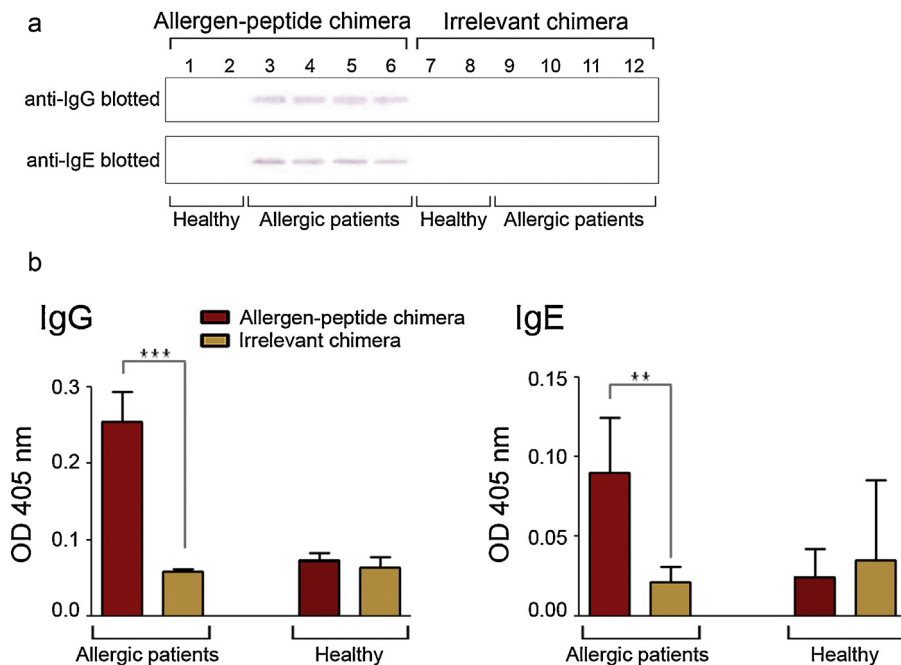


Fig. 2. The Der p 1 peptides coupled to the anti-CR1 antibody are recognized by anti-Der p 1 antibodies. (A) After the covalent conjugation of the Der p 1 peptide to the CR1 antibody, the peptide epitope retains its ability to be recognized by anti-Der p 1 antibodies. The samples (20 µg each) from the Allergen-peptide chimera (lanes 1–6) or the Irrelevant chimera (lanes 7–12) were subjected to SDS-PAGE (under non-reducing conditions using a 10% gel) and transferred to a nitrocellulose membranes. The membranes were blocked and incubated with diluted sera from allergic patients (lanes 3–6; 9–12) or healthy donors (lanes 1 and 2; 7 and 8). After washing, the membranes were incubated with anti-human IgG or IgE conjugates and developed. (B) ELISA results are shown. Allergen-peptide chimera or Irrelevant chimera coated plates were loaded with diluted sera from allergic patients or healthy donors. After the incubation the plates were washed, incubated with anti-human IgG (left panel) or IgE conjugates (right panel) and developed. Results are expressed as the mean value $\pm$ SD of triplicates (Student *t*-test; ***p* < 0.01, ****p* < 0.001).

Der p 1 epitope recognition

The efficient Der p 1 peptide–anti-CR1 antibody conjugation and the availability of allergic epitope for recognition by the anti-Der p 1 antibodies were tested by ELISA and Western blot. The Allergen-peptide and Irrelevant chimeras were coated on ELISA plates or subjected to SDS-PAGE, followed by transfer to nitrocellulose membranes. Diluted sera from allergic patients or from healthy donors were incubated with chimeric molecules loaded on ELISA plates or membranes. As shown in Fig. 2A (lanes 3–6) the IgG and IgE anti-Der p 1 patient's sera antibodies bound the Der p 1 peptide from the Allergen-peptide chimeric molecule, but did not recognize the irrelevant peptide from the control chimera (Fig. 2A, lanes 9–12). These results confirmed that the Allergen-peptide in the composition of chimera retained its conformation and was available for recognition by the Der p 1-specific antibodies. Healthy donors' sera recognized neither the Allergen nor the Irrelevant peptides (Fig. 2A, lanes 1 and 2; 7 and 8). Similar results were obtained by ELISA (Fig. 2B).

The chimeras bind to human CR1

Further on, we tested the ability of chimera constructs to bind to human CD35. First, we assessed the binding capabilities of the Allergen-peptide and Irrelevant chimeras on the human CR1-expressing LAMA-84 cell line. Both chimeras bound to CD35 in a similar way after incubation with the cells as described in materials and methods section (Fig. 3).

Second we studied the binding of Allergen-peptide chimera to B cells isolated from both allergic patients and healthy donors by showing its ability to compete with an anti-CD35 antibody. As illustrated in Fig. 4, the Allergen-peptide chimera prevented the binding of a FITC-conjugated anti-CD35 antibody to CR1 on the human B cells.

The Allergen-peptide chimeric molecule decreased the number of IgE anti-Dpt antibody-secreting plasma cells

The effects of the chimeric molecules on the number of IgE anti-Dpt antibody producing plasma cells was assessed in an ELISpot assay. PBMCs from clinically relevant allergic patients and healthy non-allergic volunteers were isolated by gradient centrifugation of heparinized blood. The cells were cultured in complete RPMI 1640 medium in the presence of increasing concentrations of the constructed chimeric molecules (ranging from 40 ng/ml to 1000 ng/ml). Cultivation of allergic donors' cells in the presence of 200 ng/ml of the Allergen-peptide chimera, resulted in a significantly reduced number of IgE anti-Dpt antibody producing plasma cells (Fig. 5). The number of Dpt-specific IgE secreting plasmacytes was partially affected in the presence of different amounts of the Irrelevant-peptide chimera as well. No anti-Dpt IgE producing plasma cells were observed when PBMCs isolated from healthy donors were used.

Allergen-peptide chimera increased the percentage of apoptotic B lymphocytes

To study the apoptotic effect of chimera treatment we cultured PBMCs from allergic patients or healthy donors for 4 days in the presence of increasing concentrations of chimeric molecules. The cells were further washed and the surface expression of phosphatidylserine was studied by Annexin-V/Propidium iodide staining and flow cytometry. High level of apoptosis was observed within CD19-gated PBMCs from allergic patients incubated with 40 ng/ml of the Der p 1-peptide chimera (Fig. 6), but not within gated T cells (not shown). At the same time, chimera treatment had no significant effect on the level of apoptosis of gated B or T cells from healthy donors (not shown).

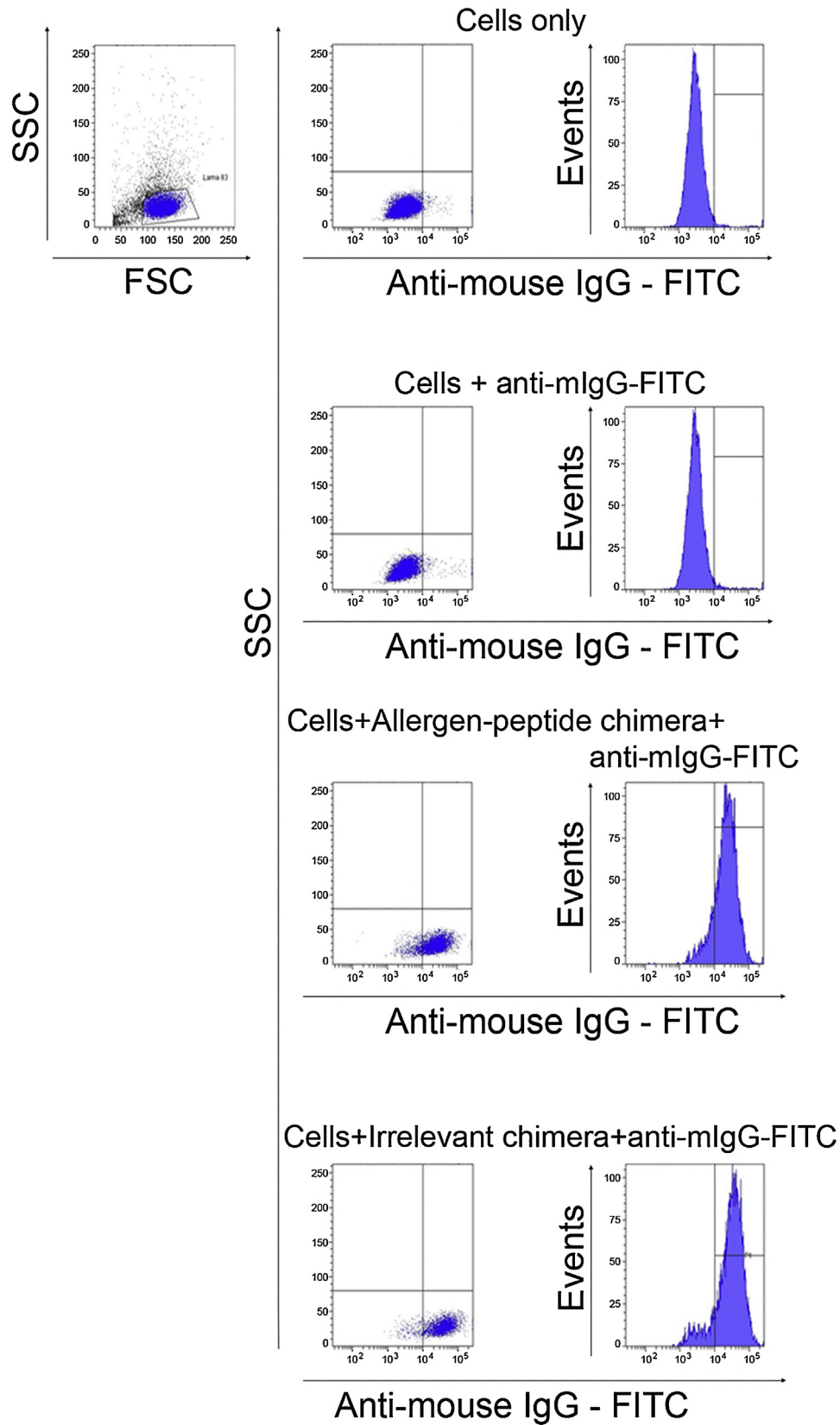


Fig. 3. Allergen-peptide and Irrelevant chimeras bind the inhibitory receptor CR1. The constructed Allergen-peptide and Irrelevant chimera bind CR1 expressed on the human cell line LAMA-84 (CR1-positive). The cells were incubated with Allergen-peptide chimera, or control chimera, or PBS only for 30 min at 4 °C followed by incubation with FITC-conjugated goat anti-mouse IgG antibody. After washing, the cells were analyzed by flow cytometry. Data are representative of at least 5 independent experiments.

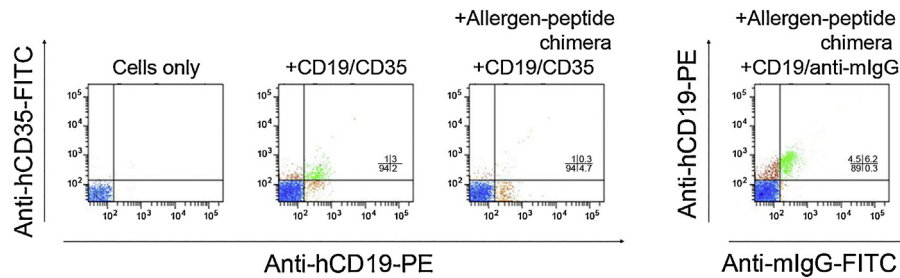


Figure 4. The Allergen-peptide chimera maintains its ability to bind to CR1 on PBMCs from Allergen-peptide patients and competes with FITC-conjugated anti-CR1 antibody for the same receptor. Flow cytometry analysis results are shown. The cells were incubated with the protein chimera, washed and incubated with either FITC-conjugated anti-human CD35 or FITC-conjugated anti-mouse IgG antibody combined with anti-human CD19-PE antibody. Data are representative of at least 5 independent experiments.

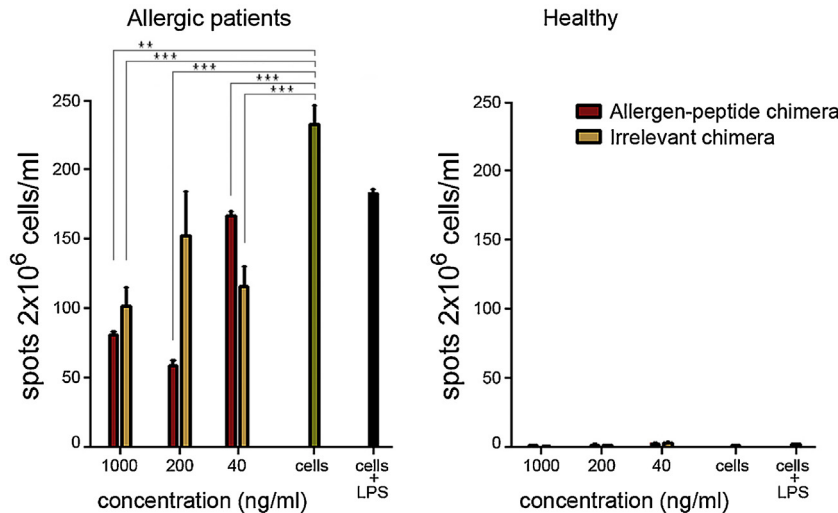


Fig. 5. Co-crosslinking of BCR and CR1 on disease-associated B lymphocytes by the Allergen-peptide chimera decreases the number of anti-Dpt IgE antibody secreting cells. PBMCs from allergic patients (left panel) or healthy donors (right panel) were cultured in the presence of the Allergen-peptide or the Irrelevant chimera and the number of plasma cells was determined by ELISpot. Control samples were cultured in LPS or in medium only. Results are expressed as the mean value $\pm$ SD of triplicates. PBMCs from each allergic patient and healthy donor were tested individually. The numbers of spots in the test-wells were compared with control wells containing Irrelevant chimera-treated or medium only-cultured cells (Student *t*-test; ** $p < 0.01$, *** $p < 0.001$). Data are representative of at least 6 experiments.

Discussion

Sensitization to house dust mite allergens and production of allergen-specific IgE antibodies induces mast cell degranulation, which contributes the development of allergic reactions and asthma. The IgE antibody production is triggered as a result of the specific recognition of allergens by B-cells antigen receptors, followed by internalization, processing and presentation. The enhanced IgE responses and inflammatory reactions during allergy progression depend on different factors as the nature of the antigen,

the exposure conditions (allergens dose, timing and route of allergen sensitization), as well as the individual genetic Th1/Th2 cytokine background (Duez et al. 2001; Gregory and Lloyd 2011).

House dust mites contain several allergens associated with the development of allergy and asthma. The main component and major allergen Der p 1 comprises a number of B and T cell epitopes which have the capacity to induce strong immune response (Duez et al. 2001; Gregory and Lloyd 2011). Based on that, small synthetic peptides containing some of these epitopes can be used for specific therapy and construction of B cell targeting molecules.

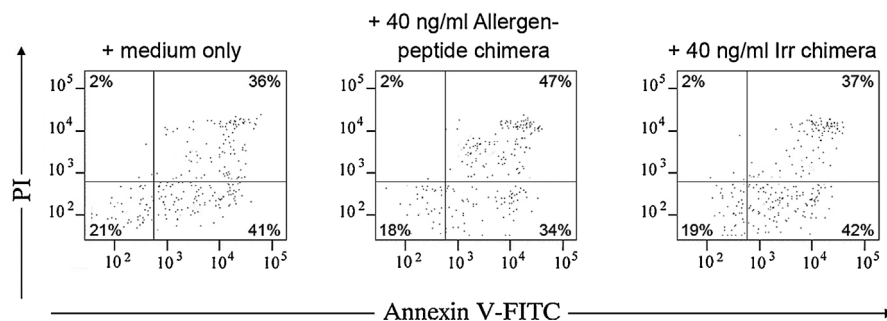


Fig. 6. The Allergen-peptide chimera induces B cell apoptosis. PBMCs from allergic patients were cultured for 4 days in complete RPMI-1640 medium only, or in the same medium containing 40 ng/ml of the Allergen-peptide chimera, or 40 ng/ml of the Irrelevant chimera. At the end of the cultivation, the cells were double-stained with Annexin V-FITC/Propidium iodide and the apoptosis of gated CD19+ cells was analyzed by flow cytometry. Percentage of stained cells is shown in each quadrant.

Allergen-specific immunotherapy (SIT) is currently a widely used strategy for clinical treatment of allergic diseases and asthma through modification of the immune response. Subcutaneous and sublingual immunotherapy are the classical routes for induction of specific T-cell tolerance, suppression of Th2 cytokine responses and production of allergen-specific blocking IgG₄ antibodies. Although efficient, SIT has many limitations due to systemic and/or local side effects and safety concerns (Keles et al. 2011; Hales et al. 2006). Novel strategies for improvement of SIT using allergen-derived peptides, recombinant and genetically modified allergens are still under investigation (Lu et al. 2012; Tsai et al. 2010).

A different approach for specific modulation of allergen-specific B cell response is the induction of negative signals by engagement of inhibitory surface receptors. The human CR1 (CD35) is a potential candidate for specific targeting because of its potency to regulate negatively the proliferation and differentiation of activated B cells after ligand binding (Jozsi et al. 2002; Wagner et al. 2006). CR1 is expressed by different human cells including erythrocytes, granulocytes, B-, T- and plasma cells, and CR1-targeting antibodies have been already used for treatment of lupus patients. King-Konert et al. have constructed the chimeric molecule ETI-104, by covalent coupling of dsDNA to a murine human CR1-specific mAb (King-Konert et al. 2004). The authors observed decreased levels of anti-dsDNA autoantibodies in SLE patients after treatment with ETI-104.

In order to suppress the proliferation and differentiation of auto-aggressive B cells in a humanized SCID murine model of SLE we have constructed a chimeric antibody molecule, consisting of a human CR1-specific antibody, coupled to the self-epitope recognized by the BCR of the auto-reactive B cells (Voynova et al. 2008; Kerekov et al. 2011a,b). Instead of native DNA we used copies of the dsDNA-mimicking decapeptide DWEYSVWLSN, which is recognized by the pathogenic anti-dsDNA antibodies (Putterman et al. 2000). The constructed protein chimeric molecule was able to cross-link cell surface BCR with the inhibitory CR1 on self-reactive DNA-specific B cells from SLE patients and selectively suppressed disease-associated autoreactive B lymphocytes. Its administration to SLE transferred-humanized SCID mice resulted in low levels of IgG anti-dsDNA antibodies and of proteinuria, suppressed IFN- γ production and preserved kidney histology.

In the present study we used a similar approach to suppress allergen-specific B cells: cross-linking the surface immunoglobulin receptors on Der p 1-specific B cells with the inhibitory CR1 by protein-engineered chimeric molecules. The chimeric antibodies were produced by coupling a monoclonal anti-human CR1 antibody to the p52-71 peptide from Dpt and would therefore bind preferentially the targeted Der p 1-specific B cells. The constructed Allergen-peptide chimeras are expected to have a higher binding avidity to the combination of the targeted surface molecules (the Der p 1-specific BCRs and the inhibitory CD35 receptors) and to perform efficient co-ligation.

They could modulate the activity of the targeted Der p 1-specific B cells by different mechanisms, including inhibition of B cell differentiation to plasma cells or suppression of the T/B lymphocyte communication. We have recently shown that a similar chimeric molecule constructed by coupling copies of a dsDNA mimotope peptide to a CR1-specific antibody suppresses specifically the activity of anti-dsDNA IgG antibody-producing cells, while it did not affect the numbers of tetanus toxoid-specific plasmocytes (Voynova et al. 2008). Indeed, the exposure of PBMCs from allergic patients to the Der p 1 peptide-derived chimeric molecules significantly reduced the number of IgE anti-Dpt antibody producing plasma cells. The partial suppression observed with the Irrelevant chimera could be explained by the possibility for interaction of the anti-CR1 antibody with any CR1 expressing targets under in vitro conditions. It is expected that the bivalent specificity and high affinity of the Allergen-peptide chimera would be more advantageous

under in vivo conditions, and limit the inhibitory effects to its relevant target only: Dpt-specific B cells. As expected, PBMCs isolated from healthy donors were not affected after incubation with the protein chimeras.

Importantly, the Allergen-peptide chimera binds only to CD19+ B cells, but not to T cells. PBMCs from allergic patients cultured in the presence of the Der p 1-peptide chimera increased the percentage of apoptotic (Annexin V/PI-double positive) B lymphocytes while incubation with the Irrelevant chimera had no significant effect.

The present study explores a different approach for preventing pathological allergen-specific IgE antibody production. Disease-related T or B cell peptide epitopes conjugated to antibody carriers can effectively down-regulate immune responses and have a promising potential for future therapeutic applications.

Competing interest

The authors declare no competing or financial interests.

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