Increased levels of IL-17A in patients with fibromyalgia

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ABSTRACT

Objectives. *The aim of this study was to evaluate the plasma levels of IL-17A in fibromyalgia patients, and to look for any correlations between this data and the concentrations of some pro- and anti-inflammatory cytokines.*

Methods. *We performed a study including 58 fibromyalgia patients and 39 healthy women matched for age and body mass index. The plasma levels of IL-17A and other pro- and anti-inflammatory cytokines were measured by using the technique of cytometric bead array (CBA). The analysis of differences between groups was performed using Mann-Whitney test and the analysis of the correlations by Spearman's correlation test.*

Results. *The analyses showed that fibromyalgia patients present increased levels of IL-17A. They also revealed that plasma concentrations of IL17A positively correlate with levels of IL-2, IL-4 and IL-10, TNF and IFNγ.*

Conclusion. *As far as we are aware, this is the first study to demonstrate increased levels of IL17A in fibromyalgia patients. The positive correlation between the levels of IL-17A and of other cytokines strengthens the hypothesis of the involvement of inflammatory mechanisms in the development of this syndrome.*

Introduction

The aetiology and physiopathology of fibromyalgia (FM) remain unclear (1), although evidences point to the involvement of the immune system in its genesis and evolution (2-5). In this study, we analysed the participation of the immune system in FM by analysing the plasma levels of IL-17A, which is a potent pro-inflammatory cytokine (6), that has been implicated in the pathogenesis of some systemic inflammatory and autoimmune diseases (7-10). In addition, we have also looked for correlations between plasmatic levels of IL-17A and other pro- and anti-inflammatory cytokines.

Materials and methods

This study was conducted on 58 women in the FM group and 39 women in the control group. Although, the American College of Rheumatology (ACR) had published new criteria of diagnostic in 2010 (11), for this study FM was diagnosed according to the previous ACR criteria (12), since the stages of research planning and recruitment of patients began before 2010, and the new criteria of diagnostic are still controversial (13-15).

The study group included only patients with primary fibromyalgia. Patients diagnosed with FM with a history or presence of: chronic inflammatory conditions (*e.g.* spondyloarthritis and ankylosing spondylitis), autoimmune diseases (*e.g.* systemic lupus erythematosus and rheumatoid arthritis), psychiatric disorders (*e.g.* major depressive disorder, schizophrenic or paranoid disorder) and or presenting infectious were excluded from the study. Also excluded were patients who had used anti-inflammatory drugs in the past six months, pregnant patients or women who were breastfeeding. This study was approved by the ethics committees of UFMG (0224.0.203.000-10) and of UNIFOR-MG (158/2010). All patients and control subjects gave written informed consent before inclusion in this study.

There were no statistically significant differences in age between the FM group (49.7±10.7 years) and the control group (50.3±7.7 years), and neither between the body mass index (BMI) in the FM group (26.3±4.5 kg/m^2) *versus* the control group (26.7±2.4 kg/m^2).

Cytokine levels were assessed using a commercial BD™ Cytometric Bead Array (CBA) $T_H1/T_H2/T_H17$ cytokine kit (BD Bioscience, San Diego, CA, USA) according to the manufacturer's protocol, and the data were acquired

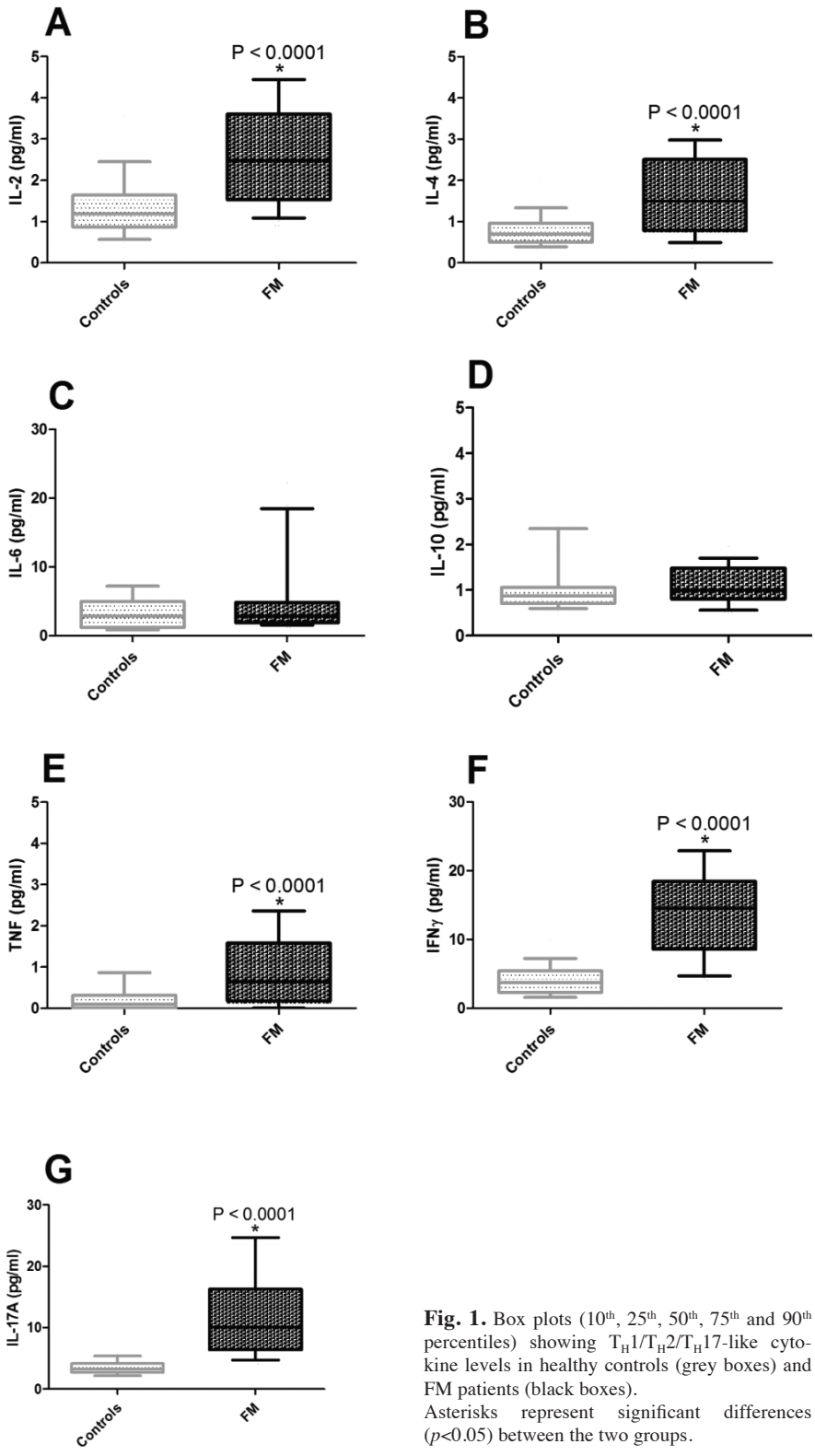

Fig. 1. Box plots (10th, 25th, 50th, 75th and 90th percentiles) showing $T_H1/T_H2/T_H17$-like cytokine levels in healthy controls (grey boxes) and FM patients (black boxes).
Asterisks represent significant differences ($p<0.05$) between the two groups.

using a FACSCalibur flow cytometer (BD Bioscience, San Jose, CA, USA). Standard curves were determined for each cytokine using a range of 0–5000 pg/mL. The quantity (pg/mL) of each respective cytokine was calculated using FlowJo 7.6.1 software (Tree Star™, Inc., Ashland, OR).

Ordinal data were analysed by Kolmogorov Smirnov, followed by a Mann-Whitney test for non-parametric data. The Spearman test was used for correlational analysis between variables. All data were analysed using GraphPad Prism 5.0 software (San Diego, CA, USA). The data were expressed as the mean ± SE. Significance was defined as $p \leq 0.05$ (α=0,05).

Results

The levels of IL-17A were significantly higher in FM patients when compared to healthy controls (Fig. 1). Besides, the levels of IL-2, TNF, IFNγ and IL-4 were also significantly higher in FM patients, when compared to controls. No significant difference was observed in IL-10 and neither in IL-6 levels.

Analyses of the Spearman correlation coefficient (Fig. 2) revealed significant positive correlations between IL-17A and IL-2 (p=0.012 and r=0.353), IL-17A and TNF (p=0.003 and r=0.396), IL-17A and IFNγ (p=0.037 and r=0.295), IL-17A and IL-4 (p=0.037 and r=0.293) and IL-17A and IL-10 (p=0.002 and r=0.419). There was no significant correlation between IL-17A and IL-6.

Discussion

This study is the first to demonstrate that FM patients present increased plasma levels of IL-17A when compared to healthy age-matched controls. IL-17A is mainly produced by T_H17 lymphocytes, but it can also be produced by natural killer cells, dendritic cells and neutrophils (16-18). The origin of IL-17A in FM patients was not addressed in this study, but it is possibly produced, at least in part, by activated T lymphocytes, since we have previously demonstrated increased numbers of these cells in FM patients (5). We have also previously demonstrated increased levels of peripheral blood $CD5^+$ and $CD5^-$ B lymphocytes in FM patients (5), what could be induced by IL-17A, as have been suggested by studies on other inflammatory conditions (19, 20).

We also demonstrate here positive correlations between IL-17A concentration and other cytokines, such as IL-2, TNF and IFNγ, what strengthens the hypothesis of the involvement of inflammatory mechanisms in the development of this syndrome. IL-2 is able to stimulate the differentiation of T_{reg}

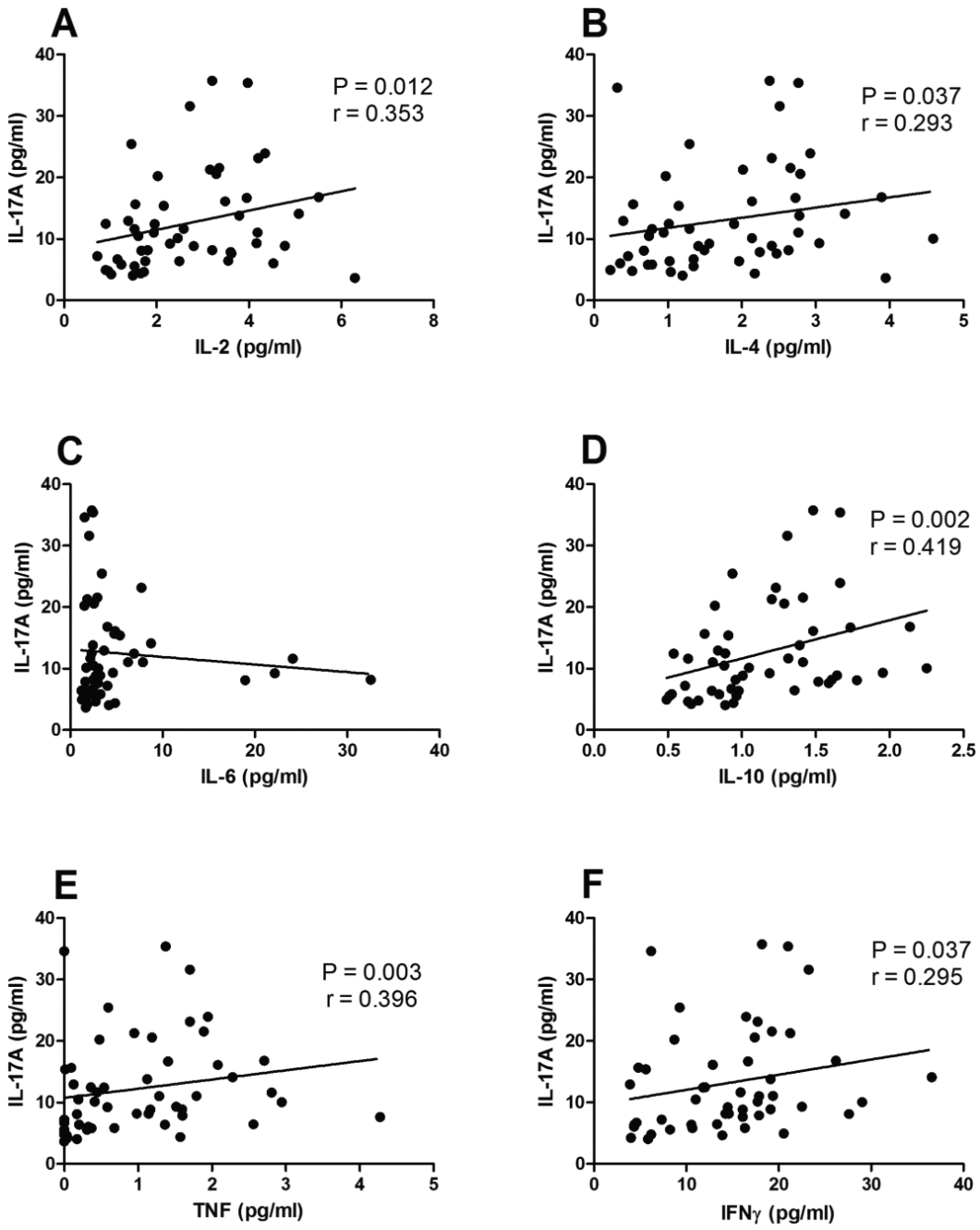

Fig. 2. Correlation between plasma levels of IL-17A *vs*. plasma levels of: IL-2 (**A**), IL-4 (**B**), IL-6 (**C**), IL-10 (D), TNF (**E**) and IFNγ (**F**) in FM patients. The significant correlations (*p*) and Spearman's correlation coefficient (r) are shown on their respective charts.

cells into T_H17 lymphocytes (21). TNF is also produced by T_H17 cells, and besides, it works in the stabilisation and maintenance of T_H17 cells (22). The association of TNF with IL-17A has been reported in several conditions characterized by chronic inflammation; these cytokines seem to act together in perpetuating the inflammatory process (10, 23). Regarding the IFNγ data, although some studies have demonstrated an inhibitory effect of IFNγ on IL-17A production (17, 24), there are others that show increased levels of both these cytokines, mainly in chronic inflammation and autoimmunity models (23, 25), likely because some cells, particularly B-cells, can co-produce them (22).

Positive correlations between IL-17A and IL-4 or IL-10, as were demonstrated here in FM patients, have also been described in some models of chronic inflammation and autoimmune diseases (26, 27). Although data from correlation analyses does not necessarily prove causation, it is tempting to speculate that our data from IL-4 and IL-10 levels could suggest an attempt of this cytokine to regulate the overproduction IL-17A and other inflammatory cytokines (28). Both IL-4 and IL-10 seem to be necessary for T_{reg} cell-mediated suppression of the T_H17 response (27).

It is known that changes in immune profile may influence the severity of main symptoms present in the FM (5, 29), and that an imbalance between pro- and anti-inflammatory cytokine levels could explain, at least in part, the induction and maintenance of symptoms

in FM patients (30, 31). Moreover, It has been shown, in other clinical conditions, that IL-17A positively correlates with indices of pain (32), depression (33) and anxiety (34), which are symptoms frequently reported by patients with FM.

Finally, we would like to emphasise the relevance of this study as being the first to demonstrate that fibromyalgia is associated with increased levels of IL-17A. The data from correlation analyses between the levels of IL17A and levels of other cytokines strengthens the hypothesis that point to the involvement of inflammatory mechanisms in the development of this syndrome. Within this perspective, future studies could be performed to clarify the effects of specific anti-inflammatory therapies of IL-17A in the quality of life and in symptoms presented by FM patients.

References

1. JAHAN F, NANJI K, QIDWAI W, QASIM R: Fibromyalgia syndrome: an overview of pathophysiology, diagnosis and management. *Oman Med J* 2012; 27: 192-5.
2. ROSS RL, JONES KD, BENNETT RM, WARD RL, DRUKER BJ, WOOD LJ: Preliminary evidence of increased pain and elevated cytokines in fibromyalgia patients with defective growth hormone response to exercise. *Open Immunol J* 2010; 3: 9-18.
3. DI FRANCO M, IANNUCCELLI C, VALESINI G: Neuroendocrine immunology of fibromyalgia. *Ann N Y Acad Sci* 2010; 1193: 84-90.
4. MENZIES V, LYON DE: Integrated review of the association of cytokines with fibromyalgia and fibromyalgia core symptoms. *Biol Res Nurs* 2010; 11: 387-94.
5. CARVALHO LS, CORREA H, SILVA GC *et al*.: May genetic factors in fibromyalgia help to identify patients with differentially altered frequencies of immune cells? *Clin Exp Immunol* 2008; 154: 346-52.
6. SONDER SU, SARET S, TANG W, STURDEVANT DE, PORCELLA SF, SIEBENLIST U: IL-17-induced NF-kappaB activation via CIKS/Act1: physiologic significance and signaling mechanisms. *J Biol Chem* 2011; 286: 12881-90.
7. ZHANG L, LI YG, LI YH *et al*.: Increased frequencies of Th22 cells as well as Th17 cells in the peripheral blood of patients with ankylosing spondylitis and rheumatoid arthritis. *PloS One* 2012; 7: e31000.
8. MIELIAUSKAITE D, DUMALAKIENE I, RUGIENE R, MACKIEWICZ Z: Expression of IL-17, IL-23 and their receptors in minor salivary glands of patients with primary Sjogren's syndrome. *Clin Dev Immunol* 2012; 2012: 187-258.
9. YOSHIZAKI A, YANABA K, IWATA Y *et al*.: Elevated serum interleukin-27 levels in patients with systemic sclerosis: association with T cell, B cell and fibroblast activation. *Ann Rheum Dis* 2011; 70: 194-200.
10. ROMERO-SANCHEZ C, JAIMES DA, LONDOÑO J *et al*.: Association between Th-17 cytokine profile and clinical features in patients with spondyloarthritis. *Clin Exp Rheumatol* 2011; 29: 828-34.
11. WOLFE F, CLAUW DJ, FITZCHARLES MA *et al*.: The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis Care Res* 2010; 62: 600-10.
12. WOLFE F, SMYTHE HA, YUNUS MB *et al*.: The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. *Arthritis Rheum* 1990; 33: 160-72.
13. SALAFFI F, SARZI-PUTTINI P: Old and new criteria for the classification and diagnosis of fibromyalgia: comparison and evaluation. *Clin Exp Rheumatol* 2012; 30 (Suppl. 74): S3-9.
14. STAUD R, PRICE DD, ROBINSON ME: The provisional diagnostic criteria for fibromyalgia: One step forward, two steps back: Comment on the article by Wolfe *et al*. *Arthritis Care Res* 2010; 62: 1675-6.
15. VANDERSCHUEREN S, VAN WAMBEKE P, MORLION B: Fibromyalgia: do not give up the tender point count too easily: comment on the article by Wolfe *et al*. *Arthritis Care Res* 2010; 62: 1675; author reply 6-8.
16. CUA DJ, TATO CM: Innate IL-17-producing cells: the sentinels of the immune system. *Nat Rev Immunol* 2010; 10: 479-89.
17. HARRINGTON LE, HATTON RD, MANGAN PR *et al*.: Interleukin 17-producing $CD4^+$ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005; 6: 1123-32.
18. PICHAVANT M, GOYA S, MEYER EH *et al*.: Ozone exposure in a mouse model induces airway hyperreactivity that requires the presence of natural killer T cells and IL-17. *J Exp Med* 2008; 205: 385-93.
19. HSU HC, YANG P, WANG J *et al*.: Interleukin 17-producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. *Nat Immunol* 2008; 9: 166-75.
20. XIE S, LI J, WANG JH *et al*.: IL-17 activates the canonical NF-kappaB signaling pathway in autoimmune B cells of BXD2 mice to upregulate the expression of regulators of G-protein signaling 16. *J Immunol* 2010; 184: 2289-96.
21. DEKNUYDT F, BIOLEY G, VALMORI D, AYYOUB M: IL-1beta and IL-2 convert human Treg into T(H)17 cells. *Clin Immunol* 2009; 131: 298-307.
22. MIOSSEC P: IL-17 and Th17 cells in human inflammatory diseases. *Microbes Infect* 2009; 11: 625-30.
23. GRIFFIN GK, NEWTON G, TARRIO ML *et al*.: IL-17 and TNF-alpha sustain neutrophil recruitment during inflammation through synergistic effects on endothelial activation. *J Immunol* 2012; 188: 6287-99.
24. CHU CQ, SWART D, ALCORN D, TOCKER J, ELKON KB: Interferon-gamma regulates susceptibility to collagen-induced arthritis through suppression of interleukin-17. *Arthritis Rheum* 2007; 56: 1145-51.
25. DOODES PD, CAO Y, HAMEL KM *et al*.: IFN-gamma regulates the requirement for IL-17 in proteoglycan-induced arthritis. *J Immunol* 2010; 184: 1552-9.
26. ANDOH A, HATA K, ARAKI Y, FUJIYAMA Y, BAMBA T: Interleukin (IL)-4 and IL-17 synergistically stimulate IL-6 secretion in human colonic myofibroblasts. *Int J Mol Med* 2002; 10: 631-4.
27. MCGEACHY MJ, BAK-JENSEN KS, CHEN Y *et al*.: TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat Immunol* 2007; 8: 1390-7.
28. WANG P, WU P, SIEGEL MI, EGAN RW, BILLAH MM: Interleukin (IL)-10 inhibits nuclear factor kappa B (NF kappa B) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. *J Biol Chem* 1995; 270: 9558-63.
29. NUGRAHA B, KORALLUS C, KIELSTEIN H, GUTENBRUNNER C: $CD3^+CD56^+$ natural killer T cells in fibromyalgia syndrome patients: association with the intensity of depression. *Clin Exp Rheumatol* 2013 Apr 2. [Epub ahead of print].
30. BAZZICHI L, ROSSI A, MASSIMETTI G *et al*.: Cytokine patterns in fibromyalgia and their correlation with clinical manifestations. *Clin Exp Rheumatol* 2007; 25: 225-30.
31. IANNUCCELLI C, DI FRANCO M, ALESSANDRI C *et al*.: Pathophysiology of fibromyalgia: a comparison with the tension-type headache, a localized pain syndrome. *Ann N Y Acad Sci* 2010; 1193: 78-83.
32. MENG X, ZHANG Y, LAO L *et al*.: Spinal interleukin-17 promotes thermal hyperalgesia and NMDA NR1 phosphorylation in an inflammatory pain rat model. *Pain* 2013; 154: 294-305.
33. CHEN Y, JIANG T, CHEN P *et al*.: Emerging tendency towards autoimmune process in major depressive patients: a novel insight from Th17 cells. *Psychiatry Res* 2011; 188: 224-30.
34. LIU Y, HO RC, MAK A: The role of interleukin (IL)-17 in anxiety and depression of patients with rheumatoid arthritis. *Int J Rheum Dis* 2012; 15: 183-7.