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Heterogeneity of the cytokinome in undifferentiated arthritis progressing to rheumatoid arthritis and its change in the course of therapy. Move toward personalized medicine

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ABSTRACT

Objectives: To conduct a comprehensive analysis of cytokine concentrations in sera and mononuclear cell supernatants in order to examine inter- and intra-individual cytokine variations in undifferentiated arthritis progressing to rheumatoid arthritis and healthy control groups.
Methods: Patients with UA (undifferentiated arthritis) developing RA (rheumatoid arthritis) (UA → RA) (n = 16) and healthy controls (n = 16) were enrolled into the study. UA → RA patients were followed up for six months since the final RA diagnosis. Cytokines IFN-γ, IL-10, TNF, IL-17A, IL-6, IL-1β, IL-2 in sera and mononuclear cell supernatants in 72 h and 120 h culture variants with- and without anti-CD3 stimulations were assayed using flow cytometric bead array.
Results: The cytokine profile of UA → RA differs from the healthy individual cytokine profile. It is possible to observe specific cytokine pattern characterizing each patient, which alters during course of disease. Specifically, we can distinguish three UA → RA cohorts: the group of patients susceptible to the therapy, characterized by the drop of cytokine levels between 1st and 3rd visit with visible decrease of cytokines in 2nd visit and then secondary slighter increase in 3rd visit; the group of patients refractory or clinically worsening on the therapy, characterized by the highest cytokine levels at 2nd visit with secondary decrease in 3rd visit; and the group of patients with variable responses to the therapy without any specific common cytokine pattern. The cytokine patterns in supernatants of PBMC stimulated anti-CD3 for 72 h and 120 h are very similar.
Conclusions: The personal profile including multiplexed cytokine patterns in serum and supernatant may be potentially used for optimization of therapy introduction and monitoring.

1. Introduction

Undifferentiated arthritis (UA) defines clinical symptoms and analytical findings that do not allow a diagnosis of any well-defined inflammatory rheumatic disease. The term describes common clinical manifestations of different potentially underlying inflammatory and immunological processes [1]. While some forms of UA will spontaneously remit, most of them will progress to a chronic arthritis, including rheumatoid arthritis (RA) as the most severe and persistent form [2]. The ability to predict the conversion from UA to RA at the earliest UA stage seems crucial for the early introduction and optimization of therapeutic scheme, especially due to the high effectiveness of very early use of disease- modifying anti- rheumatic drugs (DMARDs) [3].

Abbreviations: ACR, American College of Rheumatology; ALT, alanine aminotransferase; ANA, anti-nuclear antibodies; anti-CCP, anti-cyclic citrullinated antibodies; anti-TG, anti-thyroglobulin antibody; AST, aspartate aminotransferase; bDMARD, biologic disease modifying anti-rheumatic drugs; CBA, cytometric bead array; CBC, complete blood count; CRP, C-reactive protein; DAS28, disease activity score 28; DMARD, disease modifying anti-rheumatic drugs; EDTA, ethylene diamine-tetraacetic acid; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme linked immune spot assay; ESR, erythrocyte sedimentation rate; EULAR, European League Against Rheumatism; FACS, Fluorescence-activated cell sorting; HEp2, human epithelial cell line 2; HIV, human immunodeficiency virus; IFNγ, interferon γ; MTX, methotrexate; mHAQ, modified Health Assessment Questionnaire; PBMC, peripheral blood mononuclear cells; PCR, polimerase chain reaction; UA, undifferentiated arthritis; PE, phycoerythrin; RA, rheumatoid arthritis; RF, rheumatoid factor; SJC, swollen joint count; SSZ, sulphasalazine; Th lymphocytes, T helper lymphocytes; TJC, tender joint count; TNF, tumor necrosis factor

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There is a great concern with the placing of UA in a context of immunological mechanisms. An immunological response is effectuated by numerous immune cells and protein factors such as complement components, coagulation factors, autoantibodies, chemokines and cytokines [1]. Deregulation in cytokine network plays an undoubtedly crucial role in the pathogenesis of UA and its progression to RA. The cytokine deregulation, particularly Th1/Th2/Th22 cytokine imbalance may lead to inflammation and subsequent joint damage. Moreover, uncontrolled production of pro-inflammatory cytokines may contribute to autoimmune processes [4].

In the light of a growing need to search for new early RA biomarkers, cytokines are considered as potentially applicative. The body of evidence has already highlighted a potential utility of the cytokine assays in diagnostic and prognostic processes [4–6].

Still, our understanding of the role of cytokines in a progression of UA to RA is largely limited. However, in the light of the fact that disease-related biomarkers may be altered prior to the onset of the fully symptomatic RA [7], we decided to assess the cytokines at the stage of UA as a potentially applicative prediction biomarker of RA.

2. Material/methods

2.1. Subjects

121 people with suspected UA have been identified at Regional Hospital for Rheumatic Diseases in Sopot, Poland. All subjects underwent a wide range of laboratory tests, including: complete blood count (CBC), erythrocyte sedimentation rate (ESR), assessment of concentrations of C-reactive protein (CRP), rheumatoid factor (RF), anti-cyclic citrullinated peptide antibodies (anti-CCP), fasting blood glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine, detection of serological markers of hepatitis B, C and human immunodeficiency virus (HIV), and general urine test. Clinical assessment of their status included: tender joint count (TJC), swollen joint count (SJC), ACR tender score, ACR swollen score, disease activity score 28 (DAS28) and modified Health Assessment Questionnaire (mHAQ) were determined for all subjects. Of the 121 initially identified, 58 patients with suspected UA (mean age 43.5 ± 11.9 [mean ± SD] years) met following criteria: (1) had a minimum of one peripheral joint inflammation lasting from 2 to 4 months; (2) were not treated with disease modifying anti-rheumatic drugs (DMARDs), biological treatment (bDMARDs) nor steroids; (3) did not fulfil any of the ACR/EULAR classification criteria for any specific rheumatic disease at the time of enrolment; thus, they were diagnosed as UA. These patients were followed from 1 to 6 months which allowed for final diagnosis, at which the DMARD treatment (SSZ, MTX or Arechin) was introduced. From total of 58 UA patients, 20 were classified as having rheumatoid arthritis (UA → RA) and 16 of them (14 female, 2 male, aged 45.25 ± 11.9 years [mean ± SD]) completed the whole study procedure, participating in another two follow-up visits which occurred over three and six months since the final diagnosis. These patients constituted the study group (see Table 1).

The control group consisted of 16 clinically healthy people (all female, mean age 37.3 ± 7.6 years [mean ± SD]), with laboratory test results within normal ranges, without any symptoms of joint inflammation, with no autoimmune or chronic inflammatory diseases in personal or family history. The control individuals were examined by the same rheumatologists and they had undergone the same range of laboratory tests as the patients.

The scheme of the recruitment method was depicted in Fig. 1.

The study was approved by the Local Independent Committee for Ethics in Scientific Research at the Medical University of Gdansk and the written consent was obtained from all patients and controls.

2.2. Rationale of experimental design

Biomarkers, also known as biological markers, constitute indicators with diagnostic or prognostic utility complementing clinical markers [5]. The body of evidence unambiguously demonstrates that RA is characterized by altered T-cell cytokine profile, making it a potential powerful biomarker of RA. Based on available data, general inflammation and autoimmunity precede the onset of clinically classifiable RA; however, it is not clear where and when these processes initially begin [8]. Deane et al demonstrated pre-RA elevation of IL-6 in sera prior to anti-CCP positivity [7]. A nested case-control study performed by Kokkonen et al showed significant increase of serum IL-1β, IL-2, IL-6, TNF, IFNγ, IL-10 in individuals before disease onset compared with the levels in healthy controls [7]. Moreover, foregoing study demonstrated higher serum concentration of IL-17 in individuals before disease onset then that in patients with established RA [9]. However, involvement of cytokine network imbalance in the progression of UA to established RA, as well as in further progression of the disease, and the mechanism of observed changes remain unclear [6,10]. We chose for our study the range of cytokines produced by cells significantly involved in arthritis pathogenesis, including Th1 (IL-2, IFNγ), Th2 (IL-10, IL-6) and Th17 (IL-17A, TNF) and the pathophysiological processes related to them [6,11]. Tested cytokines are required for differentiating of naive lymphocytes toward Th1 (IFNγ), Th2 (IL-2), Th17 (IL-1β, IL-6) and Th22 (IL-6, TNF) cells respectively [10]. They are characterized by pleiotropy with predominant pro- (IL-6, IL-1β, TNF) or anti- (IL-10) inflammatory activity [10].

Thus, in order to assess capability of cytokines as the potential biomarkers of UA → RA conversion, we investigated the inter- and intra- individual variations of cytokine levels among both UA → RA and healthy control groups. We wanted to check how early these cytokine network deregulations appear and whether they develop before the fully symptomatic manifestation of RA.

The inter-individual analysis (see part 4.1) attempted to check the potential diagnostic utility of measuring cytokine concentrations in UA → RA patients by establishing whether there was any difference between them and healthy control groups.

Then, intra-individual (semi-longitudinal) analysis was performed in an attempt to observe possible changes in the cytokine secretion patterns characteristic for the disease progression (see part 4.2). The patients, initially classified as UA, were followed for 6 months since the final RA diagnosis.

Furthermore, we proposed a new methodological approach to the problem of cytokine measurements yielding results meaningful for the prediction of UA → RA conversion (see part 4.3). Thus, we measured cytokine levels in sera and supernatants of anti-CD3-stimulated and control PBMC cultures after 72 h and 120 h. We used easily accessible biological materials, potentially applicative in clinical practice

2.3. Methods

2.3.1. Preparation of peripheral blood mononuclear cells (PBMC) and cell cultures

10 ml of peripheral venous blood was obtained from each patient and control (Vacutainer™ tubes with spray coated EDTA; BD Bioscience, USA). The blood was processed within 2 h of drawing. The blood was diluted 1:1 with PBS w/o calcium and magnesium (PAN Biotech GmbH, Germany), layered onto Histopaque™ 1077 (Life Science Sigma-Aldrich, UK) and centrifuged for 30 min at 2000 rpm. Then, PBMC were washed, counted and finally loaded with 10 μM carboxyfluorescein diacetate *N*-succinimidyl ester (CFSE, Molecular Probes-Eugene, USA) according to [Witkowski 2008].

Then the cells were suspended in RPMI supplemented with 10% fetal bovine serum, 5% of 2 mM L-glutamine 1% of penicillin-streptomycin and samples of 2×10^6 PBMC × ml^{-1} were stimulated with 0.2 μg immobilized anti-CD3 antibody (purified NA/LE mouse