Immunobiology 219 (2014) 45–52

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

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

Nikola Kerekov[a], Antoaneta Michova[b], Maryia Muhtarova[b], Georgi Nikolov[b], Nikolina Mihaylova[a], Bogdan Petrunov[b], Maria Nikolova[b], Andrey Tchorbanov[a,*]

[a] The Stefan Angelov Institute of Microbiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[b] National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

ARTICLE INFO

Article history:
Received 8 July 2013
Accepted 29 July 2013
Available online 15 August 2013

Keywords:
Allergen-specific B lymphocytes
Allergy
CR1
HDM
Protein chimera

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.

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, phicoerythrin; ELISA, enzyme-linked immuno-sorbent 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.

* Corresponding author at: Stefan Angelov Institute of Microbiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Block 26, 1113 Sofia, Bulgaria. Tel.: +359 2 979 6357; fax: +359 2 870 0109.
E-mail address: tchorban@microbio.bas.bg (A. Tchorbanov).

0171-2985/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.imbio.2013.07.009

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

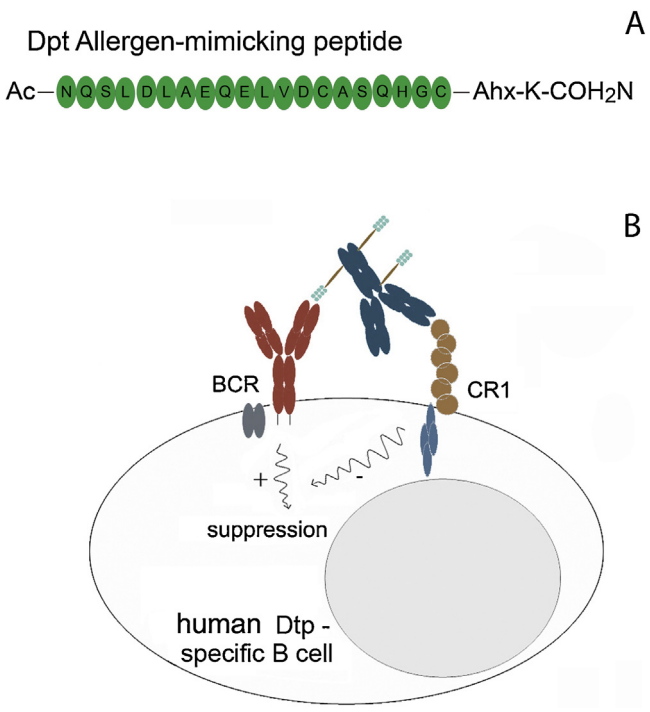

Fig. 1. Receptor co-crosslinking by a protein engineered chimeric molecule. (A) Dpt allergen-mimicking peptide. (B) Schematic view of receptor co-crosslinking.

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-NQSLDLAEQELVDCASQHGC-Ahx-K-NH_2) from Der p 1 and an irrelevant peptide (Ac-DEACLQCGSEDHQAVQNLLS-Ahx-K-NH_2), 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