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S.S. Kassymbekova*, T.A. Bukeyeva, I.E. Bishimova,
S.G. Murzageldinova, I.S. Korotetskiy

Scientific Center for Anti-Infectious Drugs, Kazakhstan, Almaty

*e-mail: s_kassymbekova@mail.ru

DIFFERENTIAL CYTOKINE RESPONSE OF IMMUNOCOMPETENT CELLS TO THE INDUCTION OF RESISTANT, REVERTANT AND SENSITIVE *ESCHERICHIA COLI*

The work is devoted to the study of the influence of the resistance phenotype of E. coli subcultures on the functionality of evolutionarily conserved pathogen-associated antigens (PAMP) and a special class of pathogen-associated molecular patterns indicating the viability of microbes (vitaPAMP) on immunocompetent human peripheral blood cells. In this work, we used the resistant E. coli strain ATCC BAA-2523, the sensitive E. coli strain ATCC 8739, and the revertant subculture of Escherichia coli obtained under experimental conditions from the E. coli strain ATCC BAA-196. Using enzyme immunoassay, we performed a comparative analysis of mononuclear (PBMC), monocytic (MON), and lymphocytic (LIM) cytokine responses to the induction of resistant, revertant, and susceptible E. coli subcultures by living and fixed cells. It turned out that the PAMP structures of subcultures of fixed E. coli cells are predominantly recognized by human PBMCs. While the pathogen-associated molecular patterns of living E. coli cells are recognized by different target cells, therefore, PBMCs primarily respond to the structures of the vitaPAMP-resistant subculture, while the structures of the revertant E. coli – human MON react to the structures of the vitaPAMP, which is a sensitive strain and PBMC – and MON respond to vitaPAMP. It has also been shown that, in response to fixed cultures of E. coli S and E. coli R, there is a decrease in the threshold level of production of IL-1β, IL-6 and TNF-α in both PBMCs and monocytes, compared with the level of cytokine production induced by live bacteria. Whereas fixed cells of revertant E. coli caused a higher production of pro-inflammatory cytokines PBMC than living cells of this culture.

Key words: Cytokines, resistant, revertant, sensitive.

С.С. Қасымбекова*, Т.А. Букеева, И.Е. Бишимова,
С.Г. Мурзагельдинова, И.С. Коротецкий

«Инфекцияға қарсы препараттардың ғылыми орталығы» АҚ, Қазақстан, Алматы қ.
*e-mail: s_kassymbekova@mail.ru

Иммунокомпетентті жасушалардың төзімді, ревертантты және сезімтал ішек таяқшаларының индукциясына дифференциалды цитокиндік реакциясы

E. coli субкультураларының төзімділік фенотипінің эволюциялық консервативті, бөгде антигендердің (PAMP) функционалдығына әсерін және адамның перифериялық қанының иммунокомпетентті жасушаларына микробтардың (vitaPAMP) өміршеңдігін білдіретін патогенге байланысты молекулалық заңдылықтардың ерекше класын зерттеуге арналған жұмыс. Жұмыс барысында ATCC BAA-196 E. coli штаммынан тәжірибелік жағдайда алынған ATCC BAA-2523 E. coli тұрақты штаммы, ATCC 8739 E. coli сезімтал штаммы және E. coli ревертантты субкультурасы пайдаланылды. Иммуноферменттік талдау арқылы E. coli төзімді, ревертантты және сезімтал субкультураларының тірі және бекітілген жасушаларын индукциялауға мононуклеарлық (PBMC), моноциттік (MON) және лимфоциттік (LYM) цитокиндік реакцияға жауаптардың салыстырмалы талдауы жүргізілді. Зерттелген E. coli субкультураларының бекітілген жасушаларының PAMP құрылымы негізінен адамның PBMC танитыны белгілі болды. Тірі E. coli жасушаларының патоген-байланысты молекулалық заңдылықтарын әртүрлі мақсатты жасушалар таниды, сондықтан төзімді субкультураның vitaPAMP құрылымдары негізінен PBMC-мен, ревертантты E. coli-MON адамның vitaPAMP құрылымына, сезімтал штаммның және PBMC-нің vitaPAMP құрылымына және MON-ға бірдей қарқындылықпен жауап береді. E. coli S және E. coli R тұрақты дақылдарына жауап ретінде тірі бактериялар қоздырған цитокиндердің өнім деңгейімен салыстырғанда PBMC-те де, моноциттерде де ИЛ-1β, ИЛ-6 және ФНО-α өнімдерінің шекті деңгейі төмендегені

көрсетілген. Ал ревертантты E. coli тұрақты жасушалары осы дақылдың тірі жасушаларына қарағанда қабынуға қарсы РВМС цитокиндерінің жоғары өндірісін тудырды.

Түйін сөздер: цитокиндер, төзімді, ревертант, сезімтал.

С.С. Касымбекова*, Т.А. Букеева, И.Е. Бишимова,
С.Г. Мурзагельдинова, И.С. Коротецкий
АО «Научный центр противоинфекционных препаратов», Казахстан, г. Алматы
*e-mail: s_kassymbekova@mail.ru

Дифференциальный цитокиновый ответ иммунокомпетентных клеток на индукцию резистентной, ревертантной и чувствительной *Escherichia coli*

Работа посвящена изучению влияния фенотипа резистентности субкультур E.coli на функциональность эволюционно-консервативных патоген-ассоциированных антигенов (PAMP) и особого класса патоген-ассоциированных молекулярных паттернов, которые обозначают жизнеспособность микробов (vitaPAMP) на иммунокомпетентные клетки периферической крови человека. В работе использовали устойчивый штамм E.coli ATCC BAA-2523, чувствительный штамм E.coli ATCC 8739 и ревертантную субкультуру E.coli, полученную в условиях эксперимента из штамма E.coli ATCC BAA-196. С помощью иммуноферментного анализа проведен сравнительный анализ мононуклеарного (PBMC), моноцитарного (MON) и лимфоцитарного (LYM) цитокинового ответа на индукцию живыми и фиксированными клетками резистентной, ревертантной и чувствительной субкультур E.coli. Оказалось, что PAMP структуры фиксированных клеток изученных субкультур E.coli преимущественно распознаются PBMC человека. Тогда как патоген-ассоциированные молекулярные паттерны живых клеток E.coli распознаются разными клетками-мишенями. Так на vitaPAMP структуры резистентной субкультуры реагируют преимущественно PBMC, на vitaPAMP структуры ревертантной E.coli – MON человека, на vitaPAMP структуры чувствительного штамма и PBMC, и MON реагируют с одинаковой интенсивностью. Показано, что в ответ на фиксированные культуры E. coli S и E. coli R происходит снижение порогового уровня продукции ИЛ-1β, ИЛ-6 и ФНО-α как в PBMC, так и в моноцитах, по сравнению с уровнем продукции цитокинов, индуцированных живыми бактериями. Тогда как фиксированные клетки ревертантной E. coli вызывали более высокую продукцию провоспалительных цитокинов PBMC, чем живые клетки этой культуры.

Ключевые слова: цитокины, резистентный, ревертантный, чувствительный.

Abbreviations

PBMC – peripheral blood mononuclear cells, MON – monocytes, PAMP – pathogen-associated molecular patterns, LYM – lymphocytes, MIC – minimum inducing concentration.

Introduction

The problem of antibiotic resistance and ways to overcome it has been actively studied for the past 30-40 years. However, in most cases, by the time bacterial infections cause symptoms and therefore require antibiotic treatment, the bacterial population is often so large that it probably includes a portion of antibiotic-resistant mutants [1]. Therefore, treatment with an antimicrobial drug alone can be expected to fail. One of the reasons why this is often not the case is the host's immune defence, which promotes bacterial clearance [2, 3]. Despite the general recognition of the important role of host defence, most studies of the dynamics of bacteria and antibiotics within the body are focused almost exclusively on the pharmacokinetics and pharmacodynamics of drugs and bacteria, without explicit consideration of the patient's immune response [4-6]. This omission of the immune response also refers to models that are specifically designed to develop treatment protocols to combat the emergence of resistant mutants [7-9].

Resistant bacteria are selected in a concentration range of drugs that is sufficient to kill a susceptible population but not sufficient to kill a (partially) resistant population. In the absence of an immune response, such treatment will lead to an increase in the number of resistant pathogens and treatment failure [10]. Scientists have shown [11-13] that the presence of an immune response can change the selection process for resistant mutants. They showed that an immune response that remains strong despite a strong drug-induced reduction in bacteria significantly reduces the emergence of resistance and mitigates the consequences of non-compliance with therapy.

Considering that a complex immunobiological reaction of the body lies at the heart of the treatment of a patient with antimicrobial drugs, it is important

to study the nature of the specificity of immune response to antibiotic-sensitive and antibiotic-resistant pathogens. The results of studying the differential role of various immunocompetent cells in the initiation of an immune response to pathogens with different antibiotic sensitivity profiles reveal the mechanisms of the relationship between infectious diseases caused by drug-resistant pathogens, induction and regulation of the innate and adaptive immune response, and antibiotics. Knowledge of this kind can open new ways to combat drug resistance of pathogens by developing more effective approaches to complex immunotherapy of infectious diseases.

The experimental conditions we have chosen allow us to perform a pairwise comparison of the cytokine response of PBMC and monocytes from the same donor to live and fixed cells of resistant, revertant and sensitive *E. coli*. Thus, this allows us to clarify the differences in PBMC and monocytic response between different subcultures of *E. coli*, as well as to assess the effect on these indicators of bacterial viability and to determine the proportion of the lymphocytic cytokine response in the total PBMC response to *E. coli*, which are distinctive in antibiotic sensitivity.

1. Materials and Methods

The experiment used peripheral blood mononuclear cells (PBMC) isolated from 6 healthy donors (with no acute diseases and severe chronic diseases) male and females aged 28 to 38 years and monocytes isolated from the PBMC fraction of the same donors by immunomagnetic separation. The non-monocyte PBMC fraction – lymphocytes (LYM) was obtained after the isolation of monocytes on an immunomagnetic separator (positive fraction – non-monocytes). All manipulations with human cells have been approved by the Ethics Commission. The work was carried out following the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human subjects.

1.1. Isolation of mononuclear fraction of cells

Heparinized human peripheral blood was premixed with 6 % dextran to precipitate erythrocytes. Incubated at room temperature for 1.5-2 hours. After the incubation time, the supernatant was precipitated at 300 g for 10 min at room temperature to obtain a cell suspension. The resulting cell suspension was fractionated on a Histopaque-1.077 density gradient (Sigma, USA) corresponding to the buoyant density of human PBMC at 4 °C at 3000 rpm for 20 min. After that PBMC were collected and washed by centrifugation at 300g for 10 min at room temperature, after which they were resuspended in RPMI-1640 culture medium containing 10 % fetal bovine serum and 2 % L-glutamine (all from Sigma, USA). In all experiments, a PBMC suspension with a percentage of viable cells greater than 90 % was used.

1.2 Isolation of monocytes by immunomagnetic separation

Isolation of monocytes from the PBMC fraction was performed using the Monocyte Isolation Kit II, following the manufacturer's protocol (Miltenyi Biotec, Germany). Isolation was carried out using the "Deplete" program, which carries out a negative selection with the release of enriched monocytes. The resulting population of monocytes was centrifuged at 300 g for 10 min at room temperature. In all experiments, a suspension of monocytes with a percentage of viable cells greater than 90 % was used.

1.3 Test cultures of microorganisms

In a study of the following strains were used: 1) resistant strain of *Escherichia coli* ATCC BAA-2523 (ATCC) was conventionally designated as "R" culture in the experiments [14], 2) a sensitive *Escherichia coli* strain ATCC 8739 (ATCC), designated as "S" culture [15] and 3) a revertant subculture of *Escherichia coli* was obtained under experimental conditions earlier [16, 17] from *Escherichia coli* ATCC BAA-196 (ATCC), producing extended-spectrum beta-lactamase. In the experiments, the subculture was conventionally designated as "Rev".

The bacteria were cultured on a solid nutrient medium until the middle of the logarithmic growth phase at a temperature of 37 ± 1 °C. Bacteria for research were prepared as described [16]. The study used the minimum inducing concentration (MIC), which was previously determined [16]. For the resistant *E. coli* R strain, the MIC value was 10^3 CFU/ml, for the sensitive *E. coli* S strain and the revertant *E. coli* Rev – 10^4 CFU/ml. The exposure time of bacterial cells to formalin was determined from the results of microbiological control of the cultural survival [16].

1.4 Co-cultivation of cells with test cultures

PBMC and monocytes were plated in 96-well flat-bottom plates (BD Falcon, USA) at a concentration of 1×10^5 cells/well. Then to PBMC and monocytes was added a certain strain of *E. coli* at a concentration of MIC, in a volume of 100 μl/well.

Immunocompetent cells and bacteria were co-cultivated in RPMI-1640 culture medium containing 10 % PBS and 2 % L-glutamine without antibiotics

for 4 hours at 37 °C, 5 % CO_2 and 95 % humidity. At the end of the incubation time, the plates were centrifuged at 300 g for 10 min at room temperature and the supernatant was collected for analysis.

1.5 Determination of cytokine concentrations

The quantitative determination of cytokines was carried out by enzyme immunoassay using commercial reagent kits for alpha-TNF-ELISA-BEST, INTERLEUKIN-6-ELISA-BEST, INTERLEUKIN-1 beta-ELISA-BEST (all from VECTOR-BEST, Russia) according to the manufacturer's instructions. Measurement of optical density and calculation of cytokine concentration was performed on a Sunrise RC.4 microplate reader (Tecan, Austria) using Magellan 2.0 software (Tecan, Austria) at a wavelength of 450 nm with a reference filter at 620 nm.

1.6 Statistical Data Processing

All studies were performed in triplicate. For all data, the arithmetic means and standard deviation from the mean were calculated. The significance of differences between experimental data was assessed using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA), using One-way ANOVA, Column Statistics, and unpaired t-test. Values of the confidence level $P > 0.05$ were considered insignificant.

2. Results and Discussion

Innate immune cells respond to a variety of stimuli, including bacterial, viral, parasitic, or fungal infections, through structurally related receptors called Toll-like receptors (TLRs). TLRs are evolutionarily conserved type I transmembrane receptors that provide a critical link between innate and adaptive immunity. TLRs can individually respond to a limited, specific number of microbial pathogen-associated molecular patterns (PAMP). VitaPAMP, a subset of PAMP specifically expressed by living microorganisms, are indicative of microbial viability. VitaPAMP elicits a strong inflammatory response to counter the heightened threat posed by living microbes compared to their dead counterparts [18]. The interaction of these structures with TLR on innate immune cells regulates the induction of more efficient adaptive immune responses [19-21]. This recognition initiates an intracellular signalling cascade, which culminates in the activation of multiple genes for the pro-inflammatory and immune response. Pro-inflammatory cytokines, in particular TNF-α, IL-1β and IL-6, provide additional signals due to the activation of co-stimulating and adhesive molecules, which are necessary for the activation of adaptive immune cells and the subsequent development of protective immune responses against infectious antigens [22, 23].

2.1 Production of IL-1β by immunocompetent cells and IL-1β inducing activity of vitaPAMP and PAMP structures of sensitive, resistant and revertant subcultures of E. coli

As a result of a comparative study of the inducibility threshold of cytokine production by human mononuclear cells, monocytes and lymphocytes under the influence of living and fixed cells, sensitive, resistant and revertant *E. coli* cultures, it was found that the level of immunogenicity of PAMP and vitaPAMP structures of these test cultures can differ significantly.

Our studies have shown that the proportion of lymphocytic cytokine response in the total PBMC response for all studied cytokines was insignificant (from 2 to 7 %) and did not depend on the viability and antibiotic sensitivity of the test cultures (data not presented).

In response to stimulation by sensitive and resistant *E. coli* living cells (Figure 1), lower production of IL-1β by monocytes was detected compared to PBMC. Induction by the revertant culture of *E. coli* showed no significant difference. Upon stimulation of immunocompetent cells by fixed *E. coli* R and *E. coli* Rev, the threshold production of IL-1β in PBMC was significantly higher than in monocytes.

The threshold production of IL-1β was significantly ($P < 0.0001$) higher in response to stimulation of immunocompetent cells (PBMC, MON) by living cells of test cultures, i.e. the vitaPAMP structures of the cultures were more immunogenic compared to their PAMP structures. An exception was the induction of PBMC *E. coli* Rev, where there was no significant difference between the PAMP and vitaPAMP structures of this strain. It was found that upon induction of PBMC IL-1β, the inducing activity of vitaPAMP structures of the sensitive strain is significantly higher than that of the revertant *E. coli* strain, moreover, the PAMP structure of this strain has significantly low immunogenicity, except for *E. coli* Rev, where a slight increase in IL-1β inducing activity of PAMP structures (Figure 2).

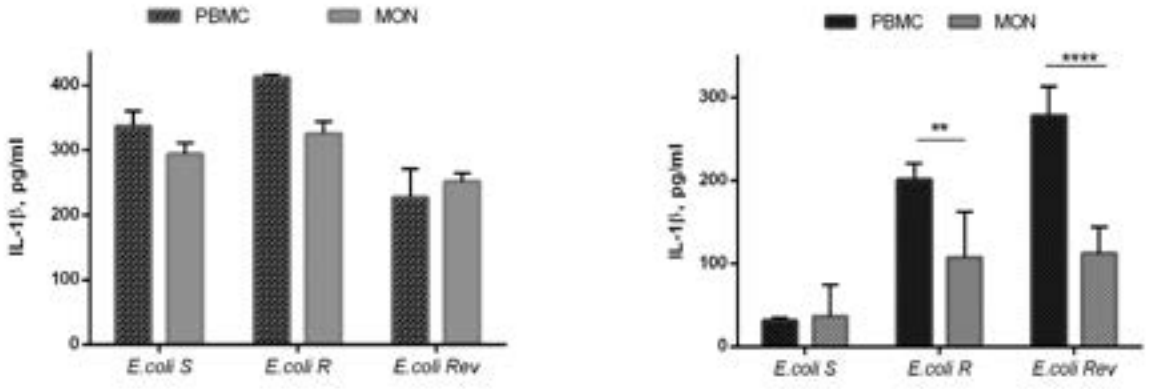


Figure 1 – IL-1β production by immunocompetent cells, induced by l iving (A) and fixed (B) cells of sensitive (S), resistant (R) and revertant (Rev) *E. coli* subcultures. All data represent means ± SEM and are significantly different comparing PBMC and MON by fixed *E. coli* Rev structures (**P = 0.004, ****P < 0.0001)

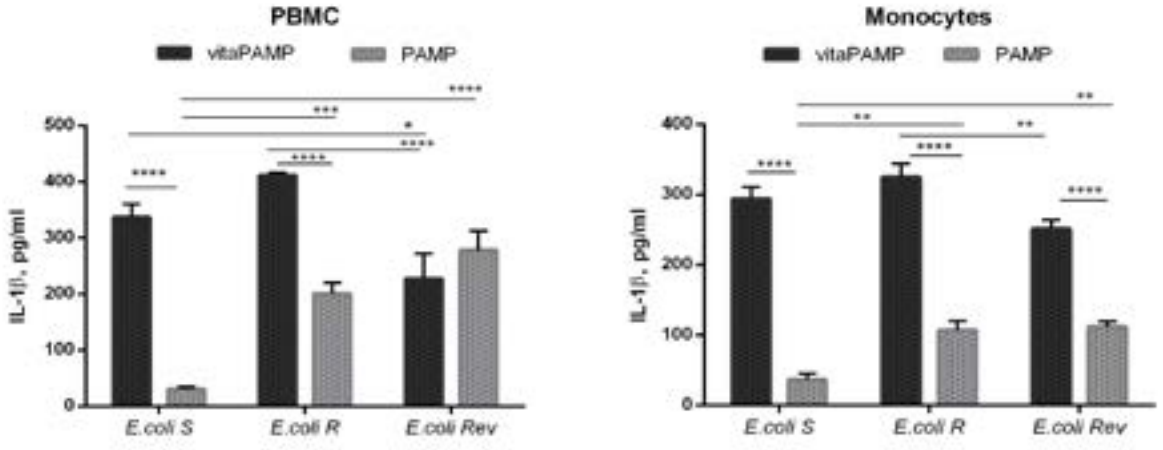
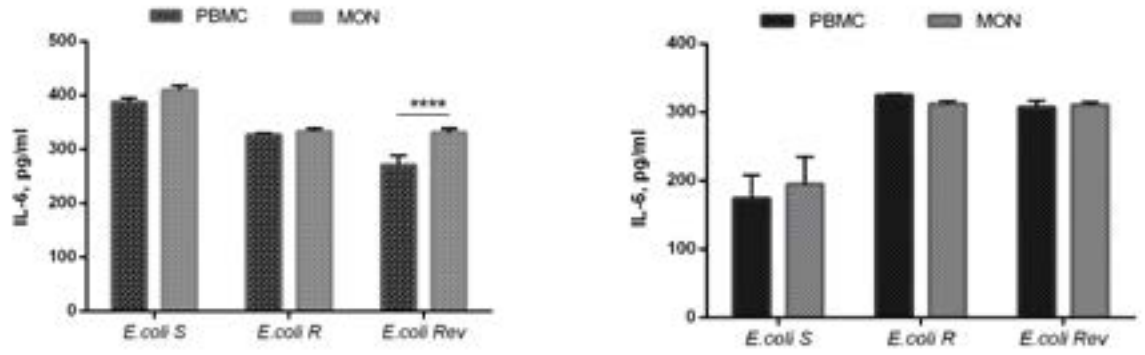


Figure 2 – IL-1β inducing activity of vitaPAMP and PAMP structures of sensitive (S), resistant (R) and revertant (Rev) subcultures of *E. coli*. All data represent means ± SEM and are significantly different comparing vitaPAMP and PAMP *E. coli* subcultures (*P = 0.04, **P < 0.005, ***P = 0.0002, ****P < 0.0001)

2.2 Production of IL-6 by immunocompetent cells and IL-6 inducing activity of vitaPAMP and PAMP structures of sensitive, resistant and revertant subcultures of E. coli

Stimulation by living cells of a sensitive and resistant *E. coli* culture caused comparable levels of IL-6 production by PBMC and MON. Whereas, the vitaPAMP structures of the revertant culture were recognized mainly by monocytes and led to a significantly (P < 0.0001) greater release of IL-6 (Figure 3). The study of the threshold production of IL-6 by immunocompetent cells under the influence of fixed cells of test cultures did not reveal a significant differential response by immunocompetent cells (Figure 3).



A B

Figure 3 – IL-6 production by immunocompetent cells, induced by living (A) and fixed (B) cells of sensitive (S), resistant (R) and revertant (Rev) *E. coli* subcultures. All data represent means ± SEM and are significantly different comparing PBMC and MON by living *E. coli* Rev structures (****P < 0.0001)

Comparison of IL-6 production by immunocompetent cells showed that IL-6 inducing activity of resistant and revertant *E. coli* cultures does not depend on cell viability. In contrast, in a susceptible strain, the loss of viability more than halves the production of IL-6 (Figure 4). IL-6 inducing activity of vitaPAMP and PAMP structures showed significant differences between susceptible, resistant and revertant cultures (Figure 4).

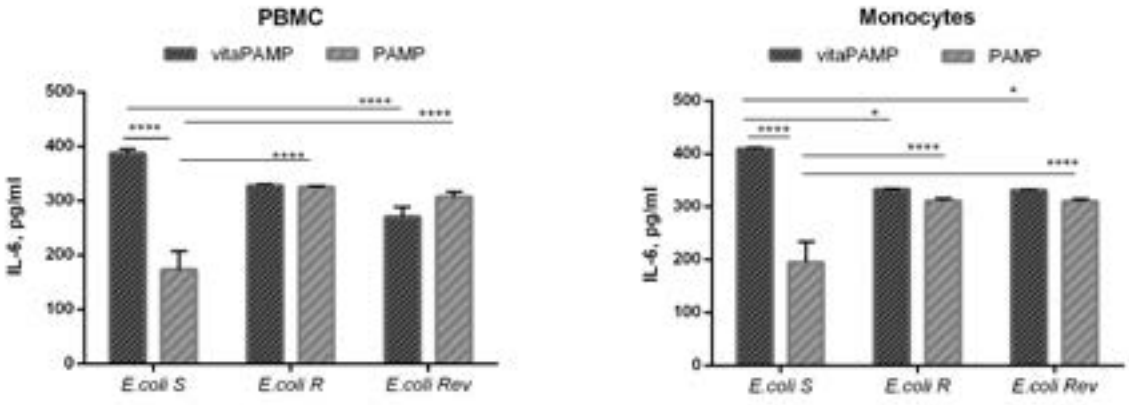


Figure 4 – IL-6 inducing activity of vitaPAMP and PAMP structures of sensitive (S), resistant (R) and revertant (Rev) *E. coli* subcultures. All data represent means ± SEM and are significantly different comparing vitaPAMP and PAMP *E. coli* subcultures (*P < 0.05, ****P < 0.0001)

2.3 Production of TNF-α by immunocompetent cells and TNF-α inducing activity of vitaPAMP and PAMP structures of sensitive, resistant and revertant subcultures of E. coli

The study of the threshold production of TNF-α by immunocompetent cells showed a reliable (P = 0.0008) differential response to the induction of PBMC and MON by living cells only by *E. coli* Rev culture (Figure 5).

Analysis of the level of TNF-α production by immunocompetent cells in response to a stimulus by living and fixed cells revealed that the vitaPAMP structures of test cultures are significantly more immunogenic than their PAMP structures (Figure 6). In contrast, the opposite picture is observed with the induction of PBMC *E. coli* Rev, where the immunogenicity of PAMP was slightly higher than with the stimulation of vitaPAMP structures of this strain (Figure 6).

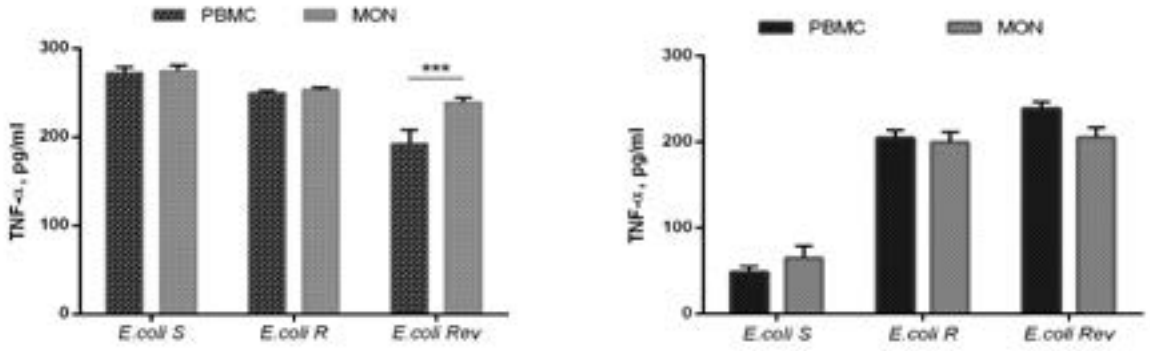


Figure 5 – TNF-α production by immunocompetent cells, induced by living (A) and fixed (B) cells of sensitive (S), resistant (R) and revertant (Rev) *E. coli* subcultures. All data represent means ± SEM and are significantly different comparing PBMC and MON by living *E. coli* Rev structures (***P = 0.0008)

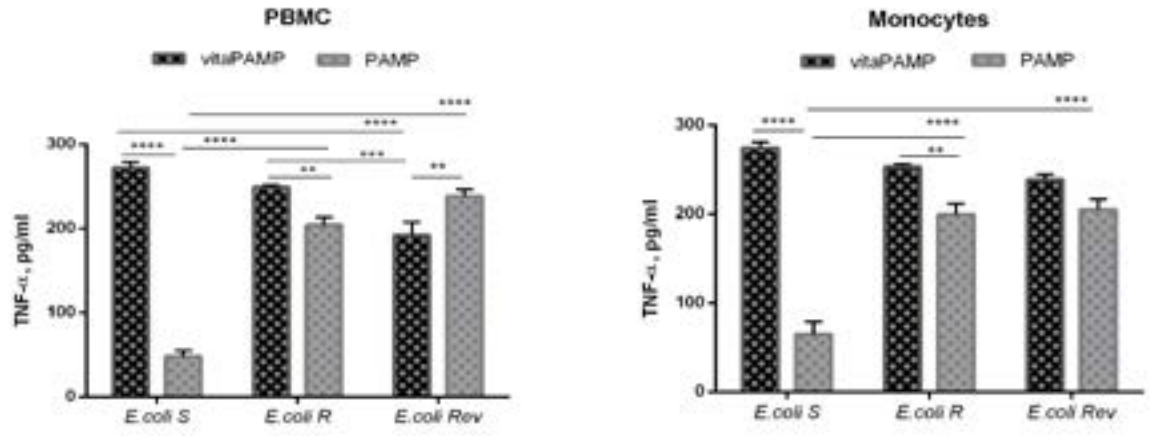


Figure 6 – TNF-α inducing activity of vitaPAMP and PAMP structures of sensitive (S), resistant (R) and revertant (Rev) *E. coli* subcultures. All data represent means ± SEM and are significantly different comparing vitaPAMP and PAMP *E. coli* subcultures (**P < 0.009, ***P = 0.0004, ****P < 0.0001)

In general, activation of the mechanisms of innate immunity and the induction of production of the main proinflammatory cytokines are known to be caused by the interaction of both PAMP and molecules associated with virulence with the PRR receptors of the cells of the natural immune system, as a result of which a protective inflammatory response is induced for an effective fight against infections [24-26]. It is also known that living bacteria induce much more pronounced immune responses than their preserved (fixed) counterparts [27, 28]. This is explained by the ability to live microorganisms to reproduce and express specialized virulence factors and additional antigens, such as mRNA (vitaPAMP), which activate a pronounced host immune response [29].

Fixed bacterial samples contain only conserved antigenic structures, of which the LPS molecule (PAMP) is the main one. If it has low immunogenicity, then such a fixed sample does not cause proper activation of immunity, as evidenced by our data on archival strains of E. coli: antibiotic-susceptible strain – *E. coli* ATCC 8739 and antibiotic-resistant strain – *E. coli* ATCC BAA-2523 (data not published).

It can be assumed that the phenomenon of the revertant subculture of *E. coli* may be associated with changes in the antigenic characteristics of bacteria. In the present study, we used an *E. coli* subculture, whose sensitivity to gentamicin was restored under experimental conditions [17]. Previously, it was shown that as a result

of long-term interaction of the FS-1 preparation with bacteria, a "new or acquired" phenotype is formed, which, possibly, as a result of changes in the genomics, proteomics, and metabolomics of bacteria, leads to a conjugate change in the expression and architectonics of immunogenic molecular structures, and reversal of antibiotic sensitivity [16].

In addition, a comparative analysis of the threshold levels of cytokines produced by immunocompetent cells in response to a stimulus from *E. coli*, which are distinct in antibiotic sensitivity, showed a different orchestration of cytokines.

To determine the proportion of each cytokine in the total cytokine response, the data obtained were converted into percentage ratios, where a shift was noted in the quantitative contribution of each cytokine to the total cytokine response by immunocompetent cells to a stimulus by life and killed cells of sensitive, resistant and revertant *E. coli* cultures (Figure 7).

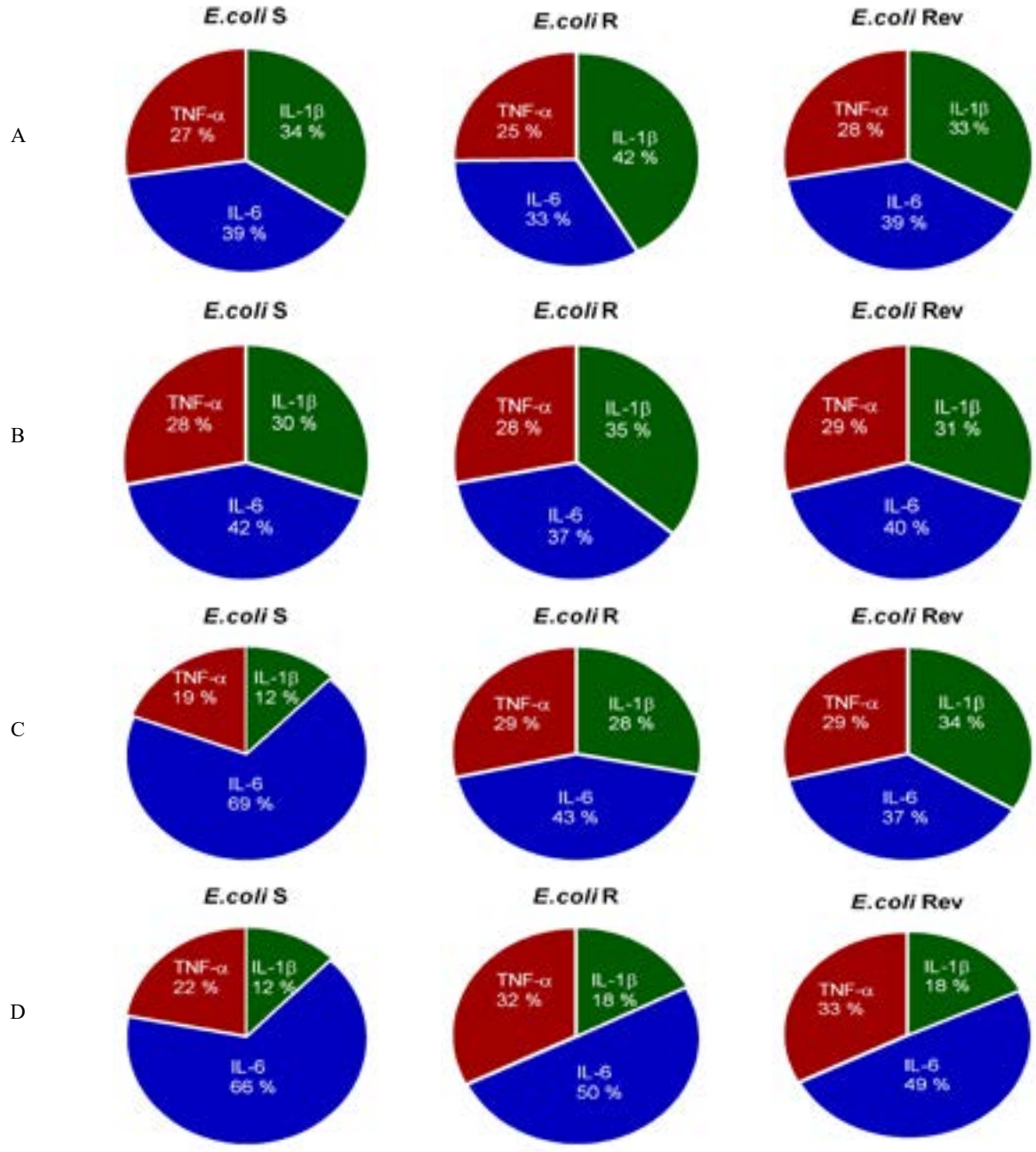


Figure 7 – Orchestration of cytokines (A) PBMC to live cells, (B) MON to live cells, (C) PBMC to fixed cells, (D) MON to fixed *E. coli* cells

It should be noted that both mononuclear cells and monocytes reacted to stimulation by living cells of sensitive *E. coli* S with emission of ~30 % (IL-1β): ~40 % (IL-6): ~30 % (TNF-α), to non-viable cells of this culture, a more anti-inflammatory response was obtained. Whereas the cytokine response obtained by immunocompetent cells to a stimulus by fixed *E. coli* R and *E. coli* Rev cells are characteristic of inflammasome inflammation.

Such a change in the levels of early mediators of the acute phase response may indicate a differential response of cellular receptors and signalling pathways of immunocompetent cells to antibiotic-resistant and sensitive bacteria.

The orchestration of the cytokine response is probably associated with the mechanisms of induction and the features of the regulation of signalling pathways. As is known, the effective and sustained action of proinflammatory cytokines depends on synergism with other cytokines and antagonism of opposing cytokines, which are often highly expressed in inflammatory foci [30].

Conclusion

Thus, the relationship between antibiotic sensitivity and the cytokine-inducing potential of *E. coli* has been studied. When studying the cellular mechanisms of induction of an innate immune response to archival living and fixed cells of sensitive and resistant strains, as well as the revertant subculture of *E. coli*, a difference in the immunogenicity of PAMP and vitaPAMP structures of test cultures was revealed. It has been shown that in response to fixed cultures of *E. coli* S and *E. coli* R, there is a decrease in the threshold level of IL-1β, IL-6 and TNF-α production, both in PBMC and in monocytes, compared to the level of cytokine production, induced by live bacteria. Whereas fixed cells of revertant *E. coli* caused a higher production of proinflammatory cytokines PBMC than living cells of this culture.

Conflict of interest

All authors have read and are familiar with the content of the article and have no conflict of interest.

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References

1 Drlica K. The mutant selection window and antimicrobial resistance // Antimicrobial Chemotherapy. – 2003. – Vol. 52. – P.11-17.

2 Pamer E.G. Immune responses to commensal and environmental microbes // Nat. Immunol. – 2007. – Vol. 8. – P. 1173-1178.

3 Happel K.I., Bagby G.J., Nelson S. Host defense and bacterial pneumonia // Semin. Respir. Crit. Care Med. – 2004. – Vol. 25, No 1. – P. 43-52.

4 Mueller M., de la Pena A., Derendorf H. Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: kill curves versus MIC // Antimicrob. Agents Chemother. – 2004. – Vol. 48, No 2. – P. 369-377.

5 DeRyke C.A., Lee S.Y., Kuti J.L., Nicolau D.P. Optimising dosing strategies of antibacterials utilising pharmacodynamic principles: impact on the development of resistance // Drugs. – 2006. – Vol. 66,

No 1. – P. 1-14.

6 Ambrose P.G., Bhavnani S.M., Rubino C.M., Louie A., Gumbo T., Forrest A. et al. Pharmacokinetics-pharmacodynamics of antimicrobial therapy: it's not just for mice anymore // Clin. Infect. Dis. – 2007. – Vol. 44, No 1. – P. 79-86.

7 Zhao X., Drlica K. A unified anti-mutant dosing strategy // Antimicrob. Chemother. – 2008. – Vol. 62, No 3. – P. 434-436.

8 Jumbe N., Louie A., Leary R., Liu W., Deziel M.R., Tam V.H. et al. Application of a mathematical model to prevent in vivo amplification of antibiotic-resistant bacterial populations during therapy // Clin. Invest. – 2003. – Vol. 112, No 2. – P. 275-285.

9 Drlica K., Zhao X.L. Is 'dosing-to-cure' appropriate in the face of antimicrobial resistance? // Rev. Med. Microbiol. – 2004. – Vol. 15, No 2. – P. 73-80.

10 Tumbarello M. et al. Predictors of mortality in patients with bloodstream infections caused by
extended-spectrum-beta-lactamase-producing Enterobacteriaceae: importance of inadequate initial antimicrobial treatment // Antimicrob. Agents Chemother. – 2007. – Vol. 51, No 6. – P. 1987-1994.

11 Kumarasamy K.K. et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study // Lancet Infect. Dis. – 2010. – Vol. 10, No 9. – P. 597-602.

12 Fleming A. Penicillin. Nobel Lectures. https://www.nobelprize.org/?p=12848&pagespeed=noscript?pagespeed=noscript. 11.08.2021.

13 World Health Organization 2012. The evolving threat of antimicrobial resistance: options for action. http://whqlibdoc.who.int/publications/2012/9789241503181_eng.pdf. 11.08.2021.

14 Cotroneo N., Rubio A., Critchley I.A., Pillar C., Pucci M.J. In vitro and in vivo characterization of tebipenem, an oral carbapenem // Antimicrob. Agents Chemother. – 2020. – Vol. 64, No 8.

15 Zhang D., Chen J., Jing Q., Chen Z., Ullah A., Jiang L., Zheng K., Yuan C. and Huang M. Development of a Potent Antimicrobial Peptide With Photodynamic Activity // Front. Microbiol. – 2021. – Vol. 12.

16 Volodina G.V., Davtyan T.K., Kulmanov M.E., Dzhumagazieva A.B., Tursunova S.K., Abekova A.O. et al. 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. – 2017. –
Vol. 8, No 522.

17 Korotetskiy I.S., Joubert M., Taukobong S. et al. Complete Genome Sequence of a Multidrug-Resistant Strain, Escherichia coli ATCC BAA-196, as a Model for Studying Induced Antibiotic Resistance Reversion // Microbiol. Resour. Announc. – 2020. – Vol. 9, No 50.

18 Mourao-Sa D., Roy S., Blander J.M. Vita-PAMPs: signatures of microbial viability // Adv. Exp. Med. Biol. – 2013. – Vol. 785. – P. 1-8.

19 Schnare M., Barton G.M., Holt A.C., Takeda K., Akira S., Medzhitov R. Toll-like receptors control activation of adaptive immune responses // Nat. Immunol. – 2001. – Vol. 2. – P. 947-950.

20 Kawai T., Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity // Immunity. – 2011. – Vol. 34. – P. 637-650.

21 Akira S., Hemmi H. Recognition of pathogen-associated molecular patterns by TLR family // Immunol. Lett. – 2003. – Vol. 85. – P. 85-95.

22 Granucci F., Feau S., Zanoni I., Pavelka N., Vizzardelli C., Raimondi G. et al. The immune response is initiated by dendritic cells via interaction with microorganisms and interleukin-2 production // J. Infect. Dis. – 2003. – Vol. 187. – P. 346-350.

23 Sharpe A.H. Mechanisms of costimulation // Immunol. Rev. – 2009. – Vol. 229. – P. 5-11.

24 Medzhitov R. Recognition of microorganisms and activation of the immune response // Nature. – 2007. – Vol. 449. – P. 819-826.

25 Blander J.M., Sander L.E. Beyond pattern recognition: five immune checkpoints for scaling the microbial threat // Nature Rev. Immunology. – 2012. – Vol. 12. – P. 215-225.

26 Akira S., Uematsu S., Takeuchi O. Pathogen recognition and innate immunity // Cell. – 2006. – Vol. 124. – P. 783-801.

27 Koenig C.H., Finger H., Hof H. Failure of killed Listeria monocytogenes vaccine to produce protective immunity // Nature. – 1982. – Vol. 297. – P. 233-234.

28 Detmer A., Glenting J. Live bacterial vaccines – a review and identification of potential hazards // Microbology Cell Fact 5. – 2006. – Vol. 5. – P. 23.

29 Sander L.E., Michael J., Mark V. Sensing prokaryotic mRNA signifies microbial viabilty and promotes immunity // Nature. – 2012. – Vol. 474. – P. 385-389.

30 Ahmed S.T., Ivashkiv L.B. Inhibition of IL-6 and IL-10 signaling and stat activation by inflammatory and stress pathways // Immunol. – 2000. – Vol. 165, No 9. – P. 5227-5237.

References

1 Ahmed S.T., Ivashkiv L.B. (2000) Inhibition of IL-6 and IL-10 signaling and stat activation by inflammatory and stress pathways. Immunol., vol. 165, no. 9, pp. 5227-5237.

2 Akira S., Hemmi H. (2003) Recognition of pathogen-associated molecular patterns by TLR family. Immunol. Lett., vol. 85, pp. 85-95.

3 Akira S., Uematsu S., Takeuchi O. (2006) Pathogen recognition and innate immunity. Cell, vol. 124, pp. 783-801.

4 Ambrose P.G., Bhavnani S.M., Rubino C.M., Louie A., Gumbo T., Forrest A. et al. (2007) Pharmacokinetics-pharmacodynamics of antimicrobial therapy: it's not just for mice anymore. Clin. Infect. Dis., vol. 44, no. 1, pp. 79-86.

5 Blander J.M., Sander L.E. (2012) Beyond pattern recognition: five immune checkpoints for scaling the microbial threat. Nature Rev. Immunology, vol. 12, pp. 215-225.

6 Cotroneo N., Rubio A., Critchley I.A., Pillar C., Pucci M.J. (2020) In vitro and in vivo characterization of tebipenem, an oral carbapenem. Antimicrob. Agents Chemother., vol. 64, no. 8.

7 DeRyke C.A., Lee S.Y., Kuti J.L., Nicolau D.P. (2006) Optimising dosing strategies of antibacterials utilising pharmacodynamic principles: impact on the development of resistance. Drugs, vol. 66, no. 1, pp. 1-14.

8 Detmer A., Glenting J. (2006) Live bacterial vaccines – a review and identification of potential hazards. Microbology Cell Fact 5, vol. 5, pp. 23.

9 Drlica K. (2003) The mutant selection window and antimicrobial resistance. Antimicrobial Chemotherapy, vol. 52, pp. 11-17.

10 Drlica K., Zhao X.L. (2004) Is 'dosing-to-cure' appropriate in the face of antimicrobial resistance? Rev. Med. Microbiol., vol. 15, no. 2, pp. 73-80.

11 Fleming A. Penicillin. Nobel Lectures. https://www.nobelprize.org/?p=12848&pagespeed=noscript?pagespeed=noscript. 11.08.2021.

12 Granucci F., Feau S., Zanoni I., Pavelka N., Vizzardelli C., Raimondi G. et al. (2003) The immune response is initiated by dendritic cells via interaction with microorganisms and interleukin-2 production. J. Infect. Dis., vol. 187, pp. 346-350.

13 Happel K.I., Bagby G.J., Nelson S. (2004) Host defense and bacterial pneumonia. Semin. Respir. Crit. Care Med., vol. 25, no. 1, pp. 43-52.

14 Jumbe N., Louie A., Leary R., Liu W., Deziel M.R., Tam V.H. et al. (2003) Application of a mathematical model to prevent in vivo amplification of antibiotic-resistant bacterial populations during therapy. Clin. Invest., vol. 112, no. 2, pp. 275-285.

15 Kawai T., Akira S. (2011) Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity, vol. 34, pp. 637-650.

16 Koenig C.H., Finger H., Hof H. (1982) Failure of killed Listeria monocytogenes vaccine to produce protective immunity. Nature, vol. 297, pp. 233-234.

17 Korotetskiy I.S., Joubert M., Taukobong S. et al. (2020) Complete Genome Sequence of a Multidrug-Resistant Strain, Escherichia coli ATCC BAA-196, as a Model for Studying Induced Antibiotic Resistance Reversion. Microbiol. Resour. Announc., vol. 9, no. 50.

18 Kumarasamy K.K. et al. (2010) Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect. Dis., vol. 10, no. 9, pp. 597-602.

19 Medzhitov R. (2007) Recognition of microorganisms and activation of the immune response. Nature, vol. 449, pp. 819-826.

20 Mourao-Sa D., Roy S., Blander J.M. (2013) Vita-PAMPs: signatures of microbial viability. Adv. Exp. Med. Biol., vol. 785, pp. 1-8.

21 Mueller M., de la Pena A., Derendorf H. (2004) Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: kill curves versus MIC. Antimicrob. Agents Chemother., vol. 48, no. 2, pp. 369-377.

22 Pamer E.G. (2007) Immune responses to commensal and environmental microbes. Nat. Immunol., vol. 8, pp. 1173-1178.

23 Sander L.E., Michael J., Mark V. (2012) Sensing prokaryotic mRNA signifies microbial viabilty and promotes immunity. Nature, vol. 474, pp. 385-389.

24 Schnare M., Barton G.M., Holt A.C., Takeda K., Akira S., Medzhitov R. (2001) Toll-like receptors control activation of adaptive immune responses. Nat. Immunol., vol. 2, pp. 947-950.

25 Sharpe A.H. (2009) Mechanisms of costimulation. Immunol. Rev., vol. 229, pp. 5-11.

26 Tumbarello M. et al. (2007) Predictors of mortality in patients with bloodstream infections caused by extended-spectrum-beta-lactamase-producing Enterobacteriaceae: importance of inadequate initial antimicrobial treatment. Antimicrob. Agents Chemother., vol. 51, no. 6, pp. 1987-1994.

27 Volodina G.V., Davtyan T.K., Kulmanov M.E., Dzhumagazieva A.B., Tursunova S.K., Abekova A.O. 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., vol. 8, no. 522.

28 World Health Organization 2012. The evolving threat of antimicrobial resistance: options for action. http://whqlibdoc.who.int/publications/2012/9789241503181_eng.pdf. 11.08.2021.

29 Zhang D, Chen J, Jing Q, Chen Z, Ullah A, Jiang L, Zheng K, Yuan C and Huang M (2021) Development of a Potent Antimicrobial Peptide With Photodynamic Activity. Front. Microbiol., vol. 12.

30 Zhao X., Drlica K. (2008) A unified anti-mutant dosing strategy. Antimicrob. Chemother., vol. 62, no. 3, pp. 434-436.