Cytogenetic parameters in acute and chronic reactive arthritis in comparison with rheumatoid arthritis

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We present preliminary data from a comparative study of cytogenetic parameters in two rheumatic diseases – rheumatoid arthritis and reactive arthritis. Cytogenetic parameters such as sister chromatid exchanges, replicative index and mitotic activity were studied in cultured peripheral blood lymphocytes from 47 patients. Clinical and immune markers of inflammation, such as erythrocyte sedimentation rate, concentration of C-reactive protein, circulating immune complexes and secretion of cytokines, were also assessed for the same set of patients. The highest rate of sister chromatid exchanges, as well as increase in other cytogenetic parameters was observed in patients with acute reactive arthritis. Statistical analysis revealed a significant correlation between the rate of sister chromatid exchanges and the type of rheumatic disease, smoking habit and markers of inflammation.

Key words: cytogenetic parameters, rheumatic disease, cytokine

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disorder of autoimmune origin, marked by synovial inflammation and destruction of bone and cartilage. Reactive arthritis (ReA) is an infection-associated arthritis, occurring mostly after infection of urogenital or gastrointestinal tract. Despite the differences in the molecular mechanisms of the diseases, both are frequently damaging and disabling disorders. Chromosomal aberrations [1] as well as gene mutations [2–4] have been detected in cells obtained from inflamed synovium of patients with some rheumatic disorders, mainly RA. Oxidative stress caused by chronic local inflammation of the joint is one of the sources of genetic damage observed in synovial cells, such as synoviocytes [5]. Only a few studies have been devoted to evaluation of systemic genetic parameters in patients with RA [6–8], while in other rheumatic disorders it was not analyzed at all.

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Cytogenetic parameters (sister chromatid exchanges (SCE), replicative index (RI) and mitotic activity (MA)) were analyzed in lymphocytes from patients with ReA and compared to these parameters of RA patients. In order to evaluate the modulatory effect of inflammation on the cytogenetic parameters of patients, several clinical and immune markers of inflammation were measured for the same set of patients. The modulatory role of clinico-pathological as well as demographic characteristics was also evaluated.

MATERIAL AND METHODS

Patients

Thirty-three patients with ReA and 19 patients with RA entered the study at the University Hospital of Vilnius Centre. Approval from the local ethical committee was obtained before the study. According to the course of disease ReA patients were divided into two groups with early acute disease (acReA; n = 22) and chronic long-standing disease (chReA; n = 11). Tender joint count (TJC), swollen joint

count (SJC) were evaluated as the clinical markers of disease activity. Conventional clinical-laboratory tests such as erythrocyte sedimentation rate (ESR), full blood count with white blood cells differentiation, serum concentration of C-reactive protein (CRP), circulating immune complexes (CIC) were used to evaluate systemic inflammation. Also, secretion of the inflammatory cytokines TNFα and IFNγ was measured in peripheral blood mononuclear cell cultures by means of two-sited ELISA as described elsewhere [9].

All patients answered the questionnaire concerning life-style, occupational and other conditions. Neither of the patients had a history of other chronic inflammatory diseases or cancer.

Cytogenetic analysis

Cytogenetic analysis was done on peripheral blood lymphocytes grown in cell culture for 72 h and specially prepared for metaphase analysis by optical microscopy. Cells were grown in RPMI 1640 containing 12% heat-inactivated fetal bovine serum, 50 μg/ml gentamycin, 2 mM L-glutamine and 10 μg/ml of 5-bromo-2′-deoxyuridine (all reagents from Sigma). Polyclonal mitogen phytohemagglutinin (PHA, 7.8 μg/ml) was used for cell activation. Cells were harvested by conventional means – hypotonised in KCl solution and then fixed 3 times in ethanol plus acetic acid. Air-dried slides were differentially stained by fluorescence plus Giemsa technique. Fifty division II metaphase cells were analyzed for SCE rate and about 200 metaphases of different divisions – for RI evaluation. The number of cells in mitosis was counted among a thousand cells to calculate MA.

Statistical analysis

Student's t test was used for comparison of different variables. Linear regression analysis was done to evaluate correlations between measured variables and cytogenetic parameters of the patients. $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

It is known that the majority of ReA patients usually recover within 3–6 months after the onset of the disease (acReA). However, in about a quarter of cases ReA becomes a chronic and severe disease (chReA) symptomatically close to RA [10]. Duration of the disease at the entry of the study was about 4 times longer in chReA as compared to acReA (Table 1). Also, the patients with chReA were statistically significantly ($P < 0.05$) older than patients with acReA. However, no significant differences in the values of clinical disease indicators (SJC, TJC) or markers of inflammation were observed between the groups with acReA and chReA in our study. Only the concentrations of inflammatory cytokines TNFα and IFNγ were significantly higher in chReA than in acReA. As expected, all markers of disease severity and inflammation were the highest in the group with RA (Table 1). Surprisingly, all cytogenetic parameters measured (SCE, RI and MA) were the highest in the group with acReA (Table 2). The SCE rate was statistically significantly ($P < 0.05$) higher in the groups with acReA as compared to RA, and exceeded that in chReA. The mean values of RI and MA were also higher in acReA as compared to chReA or RA.

Smoking is a well-known factor influencing the rate of SCE in healthy population [11, 12] and also having an impact on the incidence and severity of RA [13, 14]. We analysed the effect of smoking on cytogenetic parameters in our study groups. The number of smoking persons was the highest in the group with acReA (23%; Table 1). The patients with acReA who smoked had statistically significantly mo-

Table 1. **Demographic and clinico-pathological characteristics of patients with acute (acReA) or chronic (chReA) reactive arthritis and rheumatoid arthritis (RA)**

Patients	Age, years[a]	Disease duration, months[a]	Smokers, %	SJC[a]	TJC[a]	ESR[a], male/ female	CRP[a]	CIC[a]	TNFα[a,b], pg/ml	IFNγ[a,b], pg/ml
acReA, n = 22	31.7 ± 1.7	3.7 ± 1.6	23	1.9 ± 0.3	3.2 ± 0.4	30.5 ± 7.4 40.0 ± 10.5	1.7 ± 0.2	3.7 ± 0.3	194 ± 50	0
chReA, n = 11	44.9 ± 3.1[c]	13.8 ± 5.5[c]	18	1.8 ± 0.5	4.6 ± 1.0	44.4 ± 6.6 20.3 ± 10.6	1.7 ± 0.2	4.6 ± 0.5	424 ± 81[c]	27.3 ± 26.5
RA, n = 19	48.4 ± 3.6[c]	45.6 ± 13.0[c]	11	9.8 ± 1.3[c]	11.9 ± 1.0[c]	27.2 ± 2.8 50.6 ± 8.2	2.6 ± 0.2[c]	5.2 ± 0.8[c]	308 ± 60	4.3 ± 4.4

[a] Mean ± SEM.
[b] Data of 3 patient with acReA are missing.
[c] $P < 0.05$ as compared to acReA, Student's t test.

Table 2. **Cytogenetic parameters: sister chromatid exchange (SCE), replication index (RI) and mitotic activity (MA) of patients with acute (acReA) or chronic (chReA) reactive arthritis and rheumatoid arthritis (RA)**

Patients	SCE/cell ± SEM	RI ± SEM	MA ± SEM
acReA, n = 19	8.06 ± 0.31	2.47 ± 0.04	3.77 ± 0.48
chReA, n = 11	7.97 ± 0.42	2.32 ± 0.05[a]	3.27 ± 0.59
RA, n = 17	7.15 ± 0.29[a]	2.28 ± 0.07[a]	2.56 ± 0.48

[a] $P < 0.05$ as compared to acReA. Student's t test.

re SCE/cell than non-smokers with the same disease (9.42 ± 0.61 *versus* 7.70 ± 0.31; $P < 0.05$). Regression analysis also revealed a statistically significant ($P = 0.003$) association between the rate of SCE and the smoking habit of patients. However, smoking had no influence on the other cytogenetic parameters (RI and MI) analysed.

Regression analysis also revealed a linear correlation between the rate of SCE and two markers of inflammation. The rate of SCE directly correlated with the amount of CIC ($P = 0.028$) and inversely with ESR ($P = 0.019$). Increase in ESR indicates the presence of inflammation or infection, while CIC is a marker for autoimmunity. The detected correlations do not show a direct association between the processes of infection or inflammation and formation of genetic alterations. It is known that chronic inflammation can induce oxidative stress, which causes genetic instability and tumour formation [15]. Chromosomal aberrations as well as gene mutations [1–4] have been detected in places of chronic inflammation, *i.e*. in damaged joints from RA patients. Also, specific NO-induced deletions were detected [16] in T-cell clones from RA patients. In our study, the group of patients with acReA, which showed the highest rate of SCE, underwent a prolonged therapy with antibiotics and nonsteroidal anti-inflammatory drugs. The majority of those patients had a urogenital or gastrointestinal infection before entering the study. Acute release of endotoxins during antibiotic therapy could be responsible for the oxidative stress, a possible causative factor of cytogenetic alterations in acReA. Our previous data [17, 18] show that cytogenetic alterations can be caused also by inflammatory cytokines. TNFα in quite low concentrations increased the SCE rate in lymphocytes from healthy donors. However, our current study failed to detect any correlations between the rate of SCE and the concentrations of the inflammatory cytokines TNFα and IFNγ in RA or ReA.

To our knowledge, the present study is the first one to analyse the cytogenetic parameters in ReA. It shows an increased rate of SCE in acReA as compared to chReA or RA. The increase of cytogenetic alterations observed in acReA can be determined by multiple factors, such as smoking, inflammation-related oxidative stress, infection and its treatment-related oxidative stress, or inflammatory cytokine secretion. We continue to analyse our study group, including healthy controls in order to get more substantial results.

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CITOGENETINIŲ ŽYMENŲ ANALIZĖ ŪMAUS BEI LĖTINIO REAKTYVIOJO ARTRITO IR REUMATOIDINIO ARTRITO LIGONIŲ GRUPĖSE

S a n t r a u k a

Straipsnyje supažindinama su tyrimo, skirto reumatinių ligų molekulinių biožymenų analizei, pradiniais rezultatais. 47 pacientų, sergančių reaktyviuoju arba reumatoidiniu artritu, periferinio kraujo limfocituose autoriai tyrė citogenetinius žymenis – seserinių chromatidžių mainus, replikacijos greitį ir mitozinį aktyvumą. Kartu buvo vertinami pacientų demografiniai duomenys, analizuojami uždegiminio proceso žymenys. Didžiausias citogenetinių pažaidų kiekis aptiktas pacientų, sergančių ūminiu reaktyviuoju artritu, ląstelėse. Nustatyta tiesinė koreliacija tarp citogenetinių pažaidų kiekio ir pacientų rūkymo įpročių bei uždegiminio proceso žymenų. Padidėjęs citogenetinių pažaidų kiekis ūminio reaktyviojo artrito metu siejamas su uždegimo ir infekcijos sukeliamu oksidaciniu stresu bei intensyvia antibiotikoterapija.