

# Sister chromatid exchanges and cell proliferative abilities in cultured peripheral blood lymphocytes of patients with rheumatoid and reactive arthritis

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## Abstract

### Objective

Analysis of cytogenetic alterations in peripheral blood lymphocytes (PBL) of patients with acute and chronic reactive arthritis (AcReA and ChrReA) and rheumatoid arthritis (RA).

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### Methods

The frequencies of sister chromatid exchanges (SCE) and cell proliferative abilities were analysed in PBL from 69 patients with arthritis and 30 healthy controls. The analyses were done on metaphase chromosomes from PBL grown in cell culture for 72 hours. Cytogenetic parameters were compared among study groups and correlations with different clinical, immune and demographic characteristics were analysed.

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### Results

No significant increases in the frequencies of SCE were detected in PBL from patients with AcReA, ChrReA and RA as compared to controls. However, marked impairment of cell proliferative abilities was detected in cultured lymphocytes from patients with arthritis as compared to healthy controls. Significant associations between measures of disease activity and proliferative abilities of PBL were established. Parameters of lymphocyte proliferation were also influenced by concentration of anti-inflammatory cytokine interleukin-10 in the blood of patients.

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### Conclusions

No increased risk of genetic alterations as measured by the rate of SCE was found in patients with RA and ReA. It is most likely that impaired proliferative abilities of peripheral blood lymphocytes are related to disease activity and could reflect systemic changes in cytokines production and intracellular signal transduction.

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### Key words

Rheumatoid arthritis, reactive arthritis, IL-10, sister chromatid exchanges.

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## Introduction

Cytogenetic parameters, such as chromosome aberrations, sister chromatid exchanges (SCE) or micronuclei are detectable by cytogenetic analysis of chromosomes in metaphase. These endpoints are widely used to assess human exposure to different mutagenic and carcinogenic agents of environmental or internal origin (1). Cytogenetic alterations are frequent events in various human diseases related to chronic inflammation and oxidative stress, such as ulcerative colitis, Crohn's disease, systemic lupus erythematosus, Behçet's syndrome and different carcinomas (2-4). However in different kinds of arthritis where the significance of oxidative stress is documented, no thorough analyses of cytogenetic alterations were done. In arthritis cytogenetic endpoints were mainly used to evaluate the genotoxicity of certain anti-rheumatic medications (5-8). Rheumatoid arthritis (RA) and reactive arthritis (ReA) are common inflammatory joint diseases. Despite marked differences in causative factors and molecular mechanisms of diseases, both RA and ReA are disorders influenced by genetic and environmental factors (9, 10). Genetic alterations, such as chromosomal aberrations and gene mutations (11-13), together with evident impairments in cell cycle, apoptosis and DNA repair (14, 15) have been detected in synovial cells of rheumatic patients. Oxidative stress caused by chronic inflammation is the main source of genetic alterations detectable in arthritic synovium (16). Oxidative DNA lesions were also observed in peripheral blood cells from patients with RA and osteoarthritis (17, 18). Increased risk of lymphoproliferative malignancies established in RA and other inflammatory rheumatic diseases (19) can be related to genetic alterations in blood cells caused by durable oxidative stress.

SCE are symmetrical exchanges between sister chromatids in the chromosome which form during the processes of DNA replication and reparation (20). SCE analyses are used for monitoring of groups with increased risk of genotoxic exposure (1, 21). Previously

reported significant increases in the rates of SCE in peripheral blood lymphocytes (PBL) from patients with Behçet's disease and ankylosing spondylitis (22-24) motivate further studies of cytogenetic alterations in patients with inflammatory arthritis. In order to collect reliable data on the rates of cytogenetic alterations in the most frequent rheumatic disorders we analysed the rate of SCE and cell proliferative abilities in PBL from 69 patients with RA and ReA. The data were compared among study groups and with healthy controls. Correlations between cytogenetic parameters and clinical, immune, and demographic characteristics of analysed persons were evaluated.

## Methods

### Patients

Peripheral blood was collected from 69 patients with ReA and RA and from 30 healthy controls at the Rheumatology Department of Vilnius University Central Hospital. This was a part of a prospective study described elsewhere (25). The study was approved by the National Committee of Ethics. RA was diagnosed according to ACR, ReA – according to European Spondylarthropathy Study Group criteria. Acute reactive arthritis (AcReA) was defined as ReA which persisted up to 3 months and chronic reactive arthritis (ChrReA) – starting from 6 months.

All patients and healthy persons were asked to answer a questionnaire concerning life-style, occupational, and other exposures with suspected genotoxic effect. Main demographic and clinical characteristics including medications, and data on cigarette smoking in study groups are presented in Table I. Complete data of clinical-laboratory analysis including the analysis of cytokine production by ELISA and flow-cytometry are described elsewhere (25).

### Cytogenetic analysis

Peripheral blood (7 ml) from patients with RA, ReA, and healthy controls was collected by venipuncture into heparinised tubes. Whole peripheral blood cultures were grown in 10 ml of RPMI 1640 medium with HEPES

**Table I.** Main characteristics of the study groups.

Characteristics	Healthy controls (n = 30)	AcReA (n = 27)	ChrReA (n = 22)	RA (n = 20)
Age, years <sup>a</sup>	31 (25-44)	30 (25-37)	42.5 (33-52)	49 (35-55)
Male	10 (33%)	12 (44%)	7 (32%)	6 (30%)
Female	20 (67%)	15 (56%)	15 (68%)	14 (70%)
Disease duration, months <sup>a</sup>	0	2 (1-2)	6 (6-36)	18 (6-42)
HLA-B27+	0	12 (44%)	6 (27%)	1 (5%)
RF+	0	0 (0%)	1 (4%)	13 (65%)
Smokers	3 (10%)	5 (18%)	3 (14%)	2 (10%)
Steroid use	0	9 (33%)	6 (27%)	15 (68%)
DMARD use:				
Sulphasalazine (2 g/day)	0	6 (22%)	16 (73%)	14 (70%)
MTX (10 mg/week) or AZA (100 mg/day)	0	0	0	6 (30%)

<sup>a</sup>Median (interquartile ranges).

AcReA: acute reactive arthritis; ChrReA: chronic reactive arthritis; RA: rheumatoid arthritis; HLA: human leukocyte antigen; RF: rheumatoid factor; DMARD: disease modifying anti-rheumatic drugs; MTX: methotrexate; AZA: azathioprine.

supplemented with 12% heat-inactivated fetal bovine serum, 50 µg/ml gentamycin and 10 µg/ml 5-bromo-2'-deoxyuridine (BrdU; all reagents from Sigma). For lymphocyte activation polyclonal mitogen phytohemagglutinin (PHA, 8 µg/ml) was used. Duplicated cultures from each person were grown in 37°C for 72 hours. Colchicine (0.6 µg/ml) was added for last 3 hours to collect metaphases. Cells were harvested by conventional means: hypotonised in 0.075 M KCl solution and then fixed three times in 3:1 methanol and acetic acid. 2-4 air-dried slides were prepared from each cell culture and stained by fluorescence plus Giemsa technique (26). For each person 50 second-division metaphase cells with 44-47 chromosomes were analysed to evaluate the mean SCE number per cell. Cell proliferative abilities were evaluated using replication index (RI)

and mitotic activity (MA) of cells. RI shows the average number of divisions performed by cell in cell culture medium containing BrdU. RI of cell cultures was calculated as described elsewhere (27). The percentage of cells in mitosis among randomly distributed 1000 cells corresponds to MA.

#### Statistical analysis

For normalisation of data distribution in cytogenetic analysis natural logarithmic (*ln*) transformation of data was performed. One-way analysis of variance (ANOVA) and subsequently *post-hoc* Fisher LSD test for pair-wise comparison of cytogenetic parameters were used. Correlations between cytogenetic parameters and other variables were evaluated, and linear regression was calculated for correlated values (Statistica, version 6.0).  $P < 0.05$  was considered as statistically significant.

**Table II.** Frequency of sister chromatid exchanges (SCE) and proliferative abilities (RI and MA) in cultured peripheral blood lymphocytes from patients with inflammatory rheumatic diseases and healthy controls.

	Controls	AcReA	ChrReA	RA
SCE/cell <sup>a</sup>	7.38 ± 0.95	7.74 ± 1.35	7.61 ± 1.33	7.21 ± 1.44
RI <sup>a</sup>	2.59 ± 0.14	2.46 ± 0.16 <sup>b</sup>	2.39 ± 0.20 <sup>b</sup>	2.33 ± 0.26 <sup>b</sup>
MA <sup>a</sup>	3.82 ± 1.66	3.02 ± 1.98	2.80 ± 1.83 <sup>b</sup>	2.39 ± 1.98 <sup>b</sup>

<sup>a</sup>Means ± SD.

<sup>b</sup> $P < 0.05$  as compared to control group. For data comparison *ln* transformed values were used in one-way ANOVA and *post-hoc* Fisher LSD test.

## Results

### Cytogenetic characteristics of study groups

No difference in frequencies of SCE was detected in any of the study groups. The highest number of SCE per cell was observed in the group of patients with AcReA. However, the difference from control group was not statistically significant (Table II).

Significant difference in proliferative abilities of cells measured as RI and MA was observed in study groups ( $P < 0.01$ ; ANOVA test). RI values were statistically significantly lower in all patients groups as compared to healthy controls ( $P < 0.05$ ; Table II). Also, patients with RA and ChrReA had significantly ( $P < 0.05$ ) decreased values of MA. The most prominent decrease in proliferative abilities of lymphocytes was in the RA group. Mean MA of these cell cultures was 40% lower than in cultures from healthy controls. No significant decrease in mean MA value was observed in the AcReA group and RI in this group was statistically significantly ( $P < 0.05$ ) higher than in the RA group.

### Correlations with demographic and clinical variables

Associations between cytogenetic characteristics and other variables were analysed in the control group (healthy donors) and in overall patients group (including AcReA, ChrReA and RA). Current smokers were present in the control group (10%) and in the patients group (14.5%). Smoking only in the latter group had significant effect on the frequency of SCE. The SCE/cell value was  $8.58 \pm 1.21$  (mean ± SD) in the smoker group, and  $7.31 \pm 1.17$  in the non-smoker group ( $P < 0.05$ , *ln* values are compared).

There was no difference in cytogenetic parameters in groups of patients with AcReA and ChrReA according to HLA-B27 positivity ( $7.54 \pm 1.15$  SCE/cell in HLA-B27<sup>+</sup> group, *versus*  $7.61 \pm 1.21$  in HLA-B27<sup>-</sup> group,  $P = 0.94$ ). Similar results were obtained in RA group when cytogenetic parameters of RF positive and negative patients were compared.

Analysis of correlations between two

variables revealed significant negative correlation between RI of PBL and age of donor in arthritis patients as well as in healthy controls ( $P < 0.05$  and  $P < 0.001$ , respectively). Significant negative associations between MA of PBL and number of swollen joints ( $P < 0.05$ ), leukocyte count ( $P < 0.05$ ) and amount of circulating immune complexes ( $P < 0.005$ ) were detected in the patients group. In statistical analysis duration of disease and treatment with disease modifying anti-rheumatic drugs (DMARD, Table I) had no significant effect on cytogenetic parameters of patients.

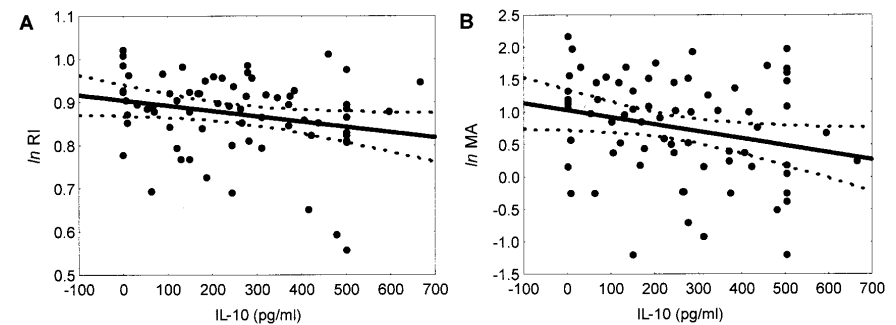
#### Correlations with cytokine production

We analysed correlations between cytogenetic variables of PBL and cytokine production in peripheral blood mononuclear cells (PBMC). Analysis of correlations between two variables revealed significant negative association between cell proliferative abilities (RI and MA) of PBL and intracellular amount of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ , calculated as the percentage of TNF- $\alpha$  positive CD3+ cells) in healthy controls ( $P < 0.05$  in both analyses). In the patients group the same variables RI and MA were inversely related ( $P < 0.05$  in both analyses) to the amount of interleukin-10 (IL-10; expressed in pg/ml) secreted by non-stimulated PBMC. In the patients group the number of SCE was inversely associated with intracellular amount of interferon- $\gamma$  (IFN- $\gamma$ ;  $P < 0.05$ ), while in healthy donors such association was not detected.

Significant association between the concentration of IL-10 and proliferative abilities of PBL from arthritis patients was also confirmed by regression analysis (Fig. 1). In multiple regression analysis, where other correlated variables were included (data not shown), only age and IL-10 concentration had significant associations with MA and RI values.

#### Discussion

Our study revealed that the number of SCE was not increased in PBL of patients with RA and ReA as compared to healthy controls. No increase in SCE



**Fig 1.** Regression plot between parameters of lymphocyte proliferation (RI and MA) and concentration of interleukin 10 in peripheral blood of patients with inflammatory rheumatic diseases. **A.**  $r_{xy} = -0.2399$  between  $\ln$  RI and concentration of IL-10 is significant ( $P = 0.042$ ). The linear regression  $\ln$  RI =  $0.90430 - 0.00012 \times \text{IL-10}$  is significant ( $r^2 = 0.058$ ,  $F(1,70) = 4.27$ ,  $P < 0.0424$ ). **B.**  $r_{xy} = -0.2465$  between  $\ln$  MA and concentration of IL-10 is significant ( $P = 0.037$ ). The linear regression  $\ln$  MA =  $1.0312 - 0.0011 \times \text{IL-10}$  is significant ( $r^2 = 0.061$ ,  $F(1,70) = 4.53$ ,  $P < 0.036$ ).

rate was detected in either acute or chronic phases of ReA. While different cytogenetic parameters have been examined in RA (4, 6-8), our study is the first which analysed cytogenetic alterations in ReA. Palmer and others (4) also did not detect increases in SCE frequencies in PBL of patients with RA. However, chromosome aberrations were observed in PBL from patients with RA in several studies (6, 7). These genetic changes were mainly related to treatment with genotoxic drugs (7) or serious disease complications, such as development of malignancy (6).

Significantly increased frequencies of SCE were recently reported in other inflammatory rheumatic disorders like ankylosing spondylitis (AS) and Behçet's disease (22-24). A higher number of SCE was detected in HLA-B27 positive AS group (22). However, in our study no significant increase in SCE frequency due to HLA-B27 positivity was observed in ReA group. The main factor significantly related to the frequency of SCE in PBL of rheumatic patients was cigarette smoking. Smoking is a well-known factor influencing SCE frequency in the population (28). Smoking also has a serious impact on the incidence and severity of RA and other rheumatic disorders (9).

It was established some years ago that the sensitivity of PBL to stimulation by mitogens like PHA is decreased in rheumatic diseases as a consequence of systemic alteration in immune response (29, 30). It is known that about 30% of

patients with RA are insensitive to mitogenic stimulation with PHA (31), response to other mitogens can be impaired as well (30). Even after stimulation with PHA lymphocytes from RA patients preferentially undergo apoptosis instead of proliferation (31). In our study proliferative characteristics of lymphocytes were measured as proliferation rate and mitotic activity after stimulation with polyclonal mitogen PHA. Proliferative abilities of cultured PBL from patients with RA and ReA were statistically significantly reduced as compared to healthy controls. Lack of responsiveness to mitogenic stimulation and impaired proliferative abilities of PBL from patients with arthritis may reflect complex alterations in signal transduction mechanisms observed in arthritis (32). Significant negative correlations between proliferative characteristics of PBL and clinical variables, such as number of swollen joints, leukocyte count and amount of circulating immune complexes, detected in our study indicates a relationship of proliferative capacities of PBL with disease activity. Age is also an important factor influencing mitogenic sensitivity of PBL from arthritis patients. Negative effect of age on proliferative abilities of PBL is established in healthy individuals (28). Cytokines produced by PBMC, especially by T lymphocytes, play an important role in regulation of immune response. Marked changes in balance of regulatory cytokines are directly related to pathogenesis of inflammatory

rheumatic diseases (33). Our study shows that in arthritis patients anti-inflammatory cytokine IL-10 can play a significant role in reduction of mitogenic responses of lymphocytes. Earlier we had detected an increased production of IL-10 in peripheral blood of RA and ReA patients (25). Further analysis revealed statistically significant negative association between the concentration of IL-10 in the blood and proliferative abilities of PBL. IL-10 is known as an immunoregulatory factor that inhibits T cell proliferation and cytokine production (34). A single IL-10 injection suppresses mitogen-induced T cell proliferation by up to 50% and causes a reduction in absolute lymphocyte count in healthy volunteers (35). As anti-rheumatic treatment with IL-10 mainly did not reach expected clinical effect additional studies on activities of cytokine in experimental models are still warranted. Permanent increase in IL-10 production observed in several rheumatic disorders can have an unfavourable effect on lymphocyte functions including defence against bacterial or viral infection and cancer. Our study revealed an apparent impairment in proliferative abilities of cultured PBL from patients with arthritis. Impaired proliferative abilities of peripheral blood lymphocytes are related to disease activity and may reflect systemic changes in cytokine production and intracellular signal transduction. It would be worth considering that anti-rheumatic biological agents should be tested for their cytogenetic and other effects in PBL cultures from patients with rheumatic diseases, where cellular responses resemble those found in pathophysiological conditions.

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