



King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa  
www.sciencedirect.com



ORIGINAL ARTICLE

# Chemokine receptors expression on peripheral CD4-lymphocytes in rheumatoid arthritis: Coexpression of CCR7 and CD95 is associated with disease activity



Alia M. Aldahlawi <sup>a,c,\*</sup>, Mohammed F. Elshal <sup>b,e</sup>, Fai T. Ashgan <sup>a</sup>, Sami Bahlas <sup>d</sup>

<sup>a</sup> Biological Sciences Department, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

<sup>b</sup> Biochemistry Department, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

<sup>c</sup> Immunology Unit, King Fahad Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

<sup>d</sup> Rheumatic Disease Unit, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

<sup>e</sup> Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute, Sadat City University, Sadat City, Egypt

Received 19 December 2014; revised 7 February 2015; accepted 8 February 2015

Available online 14 February 2015

## KEYWORDS

Rheumatoid arthritis;  
Autoimmune disease;  
Cell trafficking;  
Inflammation;  
Chemokine receptors;  
Apoptosis;  
CD95;  
Cytokines

**Abstract** Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by synovial inflammation triggered by infiltrating CD4 lymphocytes. The positioning and activation of lymphocyte in inflamed synovial tissues are dependent on a number of factors including their chemokine receptor expression profile. We aimed to investigate which chemokine receptors pattern correlate with serum cytokine levels and with disease activity. Forty patients with RA (34 female and 6 male) with age range from 21 to 68 years were included. Twenty healthy volunteers (16 female and 4 male) with matched age (range 21–48 years) were served as healthy controls (HCs). Expression of chemokine receptors (CCR5, CX3CR1 and CCR7) together with the apoptosis-related marker (CD95) was analyzed using three-color flow cytometry analysis after gating on CD4<sup>+</sup> peripheral blood lymphocytes. Plasma levels of IL-6, IL-10, IL-12 and TNF- $\alpha$  cytokines were measured in all participants using ELISA. Disease activity score (DAS28-CRP) system was assessed and active disease was defined as DAS28  $\geq$  3.2. Twenty-five (62.4%) patients were classified as active RA (ARA) and 15 (37.5%) patients with inactive RA (IRA). Percentages of CD4<sup>+</sup> lymphocytes

\* Corresponding author at: Biological Sciences Department, Faculty of Sciences, King Abdulaziz University, P.O. Box 80200, Jeddah 21589, Saudi Arabia. Tel.: +966 505357982.

E-mail address: [alia.aldahlawi@yahoo.com](mailto:alia.aldahlawi@yahoo.com) (A.M. Aldahlawi).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

<http://dx.doi.org/10.1016/j.sjbs.2015.02.011>

1319-562X © 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

expressing CD95 with either of CCR7 or CCR5 were significantly higher in ARA compared to IRA and HCs groups, while the expression of CX3CR1 on T-cells was found significantly lower in both CD95<sup>-</sup> and CD95<sup>+</sup> T-cells in RA groups than HC. Percentages of CD4<sup>+</sup>CD95<sup>+</sup>CCR7<sup>+</sup> cells correlated positively with IL-6 ( $r = 0.390$ ). Whereas CD4<sup>+</sup>CD95<sup>+</sup>CX3CR1<sup>+</sup> were negatively correlated with TNF- $\alpha$  ( $r = -0.261$ ). Correlation of CD4<sup>+</sup>CD95<sup>+</sup>CCR7<sup>+</sup> T cell subset with disease activity and inflammatory cytokines suggests a role for this cell subset in the pathogenesis of RA. Further investigation will be required to fully characterize this cell subset and its role in disease progression.

© 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by persistent inflammation affecting the musculoskeletal system that results in joints deformation and severe pain. The precise pathogenesis of RA has not been completely elucidated, but CD4<sup>+</sup> T cells are thought to play a crucial role, as it stimulate proliferation and differentiation of B-lymphocytes (Hovdenes, 1989) and participate in the induction and propagation of inflammatory responses by secreting pro-inflammatory cytokines, growth factors, and interferons (Chen et al., 2004; Perng et al., 2014).

Trafficking of CD4<sup>+</sup> T cells, and consequently their inflammatory mediators, into the synovial fluid contribute to initiation, propagation, and maintenance of chronic inflammation of synoviocytes (Berner et al., 2000; Bradfield et al., 2003). The migration of CD4<sup>+</sup> T cells as well as other immune cells to lymphoid or rheumatoid synovium is dependent on several factors including their chemokine receptors expression profile (Szekanecz et al., 2010). The chemokine receptor (CCR)5 has been reported to play key roles in the intra-articular recruitment of peripheral blood mononuclear cells (Portales et al., 2009). CCR5 upregulation was found associated with tissue and joint destruction and increased levels of CCR5 ligands (RANTES, MIP-1 $\alpha$ , and MIP-1 $\beta$ ) in the synovial fluid (Fleishaker et al., 2012). CX3CR1 is a chemokine receptor that binds to the proinflammatory chemokine fractalkine (FKN or CX3CL1). CX3CR1 has been shown to be important in inflammatory arthritis responses largely due to effects on cellular migration (Nanki et al., 2002; Tarrant et al., 2012). CCR7 was found to be important in lymphocytes homing to joints in response to the cognate ligands CCL19 and CCL21. In addition, CCR7 ligands have been detected in endothelial cells and in the perivascular infiltrate in RA synovium, suggesting their potential involvement in lymphoid neogenesis that occurs in inflamed synovial tissue (Pickens et al., 2012).

In addition to their role in lymphocytes trafficking, chemokine receptors were found crucial to the regulation of gene expression on target cells and help to control cell proliferation and apoptosis (Muller et al., 2002). Apoptosis resistance or longevity of CD4<sup>+</sup> T cells is linked to excessive infiltration of lymphocytes which is considered to exacerbate and maintain synovitis in RA (Moodley et al., 2011; Gullick et al., 2013). CD95 is a membrane receptor belonging to the tumor necrosis factor (TNF) receptor superfamily that plays a pivotal role in maintaining lymphocytes homeostasis and tolerance by mediating apoptosis in activated mature

lymphocytes (Dudley et al., 1999; Paulsen et al., 2011). Additionally CD95 was reported to participate in non-apoptotic processes including cellular activation, differentiation and induction of inflammation (Peter et al., 2007; Paulsen and Janssen, 2011; Li et al., 2014). Mutation in CD95 was found associated with autoimmune disease in lpr mice and in humans (Cui et al., 1996; Vaishnaw et al., 1999).

Therefore, we aimed to investigate whether the expression pattern of chemokine receptors and the apoptosis-related receptor CD95 on CD4<sup>+</sup> T-cells correlates with inflammatory cytokines and disease activity index in RA patients.

## 2. Material and methods

### 2.1. Patients

This study was approved by the Ethics Committee of King Abdulaziz University Hospital. The study included patients from the rheumatology outpatient clinic at the King Abdulaziz University Hospital. Forty patients with RA (34 women and 6 men) with age ranged between 21 and 68 years who fulfilled the American College of Rheumatology (ACR) 1987 criteria for the diagnosis of RA (Arnett et al., 1988) were included. Twenty healthy volunteers (16 female and 4 male) with matched age (range 21–48 years) who have no involvement for other autoimmune disease were included as healthy control (HC) group. RA patients had disease duration between 1 and 240 months. Group of patients received combined therapy of methotrexate and prednisolone ( $n = 19$ ; 7.5 mg–5.5 mg/week, 5 mg–10 mg/day), while rest of them ( $n = 21$ ) have not received any treatment for RA.

Disease activity was determined using DAS28-CRP according to previously established methods (Prevoo et al., 1995). According to DAS-28, 25 (62.5%) patients were classified with active RA (ARA) and 15 (37.5%) patients with inactive RA (IRA). The Clinical and laboratory features of subjects in ARA, IRA and HCs groups are presented in Table 1.

### 2.2. Blood samples

Ten ml blood sample was drawn from each RA and HC subjects into a BD vacutainer tube containing ethylene diamine tetra-acetic acid (EDTA) anticoagulant. Plasma samples were separated after centrifugation at 1500 rpm for 10 min, and then it was stored at  $-80^{\circ}\text{C}$  till further analysis. Fresh whole blood samples were used for flow cytometric analysis.

**Table 1** Clinical and biochemical analyses of RA patients and healthy controls.

Groups	Active RA (No. = 25)	Inactive RA (No. = 15)	HC (No. = 20)	ANOVA <i>P</i> -value
Age (years)	43.8 ± 12.0	43.1 ± 13.8	35.7 ± 7.1	–
Disease duration (months)	76.4 ± 72.6	91.6 ± 109.7	–	0.638
DAS-28	3.96 ± 0.59	2.7 ± 0.27	–	0.002
ESR (mm/h)	30.8 ± 23.09	21.1 ± 16.33	12.23 ± 5.88	0.002
CRP (mg/l)	26.0 ± 39.94	7.61 ± 5.11	4.87 ± 3.4	0.017
Anti-CCP (+ve > 50 units/ml)	16/25 (64%)	7/15 (47%)	0/20 (0%)	0.009
RF (+ve > 20 units/ml)	11/25 (44%)	9/15 (60%)	0/20 (0%)	0.045
IL-6 (pg/ml)	19.79 ± 5.45	15.81 ± 5.1	13.8 ± 5.49	0.034
IL-10 (pg/ml)	11.78 ± 3.68	11.6 ± 2.27	10.86 ± 3.78	0.881
IL-12 (pg/ml)	13.1 ± 3.92	13.39 ± 1.22	14.53 ± 2.20	0.793
TNF- $\alpha$ (pg/ml)	17.67 ± 3.17	16.01 ± 4.06	12.17 ± 3.51	0.038

Values are expressed as mean  $\pm$  SD.

### 2.3. Biochemical analyses

Erythrocyte sedimentation rate (ESR) was measured using the Westergren technique, C-reactive protein (CRP) was measured by nephelometry and CBC was counted using automated blood counter. Anti-CCP concentrations were determined using commercial kits purchased from IMTEC, Immunodiagnostica GmbH. Samples with concentrations >25 units/ml were considered positive (Karimifar et al., 2012).

### 2.4. Flow cytometric analysis

Three color flow cytometry fluorescence staining was used for the analysis of lymphocytes. The blood of patients and controls was stained with PerCP conjugated anti-CD4, PE conjugated CCR7, CCR5 or CX3CR1 and fluorescein isothiocyanate (FITC) conjugated CD95. These antibodies were purchased from R&D Systems (Minneapolis MN, USA). Antibodies or the corresponding isotopic control (IgG1, IgG2A-PE and IgG2A-Percp), were added at the concentration of 1  $\mu$ g/100  $\mu$ L of whole blood samples and incubated for 30 min, at 4 °C at the dark. Then, 3 ml of freshly prepared RBC lysis (1 $\times$ ) buffer was added, samples were mixed promptly and thoroughly to ensure complete erythrocyte lysis. Then, samples were incubated for 10 min at room temperature. Samples were centrifuged at 1500 rpm for 7 min and the supernatant was discarded. This step was repeated if the RBC still not lysis. Cells were resuspended in 400  $\mu$ L of cold PBS. The immunophenotype was assessed by triple-color cytometry. Cell fluorescence was measured using a flow cytometer (FACScalibur, Becton–Dickinson, San Jose, CA, USA) and analyzed with green (FL1), orange (FL2) or red (FL3) standard emission filters. From each sample 10,000 events were acquired. Based on side scattered (SC) versus forward scattered (FS) distribution of lymphocytes were gated from the whole PBMC population (Baumgarth and Roederer, 2000).

### 2.5. Detection of plasma cytokines

Stored plasma samples at –80 °C were used to determine the concentrations of IL-6, IL-10, IL-12 and TNF- $\alpha$  using enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions (all ELISA kits from BioLegend,

Inc., San Diego, CA, USA). All samples were measured in duplicate.

### 2.6. Statistical analysis

Values are expressed as mean  $\pm$  SD or median (range) in the tables and figures. Data were analyzed using SPSS (version 16.0, SPSS Inc., USA). Data were analyzed by one way ANOVAs, Bonferroni, Pearson correlation (*r*) and *T*-test. A *P* value < 0.05 was considered significant.

## 3. Results

Table 1 demonstrates the differences in clinical and biochemical markers of patient groups and healthy controls. No significant difference was detected in age among RA patients and healthy controls. Additionally, no significant difference was detected in disease duration between ARA and IRA patients. However, there were significant differences between studied groups in DAS-28 (*P* = 0.002), ESR (*P* = 0.002), CRP (*P* = 0.017), Anti-CCP (*P* = 0.009) and RF (*P* = 0.045). The plasma concentrations of IL-6, IL-10, IL-12 and TNF- $\alpha$  were significantly increased in patients with active RA and inactive RA compared to that of healthy subjects (Table 1). The correlations between the inflammatory parameters and cytokines and between the parameters and chemokines receptors are summarized in Table 2.

Fig. 1 shows the mean percentages of CD4<sup>+</sup> cells expressing CCR5, CCR7, CX3CR1 and CD95 in patients with ARA, IRA and HCs. Mean percentage of CD4<sup>+</sup>CCR5<sup>+</sup>CD95<sup>+</sup> and CD4<sup>+</sup>CCR5<sup>+</sup>CD95<sup>–</sup> T cells was significantly higher in ARA comparing to IRA patients and HCs (both at *P* < 0.05). On the other hand, there was no significant difference in CD4<sup>+</sup>CCR5<sup>+</sup>CD95<sup>+</sup> and CD4<sup>+</sup>CCR5<sup>+</sup>CD95<sup>–</sup> T cells between IRA and HCs (Fig. 1A). For CCR7 expression on CD4<sup>+</sup> cells (Fig. 1B), CCR7<sup>+</sup>CD95<sup>+</sup>CD4<sup>+</sup> T-lymphocytes significantly increased in ARA compared with IRA (*P* = 0.05) and HCs (*P* = 0.01). In addition, CCR7<sup>+</sup>CD95<sup>+</sup>CD4<sup>+</sup> T cells were significantly higher in IRA patients than HCs (*P* = 0.05). Whereas, no significant differences were detected between the three groups as regard CCR7<sup>+</sup>CD95<sup>–</sup>CD4<sup>+</sup> T cells. The percentages of cells expressing CX3CR1<sup>+</sup>CD4<sup>+</sup>CD95<sup>+</sup> and CX3CR1<sup>+</sup>CD4<sup>+</sup>CD95<sup>–</sup> were significantly

**Table 2** Correlations of T cell subsets with clinical and biochemical parameters.

	CD4 <sup>+</sup> CCR5 <sup>+</sup>		CD4 <sup>+</sup> CCR7 <sup>+</sup>		CD4 <sup>+</sup> CX3CR1 <sup>+</sup>	
	CD95 <sup>-</sup>	CD95 <sup>+</sup>	CD95 <sup>-</sup>	CD95 <sup>+</sup>	CD95 <sup>-</sup>	CD95 <sup>+</sup>
DAS28	0.102	0.004	0.079	0.371**	0.06	-0.197
Anti-CCP	-0.107	-0.075	-0.168	0.212	0.014	-0.079
ESR	-0.019	0.043	-0.001	0.192	-0.142	-0.056
RF	-0.106	-0.026	0.027	0.363**	-0.122	-0.04
CRP	0.146	0.022	0.206	0.052	-0.137	-0.094
IL-6	0.013	0.022	-0.038	0.331*	-0.078	-0.114
IL-10	-0.009	-0.045	0.064	-0.107	0.297*	-0.057
IL-12	0.094	0.056	0.063	-0.149	-0.031	-0.176
TNF- $\alpha$	0.098	-0.066	0.095	0.149	0.065	-0.261*

\* Correlation is significant at the 0.05 level (2-tailed).

\*\* Correlation is significant at the 0.01 level (2-tailed).

lower in ARA and IRA patients comparing to HCs (both at  $P < 0.05$ ). On the other hand, there was no significant difference in CD4<sup>+</sup> CX3CR1<sup>+</sup>CD95<sup>+</sup> and CD4<sup>+</sup> CX3CR1<sup>+</sup>CD95<sup>-</sup> T cells between ARA and IRA (Fig. 1C).

#### 4. Discussion

CD4<sup>+</sup> T cells play a crucial role in inflammatory responses affecting joints and adjacent tissues via the production of distinctive sets of inflammatory cytokines (Tak et al., 1995; Nanki and Lipsky, 2000). Recent data suggest that CD95 not only induces apoptosis, but also acts as an activator marker of CD4<sup>+</sup> T cells due to its role in regulating the activation of transcription factors and cell-cycle regulators for the induction of proliferation and cytokine production (Paulsen et al., 2011). Additionally CD95 has been found to promote proliferation of rheumatoid arthritis fibroblast-like synoviocytes and induce inflammation through a mechanism involving the activation of PI3K/Akt signaling pathway (Li et al., 2014).

These data encouraged us to investigate whether RA disease activity and inflammatory cytokines are associated with certain profile of CD95 and chemokine receptors expression on CD4<sup>+</sup> T cells. Results showed that the dual expression of CD95 with CCR7 on CD4<sup>+</sup> T cells was able to differentiate between active, inactive RA patients and healthy control (Fig. 1A). Whereas CD95<sup>+</sup>CCR5<sup>+</sup> and CD95<sup>+</sup>CX3CR1<sup>+</sup> CD4<sup>+</sup> T-cells failed to differentiate between the three studied groups significantly (Fig. 1B and C).

CCR7 was reported to be expressed only by naive and central memory T cells that control their transmigration into lymphoid tissues in response to the cognate ligands CCL19 and CCL2 (Forster et al., 2008). Furthermore, CCR7 was found critical for the generation of an adaptive T-cell response (Toka et al., 2003).

Our finding that CD4<sup>+</sup>CD95<sup>+</sup> T-lymphocytes express higher amount of CCR7 in RA compared with controls; may suggest that there is an increase in homing of this activated (CD95<sup>+</sup>) T cell subsets to either secondary lymphoid tissue or node-like synovium microstructure and hence contributing to its inflammatory environment by secreting inflammatory cytokines.

Additionally, the increased CD95<sup>+</sup>CCR7<sup>+</sup>CD4<sup>+</sup> T cells probably will result in increased apoptotic bodies and over presentation of lymphocytes' remnants that might break immune

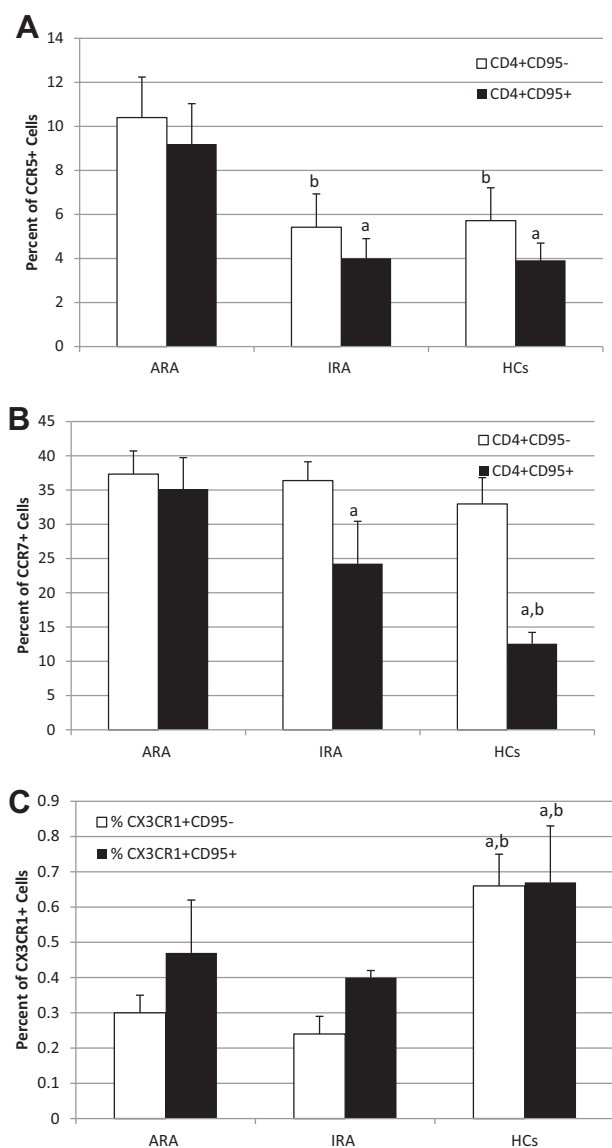
tolerance, resulting in the autoimmune phenomena or boost an already existing autoimmune response as previously reported by Lorenz et al. (2000). In line with these data our results showed significant positive correlations between CD95<sup>+</sup> CCR7<sup>+</sup>CD4<sup>+</sup> T cells with DAS28 and RF, whereas CD95<sup>-</sup> CCR7<sup>+</sup>CD4<sup>+</sup> T cell subset did not correlate with these parameters. This finding confirms that CD95<sup>+</sup>CCR7<sup>+</sup>CD4<sup>+</sup> T cell subset may contribute in the pathogenesis of RA.

CD95<sup>+</sup>CCR7<sup>+</sup>CD4<sup>+</sup> T cell subset was also found strongly correlated with IL-6. IL-6 is considered a key mediator in the inflammatory process of RA and has been found at elevated levels in the serum and synovial tissue (Smolen et al., 2014). Therefore we suggest that the positive relationships between CD95<sup>+</sup>CCR7<sup>+</sup>CD4<sup>+</sup> T cells with IL-6 may interpret their positive correlations with DAS28 and RF.

CX3CR1 and its ligand fractalkine (FKN) are strongly expressed in the chronically inflamed synovial tissue of patients with RA (Umehara et al., 2006). CX3CR1 is expressed on leukocytes and mediate not only chemotaxis, but also their firm adhesion to FKN-expressing endothelial cells even in the absence of substrates for other adhesion molecules (Fong et al., 1998). In our study we found a significant decrease in both subsets of CX3CR1<sup>+</sup>CD4<sup>+</sup> T cells compared with that of healthy control. This finding suggests that CX3CR1<sup>+</sup> cells have migrated from peripheral circulation to inflamed tissues making their percentages decreased in RA patients than healthy controls. This is also in agreement with Nanki et al. (2002) who found that the interactions of CX3CL1 and CX3CR1 might contribute to the accumulation of CX3CR1<sup>+</sup> T cells expressing type 1 cytokines and possessing cytotoxic granules in RA synovium. This conclusion is supported by the finding of negative correlations of TNF- $\alpha$  with peripheral blood CD4 lymphocytes expressing CX3CR1. TNF- $\alpha$  particularly plays a pivotal role in the pathogenesis of RA. It is present at biologically significant levels in RA synovial tissue and fluid. Moreover, the TNF- $\alpha$  level seem to parallel the severity of both inflammation and bone erosion (Matsuno et al., 2002). Accumulating evidence suggests that not only soluble TNF- $\alpha$ , but also its precursor form transmembrane TNF- $\alpha$  involved in the inflammatory response (Nakashima et al., 2010).

CCR5 is a key chemokine receptor that is highly expressed on CD4<sup>+</sup> T cells. It binds to three different chemokines CCL3 (MIP-alpha), CCL4 (MIP-beta), and CCL5 (RANTES). Ample evidence suggested that the interaction between





**Figure 1** (A–C) The mean percentages of CD4<sup>+</sup> cells expressing CCR5, CCR7, CX3CR1 and Fas in the studied groups. Values are expressed as mean percent  $\pm$  SD. \*Significant by ANOVA at  $P < 0.05$ . <sup>a</sup>Significant at  $P < 0.05$  versus active RA, <sup>b</sup>Significant at  $P < 0.05$  versus inactive RA.

CCR5 and its ligands is essential not only for attracting these CCR5<sup>+</sup> T cells but also substantial for transducing co-signals for their activation (Wu et al., 2008). In addition, CCR5 has been reported to directly regulate T-cell function in autoimmune diseases, including MS and RA (Solomon et al., 2010).

In our results there was a significant increase in the percentage of CCR5<sup>+</sup>CD4<sup>+</sup> T cells in the ARA in comparison with IRA and HCs groups. However the mean percentage of CCR5<sup>+</sup>CD4 T cells did not correlate with any of the clinical, inflammatory or the cytokine markers. This result is in accordance with the previous study by Sapir et al. (2010) who found that production of inflammatory cytokines TNF- $\alpha$ , IL-17, and IFN- $\gamma$ , are CCR5 independent and therefore likely to be mediated by the other receptors. These observations were also supported at the molecular level by the finding that CCR5

gene polymorphism do not play a major role in conferring genetic risk for, and/or protection against other autoimmune disease (Gambelunghe et al., 2004).

In conclusion, our data suggest that the percentage of CD4<sup>+</sup> T-cells coexpressing CD95 and CCR7 associate with disease activity and inflammation, and may serve as valuable index in monitoring the disease activity and the efficacy of the treatment. In addition, these data suggest that targeting this cell subset for therapeutic intervention could potentially lead to lower inflammation and disease progression in RA.

#### Conflict of interest

The authors declare no financial or commercial conflict of interest.

#### References

- Arnett, F.C., Edworthy, S.M., Bloch, D.A., McShane, D.J., Fries, J.F., Cooper, N.S., Healey, L.A., Kaplan, S.R., Liang, M.H., Luthra, H.S., Jr, T.A.M., Mitchell, D.M., Neustadt, D.H., Pinals, R.S., Schaller, J.G., Sharp, J.T., Wilder, R.L., Hunder, G.G., 1988. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum.* 31, 315–324.
- Baumgarth, N., Roederer, M., 2000. A practical approach to multi-color flow cytometry for immunophenotyping. *J. Immunol. Methods* 243, 77–97.
- Berner, B., Akca, D., Jung, T., Muller, G.A., Reuss-Borst, M.A., 2000. Analysis of Th1 and Th2 cytokines expressing CD4<sup>+</sup> and CD8<sup>+</sup> T cells in rheumatoid arthritis by flow cytometry. *J. Rheumatol.* 27, 1128–1135.
- Bradfield, P.F., Amft, N., Vernon-Wilson, E., Exley, A.E., Parsonage, G., Rainger, G.E., Nash, G.B., Thomas, A.M., Simmons, D.L., Salmon, M., Buckley, C.D., 2003. Rheumatoid fibroblast-like synoviocytes overexpress the chemokine stromal cell-derived factor 1 (CXCL12), which supports distinct patterns and rates of CD4<sup>+</sup> and CD8<sup>+</sup> T cell migration within synovial tissue. *Arthritis Rheum.* 48, 2472–2482.
- Chen, J., Zhang, X.M., Xu, Q., 2004. Involvement of lymphocytes with a Th1 cytokine profile in bone cell damage associated with MMP-9 production in collagen-induced arthritis. *Inflamm. Res.* 53, 670–679.
- Cui, H., Ju, S.T., Sherr, D.H., 1996. Functional expression of Fas (CD95) protein in autoimmune lpr mice. *Cell. Immunol.* 174, 35–41.
- Dudley, E., Hornung, F., Zheng, L., Scherer, D., Ballard, D., Lenardo, M., 1999. NF- $\kappa$ B regulates Fas/APO-1/CD95- and TCR-mediated apoptosis of T lymphocytes. *Eur. J. Immunol.* 29, 878–886.
- Fleishaker, D.L., Garcia Mejjide, J.A., Petrov, A., Kohen, M.D., Wang, X., Menon, S., Stock, T.C., Mebus, C.A., Goodrich, J.M., Mayer, H.B., Zeiher, B.G., 2012. Maraviroc, a chemokine receptor-5 antagonist, fails to demonstrate efficacy in the treatment of patients with rheumatoid arthritis in a randomized, double-blind placebo-controlled trial. *Arthritis Res. Ther.* 14, R11.
- Fong, A.M., Robinson, L.A., Steeber, D.A., Tedder, T.F., Yoshie, O., Imai, T., Patel, D.D., 1998. Fractalkine and CX3CR1 mediate a novel mechanism of leukocyte capture, firm adhesion, and activation under physiologic flow. *J. Exp. Med.* 188, 1413–1419.
- Forster, R., Davalos-Miszlitz, A.C., Rot, A., 2008. CCR7 and its ligands: balancing immunity and tolerance. *Nat. Rev. Immunol.* 8, 362–371.
- Gambelunghe, G., Ghaderi, M., Gharizadeh, B., Brozzetti, A., Tortoioli, C., Del Sindaco, P., Sanjeevi, C.B., Hjelmstrom, P.,

- Sirsjo, Nyren, P., Santeusano, F., Falorni, A., 2008. Lack of association of human chemokine receptor gene polymorphisms CCR2-64I and CCR5-Delta32 with autoimmune Addison's disease. *Eur. J. Immunogenet.* 31, 73–76.
- Gullick, N.J., Abozaid, H.S., Jayaraj, D.M., Evans, H.G., Scott, D.L., Choy, E.H., Taams, L.S., 2013. Enhanced and persistent levels of interleukin (IL)-17(+) CD4(+) T cells and serum IL-17 in patients with early inflammatory arthritis. *Clin. Exp. Immunol.* 174, 292–301.
- Hovdenes, J., 1989. B-cell growth-promoting activity in supernatants from CD4+ cells from synovial fluid and peripheral blood of patients with rheumatoid arthritis and other inflammatory arthritides. *Scand. J. Rheumatol.* 18, 385–392.
- Karimifar, M., Salesi, M., Farajzadegan, Z., 2012. The association of anti-CCP1 antibodies with disease activity score 28 (DAS-28) in rheumatoid arthritis. *Adv. Biomed. Res.* 1, 30.
- Li, X., Zhang, Z., Peng, A., He, M., Xu, J., Shen, S., Zhuang, J., Huang, X., 2014. Effect of CD95 on inflammatory response in rheumatoid arthritis fibroblast-like synoviocytes. *Cell. Immunol.* 290, 209–216.
- Lorenz, H.M., Herrmann, M., Winkler, T., Gaipl, U., Kalden, J.R., 2000. Role of apoptosis in autoimmunity. *Apoptosis* 5, 443–449.
- Matsuno, H., Yudoh, K., Katayama, R., Nakazawa, F., Uzuki, M., Sawai, T., Yonezawa, T., Saeki, Y., Panayi, G.S., Pitzalis, C., Kimura, T., 2002. The role of TNF-alpha in the pathogenesis of inflammation and joint destruction in rheumatoid arthritis (RA): a study using a human RA/SCID mouse chimera. *Rheumatology (Oxford)* 41, 329–337.
- Moodley, D., Mody, G.M., Chuturgoon, A.A., 2011. Initiation but no execution – modulation of peripheral blood lymphocyte apoptosis in rheumatoid arthritis – a potential role for heat shock protein 70. *J. Inflamm. (Lond.)* 8 (30), 12–15.
- Muller, G., Hopken, U.E., Stein, H., Lipp, M., 2002. Systemic immunoregulatory and pathogenic functions of homeostatic chemokine receptors. *J. Leukoc. Biol.* 72, 1–8.
- Nakashima, Y., Kondo, M., Harada, H., Horiuchi, T., Ishinishi, T., Jojima, H., Kuroda, K., Miyahara, H., Nagamine, R., Nakashima, H., Otsuka, T., Saikawa, I., Shono, E., Suematsu, E., Tsuru, T., Wada, K., Iwamoto, Y., 2010. Clinical evaluation of tocilizumab for patients with active rheumatoid arthritis refractory to anti-TNF biologics: tocilizumab in combination with methotrexate. *Mod. Rheumatol.* 20, 343–352.
- Nanki, T., Imai, T., Nagasaka, K., Urasaki, Y., Nonomura, Y., Taniguchi, K., Hayashida, K., Hasegawa, J., Yoshie, O., Miyasaka, N., 2002. Migration of CX3CR1-positive T cells producing type 1 cytokines and cytotoxic molecules into the synovium of patients with rheumatoid arthritis. *Arthritis Rheum.* 46, 2878–2883.
- Nanki, T., Lipsky, P.E., 2000. Cytokine, activation marker, and chemokine receptor expression by individual CD4(+) memory T cells in rheumatoid arthritis synovium. *Arthritis Res.* 2, 415–423.
- Paulsen, M., Janssen, O., 2011. Pro- and anti-apoptotic CD95 signaling in T cells. *Cell Commun. Signal.* 9, 7.
- Paulsen, M., Valentin, S., Mathew, B., Adam-Klages, S., Bertsch, U., Lavrik, I., Krammer, P.H., Kabelitz, D., Janssen, O., 2011. Modulation of CD4+ T-cell activation by CD95 co-stimulation. *Cell Death Differ.* 18, 619–631.
- Perng, O.A., Aitken, M., Rankin, A.L., Garcia, V., Kropf, E., Erikson, J., Garlick, D.S., Caton, A.J., 2014. The degree of CD4+ T cell autoreactivity determines cellular pathways underlying inflammatory arthritis. *J. Immunol.* 192, 3043–3056.
- Peter, M.E., Budd, R.C., Desbarats, J., Hedrick, S.M., Hueber, A.O., Newell, M.K., Owen, L.B., Pope, R.M., Tschopp, J., Wajant, H., Wallach, Wilttrout, R.H., Zornig, M., Lynch, D.H., 2007. The CD95 receptor: apoptosis revisited. *Cell* 129, 447–450.
- Pickens, S.R., Chamberlain, N.D., Volin, M.V., Pope, R.M., Talarico, N.E., Mandelin 2nd, A.M., Shahrara, S., 2012. Role of the CCL21 and CCR7 pathways in rheumatoid arthritis angiogenesis. *Arthritis Rheum.* 64, 2471–2481.
- Portales, P., Fabre, S., Vincent, T., Desmetz, C., Reant, B., Noel, D., Clot, J., Jorgensen, C., Corbeau, P., 2009. Peripheral blood T4 cell surface CCR5 density as a marker of activity in rheumatoid arthritis treated with anti-CD20 monoclonal antibody. *Immunology* 128, e738–e745.
- Prevo, M.L., van 't Hof, M.A., van Leeuwen, M.A., van de Putte, L.B., van Riel, P.L., 1995. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum.* 38, 44–48.
- Sapir, Y., Vitenshtein, A., Barsheshet, Y., Zohar, Y., Wildbaum, G., Karin, N., 2010. A fusion protein encoding the second extracellular domain of CCR5 arrests chemokine-induced cosignaling and effectively suppresses ongoing experimental autoimmune encephalomyelitis. *J. Immunol.* 185, 2589–2599.
- Smolen, J.S., Weinblatt, M.E., Sheng, S., Zhuang, Y., Hsu, B., 2014. Sirukumab, a human anti-interleukin-6 monoclonal antibody: a randomised, 2-part (proof-of-concept and dose-finding), phase II study in patients with active rheumatoid arthritis despite methotrexate therapy. *Ann. Rheum. Dis.* 73, 1616–1625.
- Solomon, M., Balasa, B., Sarvetnick, N., 2010. CCR2 and CCR5 chemokine receptors differentially influence the development of autoimmune diabetes in the NOD mouse. *Autoimmunity* 43, 156–163.
- Szekanecz, Z., Vegvari, A., Szabo, Z., Koch, A.E., 2010. Chemokines and chemokine receptors in arthritis. *Front. Biosci. (Schol Ed)* 2, 153–167.
- Tak, P.P., van der Lubbe, P.A., Cauli, A., Daha, M.R., Smeets, T.J., Kluin, P.M., Meinders, A.E., Yanni, G., Panayi, G.S., Breedveld, F.C., 1995. Reduction of synovial inflammation after anti-CD4 monoclonal antibody treatment in early rheumatoid arthritis. *Arthritis Rheum.* 38, 1457–1465.
- Tarrant, T.K., Liu, P., Rampersad, R.R., Esserman, D., Rothlein, L.R., Timoshchenko, R.G., McGinnis, M.W., Fitzhugh, D.J., Patel, D.D., Fong, A.M., 2012. Decreased Th17 and antigen-specific humoral responses in CX(3) CR1-deficient mice in the collagen-induced arthritis model. *Arthritis Rheum.* 64, 1379–1387.
- Toka, F.N., Gierynska, M., Rouse, B.T., 2003. Codelivery of CCR7 ligands as molecular adjuvants enhances the protective immune response against herpes simplex virus type 1. *J. Virol.* 77, 12742–12752.
- Umehara, H., Tanaka, M., Sawaki, T., Jin, Z.X., Huang, C.R., Dong, L., Kawanami, T., Karasawa, H., Masaki, Y., Fukushima, T., Hirose, Y., Okazaki, T., 2006. Fractalkine in rheumatoid arthritis and allied conditions. *Mod. Rheumatol.* 16, 124–130.
- Vaishnav, A.K., Toubi, E., Ohsako, S., Drappa, J., Buys, S., Estrada, J., Sitarz, A., Zemel, L., Chu, J.L., Elkon, K.B., 1999. The spectrum of apoptotic defects and clinical manifestations, including systemic lupus erythematosus, in humans with CD95 (Fas/APO-1) mutations. *Arthritis Rheum.* 42, 1833–1842.
- Wu, Y., Li, Y.Y., Matsushima, K., Baba, T., Mukaida, N., 2008. CCL3-CCR5 axis regulates intratumoral accumulation of leukocytes and fibroblasts and promotes angiogenesis in murine lung metastasis process. *J. Immunol.* 181, 6384–6393.