PSEUDOMONAS AERUGINOSA SIGNIFICANTLY INCREASES EXPRESSION OF RECEPTOR FOR ADVANCED GLYCATION ENDPRODUCTS (RAGE) IN THE SEPTICEMIA SUFFERING PATIENTS

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Abstract. Receptor for Advanced Glycation Endproducts (RAGE) is a cell surface receptor, which recognizes several endogenous and exogenous molecules and subsequently induces expression of several molecules including chemokines. Chemokines are members of the cytokine superfamily and participate in several immune system functions, including cell migration, inflammation, angiogenesis/angiostasis etc. CXC ligand 11 (CXCL11) is an important chemokine which participates in the induction of appropriate immune responses against microbes, including bacteria. The main mechanisms responsible to overcome septicemia are yet to be clarified. Thus, it has been hypothesized that RAGE may participate in induction of CXCL11 in response to the microbial agents. Due to the fact that immune responses play key roles in limitation of infection, it has been proposed that RAGE may inhibit spread of septicemia. Therefore, in this project mRNA levels of RAGE and CXCL11 were explored in the patients suffering from septicemia versus healthy controls. RAGE and CXCL11 expression levels in the 80 subjects, including 40 septicemia patients and 40 healthy controls were explored using Real-Time PCR technique. Accordingly, by using the specific primer against RAGE and CXCL11 in a Rotorgene vehicle the mRNA levels have been determined. The septicemia and the sources of the bacteria in the blood were diagnosed using microbial cultures. The results demonstrated that although mRNA levels for RAGE and CXCL11 did not change in the septicemia patients vs. healthy controls, mRNA levels of RAGE were significantly higher in the patients infected by Pseudomonas aeruginosa compared to those infected by other bacteria, Escherichia coli, Staphylococcus aureus, and Acinetobacter baumannii. RAGE and CXCL11 mRNA levels did not differ among male and female patients. Based on the results it seems that RAGE is a critical receptor against P. aeruginosa during septicemia and more investigations, especially on the RAGE down-stream molecules can clarify its main roles against P. aeruginosa.

Key words: septicemia, RAGE, CXCL11, gene expression.
кинов и участвуют в процессах миграции клеток, воспаления, в angiogeneze/angiostaze и т. д. СХС-лиган 11 (CXCL11) является важным хемокином, участвующим в индукции антимикробного (в том числе антибактериального) ответа. Основные механизмы, ответственные за излечение от сепсиса, еще предстоит выяснить. Было высказано предположение, что RAGE может участвовать в индукции CXCL11 в ответ на микробные агенты.

В связи с тем, что иммунный ответ играет ключевую роль в ограничении инфекции, было предложено, что RAGE может сдерживать сепсис. Поэтому в настоящем проекте были исследованы уровни мРНК RAGE и CXCL11 у пациентов, страдающих сепсисом, и проведен их сравнение с аналогичными показателями здоровых людей контрольной группы. Уровни экспрессии RAGE и CXCL11 у 80 субъектов, включая 40 пациентов с сепсисом и 40 здоровых людей из контрольной группы, были исследованы с использованием метода ПЦР в реальном времени. В амплификаторе RotorGene с использованием специфического праймера против RAGE и CXCL11 были определены уровни мРНК. Сепсис и источники бактерий в крови диагностировали с помощью культурального метода. Результаты показали, что, хотя уровни мРНК RAGE и CXCL11 не изменились у пациентов с сепсисом по сравнению со здоровыми людьми из контрольной группы, уровни мРНК RAGE и CXCL11 были значительно выше у пациентов, инфицированных Pseudomonas aeruginosa, по сравнению с пациентами, инфицированными другими бактериями: Escherichia coli, Staphylococcus aureus и Acinetobacter baumannii. Уровни мРНК RAGE и CXCL11 не различались у пациентов мужского и женского пола. На основании полученных результатов можно сделать вывод о том, что при сепсисе присутствие RAGE является критически важным фактором, а уровень CXCL11 может быть использован как маркер активности инфекции.

**Ключевые слова:** септицемия, RAGE, CXCL11, экспрессия гена.
Identification of septicemia. Due to the fact that the samples which are used in this project were obtained in our previous investigations, the infected patients were as our previous study as follow: 7 Escherichia coli, 10 Staphylococcus aureus, 17 Acinetobacter baumannii and 6 Pseudomonas aeruginosa infected patients.

Expression levels of RAGE and CXCL11. Results demonstrated that RAGE mRNA levels in the peripheral blood immune cells (PBIC) of the septicemia patients were 0.0204 (0.0048–0.2180) and in the PBIC of healthy controls were 0.1362 (0.0254–1.8975). Statistical analysis demonstrated no significant difference (p = 0.095). CXCL11 mRNA levels in the septicemia patients were 0.0483 (0.0033–0.5981) and in healthy controls were 0.3489 (0.0935–0.8956). Statistical analysis demonstrated no significant difference (p = 0.057). Fig. 1 shows the expression levels of RAGE and CXCL11 in both septicemia patients and healthy controls.

Expression levels of RAGE (p = 0.079) and CXCL11 (p = 0.208) were also not significantly different in the female when compared to male septicemia patients (Fig. 2).

Results show that, although RAGE mRNA levels (p = 0.020) were significantly in the P. aeruginosa (23.5920 [1.0118–100.2772]) infected septicemia patients than the patients with E. coli (0.0204 [3.0005–0.4805]), S. aureus (0.0342 [0.0110–0.8573]), A. baumannii (0.0087 [0.0021–0.0302]) infection, the mRNA levels of CXCL11 were not changed among the groups (p = 0.382, Fig. 3).

The results also revealed that there were a positive moderate correlation between RAGE and MDA5 mRNA levels in the septicemia patients. However, there were no significant correlations between other variables in the septicemia patients (Table).

Discussion

The results demonstrated that patients were not expressed RAGE and CXCL11 in different manner when compared to the healthy controls. However, the results revealed that the patients who infected by P. aeruginosa had higher mRNA levels of RAGE than the patients who were infected by other bacteria. Therefore, it appears that, although RAGE cannot be considered as important receptor against septic-
mia, it is important molecule to recognize P. aeruginosa in the Iranian patients who were suffered from septicemia. Interestingly, the results also confirmed that there is a significant positive correlation between MDA5 and RAGE in infected patients. As mentioned previously, MDA5 is a main intracellular sensor which induces expression of several immune related molecules such as RAGE [12]. Due to the fact that our previous results demonstrated that MDA5 and RAGE significantly increased in the septicemia patients [1], hence, it seems that the results confirmed the roles played by MDA5 to increase expression of RAGE. So, it may be hypothesized that RAGE activated immune responses against P. aeruginosa in dependent of MDA, but not RIG-1 and CXCL11. It has been documented that septicemia patients up-regulate pro-inflammatory molecules in the nuclear factor-κB (NF-κB) dependent manner [6, 13]. NF-κB is an important well-known transcription factor which is activated by several intracellular signaling pathways, including MDA5 and also RAGE dependent pathways [8, 10]. Thus, it may be concluded that the septicemia patients who were infected by P. aeruginosa expressed RAGE to overcome the bacteria infection in a positive feedback with MDA5 in NF-κB dependent.

The results also revealed that RAGE and CXCL11 mRNA levels were not changed between male and female patients and also had not correlate with age. Thus, it may be proposed that sex and age are not the critical risk factors for taking place of septicemia.

Finally, due to the results, it seems that the type of bacteria in the septicemia is a critical factor for involvement of RAGE, as an important extracellular receptor, during septicemia.

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References


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