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Modulation of PBMC-decay accelerating factor (PBMC-DAF) and cytokines in rheumatoid arthritis

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Abstract Studies have suggested that abnormal expression of complement regulatory proteins and cytokines contribute significantly to the path-physiology of rheumatoid arthritis. In this context, Decay accelerating factor (DAF) a complement regulatory protein is gaining increased attention. With the notion that immune effecter mechanisms are all interlinked and circulating peripheral blood mononuclear cells (PBMCs) should have a role in a systemic disease like rheumatoid arthritis, we studied the modulation and significance of PBMC-DAF and cytokines in RA. Seventy-five RA patients and 75 healthy controls were recruited. Expression of DAF and cytokines (IFN-γ, IL-17A and IL-10) in the PBMCs of patients and controls was determined. Correlations among DAF, cytokines, and disease activity were evaluated by standard statistical methods. The effect of IFN-γ, IL-17A, and IL-10 on the expression of DAF in patients and controls was studied in vitro. Expression of PBMC-DAF declined in patients both at mRNA and surface level and correlated negatively with the disease activity. Expression of IFN-γ also declined in patients but correlated positively with DAF and negatively with disease activity. Expression of IL-17A and IL-10 was higher in patients. The levels correlated positively with disease activity and negatively with DAF both in patients and controls. In vitro studies indicated that IFN-γ up-regulated DAF expression in PBMCs, whereas IL-17A and IL-10 had negative effect on the same. The decline in the PBMC-DAF is a contributing factor in manifestations of RA. Cytokine environment contributes to this decline. These findings brought novel insights into the complement-cytokine axis in the path-physiology of RA.

Keywords Rheumatoid arthritis · Complement regulatory protein · Cytokines · DAF · Circulating PBMCs · DAS28

Introduction

Rheumatoid arthritis is a chronic systemic inflammatory disease of unknown etiology that afflicts females at all ages in much higher proportions than the males [1]. Although the etiology of RA is not yet fully clear, exaggerated complement activity and inflammatory mechanisms of cytokines contribute significantly to tissue damage in RA [2].

In healthy people, tissues in the body are protected from complement-mediated damage by expression of multiple complement regulatory proteins that cooperate to inhibit complement activation on self-tissues [3]. While factor H is the key fluid phase complement regulatory protein for the alternative pathway, most of the complement regulatory proteins are membrane bound, distributed differentially in

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circulating cells and tissues [4]. Studies with gene knock-out mice have suggested that membrane-bound complement regulatory proteins may critically determine the sensitivity of the host tissues to complement injury in autoimmune and inflammatory disorders [5]. In systemic lupus erythematosus (SLE) patients, close interlinks between the modulation of membrane complement proteins such as CR1 and MCP expression with disease activity had been reported [6, 7].

Decay accelerating factor (DAF; CD55), a key membrane-bound complement regulatory protein, has gained much attention in context of autoimmune diseases. DAF regulates complement activation by accelerating the decay of the C3/C5-convertase of both classical and alternative pathways [8]. It is expressed ubiquitously on all circulating cells and tissues and found in the soluble form in body fluids [8]. Apart from regulation of complement cascade, recent studies also suggest role of DAF in signal transduction and modulation of T cell-mediated immune responses [9, 10]. A growing number of evidences strongly indicate disease modulating role of DAF in the animal models of autoimmune disorders [11–13]. Not nearly as many studies in humans have investigated the relationship between DAF and systemic autoimmune diseases [12, 14]. In this context, there had been only few studies on modulation of DAF in the circulating cells from RA patients and its relations with the clinical disease activity [15–17]. In view of the important role suggested for IFN-γ, IL-17A, and IL-10 in the path-physiology of RA [18–26], we aimed at gaining better insight into the complement-cytokine axis in RA.

Materials and methods

Study subjects

In this study, 75 patients (55 females and 20 males) with active RA (median age 31.8 years; range 18–50 years) were recruited from the outpatient department of All India Institute of Medical Sciences (AIIMS), New Delhi, India. Diagnosis was made in accordance with the 1987 revised classification criteria of American College of Rheumatology (ACR) [27]. The patients at the time of first visit to the hospital were naive for disease-modifying anti-rheumatic drugs (DMARD) and corticosteroid treatment. RA patients with overlap of any other systemic autoimmune diseases, diabetes mellitus, tuberculosis, hypo or hyperthyroidism, and pregnancy were excluded from the present study. Seventy-five healthy volunteers, with no history of autoimmune disorders, major infection, and other inflammatory diseases, were enrolled as controls and consisted of 58 females and 17 males (median age 29.5 years; range 20–48 years). The controls were matched as closely as possible for age, sex, and geographical lineage with the study group of the RA patients. Written consent was taken from each individual before taking blood. The Ethics committee of the All India Institute of Medical Sciences approved the study design.

Sample collection

Venous blood (10–12 ml) with or without anticoagulant (5 % EDTA) was drawn from RA patients and healthy controls. Plasma and serum samples were separated and stored at −70 °C until use. After separation of plasma, the packed cellular fractions were used for the isolation of PBMCs.

Isolation of PBMCs

PBMCs were isolated from the packed cells obtained above using Ficoll–Hypaque according to the standard procedures as described earlier [28]. PBMCs were counted in a hemocytometer, and cell viability was confirmed by trypan blue staining. The isolated cells were then further used to quantitate surface protein expression by Flow cytometry and gene quantitation by Real-time PCR.

Flow cytometry

PBMCs from control and RA were incubated with anti-human DAF antibody and also the corresponding antibody of the same isotype (AbD Serotec) for 2 h at 4 °C. After washing with PBS–BSA buffer, cells were incubated with FITC conjugated secondary antibody (AbD Serotec) for 45 min at 4 °C in dark. Stained cells were then fixed with 2 % paraformaldehyde, and surface expression of DAF was analyzed using FACS Analyser (BD FACSCanto^{TM}). Lymphocytes and monocytes have distinct light scattering properties, and each cell type was gated for measurement of fluorescence intensity independent of the other cell on the basis of forward and side light scattering properties (Online support 1) as described previously [29]. Results were expressed as mean fluorescence intensity of 10,000 cells.

Quantitation by Real-time PCR

Total RNA was isolated from cells using TRI—Reagent (Sigma-Aldrich, USA). The RNA was quantified by nanodrop (nanodrop-1000, Thermo Scientific, Wilmington, USA), and the first-strand cDNA was synthesized using 1 μg RNA, random decamers as primers and Revert Aid ^{TM} M-MuLV Reverse Transcriptase (MBI Fermentas, Hanover, MD, USA) in 20 μl reaction volume according to the

manufacturer instructions. Real-time PCR was performed on an ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA) using Syto-9 green dye (Invitrogen, NY, USA) to detect DNA during the reaction. Thermal cycling parameters consisted of an initial denaturation for 1 min at 95 °C followed by 40 cycles of 15 s at 95 °C, 15 s at an annealing temperature of 58 °C for DAF and 60 °C for IFN-γ, IL-17A, and IL-10, and extension of 30 s at 72 °C. Specificity of the PCR products was verified by melting curve analysis. β-actin was used as a housekeeping gene to normalize the expression of DAF and cytokines. The average threshold cycle (Ct) for DAF and cytokines was determined from triplicate reactions and the levels of gene expression relative to β-actin were calculated as 2^{Δ} (Ct Ref- Ct test). The sequence of specific primers used for genes DAF, IFN-γ, IL-17A and IL-10 were *DAF-F* 5′-CTT AAG GGC AGT CAA TGG TCA GAT-3′; *DAF-R* 5′-CCA CAA CAG TAC CGA CTG GAA AAT-3′; *IFN-γ-F* 5′-GTT TTG GGT TCT CTT GGC TGT TA-3′ *IFN-γ-R* 5′-AAA AGA GTT CCA TTA TCC GCT ACA TC-3′; *IL-17A–F* 5′-CCG CCA CTT GGG CTG CAT CA-3′; *IL-17A–R* 5′-AGC CGG AAG GAG TTG GGG CA-3′; *IL-10–F* 5′-GCT GGA GGA CTT TAA GGG TTA CCT-3′; *IL-10–R* 5′-CTT GAT GTC TGG GTC TTG GTT CT-3′; and *β-Actin-F* 5′-AGA AAA TCT GGC ACC ACA CC-3′; *β-Actin-R* 5′-TAG CAC AGC CTG GAT AGC AA-3′.

Cell culture

PBMCs isolated from 15 patients and 15 healthy controls were cultured in vitro at the concentration of 2×10^6 cells per well in a 6-well culture plate (Costar, USA) in the RPMI-1640 basic medium supplemented with 10 % heat inactivated FCS (BioWest, USA), Penicillin/Streptomycin (1 %) and incubated in 5 % CO_2 at 37 °C in the presence and absence of cytokines rhIFN-γ (PeproTech, USA), rhIL-17A (PeproTech, USA) and rhIL-10 (PeproTech, USA), respectively, for 6 h each. The optimum standardized doses used for rhIFN-γ, rhIL-17A, and rhIL-10 were 10, 4, and 2 ng/ml, respectively. Untreated cells served as controls. Effects of these treatments on PBMC-DAF transcript levels were studied by comparing the transcript levels of the treated cells with the untreated cells (controls). Experiments for each treatment were carried out for 15 controls and 15 patients. At the end of the treatment, the suspended cells were taken into micro centrifuge tube and pelleted by low-speed (2000 rpm) centrifugation for 10 min at 4 °C. Adhered cells in the plates were washed with PBS (pH 7.4) and pelleted. RNA was isolated from the PBMCs to determine the levels of DAF transcripts by real-time PCR as described above.

Evaluation of disease activity score 28 (DAS28) for Rheumatoid Arthritis

DAS28 was used to calculate the disease activity of the patients. The DAS 28 was calculated using patients swollen joint counts (SJC) (0-28), tender joint counts (TJC) (0-28), ESR, and global health assessment (GH) on a visual analogue scale (VAS) of 100 mm using the formula $\mathrm{DAS}_{28}(4) = 0.56 * \mathrm{sqrt}(t_{28}) + 0.28 * \mathrm{sqrt}(\mathrm{sw}_{28}) + 0.70 * \mathrm{Ln}(\mathrm{ESR}) + 0.014 * \mathrm{GH}$ as described previously [30]. DAS $28 > 5.1$ indicated high disease activity, DAS $28 < 3.2$ indicated low disease activity and DAS $28 < 2.6$ indicated remission [30].

Statistical analysis

Data were analyzed using Graph Pad Prism (Graph Pad, San Diego, CA, USA). Mann–Whitney test was utilized for comparisons between controls and patients. The correlation between the variables was assessed by applying Spearman's rho analysis, and the significance level was measured by two-tailed test. Paired sample *t* test was used to compare the effect of treatment with the untreated PBMCs in patients and controls. In all cases, $p < 0.05$ was considered significant.

Results

Characteristics of the patients

RA patients included in this study were enrolled from Medicine OPD of AIIMS. Patients were evaluated and diagnosed by the clinicians. The demographic and clinical characteristics of the patients are summarized in Table 1. Disease duration of patients ranged from 0.2 to 5 years. The levels of ESR and RF were obtained from the clinical records of the patients. All the patients were positive for RF. Out of these, 49 patients were positive for both RF and anti-cyclic citrullinated peptide (CCP) antibodies, and 16 patients were borderline positive for RF but positive for anti-CCP antibodies. Rest of the 10 patients had established clinical manifestations of RA and not reported for anti-CCP antibodies. DAS28 scores in RA patients ranged from 5.4 to 8.1 with the mean value of 6.69 ± 0.69. Thus, all the patients presented with high disease activity. At the time of clinical presentation, forty-one patients suffered from dyspnea on exertion and anemia, 7 patients reported with rheumatoid vasculitis and pericarditis, and 4 patients were diagnosed with raynaud phenomenon. All the patients suffered from fatigue. The treatment regimen initiated included non-steroid anti-inflammatory drugs, steroid anti-inflammatory drugs (prednisolone), and DMARDs

Table 1 Clinical characteristics of RA patients

Characteristics	Patients
Number (n)	(75)
Age median (range)	31.8 (18–50)
Sex ratio F/M, *n*	55 F/20 M
Duration, (range *n*) years	0.2–5
RF positive, n	75
Anti-CCP positive, n	65
DAS28 mean ± SD	6.69 ± 0.69
SJC median (range)	7.5 (1–17)
TJC median (range)	13 (5–22)
VAS mean ± SD (mm)	74.92 ± 6.17
ESR median (range, mm/h)	59 (32–146)
DMARDs (methotrexate) + NSAIDs, *n*	42
DMARDs (methotrexate, sulfasalazine), *n*	30
DMARDs (leflunomide), *n*	3

The values for variables are expressed as mean ± SD or median (range) except for number, duration of disease, sex ratio, rheumatoid factor, anti-CCP, and treatment regimen which are *n*

(methotrexate, leflunomide, and Sulfasalazine) in different combinations as appropriate for each patient (Table 1).

Expression of DAF in PBMCs of patients and controls

The levels of surface DAF expression on both lymphocytes ($p = 0.0001$) and monocytes ($p = 0.0001$) declined significantly in patients ($n = 60$) as compared to controls ($n = 60$) (Fig. 1a) and correlated inversely with DAS28 (Fig. 1b, c).

Further, significant and marked decline of DAF mRNA expression was observed in the samples from the PBMCs of RA patients as compared to controls ($p = 0.0001$; Fig. 1d). PBMC-DAF transcript also correlated inversely with the DAS28 (Fig. 1e).

Expression of IFN-γ, IL-17A, and IL-10 in PBMCs of patients and controls

The levels of IFN-γ mRNA declined significantly in patients ($n = 40$) compared with controls ($n = 40$) ($p < 0.001$; Fig. 2a). In contrast, levels of IL-17A and IL-10 mRNA increased significantly in patients compared with controls ($p < 0.0001$; Fig. 2b, c).

Inverse correlation of IFN-γ mRNA expression with DAS28 ($r = -0.39$, $p = 0.01$; Fig. 2d) was observed. However, IL-17A and IL-10 mRNA expression levels correlated positively with DAS28 ($r = 0.73$, $p = 0.0001$ and $r = 0.71$, $p = 0.0001$, respectively; Fig. 2e, f).

Correlations of PBMC-DAF with cytokines in patients and controls

PBMC-DAF and IFN-γ mRNA levels correlated positively both in patients and controls (Table 2). IL-17A and IL-10 mRNA levels correlated negatively with DAF. Observation is statistically significant (Table 2).

Effect of cytokines on PBMC-DAF expression in patients and controls

In vitro culture studies showed that IFN-γ up-regulated PBMC-DAF transcript expression by 54 % (0.54-fold) in patients ($p = 0.008$, paired *t* test) and 35.7 % (0.35-fold) in controls ($p = 0.03$, paired *t* test) as compared to the untreated cells in each group (Fig. 3a). However, IL-17A and IL-10 treatment down-regulated the PBMC-DAF transcript by 67.2 % (0.67-fold; $p = 0.0001$, paired *t* test) and 56.2 % (0.56-fold; $p = 0.0001$, paired *t* test) in patients as compared to the respective untreated cells (Fig. 3b, c). In controls, the decline by IL-17A and IL-10 was to the extent of 70.7 % (0.70-fold; $p = 0.0001$, paired *t* test) and 69.8 % (0.69-fold; $p = 0.0001$, paired *t* test) as compared to the respective untreated cells (Fig. 3b, c).

Discussion

DAF consists of four extracellular short consensus repeat (SCR) domains, linked to the membrane by a GPI anchor [8]. The major function of DAF is to protect self-cells from complement-mediated attack by accelerating the decay of the classical and alternative C3 and C5 convertases, the central amplification enzymes of the complement cascade [8]. In addition to its role as a regulator of complement activation, DAF is now known for regulating adaptive T cell responses [9, 10].

There is increasing evidence for a significant role of DAF in negatively regulating autoimmunity in both humans and experimental models [12]. However, not much is known about the role of DAF in the disease process of RA in humans. Despite the decades of investigation, the factors and mechanisms contributing to path-physiology of RA are not well elucidated.

Complement activation has been suggested as an important event in the inflammatory cascade in RA [31]. DAF, a 70-kDa single chain, regulators of complement activation (RCA) encoded complement regulatory protein is suggested to play a protective role against synovial membrane associated articular inflammation in RA by preventing complement-mediated injury [32]. Intra-articular administration of soluble form of DAF had been shown to reduce inflammation in a rat arthritis model [33]. DAF is

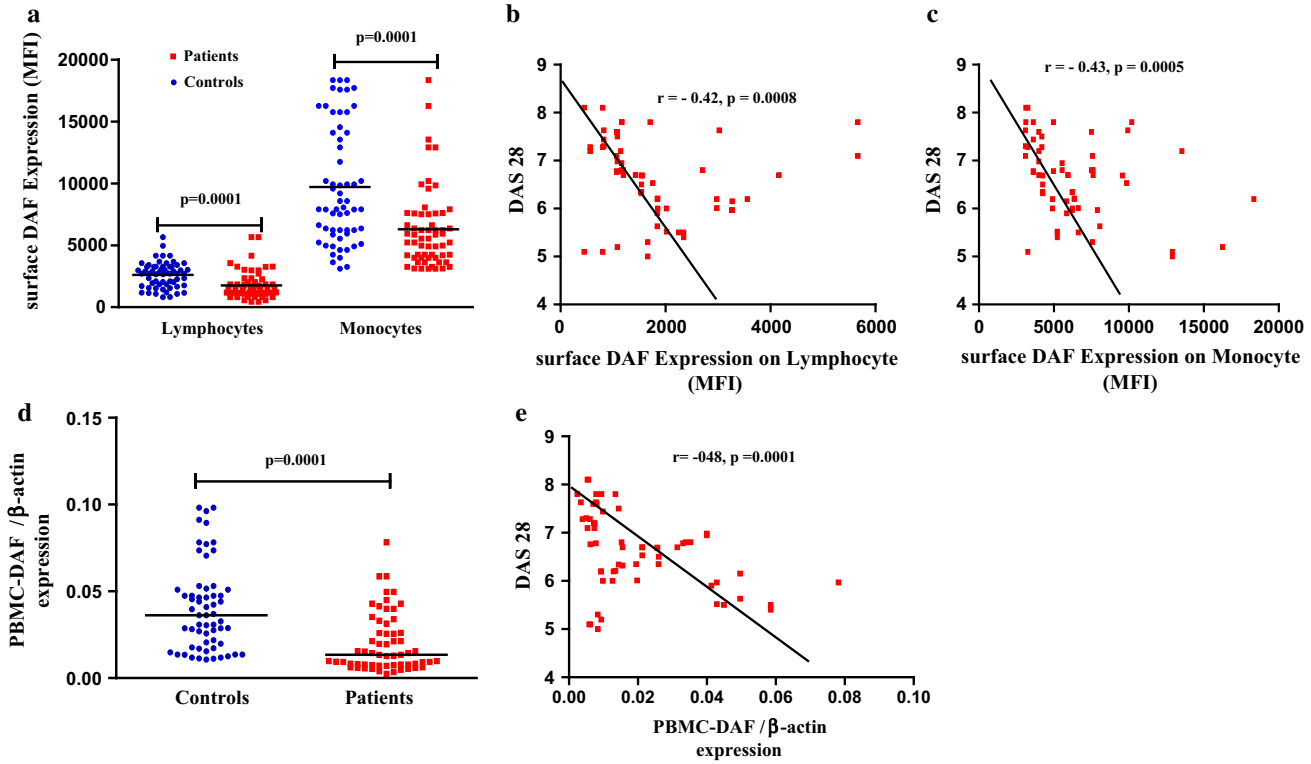

Fig. 1 Levels of PBMC-DAF expression in patients and controls and its correlation with DAS28 in RA patients. **a** The *dot plots* show the expression of surface DAF in terms of MFI for lymphocytes and monocytes in controls ($n = 60$) and RA patients ($n = 60$) as determined by flow cytometry. The values are expressed in terms of median. **b–c** The *plots* represent correlation of surface DAF expression on lymphocytes (**b**) and monocytes (**c**) with DAS28 in RA patients. **d** The plot shows the values of DAF mRNA expression normalized by β-actin expression in PBMCs of patients ($n = 60$) and controls ($n = 60$) as determined by real-time PCR. Each symbol represents one control (*circles*) or patient (*rhombus*), and the bar within each group represents the median. **e** The plot shows the correlation of PBMC-DAF mRNA expression with DAS28 in RA patients ($n = 60$). The *p* values were derived by Mann–Whitney test. The coefficient of correlation was calculated by Spearman's rho analysis and two-tailed test

widely expressed on the surface of all major circulating blood cells as well as epithelial and endothelial cells. It is also found in soluble form in plasma, cerebrospinal fluid, saliva, synovial fluid, and urine [8]. Since RA is a systemic autoimmune disorder, it had been of our interest to study the molecular alterations in circulating cells in the disease. This study remained focussed to modulation of DAF and cytokines in PBMCs.

The patients enrolled in the study showed aggressive form of the disease and presented with heterogeneous clinical manifestations. DAS28 was used as the valid and reliable disease activity index for RA [34]. All the patients were categorized in a single group as 'Patients' and healthy individuals were grouped as 'Controls.' Expression of DAF in total leukocytes declined significantly in the patients with RA compared to controls (data not shown). This decline was most significant in PBMCs, both lymphocytes and monocytes. The levels of surface DAF protein and mRNA correlated negatively with DAS28, suggesting an association of DAF with the path-physiology of RA. The decline in DAF expression in the PBMCs of the patients with RA compared to controls might contribute to the deregulation of the complement cascade and complement-mediated tissue injury along with modulation of its immune regulatory functions in RA. Several studies had reported an association of RA disease activity with C3 and C4 activation products [35–37]. Depositions of many of these complement activation products like C3c, C3b, iC3b, active C1s, and MAC have been visualized by immunohistochemistry in synovial tissue from RA patients [38–40].

An increasing number of studies in human cells demonstrated that DAF expression is modulated by cytokines such as IL-1, IL-6, TNF-α, TGF-β1, and IFN-γ, prostaglandins, and tissue-specific factors and controlled by the transcription factor SP1 [12, 41–44]. Since the expression of this regulatory protein can be modulated by various cytokines, it was logical to study the inter-relations of cytokine expression in PBMCs with DAS28 and DAF, along with their effects on DAF expression. The cytokines, namely IFN-γ, IL-17A, and IL-10, were chosen based on their reported association with RA and other inflammatory disorders [18–26].

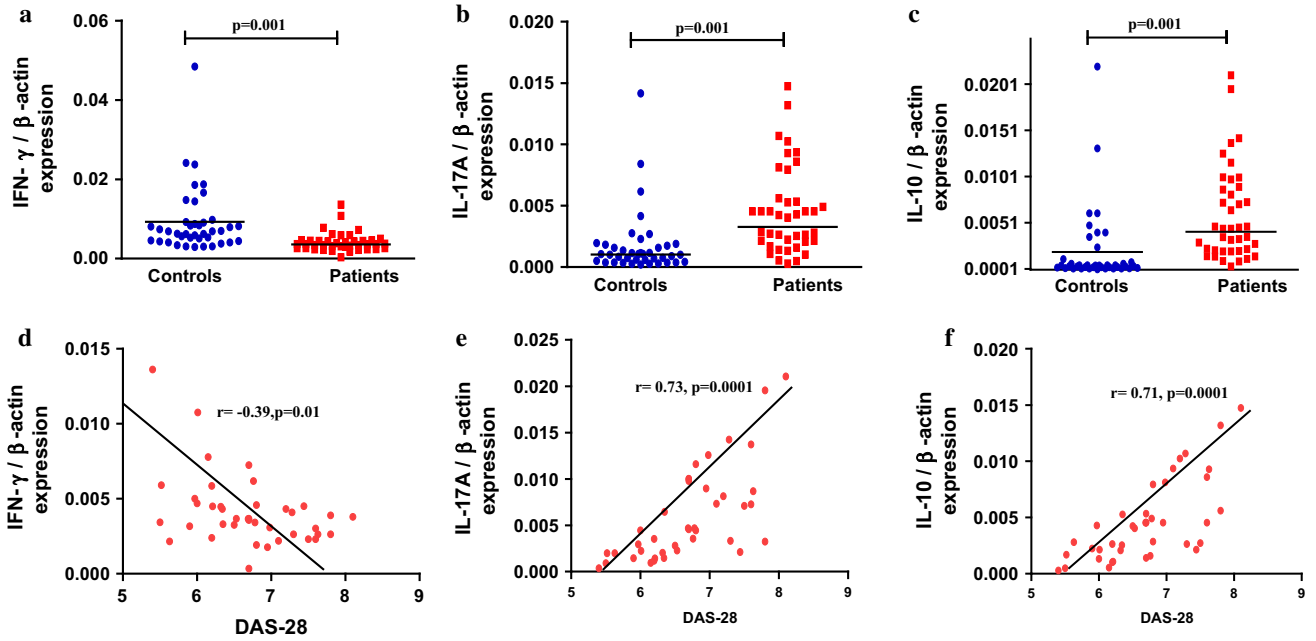

Fig. 2 Levels of IFN-γ, IL-17A, and IL-10 expression in PBMCs of patients and controls and their correlations with DAS28 in patients. The *plots* show the values of IFN-γ (**a**), IL-17A (**b**) and IL-10 (**c**) mRNA expression normalized by β-actin expression in PBMCs of patients ($n = 40$) and controls ($n = 40$) as determined by real-time PCR. The values are expressed in terms of median. Each *symbol* represents one control or patient, and the *bar* within each group represents the median. The p value was derived by Mann–Whitney test. **d** The plot shows the inverse correlation of IFN-γ mRNA expression in PBMCs with DAS28 in RA patients ($n = 40$). **e–f** The *plots* show the positive correlations of IL-17A (**e**) and IL-10 (**f**) mRNA expression in PBMCs with DAS28 in RA patients ($n = 40$). The p and r values were calculated by 2-tailed test and Spearman rho analysis, respectively

Table 2 Correlations of PBMC-DAF with IFN-γ, IL-17A, and IL-10 expression in RA patients and controls

Correlations pair	Controls ($n = 40$)	Patients ($n = 40$)
IFN-γ & PBMC-DAF	$r = 0.45$, $p = 0.003$	$r = 0.59$, p = 0.0001
IL-17A & PBMC-DAF	$r = -0.58$, $p = 0.0001$	$r = -0.41$, p = 0.008
IL-10 & PBMC-DAF	$r = -0.41$, $p = 0.007$	$r = -0.44$, p = 0.003

Shows are the correlations of DAF with IFN-γ, IL-17A, and IL-10 mRNA expression in PBMCs of RA patients ($n = 40$) and healthy controls ($n = 40$). The p and r values were calculated by 2-tailed test and Spearman rho analysis, respectively

We observed that the levels of PBMCs IFN-γ transcript declined in our patients. There are conflicting reports on the levels of IFN-γ in serum, synovial fluid, synovial cells, and peripheral blood mononuclear cells from the patients with RA [18–20]. We observed negative correlations of IFN-γ mRNA levels with DAS28 in the patients reflecting its close association with the disease. In previous reports, IFN-γ showed negative correlation with clinical markers of disease activity [20]. Some reports also suggested positive association of IFN-γ with disease activity in RA [21].

IL-17A and IL-10 mRNA levels were higher in the PBMCs of the patients compared to controls. Our finding is similar to that reported earlier in PBMCs from RA patients [45, 46]. Higher levels of these cytokines in serum, synovial fluid, and synovial cells from the patients with RA had previously been reported [19, 20, 25, 46].While IL-17A appears to mediate inflammation and joint destruction in RA, IL-10 is suggested to have a protective role because of its antagonism with the inflammatory cytokines [24–26]. Among the various cytokines, we observed positive correlations of IL-17A and IL-10 mRNA levels with DAS28. Earlier IL-17A mRNA levels in synovium and, protein levels in serum and synovial fluid showed significant positive correlations with joint damage progression, DAS28, and severe clinical course in RA patients [25, 47]. Elevated mRNA and serum protein levels of IL-10 were found to correlate with IgM–RF titres and disease activity [48, 49].

While the simultaneous decrease in the levels of the inflammatory cytokine IFN-γ and increase in the levels of the inflammatory cytokine IL-17A and anti-inflammatory cytokine IL-10 in RA may relate to their functional attributes in the disease, it had been interesting to investigate

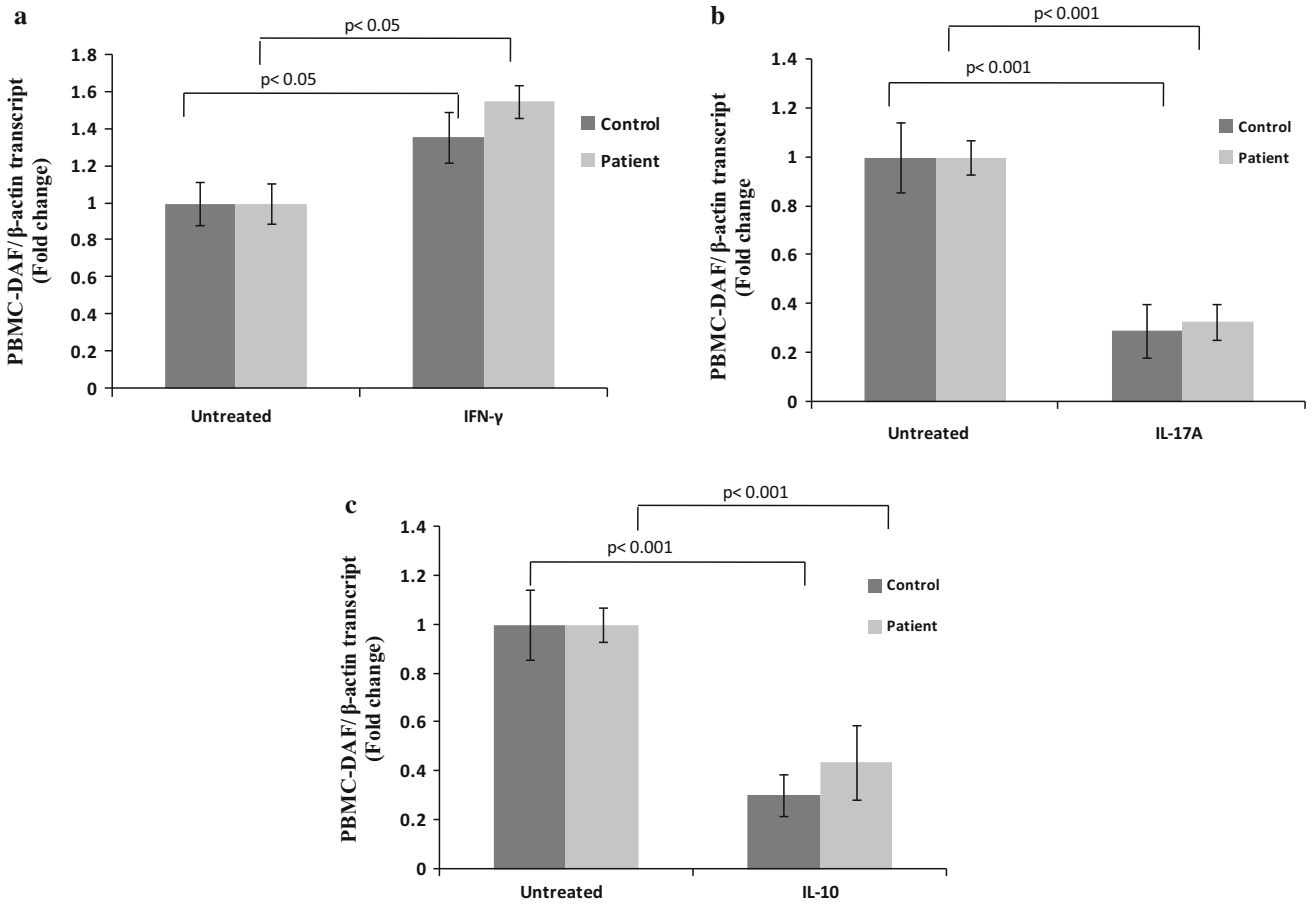

Fig. 3 Effect of cytokines IFN-γ, IL-17A and IL-10 on PBMC-DAF mRNA expression in vitro. The plots show the fold change in PBMC-DAF mRNA expression in response to treatment with 10 ng/ml IFN-γ (**a**), 4 ng/ml IL-17A (**b**) and 2 ng/ml IL-10 (**c**) in patients ($n = 15$) and controls ($n = 15$) after 6-h culture. The results are expressed as the expression ratio of PBMC-DAF transcript of treated and the untreated cells in each category. The p values were calculated for the treatment group in comparison to the control group (untreated) by paired t test

the relations of these cytokines with complement regulatory protein DAF, which also has an anti-inflammatory role.

We found that the levels of IFN-γ transcript correlated positively with L-DAF transcript both in patients and controls. This is the first report of its kind. IFN-γ had been reported to have both protective and pathogenic role in animal models of arthritis [22, 23]. Previous studies had reported up-regulation of complement regulatory proteins by IFN-γ in different cell lines and neutrophils from the SLE patients, respectively [41, 42]. All these evidences along with our observation add up a new insight into the protective role of IFN-γ against pathogenesis of RA and other autoimmune disorders. We would like to postulate that IFN-γ may also contribute to the regulation of complement cascade through the up-regulation of complement regulatory proteins [41, 42].

To further confirm this postulation, we studied the effect of IFN-γ on levels of DAF expression in the PBMCs from the healthy individuals and patients with RA by in vitro studies. The cells of the monocytes/macrophage lineage are identified as the primary targets for the effecter functions of IFN-γ [50]. We observed that IFN-γ significantly up-regulated the levels of PBMC-DAF transcript in patients. This effect was more pronounced for patients compared to controls. Our findings revealed lower levels of IFN-γ transcript in PBMCs of the patients. Elevated levels of IFN-γ in sera and synovial fluid of RA patients had previously been reported [19, 20]. Here, the source of IFN-γ could be other cells [51]. Therefore, it may be suggested that the local effect of IFN-γ on DAF expression in PBMCs is not predominating in our patients, and there must be other factors that account for decline in PBMC-DAF transcript in our patients.

IL-17 A is an inflammatory cytokine with pleiotropic function [25]. IL-17A alone can manifest the pathology of RA in IFN-γ knockout animal model [22]. Benefit of blocking IL-17A effect in RA is evidenced increasingly

[25]. We found significant inverse correlations of PBMC-DAF transcript with IL-17A levels in patients and controls. Our observation suggests a new dimension in the inflammatory effect of IL-17A which may be its antagonism with DAF expression.

This observation was further confirmed by our in vitro study which showed that IL-17A caused a significant down-regulation in the levels of PBMC-DAF transcript both in patients and controls. In the cytokine network, IFN-γ has been reported to down-regulate the expression of IL-17A [25]. A negative correlation between IFN-γ and IL-17A had been observed by us in patients (data not shown). Since we found lower level of IFN-γ expression in our patients, it may explain the higher levels of IL-17A expression observed by us. With this, we may speculate that IL-17A is one of the factors that contributed to the reduced levels of DAF transcript in patients. This notion also gets support from the fact that in patients, we found a significant negative correlation between IL-17A and PBMC-DAF transcript. The effect of IL-17A on PBMC-DAF transcript has been observed for the first time by us in RA.

While IFN-γ and IL-17A represented inflammatory cytokines that modulated the expression of DAF differentially in healthy individuals and patients with RA, it had been interesting to study the role of IL-10, the anti-inflammatory cytokine. IL-10 is known for both anti-inflammatory and immunoregulatory effects in RA [26]. We also found significant inverse correlations of PBMC-DAF transcript with IL-10 mRNA levels in patients and controls. We may speculate that compensatory increase in IL-10 is an attempt to protect the host against inflammatory damage by the injurious cytokines along with the damage that can be caused due to lower levels of DAF expression in patients with RA.

Further, by in vitro studies, it was interesting to find that IL-10 had similar down-regulating effect as IL-17A on the expression of PBMC-DAF transcript. It had been known that IL-10 down-regulates the expression of IL-17A in target cells [52]. However, here the effect was more marked in healthy individuals than patients. Our observation on parallel increase in the expression of IL-10 and IL-17A in PBMCs and their positive correlations in RA patients (data not shown) showed deviation from their normal interplay and suggested their simultaneous negative effect on PBMC-DAF expression in RA. The enhanced effect of IL-10 in control in comparison with patients in vitro may reflect to the normal role of IL-10 in healthy individuals as immune suppressor cytokine.

In summary, our findings brought valuable information on the modulation of PBMC-DAF expression in RA and its relations with cytokine environment in patients and controls. Further studies are needed to confirm our findings and their translational implications.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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