PhD thesis

Investigation of the adaptive immune response in immune-mediated diseases

Sonja Dulic MD

Tutor: Attila Balog MD, PhD

University of Szeged

Doctoral School of Interdisciplinary Medicine

Faculty of Medicine

Department of Rheumatology and Immunology

Szeged, 2018

Introduction

Rheumatoid arthritis (RA), ankylosing spondylitis (AS) and inflammatory bowel diseases (IBDs) are chronic immune-mediated diseases, in which the pathogenic role of tumor necrosis factor-α (TNF-α) can be highlighted, and consequently it is a therapeutic target too. Anti-TNF therapies significantly improve the clinical course of all three diseases. Their mechanism of action is not well characterized, and therefore there is a need for the identification of biomarkers predicting their primary or secondary inefficacy.

The balanced functionality of the adaptive immune system is fundamentally determined by the proportion and activity of T-lymphocyte subtypes. TNF-blockers have an impact on the composition of the T-cell subsets, as examined by several research groups; mainly focusing on the various effector T-helper cell lines. After the analyses of a wide range of T-lymphocyte subtypes on RA and AS patients with short-term anti-TNF treatment performed by our group, now we are comparing our data with those obtained during long-term follow-up, and we are also

complementing them with the investigation of IBD patients, another disease implicated with alterations of the adaptive immune system. This extension is feasible based upon the frequent association of IBDs with AS and other diseases within the spondyloarthritis spectrum, and upon the key role of T-cells in this disease too; furthermore, in view of the lifespan of the lymphocytes, it can be hypothesized that the detection of definitive changes requires significantly longer follow-up. From our previous results with short follow-up, we can highlight the normalization tendency of the prevalence of regulatory T-cells (Tregs) – its further study characterizes the mechanisms of the changes of the T-cell repertoire, the definite character of which develops in the long-term. We will also follow the course of the proportion of memory and activated T-cell subsets, whose function is significant for the appropriate function of the immune system; its importance is established on the assessment of the consequences of long-term biological therapies such as their inductive role in the development of malignancies or their relationship with susceptibility to infections.

Aims

1. The analysis of the T-cell repertoire in RA, AS and IBD before anti-TNF – and in RA, interleukin 6-receptor (IL6-R) blocker – therapy

2. The comparison of these experimental results with those obtained after long-term – at least three months' – biological therapy

3. The correlation of the T-cell phenotype and its changes with the clinical picture – in a disease-specific manner

4. The search for predictive biomarkers that – measured before the initiation of biological therapy – could inform about a subsequent therapeutic responsiveness

Patients

Rheumatoid arthritis

Ninety-two RA patients participated in the study, 49 of whom received anti-TNF therapy and 43 were treated with IL6-R blocker. The anti-TNF group was further divided into responder and non-responder subgroups following the EULAR response criteria. All the IL6R-blocker-treated patients were responders. Longitudinal follow-up was performed in 13 patients, starting from the biologic-naive state. As healthy controls, 30 age- and sex-matched volunteers were selected, and for disease controls, 19 newly diagnosed RA patients.

Ankylosing spondylitis

Twenty-two AS patients were involved, whose diagnosis met the modified New York criteria. Similarly to the RA study, responder (n=15) and non-responder (n=7) subgroups were distinguished following the ASAS response criteria. The healthy control group comprised 10 volunteers.

Inflammatory bowel diseases

Similarly to RA, we have applied both cross-sectional and longitudinal study designs. We have analyzed the samples of a total of 114 patients as described below. We have evaluated Crohn's disease (CD) and ulcerative colitis (UC) patients separately; as in our previous studies, responder and non-responder groups have been defined in this case too. We have prospectively followed the clinical course of UC and CD patients, 16 with each diagnosis, after the initiation of anti-TNF therapy. Repeat laboratory examinations were performed in 6 cases in each disease, after at least three months of biological therapy. In the cross-sectional part, the data of 31 CD and 16 UC patients were compared, separated according to the therapeutic response to anti-TNF. The control groups contained healthy subjects (n=30), therapy-naive patients with active disease (n=7 for both UC and CD), and patients in remission on conventional treatment (n CD=14, n UC=7).

In all three diseases, the most important demographic data, disease-specific indices, co-morbidities, laboratory and other diagnostic parameters were recorded.

Laboratory methods

We have used 15 ml of anticoagulated blood and analyzed it with flow cytometry. The following T-cell subtypes were distinguished with cell surface staining: helper T (CD4+), Th1 (CD4+CXCR3+), Th2 (CD4+CCR4+), Th17 (CD4+CCR4+CCR6+), Treg (CD4+CD25highCD127-), and naive (CD4+CD45RA+) or memory T-cells (CD4+CD45RO+). Furthermore, activated T-cells bearing various markers indicating the stage of activation – early: CD69, late: HLA-DR, intermediate: CD25 – have also been identified.

Results

Rheumatoid arthritis

During long-standing biological therapy – independent of its class – the proportion of Tregs normalizes, the prevalence of naïve CD4 and CD8 lymphocytes decreases, and that of the memory cells increases. The frequency of Th2 and Th17 cells becomes higher, most markedly in the IL6-R blocker-treated patients. Among the activated subsets, the frequency of lymphocytes bearing the late activation marker increases in the anti-TNF- and anti-IL6-R-responders, whereas decreased prevalences of CD8 T-cells with the early activation marker and of CD4CD25 subtype can be observed in both anti-TNF subgroups as compared to controls. In the IL6-R blocker-treated patients, the proportion of CD8 cells is lower than in all other patient subgroups and in the healthy population, whereas that of the CD8CD69 is higher than in all treated patients or healthy controls.

If the percentage of CD4CD69 lymphocytes at the initiation of anti-TNF is lower than 2.13%, this will predict a therapeutic response with a sensitivity of 71.4% and a

specificity of 83.3% (likelihood ratio: 4.29). The frequency of CD4CD69 and of CD8CD69 cells was higher in those anti-IL6-R-treated patients, in whom previously more anti-TNF switches had to be performed because of therapy inefficacy.

Ankylosing spondylitis

The proportion of naïve CD4 and CD8 cells was markedly lower also in this inflammatory rheumatic disease than in the healthy control group. The ratio of Tregs has normalized (similarly to RA), whereas the prevalence of Th1 and Th17 lymphocytes increased and became higher than in the healthy controls. In the anti-TNF-non-responders, the Th2 percentage was also increased as compared with the healthy population. The frequency of late activated cells was higher in responders than in the healthy subjects. When compared with our previous results with short-term follow-up, we have experienced that the above-mentioned alterations develop only after three months.

Inflammatory bowel diseases

Comparison between patients with active IBD and healthy subjects has revealed multiple differences in their immunophenotype: the prevalence of Th2, Th17, HLA-DR- and CD69-positive cells is higher, while that of naïve T-cells is lower in the IBD patients. The frequency of CD8CD69 cells in CD was higher in CD patients with newly-diagnosed active disease than in those with inactive disease treated with non-biological therapies. We have noted much less changes in the distribution of T-cell subsets during anti-TNF treatment than in the inflammatory rheumatic diseases. The CD4 percentage was lower in responder UC patients than in the non-responders. The CD8 memory cell ratio was higher in non-responder UC patients as compared with the anti-TNF naïve patients. During the prospective study, the percentage of CD8CD69 cells has decreased much more by the time of the second blood sampling in the responder UC patients than in the non-responder ones. Several cell subtype markers can be associated with the therapeutic responsiveness and/or to the disease activity. A CD4

memory subset ratio of <49.05% predicts a good therapeutic efficacy of the biological treatment in CD. The duration of the remission was directly proportional to the prevalence of CD4HLA-DR in CD, and with that of the Th2 and Th17 lymphocytes in UC.

Summary

In all three diseases, we have performed the most detailed exploration of the distribution and alterations of T-cell subsets so far. We have correlated the changes observed during biological therapies with the particular clinical presentation.

We have revealed several similarities between the investigated inflammatory rheumatic diseases, while the alterations that developed in the IBD group were less suitable to be linked with the observed autoimmune alterations. We explain it with the predominance of mucosal immune reactions, which can be detected less markedly in the peripheral blood.

The adaptive immune system reflects the disease-specific characteristic activated features (the proportion of certain effector T-helper and activated T-lymphocyte subgroups remains high, the ratio of naïve cells decreases) even after the disease has entered a long-standing quiescent state with biological treatment. However, it should be emphasized that the Treg prevalence normalizes in inflammatory rheumatic diseases during biological therapy, that is, it will be equal to values measured in the healthy controls.

We have found particular cell subtypes, especially in RA and IBD, whose prevalence carries prognostic significance, and therefore they could be used as important biological markers in the choice of a personalized therapy.

Acknowledgements:

I am grateful to my tutor, Attila Balog MD, PhD for his continuous and altruistic professional help, for his encouragement, guidance and constructive critiques, and to László Kovács MD, PhD for the organization of the unimpeded work activities. Furthermore, I thank Gergely Toldi MD, PhD and the members of his team, in particular Sava Florentina PhD, Zsófia Vásárhelyi and László Berta, and also Szilvia Pördi for their assistance in the experiments and co-authorship. I also thank Professor Tamás Molnár MD, PhD, DSc and his colleagues for the facilitation of the organization of the examinations of the IBD patients. I am indebted to my family, most importantly to Ádám Tepper JD, Katalin Dulic, Gavro Dulic and Ágnes Tepper JD for their perpetual support and encouragement.

Publications used in the thesis

I. Dulic S, Vasarhelyi Z, Sava F, Berta L, Szalay B, Toldi G, Kovacs L, Balog A. T-Cell Subsets in Rheumatoid Arthritis Patients on Long-Term Anti-TNF or IL-6 Receptor Blocker Therapy. MEDIATORS OF INFLAMMATION 2017: Paper 6894374. 19 p. (2017) **IF: 3,549**

II. Dulic S, Vasarhelyi Z, Bajnok A, Szalay B, Toldi G, Kovacs L, Balog A. The Impact of Anti-TNF Therapy on CD4+ and CD8+ Cell Subsets in Ankylosing Spondylitis. PATHOBIOLOGY 85:(3) pp. 201-210. (2018) **IF: 1,592**

Citeable conference abstracts used in the thesis

Dulic S, Toldi G, Vásárhelyi Z, Balog A. The impact of Biologic Therapies on CD4+ and CD8+ Cell Subsets in Rheumatoid Arthritis: A Long Term FollowUp Study. ANNALS OF THE RHEUMATIC DISEASES 75:(Suppl. 2.) pp. 182-183. (2016)

Dulic S, Vásárhelyi Z, Bajnok A, Szalay B, Toldi G, Kovács L, Balog A. Analysis of the T-cell subset

composition in ankylosing spondylitis patients with long-standing anti-TNF therapy. ANNALS OF THE RHEUMATIC DISEASES 76:(Suppl.2) p. 780. 1 p. (2017)

Publications not used in the thesis

Gál B, Dulic S, Kiss M, Groma G, Kovács L, Kemény L, Bata-Csörgő Z. Increased circulating anti-α6-integrin autoantibodies in psoriasis and psoriatic arthritis but not in rheumatoid arthritis JOURNAL OF DERMATOLOGY 44:(4) pp. 370-374. (2017) **IF: 2,788**

Kern A, Balog A, Dulic S, Barabas E, Kiszelak M, Vasarhelyi B. Alterations of the thrombin generation profile in rheumatoid arthritis. JOURNAL OF THROMBOSIS AND THROMBOLYSIS 41: pp. 359-364. (2016) **IF: 2,142**

Rosztoczy A, Izbeki F, Nemeth IB, Dulic S, Vadaszi K, Roka R, Gecse K, Gyokeres T, Lazar G, Tiszlavicz L, Wittmann T. Detailed esophageal function and morphological analysis shows high prevalence of gastroesophageal reflux disease and Barrett’s esophagus

in patients with cervical inlet patch. DISEASES OF THE ESOPHAGUS 25:(6) pp. 498-504. (2012) **IF: 1,642**