Tocilizumab Treatment Decreases Circulating Myeloid Dendritic Cells and Monocytes, 2 Components of the Myeloid Lineage

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ABSTRACT. ***Objective.*** Interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) are proinflammatory cytokines involved in inflammatory response. Effective TNF-α blocker treatment is associated with an increase in circulating myeloid dendritic cells (mDC), suggesting their release from inflamed synovium. Currently, *in vivo* effects of IL-6 inhibition on DC are unknown. We monitored the changes in circulating mDC and plasmacytoid DC (pDC) during tocilizumab (TCZ) therapy in patients with rheumatoid arthritis (RA).

Methods. DC subset levels were evaluated by flow cytometry in patients with RA (n = 43) and in healthy volunteers (n = 20). In patients with RA, these levels were measured before and during TCZ therapy (8 mg/kg every 4 weeks). Response to TCZ therapy was evaluated at 12 weeks. Statistical analysis was based on Mann-Whitney U tests or Wilcoxon signed-rank tests.

Results. At baseline, patients with active RA were characterized by a significantly lower level of circulating mDC and pDC compared to healthy donors. However, this difference did not correlate with any disease activity score. TCZ-treated patients who met the European League Against Rheumatism (EULAR) improvement criteria at Week 12 had significant reductions in mDC and monocyte levels as compared with EULAR nonresponders. Levels of pDC, CD4+ T cells, and CD8+ T cells remained stable during the TCZ courses, regardless of treatment response.

Conclusion. Our study reveals an unexpected reduction of circulating mDC and monocytes in patients with RA in response to TCZ therapy. In accord with reports on neutrophils and platelets decreasing during TCZ therapy, our data suggest an effect of IL-6 inhibition on cells from myeloid lineage. (J Rheumatol First Release April 1 2012; doi:10.3899/jrheum.111439)

Key Indexing Terms:
RHEUMATOID ARTHRITIS TOCILIZUMAB DENDRITIC CELLS MONOCYTES

Interleukin 6 (IL-6) is a pleiotropic cytokine involved in the initiation and maintenance of inflammatory and immune responses. IL-6 plays a central role in chronic inflammation, mainly secreted by monocytes and macrophages, and constitutes an important link between innate and adaptive immunity by mediating the T cell and B cell responses involved in inflammatory diseases[1,2,3]. Most of the biological activities assigned to IL-6 are mediated by the naturally occurring soluble IL-6 receptor (sIL-6R)[4]. The IL-6/sIL-6R complex influences leukocyte migration, activation, and apoptosis[5]. IL-6 also affects the differentiation of myeloid lineages, notably by skewing the differentiation of human monocytes away from a dendritic lineage toward a macrophage phenotype[6]. Further, IL-6 has been shown to suppress CCR7 expression in mature dendritic cells (DC) and therefore to impair chemotaxis to CCR7-activating chemokines required for recruiting DC to lymphoid tissues *in vivo*[7,8].

DC are professional antigen-presenting cells that regulate T cell responses. Two DC subsets, myeloid DC (mDC) and plasmacytoid DC (pDC), have been identified in humans. These cells play a central role in the initiation and coordination of the immune response to infectious agents and tumors. Their function is tightly controlled by the local environment through cytokine and chemokine signals. Abnormalities of DC homeostasis have been involved in the

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pathophysiology of various autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis (RA)[9]. In RA, these cells have been reported to infiltrate the synovium[10,11]. Synovial DC are more mature than DC from peripheral blood: they express various activation markers, secrete large amounts of cytokines [such as IL-12, tumor necrosis factor-α (TNF-α), and IL-6], and are able to activate both autologous T and B lymphocytes[12,13]. The presentation of arthritogenic antigens to T cells by DC could therefore contribute to the initiation as well as the perpetuation of RA. We previously showed that, among DC, mDC seem to have a prominent role in clinical disease manifestations, because their circulating numbers correlate directly with disease activity: while the percentage of mDC is increased in inflamed synovial tissue, treatment with TNF-α blockers increases peripheral blood count of mDC in patients responding well to the treatment, suggesting an mDC release from inflamed tissues[14]. Other authors have made similar observations highlighting the importance of mDC in RA pathogenesis[15,16].

Tocilizumab (TCZ) is a humanized monoclonal antihuman IL-6 receptor (IL-6R) antibody. Clinical studies have shown that inhibition of IL-6R by TCZ is effective in the treatment of RA[17,18]. The importance of IL-6 in DC homeostasis prompted us to monitor the effect of IL-6-blocking therapy on circulating pDC and mDC. We therefore conducted a prospective study in patients with active RA and investigated the effects of blocking IL-6 with TCZ on circulating monocytes and DC subsets over a 12-week study period. Our study demonstrates that patients with RA responding to TCZ treatment show a decrease in mDC, a finding that confirms the effect of TCZ on the myeloid lineage, as described with neutrophils and platelets[18]. This result suggests an opposite effect of IL-6 and TNF-α blockade on mDC homeostasis, in spite of the efficacy of both treatments.

MATERIALS AND METHODS

Study population. Forty-three patients with moderate to severe active RA (Disease Activity Score in 28 joints = DAS28 > 3.2) who fulfilled the revised classification criteria of the American College of Rheumatology for RA[19] were evaluated before and after TCZ therapy. Table 1 summarizes the characteristics of these patients. In those receiving biological agents, therapy was stopped at least 1 month prior to initiation of TCZ. TCZ (Roche Chugai) was given at a dose of 8 mg/kg intravenously every month. Most patients were on stable prednisone doses of ≤ 10 mg/day and methotrexate 7.5–15 mg/wk orally or intramuscularly. No patients were treated with a high dose of prednisone during the followup. We used the European League Against Rheumatism (EULAR) improvement criteria at Week 12 to define the response to the treatment[20]. In addition, 17 healthy blood donors were included in the study. They were matched with patients for sex and age.

Our study was approved by the local Ethics Committee, and all patients gave informed consent.

Enumeration of blood DC precursors and T cells by flow cytometry. Whole blood samples were analyzed on an FC500 CXP flow cytometer (Beckman Coulter, Villepinte, France), with 10^6 white blood cells acquired per analysis. DC subsets were measured using a DC kit from Beckman Coulter. Peripheral blood (PB) mDC and pDC subsets were defined by the concomitant lack of lineage markers, ILT3 expression, and mutually exclusive membrane expression of CD33 or CD123, respectively. Circulating T cells were defined by CD45 and CD3 expression, then divided into CD4 and CD8 T cells. Absolute numbers of blood T cells and DC precursors were calculated as the percentage of white blood cells expressed per ml of PB. Enumeration of blood T cells and DC was evaluated as published elsewhere[21].

Table 1. Baseline demographic, clinical, and biological characteristics of patients with rheumatoid arthritis treated with tocilizumab (n = 45).

Characteristic	
Age, yrs, mean ± SD	58.9 ± 14.6
Women, % (n)	75.6 (34)
Disease duration, yrs, mean ± SD	13 ± 9.9
No. previous biotherapies (%)	
0	5 (11.1)
1	8 (17.8)
2	8 (17.8)
≥ 3	24 (53.3)
Rheumatoid factor, % positive (n)	77.8 (35)
ACPA, % positive (n)	62.2 (28)
Tender joints, mean ± SD	8.9 ± 7.4
Swollen joints, mean ± SD	5.8 ± 5.9
Patient global assessment, 100 mm VAS, mean ± SD	59.4 ± 20.4
ESR, mm/h, mean ± SD	35.6 ± 26.6
Levels of CRP, mg/l, mean ± SD	26.2 ± 34.5
DAS28 (ESR), mean ± SD	5.11 ± 1.58
Patients taking methotrexate	27
Methotrexate dosage, mg/wk (range)	15 (15–20)
Patients taking prednisone	35
Prednisone dosage, mg/day (range)	9 (7–10)

ACPA: anticitrullinated protein antibodies; VAS: visual analog scale; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS28: 28-joint Disease Activity Score.

Statistical analysis. Statistical analysis was performed using the GraphPad InStat software (version 3.0a for Macintosh, GraphPad Software, La Jolla, CA, USA). Mann-Whitney U tests were used for mean comparisons between groups. The Wilcoxon signed-rank test was used for analyses of matched pairs. Correlations between DC and activity markers were assessed using linear regression, given with the r-squared correlation coefficient. P values < 0.05 were considered statistically significant.

RESULTS

Comparison of circulating DC levels in patients with RA and healthy controls. In RA pathogenesis, the effects of IL-6 overproduction on immune cells remain unclear. This cytokine could be involved in activation, differentiation, and/or migration of different immune cells such as DC, and therefore could modify their homeostasis and recruitment in the inflamed tissues[2]. In our study, to better understand the effect of IL-6R inhibition by TCZ on circulating DC, we first described the levels of these cells in 45 patients with active RA before TCZ treatment, and compared them to 17 healthy donors. Interestingly, RA peripheral blood was characterized by a global lower number of circulating DC compared to healthy donors (Table 2). The difference was significant for both DC subsets (mDC, p = 0.02, and pDC, p = 0.04).

Table 2. Comparison of dendritic cell (DC) subset levels between patients with rheumatoid arthritis (RA) and healthy controls. Values are mean ± SD).

DC Subsets	RA Patients, n = 45	Controls, n = 17	p*
Myeloid DC	9925 ± 8817	14,396 ± 6479	0.02
Plasmacytoid DC	4754 ± 3694	6825 ± 3238	0.04

* Mann-Whitney U test.

We then looked for a correlation between absolute counts of these different cells and the clinical status known to reflect disease activity [DAS28-erythrocyte sedimentation rate, DAS28-C-reactive protein (CRP), simple disease activity index (SDAI), and Crohn's disease activity index (CDAI)]. In patients with RA, we did not find any statistical correlation between DC counts and any disease activity score.

Efficacy of IL-6 inhibition on clinical and inflammatory measures. Clinical response to TCZ therapy was assessed at Week 12. The DAS28-ESR declined significantly from a mean ± SD of 5.11 ± 1.58 at baseline to 3.03 ± 1.53 at Week 12 ($p < 10^{-4}$; Figure 1A, Table 3). DAS28-CRP, SDAI, and CDAI showed similar decreases (Table 3). Most of the measurements used to calculate the scores also decreased significantly (data not shown). Further, the numbers of global leukocytes decreased during therapy with TCZ from a mean of 9357 ± 2836 cells/mm^3 at baseline to 7609 ± 2733 cells/mm^3 at Week 12 ($p = 6 \times 10^{-4}$; Figure 1B).

According to the EULAR response criteria[20], 23 patients were classified at Week 12 as good responders, 8 as moderate responders, and 9 as nonresponders. At Week 12, 15 patients achieved remission and 26 had low disease activity.

Table 3. Change in 28-joint Disease Activity Score (DAS28) under tocilizumab treatment. Week 12 data were compared to the baseline data for the 40 patients who completed the 12-week study. Each p value corresponds to the result of a Wilcoxon signed-rank test.

Score Type	Inclusion	Week 12	p
DAS28-ESR	5.2 ± 1.4	3 ± 1.5	$< 10^{-4}$
DAS28-CRP	4.9 ± 1.2	3.2 ± 1.2	$< 10^{-4}$
CDAI	27.2 ± 12.7	13.8 ± 8	$< 10^{-4}$
SDAI	29.8 ± 13.8	15 ± 9.1	$< 10^{-4}$

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; SDAI: simple Disease Activity Index; CDAI: Crohn's Disease Activity Index.

Change in DC and monocyte counts in TCZ-treated patients with RA. We have shown[14] that the level of mDC increases in patients with RA who were treated with the TNF-α blocker infliximab, reaching the level observed in healthy controls. This observation led to the monitoring of DC numbers during the course of TCZ treatment. Unexpectedly, patients with RA showed a substantial decrease in mDC (mean 9925 ± 8817 cells/ml at Day 0 vs 5349 ± 4076 cells/ml at Week 12; p = 0.04, using the Wilcoxon matched-pairs test; Figure 2A), whereas the level of blood pDC did not change significantly (4754 ± 3694 cells/ml at Day 0 vs 5605 ± 4102 cells/ml at Week 12; p = 0.54; Figure 2B).

Interestingly, we also found a decrease in monocytes from 586 ± 295 cells/mm^3 at baseline to 524 ± 271 cells/mm^3 at Week 12 ($p = 8 \times 10^{-3}$, Figure 2C). On the other hand, CD4+ and CD8+ T cell numbers remained stable, with no significant difference observed during the course of treatment (900 ± 480 CD4+ cells/mm^3 at Day 0 vs 791 ± 550 CD4+ cells/mm^3 at Week 12; p = 0.31, and 346 ± 233 CD8+ cells/mm^3 at Day 0 vs 345 ± 251 CD8+ cells/mm^3 at Week 12; p = 0.87; Figure 2D-2F).

Decrease of mDC correlates with treatment response.

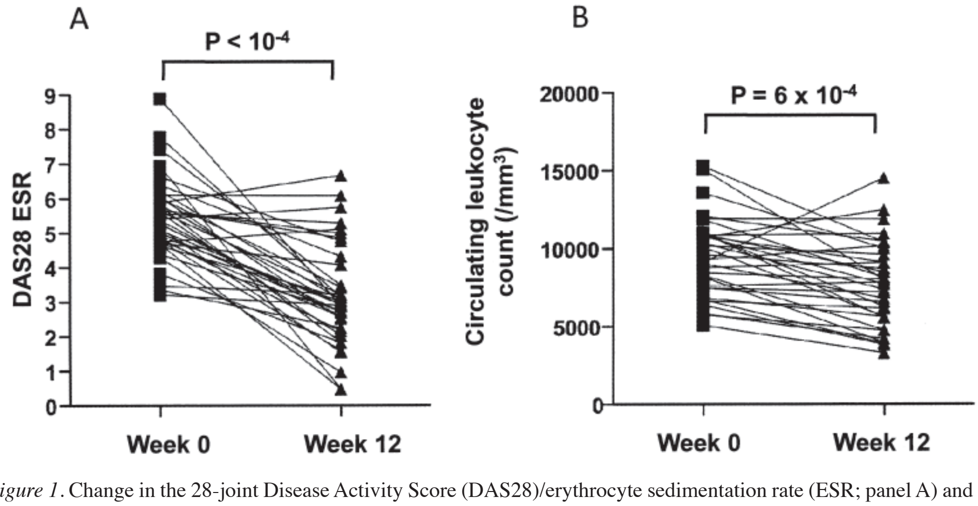

Figure 1. Change in the 28-joint Disease Activity Score (DAS28)/erythrocyte sedimentation rate (ESR; panel A) and leukocyte counts (B) over 12 weeks of treatment with tocilizumab in patients with RA. Squares indicate matched samples. P value was calculated using the Wilcoxon matched-pairs test.

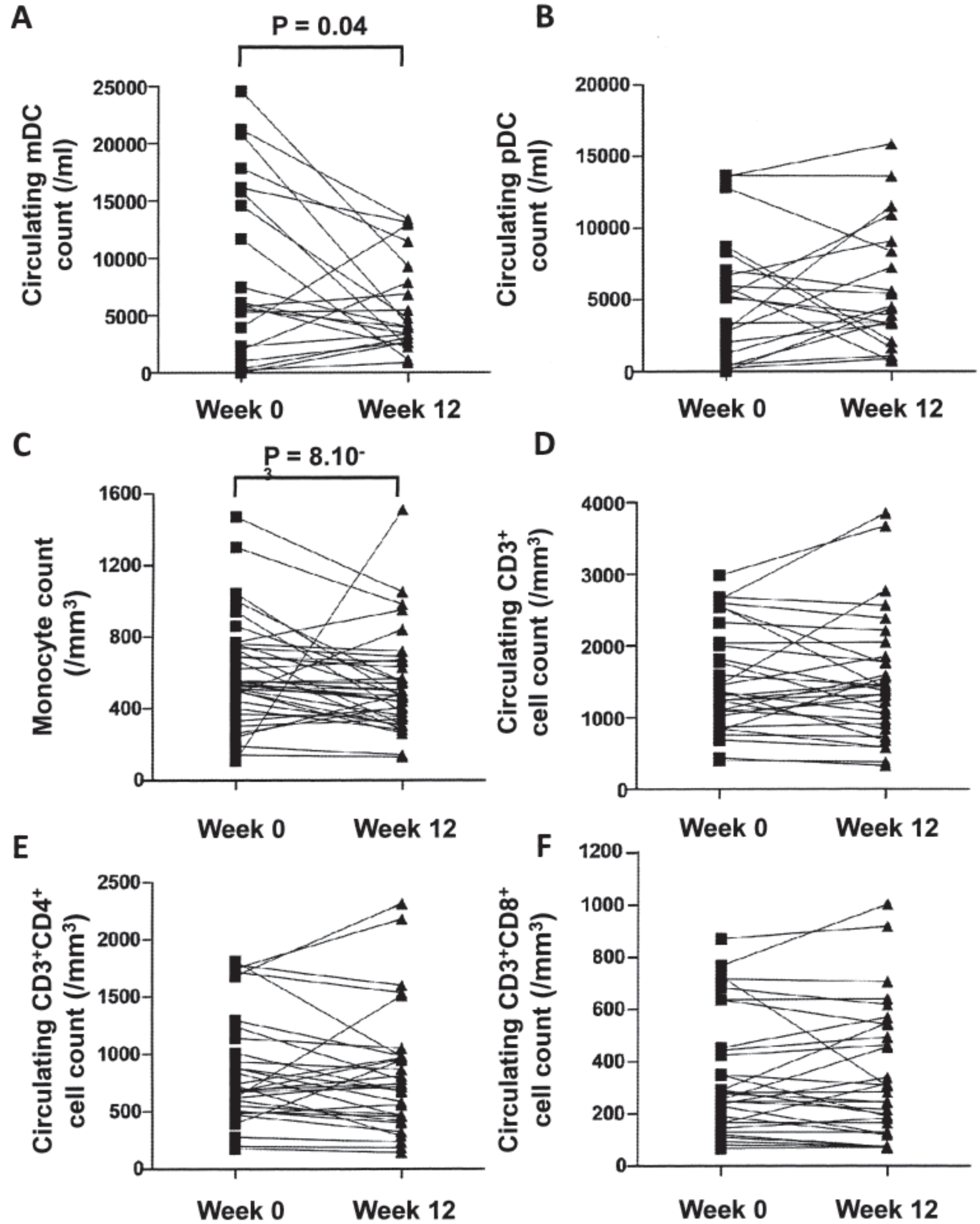

Figure 2. Change in various immune cell counts over 12 weeks of treatment with tocilizumab in patients with RA. A. Circulating myeloid dendritic cells (mDC). B. Plasmacytoid dendritic cells (pDC). C. Monocytes. D. T cells. E. CD4+ T cells. F. CD8+ T cells. Squares indicate matched samples. P value was calculated using the Wilcoxon matched-pairs test.

Levels of DC were available for 31 patients: 25 good responders and 6 nonresponders. As described, in the entire population, mDC levels in responders were significantly decreased (10,342 ± 7747 cells/ml at Day 0 vs 6430 ± 4159 cells/ml at Week 12; p = 0.03), whereas T cells and pDC levels did not change (Table 4). Further, monocyte levels in responders showed a significant decrease, from 627 ± 270 cells/mm^3 at baseline to 511 ± 206 cells/mm^3 at Week 12 (p = 7×10^{-3}). Nonresponders did not show statistically significant changes in either mDC or pDC, T cell counts, and monocytes, even though some patients showed an increase during the course of treatment. These data suggest a relationship between the fluctuations of the mDC present in the blood and the variations of disease activity.

DISCUSSION

IL-6-blocking therapy significantly reduces signs and symptoms as well as radiological progression in RA[17,22,23]. However, to date it has not been determined which of the pleiotropic IL-6 effects influence the observed clinical

Table 4. Changes in levels of monocytes, T cells, and dendritic cell (DC) counts under tocilizumab treatment. Distinction was made between responders and nonresponders at Week 12. Each p value corresponds to the result of a Wilcoxon signed-rank test in each subgroup. Values are mean ± SD except where indicated.

Phenotyping	Week 12 Responders, n = 25			Week 12 Nonresponders, n = 6		
	Day 0	Week 12	p	Day 0	Week 12	p
CD3+ total	1304 ± 696	1335 ± 809	0.73	1054 ± 692	774 ± 458	0.11
CD3+CD4+ cells	880 ± 467	900 ± 551	0.77	754 ± 550	511 ± 295	0.11
CD3+CD8+ cells	368 ± 244	378 ± 261	0.77	243 ± 140	208 ± 146	0.24
Monocytes (range)	550 (490–760)	474 (370–615)	7.10^{-3}	400 (215–603)	402 (270–555)	0.62
Myeloid DC	10,342 ± 7747	6430 ± 4159	0.03	3256 ± 5642	2368 ± 847	0.71
Plasmacytoid DC	6011 ± 4110	6438 ± 4505	0.61	2390 ± 4223	2767 ± 1472	0.71

response. We showed that patients with RA are characterized by significantly low levels of circulating mDC and pDC, consistent with our previous observation[14] and results obtained by other groups[15,16]. This finding suggested a recruitment of activated mDC to the site of inflammation, i.e., the inflamed synovium. Because IL-6 has been described as an important factor of DC migration, activation, and differentiation[6,24], we decided to investigate the influence of the anti-IL-6 monoclonal antibody TCZ on the homeostasis of DC and to correlate levels of DC subsets with the clinical efficacy of TCZ therapy. In fact, we found a decrease in circulating mDC in patients with RA who were responsive to TCZ therapy. This unexpected decrease led us to also monitor the changes in monocytes and T cells during the course of treatment. Responder patients showed a substantial decrease in monocyte count, whereas the level of CD4 and CD8 T cells did not change during TCZ therapy.

The significant decrease in circulating mDC compared to healthy donors in patients with active RA suggests that these cells play a role in RA pathogenesis. Interestingly, rheumatoid synovium is characterized by accumulation of immature and mature DC subsets perivascularly, in close association with T cell follicles[13,25,26]. Synovial fluid contains significant numbers of mDC compared to blood[14,15], suggesting a role for these cells in perpetuation of disease. However, in contrast to previous studies[14,15], we did not find any correlation between circulating numbers of these cells and RA activity. This may be due simply to the difference between RA populations analyzed a few years later; indeed, patients studied previously had more active RA (DAS28 > 6.1 and CRP > 36.2) and were free of any biological agents that could interfere with immune cell homeostasis. Further, at baseline, presence or absence of prednisone and methotrexate treatment in patients with RA was not associated with change in circulating mDC number.

As described with infliximab[14], we attempted to find an increase of mDC in patients responsive to TCZ therapy. However, we found the opposite: a significant decrease of mDC levels after 12 weeks of therapy. Interestingly, a similar finding has recently been reported in a study comparing the variation of DC after TCZ, abatacept, and infliximab treatment[27]. While no significant change in DC was shown, neither with abatacept nor infliximab treatment, TCZ therapy affected the percentage of mDC among the mononuclear cells, with a significant decrease in 5 patients after 3 infusions.

Some functions of IL-6 could explain the decrease of peripheral mDC observed in our study. IL-6 has been shown to inhibit CCR7 expression at the DC surface[7]. A potential normalization of CCR7 expression during effective IL-6 inhibition could promote the recruitment of DC to lymphoid tissues and their decrease in the periphery. Further, mDC are not the only immune cells that are decreased during TCZ therapy. Neutropenia was reported in clinical trials, and a decrease in platelets after the treatment has also been observed[18,28]. To date, different hypotheses have been suggested for the potential role of TCZ in neutrophil apoptosis or margination[29]. Together, these observations suggest an effect of TCZ on neutrophils and mDC from the same myeloid lineage. To examine this hypothesis, we monitored the levels of monocytes in TCZ-treated patients with RA and found a decrease between baseline and Week 12, suggesting a global effect of this treatment on the myeloid lineage. It will be important to examine the effect of TCZ on myeloid progenitors in future research.

We did not find any significant change during the therapy in the absolute numbers of CD4+ and CD8+ T cells. This negative result suggests the influence of IL-6 inhibition on DC hematopoiesis, but not on the effector functions of mature DC. A study *in vitro* by Santiago-Schwarz, *et al*[30] supports this result and highlights the importance of IL-6 as a cytokine of DC development. The authors described the effect of IL-6 inhibition on developmental processes associated with optimal monocyte and DC growth, in connection with the stimulatory capacity of these cells[30]. A recent *in vitro* study showed complementary findings, with no effect of IL-6 inhibition on mDC maturation/activation and alloreactive T cell proliferation[31].

Although the level of circulating mDC is reduced in the peripheral blood of patients with active RA, effective treatment by TCZ amplifies this decrease. A comparable decrease is observed with monocyte counts, whereas T cells and pDC levels remain stable, suggesting a selective effect on cells from the myeloid lineage.

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