Increased Interleukin 21 (IL-21) and IL-23 Are Associated with Increased Disease Activity and with Radiographic Status in Patients with Early Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To investigate the levels of the T helper (Th)17-related cytokines interleukin 17A (IL-17A), IL-21, and IL-23 and their association with disease activity in rheumatoid arthritis (RA).

Methods. In a longitudinal sample set from patients with early RA (< 6 months; n = 40), we measured the plasma cytokine levels of IL-17A, IL-21, and IL-23 and analyzed for correlation with disease activity in 28 joints (Disease Activity Score 28-joint count; DAS28), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and total Sharp score (TSS). In a transverse sample set of patients with chronic RA (> 8 years), using paired peripheral blood mononuclear cells and synovial fluid mononuclear cells, we investigated the cellular expression of IL-17A, IL-21, and IL-23R.

Results. Patients with early-stage RA had significantly increased plasma levels of IL-21 and IL-23, but not IL-17A, compared to patients with chronic RA and healthy volunteer controls. Plasma levels of IL-21 and IL-23 after 12 months of treatment correlated with DAS28 and ESR, but not to TSS. Changes in IL-23 plasma levels from time of diagnosis to 12 months correlated with change in DAS28 and with TSS scores at 2 years. The numbers of CD4+ T cells producing IL-21 were significantly increased in the synovial fluid of patients with chronic RA, with only marginal coexpression of IL-21 and IL-17A.

Conclusion. Our results show a significant association between plasma levels of IL-21 and IL-23 and disease activity in RA, supporting the hypothesis that IL-21 and IL-23 are important pathogenic factors of this disease. (J Rheumatol First Release August 1 2010; doi:10.3899/jrheum.100259)

Key Indexing Terms:
RHEUMATOID ARTHRITIS INFLAMMATION T LYMPHOCYTES
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Rheumatoid arthritis (RA) is a chronic autoimmune systemic disorder characterized by inflammatory responses mainly affecting the joints. Although the etiology is unknown, CD4+ T cells and their cytokines are believed to play major parts in the induction and propagation of the pathogenic inflammatory conditions[1,2]. The T cell-associated cytokines interleukin 21 (IL-21) and IL-23 have recently been proposed as important regulators of this process. This is supported by the recent discovery that both IL-21 and IL-23, produced by CD4+ T cells and dendritic cells, respectively, promote the development of the IL-17A-producing proinflammatory CD4+ T cell subset T helper (Th)17[3,4,5,6,7,8]. Further, IL-21R messenger RNA (mRNA) is reported to be increased in the synovia of patients with

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RA[9,10], and the synthesis of tumor necrosis factor-α (TNF-α), IL-6, and IL-1ß from RA synovial cells is significantly reduced if IL-21 is neutralized[9]. That IL-21 is an important factor in RA is also supported by studies in animals in which interference with IL-21 function eliminates or reverses disease progression in both mouse and rat models of arthritis[11,12].

A limited number of studies have focused on IL-23 in RA. However, one study shows that increased levels of IL-23 in plasma of patients with chronic RA are associated with disease activity[13], while another concludes that IL-23 is not abundantly present in late-stage RA[14]. Most studies of IL-23 have been carried out in mice, where obstruction of the IL-23 pathway eliminates the development of autoimmune inflammatory conditions, including collagen-induced arthritis[15,16]. This effect has principally been attributed to the inability of the mice to expand Th17 populations in the absence of IL-23[15].

In our study, we measured the levels of IL-17A, IL-21, and IL-23 in the plasma of early RA and investigated for correlation with disease activity and radiographic progression. We also examined levels of these 3 cytokines in patients with chronic RA as well as in healthy volunteer controls. In addition, we investigated the frequency of CD4+ T cells with the capacity to produce IL-17A or IL-21 in the peripheral blood (PB) and synovial fluid (SF) from patients with chronic RA. We further characterized the CD4+ T cells with regard to surface IL-23R and chemokine receptor expression.

The data we collected suggest that an IL-21/IL-23 axis is highly active in RA and that IL-21 and IL-23 are closely connected to pathogenic mechanisms in this disease.

MATERIALS AND METHODS

Collection of samples. A longitudinal set of plasma samples from patients with early-stage RA (n = 40) was obtained from the Cyclosporine, Methotrexate, Steroid in RA (CIMESTRA) study[17]. The CIMESTRA study is a multicenter, randomized, double-blinded controlled study. Patients were newly diagnosed with RA, naive to steroid and disease-modifying antirheumatic drugs (DMARD), and had had disease symptoms for less than 6 months. At entry, patients were randomized to conventional methotrexate treatment combined with an aggressive regime of intraarticular betamethasone injections, with cyclosporine (n = 20) or without (n = 20)[17]. However, the 2 treatment groups from the CIMESTRA study were considered as 1 group in our study. This is because we observed no differences regarding IL-21, IL-23, or IL-17A plasma levels between the 2 treatment regimes (data not shown). This is in agreement with the original study, which showed no major differences in clinical measurements between the 2 treatment groups[17].

Plasma samples were collected at the time of diagnosis (pretreatment, 0 month) and after 3 and 12 months of treatment. All samples were collected at the outpatient clinic at Aarhus University Hospital. The clinical characteristics were collected at the same timepoints as the plasma samples, while radiographic measurements were at the time of diagnosis and at 1, 2, 3, 4, and 5 years after diagnosis. Radiographic joint destruction was graded by the Sharp/van der Heijde scoring method, calculated as the total Sharp score (TSS). We made use of disease activity in 28 joints (Disease Activity Score 28-joint count; DAS28), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), IgM rheumatoid factor (IgM-RF), and anticyclic citrullinated peptide (anti-CCP), all recorded in the CIMESTRA study.

A cross-sectional sample set was collected from patients with chronic RA (n = 28), including plasma samples and peripheral blood mononuclear cells (PBMC) paired with SF and SF mononuclear cells (SFMC) from the outpatient clinic at Aarhus University Hospital. All the patients with chronic RA contacted the clinic because of a joint effusion and none of them received biological treatment, but all were treated with standard DMARD. All patients with chronic RA had disease duration > 8 years. All patients with RA were diagnosed in accord with the American College of Rheumatology criteria[18]. Plasma samples were also collected from controls matched for age and sex with the CIMESTRA patients [n = 29, age 56 years (range 46–64) vs patients, 58 years (52–69); p > 0.20; 62.1% vs 65% women, respectively]. All plasma and SF samples were collected in heparinized tubes and kept at –80°C until used. All samples were obtained after informed written consent according to the Danish Data Protection Agency, the local ethics committee (project numbers 20050046 and 20060012), and the Declaration of Helsinki.

ELISA. Quantification of IL-21, IL-23, and IL-17A levels in plasma and SF was done using ELISA for human IL-21 (eBioscience, San Diego, CA, USA), human IL-23 (p19/p40; eBioscience), and human IL-17A (eBioscience). All samples were diluted 1:1 with Sample Diluent supplied in the kit. All samples were analyzed in duplicates using the average of the optical density (OD) values to calculate concentrations. The minimum detection limit (cutoff) was calculated as the average value of the blanks plus 2 standard deviations, giving a cutoff value of 16 pg/ml for IL-21, 2.5 pg/ml for IL-23, and 2.9 pg/ml for IL-17A. Values below the cutoff were assigned the same value as the cutoff.

Flow cytometry. PBMC and SFMC from heparinized whole blood and SF samples were isolated using Ficoll-Paque Plus (GE Healthcare, Waukesha, WI, USA). The cells were stimulated for 4 h with 50 ng/ml phorbol 12-myristate 13-acetate (Sigma Aldrich, St. Louis, MO, USA) and 1 μg/ml ionomycin (Sigma Aldrich) in the presence of 10 μg/ml Brefeldin A (Sigma Aldrich). For surface staining, the cells were blocked with 10% heat-inactivated goat serum (Dako). For intracellular staining, cells were fixed and permeabilized using FACS Lysing solution and FACS Perm2 (BD Biosciences, San Jose, CA, USA) and then blocked with 10% heat-inactivated mouse serum (in-house production). Surface staining was done using monoclonal antibodies (mAb) to CD4 (clone MT310; Dako), CD45RO (clone PN-IM2712U; Beckman Coulter, Fullerton, CA, USA), CCR4 (clone 1G1; BD Biosciences), CCR6 (clone 11A9; BD Biosciences), CXCR3 (clone 1C6/CXCR3; BD Biosciences), CXCR4 (clone 12G5; eBioscience), CXCR5 (clone RF8B2; BD Biosciences), and biotinylated polyclonal goat anti-IL-23R (R&D Systems, Minneapolis, MN, USA) combined with streptavidin FITC (Dako). Intracellular staining was done using mAb against IL-17A (clone 64DEC17; eBioscience) and IL-21 (clone 3A3-N2; eBioscience). Viability analysis was done using 7-AAD Via-Probe (BD Biosciences). All gates on IL-21, IL-17A, IL-23R, and chemokine receptors were set using fluorescence minus-one controls. All samples were analyzed within 24 h using an FC500 with CXP software (Beckman Coulter) and FlowJo software (Tree Star Inc., Ashland, OR, USA) for analysis.

Statistics. Statistical analyses were performed using GraphPad Prism 5.0 for Mac (GraphPad Software, La Jolla, CA, USA). All data in text are expressed as medians (interquartile ranges). Grouped analyses were done by Kruskal-Wallis test with Dunn's multiple comparison test as a posthoc analysis. Individual samples were analyzed by Mann-Whitney U test for nonpaired data and Wilcoxon signed-rank test for paired data. Correlation of nonparametric paired data was tested using Spearman's rho. In all tests the level of significance was a 2-sided p value < 0.05.

RESULTS

IL-21 and IL-23 plasma levels were increased in patients with early-stage RA compared to controls. IL-21, IL-23, and

IL-17A have all been connected to the pathological processes in RA. We therefore measured the plasma levels of these 3 cytokines in a longitudinal sample set of patients with early-stage RA (n = 40; Figure 1). At the time of diagnosis the median DAS28 was 5.2 (range 4.4–6.1), dropping to 2.5 (1.7–3.4) at 3 months and 1.9 (1.5–2.8) at 12 months, indicating a high level of disease activity at the time of diagnosis, low disease activity after 3 months, and remission after 1 year.

IL-21 plasma levels were significantly elevated at the time of diagnosis as well as after 3 and 12 months compared with controls (all $p < 0.01$). Similarly, IL-23 plasma levels were also elevated at the time of diagnosis as well as after 3 and 12 months compared with controls (all $p < 0.0001$; Figure 1). In contrast, IL-17A plasma levels were low in all samples and were not elevated in RA plasma at any timepoint in comparison with controls. Median plasma levels of IL-17A were 5.2 pg/ml (range 4.7–6.3), 5.9 pg/ml (5.0–7.2), and 5.9 pg/ml (5.3–6.8) at 0, 3, and 12 months, respectively, and 5.7 pg/ml (4.7–6.8) for controls.

IL-21 and IL-23 plasma levels were decreased in chronic RA compared with early RA. Having shown that IL-21 and IL-23 plasma levels were significantly increased in patients with early-stage RA, we examined the levels of these cytokines in a cross-sectional plasma sample set from patients with chronic RA who had a minimum disease duration of 8 years and compared them with patients with early-stage RA and controls.

IL-21 plasma levels in patients with chronic RA were significantly lower than the levels in patients with early-stage RA at all timepoints measured (all $p < 0.01$) and not significantly increased compared with plasma levels in controls (Figure 1). IL-23 plasma levels in patients with chronic RA were also significantly lower than in patients with early-stage RA at the time of diagnosis and at 12 months (all $p < 0.05$), but not at 3 months after diagnosis. In contrast to IL-21, plasma levels of IL-23 in patients with chronic RA were significantly increased compared with controls ($p < 0.05$). IL-17A plasma levels in patients with chronic RA were not significantly different in comparison with patients with early-stage RA at all timepoints as well as with controls (data not shown). Median plasma levels of IL-17A were 5.8 pg/ml (range 4.8–7.3) for patients with chronic RA.

IL-21 and IL-23 plasma levels are associated with disease activity, but not with radiographic progression or status. Since IL-21 and IL-23 plasma levels were increased in the patients with early-stage RA, we investigated whether IL-21 and IL-23 plasma levels could be associated with disease activity or radiographic progression. We therefore examined the correlation between the cytokine plasma levels measured in patients with early-stage RA with a range of clinical measurements recorded for these patients.

Plasma levels of both IL-21 and IL-23 measured at 12 months correlated with DAS28 at 12 months (Table 1). No significant correlations were observed between any of the observed cytokine levels and DAS28 at time of diagnosis or

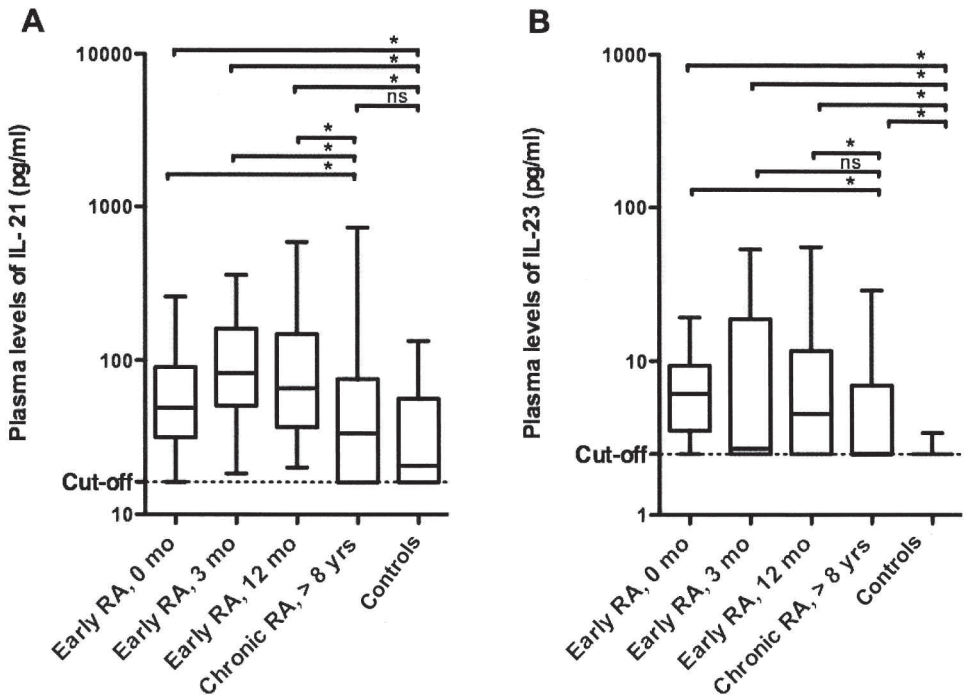

Figure 1. Cytokine levels of IL-21 and IL-23 in plasma from early-stage RA (n = 40), chronic RA (n = 28) with disease duration > 8 years, and healthy controls (n = 29). Cytokine levels in plasma from patients with early-stage RA were measured at the time of diagnosis, after 3 months, and after 12 months of treatment. Boxes represent median with interquartile range and whiskers represent 5th-95th percentile. *$p < 0.05$. ns: not statistically significant.

Table 1. Plasma levels of interleukin 21 (IL-21) and IL-23 correlate with disease activity markers in patients with early rheumatoid arthritis. Levels were measured 12 months after start of treatment.

Disease Marker	IL-21		IL-23	
	p	r	p	r
DAS28 (12 mo)	0.02*	0.40	0.01*	0.42
CRP (12 mo)	0.10	0.28	0.0050*	0.47
ESR (12 mo)	0.0023*	0.50	0.0056*	0.47

* $p < 0.05$. P represents a 2-sided p value; r represents correlation coefficient using Spearman's rho. DAS28: 28-joint count Disease Activity Score; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

at 3 months after diagnosis (data not shown). IL-21 and IL-23 plasma levels at 12 months also correlated significantly with ESR values, and IL-23 plasma levels at 12 months with CRP levels at 12 months (Table 1). Interestingly, we also observed strong intercorrelations between IL-21 and IL-23 plasma levels (IL-21 vs IL-23 at 0 month, $r = 0.82$, $p = 6.7 \times 10^{-11}$; IL-21 vs IL-23 at 3 months, $r = 0.75$, $p = 7.0 \times 10^{-8}$, and IL-21 vs IL-23 at 12 months, $r = 0.82$, $p = 1.2 \times 10^{-9}$).

With regard to radiographic status and progression, no significant correlations were found for any of the observed cytokines. Neither did the IL-21 and IL-23 correlate with level or presence of IgM-RF and CCP antibodies. No significant correlations were observed for IL-17A compared with any of these measurements (data not shown).

Changes in IL-23 plasma levels are associated with change in disease activity and with radiological status. We further evaluated whether the changes in IL-21, IL-23, and IL-17A plasma levels were connected to disease activity and radiologic status and progression. We investigated for correlation between the changes in plasma cytokine levels and disease markers and change in disease activity. The changes were calculated as the value at 12 months divided by the value at the time of diagnosis.

We observed that changes in IL-23 plasma levels correlated well with DAS28, CRP, and ESR at 12 months after start of treatment, change in DAS28, and interestingly, also with the TSS at 2 years ($p < 0.005$), and almost with TSS at 3 years ($p < 0.06$; Table 2). Changes in IL-21 plasma levels correlated significantly with ESR only at 12 months. No significant correlations were observed for changes in IL-17A plasma levels compared with these measurements (data not shown).

IL-21-producing CD4+ T cells accumulated in SF of patients with RA. Having shown that plasma levels of IL-21 and IL-23, but not IL-17A, were significantly increased in patients with RA, we evaluated the frequency and location of CD4+ T cells with the capacity to produce either IL-21 or IL-17A in patients with RA. Since IL-23 is used but not produced by CD4+ T cells, we also studied expression of surface IL-23R. For this study we used paired SFMC and PBMC samples from patients with chronic RA (n = 9). As the vast majority of CD4+ T cells in the SF from patients with RA are mature CD45RO+ T cells, all flow cytometric analyses were performed on gated CD4+CD45RO+ T cell populations.

Table 2. In patients with early rheumatoid arthritis, changes in cytokine levels from start of treatment to 12 months were calculated as the 12-month level divided by the level at 0 month and examined for correlation with disease markers.

Disease Marker	Change in IL-21		Change in IL-2	
	p	r	p	r
DAS28, 12 mo	0.11	0.27	0.012*	0.42
CRP, 12 mo	0.52	0.11	0.0096*	0.43
ESR, 12 mo	0.015*	0.41	0.0022*	0.50
Change in DAS28	0.051	0.31	0.018*	0.37
TSS 1 yr	0.099	0.28	0.080	0.29
TSS 2 yrs	0.084	0.30	0.0050*	0.47
TSS 3 yrs	0.11	0.28	0.062	0.32
TSS 4 yrs	0.22	0.24	0.11	0.16
TSS 5 yrs	0.20	0.25	0.16	0.27

* $p < 0.05$. P represents a 2-sided p value; r represents correlation coefficient using Spearman's rho. DAS28: 28-joint count Disease Activity Score; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; TSS: Total Sharp Score at the indicated time after start of treatment.

We found that the frequency of CD4+CD45RO+ T cells with the capacity to produce IL-21 was significantly increased in SF versus PB ($p < 0.05$; Figure 2). We also observed that the frequency of IL-23R-positive CD4+ T cells was increased in SF versus PB, but this increase did not reach a statistically significant level. Similarly, although the frequency of CD4+ T cells with the capacity to produce IL-17A was slightly increased in SF versus PB, this was also not statistically significant.

IL-21 and IL-17A are only marginally coexpressed in RA. IL-21 has previously been reported as a member of the Th17 cytokine repertoire. In addition, IL-23R has been reported to be a marker for Th17 cells. To investigate this further, we analyzed for coexpression of IL-17A and IL-21 together with surface expression of IL-23R in both PB and SF CD4+CD45RO+ T cells.

We observed that the majority of the CD4+CD45RO+ T cells that produced IL-21 or IL-17A were single positives in both PB and SF (Figure 3). With regard to surface expression of IL-23R by CD4+CD45RO+ T cells, this was expressed by 22% (IQR 14%–44%) in the PB and by 32% (25%–47%) in the SF. Coexpression of IL-21 and the IL-23R was observed on 24% (IQR 20%–37%) of PB and on 27% (17%–40%) of SF cells that were positive for IL-21. Similarly, coexpression of IL-17A and the IL-23R was observed on 22% (IQR 14%–44%) of PB and on 32% (25%–47%) of SF cells that were positive for IL-17A. None of the IL-17A, IL-21, and IL23R coexpression combinations were significantly altered between PB and SF.

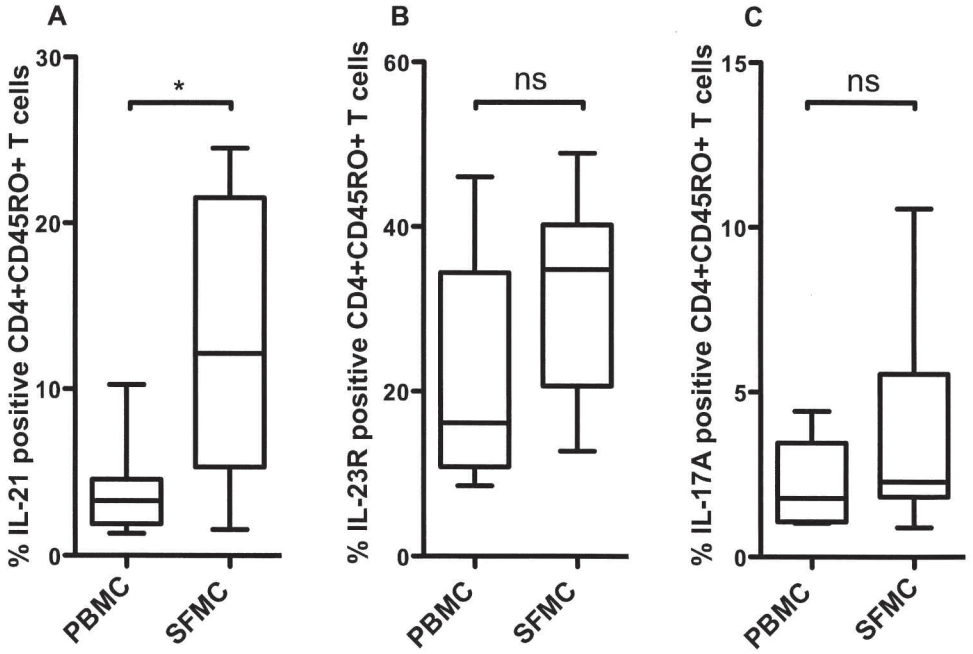

Figure 2. Expression of IL-21, IL-23R, and IL-17A by CD4+CD45+ T cells in patients with chronic RA. Paired peripheral blood mononuclear cells (PBMC) and synovial fluid mononuclear cells (SFMC) from patients with chronic RA were analyzed for surface expression of IL-23R and intracellular expression of IL-17A or IL-21 by multiparameter flow cytometry. A, B, and C show the percentage of CD4+CD45RO+ T cells that produce IL-17A or IL-21 or express IL-23R. Lines with boxes and whiskers represent medians with interquartile range (n = 9). *$p < 0.05$. ns: not statistically significant.

Characterization of IL-21- and IL-17A-producing T cells by chemokine receptor expression. Measurement of the surface expression of chemokine receptors has previously been carried out to characterize CD4+ T cell subsets. To investigate whether the IL-17A- and IL-21-producing CD4+ T cells from the patients with RA could be similarly characterized, we analyzed CD4+CD45RO+ T cells from both PB and SF for expression of a panel of chemokine receptors. The panel contained receptors that had previously been linked to either the Th17 subset or to the migration of T cells into inflamed RA tissue. These included CCR4, CCR6, CXCR3, CXCR4, and CXCR5.

Although all of these receptors were detectable on both IL-17A-producing and IL-21-producing subsets, we observed no significant difference in expression levels between IL-17A-producing and IL-21-producing CD4+ T cells (data not shown); nor did we observe a difference in chemokine receptor expression levels on these cells between PB and SF (data not shown). Thus we were unable to distinguish between IL-21-producing or IL-17A-producing cells by the expression of chemokine receptors that have previously been used to characterize the Th17 profile.

DISCUSSION

IL-21 and IL-23 have both been proposed as important factors in the pathogenesis of autoimmune diseases including RA. Both cytokines have the capacity to promote the development of the proinflammatory CD4+ T cell subset Th17 and are thus believed to contribute to inflammatory conditions. Our study showed that levels of both IL-21 and IL-23 were significantly increased in RA while IL-17A levels were unchanged. Plasma levels of IL-21 and IL-23 in early RA showed strong intercorrelations and both correlated with disease activity measured by the DAS28 12 months after the start of treatment. Moreover, changes in IL-23 plasma levels from the time of diagnosis to 12 months correlated with changes in DAS28 and the radiographic status at 2 years. Our data thus advocate that there are pathogenic mechanisms in RA that depend on or give rise to production of both IL-21 and IL-23.

Our suggestion that increased levels of IL-21 have pathogenetic relevance in RA is further supported by the increased expression of IL-21R on cells from the synovial tissue of patients with RA[10] and by the ability of IL-21 to induce synthesis of TNF-α and IL-6 from synovial T cells[9,19]. This should be seen in connection with our observation that IL-21 and IL-23 levels are strongly intercorrelated, but only levels of IL-23 are reduced during treatment. We argue that this supports our view that IL-21 could drive inflammation from a position upstream of TNF-α, IL-6, and IL-23, pointing to a key role for this cytokine in RA. The expression of IL-23R on a relatively large percentage of all

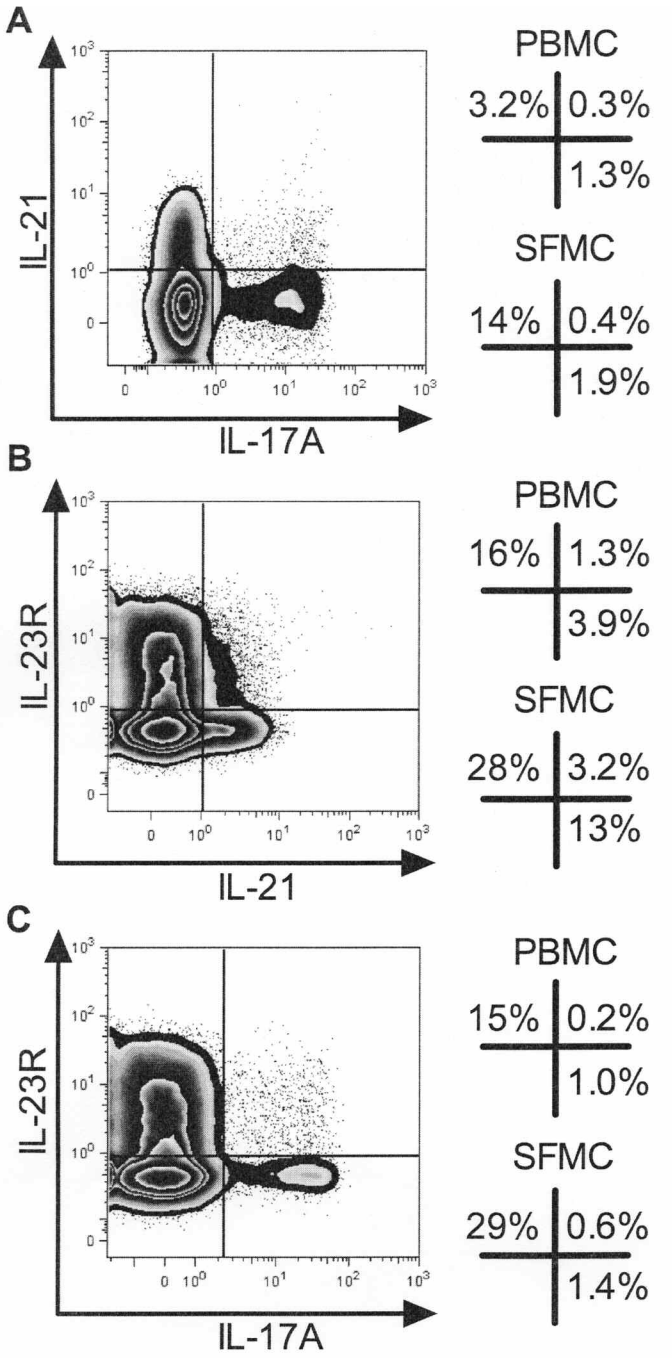

Figure 3. Expression of IL-17A, IL-21, and IL-23R in peripheral blood mononuclear cells (PBMC) and synovial fluid mononuclear cells (SFMC). Paired PBMC and SFMC were analyzed for coexpression of surface IL-23R and for intracellular contents of IL-17A and IL-21 by multiparameter flow cytometry. Expression of IL-17A, IL-21, and IL-23R gated on CD4+CD45RO+ T cells from SF of a patient with chronic RA is shown. Tables show median percentages of cells in each of the 3 quadrants (n = 9).

CD4+ T cells, including those that produce IL-21, suggests that IL-23 function is not restricted to the expansion of Th17 cells. These results are complemented in studies by Melis, *et al*, who report correlation of plasma levels of IL-23 but not IL-17A to disease activity in chronic RA[13].

We have shown that plasma levels of IL-21 and IL-23 were significantly increased in the early stages of RA, and to a lesser degree this was also the case for IL-23 in the chronic stages of RA compared with controls. Our results thus contribute to a body of evidence that T cells and their cytokines primarily dominate in early stages of arthritic diseases, only to decrease at later and chronic stages[20]. This implies that measurements of IL-21 and IL-23 could prove to be relevant biomarkers of the inflammatory processes in early RA and thus possibly guide treatment choice. In contrast to IL-21 and IL-23, the plasma levels of IL-17A remained low in both the early and chronic stages of RA. Similar observations have been reported in a study by Yamada, *et al*, who were unable to detect elevated levels of Th17 cells in the PB of patients with chronic RA compared to controls, and instead reported increased levels of interferon-γ (IFN-γ)-producing Th1 cells in the joints compared to PB[21]. By contrast, in a study by Raza, *et al*, IL-17A levels in the SF were shown to be elevated in early-stage RA but not in the later stages of RA[20]. Together, these data suggest that Th17 cells could be active in the joints of patients with early-stage RA, although not to a degree where IL-17A is detectably increased in plasma.

The increase of IL-21-producing CD4+ T cells in SF compared to PB provides evidence that these cells either home to or are induced to differentiate in the inflamed joint. Having shown that patients with early-stage RA have high plasma levels of IL-21 that correlated with disease activity and with plasma levels of IL-23, it is reasonable to expect that these IL-21-producing CD4+ T cells are important factors in the pathogenic processes in the joints of patients with RA.

Although IL-21 is reported to be primarily produced by Th17 cells[5,6,7,22], the data in our study suggest that this is not the case in RA. This is best illustrated by the lack of consistent coexpression of IL-17A and IL-21 in CD4+ T cells. Moreover, we observed increased numbers of IL-21-producing, but not of IL-17A-producing CD4+ T cells in the inflamed joints and high versus low plasma levels of IL-21 and IL-17A in patients with early-stage RA, respectively. In support of our data, Suto, *et al* recently demonstrated in mice that IL-21 was produced by a CD4+ T cell subset distinct from the Th17 subset[23]. Here, IL-21 or IL-6 alone induced the development of IL-21-producing CD4+ T cells that did not produce IFN-γ, IL-4, or IL-17A. However, IL-21 or IL-6 in combination with TGF-ß inhibited the development of IL-21-producing CD4+ T cells and instead induced development of Th17 cells[23]. Our data, combined with the data published by Suto, *et al*, support the idea that IL-21 is not produced primarily by Th17 cells, but rather by a distinct CD4+ T cell subset. Our data further suggest that this subset is important in the pathogenesis of RA.

We have shown that plasma levels of IL-21 and IL-23, but not IL-17A, were elevated in early-stage RA and corre-

lated with disease activity. Moreover, changes in levels of IL-23 correlated with changes in disease activity and with radiographic status. Finally, CD4+CD45RO+IL-21+ T cells were increased in the SF compared to PB of patients with RA, while CD4+CD45RO+IL-17A+ T cells were not. Our data thus support that IL-21 and IL-23 play important roles in the pathogenesis of RA.

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