



# Targeting CD1c-expressing classical dendritic cells to prevent thymus and activation-regulated chemokine (TARC)-mediated T-cell chemotaxis in rheumatoid arthritis

MR Hillen, FM Moret, KMG van der Wurff-Jacobs, TRDJ Radstake, CE Hack, FPJG Lafeber & JAG van Roon

To cite this article: MR Hillen, FM Moret, KMG van der Wurff-Jacobs, TRDJ Radstake, CE Hack, FPJG Lafeber & JAG van Roon (2017) Targeting CD1c-expressing classical dendritic cells to prevent thymus and activation-regulated chemokine (TARC)-mediated T-cell chemotaxis in rheumatoid arthritis, Scandinavian Journal of Rheumatology, 46:1, 11-16, DOI: [10.3109/03009742.2016.1158311](https://doi.org/10.3109/03009742.2016.1158311)

To link to this article: <http://dx.doi.org/10.3109/03009742.2016.1158311>



© 2017 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group



Published online: 02 Jun 2016.



Submit your article to this journal



Article views: 322



View related articles



View Crossmark data

# Targeting CD1c-expressing classical dendritic cells to prevent thymus and activation-regulated chemokine (TARC)-mediated T-cell chemotaxis in rheumatoid arthritis

MR Hillen<sup>1,2</sup>, FM Moret<sup>1,2</sup>, KMG van der Wurff-Jacobs<sup>1</sup>, TRDJ Radstake<sup>1,2</sup>, CE Hack<sup>1,2</sup>, FPJG Lafeber<sup>1</sup>, JAG van Roon<sup>1,2</sup>

<sup>1</sup>Department of Rheumatology and Clinical Immunology, and <sup>2</sup>Laboratory of Translational Immunology, University Medical Centre Utrecht, The Netherlands

**Objectives:** Thymus and activation-regulated chemokine (TARC) attracts cells that express the C-C chemokine receptor type 4 (CCR4), including CD4 T cells. As expression of CCR4 is increased on peripheral T cells and intra-articular interleukin (IL)-17-producing cells in patients with rheumatoid arthritis (RA), we investigated whether TARC plays a role in the attraction of T cells to the synovial compartment. In addition, we assessed the role of classical dendritic cells (cDCs) in the production of TARC in RA.

**Method:** TARC was measured in synovial fluid (SF) samples from RA and osteoarthritis (OA) patients. Spontaneous and thymic stromal lymphopoietin (TSLP)-induced TARC production by mononuclear cells (MCs) and CD1c cDCs from peripheral blood (PB) and SF was assessed. The role of TARC in CD4 T-cell migration towards cDCs was assessed and the contribution of CD1c-expressing cells to TARC production was studied.

**Results:** TARC concentrations were higher in SF of RA patients compared to OA patients. MCs from SF produced TARC spontaneously and produced more TARC upon stimulation than paired PBMCs. Blocking TARC strongly inhibited CD4 T-cell chemotaxis by TSLP-stimulated cDCs, associated with decreased production of tumour necrosis factor (TNF)- $\alpha$ . Depletion of cDCs from SFMCs strongly reduced TARC production.

**Conclusions:** TARC levels are increased in RA SF and our data indicate that this results from production by SFMCs and in particular CD1c cDCs. TARC attracts T cells and TARC secretion by MCs is crucially dependent on the presence of CD1c cDCs. Considering the potential of SF cDCs to activate T cells and induce pro-inflammatory cytokine secretion, targeting intra-articular cDCs constitutes a novel therapeutic approach in RA.

Thymus and activation-regulated chemokine (TARC/CCL17) was first identified in the thymus and was shown to attract cells expressing the C-C chemokine receptor type 4 (CCR4) (1), including natural killer (NK) cells, fibroblasts, and CD4 T cells with T-helper (Th)2, Th17, and regulatory T cell (Treg) phenotypes (2–6). TARC is released by activated platelets and is produced by HaCaT keratinocytes and specific subsets of activated T cells (7, 8). In addition, secretion was shown by epidermal Langerhans cells (9) and by activated CD11c+ classical dendritic cells [cDCs; also referred to as myeloid dendritic cells (mDCs)] in allergic conditions (10).

TARC is described as a crucial mediator in atopic diseases including atopic dermatitis and asthma (6, 11). However, TARC expression has also been described in a range of autoimmune diseases, including primary

Sjögren's syndrome, systemic lupus erythematosus, autoimmune blistering disease, juvenile idiopathic arthritis, and rheumatoid arthritis (RA) (12–16). In RA, TARC protein has been detected in plasma (15) while TARC mRNA is elevated in RA synovial tissue (17). In addition, RA patients have increased numbers of peripheral blood (PB) CD4 T cells that express CCR4 (18). Moreover, the majority of interleukin (IL)-17-positive CD4 T cells in RA synovial fluid (SF) are CCR4+ and the percentage of these cells of all mononuclear cells (MCs) is higher in the SF compared to the PB (5). These observations suggest that TARC mediates the migration of pro-inflammatory CCR4-expressing T cells into the joint in RA. Furthermore, it was previously shown that thymic stromal lymphopoietin (TSLP), a strong TARC inducer on cDCs, is increased in the RA SF (19) and that cDCs from the RA SF produce TARC ex vivo (20). The aim of this study was to evaluate TARC levels in SF of RA patients, study the role of cDCs in TARC production in RA joints, and investigate the role of TARC in the attraction of T cells in RA.

Joel AG van Roon, Department of Rheumatology and Clinical Immunology, University Medical Centre Utrecht, Lundlaan 6, Utrecht 3508 GA, The Netherlands.

E-mail: [j.vanroon@umcutrecht.nl](mailto:j.vanroon@umcutrecht.nl)

Accepted 22 February 2016

## Method

### Patients

SF samples were obtained from patients attending our outpatient clinics. RA patients were classified according to the American College of Rheumatology (ACR) criteria (21). SF from patients with osteoarthritis (OA) were used for comparison in a TARC enzyme-linked immunosorbent assay (ELISA). The medical ethical committee of the University Medical Centre Utrecht approved collection of samples in accordance with the Declaration of Helsinki. All patients gave their informed consent. The characteristics of the patients are depicted in Table 1.

### Flow cytometry

Fluorescence-activated cell sorting (FACS) and a FACS-Canto II flow cytometer (BD Bioscience, San Jose, CA, USA) were used to analyse the expression of extracellular markers on all samples. The results were analysed using FlowJo software (Tree Star, Inc, Ashland, OR, USA). To assess the number of CD1c-expressing cDCs in SF of RA patients ( $n = 17$ ), SFMCs were stained with anti-CD1c phycoerythrin (PE; Biolegend, San Diego, CA, USA) and anti-CD19 PerCP-Cy5.5 (Biolegend). CD1c+ cDCs were gated as CD19-negative and CD1c-positive cells within the live gate. The number of CD1c+ cDCs was calculated using the number of isolated SFMCs.

### Cytokine analyses

The TARC content in SF of RA patients ( $n = 100$ ) and OA patients ( $n = 50$ ) was measured with an ELISA (R&D Systems, Minneapolis, MN, USA). Samples were pretreated with hyaluronidase (20 U/mL; type IV, Sigma-Aldrich, St Louis, MO, USA). This TARC ELISA was also used to measure TARC production in MCs and CD1c-depleted MC culture supernatants. TARC production by isolated CD1c+ cDCs and T-cell

cytokine production after chemotaxis were analysed by multiplex immunoassay as described elsewhere (22).

### Cell isolation

MCs were isolated from lithium-heparinized PB and SF by density centrifugation using Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden). Prior to isolation, PB or SF was diluted 1:1 in RPMI 1640 medium (Gibco, Life Technologies, New York, NY, USA) containing penicillin (100 U/mL), streptomycin (100 µg/mL), and glutamine (2 mM) (all PAA Laboratories, Pasching, Australia). For assessment of TARC production by cDCs and chemotaxis experiments, CD19-/CD1c+ cDCs and CD4+ T cells were isolated from PBMCs by magnetic-activated cell sorting (MACS) using CD1c (BDCA-1)+ and CD4+ isolation kits (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. To confirm the efficacy of CD1c isolation and CD1c depletion, isolated cells were stained with anti-CD45 PerCP (Biolegend), anti-CD1c PE (Biolegend), and anti-CD19 FITC (Bd Biosciences, San Jose, CA, USA).

### Cell cultures

Cells were cultured in RPMI Glutamax (Gibco) supplemented with penicillin, streptomycin (both PAA Laboratories), and 10%, v/v, human AB serum (GemCell, West Sacramento, CA, USA). To compare TARC production by SFMCs with that of PBMCs, MCs were purified from paired PB and SF samples from RA ( $n = 6$ ) patients. Cells were seeded at a concentration of  $1.0 \times 10^6$ /mL in 96-well round-bottomed plates and cultured in triplicate for 72 h at 37°C in the presence of 20 ng/mL of recombinant human TSLP (R&D Systems) and/or 10 ng/mL of recombinant human IL-7 (Preprotech, Rocky Hill, NJ, USA). Supernatants were harvested and stored at -80°C. To investigate TARC production by cDCs, CD1c+ cDCs were isolated from RA patients' PB ( $n = 6$ ) and cultured in Sarstedt tubes at 37°C with 20 ng/mL TSLP (R&D) for 20 h ( $5 \times 10^5$  cells/mL). Supernatants were collected and stored at -80°C and analysed by multiplex immunoassay.

Table 1. Characteristics of RA and OA patients.

	SF TARC analysis		
	RA	OA	RA in vitro cultures
n (males/females)	100 (32/68)	50 (12/38)	35 (13/22)
Age (years), mean ± sd	56 ± 15	64 ± 10	53 ± 16
Disease duration (years), mean ± sd	17 ± 17	—	11 ± 6.3
Rheumatoid factor (no. positive)	61	—	16
ESR (mm/h), mean ± sd	44 ± 34	na	21 ± 22
No. treated with corticosteroids/DMARDs/biologics	9/51/26	na	5/14/9

RA, Rheumatoid arthritis; OA, osteoarthritis; SF, synovial fluid; TARC, thymus and activation-regulated chemokine; ESR, erythrocyte sedimentation rate; DMARD, disease-modifying anti-rheumatic drug; na, not applicable; sd, standard deviation.

### CD1c depletion

MCs were isolated from PB ( $n = 7$ ) and SF ( $n = 8$ ) of RA patients, including two paired samples. CD1c-expressing cells were depleted using anti-CD1c biotin and anti-biotin beads (Miltenyi). As control, B cells were depleted using anti-CD19 beads. In addition, the CD1c-depletion protocol was performed omitting the CD1c-biotin on SFMCs of RA patients ( $n = 4$ ) in a separate experiment. Depleted and undepleted MC fractions were cultured for 72 h in the presence of 20 ng/mL TSLP (R&D Systems) at 37°C. Supernatants were harvested and stored at -80°C until analysis of TARC levels with ELISA.

### Chemotaxis assay

CD1c+ cDCs and CD4+ T cells were isolated from PBMCs of RA patients ( $n = 6$ ) and healthy controls ( $n = 3$ ) and cell purity of subsets was confirmed by FACS. Cells were taken up in RPMI Glutamax (Gibco) supplemented with penicillin, streptomycin (both PAA Laboratories), and 10% foetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA). For each condition,  $3 \times 10^4$  cDCs were added to the lower compartment of a 24-well transwell system (Corning, New York, NY, USA), supplemented with 20 ng/mL TSLP (R&D Systems) and, where appropriate, 10 µg/mL of monoclonal anti-TARC (mouse IgG1; R&D Systems) or an equal concentration of an isotype control antibody (mouse IgG1; Amgen, Seattle, WA, USA). A well without cDCs and only culture medium was prepared as control for aspecific T-cell migration. CD4 T cells ( $5 \times 10^5$ ) were added to the upper compartment of the transwells (5.0 µm pore size, polycarbonate membrane; Corning) and cells were allowed to migrate for 20 h at 37°C. The percentage of migrated cells was determined by staining the cell fraction in the lower compartment with anti-CD3 FITC (Bd Biosciences), anti-CD19 APC (Biolegend), anti-CD1c PE (Biolegend), and anti-CD4 PerCP (Biolegend). For

analysis of the net T-cell cytokine production, the remaining cells were resuspended in 200 µL RPMI Glutamax (Gibco) supplemented with penicillin, streptomycin (both PAA Laboratories) and 10% FBS (Invitrogen) and stimulated for 24 h at 37°C with ionomycin (500 ng/mL) and phorbol myristate acetate (PMA; 50 ng/mL) (both from Sigma-Aldrich). Cytokines were measured by multiplex immunoassay. The percentage of migrated T cells was calculated using the number of cells counted in the lower compartment and the percentage of T cells therein as measured by FACS, corrected for aspecific T-cell migration. The stimulation index (SI) was calculated by dividing the measured cytokine concentration by that of the culture medium control.

### Statistical analysis

Differences in TARC content in SF between groups were determined using the Mann–Whitney U test. Correlation between TARC content in SF and the number of cDCs was calculated with Spearman's rank correlation coefficient. The Wilcoxon signed rank test was used to assess differences in TARC production, T-cell migration, and T-cell cytokine production. Statistical analyses were performed using SPSS software version 20.0 (IBM, Armonk, NY, USA). Differences were considered statistically significant at  $p < 0.05$ .

## Results

TARC levels are increased in RA SF and correlate with numbers of cDCs

TARC concentrations in the SF of RA patients were significantly increased compared to OA patients (Figure 1A). In addition, TARC levels were significantly higher in RA patients with increased systemic

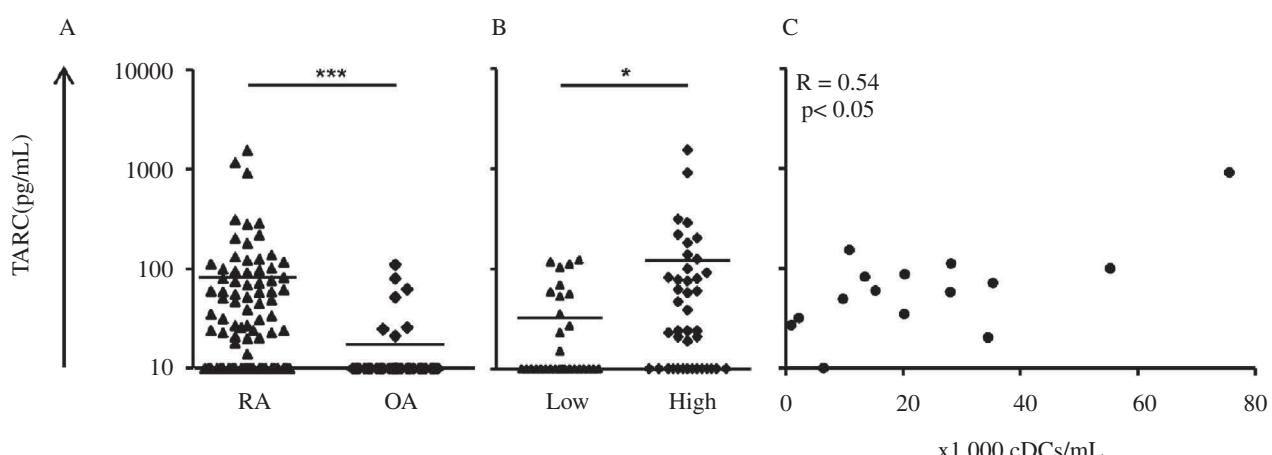


Figure 1. TARC is increased in RA SF and correlates with the number of CD1c cDCs. (A) TARC levels in SF of RA ( $n = 100$ ) patients are higher than in OA ( $n = 50$ ) patients. (B) Levels are significantly increased in RA patients with ESR  $\geq 30$  mm/h (High) compared to patients with ESR  $< 30$  mm/h (Low). (C) Numbers of CD1c+ cDCs in SF of RA patients correlate with TARC levels in SF. Levels below the detection limit were indicated as 10 pg/mL to allow plotting on a log axis, values were recorded as zero for analyses. \* $p < 0.05$ ; \*\*\* $p < 0.0001$ .

inflammation, defined as having an erythrocyte sedimentation rate (ESR)  $\geq 30$  mm/h, compared to those with low systemic inflammation (Figure 1B). As CD1c cDCs are very potent TARC producers, we investigated the relationship between TARC levels and local numbers of these cells. The number of CD1c-expressing cDCs in the SF showed a modest yet significant correlation with TARC levels, suggesting a role for these cells in intra-articular TARC production (Figure 1C). As methotrexate was previously reported to influence TARC expression in plasma of RA patients (15), we further examined the effects of therapy on intra-articular TARC levels in these samples. When the group of RA patients was divided based on therapy status, there were no significant differences in TARC levels between the untreated patients [mean  $\pm$  standard error of the mean (sem):  $42 \pm 14$  pg/mL] and those treated with biologics ( $29 \pm 10$  pg/mL,  $p = 0.58$ ) or those treated with disease-modifying anti-rheumatic drugs (DMARDs;  $80 \pm 35$  pg/mL,  $p = 0.52$ ). There was also no difference between patients treated with biologics and those treated with DMARDs ( $p = 0.79$ ).

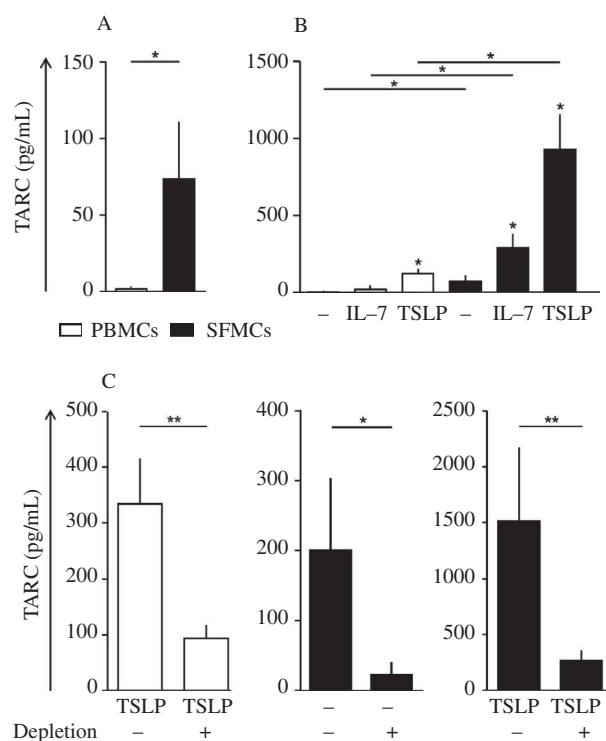


Figure 2. Increased spontaneous and stimulated TARC production by PB and SF MC fractions is crucially dependent on CD1c-expressing cells. (A) Unstimulated MCs from SF of RA patients produce increased TARC levels compared to paired MCs from the PB. Upon stimulation with TSLP, and to a lesser extent with IL-7, TARC production by PBMCs and SFMCs is greatly increased. (B) SFMCs from RA patients produce more TARC than paired PBMCs. (C) Depletion of CD1c-expressing cells from the PBMCs of RA patients robustly inhibits TARC production upon TSLP stimulation. CD1c depletion almost completely prevents spontaneous TARC production by RA SFMCs and strongly reduces TARC production by TSLP-stimulated SFMCs. Error bars indicate mean  $\pm$  sem. \* $p < 0.05$ ; \*\* $p < 0.01$ .

## MCs from SF of RA patients spontaneously produce TARC

MCs from SF samples of RA patients spontaneously produced TARC while this was not observed for paired MCs from PB, indicating that immune cells produce TARC locally in the joint (Figure 2A). We used TSLP and IL-7, both pro-inflammatory cytokines that are increased in RA SF, to investigate the induction of TARC production by activating the cDCs or T cells within the MCs, respectively. SFMCs produced significantly higher TARC levels compared to PBMCs from the same donor when stimulated with TSLP or IL-7, indicating that local MCs are more prone to produce TARC than their peripheral counterparts. TSLP induced more TARC production than IL-7. The combination of TSLP and IL-7 did not further enhance the amount of TARC produced (data not shown). This suggests that cDCs are important contributors to TARC-driven attraction of immune cells in RA. This was tested in two ways, by assessing the effect of cDC depletion on TARC production of MC populations and by analysing the capacity of TARC to induce T-cell chemotaxis.

## Depletion of CD1c CDCs from intra-articular MCs robustly prevents TARC production

To confirm the crucial role of cDCs in TARC production, we depleted MCs for these cells and assessed the effect on TARC production. Depletion of PBMCs from RA patients for CD1c-expressing cells resulted in a clear inhibition of TARC production upon TSLP stimulation compared to undepleted MCs (Figure 2C). Depletion of SFMCs for CD1c-expressing cells efficiently inhibited both spontaneous TARC production and TSLP-induced TARC production (Figure 2C). As B cells can express CD1c, we performed CD19 depletion to exclude the possibility that the effect of CD1c depletion was B-cell dependent. No differences were observed between undepleted SFMCs and CD19-depleted SFMCs in spontaneous TARC production (mean  $\pm$  sem:  $136 \pm 40.2$  pg/mL vs.  $136 \pm 68.0$  pg/mL,  $p = 1.00$ ) or TSLP-induced TARC production ( $1140 \pm 376$  pg/mL vs.  $908 \pm 369$  pg/mL,  $p = 0.88$ ). In addition, to control for influences of the MACS isolation procedure, the CD1c antibody was omitted during depletion. No differences were observed compared to undepleted MCs upon TSLP stimulation ( $395 \pm 213$  pg/mL vs.  $360 \pm 205$  pg/mL,  $p = 0.63$ ).

## TARC produced by cDCs of RA patients induces chemotaxis of T cells

We used CD1c cDCs isolated from PB to study the effect of TARC on the attraction of autologous T cells. cDCs from RA PB produced TARC when stimulated with TSLP while unstimulated PB cDCs did not (Figure 3A). When added to the lower compartment of a transwell system, TSLP-stimulated cDCs induced significantly more

autologous CD4+ T-cell migration from the upper compartment compared to unstimulated cDCs. This T-cell attraction was robustly inhibited by a neutralizing antibody against TARC (Figure 3B). No significant difference was observed when adding an isotype control antibody instead (mean  $\pm$  sem:  $6.67 \pm 1.69\%$  vs.  $5.56 \pm 1.73\%$ ,  $p = 0.31$ ). Attracted T cells were analysed for cytokine production and we observed a significant decrease in production of tumour necrosis factor (TNF)- $\alpha$ , IL-17, and interferon (IFN)- $\gamma$  upon TARC neutralization (Figure 3C), which was absent when an isotype control was used instead (data not shown). No differences in IL-10 production were observed, while IL-4 production was below the detection limit in the majority of the samples (data not shown).

## Discussion

We previously demonstrated that CD1c cDCs from RA SF are extremely potent activators of CD4 T cells, and are associated with strong proliferation and induction of Th1 and Th17 cytokine production (20). Furthermore, these RA SF cDCs are primed to produce TARC as they make it ex vivo without any stimulation (20). TSLP, which is increased in RA SF, was shown to activate cDCs from PB, and TSLP-stimulated PB DCs mimic functional and phenotypic features of intra-articular cDCs, including TARC production (19). In the current study we demonstrate that TARC is increased in SF of RA patients and that MCs from SF are primed to produce TARC. In addition, we show that CD1c cDCs are a crucial source of TARC in SF and that TARC produced by TSLP-activated PB cDCs,

used as a model for RA SF DCs, is functional and directs the migration of CD4 T cells.

Although we have not assessed the phenotype of T cells attracted by TARC in detail, we have studied the resulting cytokine expression upon in vitro activation of the attracted T-cell population. These data show a strong increase in TNF- $\alpha$  production by the T cells attracted by TSLP DCs compared to unstimulated DCs. Upon TARC blockade, TNF- $\alpha$  levels were robustly decreased, suggesting that TARC may be important in the attraction of TNF- $\alpha$ -producing T cells by TSLP DCs. In addition, TARC blockade resulted in modestly decreased production of IFN- $\gamma$  and IL-17, suggesting that some Th1 and Th17 cells are attracted by TARC in our assay. These data suggest that TARC may contribute to the attraction of T cells, which produce cytokines that drive the immunopathology in RA. However, as CCR4 is widely expressed on Th2 cells (6), it is likely that Th2 cells also migrate towards the TARC gradient, although IL-4 levels were not measurable in our assay. In addition, as a considerable proportion of FoxP3-expressing cells expresses CCR4 (4), Treg cells may also be attracted by TARC, although we did not observe differences in IL-10 production upon TARC blockade. Thus, the exact composition of the T-cell pool attracted by TARC needs to be further examined to determine the net effect of local TARC production on T-cell migration to the SF.

Although expression of CCR4 is described as a feature of Th2, Th17, and Treg cells, some studies show that CCR4-expressing cells can contain considerable percentages of IFN- $\gamma$ -producing cells (23), which may explain the reduction in IFN- $\gamma$  secretion upon TARC blockade. In the

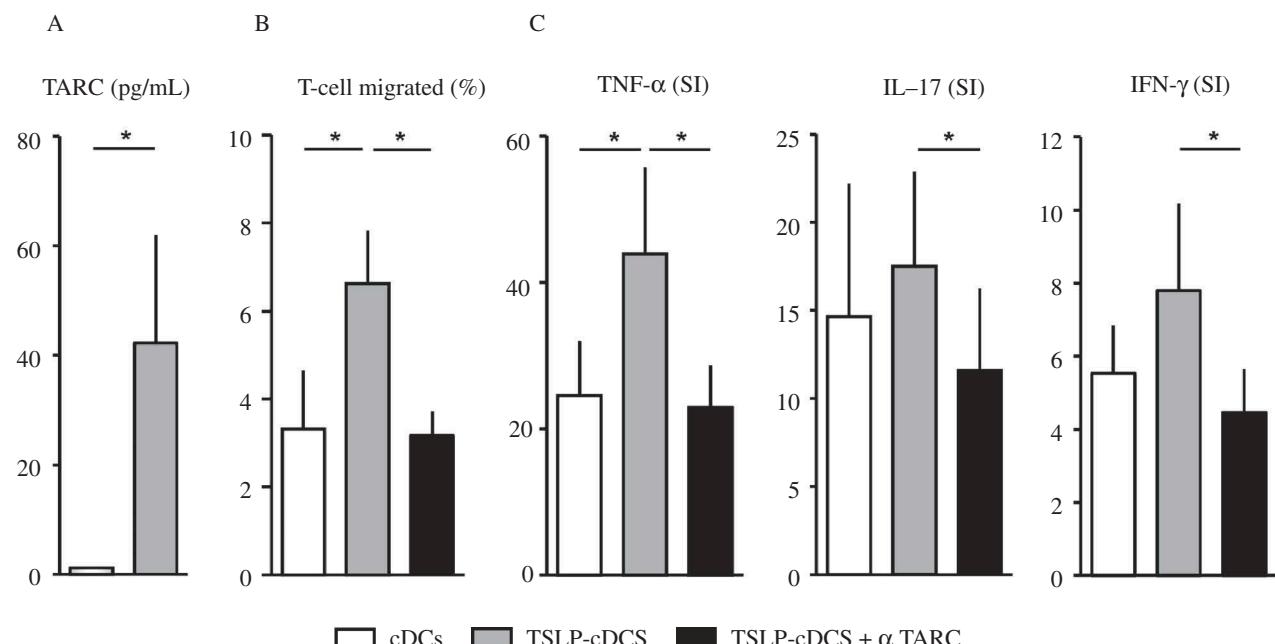


Figure 3. Chemotaxis of T cells towards activated cDCs is crucially dependent on TARC. (A) When stimulated with TSLP, cDCs show significantly increased TARC production. (B) In a transwell chemotaxis assay, TSLP-stimulated cDCs attract significantly more T cells compared to unstimulated cDCs and anti-TARC neutralizing antibody completely negates this. (C) TARC blockade inhibits attraction of T cells that produce TNF- $\alpha$ , IL-17, and IFN- $\gamma$ . Cytokine production depicted as the Stimulation Index (SI) relative to negative control without any cDCs in the lower well. Error bars indicate mean  $\pm$  sem. \*  $p < 0.05$ .

chemotaxis assay, activation of the cDCs with TSLP had no significant effect on the attraction of IL-17- and IFN- $\gamma$ -producing T cells. Nevertheless, TARC blockade significantly reduced IL-17 and IFN- $\gamma$  production. These data suggest that the minute amounts of TARC produced by unstimulated cDCs are sufficient to induce some attraction of IL-17- and IFN- $\gamma$ -producing cells. Of note, TARC can also attract fibroblasts and NK cells (2, 3), which are implicated in RA immunopathology by producing pro-inflammatory cytokines in the joint (24) and inducing tissue damage by production of catabolic enzymes (25).

The presented data indicate that TARC plays a role in the attraction of T cells to the RA SF and they also contribute to the determination of the role of TSLP in RA. As TARC mediates in vitro attraction of T cells, preventing TARC-mediated T-cell migration may prove to be beneficial for RA patients; however, this needs to be confirmed in experimental studies. CD1c cDC depletion using an antibody would be an efficacious method of preventing TARC production as shown in our experiments. Considering the potent pro-inflammatory role of cDCs as sentinel cells of the immune system, targeting these cells is an attractive approach to inhibit pathways by which they attract and activate T cells.

### Acknowledgements

This work was supported by the Dutch Arthritis Association (grant number 10-1-406).

### References

- Imai T, Yoshida T, Baba M, Nishimura M, Kakizaki M, Yoshie O. Molecular cloning of a novel T cell-directed CC chemokine expressed in thymus by signal sequence trap using Epstein-Barr virus vector. *J Biol Chem* 1996;271:21514–21.
- Inngjerdingen M, Damaj B, Maghazachi AA. Human NK cells express CC chemokine receptors 4 and 8 and respond to thymus and activation-regulated chemokine, macrophage-derived chemokine, and I-309. *J Immunol* 2000;164:4048–54.
- Kato T, Saeki H, Tsunemi Y, Shibata S, Tamaki K, Sato S. Thymus and activation-regulated chemokine (TARC)/CC chemokine ligand (CCL) 17 accelerates wound healing by enhancing fibroblast migration. *Exp Dermatol* 2011;20:669–74.
- Iellem A, Mariani M, Lang R, Recalde H, Panina-Bordignon P, Sinigaglia F, et al. Unique chemotactic response profile and specific expression of chemokine receptors Ccr4 and Ccr8 by Cd4 + Cd25 + regulatory T cells. *J Exp Med* 2001;194:847–54.
- Leipe J, Grunke M, Dechant C, Reindl C, Kerzendorf U, Schulze-Koops H, et al. Role of Th17 cells in human autoimmune arthritis. *Arthritis Rheum* 2010;62:2876–85.
- Hijnen D, De Bruin-Weller M, Oosting B, Lebre C, De Jong E, Bruijnzeel-Koomen C, et al. Serum thymus and activation-regulated chemokine (TARC) and cutaneous T cell-attracting chemokine (CTACK) levels in allergic diseases: TARC and CTACK are disease-specific markers for atopic dermatitis. *J Allergy Clin Immunol* 2004;113:334–40.
- Komine M, Kakinuma T, Kagami S, Hanakawa Y, Hashimoto K, Tamaki K. Mechanism of thymus- and activation-regulated chemokine (TARC)/CCL17 production and its modulation by roxithromycin. *J Invest Dermatol* 2005;125:491–8.
- Wirnsberger G, Hebenstreit D, Posselt G, Horejs-Hoeck J, Duschl A. IL-4 induces expression of TARC/CCL17 via two STAT6 binding sites. *Eur J Immunol* 2006;36:1882–91.
- Xiao T, Fujita H, Saeki H, Mitsui H, Sugaya M, Tada Y, et al. Thymus and activation-regulated chemokine (TARC/CCL17) produced by mouse epidermal Langerhans cells is upregulated by TNF-alpha and IL-4 and downregulated by IFN-gamma. *Cytokine* 2003;23:126–32.
- Zhou B, Comeau MR, Smedt TD, Liggitt HD, Dahl ME, Lewis DB, et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol* 2005;6:1047–53.
- Kakinuma T, Nakamura K, Wakugawa M, Mitsui H, Tada Y, Saeki H, et al. Thymus and activation-regulated chemokine in atopic dermatitis: Serum thymus and activation-regulated chemokine level is closely related with disease activity. *J Allergy Clin Immunol* 2001;107:535–41.
- Moriyama M, Hayashida J-N, Toyoshima T, Ohyama Y, Shinozaki S, Tanaka A, et al. Cytokine/chemokine profiles contribute to understanding the pathogenesis and diagnosis of primary Sjögren's syndrome. *Clin Exp Immunol* 2012;169:17–26.
- Kakinuma T, Wakugawa M, Nakamura K, Hino H, Matsushima K, Tamaki K. High level of thymus and activation-regulated chemokine in blister fluid and sera of patients with bullous pemphigoid. *Br J Dermatol* 2003;148:203–10.
- Thompson SD, Luyrink LK, Graham TB, Tsoras M, Ryan M, Passo MH, et al. Chemokine receptor CCR4 on CD4+ T cells in juvenile rheumatoid arthritis synovial fluid defines a subset of cells with increased IL-4:IFN-gamma mRNA ratios. *J Immunol* 2001;166:6899–906.
- Ho CY, Wong CK, Li EK, Tam LS, Lam CWK. Suppressive effect of combination treatment of leflunomide and methotrexate on chemokine expression in patients with rheumatoid arthritis. *Clin Exp Immunol* 2003;133:132–8.
- Hampel U, Sesselmann S, Iserovich P, Sel S, Paulsen F, Sack R. Chemokine and cytokine levels in osteoarthritis and rheumatoid arthritis synovial fluid. *J Immunol Methods* 2013;396:134–9.
- Radstake TR, van der Voort R, ten Brummelhuis M, de Waal Malefijt M, Looman M, Figdor CG, et al. Increased Expression of CCL18, CCL19, and CCL17 by Dendritic Cells from Patients with Rheumatoid Arthritis, and Regulation by Fc Gamma Receptors. *Ann Rheum Dis* 2005;64:359–67.
- Yang PT, Kasai H, Zhao LJ, Xiao WG, Tanabe F, Ito M. Increased CCR4 expression on circulating CD4+ T cells in ankylosing spondylitis, rheumatoid arthritis and systemic lupus erythematosus. *Clin Exp Immunol* 2004;138:342–7.
- Moret FM, Hack CE, van der Wurff-Jacobs KM, Radstake TR, Lafeber FP, van Roon JA. Thymic stromal lymphopoietin, a novel proinflammatory mediator in rheumatoid arthritis that potently activates CD1c+ myeloid dendritic cells to attract and stimulate T cells. *Arthritis Rheumatol* 2014;66:1176–84.
- Moret FM, Hack CE, van der Wurff-Jacobs KMG, de Jager W, Radstake TR, Lafeber FP, et al. Intra-articular CD1c-expressing myeloid dendritic cells from rheumatoid arthritis patients express a unique set of T cell-attracting chemokines and spontaneously induce Th1, Th17 and Th2 cell activity. *Arthritis Res Ther* 2013;15:R155.
- Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
- de Jager W, te Velthuis H, Prakken BJ, Kuis W, Rijkers GT. Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. *Clin Diagn Lab Immunol* 2003;10:133–9.
- Aerts NE, De Knop KJ, Leyten J, Ebo DG, Bridts CH, Weyler JJ, et al. Increased IL-17 production by peripheral T helper cells after tumour necrosis factor blockade in rheumatoid arthritis is accompanied by inhibition of migration-associated chemokine receptor expression. *Rheumatology (Oxford)* 2010;49:2264–72.
- Fogel LA, Yokoyama WM, French AR. Natural killer cells in human autoimmune disorders. *Arthritis Res Ther* 2013;15:216.
- Filer A. The fibroblast as a therapeutic target in rheumatoid arthritis. *Curr Opin Pharmacol* 2013;13:413–19.