ВЛИЯНИЕ МИКРОБНЫХ ПОЛИАМИНОВ НА ПРОДУКЦИЮ IL-10 ЛЕЙКОЦИТАМИ ПЕРИФЕРИЧЕСКОЙ КРОВИ ЗДОРОВЫХ ДОНОРОВ

Годовалов А.П., Морозов И.А.

ФГБОУ ВО «Пермский государственный медицинский университет имени академика Е.А. Вагнера» Министерства здравоохранения РФ, г. Пермь, Россия

Резюме. Как известно, полиамины бактериального происхождения, к которым относятся кадаверин и путресцин, способны разносторонне влиять на активность иммунокомпетентных клеток. В частности, такая ситуация наблюдается при длительно текущих воспалительных заболеваниях, особенно при интенсивном размножении микроорганизмов, способных к продукции полиаминов. Представляет интерес изучение продукции одного из основных противовоспалительных цитокинов – IL-10 – под влиянием бактериальных полиаминов. Для проведения исследований из периферической крови практически здоровых доноров путем градиентного центрифугирования выделяли популяцию мононуклеарных лейкоцитов. Клеточную суспензию помещали в круглодонный планшет с предварительно внесенными полиаминами в концентрациях 5, 25, 50, 75, 100 ммоль/л. В качестве контроля использовали лунки не содержащие полиаминов, по окончании инкубации в течение 72 ч при 37 °С и 5% CO_2 супернатанты стягивали и использовали для определения концентрации IL-10. В работе использовали набор для определения концентрации IL-10 с помощью иммуноферментного метода (Россия). Статистический анализ проводили с помощью программного пакета Statistica 6.0. В случае распределения приближенного к нормальному использовали критерий Стьюдента, в остальных – применяли критерий Манна–Уитни для оценки значимости различий. В ходе проведения исследований показано, что лейкоциты в присутствии конканавалина А продуцируют IL-10 в концентрации 17,13±6,08 пг/мл. Установлено, что под влиянием полиаминов бактериального происхождения продукция IL-10 усиливается только если путресцин и кадаверин в концентрациях 50 ммоль/л и выше. При низких концентрациях полиаминов значимого увеличения продукции IL-10 не выявлено. Поскольку IL-10 является противовоспалительным цитокином, для которого известен в том числе и противоболевой эффект, следует ожидать, что при увеличении его концентрации в очаге инвазии условно патогенных бактерий воспалительный процесс будет развиваться латентно, когда симптомы слабо выражены. В целом можно ожидать, что бактерии-продуценты полиаминов будут способствовать поддержанию малосимптомного воспаления.

Ключевые слова: бактериальные полиамины, IL-10, лейкоциты, конканавалин А, человек, воспаление

Адрес для переписки:

Годовалов Анатолий Петрович
ФГБОУ ВО «Пермский государственный медицинский университет имени академика Е.А. Вагнера» Министерства здравоохранения РФ
614990, Россия, г. Пермь, ул. Петропавловская, 26.
Тел.: 8 (342) 236-44-85.
E-mail: AGodovalov@gmail.com

Address for correspondence:

Anatoliy P. Godovalov
E.A. Vagner Perm State Medical University
26 Petropavlovskaya St
Perm
614990 Russian Federation
Phone: +7 (342) 236-44-85.
E-mail: AGodovalov@gmail.com

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INFLUENCE OF MICROBIAL POLYAMINES ON IL-10 PRODUCTION BY PERIPHERAL BLOOD LEUKOCYTES OF HEALTHY DONORS

Godovalov A.P., Morozov I.A.

E.A. Vagner Perm State Medical University, Perm, Russian Federation

Abstract. As is known, bacterial polyamines, which include cadaverine and putrescine, are capable of influencing the activity of immunocompetent cells in many ways. In particular, this situation is observed in long-term inflammatory diseases, especially with intensive reproduction of microorganisms capable of producing polyamines. It is of interest to study the production of one of the main anti-inflammatory cytokines, IL-10, under the influence of bacterial polyamines. For research, a population of mononuclear leukocytes was isolated from the peripheral blood of healthy donors by gradient centrifugation. The cell suspension was placed in a round-bottomed plates with preliminarily added polyamines at concentrations of 5, 25, 50, 75, and 100 μmol/L. Wells not containing polyamines were used as a control. After incubation for 72 h at 37 °C and 5% CO_2, the supernatants were harvested and used to determine the concentration of IL-10. We used a kit for determining the concentration of IL-10 using the enzyme immunoassay method (Russia). Statistical analysis was performed using the Statistica 6.0 software package. In the case of a distribution close to normal, Student's t-test was used; in the rest, the Mann–Whitney test was used to assess the significance of differences. Studies have shown that leukocytes in the presence of concanavalin A produce IL-10 at a concentration of 17.13±6.08 pg/mL. It has been established that under the influence of polyamines of bacterial origin, the production of IL-10 is enhanced only if putrescine and cadaverine are at concentrations of 50 μmol/L and higher. At low concentrations of polyamines, no significant increase in IL-10 production was detected. Since IL-10 is an anti-inflammatory cytokine, for which the analgesic effect is also known, it should be expected that with an increase in its concentration in the focus of invasion of opportunistic bacteria, the inflammatory process will develop latently, when the symptoms are mild. In general, it can be expected that polyamine-producing bacteria will contribute to the maintenance of few symptomatic inflammation.

Keywords: bacterial polyamines, IL-10, leukocytes, concanavalin A, human, inflammation

Introduction

Recently, more and more chronic forms of inflammatory diseases have been recorded, while the clinical picture is characterized by mild symptoms. This situation creates the main problem in reproduction, for example, in infertile couples, asymptomatic bacteriospermia occurs in more than 30% of cases [2]. The absence of symptoms leads to untimely diagnosis of such conditions, which further exacerbates the problem of infertility. A long-term inflammatory process ensures the persistence of microorganisms [4] with a less pronounced response of the immune system.

We have previously shown that a large variety of microorganisms is recorded in the genital tract in chronic inflammatory diseases [4]. For successful establishment in the biotope, microorganisms change the reactivity of the immune system, for example, through the production of polyamines. Thus, polyamines of bacterial origin can have a versatile effect on eukaryotic cells. Among the cytokines regulating the immune system, the study of IL-10 is of interest. IL-10 has anti-inflammatory effect, affecting mast cells, B lymphocytes, neutrophils, NK-cells and macrophages.

The aim of investigation was to evaluate the effect of bacterial diamines cadaverine and putrescine on the production of interleukin-10 in a culture of human mononuclear leukocytes.

Materials and methods

The object of the study was peripheral venous blood leukocytes obtained from 18 healthy male volunteers (mean age 24.0±0.6 years). The non-inclusion criterion was female gender, since the activity of human leukocytes is under the influence of female sex hormones, the level of which changes cyclically. The isolation of leukocytes was carried out from heparinized blood by gradient centrifugation using a ficoll-verografin mixture with a density of 1.077 g/cm^3. After collecting the interphase part, the cell suspension was stirred and washed three times.

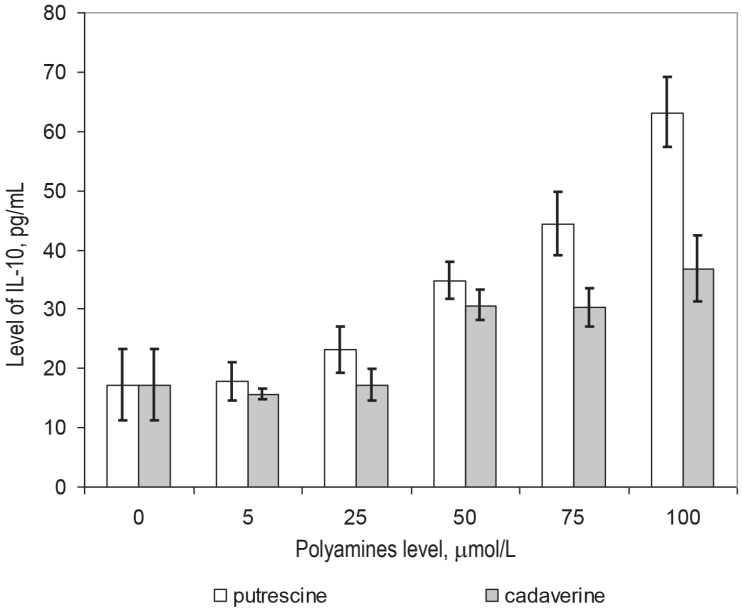

Figure 1. Influence of bacterial polyamines on the production of IL-10 by human leukocytes

The micromethod and plastic round-bottom 96-well plates were used for culturing lymphocytes. Each culture contained 2×10^5 cells in complete culture medium. The latter was prepared *ex tempore* based on medium 199 supplemented with 2 μM L-glutamine, 10 μM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 100 μg/mL gentamicin sulfate, and 10% plasma. Concanavalin A at a concentration of 5 μg/mL was used as a T-cell mitogen. Polyamines were used at final concentrations of 5, 25, 50, 75, and 100 μM/L. Cultivation was carried out in a humid atmosphere with 5% CO_2 at 37 °C for 72 hours. At the end of the incubation period, the culture liquid was pulled together and frozen for subsequent determination of the concentration of interleukin-10. Reagent kits for enzyme immunoassay for determining the concentration of IL-10 (Russia) were used.

Statistical analysis was carried out using the Statistica 6.0 software package. The arithmetic mean (M) and the standard error of the arithmetic mean (m) were calculated. The Shapiro–Wilk test was used to check the normality of the distribution. In the case of a distribution close to normal, Student's t-test was used. In the rest, the Mann–Whitney test was used to assess the significance of differences. The critical level of significance (p) when testing statistical hypotheses was taken equal to 0.05.

Results and discussion

When studying the production of IL-10 by leukocytes in the presence of Concanavalin A, it was shown that the average level of IL-10 production was 17.13±6.08 pg/mL. Under the influence of putrescine and cadverine at concentrations up to 25 μmol/L, the secretion of IL-10 does not change significantly (Figure 1). With an increase in the concentration of polyamines, an increase in the synthesis of IL-10 is observed. At the same time, putrescine at a concentration of 100 μmol/L stimulates the secretion of IL-10 2 times more than cadaverine at the same concentration.

The results obtained partly indicate a possible mechanism for the development of low-symptomatic chronic inflammatory diseases. As is known, in the focus of invasion of microorganisms, there is an increase in the concentration of polyamines, putrescine and cadaverine [1], which, by acting on the cells of the immune system, provide an increase in the concentration of IL-10. It is known that IL-10 suppresses the production of proinflammatory cytokines and nitric oxide, which significantly reduces the antimicrobial effect of leukocytes [3]. And this, in turn, is a condition for the persistence of microorganisms. Moreover, since IL-10 is an inhibitor of inflammation and the cytokine cascade, an asymptomatic clinical picture of inflammatory diseases caused by polyamine-producing microorganisms should be expected.

Conclusion

Thus, polyamines of bacterial origin, putrescine and cadaverine, affect not only the microbial community, but also immunocompetent human cells, for example, by changing the production of IL-10.

References

1. Banerji R., Kanojiya P., Patil A., Saroj S.D. Polyamines in the virulence of bacterial pathogens of respiratory tract. *Mol. Oral Microbiol., 2021, Vol. 36, no. 1, pp. 1-11.*
2. Godovalov A.P., Nikolaeva N.V., Karpunina T.I., Oborin D.A. On the assesment of the etiological significance of bacteria detected in the male genital tract. *Journal of Microbiology, Epidemiology and Immunobiology, 2022, Vol. 99. no. 4, pp. 428-435.* (In Russ.)
3. Haskó G., Kuhel D.G., Marton A., Nemeth Z.H., Deitch E.A., Szabó C. Spermine differentially regulates the production of interleukin-12 p40 and interleukin-10 and suppresses the release of the T helper 1 cytokine interferon-gamma. *Shock, 2000, Vol. 14, no. 2, pp. 144-149.*
4. Oborin D.A., Nikolaeva N.V., Godovalov A.P., Karpunina T.I. Etiology of purulent-inflammatory processes in the genital tract: suspicions of clinicians and problems of laboratory confirmation. R*ussian Clinical Laboratory Diagnostics, 2020, Vol. 65, no. 5, pp. 328-331.* (In Russ.)

Авторы:

Годовалов А.П. *— к.м.н., ведущий научный сотрудник ЦНИЛ ФГБОУ ВО «Пермский государственный медицинский университет имени академика Е.А. Вагнера» Министерства здравоохранения РФ, г. Пермь, Россия*

Морозов И.А. *— соискатель ФГБОУ ВО «Пермский государственный медицинский университет имени академика Е.А. Вагнера» Министерства здравоохранения РФ, г. Пермь, Россия*

Authors:

Godovalov A.P., *PhD (Medicine), Leading Research Associate, Central Research Laboratory, E.A. Vagner Perm State Medical University, Perm, Russian Federation*

Morozov I.A., *PhD Student, E.A. Vagner Perm State Medical University, Perm, Russian Federation*

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