

# The Effect of Antibiotic-Resistant and Sensitive *Escherichia coli* on the Production of Pro-Inflammatory Cytokine Response by Human Peripheral Blood Mononuclear Cells

Galina V. Volodina<sup>1</sup>, Tigran K. Davtyan<sup>2\*</sup>, Murat E. Kulmanov<sup>1</sup>, Ardak B. Dzhumagazieva<sup>1</sup>, Sholpan K. Tursunova<sup>1</sup>, Assima O. Abekova<sup>1</sup>, Indira E. Bishimova<sup>1</sup>, Zhansaya S. Abramova<sup>1</sup>, Roza T. Kenzhebekova<sup>1</sup>, Sabina G. Murzageldinova<sup>1</sup>, Ilya S. Korotetskiy<sup>1</sup> and Alexander I. Ilin<sup>1</sup>

<sup>1</sup>Scientific Center for Anti-Infectious Drugs, 75B Al-Farabi Ave, Almaty, Kazakhstan

<sup>2</sup>Analytical Laboratory of the Scientific Center for Drug and Medical Technology Expertise. MH RA, 49/4 Komitas Ave, Yerevan, Armenia

## Abstract

**Purpose:** A comparative study of the effect of *Escherichia coli*, differing in gentamicin sensitivity, on the production of human pro-inflammatory cytokines IL-1 $\beta$ , IL-6, TNF- $\alpha$  by human peripheral blood mononuclear cells (PBMC) was carried out to determine the threshold for cytokine response induction.

**Methods:** The multiple drug resistant *E. coli* strain ATCC-VAA-196 and its derivatives: a gentamicin-resistant *E. coli* strain R, obtained by prolonged cultivation of bacteria in the presence of antibiotics, and a gentamicin-sensitive strain of *E. coli* Rev, obtained by prolonged cultivation in the presence of new drug FS-1, were selected for this study. To determine the cytokine minimum inducing concentration (cMIC) for live (carrying DAMPs) and formalin-fixed (exposing canonical PAMPs) *E. coli* strains, PBMC were co-cultured with different concentrations of bacteria, and the level of cytokine production was measured using an enzyme immunoassay.

**Results:** The PAMP- and DAMP-mediated differences in the threshold for induction of proinflammatory cytokines IL-1 $\beta$ , IL-6, TNF- $\alpha$  production by PBMC treated with bacteria that differ in the sensitivity phenotype were found to be corresponding. cMIC for the initial resistant *E. coli* strain BAA-196 and the gentamicin-resistant *E. coli* R strain (10<sup>3</sup> CFU/ml) was ten times lower than cMIC for the gentamicin-susceptible strain *E. coli* Rev (10<sup>4</sup> CFU/ml).

**Conclusion:** The ability of the gentamicin-sensitive strain to exceedingly increase the threshold for induction of pro-inflammatory cytokine response of PBMC in comparison with the resistant strains appears to be an immunological evidence for reversion of antibiotic resistance towards restoring sensitivity to gentamicin.

**Keywords:** Antibiotic resistant and sensitive strains of *Escherichia coli*; Threshold of induction of pro-inflammatory cytokine production; Drug FS-1

## Introduction

Drug resistance of microorganisms remains an urgent and global problem of modern medicine and microbiology [1]. The present-day antibiotic therapy and arsenal of existing courses of antimicrobial treatment for infectious diseases caused by pathogens with drug resistance are currently ineffective and promote the wide spread of resistance genes in the strains of microorganisms in both the human population and environment. The search and development of new approaches and a fundamentally new generation of drugs that could increase the sensitivity of bacteria to traditional antibiotics is a perspective and promising trend in the fight against drug resistance [2]. It was shown previously that the ionic nanostructured complex formed by proteins and carbohydrates, metal salts and iodine intercalated into them, which is a new drug FS-1 [3], is capable of inducing reversion to sensitivity or reversion of resistance to antibiotics during prolonged culturing with antibiotic-resistant bacteria [4].

It should also be noted that a comparative study of the effect of resistant and sensitive bacteria on the induction and regulation of the adaptive and innate immune response can open new prospects for developing more effective approaches to complex immunotherapy of infectious diseases caused by pathogens with drug resistance. It is known that the activation of the mechanisms involved in innate immunity and induction of the production of pro-inflammatory cytokines IL-1 $\beta$ , IL-6, TNF- $\alpha$  is caused by interaction of both PAMPs (Pathogen-Associated Molecular Patterns), a complex of canonical, evolutionarily conservative microbial molecules, associated with antigenicity, and DAMPs (Danger-Associated Molecular Patterns), an

integral complex of canonical, evolutionarily conservative microbial molecules associated with antigenicity, as well as pathogen-associated or virulence-associated molecules, with pattern recognition receptors PRRs (Pattern Recognition Receptors) on cells of the innate immune system, resulting in inducing a protective inflammatory response for effective control of infections [5-7].

The purpose of this study was to compare the effect of *Escherichia coli* bacteria, differing in sensitivity to gentamicin, on the production of proinflammatory cytokines IL-1 $\beta$ , IL-6, TNF- $\alpha$  by human peripheral blood mononuclear cells and to determine the changes in the induction threshold of the cytokine response.

## Material and Methods

### Bacterial strains and cultivation

The following strains were used in the study: a) the resistant *Escherichia coli* ATCC-BAA-196 J53 pMG223p (ATCC) strain with

**\*Corresponding author:** Tigran K. Davtyan, Analytical Laboratory of the Scientific Center for Drug and Medical Technology Expertise. MH RA, 49/4 Komitas Ave, Yerevan, Armenia, Tel: +374 10 23-72-61; Fax: +374 10 23-72-61; E-mail: [tigdav@excite.com](mailto:tigdav@excite.com)

**Received:** August 08, 2017; **Accepted:** September 13, 2017; **Published:** September 25, 2017

**Citation:** Volodina GV, Davtyan TK, Kulmanov ME, Dzhumagazieva AB, Tursunova SK, et al. (2017) The Effect of Antibiotic-Resistant and Sensitive *Escherichia coli* on the Production of Pro-Inflammatory Cytokine Response by Human Peripheral Blood Mononuclear Cells. J Clin Cell Immunol 8: 522. doi: [10.4172/2155-9899.1000522](https://doi.org/10.4172/2155-9899.1000522)

**Copyright:** © 2017 Volodina GV, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

multiple drug resistance; b) the resistant *Escherichia coli* subculture (*E. coli* R) obtained by culturing *E. coli* ATCC-BAA-196 strain for 50 generations in a medium with ampicillin and gentamicin at a concentration of 40 µg/ml, which retained resistance to antibiotics; c) the gentamicin-sensitive *Escherichia coli* subculture (*E. coli* Rev) obtained under experimental conditions by culturing *E. coli* ATCC-BAA-196 strain for 50 generations in a medium with a constant content of the drug FS-1 at a<sup>1/2</sup> concentration of the minimum bactericidal concentration MBC (500 µg/ml).

For long-term serial cultivation of *E. coli* in the presence of antibiotics and FS-1,  $1.5 \times 10^6$  CFU/mL of cells in the Mueller Hinton broth (Sigma Chemical Co., St. Louis, MO) were incubated into separate tubes with total volume of 1.0 ml containing 40 µg/ml concentration of ampicillin and gentamicin or 250 µg/ml concentration of FS-1 and incubated at  $37 \pm 1^\circ\text{C}$  for 24 h for obtaining one generation. The selected concentrations for antibiotics and FS-1 represented the 1/50 and 1/2 concentrations of MBC respectively, which caused no cytotoxicity or growth suppression of initial *E. coli* ATCC-BAA-196 J53 pMG223p (ATCC) strain. The bacterial strains were propagated in the presence of selecting drugs for 50 generations and every fifth generation of each strain was tested for morphology, enzymes profiling and MIC value and cell growth in solid agar plates at  $37 \pm 1^\circ\text{C}$  for 24 h were estimated.

The bacteria were cultured in a solid nutrient medium until the middle of the log phase of growth at the temperature of  $37 \pm 1^\circ\text{C}$ . The bacteria were fixed with 37% formalin at  $37 \pm 1^\circ\text{C}$  for 20 min, followed by triple washing with PBS (phosphate-buffered saline, pH 7.2) by centrifuging for 10 min at 3,000 rpm. In the control experiments the survival of the formalin-fixed cultures was monitored. The concentrations of live and fixed bacteria from  $10^1$  CFU/ml to  $10^7$  CFU/ml were used for the co-cultivation with PMBC.

The determination of MBC and minimum inhibitory concentration (MIC) of antibiotics against the strains under study was carried out by the serial dilution method [8]. The antibiotic sensitivity of the *E. coli* Rev strain was evaluated using the disc diffusion method according to recommendations and requirements of CLSI (Performance Standards for Antimicrobial Disc Susceptibility Tests) [9].

### PBMC isolation and activation

Heparinized peripheral blood samples were obtained from 8 (4 male, 4 female) age-matched normal donors. Peripheral blood mononuclear cells (PBM) were separated from heparinized whole blood by Ficoll-Hypaque (histopaque) (Sigma Chemical Co., St. Louis, MO) density gradient centrifugation. Polyglucin (6% dextran) was added to the blood to precipitate the red blood cells. The supernatant was washed, RPMI-1640 re-suspended, and fractionated by the density gradient corresponding to the floating density of human PBMC ( $\rho=1.076$  g/ml), at  $4^\circ\text{C}$  and 3,000 rpm for 20 min. The PBMC fraction was washed by centrifugation and re-suspended in the RPMI-1640 culture medium containing 10% FCS, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin and 0.025 µg/mL amphotericin B (Sigma, USA). The percentage of viable cells was assessed by the trypan blue exclusion (Sigma, USA). Cell suspensions with viability, greater than 90%, were used in the experiments.

### Determination of cytokine minimum inducing concentration (cMIC)

As the cMIC, a lowest concentration of bacteria was taken that was able to induce statistically significant cytokine production in the threshold level above 25-30% uncertainty range of the un-stimulated PBMC negative control level [10,11]. To determine cMIC, the PBMC suspension was

dispensed into the 96-well flat-bottom plates (BD Falcon, USA) at the concentration of  $1 \times 10^5$  cells per well, and an appropriate *E. coli* strain was added at concentrations from  $10^1$  to  $10^7$  CFU/ml in a volume of 100 µl. Physiological saline was used as a control. The bacteria and PBMC were co-cultured during 4 h or 12 h incubation at  $37^\circ\text{C}$  in an atmosphere containing 5%  $\text{CO}_2$ . At the end of the incubation, the plates were centrifuged at 300-500 g for 10-15 min, and the culture supernatant was collected. Quantitative analysis of cytokines was carried out using the commercial reagent kits IL-1 beta-IFA-BEST, IL-6-IFA-BEST, alpha-TNF-IFA-BEST (Vector-Best, Novosibirsk, Russia). Optical density measurement and calculation of cytokine concentrations were performed on the Sunrise RC.4 microtiter plate reader (Tecan, Austria) with the Magelan 2.0 software (Tecan, Austria).

### Statistics

The method of dispersion analysis with parametric and non-parametric procedures was used in this study. The data reliability was assessed with the GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA), using the one-way ANOVA and unpaired t-test. The values of  $P>0.05$  were considered insignificant.

### Results

#### Antibiotic resistance of selected bacterial strains

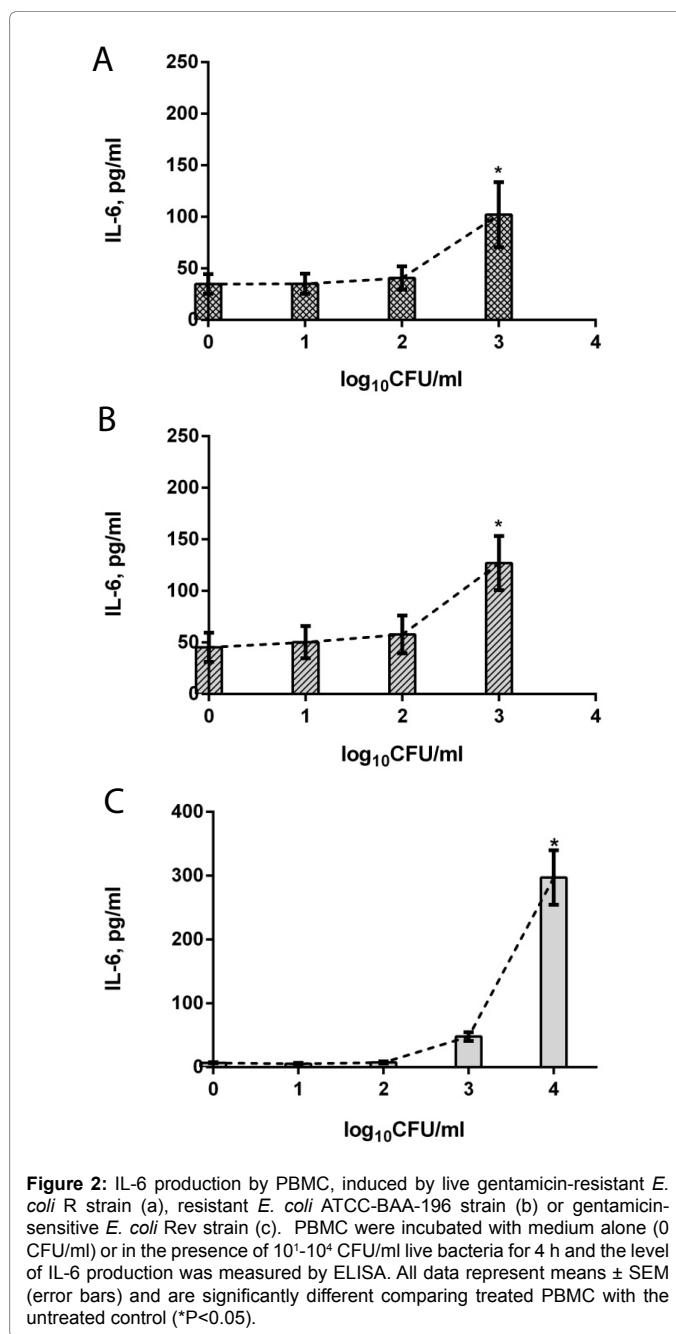
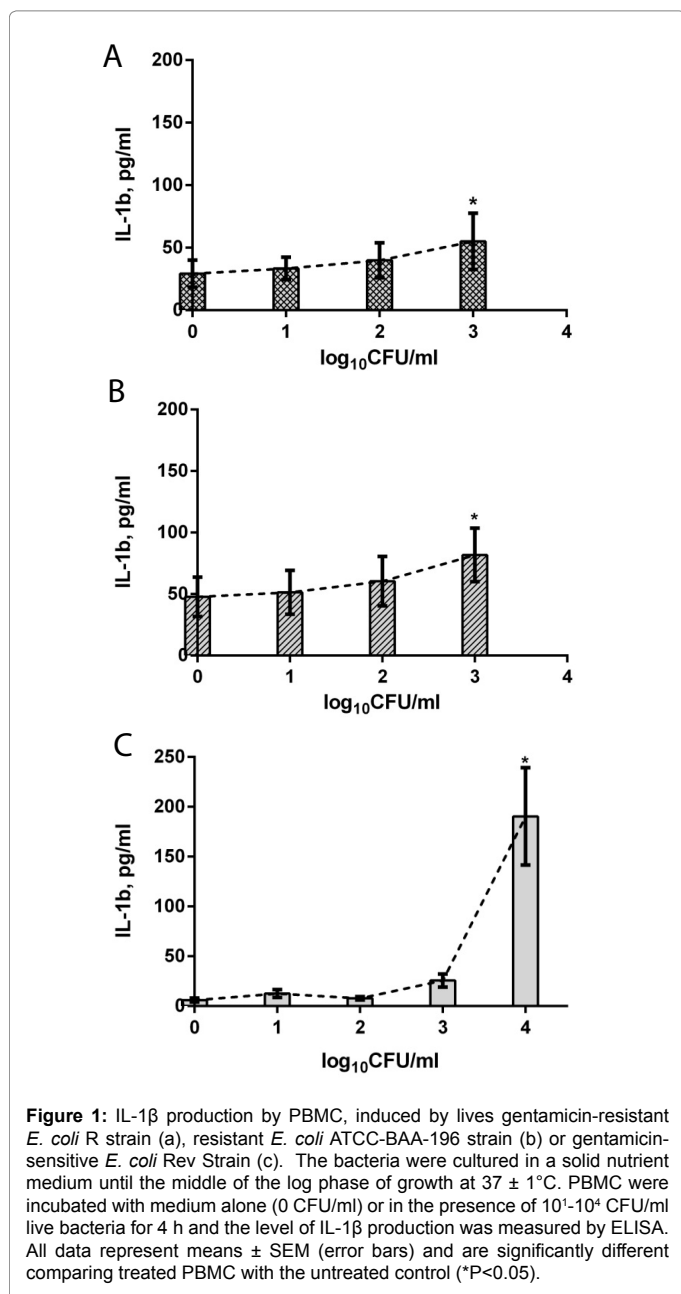
In this study, the *Escherichia coli* ATCC-BAA-196 strains were used which differ in their sensitivity to gentamicin: the resistant *E. coli* R subculture obtained by culturing the stock *E. coli* ATCC-BAA-196 strain for 50 generations in a medium with ampicillin and gentamycin, and the gentamicin-sensitive *E. coli* Rev subculture obtained by culturing the *E. coli* ATCC-BAA-196 strain for 50 generations in a medium with a constant content of the drug FS-1. The results from the MIC studies showed that for *E. coli* ATCC-BAA-196 the MBC of gentamicin was  $>2000$  µg/ml, and MIC of gentamicin was equal to 1800 µg/ml. With respect to the sensitive *E. coli* Rev strain, there was a decrease in the MBC for gentamicin to 950 µg/ml and a decrease in MIC for gentamicin to 800 µg/ml. The gentamicin MBC for *E. coli* R strain was equal to 2000 µg/ml and MIC to 1700 µg/ml respectively. These data indicates the acquisition of the sensitivity to gentamicin in *E. coli* Rev. The reversion to antibiotic sensitivity in the *E. coli* Rev strain was also determined by the disc-diffusion method, according to which the growth retardation zone to the ampicillin increased up to 15 mm and to ceftriaxone-up to 21 mm. Alongside with that, the growth retardation zone of *E. coli* R to the ampicillin was 10 mm, to ceftriaxone-17 mm.

#### DAMP-mediated differences in cytokines production by PBMC

To assess the threshold for induction of cytokine production as a result of the exposure to DAMPs, we have selected a model for co-cultivation of PBMC with live *E. coli* Rev and *E. coli* R. First, in preliminary experiments we calibrated the live bacteria-PBMC co-cultivation system by studying the time-dependence of cytokine response and found the best-fit linearity of response during 4 h incubation time. Dose-dependence preliminary studies revealed that IL-1 $\beta$ , IL-6 and TNF- $\alpha$  production by PBMC induced by live *E. coli* Rev at the concentration of  $10^1$ - $10^4$  CFU/ml had an exponential character; and at concentrations of bacteria  $10^5$  CFU/ml and higher, the production of cytokines reached to a plateau (data not shown). At  $10^1$ - $10^3$  CFU/ml concentration stock *E. coli* BAA-196 and resistant *E. coli* R strains exponentially increased the production of cytokines by PBMC, whereas at  $10^4$  CFU/ml and higher bacterial load caused starvation of PBMC cytokine response.

We observed that co-cultivation of stock *E. coli* BAA-196 strain and the gentamicin-resistant *E. coli* R strain in concentrations of  $10^1$ - $10^3$  CFU/ml with PBMC for 4 h caused a dose-dependent increase in IL-1 $\beta$ , IL-6, TNF- $\alpha$  production. As it shown on figures 1, 2 and 3, the DAMP-mediated cytokine response induced antibiotic-resistant bacteria characterized by high similarity. From the data presented in Figure 1, it follows that the resistant *E. coli* R strain and the stock *E. coli* ATCC-BAA-196 strain in concentrations of  $10^1$ - $10^2$  CFU/ml induced the production of IL-1 $\beta$  at the un-stimulated control level (0 CFU/ml). When the concentration of both *E. coli* R and *E. coli* ATCC-BAA-196 strains was raised up to  $10^3$  CFU/ml, the induction of IL-1 $\beta$  production increased ( $P < 0.05$ ) as compared to the un-stimulated PBMC (Figures 1a and 1b).

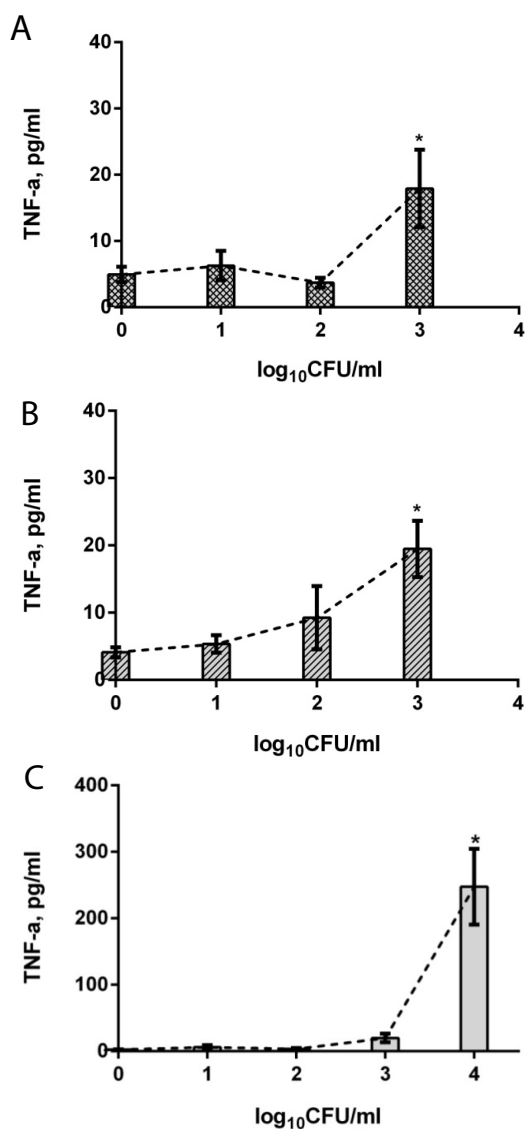
In contrast to the tested resistant strains, the gentamicin-susceptible *E. coli* Rev strain in concentrations of  $10^1$ - $10^3$  CFU/ml induced low production of IL-1 $\beta$ , which did not differ from the un-stimulated



control level (Figure 1c), however, *E. coli* Rev at the concentration of  $10^4$  CFU/ml induced an increase ( $P < 0.05$ ) in the level of IL-1 $\beta$  production relative to the untreated control (0 CFU/ml).

The production of IL-6 by PBMC as a result of the action of resistant strains *E. coli* R and the *E. coli* ATCC-BAA-196 at concentrations of  $10^1$ - $10^2$  CFU/ml was insignificant and did not differ from the untreated control level. With an increase in the concentration of *E. coli* R to  $10^3$  CFU/ml, increase in IL-6 production was observed in comparison with the control level ( $P < 0.05$ ). The strain *E. coli* ATCC-BAA-196 at the same concentration also induced ( $P < 0.05$ ) production of IL-6 (Figures 2a and 2b).

The gentamicin-sensitive *E. coli* Rev strain at concentrations of  $10^1$ - $10^2$  CFU/ml induced IL-6 production at the untreated control level; a slight and insignificant increase was observed at the concentration of  $10^3$  CFU/ml



**Figure 3:** TNF- $\alpha$  production by PBMC, induced by live gentamicin-resistant *E. coli* R strain (a), resistant *E. coli* ATCC-BAA-196 strain (b) or gentamicin-sensitive *E. coli* Rev strain (c). PBMC were incubated with medium alone (0 CFU/ml) or in the presence of  $10^1$ - $10^4$  CFU/ml live bacteria for 4 h and the level of TNF- $\alpha$  production was measured by ELISA. All data represent means  $\pm$  SEM (error bars) and are significantly different comparing treated PBMC with the untreated control (\* $P$ <0.05).

ml, and significant IL-6 production was recorded at the concentration of  $10^4$  CFU/ml ( $P$ <0.05) in relation to the untreated control level (Figure 2c).

As it follows from the Figure 3, the production of TNF- $\alpha$  in response to resistant *E. coli* R and *E. coli* ATCC-BAA-196 strains at concentrations of  $10^1$ - $10^3$  CFU/ml was insignificantly different from the untreated control level. Both, *E. coli* R and *E. coli* ATCC-BAA-196 at the concentration of  $10^3$  CFU/ml increased TNF- $\alpha$  production compared to the control level ( $P$ <0.05). The gentamicin-sensitive *E. coli* Rev strain at low concentrations of  $10^1$ - $10^3$  CFU/ml induced a baseline production of cytokine TNF- $\alpha$  at the control level, and significant induction of TNF- $\alpha$  ( $P$ <0.05) took place at  $10^4$  CFU/ml (Figure 3).

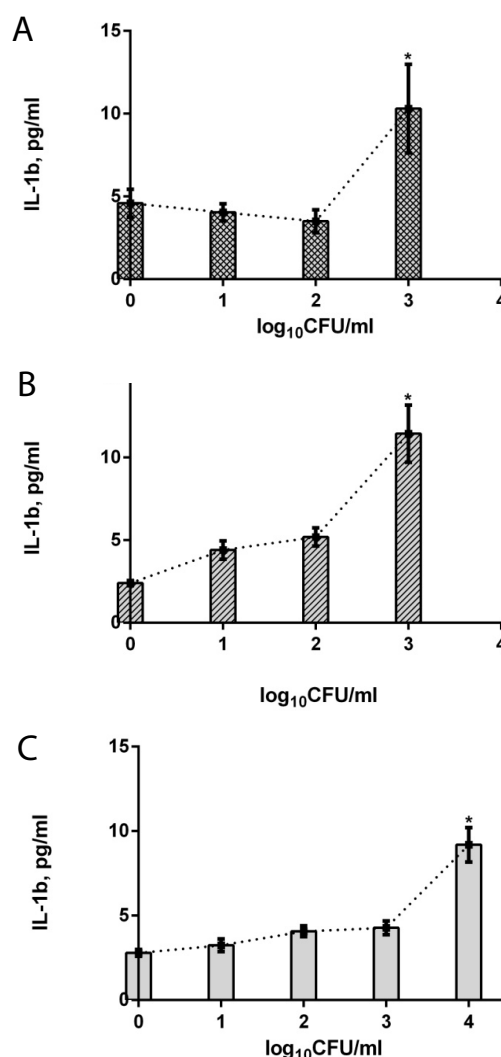
Thus, the obtained results revealed a DAMP-mediated difference in the induction threshold of IL-1 $\beta$ , IL-6, TNF- $\alpha$  production by PMBC

by live *E. coli*, differing in the gentamicin sensitivity phenotype. The cMIC of the resistant *E. coli* BAA-196 and the gentamicin resistant *E. coli* R strain ( $10^3$  CFU/ml) was at least ten times lower than cMIC of the gentamicin-sensitive *E. coli* Rev strain ( $10^4$  CFU/ml).

### PAMP-mediated differences in cytokines production by PBMC

To assess the induction threshold of cytokine production as a result of the exposure to PAMPs, we next selected a model for co-cultivation of PBMC with the formalin-fixed bacteria *E. coli* Rev and *E. coli* R and studied how *E. coli* strains induced cytokine production by PBMC.

IL-1 $\beta$  cytokine production in response to resistant *E. coli* R (Figure 4a) and *E. coli* ATCC-BAA-196 (Figure 4b) strains at concentrations



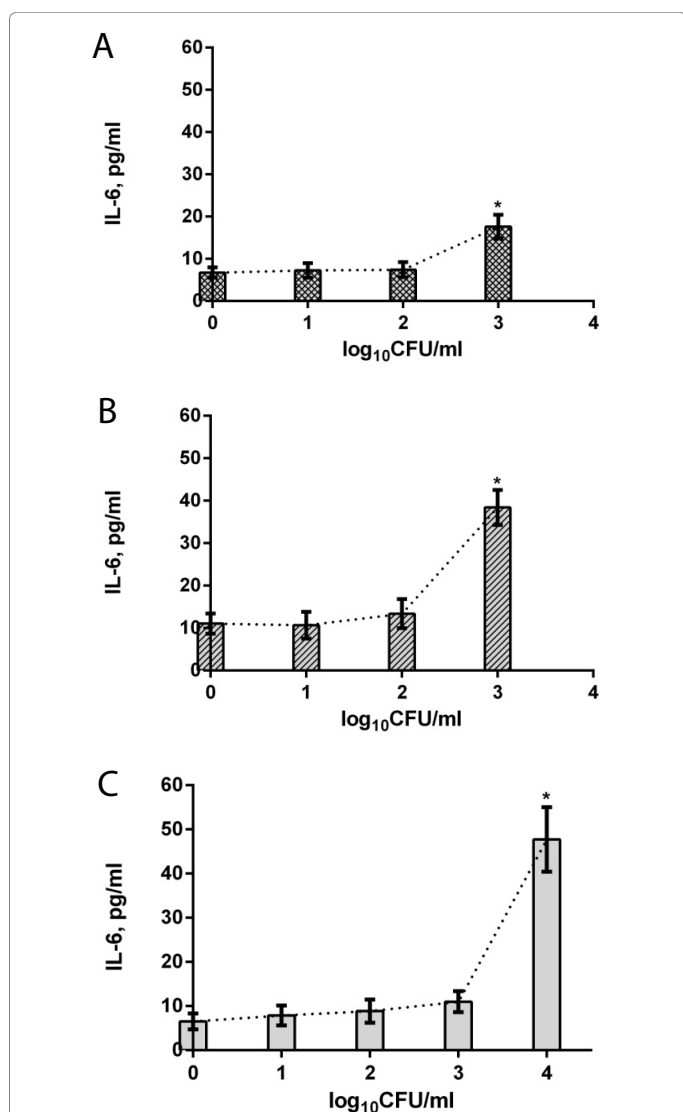
**Figure 4:** IL-1 $\beta$  production by PBMC, induced by formalin-fixed gentamicin-resistant *E. coli* R strain (a), resistant *E. coli* ATCC-BAA-196 strain (b) or gentamicin-sensitive *E. coli* Rev strain (c). The bacteria were cultured in a solid nutrient medium until the middle of the log phase of growth and were fixed with 37% formalin at  $37 \pm 1^\circ\text{C}$  for 20 min, followed by triple washing with PBS. PBMC were incubated with medium alone (0 CFU/ml) or in the presence of  $10^1$ - $10^4$  CFU/ml formalin-fixed bacteria for 4 hours and the level of IL-1 $\beta$  production was measured by ELISA. All data represent means  $\pm$  SEM (error bars) and are significantly different comparing treated PBMC with the untreated control (\* $P$ <0.05).

of  $10^1$ - $10^3$  CFU/ml was insignificant relative to the untreated control level. Fixed *E. coli* R and stock *E. coli* ATCC-BAA-196 strains at the concentration of  $10^3$  CFU/ml induced increase in IL-1 $\beta$  production ( $P < 0.05$ ). In contrast, the gentamicin-sensitive *E. coli* Rev strain (Figure 4c) induced IL-1 $\beta$  production at the control level at concentrations of  $10^1$ - $10^3$  CFU/ml, and only at the concentration of  $10^4$  CFU/ml caused significant increase in IL-1 $\beta$  ( $P < 0.05$ ).

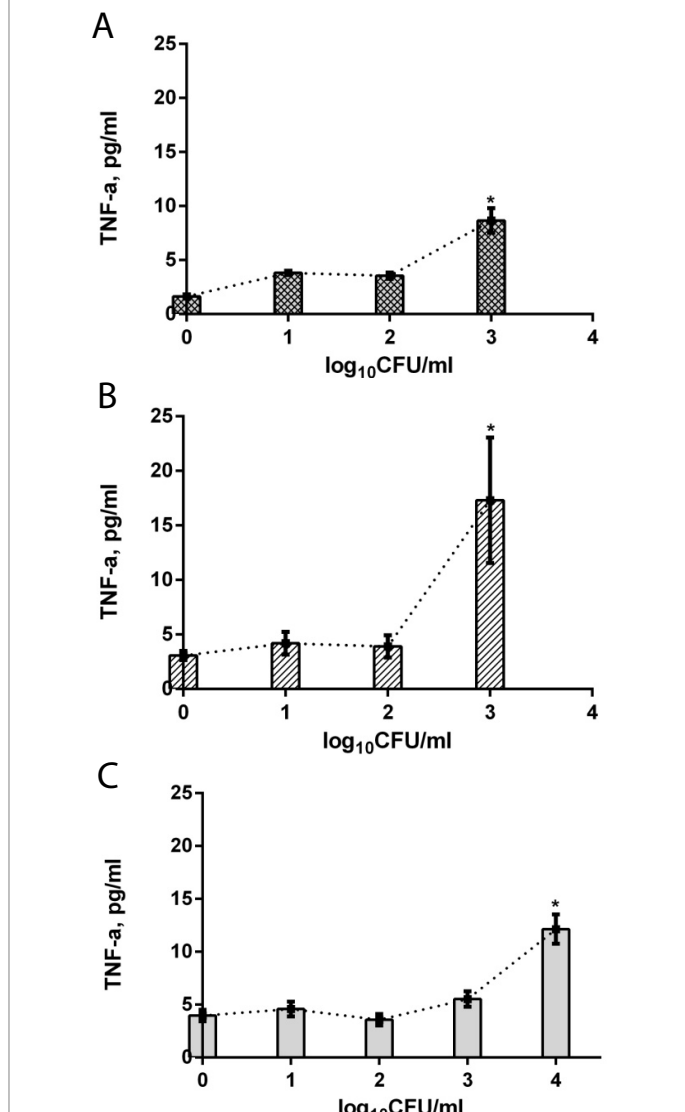
Co-cultivation of PBMC with resistant *E. coli* R (Figure 5a) and *E. coli* ATCC-BAA-196 (Figure 5b) strains at concentrations of  $10^1$ - $10^2$  CFU/ml induced IL-6 production at the control level. At the concentration of  $10^3$  CFU/ml, the *E. coli* R strain and stock resistant *E. coli* ATCC-BAA-196 strain induced increase in IL-6 production ( $P < 0.05$ ). In comparison with this, the *E. coli* Rev strain (Figure 5c) induced IL-6 production at the untreated control level at concentrations of  $10^1$ - $10^3$

CFU/ml, and at a concentration of  $10^4$  CFU/ml caused significant increase in IL-6 production ( $P < 0.05$ ).

As for TNF- $\alpha$  production, the same tendency was observed. Low concentrations of  $10^1$ - $10^2$  CFU/ml of the resistant *E. coli* R (Figure 6a) and stock resistant *E. coli* ATCC-BAA-196 (Figure 6b) strains induced insignificant production of TNF- $\alpha$  relative to the control level. The increase in the induction of TNF- $\alpha$  production ( $P < 0.05$ ) was observed in *E. coli* R at the concentration of  $10^3$  CFU/ml, the stock resistant *E. coli* ATCC-BAA-196 strain at the same concentration also induced ( $P < 0.05$ ) production of TNF- $\alpha$  (Figures 6a and 6b). *E. coli* Rev strain (Figure 6c) at concentrations of  $10^1$ - $10^3$  CFU/ml induced a slight production of TNF- $\alpha$  relative to the untreated control level, while at the concentration of  $10^4$  CFU/ml it caused the significant increase in level of TNF- $\alpha$  production ( $P < 0.05$ ).



**Figure 5:** IL-6 production by PBMC, induced by formalin-fixed gentamicin-resistant *E. coli* R strain (a), resistant *E. coli* ATCC-BAA-196 strain (b) or gentamicin-sensitive *E. coli* Rev strain (c). PBMC were incubated with medium alone (0 CFU/ml) or in the presence of  $10^1$ - $10^4$  CFU/ml formalin-fixed bacteria for 4 h and the level of IL-6 production was measured by ELISA. All data represent means  $\pm$  SEM (error bars) and are significantly different comparing treated PBMC with the untreated control (\* $P < 0.05$ ).



**Figure 6:** TNF- $\alpha$  production by PBMC, induced by formalin-fixed gentamicin-resistant *E. coli* R strain (a), resistant *E. coli* ATCC-BAA-196 strain (b) or gentamicin-sensitive *E. coli* Rev Strain (c). PBMC were incubated with medium alone (0 CFU/ml) or in the presence of  $10^1$ - $10^4$  CFU/ml formalin-fixed bacteria for 4 h and the level of TNF- $\alpha$  production was measured by ELISA. All data represent means  $\pm$  SEM (error bars) and are significantly different comparing treated PBMC with the untreated control (\* $P < 0.05$ ).

Thereby, the results of the present studies revealed similar differences between the formalin-fixed resistant and gentamicin-susceptible *E. coli* strains in relation to the induction threshold of cytokine production in response to the evolutionarily conservative molecular structures of bacteria. The cMIC of the formalin-fixed resistant *E. coli* BAA-196 and *E. coli* R strains for IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , as well as for the alive strains, was equal to 10<sup>3</sup> CFU/ml, and the cMIC of the fixed revertant *E. coli* Rev strain, as well as for the live strain, reached 10<sup>4</sup> CFU/ml.

## Discussion

The data we have obtained due to comparability of PAMP- and DAMP-mediated differences in the threshold of induction of pro-inflammatory cytokine production by PBMC seem to reflect the similarity or unidirectionality of a complex of biochemical, morphological or functional changes and coordinated expression of bacterial genes during the acquisition of antibiotic sensitivity or the formation of a reversion phenotype under the effect of a new iodine nanostructured complex FS-1. As a result of prolonged interaction of FS-1 with bacteria, a “new or acquired” phenotype is formed, which probably, due to changes in genomics, proteomics, and metabolomics of bacteria, leads to both a conjugated change in the expression and architectonics of immunogenic molecular structures and reversion to antibiotic sensitivity.

It is interesting to note that bacterial PAMPs and DAMPs differ from each other only in the availability of “additional” pathogen-associated or virulence-associated molecules (DAMP), while evolutionarily conservative molecular structures of microorganisms (PAMP) are common among *E. coli* strains in whole [12-14]. This is evidenced by the data of our comparative statistical analysis of the production level of pro-inflammatory cytokines IL-1 $\beta$ , IL-6 and TNF- $\alpha$  by PBMC stimulated with formalin-fixed and live *E. coli* ATCC-BAA-196, *E. coli* R, and *E. coli* Rev. Accordingly, the production of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  by PBMC stimulated with formalin-fixed *E. coli* ATCC-BAA-196, *E. coli* R, and *E. coli* Rev was significantly ( $P < 0.0005$ ) up to ten times lower as compared with live bacteria of the same strains, which indicates the invariance in functioning of the pattern-recognition receptors (PRR) in this experimental system. Consequently, the decrease in the potential of the immune system pro-inflammatory reaction in response to the sensitive strain of bacteria is associated with changes in the immunogenicity of both virulence-associate molecules and immunogenic factors but is not a consequence of a change in the interactions of PAMPs and DAMPs with recognizing PRR receptors in the cells of the immune system.

Summarizing the foregoing, it can be concluded that we have identified comparable PAMP- and DAMP-mediated differences in the inductance of the production of pro-inflammatory cytokines IL-1 $\beta$ , IL-6, TNF- $\alpha$  by PBMC when interacting with bacteria that differ in the sensitivity phenotype. Inflammation is a defence response of immune system to hazardous stimuli such as infections or tissue injury. However, prolonged or uncontrolled inflammation is the main cause of healthy tissue destruction and chronic pathologies. An increase in the immune system sensitivity associated with a decrease in the

threshold for induction of the pro-inflammatory reaction in response to resistant strains of bacteria can be considered as the immune system compensatory response to the changed fitness cost of the bacterial population compared to the population of the susceptible strain. Therefore, the extent of increase in the threshold for cytokine response induction by an integrated complex of canonical, evolutionarily conservative, associated with antigenicity, as well as pathogen-associated or virulence-associated molecules, indirectly determined by the cMIC value, may serve as an immunological marker of antibiotic resistance reversion.

The data we have obtained provide evidence that when a resistant stock *E. coli* BAA-196 strain was exposed to a drug FS-1, the gentamicin-sensitive strain was obtained with the microbiologically confirmed property to reverse antibiotic sensitivity. The ability of the sensitive strain to increase the threshold for pro-inflammatory cytokine induction relative to that of resistant strains appears to be an immunological evidence of resistance reversion towards reversing sensitivity to antibiotics.

## References

1. The Lancet Infectious Diseases Commission. Antibiotic resistance-the need for global solutions, 2013.
2. Hancock RE, Nijnik A, Philpott DJ (2012) Modulating immunity as a therapy for bacterial infections. *Nat Rev Microbiol* 10: 243-254.
3. Ilin AI, Kulmanov ME (2014) Antibacterial agent for treating infectious diseases of bacterial origin. Patent US20140010782 A1-2014-09-01.
4. Ilin AI, Kulmanov ME, Korotetskiy IS, Islamov RA, Akhmetova GK, et al. (2017) Genomic insight into mechanisms of reversion of antibiotic resistance in multidrug resistant *Mycobacterium tuberculosis* induced by a nanomolecular iodine-containing complex FS-1. *Front Cell Infect Microbiol* 7:151.
5. Medzhitov R (2007) Recognition of microorganisms and activation of the immune response. *Nature* 449: 819-826.
6. Ruslan Medzhitov (2007) Recognition of microorganisms and activation of the immune response. *Nature* 449: 819-826.
7. Blander JM, Sander LE (2012) Beyond pattern recognition: five immune checkpoints for scaling the microbial threat. *Nature Rev Immunology* 12: 215-225.
8. Akira S1, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. *Cell* 124: 783-801.
9. M07-A9 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Ninth Edition, 32: 88; 2012.
10. M02-A11 Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eleventh Edition, 32: 76, 2012.
11. Hahn CL, Best AM, Tew JG (2000) Cytokine induction by *Streptococcus mutans* and pulpal pathogenesis. *Infect Immun* 68: 6785-6789.
12. Hahn CL, Best AM, Tew JG (2000) Cytokine induction by *Streptococcus mutans* and pulpal pathogenesis. *Infect Immun* 68: 6785-6789.
13. Elson G, Dunn-Siegrist I, Daubeuf B, Pugin J (2007) Contribution of Toll-like receptors to the innate immune response to Gram-negative and Gram-positive bacteria. *Blood. Immunol* 109: 1574-1583.
14. Tang D, Kang R, Coyne CB, Zeh HJ, Lotze MT (2012) PAMPs and DAMPs: signal 0s that spur autophagy and immunity. *Rev* 249: 158-175.