HIRSZFELD INSTITUTE OF
IMMUNOLOGY AND EXPERIMENTAL THERAPY
POLISH ACADEMY OF SCIENCES

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The role of polymorphisms and expressions of the receptors from the CD94/NKG2 family and the HLA-E molecule in pathogenesis of the rheumatoid arthritis

Summary

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Wrocław, 2018

Rheumatoid arthritis (RA) is a multisystem autoimmune disease characterized by inflammatory synovitis leading to joint destruction. Natural killer (NK) cells and T cells participate in pathogenesis of RA. The function of these cells is modulated by several receptors. The cytotoxic activity and cytokine release are controlled by the balance between the inhibitory and the activating receptors of NK and T cells. The activating NK and T cell responses can be modulated by CD94/NKG2 molecules belonging to the C-type lectin like family. These molecules mediate either the inhibition or the augmentation of cytotoxicity, as well as the generation of the proinflammatory cytokines by NK and T cells. An imbalance in cytotoxic activity and cytokine production has been implicated in pathogenesis of RA. Therefore, the inhibitory and activating CD94/NKG2 receptors and their corresponding ligands may play a potential role in the pathogenesis of RA.

The aims of this research were (1) to examine potential relationships between genetic variants of *CD94/NKG2* receptors as well as *HLA-E* molecule and RA development, clinical outcome of anti-TNF-α therapy and laboratory and clinical parameters of RA activity; (2) to examine potential relationships between protein expression levels of the CD94/NKG2 receptors as well as HLA-E molecule and RA development.

A total of 303 RA patients receiving anti-TNF-α therapy from Department of Rheumatology and Internal Medicine of Wroclaw Medical University as well as Clinical Department of Rheumatology and Connective Tissue Diseases of Hospital University Number 2 Jana Biziela in Bydgoszcz were enrolled to the study. The control group consisted of 238 healthy blood donors recruited from Blood Bank of Wroclaw.

Genotyping for studied polymorphisms were performed using real-time PCR employing LightSNiP as well as TaqMan assays. The analyses of surface expression of investigated molecules were investigated for 34 RA patients and 24 healthy individuals. A number of cells expressing HLA-E, NKG2A, NKG2C and NKG2D molecules was measured using flow cytometry. Surface expression of NKG2A, NKG2C and NKG2D was examined on following cells: NK (CD3-CD56+), NKT (CD3+CD56+) and T (CD3+CD56-) comprising CD8+, CD4+, CD28+, γδ1+ and γδ2+ cells. Expression of HLA-E molecule was analysed on T (CD3+CD4+) cells, B (CD19+) cells and monocytes (CD14+).

The *HLA-E* rs1264457 polymorphism was associated with predisposition to RA as well as clinical outcome of anti-TNF-α treatment at 12th week in female patients. The frequency of the *HLA-E**01:01/01:01 genotype was significantly decreased in female RA patients in

comparison to healthy female individuals (p = 0.031). The presence of the *HLA-E**01:01/01:01 genotype among female patients correlated with better response after 12 weeks of anti-TNF-α treatment in comparison to other genotypes (p = 0.014). The *HLA-E**01:03/01:03 genotype was also overrepresented among non-responding female patients in comparison to *HLA-E**01:01/01:01 genotype (p = 0.021). With regard to *HLA-E* rs1059510 genetic variant, significant relationships with efficacy of anti-TNF-α therapy after 12 weeks were observed. Patients carrying *HLA-E**01:03:01/01:03:01 genotype achieved significantly better responses to therapy in comparison to patients with other genotypes (p = 0.009).

The *CD94* rs2302489 polymorphism was associated with risk of RA development, presence of anti-CCP antibodies and response to TNF-α blockade therapy among patients. The frequency of the *CD94* rs2302489 AA genotype was significantly decreased in RA patients compared to controls (p = 0.016). The *CD94* rs2302489 AA homozygotes were also more common among patients negative to anti-cyclic citrullinated peptide (anti-CCP) antibodies (p = 0.001) as compared to anti-CCP-positive patients and the presence of the *CD94* rs2302489 allele A was associated with lack of anti-CCP antibodies (p = 0.0005). In addition, the *CD94* rs2302489 TT genotype was overrepresented in patients exhibiting worse response to therapy at 12^{th} week (p = 0.017).

With respect to *NKG2A* genetic variants, a significant relationship was observed between *NKG2A* rs7301582 polymorphism and efficacy of anti-TNF-α treatment. Lack of response after 12 weeks was more frequent among patients carrying the *NKG2A* rs7301582 C allele (p = 0.019) or the CC genotype (p = 0.035) in comparison to allele T or CT/TT genotypes. The analyses performed for the *NKG2A* rs2734414 and rs2734440 polymorphisms failed to demonstrate any significant associations in relation to efficacy of anti-TNF-α treatment or predisposition to RA development.

Within *NKG2D* gene following genetic variants were studied: rs2255336, rs1049174 and rs1154831. Both the *NKG2D* rs225336 and rs1049174 polymorphisms were significantly associated with efficacy of TNF-α inhibitors at 12^{th} week. Inefficiency of therapy was more frequently observed among patients carrying the *NKG2D* rs2255336 GG genotype as compared to other genotypes (p = 0.003). There was also significant increase in a frequency of the *NKG2D* rs2255336 G allele among patients non-responding to anti-TNF-α treatment (p = 0.002). On the other hand, patients with the heterozygous *NKG2D* rs2255336 AG

genotype achieved significantly better responses to anti-TNF-α therapy than patients bearing the homozygous genotypes (p = 0.010). With respect to *NKG2D* rs1049174 polymorphism, the C allele was overrepresented among patients characterized with no response to treatment after 12 weeks (p = 0.031). Presence of the *NKG2D* rs1049174 CC genotype in patients correlated with anti-TNF-α treatment failure as compared to other genotypes (p = 0.004).
In addition, heterozygous *NKG2D* rs1049174 CG genotype among patients was associated with good responses to the treatment in comparison to other genotypes (p = 0.002). On the other hand, increased frequency of *NKG2D* rs1049174 CG genotype has been observed in patients as compared to healthy individuals (p = 0.005).

Significant differences between patients and healthy controls have been also detected with regard to surface expression of receptors from CD94/NKG2 family. The percentages of NK cells expressing CD94 molecule in RA patients were significantly higher than that in the controls (p = 0.0350). On the other hand, RA patients had a lower frequency of T CD3+CD94+ cells than healthy individuals (p = 0.0021). A significantly decreased frequencies of T cells as well as NKT cells positive for NKG2A and NKG2C receptors have been also observed in RA patients as compared to controls (T: p = 0.0159 and p = 0.0030; NKT: p = 0.0386 and p = 0.0433).

Furthermore, among RA patients a significant increase in a frequency of NKG2D positive cells was observed in NK, NKT as well as T cell populations as compared to healthy individuals (NK: p = 0.0207; T: p = 0.0386; NKT: p = 0.0034). The frequencies of T CD3+CD8+ as well as T CD3+CD8+CD28+ cells expressing NKG2D receptor were significantly increased in patients as compared to healthy controls (p = 0.0386 and p = 0.0449).

No significant differences in frequencies of T CD3+CD4+CD28+ as well as T CD3+CD4+CD28- were observed between patients and controls. However, the percentage of CD3+CD8+CD28- cells was significantly higher than that in healthy individuals (p = 0,0012). Moreover, significantly increased proportion of CD3+CD8+CD28-NKG2D+ cells was observed in patients as compared to controls (p = 0,0212).

The percentage of T γδ2 cells were significantly lower in RA patients than in controls (p = 0.0028). Moreover, in RA patients there were significantly increased proportions of T

γδ1 and T γδ2 cells positive for NKG2D receptor as compared to controls have been detected (p = 0.0296 and p = 0.0183, respectively).

The obtained results imply a potential involvement of the receptors from CD94/NKG2 family and HLA-E molecule in RA development.