CYTOKINE AND CHEMOKINE MRNA EXPRESSION DURING *IN VITRO* INFECTION OF PHAGOCYTIC CELLS – CURRENT STATE OF STUDY

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The aim of this work is to analyze the expression of eukaryotic genome of the phagocytic cell after infection with microorganism, in particular after infection with *Francisella tularensis*. Mouse macrophage cell line J774 is used in experiments as a model of phagocytic cell.

The first part of activities which should lead to reach the aim was selecting of suitable markers of the infection. The expression of these markers should be affected by the infection of phagocytes. We chose some cytokines, chemokines and also enzymes.

Table 1

The list of cytokines, chemokines and enzymes selected for characterization of infection-induced immune response. Symbol of the marker, description and alternate symbol were copied from NCBI database (http://www.ncbi.nlm.nih.gov/).

name of the marker	symbol of the marker (NCBI database)	description (NCBI database)	alternate symbol (NCBI database)
cytokines			
TNF-α	Tnf	tumor necrosis factor	DIF, Tnfa, TNFSF2, TNFalpha, TNF-alpha
IL-6	Il6	interleukin 6	Il-6
IL-12b	Il12b	interleukin 12b	p40, Il-12b, Il12p40, Il-12p40, IL-12 p 40
GM-CSF	Csf2	colony stimulating factor 2 (granulocyte-macrophage)	Csfgm, Gm-CSf
IL-1 β	Il1b	interleukin 1 beta	IL-1B, Il-1b, IL-1beta
IL-18	Il18	interleukin 18	Igif, Il-18
IL-10	Il10	interleukin 10	CSIF (cytokine synthesis inhibitory factor), Il-10
IFN-γ	Ifng	interferon gamma	Ifg, IFN-g, IFN-gamma
TGF-β	Tgfb1	transforming growth factor, beta 1	Tgfb, Tgfb-1, TGF-beta1
IL-13	Il13	interleukin 13	Il-13
chemokines			
MIP-1γ	Scya9	small inducible cytokine A9	CCF18, MRP-2, MIP-1 gamma
MIP-2	Scyb2	small inducible cytokine subfamily, member 2	Mip2, Scyb, MIP-2
MCP-5	Scya12	small inducible cytokine A12	MCP-5
RANTES	Scya5	small inducible cytokine A5	RANTES, MuRantes
enzymes			
iNOS	Nos2	nitric oxide synthase 2, inducible, macrophage	iNOS, Nos-2, NOS-II
p47 phox	Ncf1	neutrophil cytosolic factor 1	Ncf-1, p47phox
p22 phox	Cyba	cytochrome b-245, alpha polypeptide	NADPH oxidase subunit (47 kD)
gp91 phox	Cybb	cytochrome b-245, beta polypeptide	b558, p22 phox cytochrome beta-558

There are proinflammatory cytokines like TNF--α, IL-6, IL-12b, IL-1 β, IL-18 and GM-CSF, which are produced after activation of the macrophage (Vankayalapati et al. 2001, Golovliov et al. 1995, Yamamoto et al. 1994). There are also some supressory cytokines like TGF-β, IL-13 and IL-10 (Tripp et al. 1993) . Their production could be triggered after infection with pathogen to help him survive in the hostile environment of the phagocyte. We chose also MIP-1 γ, MIP-2, MCP-5, RANTES – chemokines, which are produced by macrophages after infection to attract other kinds of leukocytes like lymphocytes and neutrophils to the site of inflammation.The group of the enzymes contains markers which are connected with killing activity of macrophage after infection. Inducible nitric oxide synthase (iNOS) is responsible for the production of nitric oxide after infection (Fang 1997). Phoxes (p47, p22 and gp91) belong to the group of proteins which create the enzyme complex called NADPH oxidase. It catalyzes the production of superoxide. Both p22 and gp91 create transmembrane heterodimeric part of NADPH oxidase called cytochrome b558. Protein p47 is one of the cytosolic parts of NADPH oxidase (Babior 1999).

The first part of experiments was done with the noninfected cells to find if there is some constitutive expression. Mouse macrophage cell line J774 was cultivated in the Department of Clinical Immunology and Alergy. The cells were harvested in the 4, 10 and 15 passage.

The expression of the markers was characterized at the level of mRNA using RT-PCR method. The total RNA was isolated with the RNeasy Mini kit (Qiagen) from the samples of the cells. There were about 3.10^6 cells in each sample. The concentration and purity of RNA was measured in a spectrophotometer. Then 2 μg of RNA was reverse transcribed into cDNA. After that 5 μl of cDNA was put in PCR amplification with specific primers. The PCR products were loaded onto an 1,5% agarose gel, separated electrophoretically and stained with ethidium bromide.

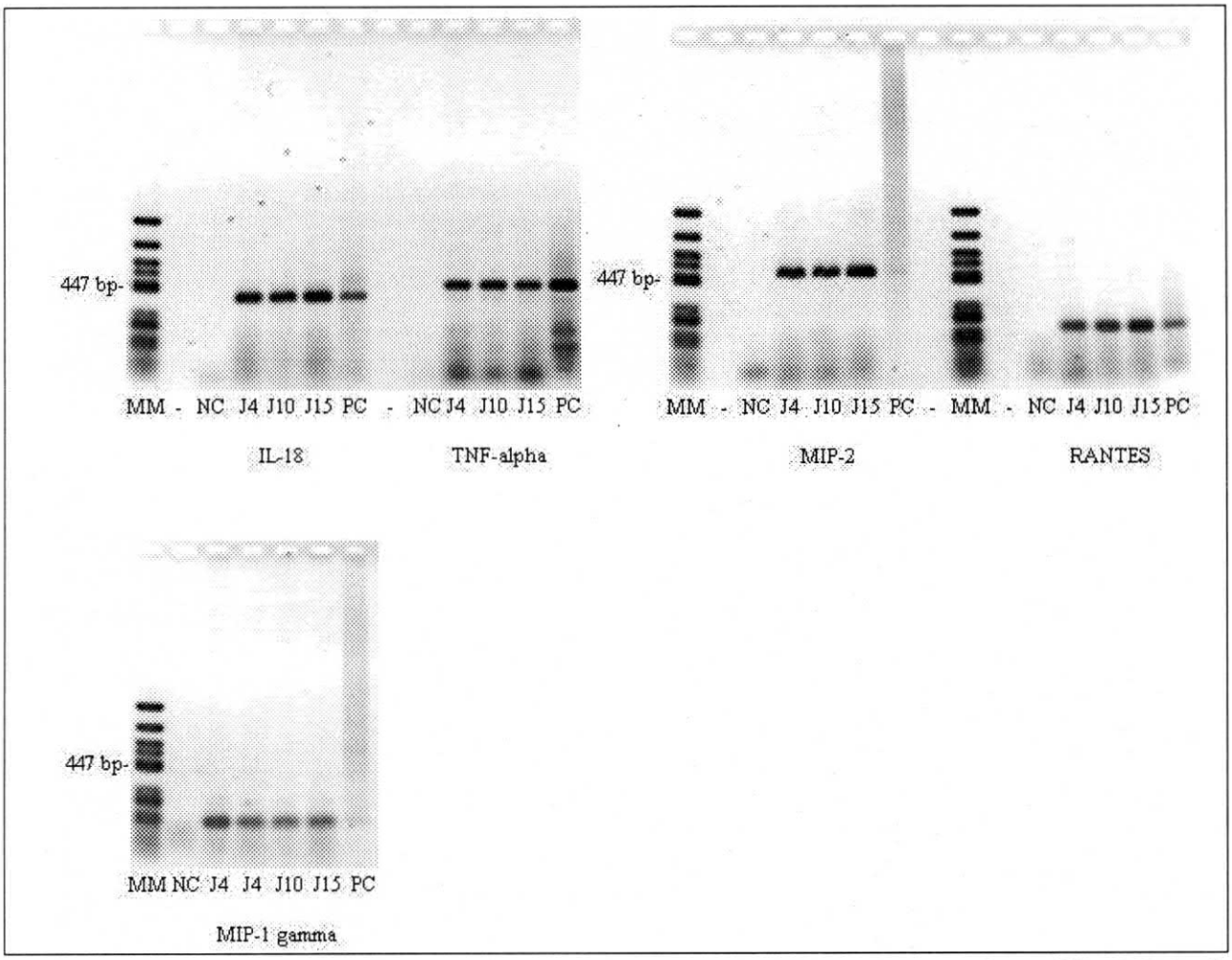

Figure 1. Expression of IL-18, TNF-alpha, MIP-2, RANTES and MIP-1 gamma mRNA in mouse macrophage cell line J774 - cells cultivated without infection. MM - molecular weight marker, NC - negative control, J4 - 4th passage, J10 - 10th passage, J15 - 15th passage, PC - positive control.

It seems that the results of the expression of the markers in noninfected cells can be divided into groups according to presence of the expression in different passages and the intensity of the expression in the passages. The expression of IL-18, TNF--alpha, MIP-2, RANTES and MIP-1 gamma were detected in all three passages. The intensity of the expression was similar in each passage.

The phoxes mRNA also seems to be expressed in all passages. The PCR amplification with p22 phox and gp91 phox gave more than one product.

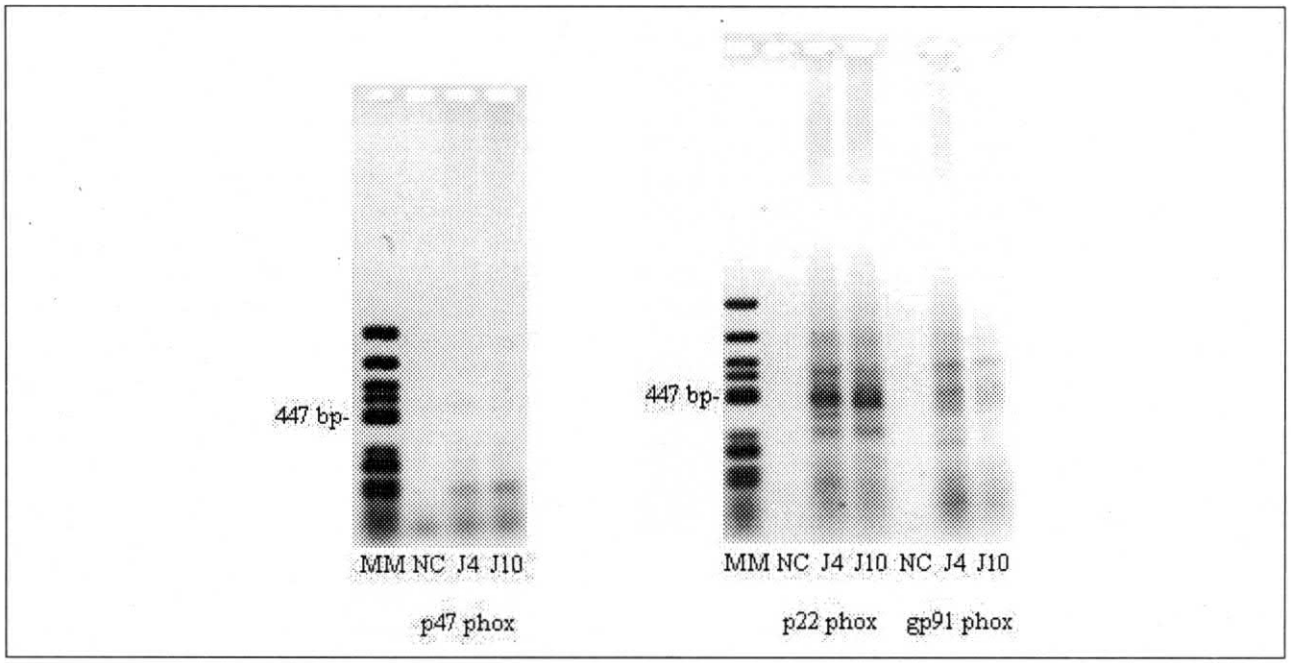

Figure 2. Expression of p47 phox, p22 phox and gp91 phox mRNA in mouse macrophage cell line J774 - cells cultivated without infection. MM - molecular weight marker, NC - negative control, J4 - 4th passage, J10 - 10th passage, PC - positive control.

If it can not be adjusted by optimalization of the conditions of the PCR reaction, another primers will have to be found. The second group contains markers which gave negative or different results among passages. I got such results with IL-12b, IL-6, GM-CSF, IL-10, iNOS, IL-1 beta and MCP-5. The intensity of their expression differ among passages or was detected only in some of the passages. All these experiments have not been finished yet. The results mentioned here are summarized in the Table 2.

In the next future is necessary to finish the experiments with the noninfected cells and examine the cells after infection with *Francisella tularensis*. It is probable that the infection can trigger expression of some markers, which gave negative results with noninfected cells. We are planning to exploit QRT--PCR technique to monitore the alterations in the expression during infection to catch even slight differences. We are also planning to approach cDNA arrays technique to be able to identify wider range of markers affected by infection.

Table 2

The summary of the recent RT-PCR results.

name of the marker	J774 – 4th passage	J774 – 10th passage	J774 – 15th passage
IL-18	+	+	+
TNF-alpha	+	+	+
MIP-2	+	+	+
RANTES	+	+	+
MIP-1 gamma	+	+	+
IL-12b	-	-	-
IL-6	-/-/-	-/+	+
GM-CSF	-/-	-/+	-
IL-10	+	+	+
iNOS	-	-	+
IL-1 beta	+	-	+
MCP-5	+	+	+

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