

The Cellular Source of IL-17A in the Pathogenesis of Inflammatory Joint Diseases

COJOCARU-GOFITA I.R.⁽¹⁾, CIUREA PAULINA⁽¹⁾, YEREMENKO N.G.⁽²⁾

⁽¹⁾ Rheumatology Department, University of Medicine and Pharmacy of Craiova, România;

⁽²⁾ Department of Clinical Immunology and Rheumatology, "Academic Medical Center" University of Amsterdam, The Netherlands

ABSTRACT Inflammatory joint diseases comprise a various range of maladies such as: rheumatoid arthritis, psoriatic arthritis or ankylosing spondylitis. One of their common pathogenical features is the implication of T_H1/2 cytokines(1). In addition to the well-established T_H1 and 2 cytokines there has been found a new family of cytokines and their receptors – IL-17 - which was thought to be produced mainly by the T_H17 subset(1, 2). Recent findings suggest that this highly pro- inflammatory cytokine could be produced by various other cell subtypes such as mast cells(3) (4)and neutrophils. The novelty brought by the intensive research upon IL-17 in human inflammatory joint diseases may bring into clinical practice a broader therapeutical range of options for these patients(5, 6).

KEY WORDS rheumatoid arthritis; psoriatic arthritis; ankylosing spondylitis; pro- inflammatory cytokines

Introduction

Text The intensively studied T_H subsets (T_H1 and T_H2) were first described in 1986 (5-16) due to their distinct cytokine pannels, T_H1 being the cell-source of interferon – γ and tumor necrosis factor therefore being involved in cell-mediated immunity whereas T_H 2 produce mainly IL-4, IL-5, IL-13 (7) playing a key-role in humoral immunity. This paradigm became more and more elaborate over the next 25 years. When the IL12 - p40 subunit was recognized to be a part of the IL-23 structure together with IL-23p19 and further on that IL23(17) is crucial in the stabilization of the cell population that produces IL-17A it became obvious that another CD4+ T cell was the main source of the newly found cytokine and this discovery revealed a completely new T_H subset, named T_H17 and it was first described as such in 1995(18). The IL-17A is also classically known to be produced by other cells such as CD8+ T cells, $\gamma\delta$ T cells, NK cells, NKT cells, LTi cells(19) but also MCT+(4) and CD15+ cells.

In humans, T_H17 polarization is dependent on RORC2 (the homologue of the ROR γ t – found in murine models)(20). Apart from IL-17 (A and F) TH17 were proved to produce IL-21, IL-22, IL-26 and CCL20 all being involved in the pathogenesis of inflammatory arthritides. There were many studies in the field of rheumatology which concerned the IL-17 production mainly in periphery but very few focused on local synovial fluid and tissue.

A 2-year prospective study which analyzed rheumatoid arthritis synovial samples showed that both IL-17 and TNF – α mRNA levels are prognostic factors for worse outcomes in the RA patient. While some research reports showed clear

involvement of TH17 cells in producing IL-17 in inflammatory diseases there were others recently published which undoubtedly prove other cell populations to be the main producers of the IL-17A.(21)

The IL-17A and IL-17A receptor

IL- 17 A is a 17 kDa protein - the first known member of the IL-17 cytokine family which now consists of 6 members found through genomic sequencing: IL-17A, IL-17B, IL-C, IL-17D, IL-17E (also named IL-25) and IL-17F(22). The IL-17A was first identified in a murine T cell library through subtractive hybridization screen. The amino acid structure of the A form is most similar to the F type which can adopt a three-dimensional cysteine-knot form and is the only IL-17 form which has been crystallized and used for structural studies(23). IL-17 A and F are both homodimers but can also take the shape of IL-17 A- IL-17F heterodimers although in human studies it is yet unknown which form is the predominant one. Also the genes encoding these two IL-17 subtypes were found on the same chromosome in both humans and mice(24).

The IL-17 receptors include 5 different subunits, alphabetically named A –E are involved in triggering NF- κ B and JUN amino terminal kinase signaling pathways through TRAF6 (Tumor-necrosis factor receptor associated factor 6)(25). All 5 IL-17 receptor units encoding genes are to be found in humans at the chromosome 3 level and they are all single trans-membrane proteins with variable size at an average of 680 amino acids.

IL-17 A also known as CTLA8 can make use of both IL-17RA and C exerting its main function as both a homo and a heterodimer in autoimmune pathology(26) (human rheumatoid arthritis, systemic lupus erythematosus and allograft rejection), neutrophil recruitment and immune reactions against extra cellular pathogens(27). IL – 17 B, C and D may be described as pro-inflammatory cytokines but they have not been precisely described so far and they can be found ubiquitously throughout the human cells while the IL-17 F appears to be synthesized at the same cellular level as the A form. IL-17E is produced within various epithelial cells and also macrophages, NKT, T_H2 or mast cells and it can firmly induce T_H2 cell responses and can repress T_H17 driven responses(28). There is another form of IL-17 observed in the *Herpes virus saimiri* named vIL-17 which uses the same receptor – IL-17RA as the human IL-17 but its functions are far from being revealed(29).

IL-17RA which is mainly expressed in haematopoietic tissues shows a lower affinity towards IL-17A than the concentration needed to induce the proper response which became the clear proof that this receptor – by far the largest of the family – needs a second subunit to bind ligands and thus elicit signalling(11). This receptor which is also by itself a common signalling unit used by various ligands pairs with IL-17RC in order to facilitate the actions of both IL-17A and F resembling to other shared cytokine receptors such as gp130 (for the IL-6) family(6). Its role is extremely important since it reduces its surface expression after binding to IL -17A which indicates that it might internalize its ligand and clear it in this manner from the inflammatory site.

IL-17A in Rheumatoid Arthritis

Rheumatoid arthritis is a chronic inflammatory process which involves the synovial membrane which will induce proinflammatory cytokine production further on leading to protease activity and both bone and cartilage destruction. In rodent models it was proven that even a single intra-articular IL-17A injection in previously healthy subjects can trigger cartilage damage and constant injection can produce massive inflammatory cell migration and even bone erosions. From another point of view using monoclonal antibodies directed against IL-17 and its receptors provided protection from joint inflammation.

Initial clinical studies which included RA patients high levels of IL-17 in the synovial biopsies and fluid (as compared to osteoarthritis) was directly correlated with both activity and

severity of the disease. The activity of IL-17 was measured by an assay where the supernatant of RA synovial biopsies induced IL-6 secretion in human RA synoviocytes. Further on in 2010 there were three clinical trials which analysed the efficacy and safety of the human IL-17 specific IgG1 κ monoclonal antibody in a cohort of 104 patients with rheumatoid arthritis, chronic plaque-type psoriasis and non-infectious uveitis and it concluded that even after two doses there were variable yet clinically relevant positive responses.

It became clear that IL-17 is deeply involved in the pathogenesis of joint inflammation but the fact that its main cell source is the T_H17 cell population was not argued until the past 2 years when a research paper showed that the main IL-17 expressing cells in RA synovium are mast cell triptase positive and that may constitute a solid proof that at least at synovial level mast cells are the sole IL-17 producing cells.

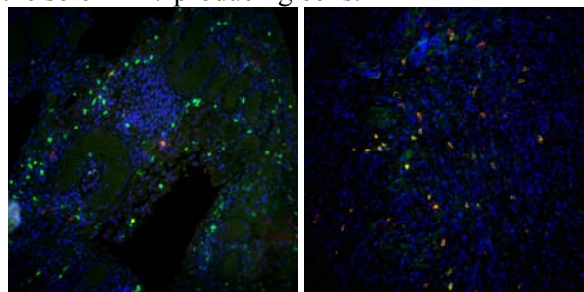


Fig. 1 IL-17 expression and production by synovial mast cells. Double immunofluorescence for anti-IL-17 (green) and mast cell tryptase (red) in rheumatoid arthritis (RA) (A) and spondyloarthritis (SpA) synovitis (B) – 25x magnification

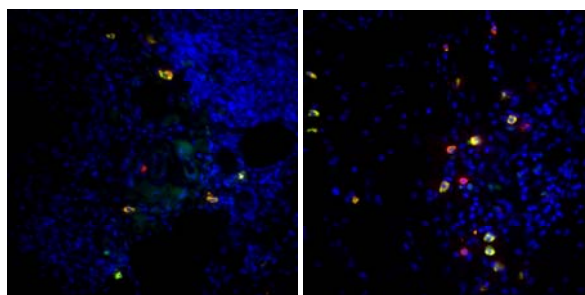


Fig.1 (larger magnification, 40x)

Unpublished data (by Yeremenko N.G et al.) which describe the contribution of IL-17 expressing mast cells in synovial inflammation in spondyloarthritis shows the morphology of the IL-17A and MCT double positive cells in both rheumatoid arthritis and spondyloarthritis patients (Fig.1) through immunofluorescence techniques. Apart from that the same authors observe the presence of another cell type IL17A+CD15+ which seem of granulocytic nature, most likely neutrophils which also produce IL-17A. They also

conquer that there is an even larger increase in IL-17 producing cells in a TNF-independent manner in spondyloarthritis versus rheumatoid arthritis.

The role of mast cells in inflammatory joint disease

Mast cells express predominantly immunological related functions being multifunctional effector cells and they do contain a large range of granules sharing common features in all mammalian organisms including their membrane receptors (FcεR1)(30). In their granules they store a large variety of biologically active amines, cytokines, serine proteases or proteoglycans(31).

Various studies promote the role of mast cells in the pathogenesis of different inflammatory disease, for instance in psoriasis the release of the IL-33(32) (one of the recent found members of the IL-1 cytokine family) increases the SP – stimulated VEGF from skin MCs. In the attempt to characterize the IL-17 producing cells in extra-articular sites affected by spondyloarthritis it has been recently showed that in psoriatic skin there is a massive infiltration of mast cells which produce IL-17 (unpublished data) (Fig.2). The same data were obtained while analyzing gut samples from patients diagnosed with both Crohn's disease and ulcerative colitis. Also in multiple sclerosis MCs are considered an attractive therapeutic target since they can release cytokines which selectively induce T cell activation or even present myelin antigens to T cells(33).

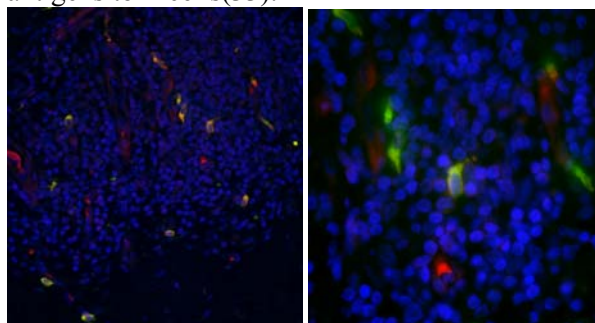


Fig.2 – psoriatic skin Psoriatic skin – double staining IL-17 (green) and MCT (red)

DAPI – blue, A – 25x magnification, B - 40X magnification

In rheumatoid arthritis mast cells can be found in high numbers around blood vessels and in the sublining area in sites of cartilage erosions or in the synovial fluid, otherwise the percentage of mast cells in normal synovium hardly reaches 5% of all cells(34). Their recruitment in RA is due to chemotactic factors such as SCF (stem-cell factor) also known as c-kit ligand produced by fibroblasts, stromal cells and endothelial cells. Lee et al. showed in an animal model that subjects

lacking MCs become resistant to serum induced erosive arthritis(35).

At the articular sites in RA MCs synthesize inflammatory cytokines such as: IL-6, IL-8, and TNF α whose expression depends on the nuclear factor- NF κ B – in this manner MCs become important in lymphocyte chemotaxis and infiltration of the synovial areas(36). In the presence of IL-6 and TGF β MCs are needed for the T_H17 cell line production. Mast cells also trigger macrophages to enhance IL-1 production and in RA(37), heparin and histamine secreted from their granules activate local macrophages to produce both IL-1 and TNF α .

MCs can produce themselves a multitude of mediators which influence fibroblasts such as VEGF, PDGF, FGF-2 and synovial fibroblasts themselves express high levels of SCF(38) necessary for MCs to undergo differentiation and maturation processes during inflammation. As far as angiogenesis is concerned in RA mast cells seem to induce it through the tryptase and chymase enzymes(39) They also mediate the production of matrix metalloproteinases by type-B synoviocytes and chondrocytes which are highly involved in bone remodeling and cartilage degradation (40).

Neutrophils as IL-17 producing cells

Until recently most of the researchers did not value neutrophils as IL-17 producing cells since they were proved to be a major recruitment target for both IL-17A (41) and F activation in innate cells. First of all animal models have already changed that opinion when a murine model of the ischemia-reperfusion injury since only as little as 3 hours after the injury neutrophils are present in huge numbers at the local site and that seems to be an IL-23 dependent process(42). Also another model proved that Bordetella pertussis infection can induce the IL-17 production from infiltrating granulocytes and also T lymphocytes or macrophages.

Another recent paper from 2008 detected IL-17RA mRNA in circulating neutrophils of healthy donors thus indicating that the IL-17 receptor may be constitutively expressed on the circulating neutrophil lineage(43). As regarding neutrophils presence and function in arthritis there was a recent study upon the profile of neutrophils in collagen induced arthritis (CIA) in an animal model which showed significantly high numbers in the knees and digits of DBA/IJ mice submitted to CIA in comparison to non-arthritic animals and it also suggests that mast cells may be the modulators of the neutrophil influx in arthritis.

There are though only few scientific data regarding the IL-17 production of neutrophils but at least one shows in a immunohistochemical analysis the co localization of CD15 and IL-17 antibodies in the synovial tissues of both rheumatoid arthritis and spondylarthritis with a higher rate in the SpA patients (Fig.3). Although the mechanism behind this process is still to be elucidated there are strong evidence from clinical trials using anti-IL-17 antibodies to treat psoriatic arthritis and spondyloarthritis that show efficacy and safety in lowering the IL-17 production in this patients yet again in a TNF-independent manner. Looking further on in the extraarticular sites affected in certain arthritis site such as psoriatic skin or intestine of Crohn and ulcerative colitis the same authors find a high rate of neutrophils which are also positive for anti-IL-17 antibody immune staining.

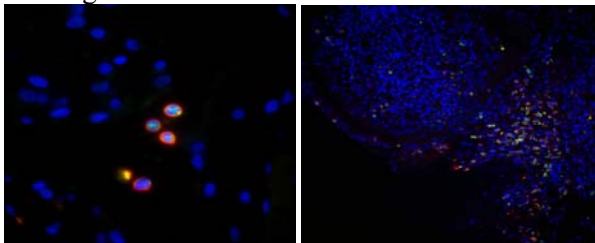


Fig.3 – CD15 and IL-17 in synovium IL-17 expression by neutrophils. Double immunofluorescence for anti- IL-17 (green) and CD15 (red) in the synovium of spondyloarthritis (SpA) patients (A) 100x (B) – 25x magnification

References

- Serrano Hernandez A. [Helper (TH1, TH2, TH17) and regulatory cells (Treg, TH3, NKT) in rheumatoid arthritis]. *Reumatol Clin* 2009;5S1:1-5.
- Shahrara S, Pickens SR, Dorfleitner A, Pope RM. IL-17 induces monocyte migration in rheumatoid arthritis. *J Immunol* 2009;182(6):3884-91.
- Yeremenko N, Baeten D. IL-17 in spondyloarthritis: is the T-party over? *Arthritis Res Ther*;13(3):115.
- Hueber AJ, Asquith DL, Miller AM, Reilly J, Kerr S, Leipe J, et al. Mast cells express IL-17A in rheumatoid arthritis synovium. *J Immunol*;184(7):3336-40.
- Miossec P. IL-17 in rheumatoid arthritis: a new target for treatment or just another cytokine? *Joint Bone Spine* 2004;71(2):87-90.
- Hwang SY, Kim JY, Kim KW, Park MK, Moon Y, Kim WU, et al. IL-17 induces production of IL-6 and IL-8 in rheumatoid arthritis synovial fibroblasts via NF-kappaB- and PI3-kinase/Akt-dependent pathways. *Arthritis Res Ther* 2004;6(2):R120-8.
- Hussein MR, Fathi NA, El-Din AM, Hassan HI, Abdullah F, Al-Hakeem E, et al. Alterations of the CD4(+), CD8 (+) T cell subsets, interleukins-1beta, IL-10, IL-17, tumor necrosis factor-alpha and soluble intercellular adhesion molecule-1 in rheumatoid arthritis and osteoarthritis: preliminary observations. *Pathol Oncol Res* 2008;14(3):321-8.
- Kim HR, Cho ML, Kim KW, Juhn JY, Hwang SY, Yoon CH, et al. Up-regulation of IL-23p19 expression in rheumatoid arthritis synovial fibroblasts by IL-17 through PI3-kinase-, NF-kappaB- and p38 MAPK-dependent signalling pathways. *Rheumatology (Oxford)* 2007;46(1):57-64.
- Furuya T, Hakoda M, Ichikawa N, Higami K, Nanke Y, Yago T, et al. Associations between HLA-DRB1, RANK, RANKL, OPG, and IL-17 genotypes and disease severity phenotypes in Japanese patients with early rheumatoid arthritis. *Clin Rheumatol* 2007;26(12):2137-41.
- Ryu S, Lee JH, Kim SI. IL-17 increased the production of vascular endothelial growth factor in rheumatoid arthritis synoviocytes. *Clin Rheumatol* 2006;25(1):16-20.
- Hwang SY, Kim HY. Expression of IL-17 homologs and their receptors in the synovial cells of rheumatoid arthritis patients. *Mol Cells* 2005;19(2):180-4.
- Kehlen A, Thiele K, Riemann D, Langner J. Expression, modulation and signalling of IL-17 receptor in fibroblast-like synoviocytes of patients with rheumatoid arthritis. *Clin Exp Immunol* 2002;127(3):539-46.
- Chabaud M, Page G, Miossec P. Enhancing effect of IL-1, IL-17, and TNF-alpha on macrophage inflammatory protein-3alpha production in rheumatoid arthritis: regulation by soluble receptors and Th2 cytokines. *J Immunol* 2001;167(10):6015-20.
- Chabaud M, Lubberts E, Joosten L, van Den Berg W, Miossec P. IL-17 derived from juxta-articular bone and synovium contributes to joint degradation in rheumatoid arthritis. *Arthritis Res* 2001;3(3):168-77.
- Ziolkowska M, Koc A, Luszczkiewicz G, Ksiezopolska-Pietrzak K, Klimczak E, Chwalinska-Sadowska H, et al. High levels of IL-17 in rheumatoid arthritis patients: IL-15 triggers in vitro IL-17 production via cyclosporin A-sensitive mechanism. *J Immunol* 2000;164(5):2832-8.
- Ziegler A, Uchanska-Ziegler B. Rheumatoid arthritis etiology and HLA-linked odorant receptor gene polymorphisms: comment on the article by Klareskog et al. *Arthritis Rheum* 2006;54(8):2705-6.
- Duvallet E, Semerano L, Assier E, Falgarone G, Boissier MC. Interleukin-23: A key cytokine in inflammatory diseases. *Ann Med*.
- Yao Z, Painter SL, Fanslow WC, Ulrich D, Macduff BM, Spriggs MK, et al. Human IL-17: a novel cytokine derived from T cells. *J Immunol* 1995;155(12):5483-6.
- Tanaka S, Yoshimoto T, Naka T, Nakae S, Iwakura Y, Cua D, et al. Natural occurring IL-17 producing T cells regulate the initial phase of neutrophil mediated airway responses. *J Immunol*;183(11):7523-30.
- Broussard DR, Lozano MM, Dudley JP, Rorgamma (Rorc) is a common integration site in type B leukemogenic virus-induced T-cell lymphomas. *J Virol* 2004;78(9):4943-6.
- Kirkham BW, Lassere MN, Edmonds JP, Juhasz KM, Bird PA, Lee CS, et al. Synovial membrane cytokine expression is predictive of joint damage progression in rheumatoid arthritis: a two-year prospective study (the DAMAGE study cohort). *Arthritis Rheum* 2006;54(4):1122-31.

22. Sarkar S, Fox DA. Targeting IL-17 and Th17 cells in rheumatoid arthritis. *Rheum Dis Clin North Am*;36(2):345-66.
23. Paradowska-Gorycka A, Wojtecka-Lukasik E, Trefler J, Wojciechowska B, Lacki JK, Maslinski S. Association between IL-17F gene polymorphisms and susceptibility to and severity of rheumatoid arthritis (RA). *Scand J Immunol*;72(2):134-41.
24. Gaffen SL, Kramer JM, Yu JJ, Shen F. The IL-17 cytokine family. *Vitam Horm*;74:255-82.
25. Kramer JM, Yi L, Shen F, Maitra A, Jiao X, Jin T, et al. Evidence for ligand-independent multimerization of the IL-17 receptor. *J Immunol*;176(2):711-5.
26. Cho ML, Jung YO, Kim KW, Park MK, Oh HJ, Ju JH, et al. IL-17 induces the production of IL-16 in rheumatoid arthritis. *Exp Mol Med* 2008;40(2):237-45.
27. Witowski J, Pawlaczyk K, Breborowicz A, Scheuren A, Kuzlan-Pawlaczyk M, Wisniewska J, et al. IL-17 stimulates intraperitoneal neutrophil infiltration through the release of GRO alpha chemokine from mesothelial cells. *J Immunol*;165(10):5814-21.
28. Zimmerman DH, Taylor P, Bendele A, Carambula R, Duzant Y, Lowe V, et al. CEL-2000: A therapeutic vaccine for rheumatoid arthritis arrests disease development and alters serum cytokine/chemokine patterns in the bovine collagen type II induced arthritis in the DBA mouse model. *Int Immunopharmacol* 2010;10(4):412-21.
29. Mathur AN, Chang HC, Zisoulis DG, Stritesky GL, Yu Q, O'Malley JT, et al. Stat3 and Stat4 direct development of IL-17-secreting Th cells. *J Immunol* 2007;178(8):4901-7.
30. McInnes IB. Leukotrienes, mast cells, and T cells. *Arthritis Res Ther*;5(6):288-9.
31. Gotis-Graham I, McNeil HP. Mast cell responses in rheumatoid synovium. Association of the MCTC subset with matrix turnover and clinical progression. *Arthritis Rheum*;40(3):479-89.
32. Tedla N, Gibson K, McNeil HP, Cosman D, Borges L, Arm JP. The co-expression of activating and inhibitory leukocyte immunoglobulin-like receptors in rheumatoid synovium. *Am J Pathol*;160(2):425-31.
33. Godfrey HP, Ilardi C, Engber W, Graziano FM. Quantitation of human synovial mast cells in rheumatoid arthritis and other rheumatic diseases. *Arthritis Rheum*;27(8):852-6.
34. McCrea PC. Tissue mast cells in the bone marrow in rheumatoid arthritis. *Ann Rheum Dis*;20:83-5.
35. Lee JH, Kim JW, Kim do K, Kim HS, Park HJ, Park DK, et al. The Src family kinase Fgr is critical for activation of mast cells and IgE-mediated anaphylaxis in mice. *J Immunol*;187(4):1807-15.
36. Kobayashi Y, Okunishi H. Mast cells as a target of rheumatoid arthritis treatment. *Jpn J Pharmacol*;90(1):7-11.
37. Crisp AJ. Mast cells in rheumatoid arthritis. *J R Soc Med*;77(6):450-1.
38. Ruschpler P, Lorenz P, Eichler W, Koczan D, Hanel C, Scholz R, et al. High CXCR3 expression in synovial mast cells associated with CXCL9 and CXCL10 expression in inflammatory synovial tissues of patients with rheumatoid arthritis. *Arthritis Res Ther*;5(5):R241-52.
39. Kiener HP, Baghestanian M, Dominkus M, Walchshofer S, Ghannadan M, Willheim M, et al. Expression of the C5a receptor (CD88) on synovial mast cells in patients with rheumatoid arthritis. *Arthritis Rheum*;41(2):233-45.
40. Timmer TC, Baltus B, Vondenhoff M, Huizinga TW, Tak PP, Verweij CL, et al. Inflammation and ectopic lymphoid structures in rheumatoid arthritis synovial tissues dissected by genomics technology: identification of the interleukin-7 signaling pathway in tissues with lymphoid neogenesis. *Arthritis Rheum*;56(8):2492-502.
41. Kasama T, Kobayashi K, Yajima N, Shiozawa F, Yoda Y, Takeuchi HT, et al. Expression of vascular endothelial growth factor by synovial fluid neutrophils in rheumatoid arthritis (RA). *Clin Exp Immunol*;121(3):533-8.
42. Ohtsu S, Yagi H, Nakamura M, Ishii T, Kayaba S, Soga H, et al. Enhanced neutrophilic granulopoiesis in rheumatoid arthritis. Involvement of neutrophils in disease progression. *J Rheumatol*;27(6):1341-51.
43. Edwards SW, Hallett MB. Seeing the wood for the trees: the forgotten role of neutrophils in rheumatoid arthritis. *Immunol Today*;18(7):320-4.

Correspondence Adress: Cojocaru-Gofita I.R. MD, Ph D Student Rheumatology Department, University of Medicine and Pharmacy of Craiova, România, Str Petru Rares nr. 4, 200456, Craiova, Dolj, Romania