Association of Rheumatoid Arthritis with Ergothioneine Levels in Red Blood Cells: A Case Control Study

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ABSTRACT. ***Objective.*** The dietary thiol compound and erythrocyte ingredient ergothioneine (ET) is the preferential physiological substrate of the organic cation transporter OCTN1, found to be associated with rheumatoid arthritis (RA) in genetic studies, but the biological roles of ET and OCTN1 are unclear. We investigated the association between ET concentrations in peripheral blood erythrocytes and the occurrence of RA.

Methods. Erythrocyte ET concentrations in patients with mildly active RA (n = 73) were compared to ET levels in patients with coronary heart disease (CHD; n = 62) and osteoarthritis (OA; n = 148), serving as non-RA chronic inflammatory disease controls. Correlation of ET levels in erythrocytes with levels of ET and OCTN1 mRNA in CD14+ monocytes was determined in 10 healthy subjects.

Results. Erythrocyte ET levels were significantly higher in patients with RA, with a median (interquartile range) of 12.6 μmole/l of erythrocytes (IQR 8.1–18.3), compared to 7.7 (IQR 5.0–12.0; $p < 0.001$) in CHD and 7.8 (IQR 4.8–12.8; $p < 0.001$) in OA. The prevalence of RA compared to non-RA controls increased with increasing blood ET concentrations, with an odds ratio of 0.23 (95% CI 0.13–0.41; $p < 0.001$) in the lowest quartile of RA erythrocyte ET levels to 3.11 (95% CI 1.54–6.29; $p = 0.002$) in the highest quartile. The group differences in ET values were maintained after adjustment for disease-related anthropometric and clinical variables (age, sex, body mass index, smoking, duration of disease, hemoglobin, C-reactive protein, and medication) and were also independent of erythrocyte glutathione levels and of polymorphisms of the OCTN1 gene. ET levels in erythrocytes were linearly correlated with ET concentrations ($R^2 = 0.936$, $p < 0.001$) and OCTN1 mRNA levels ($R^2 = 0.946$, $p < 0.001$) in CD14+ cells.

Conclusion. Mildly active cases of RA are associated with an unexplained high level of ET in red blood cells. (J Rheumatol 2006;33:2139–45)

Key Indexing Terms:
ERGOTHIONEINE ORGANIC CATION TRANSPORTER OCTN1 ERYTHROCYTES

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory autoimmune disease of unknown etiology with a worldwide prevalence of 0.8%[1] that primarily affects the synovial tissue of the peripheral diarthrodial joints. Synovial inflammation and joint destruction are mediated by type-1 helper T cell-dependent production of proinflammatory cytokines [particularly tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1), and IL-6] in monocytes, macrophages, and synovial fibroblasts. Aberrant proliferation of resident synoviocytes and exposure to persistent oxidative stress are thought to be central to pathophysiology in the early stages of RA[2-4].

Heritability analysis suggests that about 60% of a population's predisposition to RA can be accounted for by genetic factors. In genetic studies, RA is linked to major histocompatibility complex class II (HLA) antigens[5]. However, non-HLA disease-association genes have also been identified. The SLC22A4 gene coding the organic cation transporter OCTN1 was found to be strongly associated with RA[6,7]. OCTN1 is highly expressed in hematological and immunological tissues delineating from myeloid precursor cells, such as erythroblasts and monocytes[8].

Recently, L-ergothioneine (ET), a naturally occurring water-soluble thiol compound of dietary origin, was determined as the preferential physiological substrate of OCTN1, which was 100-fold more effectively transported than the previously suggested physiological substrate L-carnitine[9]. ET is exclusively synthesized in some fungi and bacteria[10]. Main dietary sources of ET are meat, grains, and some mushrooms,

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and it has been reported to be present in several mammalian tissues including erythrocytes[10,11]. ET has been proposed to exert protective antioxidant properties, possibly by scavenging radicals, quenching of reactive oxygen intermediates, or formation of complexes with divalent transition metals[12,13], but to date no biological role has been assigned to ET. Moreover, ET markedly differs in its antioxidative potency from other physiological thiol compounds, such as the principal water-soluble intracellular antioxidant glutathione. Unlike glutathione, ET is stable in its reduced form, and therefore at physiological conditions the redox equilibrium between ET and ET disulfide has been found to lie completely in the reduced state[13].

Based on reports of RA risk-associated polymorphisms in the ET-specific transporter gene SLC22A4, the potentially functional role of ET in RA, and the prevailing occurrence of ET in erythrocytes, we investigated the ET concentrations in peripheral blood erythrocytes of patients with RA. We conducted a case-control study comparing ET levels in erythrocytes of patients with RA to those of patients with coronary heart disease (CHD) and patients with osteoarthritis (OA). CHD[14] and OA[15,16] share common inflammatory components with RA in the disease process, including key roles of tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1), and IL-6 and a close correlation of serum markers of systemic inflammation such as C-reactive protein (CRP) with disease activity[17-22], and thus patients with CHD and OA were used as non-RA chronic inflammatory disease controls.

MATERIALS AND METHODS

Study population. Between June 2003 and August 2004, unrelated Caucasian adult patients (age range 18–80 yrs) with RA (n = 73), CHD (n = 62), and OA (n = 148) were consecutively and randomly enrolled from a community sample referred to the Internal Medicine Clinics I, II, and III and the Department of Orthopedic Surgery, University Hospital Cologne.

RA was diagnosed according to the 1987 American College of Rheumatology (ACR) criteria[23]. RA activity was determined using the Disease Activity Score (DAS28) index; only patients with low disease activity (DAS28 ≤ 3.2) were included. CHD was angiographically diagnosed by stenosis > 30% in at least one major coronary artery; only patients with moderate to high disease activity (CCS grade ≥ 2) were included. OA of knee or hip was clinically and radiographically diagnosed according to the 1986 ACR criteria[24,25]; only patients with erosive OA were included. The diagnoses were mutually exclusive. All patients were actively treated following established therapeutic guidelines. Exclusion criteria were known autoimmune diseases (other than RA), acute myocardial infarction, valvular heart disease, cardiomyopathy, stroke within the last 3 months, active bleeding, oral anticoagulant therapy within the previous month, hepatic and renal disorders, hematopoietic disorders, cancer, infectious diseases, trauma or surgery within the last 3 months, and a body mass index (BMI) > 35 or < 20 kg/m^2.

The study was approved by the ethics committee of the Medical Faculty of the University of Cologne. All participants were unpaid volunteers and gave written informed consent.

Laboratory analyses. Peripheral blood (10 ml) was drawn under standardized conditions after an overnight fast. ET was determined by liquid chromatography electrospray-tandem mass spectrometry (LC-ESI-MS/MS; TSQ Quantum, Thermo Electron, Dreieich, Germany) in washed erythrocytes obtained within 24 h after sampling from whole blood anticoagulated with EDTA. Storage of EDTA blood samples for up to 48 h did not lead to changes in ET concentrations. The recovery after 48 h was 99.0 ± 3.7% (mean ± SD) compared to the ET concentrations measured directly after blood sampling (p = 0.69; n = 5). Longer storage times resulted in decreased erythrocyte ET contents due to progressive hemolysis. For quantification, erythrocyte samples were spiked with ET (0, 5, 10, 30, 60 μmol/l), and after precipitation with acetonitrile the precursor ion $[M+H]^+ \rightarrow$ product ion transition m/z 230 → 127 was monitored. ET concentrations are given in micromoles per liter of erythrocytes. The intraassay and interassay coefficients of variation were 7.2% and 8.0%, respectively. The possible formation of ET disulfide and complexes of ET with copper(II) was assessed by monitoring the m/z 457 → 310 and m/z 520 → 417 transitions, respectively. In 88 blood samples with red blood cell (RBC) ET levels between 2 and 29 μmol/l, the presence of ET was also analyzed in the plasma after precipitation with acetonitrile; the ET concentration did not exceed the lower limit of detection of 0.1 μmol/l in any plasma sample. The periodic variability of RBC ET levels was monitored by repeated determinations in fasting blood samples from 5 healthy subjects drawn after 0, 2, 4, 8, and 12 months. Additionally, in 36 patients with RA and 22 patients with OA the erythrocyte content of reduced and oxidized glutathione was determined immediately after blood sampling to prevent further oxidation by monitoring the m/z 308 → 162 and m/z 613 → 231 transitions, respectively, in positive ESI-MS/MS mode. For all other biologic markers, plasma and RBC were stored at –80°C until analysis. Rheumatoid factor (RF) was measured in serum with a latex-particle enhanced immunoassay (Roche Diagnostics, Mannheim, Germany). All analyses were done blinded to the group status of the subjects. No data were lost.

From 10 healthy volunteers with no family history of autoimmune disease or CHD (7 women, 3 men, ages 22–38 yrs), 50–300 ml of blood (dependent on total leukocyte count) was drawn under standardized conditions after 8 h fast, and CD14+, CD4+, and CD8+ cells were fractionated from peripheral blood mononuclear cells using immunomagnetic beads (Dynabeads; Dynal Biotech GmbH, Hamburg, Germany). Total RNA was isolated using the RNEasy isolation kit (Qiagen, Hilden, Germany) and reverse-transcribed to cDNA using the Superscript First-Strand Synthesis System (Invitrogen, Karlsruhe, Germany). Expression of OCTN1 mRNA was determined by quantitative real-time polymerase chain reaction (LightCycler, Roche Diagnostics) relative to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase using TaqMan probes (Applied Biosystems, Weiterstadt, Germany), and quantified as described[26]. ET concentrations were determined in erythrocytes and CD14+, CD4+, and CD8+ cells by LC-ESI-MS/MS.

Genotyping of functional single nucleotide polymorphisms (SNP) in intron 1 (rs3792876) and exon 9 (rs1050152) of SLC22A4 was performed from genomic DNA by Taq dideoxy-terminator cycle sequencing (ABI Prism BigDye Terminator v2.0 cycle sequencing kit; Applied Biosystems, Foster City, CA, USA) using sequence-specific oligonucleotide primers.

Statistical analyses. Discrete data are presented as frequencies and percentages, and continuous variables are expressed as arithmetic means ± standard deviation (SD). Variables with a skewed distribution were also presented as medians with interquartile ranges (IQR). The significance of differences between 2 groups was assessed with Student's t test in the case of normal distribution or Wilcoxon rank-sum test for non-normal distribution, and the significance of differences in proportions was tested with the chi-square statistic. Differences between more than 2 groups were analyzed by one-way ANOVA (normal distribution) or one-way ANOVA on ranks (skewed distribution), and adjustments for multiple comparisons were made according to Bonferroni's or Dunn's method, respectively. Correlation between continuous variables was assessed by Pearson's test (normal distribution) or Spearman rank order test (skewed distribution). Multiple linear regression analysis was conducted to evaluate prediction of blood ET concentrations by anthropometric or clinical variables. Categorial variables were transformed into dichotomous dummy variables, skewed continuous variables were logarithmically transformed into normal distributions. Absence of multicollinearity was assessed by the variance inflation method. How well the correlation or regression model describes the data was measured by the R^2 coefficient of determination. A 2-tailed p value < 0.05 was considered a significant difference.

Fisher's exact test was performed and odds ratios were calculated to

assess the frequency of RA patients compared to non-RA patients across the quartiles of erythrocyte ET concentrations.

The required sample size was calculated prospectively on the basis of a previous pilot study in which we had enrolled 30 healthy volunteers (19 women, 11 men, ages 21 to 35 yrs). ET concentrations in RBC showed a log-normal distribution, with a mean (SD) of 9.8 ± 7.2, a median of 8.2, and IQR 5.6 to 10.4 μmol/l. To detect a minimal difference in means of 2.2 μmol/l (i.e., a mean < 7.5 or > 12.0 μmol/l) at a within-group SD of 8 μmol/l with a desired power of 80% and a significance level of 95%, a sample size of at least 120 cases (divided into 60 RA and 60 CHD patients) and 120 controls was calculated by ANOVA a priori analysis after logarithmic transformation.

Based on published minor allele frequencies of 36% for rs3792876 and 44% for rs1050152, the sample size was also sufficient to detect an association of a SNP with RA compared to OA or CHD with a power of at least 70% and a significance level of 95% assuming an odds ratio of 2 for the susceptible allele as calculated by chi-square test.

RESULTS

Main anthropometric and clinical characteristics of the 3 patient groups are given in Table 1. There were no significant differences between the RA and the OA control group, while the CHD group included a higher proportion of men and more older patients, reflecting the epidemiologic differences in sex and age distribution between these diseases. Additionally, a higher proportion of CHD patients received nonsteroidal anti-inflammatory drugs (NSAID), which always included low-dose aspirin. In RA and OA patients diclofenac was the predominant NSAID.

ET concentrations in RBC were log-normally distributed among all groups, ranging from 0.2 to 49.4 μmol/l. ET levels in RA patients were significantly higher, with a mean (SD) 14.2 ± 8.1 μmol/l, median 12.6 μmol/l (IQR 8.1–18.3), compared to CHD patients with a mean (SD) 9.9 ± 7.5 μmol/l, median 7.7 (IQR 5.0–12.0; p < 0.001), and to the OA control group with a mean (SD) 10.0 ± 8.0 μmol/l, median 7.8 (IQR 4.8–12.8; p < 0.001), while there was no difference between CHD and OA controls (p = 0.965). Notably, RBC ET levels of CHD and OA patients were also not different from the pilot sample of 30 healthy volunteers (p = 0.985) that showed no signs of inflammation (CRP < 0.5 mg/l). ET concentrations were independent of hemoglobin levels in both groups (RA: R^2 = 0.019, p = 0.640; OA: R^2 = 0.016, p = 0.523; CHD: R^2 = 0.0005, p = 0.864), and no signs of RA-associated anemia or decreased erythrocyte survival were noted compared to patients with OA or CHD. Hence, the differences between RA patients and the other groups were maintained when ET concentrations were adjusted to the hemoglobin levels (Figure 1).

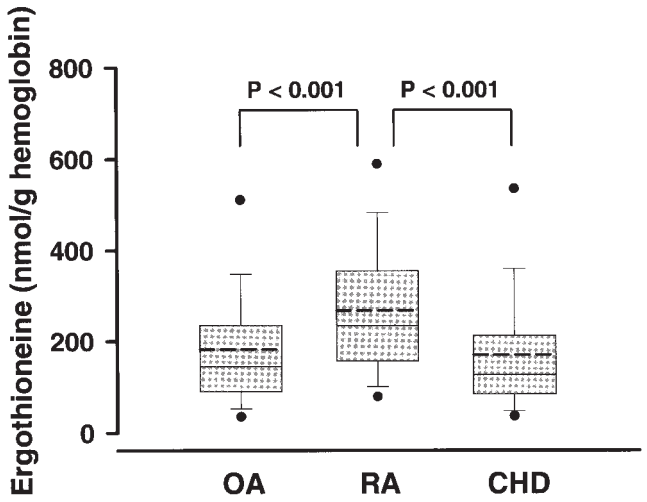

Figure 1. Ergothioneine concentrations in erythrocytes of patients with RA (n = 73), coronary heart disease (CHD; n = 62), and OA (n = 148) relative to hemoglobin levels. Values are nmol ergothioneine/g hemoglobin. Each box represents the arithmetic mean (broken line), median, and interquartile range (horizontal lines). Whiskers show 10th/90th percentiles; circles the 5th/95th percentiles.

Table 1. Anthropometric and clinical characteristics of participants.

Characteristic	RA, n = 73	OA, n = 148	CHD, n = 62	p for Difference, RA/OA	p for Difference, RA/CHD
Age, yrs	52.9 ± 15.0	57.5 ± 16.4	63.5 ± 9.9	0.15	< 0.001
Body height, cm	167 ± 11.0	168 ± 10.0	173 ± 9.0	0.74	0.11
Body weight, kg	75.9 ± 16.8	76.2 ± 18.7	79.1 ± 14.9	0.90	0.13
Body mass index, kg/m^2	26.9 ± 4.7	26.8 ± 4.7	27.0 ± 4.6	0.53	0.25
Men, %	39.7	41.2	64.5	0.95	< 0.001
Smokers, %	21.9	20.9	22.6	0.99	0.91
Disease duration, yrs*	6.7 ± 6.4	6.3 ± 7.0	6.4 ± 6.3	0.71	0.73
Hemoglobin, g/dl	13.4 ± 1.6	13.3 ± 2.1	14.2 ± 1.4	0.82	< 0.001
Hematocrit, %	41.5 ± 3.8	39.6 ± 5.8	41.6 ± 3.7	0.17	0.41
Erythrocytes (× 10^6/μl)	4.5 ± 0.4	4.3 ± 0.7	4.6 ± 0.5	0.25	0.38
Serum CRP, mg/l*	12.3 ± 16.7	14.7 ± 27.2	13.3 ± 32.9	0.80	0.13
NSAID use, %	56.1	52.0	79.0	0.66	0.009
Rheumatoid factor, kU/l*	248 ± 391	ND	ND		
DMARD use, %	97.3	0	0		

Values are arithmetic means ± standard deviation. * Non-normally distributed variables. Body mass is calculated as weight kg/height m^2. Smokers are self-reported actual smokers (≥ 1 cigarette per day). NSAID: nonsteroidal antirheumatic drugs; DMARD: disease modifying antirheumatic drugs; ND: not determined, i.e., data were available in less than 50% of patients.

Figure 2 depicts the odds ratios for the frequency of RA compared to non-RA patients (OA and CHD) across the quartiles of ET erythrocyte concentrations observed in the RA group. The prevalence of RA patients in the highest quartile of ET concentrations (> 352.6 nmol/g hemoglobin) was about 3-fold of that in non-RA controls, while the prevalence of RA in the lowest quartile of ET concentrations (< 156.6 nmol/g hemoglobin) was about one-fourth of that in the controls. The difference in RA prevalence between the lowest and highest ET quartiles and the increasing RA prevalence across quartiles 1 to 4 were significant ($p < 0.001$ for both comparisons).

Age, sex, BMI, smoking status, duration of disease, CRP, and use of NSAID were independent of RBC ET concentrations in either group by multiple linear regression analysis. Aggregate measures of the coefficient of determination and the corresponding p value were $R^2 = 0.024$, $p = 0.801$ for RA; $R^2 = 0.018$, $p = 0.763$ for OA; and $R^2 = 0.035$, $p = 0.840$ for CHD. Less than 3.5% of the variance in ET levels was predicted by the values of all independent variables together, and none of the partial correlations attained statistical significance ($p > 0.23$), thus adjustment for these variables did not interfere with outcome measures.

In the RA group, use of biologic disease modifying antirheumatic drugs (DMARD) that target the proinflammatory cytokines TNF-α (infliximab, etanercept, and adalimumab) or IL-1 (anakinra) were not associated with different ET concentrations when compared to the use of conventional DMARD (methotrexate, sulfasalazine, and hydroxychloroquine). For treatment with biologic DMARD (28 patients), we determined a mean RBC ET concentration (SD) of 14.3 ± 7.4, median 12.2, and IQR 8.0–18.8 μmol/l; for treatment with conventional DMARD (43 patients) a mean of 14.1 ± 8.6, median 12.6, and IQR 7.9–17.9 μmol/l ($p = 0.738$). Moreover, among patients with RA, serum levels of RF were not correlated with ET concentrations ($R^2 = 0.007$, $p = 0.498$).

To assess the contribution of ET to the antioxidant thiol defense system, we examined the correlation of erythrocyte reduced (GSH) and oxidized (GSSG) glutathione levels with ET concentrations and the occurrence of oxidation products or metal ion complexes of ET. In the RA group, GSH levels (mean ± SD) were lower and GSSG levels were higher than in the OA group (GSH: 1219 ± 1050 vs 1808 ± 833 μmol/l, respectively, $p = 0.046$; GSSG: 61 ± 44 vs 36 ± 25 μmol/l, $p = 0.037$). RBC levels of GSH and GSSG and the GSH/GSSG ratio were independent of the RBC ET content in both groups ($R^2 < 0.010$, $p > 0.680$). Although we were able to generate ET disulfide or copper complexes of ET under nonphysiological experimental conditions, such species were not detected in any of the erythrocyte or plasma samples.

Table 2 shows the distribution of suggested autoimmune disease related polymorphisms in the SLC22A4 gene among the 3 patient groups. There was no significant association of the allelic variants with RA when compared to CHD or OA groups. Further, the SLC22A4 genotype was independent of erythrocyte ET concentrations in all groups according to multiple linear regression analysis — RA: $R^2 = 0.021$ ($p = 0.682$); OA: $R^2 = 0.006$ ($p = 0.912$); and CHD: $R^2 = 0.015$ ($p = 0.835$).

To assess whether the erythrocyte ET content might be associated with the ET content in OCTN1-expressing immune cells, the correlation of ET concentration in RBC with ET concentration and OCTN1 mRNA expression in CD14+ blood cells was determined in a group of healthy volunteers. Figures 3A and 3B show that there was a strong linear association between ET levels in erythrocytes and the ET concentration and OCTN1 mRNA expression in CD14+ cells ($R^2 = 0.936$, $p < 0.001$ and $R^2 = 0.946$, $p < 0.001$, respectively); that is, more than 90% of the variance in the variables was explained by linear correlation. Consistently, no ET was detected in the CD4+ T helper and CD8+ T suppressor cells that were lacking OCTN1 expression.

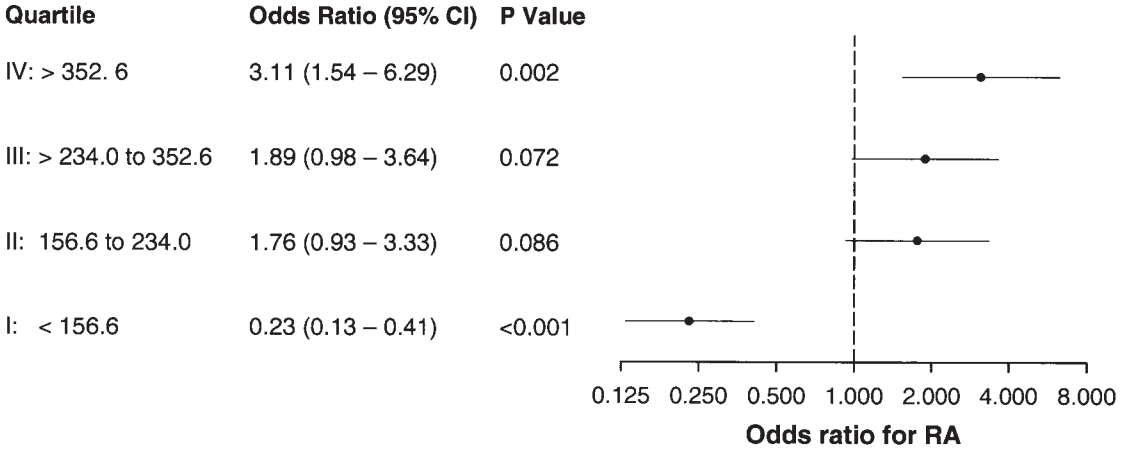

Figure 2. Odds ratios for the frequency of patients with RA compared to patients without RA according to quartiles of ergothioneine erythrocyte concentrations obtained in the RA group. Quartiles of ergothioneine concentrations are expressed in nmol ergothioneine/g hemoglobin. Quartile thresholds represent 25th, 50th, and 75th percentiles of ergothioneine distribution among RA patients.

Table 2. Allelic frequencies of single nucleotide polymorphisms (SNP) of SLC22A4. rs3792876 denotes in intron 1 leading to impaired binding of the transcription regulator RUNX-1 associated with RA in a Japanese population[6]. rs1050152 denotes SNP in exon 9 leading to the amino acid substitution L503F associated with Crohn's disease in Caucasian populations[33,42].

Polymorphism	RA, %	OA, %	CHD, %	p for Difference, RA/OA	p for Difference, RA/CHD
rs3792876 (C→T transition)	4.1	6.1	7.3	0.818	0.390
rs1050152 (C→T transition)	42.4	40.2	40.3	0.725	0.816

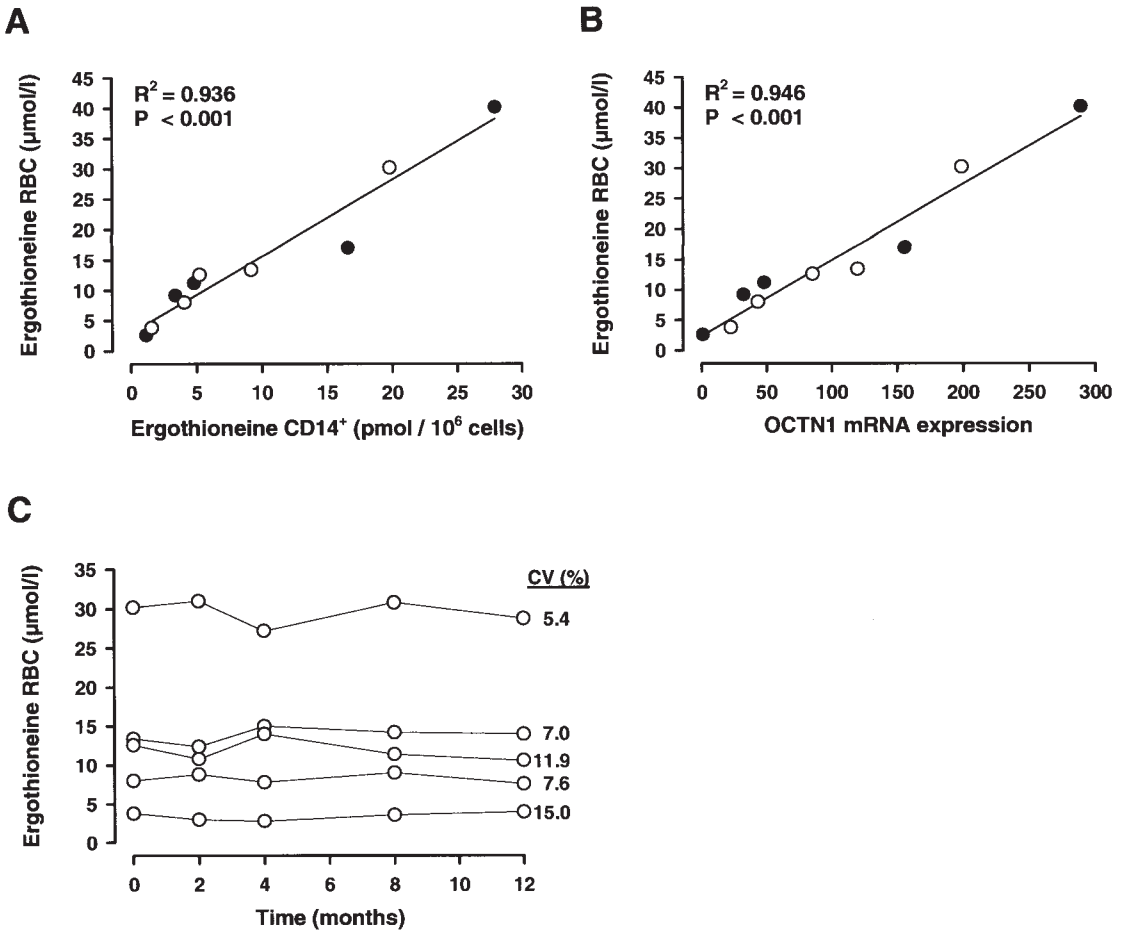

Figure 3. Linear correlation of ergothioneine concentrations in red blood cells (RBC) with the ergothioneine concentration in CD14+ cells (A), and mRNA levels of organic cation transporter OCTN1 in CD14+ cells (B) in 10 healthy subjects. Periodic variability of RBC ergothioneine concentrations (C). Ergothioneine concentrations in RBC are reported in µmole/l of RBC, and in CD14+ cells in picomoles per million cells. OCTN1 mRNA expression was normalized to expression of the housekeeping gene gyceraldehyde-3-phosphate dehydrogenase, and given in factors of the lowest mRNA level (= 1). ○: Individuals with recurrent measurements of RBC ergothioneine content over a period of 12 months. CV: coefficient of variation in percentage of the individual means.

In contrast to the high between-subject variability, the within-subject variability in RBC ET content as determined by recurrent ET measurements over a period of 1 year was extremely low, approximating the interassay coefficient of variation of the detection method (Figure 3C).

DISCUSSION

We observed that erythrocyte ET concentrations are higher in patients with RA with mild disease activity compared to patients with non-RA chronic inflammatory diseases or healthy individuals, with an increase of the RA prevalence

with increasing ET levels. The differences in ET erythrocyte concentrations between RA and non-RA patient groups were not explained by differences in anthropometric or clinical features. In particular, the ET concentration was independent of hemoglobin levels, disease duration, medication, and general inflammation (indicated by CRP), suggesting that elevated ET levels are independently associated with RA.

Genetic polymorphisms in the SLC22A4 gene thought to be implicated in the etiology of RA and Crohn's disease[6,33] — a SNP in intron 1 that impairs binding of the transcription activator RUNX-1, and a SNP in exon 9 that was found to moderately increase ET transport efficiency[29] — were not associated with RA in our study. This is in keeping with results in Caucasian populations[27,34] that also showed no associations between the suggested susceptible variants of SLC22A4 and RA. Analysis of additional SNP found in SLC22A4 and haplotypes similarly revealed no evidence for association with RA[27,34]. Consequently, these polymorphisms could not explain the intersubject variability in erythrocyte ET content. Rather, the observations of high interindividual heterogeneity in SLC22A4 mRNA expression in conjunction with the strong linear correlation with intracellular ET levels indicate that the ET content is essentially determined by (RUNX-1-independent) transcriptional regulation of SLC22A4. The exact mechanisms of SLC22A4 regulation and the reason for the high interindividual variability in expression remain to be elucidated.

We investigated whether the high ET levels in patients with RA might result from the inflammation. However, our findings do not support this hypothesis. First, markers of inflammatory intensity (RF and CRP) and treatment with agents neutralizing proinflammatory cytokines (TNF-α and IL-1) were independent of ET concentrations. Second, despite lower ET levels in the CHD and OA groups the extent of systemic inflammation as expressed by CRP was not different from the RA group, which may be attributed to the selection of CHD and OA patients with high disease activities, while RA patients were characterized by low disease activity scores. The validity of this conclusion may be limited, since we did not determine the differences in inflammatory cytokines or other blood indicators of inflammation. However, serum levels of the acute-phase reactant CRP were found to be well correlated with clinical disease activity in RA[20-22], CHD[17], and OA[18,19]. Third, there were no differences in ET concentrations between the healthy reference group and the OA and CHD groups. Finally, in healthy subjects interindividual differences in SLC22A4 mRNA expression comprised a factor of 300, indicating that inflammatory stimuli are not crucial for OCTN1 transcriptional regulation. Hence, the higher ET levels in RA patients are probably not a consequence of the disease but may be a predisposing factor for the development of RA.

We have previously reported that the intracellular uptake of ET is essentially dependent on the presence of the organic cation transporter OCTN1, a highly efficient and specific ET transporter[9,29]. Failure to detect ET in plasma of fasting subjects thus argues for rapid OCTN1-mediated clearance (elimination or intracellular uptake) from the circulation. OCTN1 was found to be highly expressed in cells of the myeloid lineage, including the erythroid progenitor cells of bone marrow[8,9], while functional expression of OCTN1 was absent in circulating mature erythrocytes and reticulocytes, as indicated by the lack[10] of or only very slow diffusion-controlled uptake[30] of ET into RBC in incubation experiments. Although we did not determine the OCTN1 mRNA or protein expression in the developmental stages of the erythroid lineage, these findings suggest that the ET of erythrocytes originates from accumulation in OCTN1-expressing myeloid progenitor cells, and persists unchanged in the erythrocytes due to the absence of transport mechanisms and its high chemical stability[13,31]. Yet the ET content of peripheral RBC is probably in good agreement with the ET content in OCTN1-expressing myeloid cells, since it was closely correlated with the ET concentration and the expression level of OCTN1 mRNA in CD14+ blood monocytes.

The supply of ET is restricted to the exogenous uptake with nutrition. Nevertheless, our time series data and previous investigations[32] with recurrent ET determinations in the same individuals revealed that actual dietary intake had no apparent effect on erythrocyte ET levels. This may be attributed to the long half-life of the erythrocytes, with a daily turnover rate of 0.8%, suggesting that only changes in mean dietary ET uptake over long periods would result in significant changes of intracellular ET concentrations.

In keeping with our findings several studies, but not others[37-40], have shown decreased blood activities of antioxidant enzymes like glutathione peroxidase, glutathione reductase, or catalase and a depletion of GSH levels in RA[35,36]. Since ET has been proposed to exert protective antioxidant properties[12], we examined whether the elevation of intracellular ET levels may reflect a counterregulatory response to increased oxidative stress. However, the lack of positive or negative associations between erythrocyte GSH (or GSSG) and ET levels and the absence of oxidation products or metal complexes of ET in erythrocytes suggests that ET has little or no antioxidant effects, and that regulation of ET is independent of the oxidative state. In contrast, we recently reported that ET exhibited stimulatory effects on proliferation of OCTN1-expressing cells, whereas GSH elicited antiproliferative effects[29]. A prominent feature in the pathophysiology of RA is an increased proliferation of synoviocytes — the synovial lining becomes hyperplastic and begins to invade and destroy adjacent cartilage and bone[41]. Hence, ET may accelerate transformation of resident synovial cells into a proliferative synovitis, thus promoting progression of disease.

Further studies are needed to examine the biological mechanism of ergothioneine and the organic cation transporter OCTN1 in the pathogenesis of RA. Extending our study, it would be of particular interest to compare patients with active

RA disease with active non-RA inflammatory rheumatic controls to determine whether the elevation of blood ET levels corresponds with RA disease activity and is specific to RA.

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