

Age-related differences in the cytokine profile in the lungs of Syrian hamsters (*Mesocricetus auratus*) infected with SARS-CoV-2 virus

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Abstract. Cytokine profile and age play a significant role in COVID-19. A hyperinflammatory response caused by proinflammatory cytokines can lead to cytokine storm and tissue damage. And the levels of interferons, which play an essential role in antiviral immunity, decrease with age. In this regard, there is a need to study the age-related characteristics of COVID-19. Syrian hamsters (*Mesocricetus auratus*) are the primary model objects for studying the SARS-CoV-2 infectious process. Classic laboratory animals such as mice and rats are not infected by this virus. Current research aimed to examine and compare the cytokine profile in the lungs of young (5-month-old) and aging (18-month-old) individuals of Syrian hamsters infected with the SARS-CoV-2 virus. The levels of cytokines were studied by ELISA: TNF- α , as the main proinflammatory cytokine; IL-6, as the predictor of severe COVID-19; INF- α and INF- γ , which are important components of antiviral immunity. The study showed that during COVID-19, the level of the proinflammatory marker TNF- α significantly increased in 5-month-old hamsters, while IL-6 increased in 18-month-old hamsters. Interferon levels were higher in young, healthy animals than in healthy, aging animals.

1 Introduction

In most cases, the course of COVID-19 is mild. [1]. However, several factors contribute to the severe course of this disease. Chronic disease, age, and cytokine profile may play a significant role in the duration and severity of COVID-19 [2, 3]. Dysregulation of the immune system may occur with age and be associated with changes in the subpopulations of immune cells [4]. It can contribute to severe disease or high mortality in infectious diseases. In addition, the effectiveness of vaccination decreases [5]. At the same time, levels of proinflammatory cytokines may increase with age.

This study focused on the role of four cytokines: TNF- α , IL-6, IFN- α , and IFN- γ . TNF- α is a major proinflammatory cytokine, which increases in response to a wide range of distress factors, including pathogens. IL-6 is also distinguished by its multifunctionality.

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However, elevated levels are associated with severe COVID-19 in aging people [6]. Several respiratory diseases, progression of cancer, depressive disorders, and autoimmune diseases are also associated with high levels of IL-6 [7-9]. However, the mechanism of the pathological effect of IL-6 on the body remains not fully understood. The likely reason is the large number of target cells and the multifunctionality of this interleukin. The importance of interferons in protection against viral pathogens is also important. INF- α and INF- γ are responsible for viral clearance and prevention of the development of pathological immune-mediated reactions. With age, immune cells, due to accumulated defects, lose the ability to produce interferons at the same level, which causes dysregulation of antiviral immunity [10]. However, their hypersecretion can also cause uncontrolled inflammatory reactions, leading to tissue damage [11]. Studying the cytokine profile in the lungs of Syrian hamsters under normal conditions and when infected with SARS-CoV-2 will help both in the study of age-related changes in this model and in the study of COVID-19.

2 Materials and Methods

2.1 Animal keeping

The research used outbred Syrian hamsters (*Mesocricetus auratus*) of both sexes, 5 and 18 months old. A total of 40 animals were involved in the study. Four groups of 10 animals (five females and five males per group) each were formed: 5-month-old control, 18-month-old control, 5-month-old infected with SARS-CoV-2, 18-month-old infected with SARS-CoV-2. All animals were kept in the Central Reference Laboratory (CRL) vivarium of the Masgut Aikimbayev's National Scientific Center of Especially Dangerous Infections (NSCEDI, Almaty, Kazakhstan). Animals were kept in individually ventilated cages (IVC) with HEPA filter (Allentown, USA) on special autoclaved SSNIFF bedding (Spezialdaten GmbH, Germany). The ambient temperature was $21 \pm 2^\circ\text{C}$, humidity $50 \pm 10\%$, and day/night cycle 10:14. The diet included the standardized complete food SSNIFF for hamsters (cat. no. V 2144-000) (Spezialdaten GmbH, Germany). Water and food *ad libitum*. The present study was conducted by national and international laws and guidelines for the handling of laboratory animals. The Institutional Committee of Keeping and Use of Laboratory Animals of the NSCEDI approved the study by the protocol № 14, dated December 09, 2022.

2.2 Pathogen

The study used the patented strain of coronavirus infection hCoV-19/Kazakhstan/KazNAU-NSCEDI-481/2020 (hCoV-19) (NSCEDI, The Republic of Kazakhstan) – a genetic feature of the strain: mutation D614G in the S protein [12]. The complete protein sequence is published in the GISAID database under EPI_ISL_514093. The virus was cultivated on a monolayer cell line Vero E6 in DMEM medium with the addition of 2% bovine fetal serum and an antibiotic-antimycotic solution (penicillin 10,000 U, streptomycin 10 mg and amphotericin B 25 mg) (Gibco, USA) at a temperature of 37°C in a CO₂ incubator in an atmosphere of 5% CO₂ for three days. The virus was injected into animals intranasally.

2.3 ELISA analysis in lungs

On the third day after infection, the animals were euthanized using CO₂ inhalation, after which cervical dislocation was performed. Afterward, an autopsy was performed, and pieces of the lungs were removed. Lung tissue was homogenized in a ratio of 1 to 10 PBS

(Sigma, USA) with protease inhibitor A32965 (Thermo Scientific, USA) to study the level of cytokines. Cytokines were measured by quantitative sandwich ELISA using hamster kits from MyBioSource (USA) for TNF- α (MBS7606475), IL-6 (MBS700950), IFN- α (MBS010919) and IFN- γ (MBS010146). Cytokine levels are given in picograms per gram (pg/g).

2.4 Biosafety

All work was done in an ISO 35001-certified Animal Biological Level Safety 3 (ABSL3) laboratory in the NSCDEI CRL, in a biological safety cabinet SG404, class II, type A2 (Baker, USA). A CRL biosafety officer participated in all work. The Biological Safety Committee approved the study by the protocol № 3, dated August 9, 2022.

2.5 Statistical analysis

All quantitative results are presented as mean $\pm$ SD. The analysis used the Kruskal-Wallis test and Dunn's post hoc test. Differences were considered significant for p-values less than 0.05. Statistical tests were performed in GraphPad Prism version 9.0 (GraphPad Software, La Jolla, CA, USA).

3 Results and Discussion

Signs of aging in 18-month-old hamsters were expressed in the formation of foci of alopecia and the manifestation of cystic liver disease, which we had previously shown in a previous study [13]. Also, within three days after infection, signs of the disease were observed: decreased activity and weight loss. At autopsy, pathological lesions of the lungs were noted at the macroscopic level. Lung pieces were removed to determine TNF- α , IL-6, IFN- α , and IFN- γ levels. Based on the results of the ELISA analysis, the concentration of cytokines in the tissue was determined.

It was observed that TNF- α levels were significantly increased in both males ($p \leq 0.0001$) and females ($p \leq 0.0001$) from the groups with young animals infected with SARS-CoV-2 (Fig. 1 A, D). An increase in IL-6 levels was observed in infected aged males compared to control young ($p \leq 0.05$) and aged controls ($p \leq 0.01$) (Fig. 1 C). A similar pattern was observed in females compared to control young animals ($p \leq 0.05$) (Fig. 1 D).

When studying interferons, an increase in the level of these cytokines was noted in young animals in control groups. IFN- α was increased in young opposite old individuals in both males ($p \leq 0.05$) and females ($p \leq 0.05$) (Fig. 2 A, B). A similar phenomenon was noted in the IFN- γ (C-D) study, where cytokine levels were higher in healthy young animals compared to healthy old animals, both in the case of males ($p \leq 0.01$) and females ($p \leq 0.01$). In males, there was an increase in IFN- γ in infected young individuals compared to old ones ($p \leq 0.05$), (Fig. 2 C).

When infected with the SARS-CoV-2 virus, the level of TNF- α increased in 5-month-old individuals. It has previously been shown that younger patients without comorbid conditions had higher levels of TNF- α [2]. At the same time, an increase in the concentration of IL-6, as the main predictor of severe COVID-19, was noted in 18-month-old individuals. IL-6 is produced by macrophages, fibroblasts, and T cells in response to the detection of viral particles by pathogen-recognition receptors (PRRs) [14]. Also, IL-6 is a marker of tissue damage, and its level increases in response to damage-associated molecular patterns (DAMPs), which are released from dying cells. Damage to vascular endothelial cells and alveolar epithelium in Acute Respiratory Distress Syndrome and

NETosis products cause massive formation of DAMPs, which may be the main factor leading to an increase in the level of this cytokine.

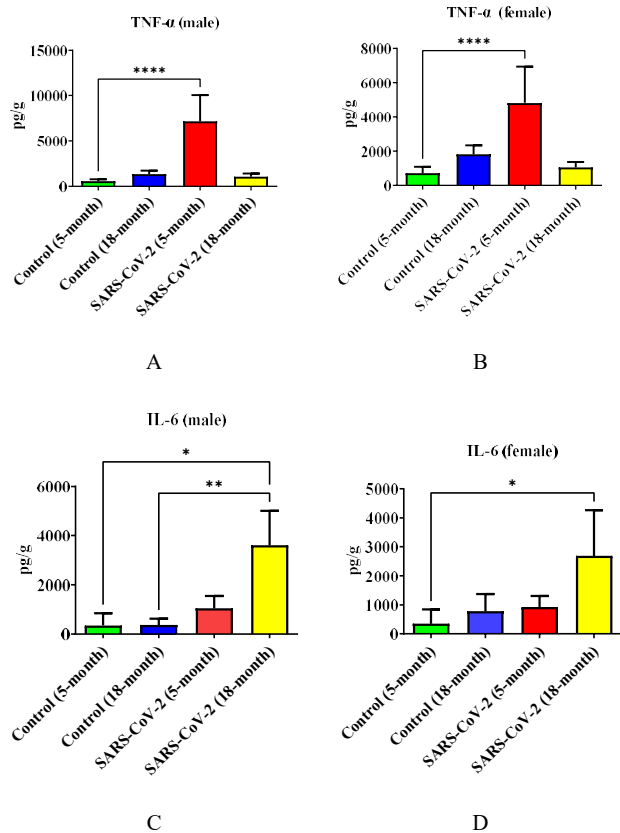


Fig. 1. ELISA results for TNF-α and IL-6 in Syrian hamsters infected with SARS-CoV-2 lungs. TNF-α levels have increased in young infected 5-month-old animals (A, B). Meanwhile, in aging 18-month-old individuals, there was an increase in IL-6 levels (C, D). Asterisks indicate the significance level as follows: *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001.

The study showed that the level of interferons in Syrian hamsters decreases with age. Type I interferons, which include IFN-α, are produced by plasmacytic dendritic cells. These cells reduce their functional activity with age, depleting their pool [10, 15]. Increased reactive oxygen species and cellular damage in senescent cells initiate defects in the expression of interferon regulatory factor 7 (IRF7), which regulates the production of interferons [16]. Decreased IFN-α levels lead to further uncontrolled immune reactions. The main one is the excessive recruitment of neutrophils, which trigger the NETosis program in response to viral infection. [17]. Neutrophil extracellular traps (NETs) are web-like nets consisting of intracellular components of neutrophils, the main task of which is to neutralize pathogens [18]. However, excessive formation of these products leads to tissue damage. Type II interferons, of which IFN-γ is the only member, are released by the Natural Killer cells and T cells during the viral infection [19, 20]. The antiviral response mediated by these cells is directly dependent on type I interferons. As a result of the disruption of their production, the level of IFN-γ will also decrease.

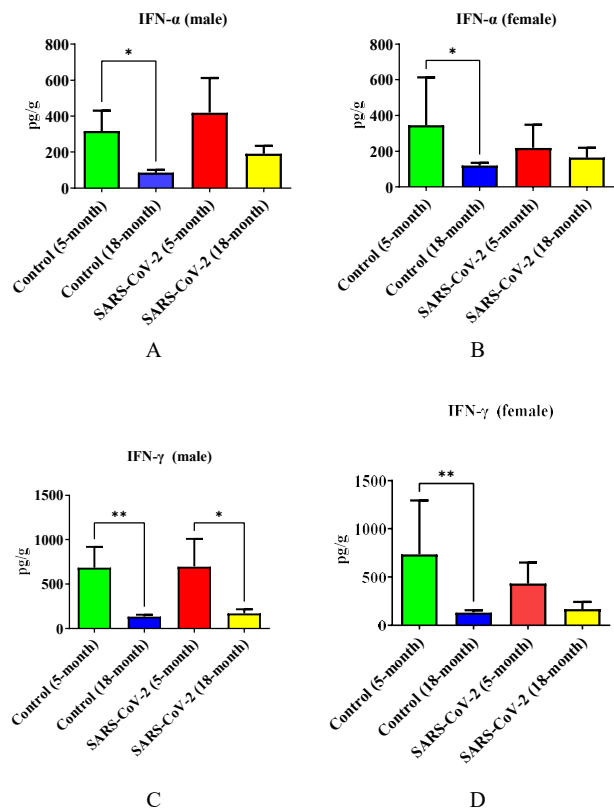


Fig. 2. ELISA results for IFN-α and IFN-γ in Syrian hamsters infected with SARS-CoV-2 lungs. Interferon levels were lower in 18-month-old animals (A-D). There were no differences in levels between infected animals, except INF- γ in males (C). Asterisks indicate the level of significance as follows: *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.

4 Conclusion

This study examined several immune response characteristics in Syrian hamsters infected with SARS-CoV-2. For these purposes, the cytokine profile in the lungs of 5- and 18-month-old individuals was studied for the levels of pro-inflammatory cytokines - TNF-α and Il-6, as well as IFN-α and IFN-γ, responsible for antiviral immunity. The data obtained show that 18-month-old Syrian hamsters exhibit a state of immunosenescence. Along with external signs of aging, dysregulation of antiviral immunity with age was noted in animals. Levels of IFN-α and IFN-γ were lower in 18-month-olds compared to 5-month-olds. This condition can lead to a more severe course of COVID-19. This is evidenced by an increase in Il-6 in a group of 18-month-old animals infected with SARS-CoV-2. At the same time, during infection in young individuals, the concentration of TNF-α increased significantly. The different cytokine profiles among Syrian hamsters' different age groups may indicate different immune response activation pathways during SARS-CoV-2 infection. At the same time, a decrease in the level of specific cytokines, mediated by immunosenescence, creates an unfavorable background for the course of the disease. A probable reason for this may be the depletion of the pool of immune cells and the accumulation of defects in them, leading to decreased functional activity and, consequently, the release of their products. Suppression of some immune mechanisms can lead to hyperactivation of others, which

ultimately leads to uncontrolled immune reactions, tissue damage, and severe COVID-19. Future research requires studying and specifying the mechanisms leading to decreased cytokine levels in Syrian hamsters. Generally, the data obtained is similar to such observed in human patients, what can probably be applied in further studies of COVID-19 and its treatment methods using the aging Syrian hamster model. Also, observation data can be considered when studying the course of other infectious diseases and assessing their severity, considering age.

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Authors' contribution

F.G.I. prepared the manuscript, executed the study, and interpreted the study; I.R.A. contributed to the concept and study design.

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