Received: 15 September 2022 | Revised: 22 November 2022 | Accepted: 29 November 2022

DOI: 10.1111/sji.13241

Scandinavian Journal of Immunology An International Monthly Journal WILEY

ORIGINAL ARTICLE

Targeted suppression of Dpt-specific B cells in humanized Rag2- γc- mouse model of HDM allergy

Nikola Ralchev Ralchev[1] | **Nikola Kerekov**[1] | **Nikolina Mihaylova**[1] | **Mariann Kremlitzka**[2] | **Diana Hristova**[3] | **Julian Dzhorev**[4] | **Anna Erdei**[2,5] | **Andrey Ivanov Tchorbanov**[1,6]

[1]The Stefan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

[2]MTA-ELTE Immunology Research Group, Eötvös Loránd University, Budapest, Hungary

[3]Allergology Clinic, Alexander's University Hospital, Sofia, Bulgaria

[4]Biosystems Ltd, Sofia, Bulgaria

[5]Department of Immunology, Eötvös Loránd University, Budapest, Hungary

[6]National Institute of Immunology, Sofia, Bulgaria

Correspondence
Andrey Ivanov Tchorbanov, Stefan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Block 26, 1113 Sofia, Bulgaria.
Email: tchorban@microbio.bas.bg

Funding information
Bulgarian National Science Fund, Grant/Award Number: DO 02-312/08; Hungarian National Science Fund, Grant/Award Number: K112011; TÁMOP, Grant/Award Number: 4.2.1./B-09/1/KMR-2010-0003

Abstract

Der p 1 is one of the major allergenic molecules of *Dermatophagoides pteronyssinus*, causing house dust mite (HDM) allergy. The pathological B cells produce allergen-specific IgE antibodies that mediate the hypersensitivity reaction, therefore the selective elimination of these B cells is a legitimate therapeutic goal in allergy. Chimeric molecule Dp51-72 able to cross-link B cell inhibitory complement receptor type 1 and BCR on Der p 1-specific B cells was constructed. The signalling capabilities of this molecule have been tested on human B cells. A humanized mouse model of HDM allergy has been used to test the in vivo effects of the chimeric molecule administration. Administering the chimeric molecule to immunodeficient Rag2- γc- mice transferred with PBMCs from allergic patients resulted in reduction of allergen-specific IgE antibodies in the sera, and reduced infiltration of immune cells in lung histology preparations. Reduced numbers of human $CD45^+$ and $CD4^+$ cells in the lungs as well as inhibition of mast cell degranulation were also observed. The treatment with Dp51-72 chimera significantly decreased the local levels of anti-Dpt IgE antibodies in the bronchoalveolar lavage fluid (BALF). The binding of the chimeric molecule to tonsillar B cells triggers the tyrosine phosphorylation of 30–32 kDa protein, which is most likely involved in the inhibitory process. Administration of constructed chimeric molecules to humanized mice with developed inflammation resulted in specific suppression of disease-associated IgE antibody-producing cells and preserved lung histology. This effective approach could be further developed into a therapeutic agent for treatment of patients with HDM allergy.

1 | INTRODUCTION

Hypersensitivity to house dust mites (HDM) is one of the most prevalent allergic reactions in humans; more than 50% of all asthmatics worldwide are sensitized to HDM-borne aeroallergens.[1,2] *Dermatophagoides pteronyssinus* (Dpt) is a widely distributed mite species which is responsible for most of the HDM allergic reactions in the

© 2022 The Scandinavian Foundation for Immunology.

13653083, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/sji.13241 by University Of Liverpool, Wiley Online Library on [28/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

industrialized world.[2] In atopic patients, HDM-derived allergens activate pathological Dpt-specific B cells, which develop into IgE-producing plasma cells.

The generation of allergen-specific IgE antibodies is considered to be of upmost importance in allergies, in which a small quantity of the allergen in the environment has the capacity to elicit very strong hypersensitivity reactions. The administration of the humanized anti-IgE monoclonal antibodies omalizumab or ligelizumab to allergic patients or a mouse model results in a rapid reduction of IgE serum levels and ameliorates most of the symptoms in patients with allergic asthma, rhinoconjunctivitis and atopic dermatitis.[3,4] Reduction of serum IgE levels leads to significant down-modulation of the FcεRI receptor expression on mast cells, which further decreases the amount of FcεRI-IgE complexes on the surface of the effector cells and renders them less sensitive to allergen stimulation.[5] These results highlight the importance of IgE, thus the allergen-specific B lymphocytes represent a logical target for selective therapeutic intervention.

Human complement receptor type 1 (CR1, CD35) is expressed either alone or in complex with complement receptor type 2 (CR2) on the surface of B cells.[6] It had been shown earlier that CR1 delivers strong inhibitory signals in human B lymphocytes, resulting in an efficient reduction of proliferation and differentiation to antibody-producing plasma cells.[7,8] Hence, human CR1 could be a promising candidate for specific targeting of pathogenic B cells.

We have previously constructed a protein-engineered chimeric molecule consisting of a DNA-mimotope peptide DWEYSVWLSN coupled to a monoclonal anti-human CR1 antibody, which binds to human B cells. We showed that this chimera cross-links cell surface immunoglobulin and CR1 on dsDNA-specific B cells from lupus patients, and suppresses anti-dsDNA IgG antibody-secreting plasma cells with the same specificity.[9,10]

As a logical approach we have constructed another chimeric molecule with the aim of selectively silencing pathological Der p 1-specific B cells as producers of IgE antibodies. HDM-allergic individuals develop hypersensitivity to different mite proteins, but the dominating allergen is the 25 kDa glycoprotein Der p 1 found in mite faeces (consisting of 222 amino acids). Together with Der p 2, these two major allergens are responsible for 50%–60% of IgE-binding reactivity of sera from HDM-reactive subjects.[11,12] In this sequence, four potential B cell epitopes have been predicted by 3D modelling and bioinformatics techniques and their IgE-dependent biological activities have been tested.[13–15] We used one of these epitopes (p52-71) and coupled it to a monoclonal anti-human CR1 antibody to construct a chimeric molecule, able to recognize Der p 1-specific B cells.[16] We showed that the engineered antibody could selectively decrease the number of anti-Dpt IgE-secreting B cells when administered in vitro to peripheral blood mononuclear cells (PBMCs) from HDM-allergic patients.

Rag2- γc- and NOD-SCID gamma (NSG) immunodeficient mice lack mature T, B and NK-cells and are perfect recipients of xenogenic cells. As a model host for therapeutic investigations, the animals can develop disease symptoms when humanized with pathological donor cells.[17–20] We have previously developed an autoimmune model in NSG mice reconstituted with PBMCs from systemic lupus erythematosus (SLE) patients.[21] The humanized animals showed presence of autoantibodies against dsDNA as well as human immunoglobulin depositions in the renal glomeruli. Administration of a monoclonal anti-Annexin A1 antibody to the sick humanized NSG mice prevented the appearance of anti-dsDNA antibodies, proteinuria and glomerular deposition of human IgG-immune complexes observed in the control animals.

Although there are plenty of studies that use animal models of allergy and asthma, these data cannot be directly extrapolated to humans.[22–24] Humanized models have been successfully used to study specific aspects of human allergy and asthma and are especially useful in evaluating the role of T and B cells in in vivo models of the disease. Therefore, we established a humanized model of HDM allergy in order to study the effect of a chimeric antibody, composed of a Der p 1-derieved peptide sequence (p52-71) and the 3D9 monoclonal antibody. This protein-engineered molecule should target the allergen-reactive human B cells in the Rag2- γc- mice transferred with PBMCs derived from HDM allergy patients by engaging their Der p 1-specific B cell receptor (BCR) and to selectively silence them. We show here that the engineered construct decreases the number of anti-Dpt IgE-secreting B cells in Rag2- γc- mice by delivering a strong inhibitory signal through the surface CR1 receptors. Based on our results we hypothesize that the chimeric molecule might be developed into a therapeutic agent, which could ameliorate the manifestations of allergic inflammatory response in patients with HDM allergy.

2 | MATERIALS AND METHODS

2.1 | Monoclonal antibodies

Purified mouse 3D9 IgG1 monoclonal antibody (mAb) specific to human CR1 was prepared using the mouse 3D9 hybridoma as described.[10]

FITC–conjugated anti-human CD45 (Immunotech, clone J.33; #0782), anti-human CD3-PeCy7 (Tonbo Biosciences, clone SK7; #60-0036-T100), anti-human CD4-APC (eBiosciences, clone OKT4; #17-0048-42), anti-human CD19-eFlour450 (eBiosciences, clone SJ25C1; #48-0198-42), PE-conjugated anti-human CD8a (eBiosciences, clone

13653083, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/sji.13241 by University Of Liverpool, Wiley Online Library on [28/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

RPA-T8; #12-0088-42), anti-human CD19 (Immunotools, Friesoythe, Germany; clone LT19, #21270191), Alexa 647-conjugated anti-mouse IgG (Life Technologies, Budapest, Hungary; #A-21235), and anti-human CD35 and PE-conjugated anti-human CD35 (clone 3D9, kindly provided by Dr. J. Prechl, Diagnosticum zrt, Budapest, Hungary), were used for fluorescence-activated cell sorting (FACS) experiments. Mouse IgG1 kappa-PE (#12–4714-42, clone P3.6.2.8.1), IgG1 kappa-FITC (#11–4714-81, clone P3.6.2.8.1), IgG1 kappa-eFluor 450 (#48-4714-82, clone P3.6.2.8.1), IgG2b kappa-APC (#17-4732-81, clone eBMG2b), and IgG1 kappa-PECy7 (#25–4714-42, clone P3.6.2.8.1), (all from eBioscience) were used as isotype controls.

2.2 | Construction of chimeric antibody molecules

One selected peptide from the Der p 1 molecule (p52-71, Ac-NQSLDLAEQELVDCASQHGC-Ahx-K-NH_2), as well as an irrelevant peptide, containing the same number of randomized amino acids (Ac-DEACLQCGSED HQAVQNLLS-Ahx-K-NH_2), were synthesized using Fmoc chemistry on resin with >96% purity from Caslo Laboratory (Lyngby, Denmark).

The chimeric antibody molecules were constituted by separated conjugation of peptides to the anti-CD35 mAb 3D9 using the zero-length crosslinking agent EDC (1-ethy l-3-[3′-dimethylaminopropyl] carbodiimide.HCl), (Sigma-Aldrich, Taufkirchen, Germany; #E6383) as previously described.[16] The resulted engineered protein molecules: an Dp51-72 chimera (consisting of the p52-71) and a control chimera (consisting of the irrelevant peptide) were dialysed against PBS, concentrated by ultrafiltration and used in the next experiments.

2.3 | Allergic patients and healthy blood donors

The study comprised 14 recently diagnosed untreated patients with bronchial asthma and/or allergic rhinitis, sensitive to Dpt (female to male ratio 4:10; aged [min–max] 16÷40). 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 a high titre of allergen-specific IgE antibodies. The control group consisted of six age- and sex-matched healthy donors. Permission from the local Ethics Committee at the Institute of Microbiology, Sofia, Bulgaria (Prot. №2/January 14, 2020) and informed consent were obtained in all cases. The study was conducted according to the Declaration of Helsinki.

Peripheral blood mononuclear cells from patients and controls were isolated using Pancoll gradient solution (PAN Biotech, Aidenbach, Germany; # P04-60225).

2.4 | Mice

Female 8-weeks-old Rag2- γc- (C;129S4-Rag2tm1.1Flv Il2rgtm1.1Flv/J) mice were obtained from The Jackson Laboratory (Bar Harbor, ME; #014593). The animals were raised under specific pathogen-free (SPF) conditions. All manipulations were approved by the Animal Care Commission at the Institute of Microbiology in accordance with the EU Directive 2010/63/EU for animal experiments and national regulations.

2.5 | Isolation of tonsillar B cells

Tonsils from children undergone routine tonsillectomy were obtained from the Saint Istvan and Saint Laszlo Hospital in Budapest, Hungary, after obtaining written informed consent according to the Declaration of Helsinki. Tonsillar mononuclear cells were isolated as previously described.[25] $CD19^+$ B cell purity was verified by flow cytometry and was higher than 95% in each case.

2.6 | Flow cytometry analysis

The isolated B cells (1×10^6 cells/ml) were double-washed with PBS and incubated with the Dp51-72 chimera, or with unconjugated 3D9 mAb (1 μg/10^6 cells), or with PBS alone for 30 minutes at 4°C, followed by two washes and incubation with Alexa 647-conjugated anti-mouse IgG for 30 minutes at 4°C.

In a separate experiment, the isolated tonsillar B cells were incubated with the Dp51-72 chimera, or with unconjugated 3D9 mAb, or with PBS alone for 30 minutes at 4°C, followed by two washes. A second incubation with PE-conjugated anti-human CD35 antibody for 30 minutes at 4°C was performed.

Ten thousand cells were analysed from each sample with a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) using the CellQuestPro software.

2.7 | Tyrosine phosphorylation of B cell signalling molecules

Tonsillar B cells were resuspended in serum-free RPMI medium supplemented with gentamicin and pretreated with Dp51-72 chimera for 20 min. followed by 5 min.

13653083, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/sji.13241 by University Of Liverpool, Wiley Online Library on [28/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

stimulation with $F(ab')_2$ fragment of goat anti-human IgG/A/M (Jackson ImmunoResearch, Newmarket, UK; #109–005-064) at 37°C. Cells cultivated in medium only were used as controls. After activation and extraction in lysis buffer (50 mM HEPES (Sigma-Aldrich, #H3375) (pH 7.4), 1% Triton X-100 (Sigma-Aldrich, # T8787), 100 mM sodium fluoride (Sigma-Aldrich, #201154), 10 mM EDTA (Sigma-Aldrich, #431788), 2 mM sodium orthovanadate (Sigma-Aldrich, #S6508), 10 mM sodium pyrophosphate (Sigma-Aldrich, #221368), 1% glycerol (Sigma-Aldrich, #G5516), 2 μg/ml aprotinin (Sigma-Aldrich, #A1153), 2 μg/ml pepstatin (Sigma-Aldrich, #P5318), 5 μg/ml leupeptin (Sigma-Aldrich, #L2884) and 2 mM phenylmethanesulfonyl fluoride (Sigma-Aldrich, # P7626)), samples were pelleted for 15 minutes at 4°C. The total protein concentration was measured by the Bicinchoninic Acid Kit (Sigma-Aldrich; #BCA1-1KT). The proteins were boiled in reducing sample buffer, subjected to a 10% sodium dodecyl sulphate polyacrilamid gel electrophoresis (Mini-Protean II cell, Bio-Rad, Hercules, CA, USA) and transferred to nitrocellulose membranes (Bio-Rad Laboratories, #1620115).

After blocking with 5% milk powder in Tris-buffered saline supplemented with 0.05% Tween 20 for 1 hour, the membranes were incubated with the relevant primary antibodies: anti-phospo-Tyr (PY-20, BD Transduction Laboratories; #610000), anti-phospho-ERK (anti-P-ERK, Cell Signaling Technology, Danvers, MA, USA; #4377), anti-phospo-Syk (anti-P-Syk, Santa Cruz Biotehnology, Dallas, TX, USA; #sc-51 703) or anti-β-actin (Cell Signaling Technology; #4967) overnight, at 4°C. Membranes were then incubated with HRP-conjugated rabbit anti-mouse IgG (Santa Cruz Biotechnology; #sc-525 409) or HRP-conjugated goat anti-rabbit Igs (Dako, Glostrup, Denmark; #P0448) secondary Abs. Bound antibodies were visualized by the enhanced chemiluminescent method (Millipore, Bedford, MA, USA; #WBKLS0100) and analysed with the ImageJ software. Relative intensity (representing the phosphorylation level of individual signalling molecules) was calculated by dividing the peak densitometry values of the target protein by the peak values of the loading control. The signal of untreated cells, representing background was subtracted from the signal of the individual samples and the final values were presented as graphs using arbitrary units (AU).

2.8 | Cell transfer

Groups of female Rag2- γc- mice (n = 2 to 5 per donor, total 40) were used for human cell transfer. PBMCs, obtained from Dpt-sensitive patients or healthy donors were isolated as described. The cells from each donor were separated into equal parts and a total of 1×10^7 cells were transferred intraperitoneal (i.p.) to each Rag2- γc- mice. Details are presented on Figure 1A.

2.9 | Treatment schedule for the humanized Rag2- γc- model of HDM allergy

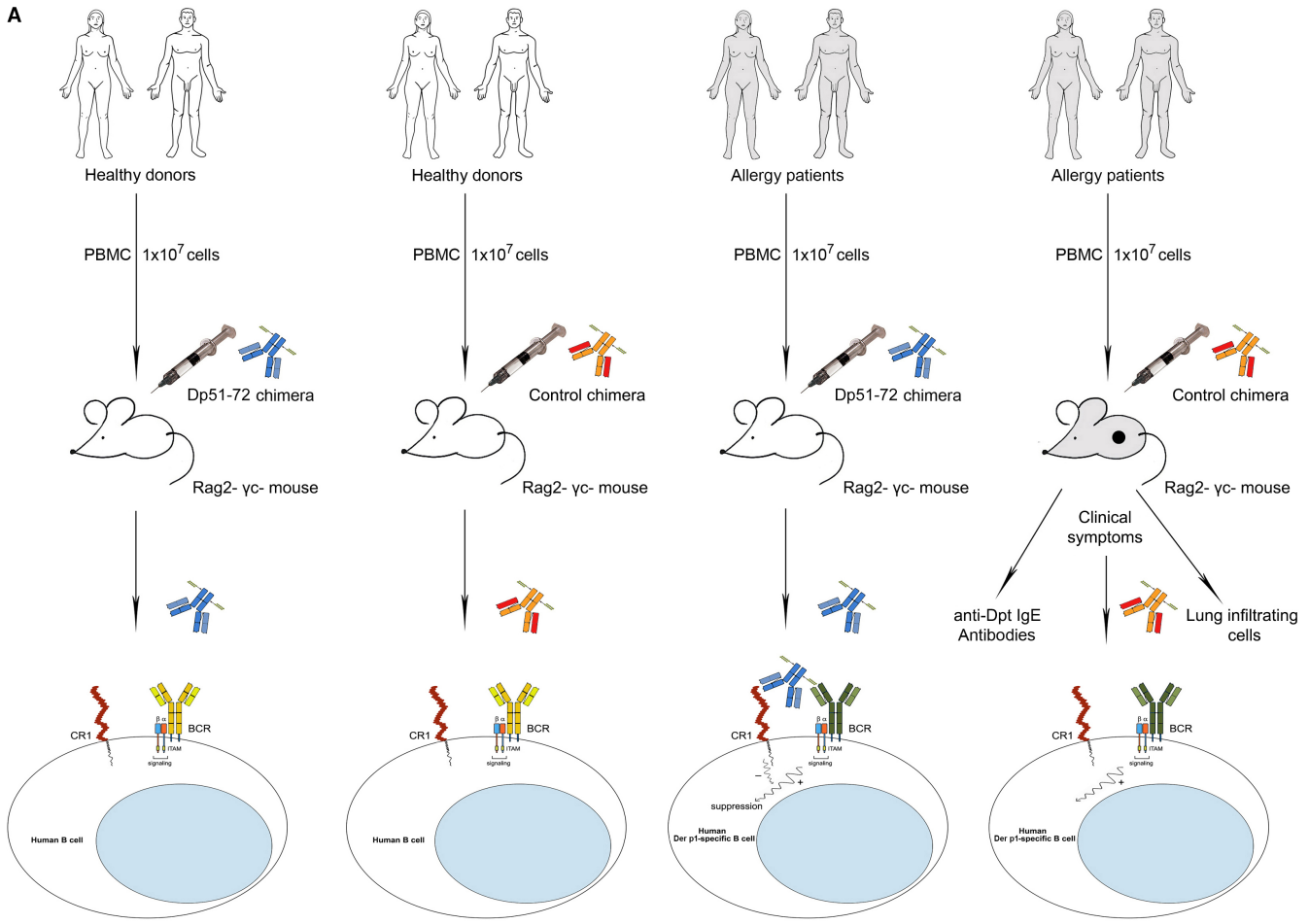
Each group of Rag2- γc- mice transferred with PBMCs from allergic patients or healthy donors was separated into two subgroups. The first subgroup was injected intravenous (i.v.) once a week in the course of 4 weeks with 50 μg per mouse of the Dp51-72 chimera and the first treatment started at day 3 after the PBMCs transfer. The second group was injected with control chimera. On days 0 and 17, after the transfer of the human PBMCs, the mice were sensitized with 100 μg/mouse of HDM allergen extract (Citeq biologics, Groningen, Netherlands; #02.01.85) adsorbed on 100 μg alum (kindly provided by National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria). Three times a week after the administration of chimeras the animals were stimulated with intranasal administration of 25 μg/mouse HDM allergen in a final volume of 35 μl/mouse. The detailed treatment schedule is presented in Figure 1B.

Every week the animals from all groups were bled from the retro-orbital sinus and the collected sera were kept frozen at −70°C. Bronchoalveolar lavage fluid (BALF) was collected at the end point by introducing 1 ml ice cold PBS into the lungs followed by centrifugation at 600 g.

2.10 | Detection of anti-Dpt IgE, IgA, IgG and total IgE antibodies

Serum and BALF levels of IgE anti-Dpt antibodies were evaluated by enzyme-linked immunosorbent assay (ELISA). Opaque 96-well flat-bottom plates (Greiner Bio-One, Kremsmünster, Austria; #675076) were coated with 100 μg/mL Dpt extract diluted in 50 mM carbonate buffer (pH 9.6) and incubated overnight at 4°C. The plates were further blocked with 1% bovine serum albumin (BSA) in Tween 20-PBS for 2 hours at room temperature (RT) and incubated with sera from treated humanized Rag2- γc- mice diluted in Tween 20-PBS + 0.1% BSA for 2 hours at RT. Later, the plates were washed and incubated for two more hours with biotin-conjugated anti-human IgE antibody (Kirkegaard & Perry Laboratories, Gaithersburg, Maryland, US; #16-10-04), followed by incubation with streptavidin-conjugated HRP (Sigma-Aldrich, #S5512) for 40 min/RT. The reaction was revealed with ultrasensitive ADHP

13653083, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/sji.13241 by University Of Liverpool, Wiley Online Library on [28/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License



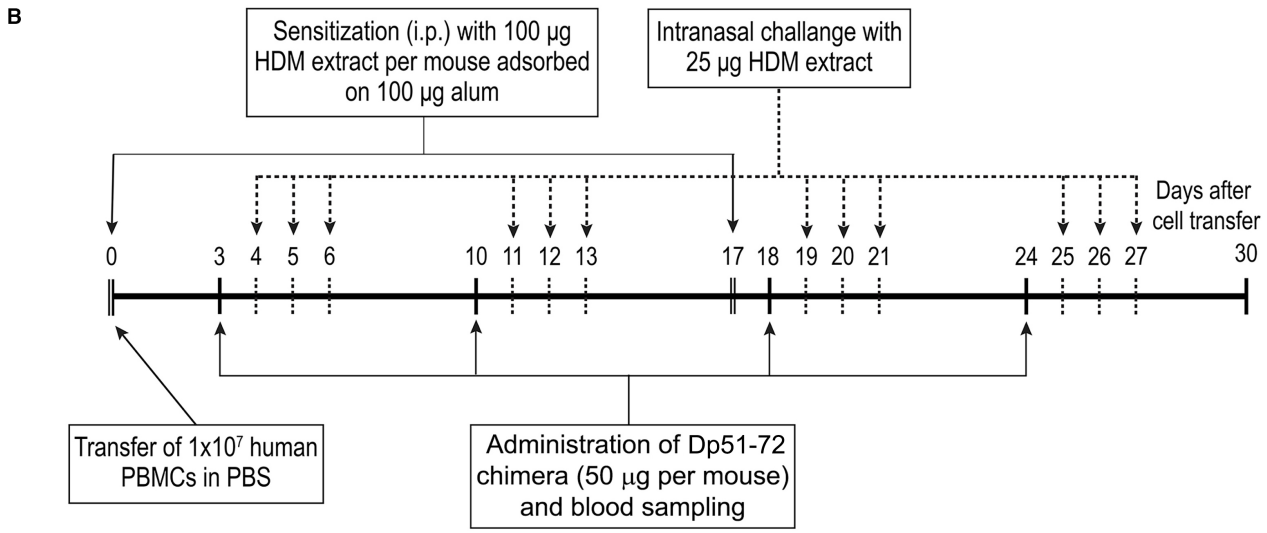


FIGURE 1 Scheme of PBMCs transfer from HDM allergy patients and healthy donors to Rag2- γc- mice and the experimental therapeutic design (A). Development of humanized Rag2- γc- model of HDM allergy and the treatment schedule with protein chimeric molecule (B)

(10-Acetyl-3, 7-dihydroxyphenoxazine) SensoLyte substrate for peroxidase (Anaspec, Fremond, US; #AS-71111). Plates were read on automatic fluorescence reader (BMG Labtech, Offenburg, Germany). The fluorescence substrate was excited on 530 nm, and the resulting fluorescence signal was detected on 590 nm.

13653083, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/sji.13241 by University Of Liverpool, Wiley Online Library on [28/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

BALF levels of anti-Dpt IgA and anti-Dpt IgG antibodies were evaluated by ELISA using the same coating and blocking strategy followed by incubation with anti-human IgA (Southern Biotech, Birmingham, AL, USA; #2050-04) and IgG (Sigma-Aldrich, #A-1543) antibodies coupled with alkaline phosphatase. The reaction was developed with pNPP substrate (Calbiochem, San Diego, CA, USA; #4876) and measured at 405 nm. Total human IgE in BALF was measured using total IgE ELISA kit (Euroimmun, Lübeck, Germany; #EV 3840-9601 E) following the manufacturer's protocol.

2.11 | β-hexosaminidase activity assay and total protein measurements

The activity of β-hexosaminidase in BALF was investigated as a marker of mast cell degranulation in the lungs. The enzymatic reaction was started by mixing 50 µl BALF and 50 µl 5 mM p-nitrophenyl N-acetyl-b-d-glucosaminide (Carl Roth, Karlsruhe, Germany; #4062.2) (in 50 mM trisodium citrate buffer, pH 4.5, Sigma-Aldrich, #S1804). After 2 h incubation at 37°C the reaction was stopped by adding 200 µL 0.2 M glycine-NaOH (pH 10.6), (Riedel-de-Haën, #15527; Sigma-Aldrich, #S5881). Optical density was measured at 405 nm.

Total protein concentration in BALF was measured using Bradford reagent (Sigma-Aldrich, #B6916) following the manufacturer's protocol.

2.12 | Phenotyping of lung infiltrates

The lungs of humanized Rag2- γc- mice were analysed for the presence of human lymphocytes by FACS analysis. The cells were isolated from the lungs together with histology using digestion buffer 2 mg/ml collagenase D (Sigma-Aldrich; #11088858001) and 0.1 mg/ml DNase I (Sigma-Aldrich; #10104159001) at 37°C for 45 minutes. After two washing steps the lymphocytes were stained with antibody cocktail for phenotyping. The cells were gated with anti-human CD45-FITC antibody, and T -cells were stained with anti-human CD3-PeCy7, anti-human CD4-APC and anti-human CD8a-PE antibodies. B-cells were stained with anti-human CD19-eFlour450. Thirty thousand CD45 positive cells were analysed from each sample with a BD LSR II flow cytometer using the Diva 6.1.1. software (BD Biosciences, San Jose, CA).

2.13 | Histology

Lungs from humanized Rag2- γc- mice were isolated and fixed in 10% formalin (Sigma-Aldrich; #HT501128) overnight. Fixed lungs were dehydrated in ethanol and xylene substitute (Tissue-clear, Sakura Finetek Europe; #1426) followed by embedding in paraffin (Paraplast, Sigma-Aldrich, #P3558). Paraffin sections were analysed with standard haematoxylin/eosin (H&E) (Sigma-Aldrich; #GHS216; #HT110) staining technique. The slides were observed using light microscopy (40×, Nikon Eclipse E100). Perivascular and peribronchial inflammation score analysis was performed with ImageJ software. The score was calculated using the formula: *area with cell infiltrates around vessels/area of the vessels*.

2.14 | Statistical analysis

All statistical analyses were performed with GraphPad Prism 5 software (San Diego, CA). The two-way ANOVA test was used to determine differences between each two groups in serum analysis. Unpaired or paired *t* test was used for all other experiments. Values in the figures correspond to mean ± SD. A value of $p < 0.05$ was considered as statistically significant.

3 | RESULTS

3.1 | The chimeric molecule binds to human CR1 expressed on tonsillar B cells

We have previously demonstrated by flow cytometry the potency of the Dp51-72 chimera to bind to CD35 expressed by human B cells isolated from blood, as well as with the LAMA-84 cell line.[16] In order to study the ability of the chimeric molecules to engage CR1 on tonsillar B cells isolated from healthy donors, we assessed its binding capacity in competition with the PE-conjugated anti-CD35 antibody or with an Alexa647-conjugated anti-mouse IgG antibody. We excluded the theoretical possibility of an antigen-binding site of the parent antibody to be affected during the conjugation process with the Der p 1-derived peptides. As shown in Figure 2A upper panel, the Dp51-72 chimera inhibited the binding of the PE-conjugated anti-CR1 antibody to the complement receptor on tonsillar B cells. Furthermore, both the Dp51-72 chimera and the unconjugated anti-CR1 antibody bound to targeted receptor CD35 on human B cells in the same manner (Figure 2A, lower panel).

3.2 | The Dp51-72 chimera triggers signalling pathways through CR1

The ability of the constructed Dp51-72 chimeric molecule to induce intracellular signalling via the inhibitory

13653083, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/sji.13241 by University Of Liverpool, Wiley Online Library on [28/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

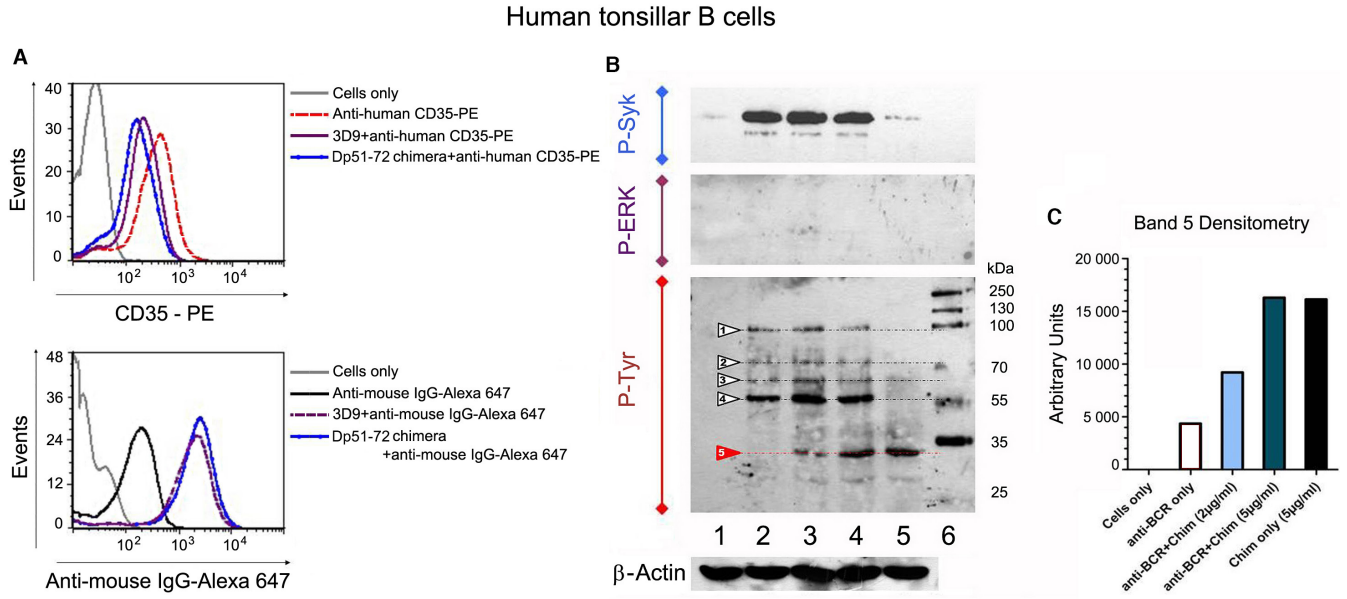


FIGURE 2 Dp51-72 chimera binds to CD35 on human tonsillar B cells and trigger signal transduction through CR1. (A) Dp51-72 chimera binds to CD35 on human tonsillar B cells and competes with PE-conjugated anti-CR1 antibody for the same receptor. The isolated B lymphocytes were incubated with Dp51-72 chimera, or with unconjugated 3D9 MoAb, or PBS only for 30 min at 4°C followed by incubation either with PE-conjugated anti-human CD35 antibody (upper panel), or Alexa647-conjugated anti-mouse IgG antibody (lower panel). After washing, the cells were analysed by flow cytometry. Data are representative of at least 5 independent experiments. (B) Dp51-72 chimeric molecules trigger signal transduction through CR1. Isolated human tonsillar B cells were incubated in the presence of the Dp51-72 chimera at 37°C with or without an anti-IgG/M/A stimulation. Cells cultivated in medium only were used as controls. The cells were lysed, centrifuged and the supernatants were subjected to Western blotting with an anti-phospho-tyrosine, or with anti-phospho-ERK, or with anti-phospho-Syk antibodies and were developed using the ECL technique. Different bands in the p-tyrosine blot are marked with numbered arrows with band №5 marked in red. Lanes: (1) Untreated cells; (2) anti-BCR stimulus (5 μg/ml) only; (3) anti-BCR + Dp51-72 chimera (2 μg/ml); (4) anti-BCR + Dp51-72 chimera (5 μg/ml); (5) Dp51-72 chimera (5 μg/ml) only; and (6) a protein ladder. For normalization the same membrane was re-probed with anti-β-actin antibody (bottom). Data shown are representative of three independent experiments. (C) Intensity of the anti-phospho-tyrosine blotted band 5 from individual lanes was measured after ECL development by densitometry.

cell surface CR1 was assessed using tonsillar B cells. Antibodies against several phosphorylated molecules involved in intracellular signalling, such as phospho-ERK and phospo-Syk, as well as the antibody to phospo-Tyr were tested to assess general phosphorylation pattern.

As shown in Figure 2B (upper panel), phosphorylation of Syk was strong and identical in all samples where the cells were stimulated by anti-BCR either alone (Lane 2) or pretreated with the two concentrations of the Dp51-72 chimera (Lanes 3 and 4), in contrast to the cells treated with the Dp51-72 chimera alone (Lane 5).

Using the anti-ERK antibody no signal was detected in any of the probes after the same treatment (Figure 2B, middle panel).

The total tyrosine phosphorylation pattern of the same samples was much more diverse (Figure 2B, lower panel). Strong Tyr phosphorylation of a protein with an estimated molecular mass of 30–32 kDa was detected after incubation of cells treated with Dp51-72 chimera with or without anti-BCR stimulation. Interestingly, this band appears even in the absence of the BCR-signal, which suggests that the phosphorylation of this protein depends on ligation of CR1 via the chimeric molecule. No signs of tyrosine phosphorylation events were seen in the untreated cells.

Densitometry analysis of the main bands (pointed by arrows) detected on the anti-p-Tyr blot was performed and the results are presented in Table 1. The graphic representation of the differences in the intensity of the auxiliary band 5 corresponding to the 30–32 kDa protein is shown in Figure 2C.

3.3 | Effects of the Dp51-72 chimeric molecule in humanized Rag2- γc- mice

Isolated PBMCs obtained from diagnosed untreated allergic patients, sensitive to Dpt with high levels of anti-Dpt IgE antibodies, or from healthy donors were transferred to female Rag2- γc- mice. The successfully engrafted Rag2- γc- mice were treated either with the Dp51-72 or the control chimeras to assess in vivo the Dpt-specific B cell suppression. The animals were weighed and monitored throughout the experiment, and no signs of graft-versus-host reaction were observed (data not shown).

13653083, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/sji.13241 by University Of Liverpool, Wiley Online Library on [28/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Since the elevation of serum anti-Dpt IgE antibodies is a distinctive feature of HDM allergy, we assessed the allergen-specific IgE levels in the sera of differently treated animals. As shown in Figure 3, the administration of the Dp51-72 chimeric molecule to Rag2- γc- mice transferred with PBMCs from HDM-allergic patients resulted in partial suppression of the anti-Dpt IgE antibodies. Lower levels of human anti-Dpt IgE antibodies were detected in the sera of Dp51-72 chimera-treated mice in the beginning of the observation period (Figure 3, left panel). At Week 4 of treatment the anti-Dpt IgE levels were not detectable.

The control group of Rag2- γc- mice transferred with PBMCs from healthy donors had low levels of human anti-Dpt IgE antibodies which were not affected by the administration of the Dp51-72 chimera (Figure 3, right panel).

BAL fluid was analysed to identify the local immune response in HDM allergy model. The treatment with Dp51-72 chimera significantly decreased the levels of human anti-Dpt IgE antibodies compared to the control chimera (Figure 4A). There was no change in the levels of allergen-specific IgA, IgG and total IgE antibodies (Figure 4B–D). Total protein concentration in BALF was used as a marker of vascular permeability.[26] Administration of Dp51-72 chimera reduced the protein exudate in BALF (Figure 4E). Mast cell degranulation in the lungs was evaluated by measuring β-hexosaminidase activity in BALF.[27] The β-hexosaminidase release in the airways was significantly inhibited in the Dp51-72 chimera group (Figure 4F).

To detect lymphocyte infiltration and verify the allergic reaction, the right lung lobes from all animals were used for preparation of cell suspension, and the lung microenvironment was investigated by flow cytometry. It was found a reduced number of human CD45, CD3, CD4 and CD19 cells in the group treated with Dp51-72 chimera compared to control chimera treated animals, while the number of infiltrated CD8 T cells was significantly increased in the allergic chimera treated group than in controls (Figure 5).

At the end of the in vivo experiments, the left lungs from all animals were used for histopathological analysis

TABLE 1 Densitometry of the individual bands on the anti-phosphotyrosine blot

Bands	Cells only	Anti-BCR only	Anti-BCR + Chimera (2 μg/ml)	Anti-BCR + Chimera (5 μg/ml)	Chimera only (5 μg/ml)
Band 1	0	9778.3	2647.9	1857.7	0
Band 2	0	7946.3	9420.2	1310.6	0
Band 3	0	1261.0	1422.3	1399.3	0
Band 4	0	10 262.7	15 726.1	13 708.5	1285.9
Band 5	0	4356.4	9213.3	16 297.7	16 133.5

Note: Data shown are representative of 3 independent experiments.

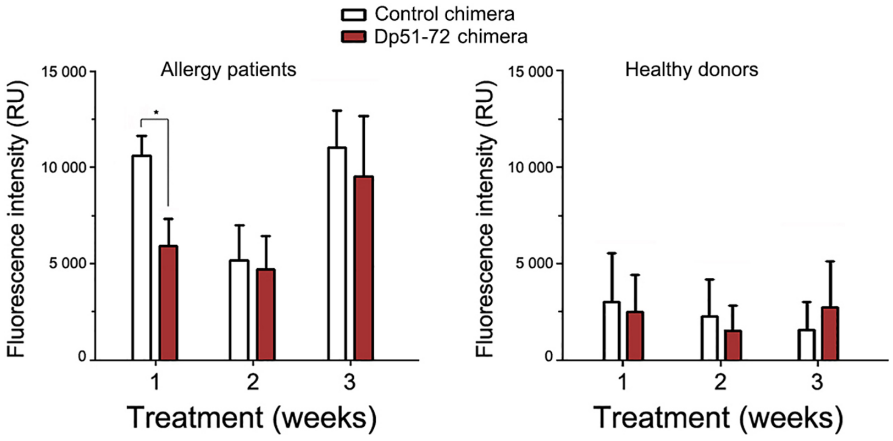


FIGURE 3 Administration of Dp51-72 chimera decreases anti-Dpt IgE antibody production in humanized HDM allergy-Rag2- γc- mice. Isolated PBMCs from 10 allergic patients and 6 healthy donors were used for cell transfer into Rag2- γc- mice. Each group of allergic or healthy donor-transferred Rag2- γc- mice was separated into two subgroups that were treated weekly with either Dp51-72 chimera (50 μg/mouse i.v.), or control chimera. Anti-Dpt IgE antibody values were evaluated by fluorescence ELISA. Data are represented as mean ± SD of 12–18 mice per group; p values are calculated using the two-way ANOVA test to determine differences between the Dp51-72 chimera-treated groups (*$p < 0.05$) in comparison to control chimera-injected mice

13653083, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/sji.13241 by University Of Liverpool, Wiley Online Library on [28/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

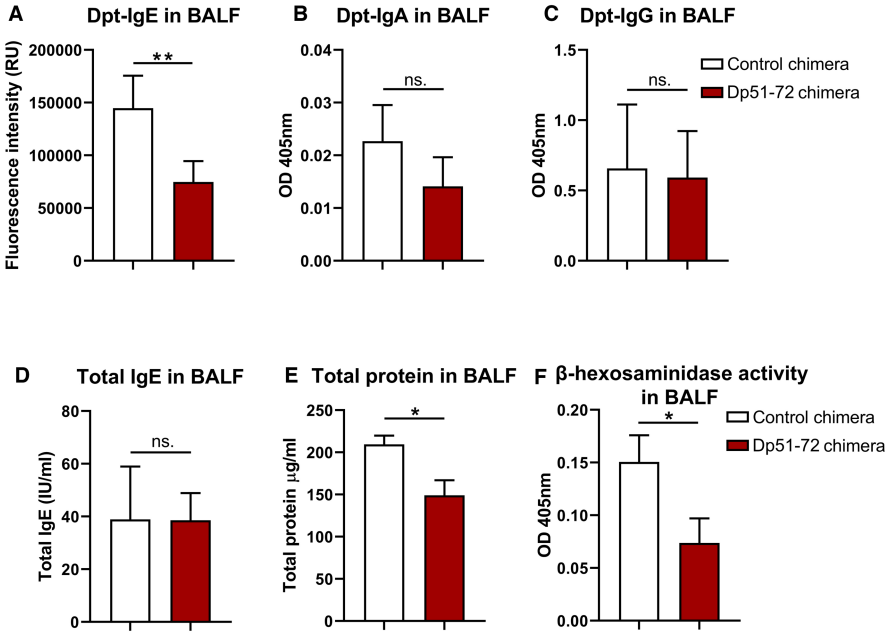


FIGURE 4 Analyses of BAL fluid. Two Rag2- γc- mice were engrafted with PBMC from each individual HDM allergy patient (n = 4), and treated with control chimera, or with Dp51-72 chimera. Each sample represents BALF from individual animal. (A) Anti-Dpt IgE levels in BALF were measured by fluorescent ELISA. Anti-Dpt IgA (B) and anti-Dpt IgG (C) levels in BALF were measured by ELISA. The concentration of total IgE (D) and total proteins (E) in BALF was evaluated. The activity of β-hexosaminidase in BALF was measured as an indication of mast cell degranulation (F). Data are represented as mean ± SEM of 4 mice per group; p values are calculated using the paired *t* test to determine differences between the Dp51-72 chimera-treated group (*$p < 0.05$; **$p < 0.01$) in comparison to control chimera-injected mice

by staining with haematoxylin/eosin. As illustrated in Figure 6, we observed perivascular and peribronchial inflammatory cell infiltrates in the lungs of control chimera-treated mice transferred with PBMCs from allergic patients after intranasal HDM challenge. In contrast, such a strong pathological inflammation was not detected in the lungs of humanized mice, treated with the Dp51-72 chimera and significantly less cell infiltrates were observed.

4 | DISCUSSION

Generation of high-affinity IgE antibodies is considered to be a key step in allergies in which a small amount of the allergen in the environment has the capacity to elicit a very strong hypersensitivity response. It is already shown that there is a correlation between serum IgE levels and the number of FcεRI expressed on basophils and mast cells.[1,2] Therefore, elimination of circulating IgE or IgE-producing B cells as pathological factors for the inflammatory cascade, could be a promising therapeutic approach.

Today, only few approaches using antibodies are accepted now to treat allergic patients and reduce the allergic reaction. Allergy and allergic asthma patients treated with omalizumab exhibit a reduction in tissue eosinophilia and in secretion of inflammatory mediators, as well as a decrease in the number of IgE-positive and FcεRI-positive cells in the submucosa. They also show a decrease in the number of IL-4-producing cells, and a reduced overall number of activated CD4 and CD8 T lymphocytes in the respiratory tract. Omalizumab is approved for treatment of severe persistent allergic asthma, especially in patients who do not respond to conventional therapies.[28,29] As an alternative approach for the treatment of allergy, epitope-specific therapeutic strategies have been developed. Chemically-modified allergen epitopes as well as a variety of carriers have been tested for immune response modulation, suppression of IgE production or induction of tolerance.[30,31]

A further plausible approach is the inhibition of IgE production by B cells which specifically recognize the allergen. This is the strategy we utilized via targeting the inhibitory CR1 and the allergen-specific B cell by a construct which is able to cross-link both cell membrane structures. It was proven that such an artificial bi-specific chimeric molecule carrying an anti-CR1 antibody would recognize preferentially the targeted disease-associated B cells instead of other CR1-positive cells.[9,10]

The inhibitory function of human CR1 has been reported by several authors.[7,9,10,25] Józsi et al. demonstrated that aggregated C3, which mimics C3b, the natural ligand of CR1 results in a reduction in the level of free Ca^{2+} and inhibits phosphorylation of cytoplasmic proteins after

13653083, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/sji.13241 by University Of Liverpool, Wiley Online Library on [28/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

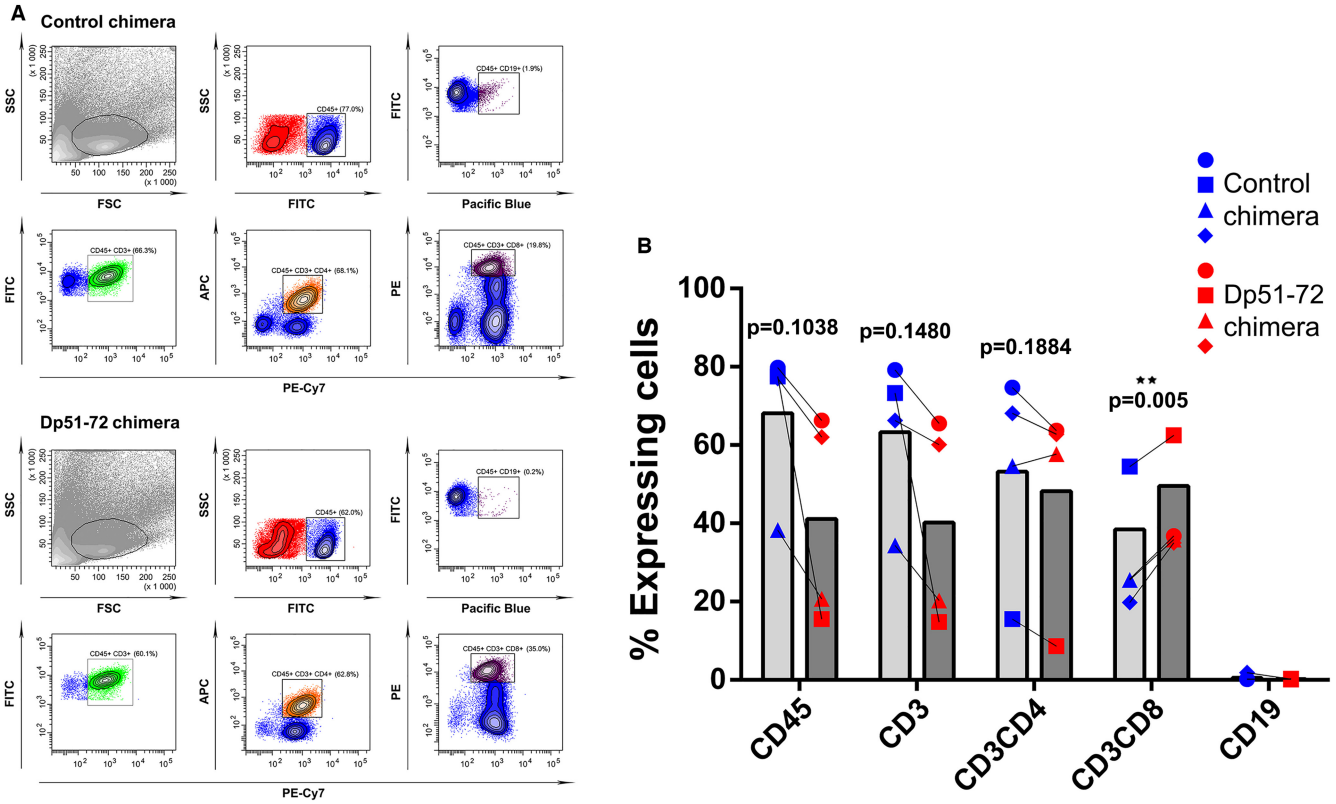


FIGURE 5 Quantitative analyses and phenotyping of lung infiltrates. (A) Lungs from all animals were excised and the single-cell suspensions derived from the lungs were incubated with one of the following anti-human antibody combination through CD45-FITC: CD3-PeCy7, CD3-PeCy7/CD4-APC, CD3-PeCy7/CD8a-PE, or CD19-eFlour450. Thirty thousand CD45-positive cells were analysed from each sample by flow cytometry. (B) The extracted results are presented graphically as the percentage of total immune cells. Each shape represented cells from single animal engrafted by HDM allergy patient (n = 4), treated with control chimera (blue), or with Dp51-72 chimera (red). Data are represented as values of 4 mice per group; p values are calculated using the paired *t* test to determine differences between the Dp51-72 chimera-treated group (**$p < 0.01$) in comparison to control chimera-injected mice.

engagement of CR1 on the BCR-activated lymphocytes.[7] This inhibitory capacity of CR1 has been demonstrated in SLE-patients, too.[8,10,32]

Der p 1, the major allergen of house dust mites, comprises several B and T cell epitopes involved in the induction of allergic immune response.[13–15] For the construction of our protein-engineered chimeric molecule targeting pathological Dpt-specific B cells we have used a small synthetic peptide (p52-71) containing one of the B cell epitopes from Der p 1 coupled to an antibody specific to the CR1. This immunodominant peptide has been proven as effective inducer of cell activation and expressed its biological activity only on $Fc\varepsilon R^+$-bearing cells and histamine release from Dpt-sensitive patients.[14] The Der p 1 epitope recognition by the anti-Der p 1 IgG and IgE antibodies after peptide-antibody conjugation has been tested already by ELISA and Western blot.[16]

Despite these convincing data, the molecular mechanism of the intracellular signalling process initiated by CR1 is still unknown. Since its cytoplasmic tail is very short, it can be assumed that the receptor molecule is unable to transmit signals on its own. The mechanism of CR1 signalling is still not investigated, and thus far no molecule has been identified to be involved in the CR1-mediated blocking of BCR-induced functions in human B cells. Previously, the potential of a chimeric molecule consisting of a human CD35-specific antibody and self-epitopes to provide signals through the inhibitory CR1 was proven using the CD35-expressing cell line, U937. We did not observe any interaction between the chimera and the human Fcγ receptors.[16] In the present study, we used primary B cells isolated from tonsils. We found that the Dp51-72 chimera triggers a BCR-independent signal through CR1, which resulted in the tyrosine phosphorylation of a protein with an approximate molecular weight of 30–32 kDa (Figure 2). These data suggest the existence of a so far-unravelled inhibitory pathway in human B cells, which is initiated by the ligation of CR1. The identification of this molecule and its role in the inhibitory process is under investigation in our laboratories. Nevertheless, inhibition through CD35 provides an additional regulatory checkpoint by which immune complexes

13653083, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/sji.13241 by University Of Liverpool, Wiley Online Library on [28/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

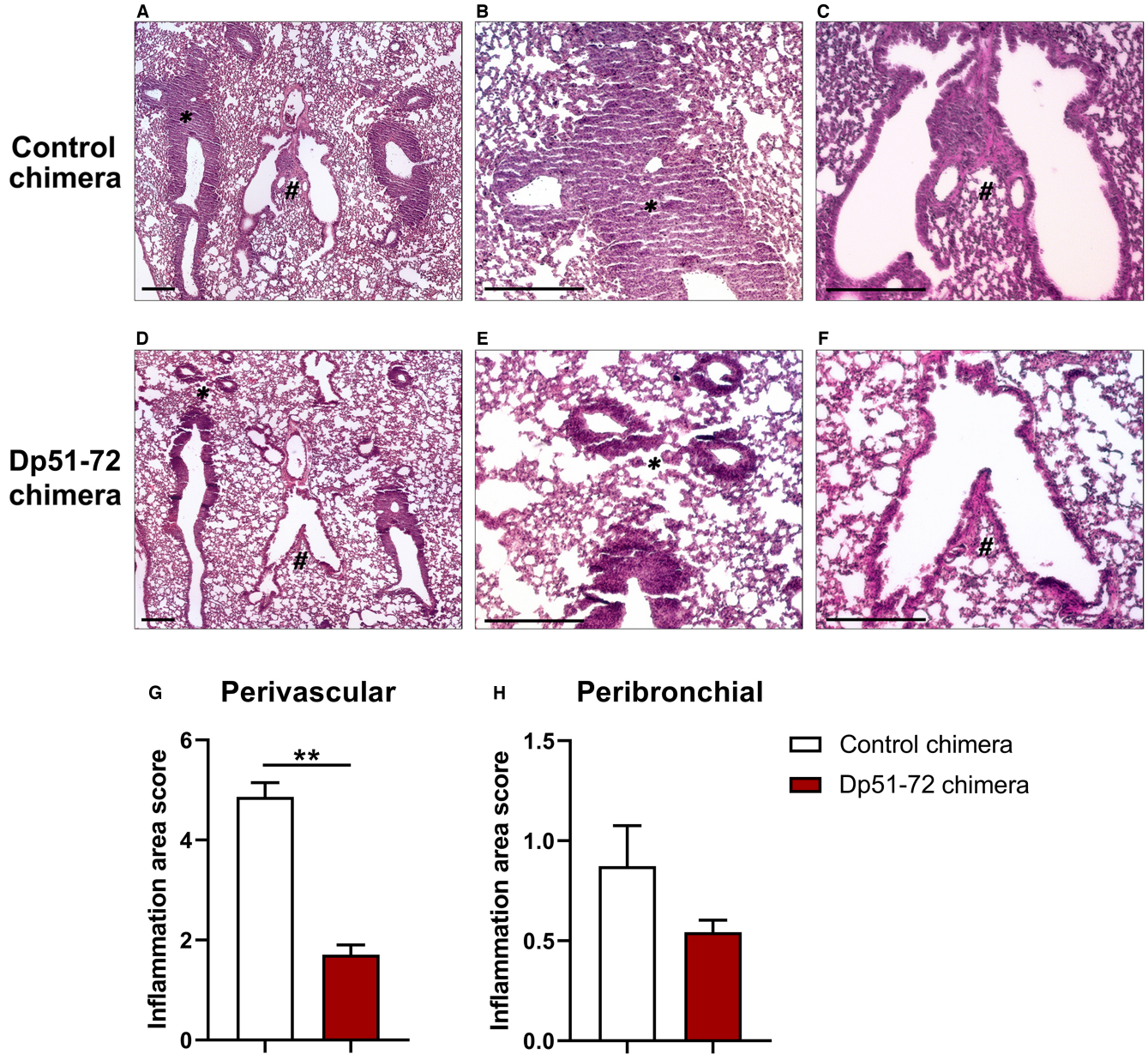


FIGURE 6 Lung histology of the humanized Rag2- γc- mice. Histological examination of paraffin-embedded lung sections from humanized Rag2- γc- mice engrafted with PBMCs from HDM-allergy patients and treated with the control chimera (A–C), or with the Dp51-72 chimera (D–F). Perivascular (*) and peribronchial (#) inflammatory infiltrates were detected in the lungs of humanized mice after intranasal HDM challenge (25 μg/mouse). Standard haematoxylin/eosin staining technique was used. Representative images of 4 animals are shown. Scale bar, 250 μm. Perivascular (G) and peribronchial (H) inflammation score was evaluated. Data are represented as mean ± SD of 4 mice per group; p values are calculated using the unpaired *t* test to determine differences between the Dp51-72 chimera-treated group (**$p < 0.01$) in comparison to control chimera-injected mice.

and C3 degradation products could restrict the activation and function of B cells.

Immunodeficient SCID, NSG and Rag2- γc- mice do not develop functional T, B (and NK) lymphocytes, which makes them tolerant to transplantation of xenogenic cells like human lymphoid cells, and allows limited differentiation and maturation capability.[17,18] It has been reported that SCID mice transferred with PBMCs from HDM-sensitized patients and exposed to intranasal stimulation with Dpt allergen developed allergic reaction similar to the one in humans: with T_H2-mediated response, production of allergen-specific IgE and respiratory inflammation reactions. Transfer of 1×10^7 PBMCs are shown to be enough to elicit a detectable amount of human IgE.[33–35] Passive immunization of SCID mice with Dpt-sensitized donor serum reveals that human IgE binds to murine mast cells, and allergen challenge leads to immediate type-1 immune response.[36]

13653083, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/sji.13241 by University Of Liverpool, Wiley Online Library on [28/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

In an attempt to selectively eliminate or suppress the Der p 1-specific B lymphocytes in vivo we have utilized a model of human HDM allergy in humanized Rag2- γc- mice based on HDM sensitization and challenge protocols for intact Balb/c mice.[37] We have shown earlier that Der p 1-peptide chimeric molecules are able to cross-link Der p 1-specific BCR with CR1 on the allergen-specific B cells from HDM allergy patients, resulting in the significant reduction of anti-Dpt IgE antibody-producing plasma cells in vitro.[16] It would be expected that the specific therapy with the constructed chimeric antibody has to be more advantageous under in vivo conditions based on its bivalent specificity and hence could selectively suppress the pathological Dpt-specific B cells and the production of anti-Dpt IgE antibodies. Basically, the IgE antibody levels are very low in human sera, but the levels of Dpt-specific IgE in humanized mice are extremely low and hardly detectable. We found that in the Rag2-γc- mice reconstituted with PBMCs from HDM allergy patients and treated with Dp51-72 chimera there was a reduction in the levels of Dpt-specific IgE antibodies in the sera and in the number of human CD45, CD3, CD4 and CD19 cells compared to control chimera treated animals. In contrast, significantly higher number of human CD8 T cells were found in Dp51-72 chimera-treated mice and some data suggested the role of increased human IFN-γ-producing CD8 T cell population being suppressors of T_H2 immune response.[38]

Indeed, with the use of a single epitope (p52-71) for chimera construction, it was not possible to suppress all Der p 1-specific B cells recognizing the variety of Der p 1 epitopes. The significant anti-Der p 1 IgE decrease may have resulted from the phenomenon of epitope spreading, implicated in allergy and autoimmunity. Using Fel d 1-derived peptide vaccine has induced an immune response to epitopes of Fel d 1 not included in the vaccine.[39] The same explanation could be relevant for the significant decrease of the other followed clinical parameters.

Monitoring of the local immune response in BAL fluid after treatment with Dp51-72 chimera showed significantly decreased levels of human anti-Dpt IgE antibodies and no change in the levels of allergen-specific IgA, IgG and total IgE antibodies compared to the control chimera. Significantly reduced levels of β-hexosaminidase release and total protein concentration were also found in BAL fluid after Dp51-72 chimera therapy, assuming a local inhibition of mast cell degranulation and vascular permeability.

Dp51-72 chimera probably inhibits the differentiation of B cells to plasmablasts, but seems some existing Dpt-specific antibody-producing plasmablasts are not affected because of the lack of BCR. It was already shown that the control chimera have not suppressive effect on the Dpt-specific cells.[16] Importantly, there was also less pathological cell infiltration in the lungs of these animals. It seems that the targeted down-modulation of Dpt-specific B cells disrupt also their antigen-presenting functions, resulting in resolution of the lung inflammation.

In the present study, we explored a protein-engineered CR1-specific chimeric mAb in a Rag2- γc- model of human HDM allergy with the aim to test a possible novel approach for specific therapy. The activity of the targeted Der p 1-specific B cells could be suppressed either by blocking the B cell differentiation to IgE-secreting plasmocytes or by restriction of the pathological T/B lymphocyte communication. Using these chimeric molecules in humanized model systems, we demonstrate that this approach is effective and should be further developed with the aim to diminish the production of pathological IgE antibodies and the histopathological changes in the lungs.

AUTHOR CONTRIBUTIONS

A.T., N.M. and A.E. designed the experiments. N.R., N.K., N.M., M.K., D.H. and J.D. performed the experiments. A.T., N.M. and A.E. analysed the data. N.R., N.K., A.E. and A.T. wrote and edited the paper.

ACKNOWLEDGMENT

The work was supported by grant DO 02–312/08 from National Science Fund, Bulgaria. The financial support from the Hungarian National Science Fund (OTKA) grant K112011, by TÁMOP 4.2.1./B-09/1/KMR-2010-0003, and the support from the Hungarian Academy of Sciences are gratefully acknowledged.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Nikola Ralchev Ralchev https://orcid.org/0000-0002-9046-5914
Nikola Kerekov https://orcid.org/0000-0002-0475-8682
Nikolina Mihaylova https://orcid.org/0000-0001-8582-9296
Mariann Kremlitzka https://orcid.org/0000-0001-6447-2750
Diana Hristova https://orcid.org/0000-0002-9710-1312
Julian Dzhorev https://orcid.org/0000-0003-1841-5653
Anna Erdei https://orcid.org/0000-0002-3622-6680

13653083, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/sji.13241 by University Of Liverpool, Wiley Online Library on [28/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Andrey Ivanov Tchorbanov https://orcid.org/0000-0002-9161-3325

REFERENCES

1. Gaffin JM, Phipatanakul W. The role of indoor allergens in the development of asthma. *Curr Opin Allergy Clin Immunol*. 2009;9(2):128-135.
2. Calderón MA, Linneberg A, Kleine-Tebbe J, et al. Respiratory allergy caused by house dust mites: what do we really know? *J Allergy Clin Immunol*. 2015;136(1):38-48.
3. D'Amato G, Stanziola A, Sanduzzi A, et al. Treating severe allergic asthma with anti-IgE monoclonal antibody (omalizumab): a review. *Multidiscip Respir Med*. 2014;9:23. doi:10.1186/2049-6958-9-23
4. Normansell R, Walker S, Milan SJ, Walters EH, Nair P. Omalizumab for asthma in adults and children. *Cochrane Database Syst Rev*. 2014;1:CD003559.
5. Gasser P, Tarchevskaya SS, Guntern P, et al. The mechanistic and functional profile of the therapeutic anti-IgE antibody ligelizumab differs from omalizumab. *Nat Commun*. 2020;11:165. doi:10.1038/s41467-019-13815-w
6. Roozendaal R, Carroll MC. Complement receptors CD21 and CD35 in humoral immunity. *Immunol Rev*. 2007;219:157-166.
7. Jozsi M, Prechl J, Bajtay Z, Erdei A. Complement receptor type 1 (CD35) mediates inhibitory signals in human B lymphocytes. *J Immunol*. 2002;168:2782-2788.
8. Kremlitzka M, Polgár A, Fülöp L, Kiss E, Poór G, Erdei A. Complement receptor type 1 (CR1, CD35) is a potent inhibitor of B-cell functions in rheumatoid arthritis patients. *Int Immunol*. 2013;25:25-33.
9. Nikolova K, Mihaylova N, Voynova E, et al. Re-establishing tolerance to DNA in humanized and murine models of SLE. *Autoimmun Rev*. 2010;9:499-502.
10. Kerekov N, Mihaylova N, Grozdev I, et al. Elimination of autoreactive B cells in humanized SCID mouse model of SLE. *Eur J Immunol*. 2011;41:3301-3311.
11. Reithofer M, Jahn-Schmid B. Allergens with protease activity from house dust mites. *Int J Mol Sci*. 2017;18(7):1368. doi:10.3390/ijms18071368
12. Waldron R, McGowan J, Gordon N, McCarthy C, Mitchell EB, Fitzpatrick DA. Proteome and allergenome of the European house dust mite *Dermatophagoides pteronyssinus*. *PLoS One*. 2019;14(5):e0216171. doi:10.1371/journal.pone.0216171
13. Duez C, Gras-Masse H, Hammad H, et al. Modulation of the allergen-induced human IgE response in hu-SCID mice: inhibitory effect of human recombinant IFN-gamma and allergen-derived lipopeptide. *Eur Cytokine Netw*. 2001;12(3):453-461.
14. Cui Y. Immunoglobulin E-binding epitopes of mite allergens: from characterization to immunotherapy. *Clin Rev Allergy Immunol*. 2014;47:344-35314.
15. Osinski T, Pomés A, Majorek KA, et al. Structural analysis of Der p 1–antibody complexes and comparison with complexes of proteins or peptides with monoclonal antibodies. *J Immunol*. 2015;195(1):307-316.
16. Kerekov N, Michova A, Muhtarova M, et al. Suppression of allergen-specific B lymphocytes by chimeric protein-engineered antibodies. *Immunobiology*. 2014;219(1):45-52.
17. Ito R, Takahashi T, Katano I, Ito M. Current advances in humanized mouse models. *Cell Mol Immunol*. 2012;9:208-214.
18. Song J, Willinger T, Rongvaux A, et al. A mouse model for the human pathogen salmonella typhi. *Cell Host Microbe*. 2010;8(4):369-376.
19. Koboziev I, Jones-Hall Y, Valentine JF, Webb CR, Furr KL, Grisham MB. Use of humanized mice to study the pathogenesis of autoimmune and inflammatory diseases. *Inflamm Bowel Dis*. 2015;21:1652-1673.
20. Brehm MA, Racki WJ, Leif J, et al. Engraftment of human HSCs in nonirradiated newborn NOD-scid IL2rγ^{null} mice is enhanced by transgenic expression of membrane-bound human SCF. *Blood*. 2012;119:2778-2788.
21. Mihaylova N, Chipinski P, Bradyanova S, et al. Suppression of autoreactive T and B lymphocytes by anti-annexin A1 antibody in humanized NSG murine model of systemic lupus erythematosus. *Clin Exp Immonol*. 2019;199:278-293.
22. Aun MV, Bonamichi-Santos R, Arantes-Costa FM, Kalil J, Giavina-Bianchi P. Animal models of asthma: utility and limitations. *J Asthma Allergy*. 2017;10:293-301.
23. Nials AT, Uddin S. Mouse models of allergic asthma: acute and chronic allergen challenge. *Dis Model Mech*. 2008;1(4–5):213-220.
24. Chapman DG, Tully JE, Nolin JD, Janssen-Heininger YM, Irvin CG. Animal models of allergic airways disease: where are we and where to next? *J Cell Biochem*. 2014;115(12):2055-2064.
25. Mácsik-Valent B, Nagy K, Fazekas L, Erdei A. Complement receptor type 1 (CR1, CD35), the inhibitor of BCR-mediated human B cell activation, differentially regulates TLR7, and TLR9 induced responses. *Front Immunol*. 2019;10:1493. doi:10.3389/fimmu.2019.01493
26. Yang J, Ramirez Moral I, van't Veer C, et al. Complement factor C5 inhibition reduces type 2 responses without affecting group 2 innate lymphoid cells in a house dust mite induced murine asthma model. *Respir Res*. 2019;20(1):165.
27. Shou Q, Lang J, Jin L, et al. Total glucosides of peony improve ovalbumin-induced allergic asthma by inhibiting mast cell degranulation. *J Ethnopharmacol*. 2019;244:112136.
28. Ling MF, Luster AD. Allergen-specific CD4(+) T cells in human asthma. *Ann Am Thorac Soc*. 2016;13(Suppl 1):S25-S30. doi:10.1513/AnnalsATS.201507-431MG
29. Hanania NA, Alpan O, Hamilos DL, et al. Omalizumab in severe allergic asthma inadequately controlled with standard therapy: a randomized trial. *Ann Intern Med*. 2011;154(9):573-582.
30. Valenta R, Campana R, Marth K, Van Hage M. Allergen-specific immunotherapy: from therapeutic vaccines to prophylactic approaches. *J Intern Med*. 2012;272(2):144-157.
31. Rajakulendran M, Tham EH, Soh JY, Van Bever HP. Novel strategies in immunotherapy for allergic diseases. *Asia Pac Allergy*. 2018;8(2):e14. doi:10.5415/apallergy.2018.8.e14
32. Kremlitzka M, Mácsik-Valent B, Polgár A, Kiss E, Poór G, Erdei A. Complement receptor type 1 suppresses human B cell functions in SLE patients. *J Immunol Res*. 2016;2016:5758192. doi:10.1155/2016/5758192
33. Ito R, Maruoka S, Soda K, et al. A humanized mouse model to study asthmatic airway inflammation via the human IL-33/IL-13 axis. *JCI Insight*. 2018;3(21):e121580. doi:10.1172/jci.insight.121580
34. Sonar SS, Hsu YM, Conrad ML, et al. Antagonism of TIM-1 blocks the development of disease in a humanized mouse model of allergic asthma. *J Clin Invest*. 2010;120(8):2767-2781.

13653083, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/sji.13241 by University Of Liverpool, Wiley Online Library on [28/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

35. Duez C, Tsicopoulos A, Janin A, et al. An in vivo model of allergic inflammation: pulmonary human cell infiltrate in allergen-challenged allergic hu-SCID mice. *Eur J Immunol*. 1996;26(5):1088-1093.
36. Herz U, Botchkarev VA, Paus R, Renz H. Increased airway responsiveness, allergy-type-I skin responses and systemic anaphylaxis in a humanized-severe combined immuno-deficiency mouse model. *Clin Exp Allergy*. 2004;34(3):478-487.
37. Woo LN, Guo WY, Wang X, et al. A 4-week model of house dust mite (HDM) induced allergic airways inflammation with airway remodeling. *Sci Rep*. 2018;8:6925. doi:10.1038/s41598-018-24574-x
38. Leggat JA, Gibbons DL, Haque SF, et al. Innate responsiveness of CD8 memory T-cell populations nonspecifically inhibits allergic sensitization. *J Allergy Clin Immunol*. 2008;122(5):1014-1021.e4.
39. Woodfolk JA. High-dose allergen exposure leads to tolerance. *Clin Rev Allergy Immunol*. 2005;28(1):43-58. doi:10.1385/CRIAI:28:1:043

How to cite this article: Ralchev NR, Kerekov N, Mihaylova N, et al. Targeted suppression of Dpt-specific B cells in humanized Rag2- γc- mouse model of HDM allergy. *Scand J Immunol*. 2023;97:e13241. doi:10.1111/sji.13241

13653083, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/sji.13241 by University Of Liverpool, Wiley Online Library on [28/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License