Trakia Journal of Sciences, Vol. 6, No. 1, pp 7-11, 2008

Available online at:
http://www.uni-sz.bg

ISSN 1312-1723 (print)
ISSN 1313-3551 (online)

Original Contribution

DYNAMICS IN EXPRESSION OF THE IL-12 RELATED CYTOKINE TRANSCRIPTS OF *IL-12A*, *IL-12B* AND *IL-23* AFTER STIMULATION OF HUMAN PBMC

S. Stanilova, L. Miteva

Department of Molecular Biology, Immunology and Genetics; Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

The proper balance between IL-12-related cytokines controls the appearance of normal and pathological Th1-mediated immune response. In this study we examined the inducible IL-12p40, IL-12p35 and IL-23p19 mRNA expression in PBMC from healthy donors in response to different stimuli at 3, 6 and 9 hours. All stimuli tested up-regulated mRNA expression for IL-12p40, followed by IL-23p19 at 6th hour. The dynamics in transcription of each gene followed specific profile depending on the stimulus. The earlier and longer expression of all three genes was detected after LPS stimulation. We conclude that the differences of mRNA expression of IL-12-related cytokine transcripts are responsible at least for different quantity of IL-12p70, IL-12p80 and IL-23, which determine the special feature of developed immune response.

Key words: qRT-PCR; IL-12p40; IL-12p35; IL-23; IL-23p19.

INTRODUCTION

Activation and differentiation of the immune cells upon antigen stimulation are regulated through changes in gene expression, including genes of regulatory cytokines Depending on dynamics of expression of regulatory cytokines IL-12 and IL-10 from stimulated antigen-presenting cells (macrophages and dendritic cells) the immune response has driven towards humoral or cellular pattern.

It is well known that IL-12p70 is the cytokine responsible for the differentiation of Th cell to IFN-gamma producing Th1 cells and development of Th1 mediated immune response (1, 2, 3). Moreover some authors reported that latest discovered IL-12 related cytokines - IL-23 and IL-27 are involved in pathological Th1-mediated immune response in some diseases (4, 5). IL-23 can enhance the proliferation of memory T cells and production of IFN-gamma from activated T cells.

The cytokine IL-12 and IL-23 are heterodimers, which share the same subunit – IL-12p40, expressed from IL-12B gene (*IL-12B*). Other subunits for IL-12p70 and IL-23 are p35 and p19 subunits, produced respectively from *IL-12A* and *IL-23* genes (4). Moreover IL-12p40 subunit forms two heterodimers: IL-12p70 (p40/p35) and IL-23 (p40/p19), as well as monomer IL-12p40 and homodimer IL-12p80.

The expression of *IL-12A*, *IL-12B* and *IL-23* genes is regulated by different agents and involves specific intracellular signalling pathways. While the p35 gene is expressed in most cells, p40 expression is restricted to a few cell types, such as dendritic cells, monocytes, and macrophages (6). The cytokine IL-12p70, IL-12p40 and IL-23 production from macrophages and dendritic cells affect the type of both normal and pathological immune response (7, 8). That is why we decided to investigate the dynamics of *IL-12A*, *IL-12B* and *IL*-23 gene expression in PBMC after stimulation with bacterial and plants immunomodulators by quantitative RT-PCR.

*** Correspondence to**: *S. Stanilova, Department of Molecular Biology, Immunology and Genetics; Faculty of Medicine, Trakia University, Stara Zagora; Bulgaria; stanilova@mf.uni-sz.bg*

MATERIALS AND METHODS

Blood donors and PBMC isolation

4 healthy volunteers were recruited for this study. The informed consent was obtained from each participant. The peripheral venous blood (10 ml) was collected in sterile tubes with ethylenediamine tetraacetic acid (EDTA). Peripheral blood mononuclear cells (PBMC) were isolated by Histopaque-1077 density gradient centrifugation. The interface containing PBMC was harvested and washed twice with cold RPMI-1640 medium. After washing the cell pellet was re-suspended in the same medium. PBMC viability was tested with trypan blue exclusion; cells were counted and adjusted to 5x106 PBMC/ml in RPMI-1640.

Cell cultures and stimulation

2 x 10^6 PBMC were cultured in sterile polystyrene tubes containing 2 ml RPMI 1640. The cultures were supplemented with: 10% FBS, 100 U/ml penicillin, 100 μg/ml gentamicin and 0.3 mg/ml L-glutamine. The cells were stimulated with: 30 μg/ml C3bgp, 1 μg/ml LPS and 20 μg/ml PHA. Cultures were incubated at 37°C for 3, 6 or 9 h.

RNA isolation

After incubation, cells were washed with PBS and re-suspended in Lysis Solution RL supplied with a column-based innuPREP blood RNA isolation kit AJ Roboscreen (Leipzig; Germany). The following steps for total RNA isolation were performed according to manufacturer's instructions. The total RNA was quantified by spectrophotometric analysis. To remove traces of genomic DNA, total RNA (1 μg) was treated with RNase-free DNase I (Fermentas) following the manufacturer's instruction.

Reverse transcription

Synthesis of cDNA was performed manually according to manufacturer's instructions with High-Capacity cDNA Archive kit (Applied Biosystems, USA) that uses random primers and MutliScribe TM MuLV reverse transcriptase enzyme. Incubation conditions for reverse transcription was 10 min at 25°C followed by 2 hours at 37°C and was performed on a GeneAmp PCR System 9700 (Applied Biosystems, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed on a 7500 Real - Time PCR System (Applied Biosystems, Foster City, CA, USA). The following validated PCR primers and TaqMan MGB probes (6FAM-labeled) were used: IL-12p40 (assay ID: Hs00233688_m1); IL-12p35 (Hs00168405_m1) and IL-23p19 (Hs00372324_m1). As endogenous control we used eukaryotic 18S ribosomal RNA (Hs99999903_m1).

An aliquot of 5μl of the RT reaction was amplified in duplicate in final volume of 25μl of TaqMan Universal PCR Master Mix and Gene Expression Assay mix, containing specific forward and reverse primers and labelled probes for target genes and endogenous control (Applied Biosystems, USA). The thermocycling conditions were: an initial 10 min incubation at 95°C followed by 40 cycles of denaturation for 15s at 95°C and extension for 1 min at 60°C. PCR data were collected with Sequence Detection System software, version 1.3.1.

Relative quantitative evaluation of cytokine mRNAs was performed by the comparative ΔΔCt method. The mean ΔCt obtained in non-stimulated PBMC for each cytokine mRNA was used as calibrator. The results are presented as an n-fold difference relative to calibrator ($RQ=2^{-\Delta\Delta Ct}$).

RESULTS

Effects of 3, 6 and 9 hours stimulation on IL-12A, IL-12B and IL-23 mRNA expression in human PBMC

The results from relative quantification of IL-12p40, IL-12p35 and IL-23p19 transcripts in non-stimulated and 3rd, 6th and 9th hour stimulated normal PBMC are presented on **Figure 1**.

The highest level of expression in stimulated PBMC was observed for *IL-12A* at 3rd hour after LPS stimulation, followed by rapid decrease of mRNA at 6th h. The highest level of *IL-12A* expression after PHA stimulation was detected at 6th h. *IL-12B* expression was increased up to 9-fold in stimulated PBMC than non-stimulated at 6th h regardless of stimuli used. IL-23p19 mRNA expression was increased up to 3.4-fold after stimulation, also at 6th h and independently of stimuli used. Only LPS stimulation resulted in continuous expression of *IL-12B* and *IL-23* at 9 hours.

Effects of C3bgp, LPS and PHA on IL-12A, IL-12B and IL-23 mRNA expression in human PBMC

The data for mRNA expression from the three studied genes at the same hour demonstrated preferentially expressed gene and dynamics of the formed dimers upon stimulation as shown on **Figure 2.** Our results demonstrated that at 3rd h the expression of IL-12A and IL-12B were higher and equivalent unlike IL-23 expression. All stimuli used provoke the same expression at 6th hour, and demonstrates that at this hour there are no stimuli dependent differences. Interestingly at 9th hour the expression only of *IL-12B* after LPS stimulation remained higher. The earlier and longer expression of all three genes was detected after LPS stimulation only.

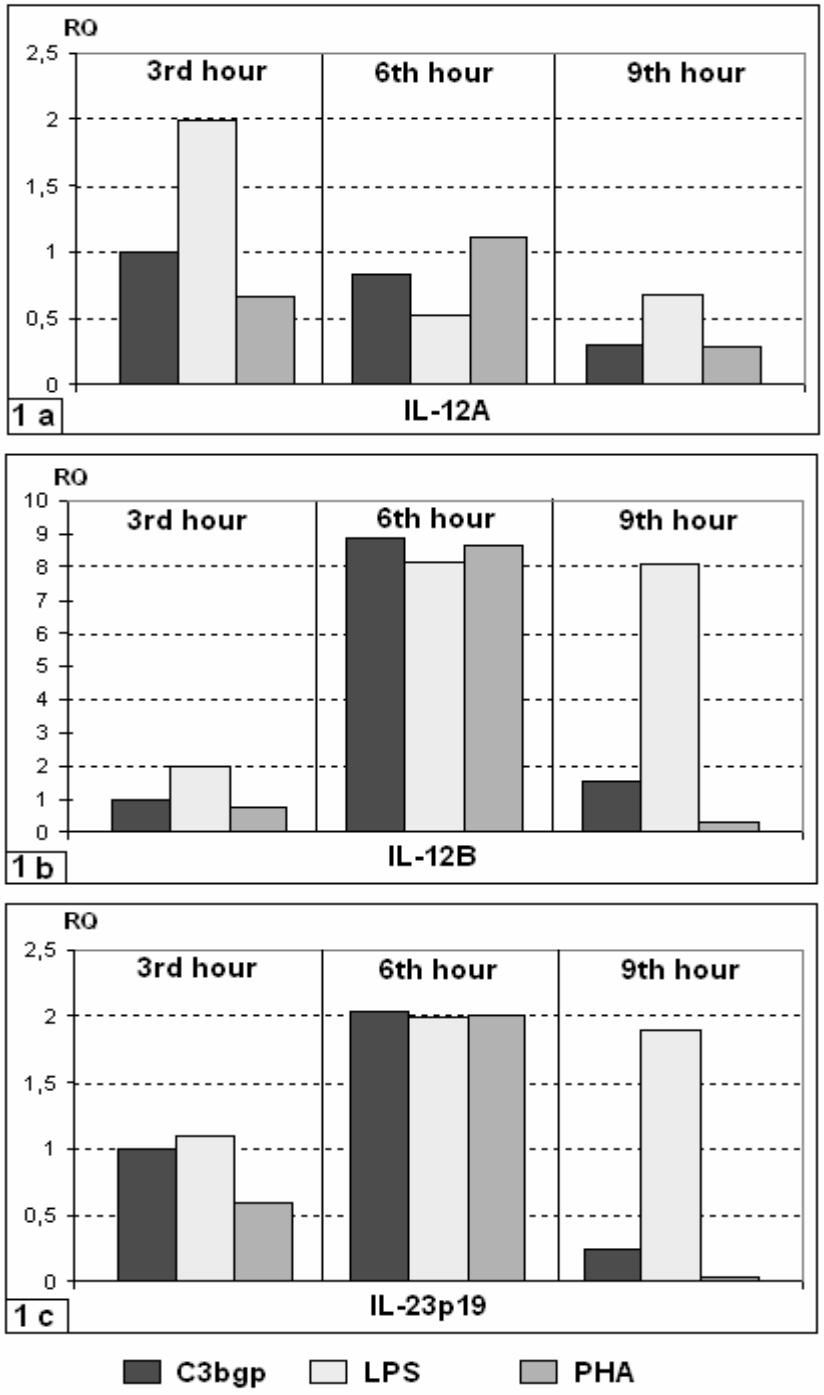

Figure 1. *Changes of IL-12A, IL-12B u IL-23 gene expression depends on hours of stimulation. The results are shown for 1 from 4 donors and were expressed in relative quantification units (RQ fold difference in comparison to non-stimulated cells), after normalization to endogenous control 18S rRNA*

DISSCUSION

Early in the antigen stimulation, phagocytic cells produced IL-12-related cytokines that provides an important function in mounting the protective or harmful Th1 adaptive immune response (4, 9). Human PBMC produced high level of IL-12 after stimulation with bacteria or bacterial product. The cells that produce the IL-12p70 heterodimer secrete

the monomer p40 at an excess of many folds above the heterodimer. Moreover, the individual differences in cytokine production due to the level of expression are well described. Obviously, the expression of *IL-12B*, *IL-12A* and *IL-23* genes as a result of intracellular signal pathway activation after stimulation is a precisely regulated process. One reason for different level of expression might be the promoter sequences of *IL-12B*, *IL-12A* and *IL-23* genes and their binding to activated transcription factors (6, 10).

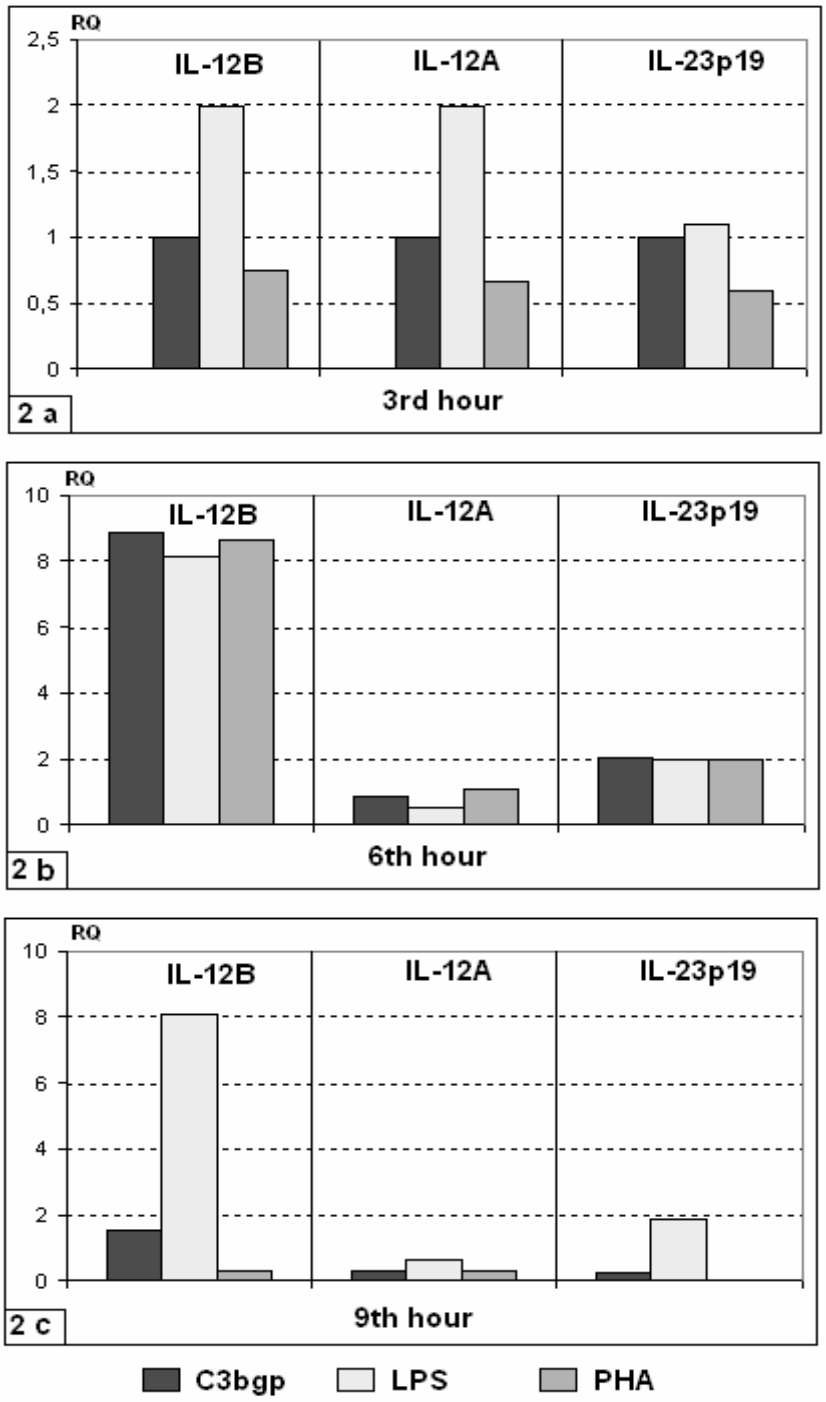

Figure 2. *Changes of IL-12A, IL-12B u IL-23 gene expression depends on stimuli used. The results are shown for 1 from 4 donors and were expressed in relative quantification units (RQ fold difference in comparison to non-stimulated cells), after normalization to endogenous control 18S rRNA*

The results from our study demonstrated that the dynamics of mRNA quantity, expressed by *IL-12B*, *IL-12A* and *IL-23* genes in human PBMC are dependent on stimuli used. Plant immunomodulators (C3bgp and PHA) induced higher and equivalent expression of the three genes at 6th hour stimulation, until LPS induced two-fold higher expression of *IL-12B* and *IL-12A* compared to IL-23 at 3rd hour. Moreover, LPS induced expression of *IL-12B* retained at higher level for 6th and 9th hour stimulation. These results for synthesis

of *IL-12A* mRNA after LPS stimulation are in some disagreement with the results from other authors that LPS stimulation leads to synthesis mainly of p40 subunit, but not p35. For higher production of p35 and IL-12p70 heterodimer from antigen presenting cells is through a combination of bacterial stimulus and IFN-γ (5). A proper explanation could be the existing post-transcriptional regulation of protein synthesis and mRNA stability as well as the target cells used. Similar results for the differences of *IL-12B*, *IL-12A* and *IL-23* expression in patients with ulcerative colitis and Cron'disease are reported by Schmidt et al., (11). We suppose that after stimulation the transduction of signal and consequence forming of complex of activated transcription factors is responsible for the level of gene transcription.

Our results demonstrated also that comparatively the expression of *IL-12B* is the highest, while the expression of *IL-12A* is lower in PBMC, independently of stimulus used. We assume that the observed differences in the expression of *IL-12B*, *IL-12A* and *IL-23* are responsible for dynamics of the production of IL-12p70, IL-12p80, and IL-23 cytokines after stimulation. Each of IL-12 related cytokines has specific immunological activity: while IL-12 and IL-23 as pro-inflammatory cytokines preferentially regulate the proliferation of Th1 cell clones and induced IFN- γ production, data for function of IL-12p80 homodimer show that it has an opposite function mainly as inductor of humoral response (7, 8, 12). Thus the secreted IL-12 related cytokine affected the development and magnitude of normal or pathological Th1 immune response.

REFERENCES

1. Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Ann. Rev. Immunol. 1995; 13: 251-256.
2. Heufler C, Koch F, Stanzl U, Topar G, Wysocka M, Trinchieri G, En A, Steinman RM, Romani N, Romani N, Shuler G: Interleukin-12 is produced by dendritic cells and mediates T helper 1 development as well as interferon-gamma production by T helper 1 cells. Eur J Immunol 1996; 26:659-68.
3. Trinchieri, G. Interleukin – 12 and the Regulation of Innate Resistance and Adaptive Immunity. *Nature Reviews/Immunology,* 3: 133 – 146, 2003.
4. Hölscher C. The power of combinatorial immunology: IL-12 and IL-12-related dimeric cytokines in infectious diseases. *Med. Microbiol. Immunol*. 2004; 193:1-17.
5. Ma X, Trinchieri. Regulation of IL-12 poduction in antigen-presenting cells. Adv. Immunol, 2001,79: 55-92.
6. Utsug, M, Dobashi K, Ishizuka T, Kawata T, Hisada T, Shimizu Y, Ono A and Mori M. Rac 1 negatively regulates lipopolysaccharide-induced IL-23p19 expression in human macrophages and dendritic cells and NF-κB p65 trans activation plays a novel role. J. Immunol. 2006; 177: 4550-4557.
7. Gutcher I and Becher B. APC-derived cytokines and T cell polarization in autoimmune inflammation. *J. Clin. Invest*. 2007; 117: 1119-1127.
8. Lankford RSC, and Fricht MD. A unique role for IL-23 in promoting cellular immunity. *J. Leukoc. Biol*. 2003; 73: 49-56.
9. Ma X-T, Zhang X-J, Zhang B, Geng Y-Q, Lin Y-M, Li G, Wu K-F. Expression and regulation of interleukin-23 subunits in human peripheral blood mononuclear cells and hematopoietic cell lines in response to various inducers. Cell Biol. Int. 2004; 28: 689-697.
10. Ma W, Gee K, Lim W, Chambers K, Angel JB, Kozlowski M, and Kumar A. Dexamethasone inhibits IL-12p40 production in lipopolysaccharide-stimulated human monocytic cells by down-regulating the activity of c-Jun N-terminal kinase, the Activation protein-1 and NF-κB transcription factors. J. Immunol. 2004; 172: 318-330.
11. Schmidt C, Giese T, Ludwig B, Mueller-Molaian I, Marth T, Zeuzem S, Meuer SC, Stallmach A. Expression of interleukin-12-related cytokine transcripts in inflammatory bowel disease: elevated interleukin-23p19 and interleukin-27, 28 in Crohn's disease but not in ulcerative colitis. Inflamm Bowel Dis. 2005, 1:16-23
12. Heinzel FP, Hujer AM, Ahmed FN and Rerko RM. In vivo production and function of IL-12p40 homodimers. *J. Immunol*. 1997; 158: 4381-4388.