Expression of interferon-stimulated genes in patients with rheumatoid arthritis on anti-B-cell therapy (preliminary results)

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Objective: to evaluate the expression of interferon-stimulated genes (ISG) — interferon (IFN) signature — in patients with rheumatoid arthritis (RA) and its dynamics during anti-B-cell therapy.

Patients and methods. We examined 20 patients with RA who received two infusions of the biosimilar rituximab (RTM) Acellbia* in a total dose of 1200 mg. Five genes were selected to evaluate IFN signature: IFI44L, MX1, IFIT1, RSAD2, EPSTI1. The expression of IFI44L and IFIT1 could not be determined for technical reasons, and further analysis included three genes — MX1, EPSTI1, RSAD2. IFN signature was calculated as the average value of the expression of three selected genes (IFN-score).

Results and discussion. The initial expression level of MX1 was 11.48 (5.45–19.38), EPSTI1 — 12.83 (5.62–19.64), RSAD2 — 5.16 (2.73–10.4) and IFN-score — 10.3 (5.18–17.12), in patients with RA it was statistically significantly higher than in healthy donors: 1.26 (0.73–1.6); 1.06 (0.81–1.48); 0.93 (0.72–1.19) and 1.09 (0.92–1.42), respectively (p<0.05). The IFN-score was high in 15 (75%) patients, low in 5 (25%). The use of RTM was accompanied by a statistically significant decrease in disease activity and the level of acute phase parameters (ESR, CRP) after 12 and 24 weeks of therapy (p<0.05). In the group as a whole, as well as in patients with a moderate effect of therapy or its absence, by the 24th week of treatment, an increase in the expression of RSAD2 (p<0.05) and a tendency to an increase in the IFN-score level (p=0.06) were observed.

Conclusion. In patients with RA, an increased expression of ISH was found compared to healthy donors. An increase in the expression of RSAD2 and IFN-score is observed both in patients with a satisfactory effect of RTM and with no effect. The obtained results can be important for predicting the course of the disease and personalizing therapy.

Key words: rheumatoid arthritis; interferon-stimulated genes; interferon signature; disease activity; the effectiveness of therapy.

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Rheumatoid arthritis (RA) belongs to a broad class of immune-mediated inflammatory rheumatic diseases (IIRDs), which stem from abnormal immunological tolerance to one’s own tissues leading to inflammation and irreversible organ damage [1]. Lately, it has been proposed that an important role in the pathogenesis of IIRDs is played by the so-called interferonopathies, i.e., abnormal regulation of type I interferon (IFN) production. The assessment of these abnormalities may be useful for determining the clinical disease phenotypes and predicting treatment outcomes.

IFNs are a group of molecules with pleiotropic effects on the immune system, which ensure the interaction between innate and adaptive immune responses [2, 3]. There are type I, II and III IFNs with different properties and structures, produced by different cells [3]. Type I IFN is the largest group, which includes IFNα, β, ω, ε, κ; the best known are IFNα and β. Type II IFNs include IFNγ; type III IFNs include IFNγ1, γ2, γ3, γ4. Type I and III IFNs activate intracellular signaling pathways mediating antiviral and anti-tumor immune response [2–5]. Type I IFNs are primarily produced by plasmacytoid dendritic cells (DCs) [3]. Plasmacytoid DCs produce type I IFNs after the interaction of virus antigens or endogenous nucleic acids with pattern recognition receptors (PRR) or toll-like receptors (TLR), predominantly type 7 or 9 [6]. Type I IFNs act on all nucleated cells for the inhibition of viral replication and have immune-stimulating properties, including those related to myeloid DC maturation induction and activation, Th1 polarization of the immune response, B cell activation, antibody production and immunoglobulin class switching [7–10]. Type I IFN activity is usually assessed based on the expression of IFN-stimulated genes (ISG), which is called IFN-signature [7–9, 11]. Unlike type I IFNs, type II IFNs induce the expression of other genes primarily produced by NK cells and certain T cell subpopulations. The main role of type II IFN is to regulate certain aspects of the immune reaction: phagocytosis and antigen presentation [11].

The importance of type I IFN hyperproduction in the pathogenesis of IHRDs was confirmed both in laboratory animal models of rheumatic diseases (RDs) and in patients with hereditary monogenic disorders with a specific inflammatory phenotype, which, in 2011, were proposed to be merged into a group of congenital type I interferonopathies [12–14].

In patients with RA, type I IFNs can potentially become prognostic biomarkers of response to biological therapy. A series of papers demonstrated that a low expression of the type I IFN...
Table 1. Clinical and immunological characteristics of RA patients (n=20)

| Parameter                  | Value                        |
|----------------------------|------------------------------|
| Sex, n (%): males/females  | 2 (10)/18 (90)               |
| Age, years, Me [25th; 75th percentile] | 61.5 [54.0; 66.5]               |
| Disease duration, months, Me [25th; 75th percentile] | 39.5 [20.0; 84.0]               |
| Radiographic stage, n (%):   |                              |
| I                          | 2 (10)                       |
| II                         | 13 (65)                      |
| III                        | 4 (20)                       |
| IV                         | 1 (5)                        |
| PK, n (%):                  |                              |
| I                          | 4 (20)                       |
| II                         | 11 (55)                      |
| III                        | 5 (25)                       |
| IV                         | 0                            |
| DAS28, Me [25th; 75th percentile] | 5.6 [4.9; 6.8]               |
| HAQ, Me [25th; 75th percentile] | 1.7 [1.2; 2.3]               |
| ESR (Westergren), mm/h, Me [25th; 75th percentile] | 45.0 [19.5; 80.0]               |
| CRP, mg/mL, Me [25th; 75th percentile] | 12.3 [8.9; 42.5]               |
| IgM RF, IU/mL, Me [25th; 75th percentile] | 197.0 [83.2; 492.5]              |
| IgM RF, n (%): positive     | 18 (90)                      |
| negative                   | 2 (10)                       |
| ACCPA, U/ml, Me [25th; 75th percentile] | 161.8 [98.3; 300.0]              |
| ACCPA positive, n (%)       | 20 (100)                     |

Note. HAQ = Health Assessment Questionnaire.

system prior to rituximab (RTM) therapy is associated with high efficacy of this drug [15, 16].

The goal of this study was to evaluate ISG expression in RA patients and its changes with anti-B cell therapy.

Patients and methods. A total of 20 patients with a confirmed RA diagnosis according to ACR/EULAR criteria (American College of Rheumatology/European Alliance of Associations for Rheumatology 2010) followed up at V.A. Nasonova Research Institute of Rheumatology were investigated, most of whom were female, middle-aged, with long-term disease (median, Me 39.5 months), with positive IgM rheumatoid factor (RF) and anticyclic citrullinated peptide antibodies (ACCPA), high inflammatory activity, radiographic stage II or III, functional class (FC) II, and moderate disability status (Table 1).

Prior to starting anti-B cell therapy, patients received methotrexate (MTX) at stable and moderate disability status (Table 1). Prior to starting anti-B cell therapy, radiographic stage II or III, functional class (FC) II, cyclic citrullinated peptide antibodies (ACCPA), high inflammatory activity, were considered statistically significant at p<0.05.

Results. Prior to RTM therapy, DAS28 (5.6 [4.9; 6.8]), SDAI (27.17 [23.08; 39.9]) and CDAI (26.6 [22.25; 37]) scores indicated high RA activity. A reduction in disease activity was observed at 12 and 24 weeks of therapy (p<0.05; Table 2). At week 24 of RTM therapy, a good response according to EULAR criteria was observed in 5 patients, a moderate response in 12 patients and no response in 3 patients; DAS28 remission (<2.6) was achieved in 4 (20%) patients; SDAI remission (<3.3) was achieved in 2 (10%) patients; CDAI remission (<2.8) was achieved in 1 (5%) patient. At week 12 of the study, 20% improvement in ACR was observed in 70% of patients, ACR50 in 55% of patients, ACR70 in 5% of patients; CDAI remission (≤2.8) was achieved in 1 (5%) patient. At week 24 these parameters were 75, 45 and 15%, respectively.

Baseline expression levels in RA patients were 11.48 [5.45; 19.38] for MX1, 12.83 [5.62; 19.64] for EPSTI1, and 5.16 [2.73; 10.4] for RSAD2 and were statistically significantly higher than in healthy donors: 1.26 [0.73; 1.6], 1.06 [0.81; 1.48] and 0.93 [0.72; 1.19], respectively (p<0.05). The IFN score in RA patients was statistically significantly higher than in healthy donors: 1.26 [0.73; 1.6], 1.06 [0.81; 1.48] and 0.93 [0.72; 1.19], respectively (p<0.05). The IFN signature was detected in 15 (75%) patients and was not different from healthy donors in 5 (15%) patients.
In the group in general, and in patients with moderate or lack of treatment efficacy in particular, an increase in RSAD2 expression (p<0.05) and a trend of increasing IFN score (p=0.06) were observed at Week 24 of therapy. Changes in expression were statistically significant in patients with good response to therapy which is likely related to the small number of patients in this group (p>0.05; Table 2).

In patients with no IFN signature (n=5), the reduction in disease activity was more pronounced at Week 24 than in the group of patients with IFN signature: ΔDAS28 = 3.45 [2.94; 3.69] and 1.02 [0.5; 2.02], respectively (p<0.05). All patients who did not respond to therapy had an increased ISG expression.

Discussion. These results indicate a higher ISG expression in RA patients compared with healthy donors: IFN signature was detected in 75% of these patients. Literature data suggest that IFN signature is detected in the peripheral blood of more than 50% of RA patients and can also be found at the preclinical stage of the disease [18, 19]. The relative ISG expression in RA patients is lower compared with patients with systemic lupus erythematosus (SLE) or other IIRDs [20]; however, certain genes associated with increased activation of type I IFN system in SLE (IRF5, IRAK1, STAT4 and PTPN22) are also associated with a risk of RA [20]. Identification of a specific polymorphism correlates with the risk of some RDs, which may suggest that a large group of disorders may share the same pathogenetic mechanisms [20].

The IFN signature can be a potential predictive marker of response to biological therapy. Several studies demonstrated that a low expression of the type I IFN system prior to RTM therapy is associated with a high efficacy of this treatment [15, 16]. R.M. Thurlings et al. [15] analyzed the expression of type I IFN in two cohorts of RA patients treated with RTM (n=20 and n=31, respectively). Depending on the level of type I IFN expression in mononuclear cells, all patients were divided into two groups: with high and low IFN levels. A more pronounced reduction in the disease activity according to DAS28 was observed in the group with low IFN levels, and response to RTM therapy according to EULAR criteria was also observed more frequently in these patients. The authors concluded that there is an inverse relationship between the efficacy of RTM therapy and type I IFN expression. Similar results were obtained by H.G. Raterman et al. [16], who investigated the expression of several genes (LY6E, HERC5, IFI44L, ISG15, MxA, MxB, EPSTI1 and RSAD2) in the peripheral blood of RA patients using real-time PCR. ROC-analysis showed that efficacy of RTM therapy can be predicted with a probability of 87% using the baseline expression of the genes associated with type I IFN system (authors proposed several gene combinations: EPSTI1, RSAD2 and MxA; HERC5, RSAD2, MxA and LY6E; HERC5, IFI44L, EPSTI1, RSAD2, MxA and LY6E).

We obtained similar evidence of an inverse correlation of the IFN signature level with the efficacy of RTM therapy: in the absence of IFN signature, a more pronounced reduction in the disease activity was observed at week 24 of therapy compared with IFN signature presence: ΔDAS28 3.45 [2.94; 3.69] and 1.02 [0.5; 2.02], respectively (p<0.05). In the group of patients with moderate efficacy of RTM therapy or lack of efficacy, an increase in ISG expression was observed at Week 24, whereas changes in this parameter were not statistically significant in patients with a good response to RTM therapy.

Assessment of IFNα/IFNβ ratio may be useful for predicting the efficacy of tumor necrosis factor α inhibitors [21, 22]. T. Wampler Muskardin et al. [22] demonstrated that a higher baseline level of IFNβ was associated with lack of response to therapy (p=0.013). According to ROC-analysis results, an IFNβ/IFNα ratio >1.3 allows to predict lack of response to therapy (odds ratio 6.67; p=0.018) with a sensitivity of 77% and specificity of 45%.

The causes of the differences in IFNα/IFNβ ratios in the bloodstream are not known. IFNα predominates in SLE, whereas IFNβ predominates in RA [22, 23]. This phenomenon remains unclear, especially considering various anti-inflammatory effects of IFNβ and the lack of improvement with recombinant IFNβ therapy in RA patients, as well as worse response to TNFα inhibitors in patients with higher levels of this type of IFN. Considering the complex regulation of type I IFN signaling, it can be assumed that IFNβ effects likely depend on the amount, duration, location of activity (peripheral blood or tissues) and other factors.

Conclusions. Thus, the foregoing results suggest an increased ISG expression in patients with RA compared with healthy donors. IFN signature assessment may help to predict the efficacy of treatment with genetically engineered biological drugs and develop personalized management strategies. However, further studies are needed in different patient groups for a better understanding of the role of type I IFN system in the pathogenesis of RA.

Table 2. Dynamics of disease activity and ISH expression during RTM therapy, Me [25; 75th percentile]

| Parameter | Overall group | Moderate response/ no response at Week 24 (n=15) | Good response at Week 24 (n=5) |
|-----------|--------------|-----------------------------------------------|-------------------------------|
| DAS28     | baseline     | 5.6 [4.9; 6.8]                               | 5.6 [5.2; 6.57]               |
|           | 12 weeks    | 4.28 [3.24; 4.75]                            | 4.4 [3.5; 5.05]*              |
|           | 24 weeks    | 4.14 [3.11; 4.66]                            | 4.47 [3.8; 4.8]*              |
| ESR, mm/h | baseline     | 45.0 [19.5; 80.0]                             | 50.6 [14.0; 87.6]             |
|           | 12 weeks    | 20.0 [16.0; 38.0]*                            | 22.0 [18.0; 40.0]*            |
|           | 24 weeks    | 21.5 [12.0; 31.0]*                            | 28.0 [14.0; 36.0]*            |
| CRP, mg/L | baseline     | 12.3 [8.9; 45.2]                              | 14.4 [9.2; 46.0]              |
|           | 12 weeks    | 4.9 [2.2; 11.3]*                              | 5.7 [2.4; 13.3]*              |
|           | 24 weeks    | 4.9 [2.3; 21.9]*                              | 10.4 [2.7; 24.1]*             |
| EPSTI1    | baseline     | 2.38 [1.5; 6.2]                               | 13.1 [5.4; 19.9]              |
|           | 12 weeks    | 18.3 [5.6; 19.6]                              | 13.7 [5.4; 19.9]              |
|           | 24 weeks    | 14.3 [3.3; 43.9]                              | 10.4 [2.9; 45.9]              |
| RSAD2     | baseline     | 5.16 [2.73; 10.4]                             | 5.12 [2.3; 9.7]               |
|           | 12 weeks    | 14.97 [5.04; 42.1]*                           | 14.6 [13.3; 43.9]*            |
|           | 24 weeks    | 11.40 [5.15; 19.58]                           | 10.6 [5.3; 18.8]              |
| MX1       | baseline     | 12.40 [3.4; 49.1]                             | 10.4 [3.2; 80.4]              |
|           | 12 weeks    | 10.3 [5.18; 17.12]                            | 11.7 [7.4; 18.9]              |
|           | 24 weeks    | 16.5 [5.05; 55.8]                             | 14.8 [2.8; 60.8], p<0.06      |

*p<0.05 versus baseline; *p=0.05 for moderate response/no response versus good response groups
ORIGINAL INVESTIGATIONS

REFERENCES

1. Nasonov EL, Karateev DE, Balabanova RM. Rheumatoid arthritis. In: Nasonov EL, Nasonova VA, editors. Rheumatology. National Academic Publishing. Moscow: Egor-Media; 2008. C. 290-331.

2. Nasonov EL, Avdeeva AS. Immunomodu- latory autoimmune disorders, associated with interferon type I: new data. Scientific Practice Rheumatology. 2019;57(4):452-61.

3. Nasonov EL, Avdeeva AS. Immuno- inflammatory Rheumatic Diseases Associated With Type I Interferon: New Evidence. Naucho-prakticheskaya revmatologiya. 2019;57(4):452-61. (In Russ.).

4. De Weer ND, Nguyen T. The interferons and their receptors—distribution and regulation. Immunol Cell Biol. 2012 May;90(3): 483-91. doi: 10.1038/icb.2012.9.

5. Kotenko SV, Gallagher G, Baurin VV, et al. IFN-λs mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol. 2003 Jan;4(1):69-77. doi: 10.1038/nej.2012.9.

6. Ank N, West H, Bartholdy C, et al. Lambda interferon (IFN-λs) and type III IFN, is induced by viruses and IFNs and displays potent antiviral activity against some virus infections in vivo. J Virol. 2006 May;80(9): 4501-9. doi: 10.1128/JVI.009.4501- 4509.2006.

7. Honda K, Takaoka A, Taniguchi T. Type I interferon gene induction by the interferon regulatory factor family of transcription factors. Immunity. 2006 Sep;25(3):349-60. doi: 10.1016/j.immuni.2006.08.009.

8. Jego G, Palucka AK, Blanck JP, et al. Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. Immunity. 2003 Aug; 19(2):225-34. doi: 10.1016/s1074-7613 (03)00208-5.

9. Longhi MP, Trumpelher C, Idojaga J, et al. Dendritic cells require a systemic type I interferon response to mature and induce CD4+ Th1 immunity with poly IC as adjuvant. J Exp Med. 2009 Jul 6;206(7):1589-602. doi: 10.1084/jem.20090247.

10. Epub 2009 Jun 29.

11. Green DS, Young HA, Valencia JC. Current prospects of type II interferon signaling and autoimmunity. J Biol Chem. 2017 Aug 25:2929(4):13925-33. doi: 10.1074/jbc.R117.774745. Epub 2017 Jun 26.

12. Le Bon A, Thompson C, Kamphuis E, et al. Cutting edge: enhancement of antibody responses through direct stimulation of B and T cells by type I IFN. J Immunol. 2006 Feb 15;176(4):2074-8. doi: 10.4049/jimmunol.176.6.2074.

13. Prazsar A, Emery P, Vital EM. Type I interferon-mediated autoimmune diseases: pathogenesis, diagnosis and targeted therapy. Rheumatology (Oxford). 2017 Oct 1;56(10): 1662-75. doi: 10.1093/rheumatology/kew431.

14. Crow YJ. Type I interferonopathies: a novel set of inborn errors of immunity: type I interferonopathies. Ann N Y Acad Sci. 2011 Nov;1238:91-8. doi: 10.1111/j.1749-6632.2011.06220.x.

15. Ioannou Y, Isenberg DA. Current evidence for the induction of autoimmune rheumatic manifestations by cytokine therapy. Arthritis Rheum. 2000 Jul;43(7):1431-42. doi: 10.1002/1529-0131(200007)43<1431::AID-ANR3>3.0.CO;2-E.

16. Thrulings RM, Boumans M, Tekstra J, et al. Relationship between the type I interferon signature and the response to rituximab in rheumatoid arthritis patients. Arthritis Rheum. 2010 Dec;62(12):3607-14. doi: 10.1002/art.27702.

17. Katemina EV, Poole AR, EM Zaitseva, et al. Differences in mTOR (mammalian target of rapamycin) gene expression in the peripheral blood and articular cartilages of osteoarthritis patients and disease activity. Arthritis. 2013;2013:461486. doi: 10.1155/2013/461486. Epub 2013 Jun 25.

18. Lubbers J, Brink M, van de Stadt LA, et al. The type I IFN signature as a biomarker of preclinical rheumatoid arthritis. Ann Rheum Dis. 2015 Mar;74(3):776-80. doi: 10.1136/annrheumdis-2012-202753.

19. Epub 2013 Feb 23.

20. Van der Pauw Kraan TC, Wijbrandts CA, van Baarsen LG, et al. Rheumatoid arthritis subtypes identified by genomic profiling of peripheral blood cells: assignment of a type I interferon signature in a subgroup of patients. Ann Rheum Dis. 2007 Aug;66(8): 1008-14. doi: 10.1136/ard.2006.063412.

21. Higgs BW, Liu Z, White B, et al. Patients with systemic lupus erythematosus, myositis, rheumatoid arthritis and scleroderma share activation of a common type I interferon pathway. Ann Rheum Dis. 2011 Nov;70(11): 2029-36. doi: 10.1136/ard.2011.150326.

22. Mavragani CP, La DT, Stohl W, Crow MK. Association of the response to tumor necrosis factor antagonists with plasma type I interferon activity and interferon-β/α ratios in rheumatoid arthritis patients: a post hoc analysis of a predominantly Hispanic cohort. Arthritis Rheum. 2010 Feb;62(2):392-401. doi: 10.1002/art.27226.

23. Wampler Muskardin T, Vashisht P, Dorschner JM, et al. Increased pretreatment serum IFN-β/α ratio predicts non-response to tumour necrosis factor α inhibition in rheumatoid arthritis. Ann Rheum Dis. 2016 Oct;75(10):1757-62. doi: 10.1136/annrheumdis-2015-208001.

Conflict of Interest Statement

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