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SCIENTIFIC CONCEPTS OF CYTOKINE GENE EXPRESSION

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Abstract
Cytokines are signalling molecules of the immune system. The level of expression of these proteins has an important diagnostic value for the selection of immunocorrective therapy and prediction of the intensity and direction of the immune response. In order to determine which cytokines are synthesised and in what quantity, the PCR method is widely used.

Keywords: polymerase chain reaction, natural DNA replication, fragments, molecular biology.

Introduction

Polymerase chain reaction is a technique that mimics natural DNA replication and allows the detection of a few specific DNA molecules in the presence of millions of other molecules. The discovery of the polymerase chain reaction method was one of the most outstanding developments in the field of molecular biology in recent decades. The method is based on multiple selective copying of a certain DNA strand using enzymes under artificial conditions (in vitro). In this case, only that section is copied that fulfils the specified conditions and only if it is present in the sample under study. PCR amplifies relatively short stretches of DNA. In a conventional PCR process, the length of the copied DNA fragments is no more than 3000 base pairs. With the help of a mixture of different polymerases, using additives and under certain conditions, the length of a PCR fragment can reach 20-40 thousand nucleotide pairs.
PCR analysis in the laboratory is performed in three steps:

1. DNA extraction
2. amplification of DNA fragments
3. Detection of DNA amplification products

DNA extraction is the initial stage of PCR diagnostics, the essence of which is as follows: the doctor takes the patient's material for examination and subjects it to special processing. During processing, the DNA double helix is split into individual strands. A special liquid is added to the patient's material to dissolve organic substances that interfere with the "purity" of the reaction. This removes lipids, amino acids, peptides, carbohydrates, proteins and polysaccharides. As a result, DNA or RNA is formed [3,5,.7,9,10].
DNA amplification and, consequently, the whole principle of PCR reaction is based on the process of DNA amplification, which is natural for all living things - DNA replication, which is carried out by doubling a single DNA strand.

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Starting with a single fragment of DNA, the doctor-laborator copies it and increases the number of copies in chain reaction mode: after the first cycle you already have 2 fragments, after the second cycle - 4, after the third - 8, after the fourth - 16, then 32, 64, 128, 256.... With each cycle, the number of copies doubles, so that after twenty cycles the count is in the millions and after thirty cycles it is in the billions. The cycle lasts for a few minutes and is reduced to a certain change of temperature regime in a very small chemical reactor. Here, all the necessary synthesis components, especially nucleotides A, G, T and CD, are in sufficient quantity in the solution, and subtle preparatory chemical operations are carried out so that an exact copy is immediately taken from each finished piece of DNA, and then from this copy a copy again, which is what the branched chain reaction is all about. By attaching primers to the DNA strand, two short, double-stranded DNA helices are formed that are necessary for the synthesis of future DNA [1,2,4,6,8].

The synthesis of a new strand occurs by completing each of the two strands of DNA. The amplification process is carried out by a specific site, DNA polymerase, which gave its name to the laboratory method. The polymerase acts as a catalyst for the reaction and monitors the sequential attachment of nucleotide bases to the growing new DNA strand.

Thus, DNA amplification is the repeated increase in the number of DNA copies that are specific. All multiple amplification steps occur at different temperatures. PCR analysis is performed using specially programmable equipment - PCR thermostat or amplifier, which automatically performs temperature change. Amplification is carried out according to a preset programme corresponding to the type of infection to be detected. Depending on the programme and the type of infection to be detected, the automated PCR process takes from 2 to 3 hours [7,9].

In the process of detection of amplification products, the resulting mixture of amplification products is separated. Special solutions are added to the mixture, which give the DNA fragments the ability to fluoresce - to reflect orange-red luminous stripes.

The study of genes controlling the activity of cytokines that mediate inflammation is one of the important tasks in unravelling the pathogenetic links in the initiation and course of diseases, and early detection of predisposition to diseases. Knowledge of their role in the pathogenesis of many diseases allows, on the one hand, to predict the risk of pathology development or severity of its course, on the other hand, to individually select specific therapy for a particular patient [1,2].

The genetic control of proinflammatory cytokine expression is widely studied. Let us consider the functional polymorphism of the TNFA gene. The TNFA gene is located on the sixth chromosome (6p21.3) in the locus encoding molecules of the major histocompatibility complex of the first (HLA-A, B, C) and second classes (HLA-DP, DQ, DR). The location in the middle part of the genome determines the great variability of the locus, in particular, the promoter zone of the TNFA gene includes eight polymorphic sites with single nucleotide substitutions: -1031T/C, -863C/A, -857C/T, -575G/A, -376G/A, -308G/A, -244G/A, -238G/A. However, two are considered the most significant for humans. These are single nucleotide substitutions of guanine for adenine at positions: -308 (GRA) and -238 (GRA), which cause changes in the level of TNFα production, i.e. they are functional. Positions -308 and -238 are located at the promoter, which affects the ability of transcription factors to bind to this part of

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the gene and thus affect the rate of transcription. The -308 polymorphism increases the transcriptional activity of the TNFA gene and, consequently, cytokine production. The most active transcription of the polymorphic TNFA gene (-308*A) is in macrophages: in them it is 5 times higher than the transcription of the normal gene -308*G. Taking into account the fact that macrophages are the main source of TNFα, their genetically determined ability for increased production of the proinflammatory cytokine may be reflected in the development of inflammatory and immune reactions of the organism [5,7,8].

Another polymorphic region of the TNFA gene that affects cytokine production is position -238. However, in this case, the replacement of guanine by adenine leads not to an increase but to a decrease in protein production. So, stimulation of whole blood cells with lipopolysaccharide showed that cells with genotype -238GA synthesise 1.5 times less TNFα than cells with genotype -238GG [1,5,7,9].

In rheumatoid arthritis, which we will discuss in Chapter 3, the proinflammatory cytokines IL-1β and TNFα play a leading role in pathogenesis. The study revealed that patients with the -308G/A genotype of the TNFA gene have a more severe course of RA than those carrying the G/G genotype. Patients with the G/A allele had an earlier onset of the disease, higher activity, and a greater number of erosions. At the same time, in other patient populations, the allelic variant of the TNFA gene did not affect the severity and course of RA and was not associated with the disease. The study of IL1B gene polymorphism at the point (+3953 RT) of exon 5 revealed that the T/T genotype (A2A2 allele) is associated with more active RA compared to the C/C and C/T genotypes. From other data, the presence of the T allele at this point is known to be associated with a greater number of erosions in RA and high in vitro gene expression [2,3,4,9,10].

One of the most negative consequences of bacterial infection is septic shock. The main endogenous mediator of septic shock development is TNFα. In high concentrations, it can cause endothelial activation leading to vasodilation and blood pressure drop, disseminated intravascular coagulation (DIC), multi-organ failure, impaired thermoregulation, which in total leads to lethal outcome. Genotyping of children showed that the presence of at least one copy of the high-producing allele -308*A in a child's genotype increases the probability of mortality by 2.5 times. Mortality of children with polymorphic genotype -308 (AG, AA) was 3 times higher compared to carriers of the homozygous variant (-308 GG) of the TNFA gene.

The same applies to asthma: the substitution of G for A in position-308 is associated with increased production of this cytokine, which is associated with the risk of autoimmune pathology. Also the revealed correlation of the increased frequency of IL-10 -592A allele with the severity of sepsis, development of multi-organ failure and high probability of lethal outcome [1,2,7,8].

In HIV-infected patients, there are a number of differences with the control group of healthy people in the frequency of combinations of allelic variants of cytokine genes, associated with the appearance of the A/A homozygous variant of the IL-10 gene (RR = 2.36), not detected in healthy individuals, and the absence of the homozygous A/A variant of the TNFA gene (RR = -12.25), found in 4% of healthy women. The frequency of the A/G variant of the TNFA gene was four times higher in patients (RR = 4.67) due to the reduced frequency of both homozygous variants [4,5,6].

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The IL1RN gene encodes IL-1RA and is localised in chromosome 2. Carriage of the IL1RN*2 allele is associated with increased levels of circulating IL-1RA and mRNA expression during inflammation. The influence of IL1B (encoding IL-1β) and IL1RN gene polymorphisms on the nature of inflammation can be described in the form of the following trends: carriers of non-mutant variants of these genes determine adequate production of the corresponding proteins and regulation of IL-1 system functioning; in carriers of genetically determined bias towards IL-1β production inflammation is more acute, in carriers of genetically determined bias towards IL-1RA production inflammatory response is more prolonged, which may be the cause of chronicity of inflammation. Plasma IL-1RA levels are coordinately and co-regulated by the IL1B and IL1RN genes, and IL1RN*2 carriers are responsible for elevated levels of both circulating IL-1RA and IL-1β, whose increased activation of expression and production is a consequence of IL-1RA overproduction. According to this version, when the inflammatory response is realised in carriers of genetically determined bias towards IL-1RA production, the amount of this protein is greater than necessary for adequate realisation of inflammation, which causes compensatory formation of even more IL-1β. At the same time, more IL-1RA is also produced in response. Thus, carrying combinations of IL1B and IL1RN genes, which determine the preponderance of IL-1RA production, leads to a longer inflammatory response [1,5,6,7].

Thus, studies of gene polymorphism as a factor of genetic predisposition to various human diseases open new horizons in identifying risk groups and selecting the most optimal therapy for each patient. In the future, we can expect the emergence of preventive methods to correct predisposition to a wide range of diseases.

References

1. Shamsiddinova D. K., Kudratova Z. E. Genetikaning turli patologiyalar rivojlanishidagi ahamiyati //Journal of new century innovations. – 2024. – T. 51. – №. 2. – C. 198-201.
2. Kudratova Z. E., Shamsiddinova D. K. New insights into the etiopathogenesis of type 1 diabetes mellitus //Western European Journal of Medicine and Medical Science. – 2024. – T. 2. – №. 4. – C. 81-84.
3. Qudratova Z. E. et al. Qalqonsimon bez kasalliklarining zamonaviy klinik laborator tashxisot usullarI //Journal of new century innovations. – 2024. – T. 49. – №. 1. – C. 38-40.
4. Kudratova Z. E. et al. Modern methods of laboratory diagnostics of pyelonephritis //Web of Medicine: Journal of Medicine, Practice and Nursing. – 2024. – T. 2. – №. 1. – C. 38-40.
5. Muhamadiyeva L. A., Kudratova Z. E., Sirojeddinova S. Pastki nafas yo'llari patologiyasining rivojlanishida atipik mikrofloraning roli va zamonaviy diagnostikasi //Tadqiqotlar. Uz. – 2024. – T. 37. – №. 3. – C. 135-139.
6. Исомадинова Л. К., Даминов Ф. А. Современная лабораторная диагностика хронического пиелонефрита у детей //Journal of new century innovations. – 2024. – Т. 49. – №. 2. – С. 112-116.
7. Isomadinova L. K., Daminov F. A. Glomerulonefrit kasalligida sitokinlar ahamiyati //Journal of new century innovations. – 2024. – T. 49. – №. 2. – C. 117-120.

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8. Isomadinova L. K., Qudratova Z. E., Shamsiddinova D. K. Samarqand viloyatida urotiliaz kasalligi klinik-kechishining o'ziga xos xususiyatlari //Центральноазиатский журнал образования и инноваций. – 2023. – Т. 2. – №. 10. – С. 51-53.
9. Isomadinova L. K., Qudratova Z. E., Sh B. F. Virusli gepatit b fonida Covid-19 ning klinik laborator kechish xususiyatlari //Journal of new century innovations. – 2023. – Т. 30. – №. 3. – С. 60-65.
10. Isomadinova L. K., Yulayeva I. A. Buyraklar kasalliklarning zamonaviy diagnostikasi //Центральноазиатский журнал образования и инноваций. – 2023. – Т. 2. – №. 10 Part 3. – С. 36-39.

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