Ph. D. Thesis

Screening of autoantibody profile and B cells' epitopes by citrullinated peptides in rheumatoid arthritis and study the patomechanism in the animal model of the disease

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Introduction

Rheumatoid arthritis (RA) is a chronic, autoimmune disorder characterized by inflammation and destruction of the joints caused by self-specific pathological immune response against certain synovial proteins. The tendency to develop rheumatoid arthritis is genetically inherited, additionally; several environmental factors (e.g. smoking), hormonal effects and infectious agents are involved in the development of the disease. There is an unmet need for novel diagnostic possibilities and disease markers that should potentiate early diagnosis and help to find the relevant treatment. Anti-citrullinated protein autoantibodies (ACPA) are specific and sensitive markers for RA, however the commercially available test kits do not cover the entire spectrum of relevant autoantibodies.

Due to its similar immunological characteristics involving high level of auto-antibodies and collagen specific T-cells collagen-induced arthritis (CIA) obtained by immunizing DBA/1 mice with bovine type II collagen (CII) is a widely used model of human rheumatoid arthritis (RA). In this arthritis model anti-CII antibodies are regarded as important factors in the development of arthritis, as the transfer of sera from diseased

mice can induce CIA in healthy DBA/1 recipients. IgG containing immune complexes that can bind to FcγR are crucial players in the pathogenesis of arthritis; as they were shown to have a regulatory role in both the central and the effector phase of CIA.

Aims

Our aims were:

A, to investigate citrulline and arginine containing synthetic peptides corresponding to short sequences of known proteins playing a role in RA in order to find new diagnostic possibilities, and to identify new, citrulline containing peptides that could be useful not only in the diagnosis but also applicable to develop personalized therapy in the future

B, to reveal the role of immunocomplexes in the setting and on the course of the disease in the CIA model.

Questions, A:

- Are the citrulline containing peptides derived from filaggrin, vimentin and collagen appropriate to determine the autoantibody profile, and whether applying the citrulline-peptide panel would provide

additional information compared to the available diagnostic kits?

- Do the autoreactive B-cell receptors recognize the previously identified citrulline containing peptides?
- Are these citrulline-containing peptides appropriate for testing autoantibody production in vitro?

B, Our further aim was to analyze role of immunocomplex-mediated processes in the CIA model. We had four groups of tested mice: the non-treated CIA group, the collagen (CII) peptid tetramer treated group, the FcγRII/III specific, 2.4G2 single-chain antibody tetramer treated group, and the 'immunocomplex' (CII-peptide - 2.4G2) treated animals.

Questions, B:

- Is there any difference between various groups of mice treated with antibody or peptide tetramers, or with "immunocomplexes" regarding the onset and the course of the disease?
- Which cell types bind the complexes *in vitro* and *in vivo*?
- How the complexes influence the collagen-specific and collagen-peptide-specific antibody production?

- How the cytokine and chemokine secretion is modified upon treatments?

Methods

- Multipin ELISA, indirect ELISA, competitive ELISA
- ELISpot
- purification of human B-cells
- flow cytometry (FACS)
- induction of CIA
- preparation of recombinant protein
- confocal microscopy
- cytokine array

Results

- Our citrulline-peptide panel can detect RA patient diagnosed as CCP negative with commercial tests.
- We have shown that there is a cross-reaction between vimentin protein and filaggrin peptide, however, the vimentin epitope peptide does not cross-react with the filaggrin epitope.

- With our citrulline-peptide-panel, autoreactive B-cells are detectable by flow cytometry.
- The citrulline-peptide-panel is also applicable for measuring *in vitro* autoantibody production.
- Citrulline-peptide stimuli might induce cytokine production.
- Examining the CIA model, we observed that CII peptide containing artificial immunocomplexes accelerated the disease and elevated disease scores; furthermore, also elevated the level of collagen peptide specific IgG2a and enhanced the production of various inflammatory cytokines and chemokines.
- We have shown that inflammatory IgG2a containing immunocomplexes have major role in maintenance of inflammation and trigger positive feedback of autoreactivity.

Discussion

We have characterized a citrulline-peptide-panel containing filaggrin, vimentin and collagen peptides that has similar sensitivity and specificity values then commercially available tests. Furthermore, the panel is able to detect almost 60% of patients from the CCP negative group. The numbers of recognized peptides has shown a strong association with CCP2 and MCV titers. We showed that antibodies reacting with the filaggrin peptide do not cross-react with the immunodominant epitope of vimentin; indicating that the autoimmune response is polyclonal, however, the MCV protein-specific antibodies do recognize the filaggrin peptide. With this citrulline peptide panel we are able to detect autoreactive B cells by flow cytometry and also peptide specific antibody producing cells via ELISpot. PBMC from RA patients treated with citrullinated peptides in vitro secreted pro-inflammatory cytokines such as IFNγ and IL-17 in about 50 % of cases, while cells from an other group of patients produced anti-inflammatory IL-10. Taking all together these data, we conclude that the citrulline peptide panel is applicable for diagnostic purpose, and may be applied to develop personalized therapy in the future.

The collagen induced arthritis (CIA) in mice is one of the best models for the human disease. We have studied the effect of complexes composed of collagen peptide and FcγRII/III specific single chain antibody 2.4G2. In the four experimental groups (2.4G2 scFv-CII-peptide complexes, CII-peptide 'tetramer's or 2.4G2 scFv 'tetramer's) of mice, surprisingly, we found that all molecular constructs aggravated disease activity. FcγRII/III targeted CII-peptide complexes enhanced the synthesis of the inflammatory IgG2a isotype, which is associated with a Th1 response. We suggest that 2.4G2 scFv-CII-peptide complexes and the *in vivo* formed IgG2a -CII-peptide immunocomplexes may bind to FcγRIII and/or to FcγRIV, inducing the secretion of IL-23, which in turn trigger Th17 cells. Taking all together these data indicate that the administration of FcγRII/III targeted CII-peptide complexes into collagen primed DBA/1 mice enhances the inflammatory Th1 driven IgG2a response. Thus we conclude that inflammatory IgG2a containing immune complexes have major role in maintenance of inflammation and trigger positive feedback of autoreactivity.

Publications

E. Szarka, F. Babos, A. Magyar, K. Huber, Z. Szittner, K. Papp, J. Prechl, J. Pozsgay, G.Nagy, B. Rojkovich, T. Gáti, J Kelemen, Z. Baka, M. Brózik, B. Pazár, G. Poór, F. Hudecz and G. Sármay

Recognition of new citrulline containing peptide epitopes by autoantibodies produced *in vivo* and *in vitro* by B cells of Rheumatoid arthritis patients **IMMUNOLOGY** (2013) 141(2):181-91. IF: 3.705

F. Babos, E. Szarka, G. Nagy, Z. Majer, G. Sármay, A. Magyar, F. Hudecz

The role of *N*- or *C*-terminal biotinylation in antibody recognition of citrullin containing filaggrin epitope peptides in Rheumatoid arthritis **BIOCONJUGATE CHEMISTRY 24:** (5), *pp 817–827* (2013) IF: 4.58

Szarka E*, Neer Z*, Balogh P, Adori M, Angyal A, Prechl J, Kiss E, Kovesdi D, Sarmay G

Exacerbation of collagen induced arthritis by Fcgamma receptor targeted collagen peptide due to enhanced inflammatory chemokine and cytokine production.

***BIOLOGICS: TARGETS & THERAPY* 6:** *pp. 101-115.* (2012) (*: megosztott első szerzők)

Baka Z, Barta P, Losonczy G, Krenacs T, Papay J, Szarka E, Sarmay G, Babos F, Magyar A, Geher P, Buzas EI, Nagy G
Specific expression of PAD4 and citrullinated proteins in lung cancer is not associated with anti-CCP antibody production ***INTERNATIONAL IMMUNOLOGY* 23:**(6) *pp. 405-414.* (2011) IF: 3.301

Harre U, Georgess D, Bang H, Bozec A, Axmann R, Ossipova E, Jakobsson PJ, Baum W, Nimmerjahn F, Szarka E, Sarmay G, Krumbholz G, Neumann E, Toes R, Scherer HU, Catrina AI, Klareskog L, Jurdic P, Schett G
Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. ***JOURNAL OF CLINICAL INVESTIGATION* 122:**(5) *pp. 1791-1802.* (2012) IF: 14.152