## Acid sphingomyelinase activity is elevated in the serum of rheumatoid arthritis patients, suppressed by anti-TNF-α treatment

Sirs,

The conserved stress enzyme acid sphingomyelinase (SMPD1) is a key enzyme regulating cell proliferation, differentiation, autophagy, and apoptosis (1) and may be involved in immunological processes during acute and chronic inflammation (2). The enzyme is held responsible for the breakdown of sphingomyelin to ceramide resulting in lipid raft formation and initiating signal transduction pathways by clustering several receptors due to ligand stimulation (1). Rheumatoid arthritis (RA), a chronic autoimmune disease characterised by polyarticular inflammation, shows infiltrations of the synovial tissue with inflammatory cells (3, 4). As a sign of the systemic character of the disease, extra-articular manifestations including the eye, heart, lung, and nervous system are present in about 40% of RA patients and these manifestations are associated with a high risk for premature death (5). TNF- $\alpha$  and IL-1 $\beta$ are principal mediators involved in the overwhelming cytokine cascade and are used as targets for clinically common antibody therapy (6). Recently, it has been demonstrated that acid sphingomyelinase may play a key role in the pathophysiology of RA and that its genetic as well as pharmacological inhibition results in an improved outcome in a preclinical model of RA (7). Furthermore, its product and mediator ceramide has been found to be increased in the synovial fluid of RA patients (8).

Supporting these data, we demonstrate significantly increased serum activity levels of SMPD1 in RA patients (median: RA 176.5 pMol/ml\*h-1, n=44) as compared to healthy controls (median: HC 134.0 pMol/ml\*h-1, n=25; 1.32-fold increase). However, no correlations were observed between serum SMPD1 activity and clinical parameters of the patients [e.g. Disease Activity Score 28, erythrocyte sedimentation rate (ESR), the levels of C-reactive protein or the number of criteria defined by the American Rheumatism Association (ARA; now American College of Rheumatology (ACR)]. The influence of specific TNF- $\alpha$  blockade on the serum levels of SMPD1 was analysed in a separate patient cohort treated

**Table I.** Clinical and demographic parameters from a total of 52 RA patients and 25 healthy volunteers. Previous treatment with the anti-TNF- $\alpha$  biologics infliximab or etanercept was an exclusion criterion for these patient groups. Data are presented as mean  $\pm$  standard error of the mean (SEM).

	RA (serum)	RA (α TNF/MTX)	Healthy controls
n.	44	8	25
Age [years]	$57.0 \pm 2.0$	$54.1 \pm 4.3$	$31.4 \pm 1.6$
Disease duration [years]	$13.1 \pm 1.5$	$12.8 \pm 3.8$	-
Rheumatoid factor [U/mL;	73.5 ± 22.5	92.8 ± 35.2	n.d.
positive/negative]	33/11	6/2	n.d.
ESR [mm/h]	$23.3 \pm 2.5$	$28.6 \pm 9.9$	n.d.
C-reactive protein [mg/L]	$17.1 \pm 3.3$	$10.2 \pm 2.6$	n.d.
Number of ARA criteria (RA)	$4.6 \pm 0.3$	$4.3 \pm 0.6$	-
Leukocytes [Gpt/L]	8.1 0.4	8.0 ± 0.6	n.d.

n.d.: not determined.



Fig. 1. A: SMPD1 activity in serum samples of healthy controls (HC; n=25) and RA patients (RA; n=44). In comparison to healthy controls activity of SMPD1 was significantly increased in the serum of RA patients (Mann-Whitney-U-test;  $p \le 0.001$ ).

**B:** Serum SMPD1 activity in patients undergoing specific TNF- $\alpha$  blockade by adalimumab (baseline and 2 weeks, n=8; 20 mg weekly or 40 mg biweekly for a total of 2 years) was strongly and significantly suppressed within one year after initiation of treatment (3 months and 1 year, n=5 each; Mann-Whitney-U-test;  $p \le 0.05$ ).

with the anti-TNF- $\alpha$  antibody adalimumab (Humira®; n=8; 20 mg weekly or 40 mg biweekly for a total of 2 years). Prior to adalimumab treatment, none of these patients had received any other biologic agent and concurrently received standard anti-rheumatic therapy with acetylsalicylic acid (100 mg), sulfasalazine (500 mg), and/or prednisolone (5-7.5 mg). Baseline levels of serum SMPD1 in RA patients prior to therapy revealed a serum activity of 266.1 pMol/ml\*h-1, indicating a high systemic inflammation level in the treated patient cohort. Two weeks after initiation of anti-TNF-α therapy, the activity of SMPD1 in the serum of RA patients was not yet significantly reduced (median: 258.5 pMol/ml\*h-1). Within 1 year after the initiation of therapy, however, serum SMPD1 activity levels stepwise and significantly decreased (median: 179.2 pMol/ml\*h-1 at 3 months and 120.3 pMol/ml\*h-1 at 1 year; n=5; both p=0.043) to the levels determined in healthy controls (dotted line). This was further supported by a significant correlation between the decrease of serum SMPD1 activity levels in anti-TNF-atreated RA patients and the therapeutic efficacy (as defined by the ACR 20, ACR 50, and ACR 70 response criteria; r=0.550, p=0.006, n=24).

According to previous reports, SMPD1 knock-out animals demonstrated less pronounced joint swelling and levels of pro-inflammatory cytokines in arthritic joints despite normal T-cell function (7). Accordingly, Beckmann et al. discussed defects in lymphocyte adhesion caused by SMPD1 deficiency in immune or endothelial cells (7, 9). However, the exact mechanisms remain unresolved. The increased enzyme levels in RA patients are likely a response to the ongoing systemic inflammation. Treatment with anti-TNF-a antibodies in RA patients with high serum levels of SMPD1 resulted in a successful inhibition of enzyme activity, suggesting its association to the systemic inflammatory status of the patients. Therefore, the serum levels of SMPD1 may be suitable as a biomarker to monitor disease activity in RA patients. In a small-sized observational trial, Hanaoka et al. described a 1.4-fold increase (present study 1.32-fold increase) of serum SMPD1-activity in patients with RA, which was weakly correlated with P-selectin, but not with other measures of disease activity, or the functional status and quality of life (10). This is in agreement with the occurrence of elevated SMPD1 activity levels in other acute and chronic inflammatory diseases, such as sepsis (11), diabetes (12), and cardiac heart failure (13). Therefore, its enzyme activity may rather be a general indicator for immune activation than an RA-specific marker. As a limitation, this study lacks information regarding the SMPD1 activity in other rheumatic diseases as disease controls.

In conclusion, little is currently known about the role of SMPD1 in the pathophysiology of systemic RA. Therefore, further studies are warranted to clarify the role of SMPD1 and its potential as a therapeutic target. Furthermore, the enzyme activity could be used as a biomarker to monitor disease activity and therapeutic success, especially in the case of treatment with anti-TNF- $\alpha$  antibodies.

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