Cytokine 96 (2017) 41–48

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Parvovirus B19 infection modulates the levels of cytokines in the plasma of rheumatoid arthritis patients

Milda Naciute [a], Diana Mieliauskaite [a], Rita Rugiene [a,b], Gabriele Maciunaite [a], Mykolas Mauricas [a], Modra Murovska [c], Irute Girkontaite [a,*]

[a] Department of Immunology, State Research Institute Centre for Innovative Medicine, Santariskiu 5, Vilnius LT 08406, Lithuania
[b] Centre of Rheumatology, Vilnius University, Santariskiu Street 2, LT-08661 Vilnius, Lithuania
[c] A. Kirchenstein Institute of Microbiology and Virology, Riga Stradins University, Ratsupites St. 5, Riga LV1067, Latvia

ARTICLE INFO

Article history:
Received 22 November 2016
Received in revised form 5 January 2017
Accepted 2 March 2017

Keywords:
Parvovirus B19
Rheumatoid arthritis
IFN$_\gamma$
IL-4

ABSTRACT

Background: Parvovirus B19 (B19V) infection is associated with various autoimmune diseases. We investigated the levels of pro-inflammatory (IFN$_\gamma$, TNFα, IL-2, IL-12) and anti-inflammatory (IL-4, IL-10) cytokines in the plasma of B19V DNA positive (B19$^+$) and negative (B19$^-$) rheumatoid arthritis (RA) patients in comparison with the control group (healthy persons).

Methods: Blood samples were collected from 118 patients with RA and 49 healthy voluntaries. B19V sequence was determined in whole blood and cell-free plasma DNA by nested PCR. The levels of cytokines in the plasma and cell culture medium from Concanavalin A (ConA) or B19V VP1 protein stimulated PBMC were determined by ELISA.

Results: The levels of IL-4, IL-10, IL-12, IL-2 and TNFα were higher in plasma of RA patients in comparison with control persons. B19$^+$ controls and RA patients had lower levels of IFN$_\gamma$ in comparison with B19$^-$ controls and RA patients.

Within RA patients the plasma levels of IFN$_\gamma$ were lower in patients with low RA disease activity or remission. Plasma level of IL-4 was increased and IL-10 level was decreased in B19$^+$ RA patients in comparison with B19$^-$ RA patients and did not differ between B19$^+$ and B19$^-$ controls. B19V infection did not affect plasma levels of IL-12, IL-2, and TNFα.

ConA and B19 VP1 protein stimulated PBMC from RA patients produced less IFN$_\gamma$ than stimulated PBMC from the healthy controls.

Conclusions: B19V infection could differently modulate the amount of cytokines in the plasma of healthy persons and RA patients. Decreased production of IFN$_\gamma$ and raised level of plasma IL-4 in RA patients could lower antiviral clearance.

1. Introduction

Rheumatoid arthritis (RA) is a complex, chronic autoimmune disease that associates with progressive disabling, major systemic complications, early death and increased socioeconomic problems. It is characterized by synovial tissue inflammation and hyperplasia, autoantibody production, cartilage and bone destruction and systemic features such as joint deformities, pulmonary, cardiovascular and dermatological disorders [1].

Human parvovirus B19 (B19V) is a small single-stranded DNA virus that belongs to *Parvoviridae* family. B19V mainly infects human erythroid progenitor cells. However, recent clinical studies indicate that B19V could persist in other cells [2–4]. B19V DNA is found in peripheral blood and synovial fluid cells [5], and VP-1 protein is detected in synovial cells including lymphocytes, macrophages and neutrophils [6].

B19V infection more frequently is found in patients with such autoimmune diseases as juvenile rheumatoid arthritis [7], systemic lupus erythematosus [8], Sjogren's syndrome [9], autoimmune thyroiditis [10,11], and chronic fatigue syndrome [12].

Abbreviations: RA, rheumatoid arthritis; B19V, parvovirus B19; B19$^+$, parvovirus B19 DNA positive; B19$^-$, parvovirus B19 DNA negative; WBC, white blood cells; HgB, hemoglobin; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; anti-CCP, cyclic citrullinated peptide antibody; DAS28, disease activity score; RF, rheumatoid factor; IL, interleukin; IFN-γ, interferon gamma.

* Corresponding author at: Department of Immunology, State Research Institute Centre for Innovative Medicine, Santariskiu 5, Vilnius LT 08406, Lithuania.
E-mail address: igirkontaite@yahoo.com (I. Girkontaite).

http://dx.doi.org/10.1016/j.cyto.2017.03.002
1043-4666/

Parvovirus B19 is often associated with chronic arthritis, although etiologic associations with rheumatoid arthritis are conflicting. According to some publications [13,14]. B19V is not associated with rheumatic disease manifestation. Although there is the evidence based on the fact that parvovirus B19 infection may play certain role in the susceptibility to RA. The prevalence of parvovirus B19 DNA is significantly higher in patients with RA than in controls [5,15–17]. Both B19V DNA and B19V VP1/VP2 proteins in synovial specimens of RA patients have been detected in higher frequency than in control individuals [6,18]. Kakurina et al. have shown that the highest disease activity was observed in RA patients with active B19V infection [19]. The evidence that B19V could be involved in RA pathogenesis has been shown also in animal models. B19V NS1 transgenic mice are susceptible to type II collagen (CII)-induced arthritis. NS1 is expressed in synovial cells on the articular lesions that were histologically characteristic of granulomatous synovitis and pannus formation in cartilage and bone [20].

In this study we investigated whether B19V infection could modulate the levels of cytokines in the plasma of RA patients.

2. Materials and methods

2.1. Participants of the study

Participants of the study were selected from patients seen at the Vilnius University hospital Santariskiu clinics. All patients underwent an extensive medical examination, whole blood analysis and extensive serologic evaluations which included test for the white blood cells (WBC), hemoglobin (HgB), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), cyclic citrullinated peptide antibody (anti-CCP), rheumatoid factor (RF) and disease activity score (DAS28). Blood samples were collected in vacutainers with K_2EDTA and processed within 4 h of collection. B19V genomic DNA was determined in the whole blood and cell-free plasma using nested PCR. The individuals with the presence of B19V DNA (independently in whole blood or cell-free plasma) were named as $B19^+$. Similarly, the persons without the presence of B19V DNA were named as $B19^-$. Totally 118 RA patients were enrolled in this investigation. 30 of them (25.4%) were $B19^+$. From 49 age and sex matched healthy voluntaries in 9 (18.4%) B19V DNA was detected. From $B19^+$ persons 6 RA and 8 control group individuals had persistent infection in latent phase; 22 RA and none of control group individuals had persistent infection in active phase; one RA patient had acute or persistent infection in active phase; one RA and one control group individual had acute infection. From $B19^-$ persons 76 RA and 32 control group individuals had past infection; 12 RA and 8 control individuals were without infection. More detail description of the patients is previously published [21]. This study was approved by the Vilnius Regional Biomedical Research Ethics Committee.

2.2. Determination of the cytokine concentration in the plasma and cell culture supernatant

The plasma levels of interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 10 (IL-10), interleukin 12 (IL-12), tumor necrosis factor alpha (TNFα) were detected using human ELISA MAX™ Standart Sets (Biolegend, San Diego, USA) according manufacture's recommendations. Interferon gamma (IFNγ) level was detected by two site ELISA using home-made murine monoclonal antibodies to human IFNγ and recombinant human IFNγ (Life Technologies) as the standard as described before [22]. The plasma for ELISA was diluted 1:3. The cell culture supernatants were diluted 1:5 for IFNγ determination and used undiluted for determination of other cytokines. The results were calculated using "Gen5" software.

2.3. Cell culture

Peripheral blood mononuclear cells (PBMC) were purified using Lymphoprep™ (Fresenius Kabi Norge AS, Norway) gradient centrifugation. The cells were frozen in medium containing 10% of fetal calf serum (FCS) and 10% of DMSO and stored in liquid nitrogen until used for analysis. PBMC were thawed, diluted in CHO-S-SFM II medium (Gibco) with 2 mM glutamine (Biochrom), 100 U/ml penicillin and 100 μg/ml streptomycin (HyClon), 50 μM 2-mercapthoethanol and 3% of human plasma (pooled from healthy persons) and plated into 96-well round-bottom tissue culture plates 2×10^5 cells per well. The cells were unstimulated or stimulated with 5 μg/ml of Concanavalin A (ConA) (Calbiochem), 1 μg/ml of B19V VP1 recombinant protein (Fitzgerald Industries International). The cells were incubated 48 h at 37 °C in 5% of CO_2 atmosphere, after that cell supernatants removed and the levels of cytokines determined immediately or supernatants stored at −80 °C until analysis.

2.4. Statistical analysis

Mann-Whitney *U* test was used to compare two groups of patients with non-normal distribution of data. Fisher's exact test was used to calculate rates and proportions between the groups. $P < 0.05$ was considered as significant.

3. Results

3.1. The amount of cytokines in the plasma of RA patients and healthy control individuals

The levels of IFNγ, IL-4, IL-10, IL-12, IL-2 and TNFα were measured in plasma of $B19^+$ and $B19^-$ RA patients and age and sex mashed healthy persons (controls).

3.1.1. IFNγ

The averages of IFNγ concentrations in the plasma of $B19^-$ RA patients and $B19^-$ controls did not differ significantly (Fig. 1A). Nevertheless, IFNγ levels in RA patients group were very widely scattered and a part of the $B19^-$ RA patients had very high plasma IFNγ level (up to 250 pg/ml) compared with control persons (up to 130 pg/ml). IFNγ concentrations in the plasma from RA $B19^+$ (70.5 ± 83.5 pg/ml) and control $B19^+$ (49.4 ± 12.9 pg/ml) people were significantly ($p < 0.05$) lower than in plasma from RA $B19^-$ (85.0 ± 64.7 pg/ml) and control $B19^-$ (76.1 ± 33.1 pg/ml) people, respectively (Fig. 1A). Next the amount of IFNγ was measured in RA patients with and without B19V infection markers in connection with clinical parameters (Fig. 1B). $B19^+$ and $B19^-$ RA patients were divided according disease activity score - DAS28 (DAS28 < 5.2 and DAS28 > 5.2), disease duration (up to 10 years and over 10 years), used therapy (without and with immunotherapy), erythrocyte sedimentation rate – ESR (normal and increased), hemoglobin level – HgB (low and normal), and C-reactive protein - CRP (normal and increased). The significant decrease of plasma IFNγ levels were detected in RA $B19^+$ patients in comparison with RA $B19^-$ patients when DAS28 < 5.2 (34.1 ± 21.2 vs. 76.4 ± 46.5 pg/ml), disease duration over 10 years (58.8 ± 71.5 vs 88.3 ± 54.7 pg/ml), used immunotherapy (36.2 ± 11.8 vs 80.4 ± 44.7 pg/ml), normal ESR (34.4 ± 27.3 vs 71.4 ± 41.3 pg/ml) and low HgB (36.6 ± 24 vs 73.3 ± 42.1 pg/ml). IFNγ concentrations (pg/ml) in the plasma did not differ in RA $B19^+$ and RA $B19^-$ patients that had DAS > 5.2 (67.0 ± 52.8 and 86.34 ± 55.1), higher than normal ESR (91.3 ± 97.8 and 85.5 ± 54.1), lower than normal HgB (100.1 ± 104.7 and 90.6 ± 58.7), in patients who were without