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*Original Article*

# T-cell immunoglobulin and mucin domain 3 is upregulated in rheumatoid arthritis, but insufficient in controlling inflammation

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**Abstract:** Objectives: Rheumatoid arthritis (RA) is a chronic autoimmune disease, that involves both pro- and anti-inflammatory mechanisms. The purpose of the present study is to investigate T-cell immunoglobulin and mucin domain 3 (Tim-3) in RA. Methods: Plasma levels of soluble (s) Tim-3 in early RA (n=98), were followed, to evaluate association with treatment and disease activity, acquired from a prospective collected biobank (clinicaltrials.gov (NCT00660647)). We also investigate the influence of Tim-3 on spontaneous cytokine production in synovial fluid mononuclear cells (SFMC) from RA patients after addition of neutralizing anti-Tim-3's antibodies, either alone or in combination with neutralizing anti-Programmed Cell death protein 1 (PD-1) antibodies. Results: Long-time stimulated CD4 T-cells expressed high levels of Tim-3, but tended to decrease their PD-1 expression. Tim-3 expression was exclusively seen co-expressed with PD-1 by CD3, CD4, CD45RO positive cells in the inflamed RA joint. Addition of neutralizing Tim-3 antibodies increased the secretion of IFNγ and MCP-1, in SFMC cultures from RA. Whereas neutralizing anti-PD-1 antibodies showed a broader impact on cytokine production. Finally, we observed that soluble Tim-3 is increased in plasma and is associated with disease activity in early RA. Conclusion: Taken together, our findings indicate disease-suppressive functions of Tim-3 in RA.

**Keywords:** Rheumatoid arthritis, Tim-3, anti-TNF-alfa, DAS28CRP

## Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease dominated by continuous T cell activation leading to joint destruction [1]. In recent years it has become apparent that co-inhibitory receptors (CIRs), like Programmed cell death protein 1 (PD-1), Lymphocyte Activation Gene 3 (LAG-3) and Cytotoxic T-Lymphocyte associated Antigen 4 (CTLA-4), are important in modulating the immune system and thus the development and progression of autoimmune diseases. This is confirmed by the increased likelihood of developing chronic inflammatory conditions, including RA-like disease, in response to antibody treatments that inhibit the function of CIRs [2, 3]. The importance of CIRs in RA, is supported by the use of CTLA-4: Fc (Abatacept), in RA [4]. Abatacept is most efficient for inducing remission early in disease, supporting that effector T cell activity, involving CIR's is most active in early phases of the disease [5].

T-cell immunoglobulin and mucin domain 3 (Tim-3) is a transmembrane protein that is recognized as a CIR [6]. Tim-3 is primarily expressed by activated T cells, including $CD4^+$ and $CD8^+$ T cells, but also by monocytes and dendritic cells [7, 8]. Among T cells, Tim-3 is associated with exhausted T cells. Exhausted T cells, both $CD4^+$ and $CD8^+$, develop during chronic immune stim-

ulation and are characterized by downregulation of transcription factors and loss of effector function. This includes loss of cytokine production in a stepwise manner. First IL-2, then TNFα and lastly the ability to produce IFNγ is lost, as cells advance towards a more severe exhaustion profile [9]. Upregulation of multiple CIRs is another characteristic feature that marks exhausted T cells. Particularly, high expression of PD-1, often co-expressed with other CIRs has been suggested to identify exhausted T cells [9-12]. Since the proportion of exhausted T cells has showed to predict fewer flares and a better prognosis in many autoimmune diseases, more knowledge regarding the role of CIRs, including Tim-3 and its mechanism of action, is essential for a better understanding and treatment of autoimmunity [13].

The co-inhibitory property of Tim-3 is first and foremost described as supressing Th1, but to some extent also Th17 cell responses. This includes induction of Th1 apoptosis upon interaction between Tim-3 and Galectin-9 (Gal-9) [14, 15]. Apart from Gal-9, Tim-3 has three other known ligands; Carcinoemryonic Antigen-related Cell Adhesion Molecule 1 (CEACAM-1), High Mobility Group Box 1 (HMGB1) and phosphatidylserine (PtsSer). These are less studied, but like Gal-9, they seem to promote an inhibitory immune response upon interaction with Tim-3 [16].

Besides expression of transmembrane Tim-3, shedding of Tim-3 (sTim-3) by A Disintegrin and Metalloprotease (ADAM) 10 and ADAM-17 has been described [17, 18]. Elevated plasma soluble (s)Tim-3 is reported in patients with both Human Immunodeficiency Virus type 1 (HIV) infection or Systemic Lupus Erythematosus (SLE). It has been proposed that sTim-3 counteracts the function of the transmembrane Tim-3, as suggested for others CIRs [18-20].

In RA patients, increased expression of Tim-3 by $CD4^+$ T cells, $CD8^+$ T cells and monocytes is reported [21, 22]. In several cross-sectional studies the expression of Tim-3 on T cells has been shown to correlate inversely with disease activity assessed by Disease Activity Score 28 CRP (DAS28CRP) [21, 23, 24], suggesting that the presence of T cells with increased Tim-3 expression is associated with a more favourable disease profile. Despite these associations, it remains to be fully elucidated if Tim-3 modulates the inflammatory response in RA. Thus, the purpose of the present study is to clarify Tim-3's role in an RA environment, and to investigate sTim-3 as a new potential biomarker for the disease.

## Materials and methods

### *Patient material*

From chronic RA (cRA) patients, who presented with disease flare, with swollen joint, peripheral blood (PB) and paired synovial fluid (SF) were collected. SF was collected by experienced rheumatologists during therapeutic joint aspiration. All of these patients had RA for more than 8 years and received a mixture of DMARD. From PB and SF, peripheral blood mononuclear cells (PBMC) and synovial fluid mononuclear cells (SFMC) were isolated and stabilized in EDTA followed by Ficoll gradient centrifugation (n=8 for flow, n=9 for SFMC cultures). Furthermore, plasma and cell free synovial fluid were isolated from cRA samples (n=17). At the time of sample collection, DAS28CRP was recorded for all cRA patients. Plasma samples from early RA (eRA) patients (n=98) were included from the OPERA cohort (OPtimized treatment in Early RA). The study is described in detail elsewhere [25]. In short; patients were treatment-naïve, with symptoms less than 3 months. All patients fulfilled the ACR 1987 criteria for RA. Patients are randomized into treatment with conventional disease modifying anti-rheumatic drug (DMARD) + placebo or conventional DMARD + anti-TNFα treatment. From each patient, plasma samples at baseline, after 3 months and after 12 months of treatment were analysed. At time of all plasma samples and after 2 years of treatment, clinical data were collected including DAS-28CRP, Visual Analog Scale (VAS) and Total Sharp Score (TSS). All patients provided written informed content to participate in the study. The Danish Data Protection Agency and the Ethics Committee at Region Midt approved the OPERA study (20070008) and the collection of synovial fluid and peripheral blood from chronic RA patients for isolation of plasma, SFMCs and PBMCs (20121329).

PBMC from healthy volunteers (HV) (n=6) were collected and treated in the same way as cells from cRA patients. Furthermore, plasma from HVs (n=44) were collected. HV were included

**Table 1.** Characteristics of patients with early rheumatoid arthritis (eRA), chronic RA (cRA) and healthy volunteers (HV)

| | Group characteristics | | |
|---|---|---|---|
| | eRA | cRA | HV |
| Flow cytometry | | | |
| Number of subjects | - | 8 | 6 |
| Male/female | - | 4/4 | 2/4 |
| Median age (range) | - | 55 (25-64) | 58 (23-65) |
| Culture | | | |
| Number of subjects | - | 9 | - |
| ELISA (sTim-3) | | | |
| Number of subjects | 98 | 17 | 44 |
| Male/female | 25/73 | 7/10 | 16/28 |
| Median age (range) | 56 (18-86) | 48 (24-71) | 49 (22-74) |

based on similarities in age and gender as cRA and eRA patients, respectively (**Table 1**). All HV samples were obtained from an established collaboration with the Danish Blood Bank at Aarhus University Hospital. Cells were stored at -150°C. Plasma and synovial fluid samples were stored at -80°C.

*Long-term in vitro stimulation of CD4 T cells*

$CD4^+$ T cells were isolated from HV PBMC, by negative selection using EasySep Human $CD4^+$ T Cell Isolation Kit (Stemcell). Approximately $3\times10^5$ isolated $CD4^+$ T cells/well were seeded in a 48-well plate and activated, either transiently or persistently, with Dynabeads Human T-activator CD3/CD28 (Thermo Fisher Scientific) in a bead-to-cell ratio of 1:2 and cultured in RPMI (Sigma-Aldrich) supplemented with 10% FCS, 10 mM HEPES (Gibco), 2 mM glutamine (Gibco), and 1 ng/ml of recombinant human IL-2 (Sigma-Aldrich). Cell culture media was refreshed every 3-4 day. Prior to staining for flow cytometry, the cultured $CD4^+$ T cells were blocked with Human IgG (CSL Bering) in a concentration of 50 µg/ml for 15 minutes.

*Flow cytometry*

The cells from long-term stimulation cultures were subsequently stained with the following antibodies αCD3-FITC (UCHT1), αCD25-Alexa Flour 700 (BC96), αTim-3-Brilliant Violet 711 (F38-2E2), αCTLA-4-PerCP-Cy5.5 (L3D10), αLAG-3-Brilliant Violet 421 (11C3C65), αPD-1-PE (EH12.2H7) (all BioLegend), αCD4-PE-CF594 (RPA-T4), αCD127-Brilliant Violet 786 (HIL-7R-M21) (both BD Horizon), and LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (Life Technologies). All antibodies were titrated prior to use. OneComp eBeads (eBioscience) was stained with each antibody individually and used to compensate for spectral overlap. Data were acquired on a BD LSR Fortessa flow cytometer (BD Biosciences) and processed in FlowJo (Tree Star). PBMC and SFMC from cRA patients and HV were stained with a 7-color panel including following antibodies; αCD3-FITC (UCHT1), αCD4-BV605 (RPA-T4), αCD45RO-APC (UCHL1), αCD14-Brilliant Violet 785 (M5E2), αTim-3-Brilliant Violet 510 (F38-2E2), αPD-1-PE (EH12.2H7) (all BioLegend) and LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (Life Technologies), in Brilliant Stain Buffer (cat: 563794, BD Biosciences) in combination with stain/wash buffer (PBS + 0.5% BSA + 0.09% $NaN_3$ + 2 mM EDTA). Fc block with 10 µg/ml human IgG (Beriglobin, CSL Behring) was included [26]. Cells were analysed on the Novocyte Flow Cytometer (ACEA Bioscience). Doublets and dead cells were excluded, and memory Th cells were identified by CD3, CD4 and CD45RO gates. Fluorescence Minus One (FMO) was included for Tim-3 and PD-1. Data was analysed using FlowJo Version 10.5.3.

*Cell cultures*

SFMC from cRA patients were thawed and cultured in T cell media (RPMI + 10% FCS + 1% HEPES, 1% Glutamax, 1% Penicillin/Streptamycin, 15 µg/mL Gentamycin) for 48 hours with 10 µg/mL of one of the respective compounds: αTim-3 antibody (Biolegend, clone: F38-2E2) and/or αPD-1 antibody (Keytruda®, MSD). Stimulation with Dynabeads Human T-activator CD3/CD28 (Thermo Fisher Scientific) was used as a positive control. For each patient, an untreated control (cells that were treated and cultured similarly but without any antibody treatment) were included. Cells were visualized with microscopy at day 0, after 24 hours, and after 48 hours. After 48 hours the supernatants were harvested and frozen at -80°C.

*Mesoscale V-plex*

Supernatants from the previously mentioned SFMC cultures were thawed and IFNγ, IL-1β,

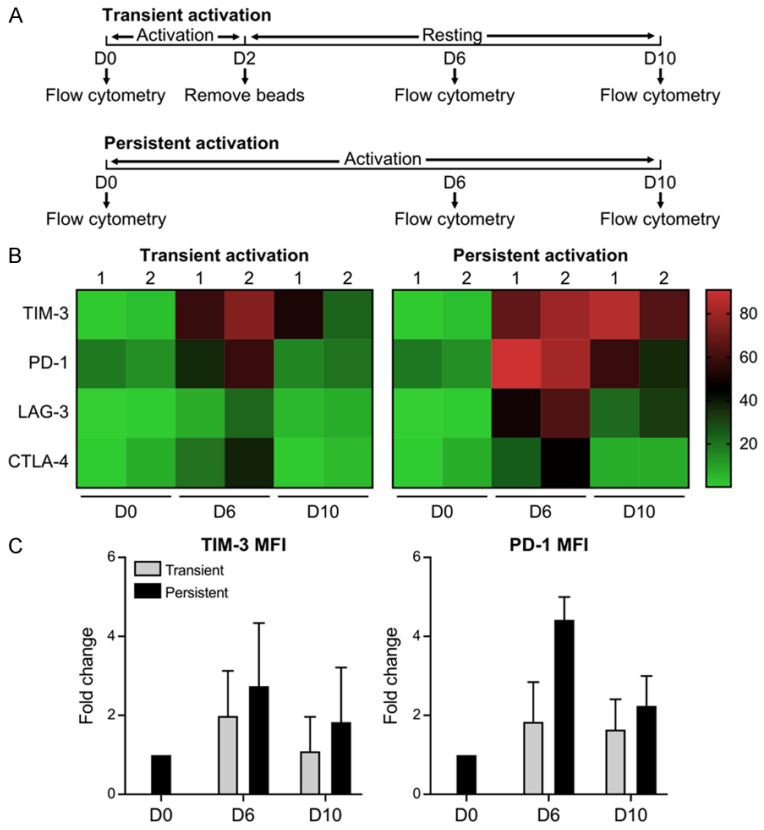


**Figure 1.** Increased Tim-3 expression after persistent T cell activation. A. Transient and persistent activation of CD4$^+$ T cells stimulation of T cells through the CD3/CD28 axis, results in sustained Tim-3 expression. B. Heatmap showing the frequency of CD3$^+$CD4$^+$ cells with increased expression of the indicated surface receptors at different time-points. D = days. C. Median fluorescence intensity (MFI) of Tim-3 and PD-1 respectively, in either transiently or persistently activated CD4$^+$ T cell cultures at the indicated time-points. Data are normalized to the MFI at D0 and show mean + SD.

IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and TNFα were measured by V-plex (Meso Scale Discovery®, cat: K15049D-1, V-PLEX® Proinflammatory Panel 1 (human)) according to manufactures protocol with samples diluted 1:2.

*Enzyme linked immunosorbent assay (ELISA)*

*MCP-1:* Supernatants from the SFMC cultures were thawed and MCP-1 was quantified as described by manufactures protocol (Biolegend, cat: 438804, Human MCP-1/CCL2). Samples were diluted 1:4.

*Tim-3:* Quantification of sTim-3 concentration in plasma from both eRA and cRA patients, SF from cRA, and plasma from HV were assessed by a human Tim-3 ELISA kit (R&D systems, cat: DY2365). The kit was used in accordance with the manufacturer's instructions with the following modifications: The kit was optimized for interfering antibodies by blocking the plates with skimmed milk (5% in 0.5% BSA in PBS) and pre-incubation of all samples and standards with mouse, bovine and goat IgG antibody (10 µg/ml mouse, 30 µg/ml bovine, 30 µg/ml goat) [27]. Plasma samples were diluted 1:8, SF 1:50, and HV plasma were diluted 1:4. Linearity between dilutions was confirmed for SF and plasma of both HV and RA patients (data not shown). For both MCP-1 and sTim-3 ELISA, the respective minimum detection limits (cut-offs) were calculated as two standard deviations of the blanks, respectively 0.01 ng/ml and 0.61 ng/ml. The optical density (OD) was measured at 450 nm with a reference at 570 nm using Thermo Scientific Multiscan GO reader.

*Statistical analysis*

For normally distributed data, students t-test was applied, whereas data that did not fit a normal distribution were analysed with either Wilcoxon rank sum test or Mann-Whitney U test. The normality assumption was tested throughout the study by the use of QQ normality plots. Spearman correlation ($\rho$) were used to investigate relations between sTim-3 and clinical follow up status. *P*-values <0.05 were considered significant.

## Results

*Long-term stimulation of CD4 T cells results in sustained Tim-3 expression*

The expression of Tim-3 on activated CD4$^+$ T cells over time, was addressed by stimulating CD4$^+$ T cells transiently (48 hrs), or persistently (10 days), and examining their surface expression of multiple co-inhibitory receptors associated with T cell exhaustion (**Figure 1**). Following

persistent activation, the majority of the cells expressed both Tim-3 and PD-1 after 6 days of activation (**Figure 1**). Persistently activated $CD4^+$ T cells only showed sustained expression of Tim-3 over time, whereas the cells gradually decreased their expression of PD-1, CTLA-4 and LAG-3. The median fluorescence intensity (MFI) of Tim-3 remained high after 10 days of persistent stimulation. By contrast, expression of PD-1 peaked at day 6 and decreased slightly at day 10 (**Figure 1**).

*High percentage of Tim-3$^+$ T cells at the active site of inflammation in RA*

In cRA patients, the percentage of Tim-$3^+$ memory Th cells ($CD3^+CD4^+CD45RO^+$) in the SF was significantly increased, compared with autologous PBMC ($P<0.01$) and PBMC from HV ($P<0.05$) (**Figure 2**). $CD3^+CD4^-CD45RO^+$ cells, most likely activated Tc cells, from SF showed a similar profile. In the SF, more than 80% of Tim-$3^+$ memory Th cells also co-expressed PD-1 (**Figure 2**). This was significantly higher than in the blood of both cRA and HV, ($P<0.001$ and $P<0.01$, respectively) (**Figure 2**). Analysing MFI showed similar results (data not shown).

We also examined associations between Tim-3 expression and disease activity. We observed a tendency towards an inverse association between percentage of memory Th cells co-expressing Tim-3 and PD-1 in the SF and DAS28CRP ($r^2=0.487$, $P=0.05$). Among $CD3^+CD4^-CD45RO^+$ cells from SF, Tim-3 and PD-1 co-expression were not associated with DAS28CRP ($r^2=0.009$).

*Blocking Tim-3 and PD-1 in an RA environment increase cytokine production*

This led us to examine the effect of blocking Tim-3 and PD-1 in an *ex vivo* RA model. In the SFMC culture, neutralizing Tim-3 and PD-1 antibodies enhanced the inflammatory response compared with untreated samples. A significant increase in IFNγ and MCP-1 production was detected when blocking Tim-3 alone ($P<0.05$, $P<0.01$) (**Figure 3**). In general, blocking PD-1 alone triggered a broader spectrum of the measured cytokines to increase in concentration, including IFNγ, IL-10, IL-13, IL-12p70, TNFα and MCP-1 (**Figure 3**). Blocking both Tim-3 and PD-1 did not synergistically affect the proinflammatory response.

*sTim-3 is highly increased in RA synovial fluid*

We then turned to examine sTim-3 in patients with cRA. In SF from patients with cRA, the sTim-3 concentration was significantly elevated compared with paired plasma concentrations and plasma from HV (both $P<0.0001$) (**Figure 4**). No significant correlation was observed between plasma and autologous SF concentrations of sTim-3.

*Plasma sTim-3 is elevated in RA and associated with clinical disease activity and radiographic progression*

In eRA patients, plasma sTim-3 concentration was increased at baseline (0 month) compared with HV ($P<0.0001$) (**Figure 4**). After 3 months of treatment, the plasma sTim-3 concentration decreased significantly ($P<0.0001$), though the mean concentration among eRA patients was still significantly increased compared with HV ($P<0.0001$). Even after one year of treatment, using a treat-to-target protocol, plasma sTim-3 declined, but remained elevated in eRA patients and did not decline to the sTim-3 plasma levels of HV (**Figure 4**).

Patients with eRA were treated with either DMARD only, or DMARD in combination with anti-TNFα antibodies (Adalimumab), but difference in treatment was not related to change in plasma sTim-3 over time (data not shown).

In eRA, plasma sTim-3 concentration at baseline correlated with baseline disease activity assessed by DAS28CRP, swollen joints (count 28) and painful joints (count 28). The correlation between baseline sTim-3 and DAS28CRP remained at 24 months of treatment (**Table 2**). No other correlations between baseline sTim-3 concentration and paraclinical or clinical outcome were observed, neither anti-CCP levels nor IgM-RF.

We divided eRA patients into two groups based on plasma sTim-3 concentration at baseline: A *low* sTim-3 group that did not exceed the highest sTim-3 concentration detected among HV and a *high* sTim-3 group, whose plasma sTim-3 concentrations all were above the highest sTim-3 concentration detected among HV. Of all patients categorised to the *low* sTim-3 group, 80% were in remission after two years of treatment, whereas only 65% of patients in the

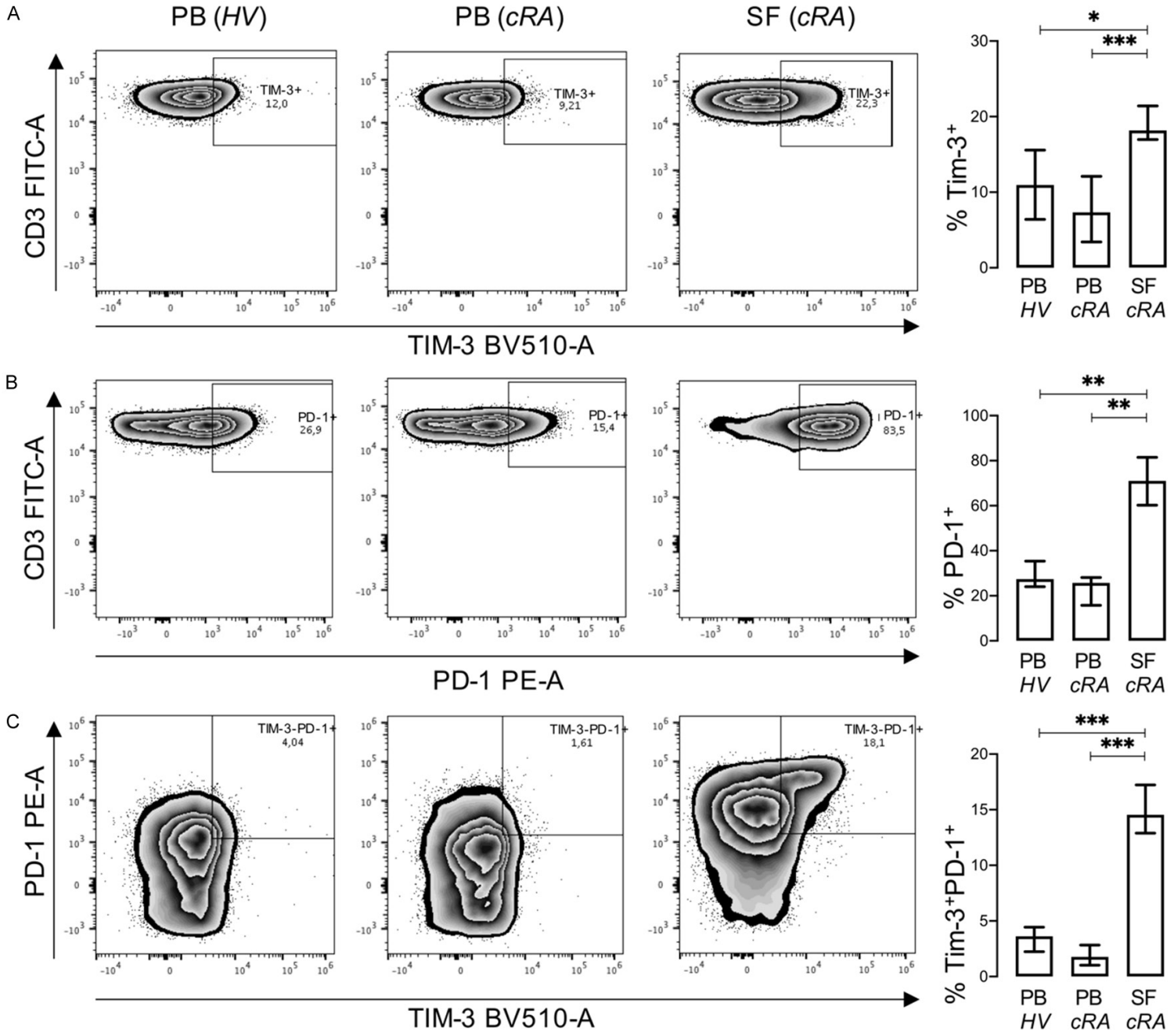
A
PB (HV)
PB (cRA)
SF (cRA)
CD3 FITC-A
TIM-3 BV510-A
TIM-3+ 12,0
TIM-3+ 9,21
TIM-3+ 22,3
% Tim-3+
PB HV
PB cRA
SF cRA
B
PD-1+ 26,9
PD-1+ 15,4
PD-1+ 83,5
PD-1 PE-A
% PD-1+
C
TIM-3-PD-1+ 4,04
TIM-3-PD-1+ 1,61
TIM-3-PD-1+ 18,1
% Tim-3+PD-1+

**Figure 2.** Increased percentage of Tim-3$^+$ and PD-1$^+$ cells among CD3$^+$CD4$^+$CD45RO$^+$ in the synovial fluid (SF) of chronic RA (cRA) patients (n=8) compared with peripheral blood (PB) and HV PB (n=6). The majority of Tim-3$^+$ cells from SF co-express high levels of PD-1. Column graphs show percentage of positive gated cells as median (IQR). $^*$P<0.05, $^{**}$P<0.01, $^{***}$P<0.001.

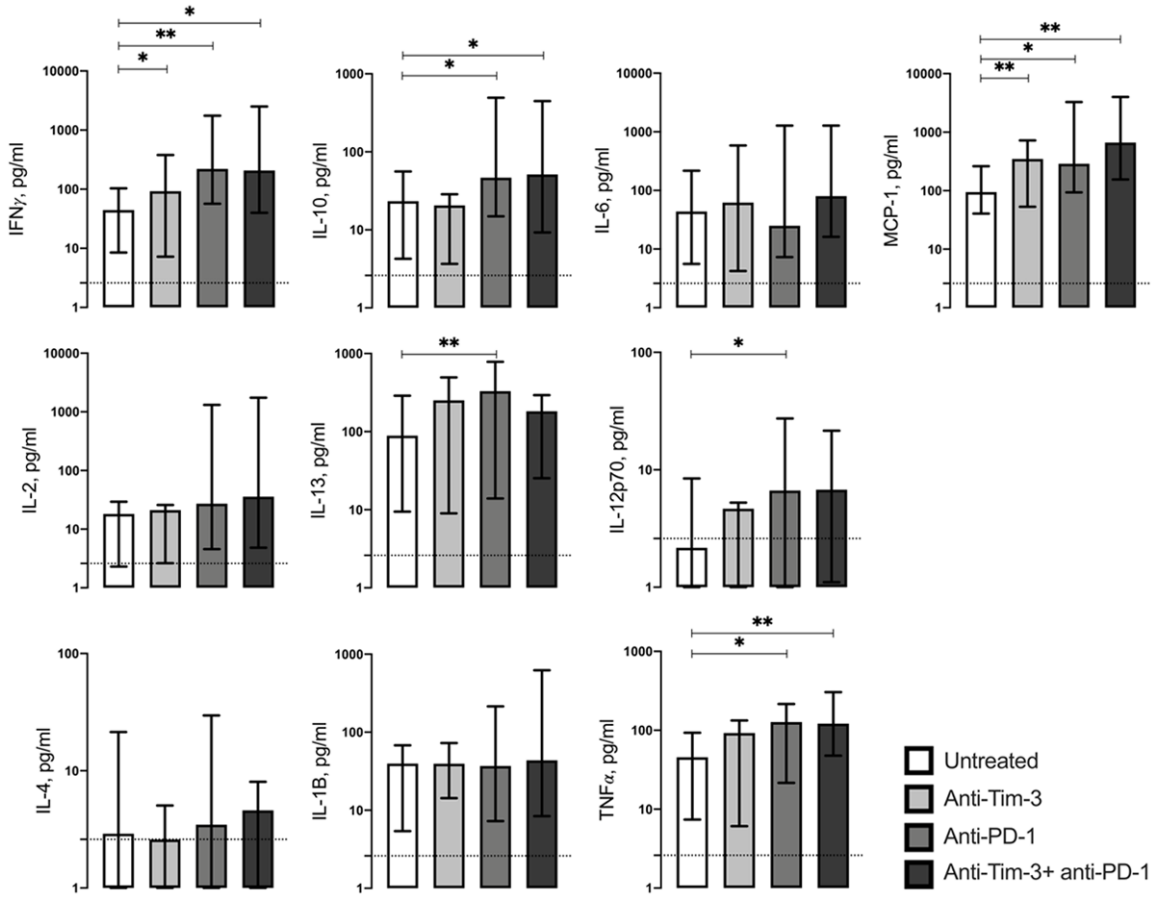


**Figure 3.** SFMC were cultured for 48h from chronic RA (cRA) (n=9) with and without added neutralizing antibodies toward TIM3 and PD-1. Supernatants were collected and cytokines were measured by V-plex and ELISA for MCP-1. 0: untreated sample; I: anti-Tim-3 antibody (10 μg/ml); II: anti-PD-1 antibody (10 μg/ml); III: anti-Tim-3 (5 μg/ml) + anti-PD-1 (5 μg/ml) antibodies. Data are shown as median, bars are IQR. All significant results are shown. $^*$P<0.05, $^{**}$P<0.01.

*high* sTim-3 would be in remission after two years of treatment (remission defined as DAS28CRP ≤2.6).

To examine if sTim-3 plasma levels were associated with progression in joint destruction, eRA were divided into patients with *no progression* or *progression*. We defined progression as increase in TSS after 24 months of treatment. Baseline levels of sTim-3 were not related to progression in TSS. However, the *progression* group, showed a greater decrease in plasma sTim-3 concentration the first three months of treatment ($\Delta$sTim-3$^{3\text{months-baseline}}$) compared with the *no progression group* (P<0.05) (**Figure 5**).

## Discussion

Tim-3, together with PD-1, is recognized as central CIRs in the immune reaction. Here we show that Tim-3 is highly expressed by memory-prone Th cells in RA and that both Tim-3 and PD-1 play an active role within the inflamed RA joint. Furthermore, we show that sTim-3 can be detected in RA and is associated with disease activity and progression.

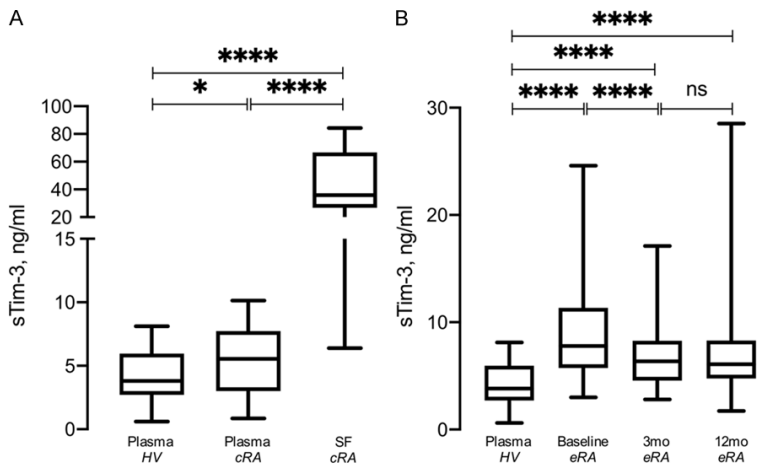


**Figure 4.** Increased sTim-3 in synovial fluid (SF) and in plasma from RA patients (A, B). Plasma sTim-3 decreases in early RA (eRA, n=98) after 3 months of treatment (B). Chronic RA (cRA, n=17), healthy volunteers (HV, n=44). $^{*}$P<0.05, $^{****}$P<0.0001.

It has been described earlier that a higher degree of T cell exhaustion in autoimmune diseases is associated with a better prognosis and fewer flares in RA [13]. All cRA patients included in our analyses did indeed present with disease flare (DAS28CRP >2.6), meaning that their "exhaustion profile" was not sufficient to prevent disease flares or disease activity. However, we did observe a tendency towards an inverse association between percentage of double positive (Tim-3$^{+}$PD-1$^{+}$) memory Th cells in the SF and the severity of disease activity assessed by DAS28CRP. This supports that a high prevalence of Tim-3$^{+}$PD-1$^{+}$ memory Th cells in the inflamed joint, is associated with lower disease activity in RA, as reported earlier [21, 23, 24].

Exhausted T cells and the CIRs associated herewith, are acknowledged as important mediators in immunological and rheumatological research. It still remains to be elucidated to what extend Tim-3, alone and in cooperation with PD-1, impacts the inflammatory environment in RA. As most T-cell driven animal models are comparable with an immunization, or hypersensitivity type IV reaction, without formation of anti-CCP antibodies or IgM-RF, we turned to an *ex vivo* cultured RA synovial cell model. It is a unique model representing mononuclear cells from actual diseased tissue taken from the site of pathology and dominated by monocytes and T cells. It is driven by the spontaneous endogenous production of a diversity of pro-inflammatory factors without the addition of exogenous stimulation.

Using this setup, we confirmed that Tim-3 indeed regulates the Th1 associated cytokines response in RA, as IFNγ and MCP-1 increased significantly after blocking Tim3. The *ex vivo* study also confirmed that PD-1 have a broader immunosuppressive role than Tim-3, as neutralisation of PD-1 resulted in increased cytokine production of many different cytokines. Interestingly, IL-2 production was not affected by neither of the two blocking agents, and TNFα only increased after PD-1 blockade. Our present finding supports cancer studies of a stepwise loss of cytokine production characteristic for exhausted T cells. T cells that express Tim-3, and also express high levels of PD-1, are at an "exhaustion step" where especially IL-2, but also TNFα production, cannot be recovered by blocking Tim-3.

Measurement of sTim-3 is presently not used in a clinical setup. We found an elevated plasma sTim-3 concentration, both in eRA and cRA patients compared with HV. This is in agreement of a recent study [28, 29], and with reports from SLE and HIV, suggesting that sTim-3 is upregulated in diseases with chronic immune load [18, 19]. We observed that in eRA a lower plasma sTim-3 concentration at diagnosis predicts a more favourable prognosis in terms of DAS28CRP. That a higher concentration of sTim-3 in plasma indicates a poorer prognosis, may seem counterintuitive. It is, however, in line with our observation that Tim-3 is expressed following TCR stimulation. Lower plasma sTim-3 concentration, could then be a reflection of lower degree of T-cell activity early in disease. Another explanation could be that cleavage of Tim-3 directly drives the T cells to a less exhausted profile. sA similar phenomenon has been reported for LAG-3 [20]. Finally, sTim-3 is able to bind soluble Gal-9, thereby shielding its contact to membrane expressed Tim-3 and influencing osteoclastogenesis [30]. Either way, these explanations attribute plasma sTim-

**Table 2.** Correlation betweeen baseline plasma sTim-3 concentration and clinical and paraclinical follow up on early RA (eRA) patients (n=98)

| | Clinical and paraclinical characteristics | | | Correlations between baseline sTim-3 plasma concentration and clinical/ paraclinical data | | |
|---|---|---|---|---|---|---|
| | Median (IQR) | | | ρ (*p*-value) | | |
| | *Baseline* | *3 months* | *2 years* | *Baseline* | *3 months* | *2 years* |
| CRP | 14 (7-35) | 7 (7-8.8) | 7 (7-7) | 0.14 (0.19) | -0.05 (0.61) | 0.13 (0.23) |
| DAS28CRP | 5.7 (5.1-6.4) | 2.1 (1.8-3.2) | 2 (1.8-2.8) | 0.28 (0.005)** | -0.01 (0.92) | 0.24 (0.02)* |
| Painful joints count 28 | 12 (8-15.5) | 0 (0-2) | 0 (0-1) | 0.21 (0.04)* | 0.01 (0.90) | 0.08 (0.47) |
| Swollen joints count 28 | 10 (7-14.5) | 0 (0-0) | 0 (0-0) | 0.29 (0.003)** | -0.02 (0.88) | -0.02 (0.8) |
| TSS | 1 (0-4) | - | 3.5 (1-6) | 0.17 (0.1) | - | -0.00 (1) |

*P<0.05, **P<0.01. C-Reactive Protein (CRP) (mg/L), Disease Activity Score 28 CRP (DAS28CRP), Total Sharp Score (TSS). Correlation was carried out by Spearman rho (ρ).

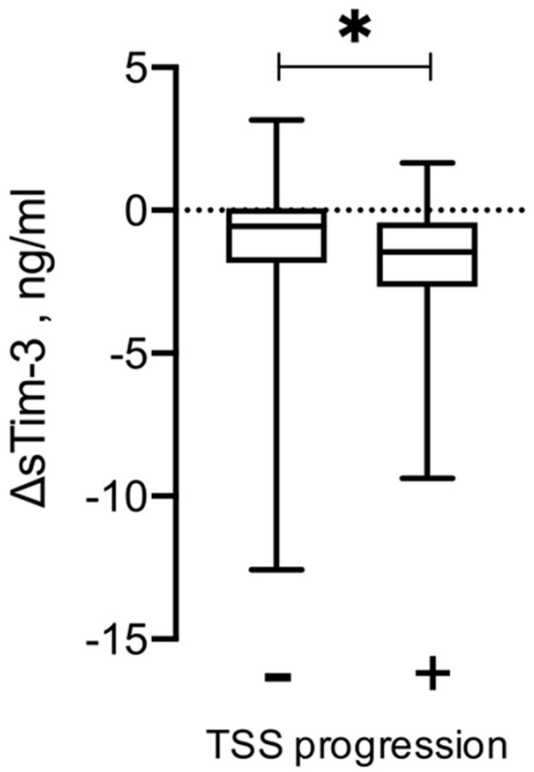


**Figure 5.** Plasma concentrations of soluble (s) Tim-3 was examined in patients with early RA (eRA) at baseline and after 3 months (n=98). These were treated by a treat-to-target strategy for 2 years. Patients were divided into those who showed radiographic progression (+, n=55) or no progression (-, n=43) measured by their change in Total Sharp Score after 2 years of treatment TSS. Patients with progression had a greater reduction in sTim-3 from baseline to 3 months (ΔTim-3) compared with patients that did not progress in TSS. *P<0.05.

3 to be directly involved in the disease pathogenesis.

In conclusion, Tim-3 expression is upregulated on activated Th cells and acts as a regulator of IFNγ and MCP-1 production in RA. Furthermore, the soluble form of Tim-3 is associated with disease activity and long-term outcome. Our findings support the importance of CIR in the plethora of immunological factors that influences autoimmune diseases like RA. In this perspective, Tim-3 seems to play a minor role.

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## Disclosure of conflict of interest

None.

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