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Upregulated A20 (TNFAIP3) expression during respiratory syncytial virus infection in mice

Bahman Aghcheli [1], Romina Yavarinamini [2], Alireza Tahamtan [3,4*]

1. Infectious Disease Research Center, Gonabad University of Medical Sciences, Gonabad, Iran
2. Taleghani Children's Hospital, Golestan University of Medical Sciences, Gorgan, Iran
3. Department of Microbiology, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran
4. Infectious Diseases Research Center, Golestan University of Medical Sciences, Gorgan, Iran

* Correspondence: Alireza Tahamtan. Department of Microbiology, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran. Tel: +981732255331; Email: Alireza.tmn@gmail.com

Abstract

Background: Severe lower respiratory tract infections in infants and young children are frequently caused by respiratory syncytial virus (RSV), with the degree of illness strongly associated with disproportionate inflammatory activity. The signaling protein A20 (TNFAIP3) functions to inhibit NF-κB pathway activation, suggesting a possible role in tempering RSV-triggered lung inflammation. In this study, we assessed how RSV infection alters A20 gene expression in the lungs using a mouse model system.

Methods: Of the twelve female BALB/c mice allocated for the study, half were administered RSV intranasally at a concentration of 3 × 106 plaque-forming units (PFU), while the remaining six served as uninfected controls. All animals were humanely euthanized five days post-infection. Upon collection, lung tissue samples were immediately processed. The relative expression levels of messenger RNA (mRNA) for the TNFAIP3 gene, which encodes the A20 protein, were subsequently quantified using real-time reverse transcription polymerase chain reaction (RT-PCR).

Results: Analysis by quantitative PCR revealed that A20 expression was significantly higher in the lungs of RSV-infected mice compared with uninfected controls at day 5 post-infection (P = 0.0048).

Conclusion: The upregulation of A20 in RSV-infected mice suggests its potential role in modulating post-viral pulmonary inflammation.

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Introduction

Respiratory syncytial virus (RSV) represents a leading cause of acute lower respiratory tract illness in infants and young children, frequently resulting in clinical diagnoses of bronchiolitis and pneumonia (1). Infection initiates within the respiratory epithelium, provoking a robust inflammatory cascade. This response is characterized by the secretion of numerous pro-inflammatory signaling molecules, which in turn mediate the influx of innate immune cells, including macrophages and monocytes, into pulmonary tissue (2). Although this immunological activity is crucial for controlling viral replication, an overly aggressive or dysregulated response can contribute to significant tissue pathology and exacerbate clinical outcomes. Consequently, the central challenge in RSV immunopathology lies in achieving effective viral clearance while minimizing collateral inflammatory damage (3).

The protein A20, produced by the TNFAIP3 gene, acts as a vital regulator of inflammatory and immune signaling pathways. Its primary function is to serve as a negative feedback regulator, dampening activity in the NF-κB pathway - a key signaling cascade that activates genes responsible for the production of inflammatory mediators. Through this modulation, A20 plays an essential role in restraining excessive immune responses and maintaining tissue homeostasis (4). Impairments in A20 function are associated with numerous inflammatory and autoimmune disorders, underscoring its significance in limiting immune-related tissue injury during infections (5). Considering its fundamental role as a molecular checkpoint against inflammation, we hypothesized that RSV infection would alter A20 expression levels. Accordingly, this study was conducted to examine how RSV infection influences the expression of the A20 gene in the lungs of mice.

Methods

This laboratory experiment investigated differences between mice infected with respiratory syncytial virus (RSV) and a non-infected control group. Female BALB/c mice, aged five to seven weeks and weighing 15 - 18 grams, were used in the study. A total of twelve mice were obtained from the Pasteur Institute in Karaj, Iran, and were equally divided into two groups of six: One RSV-infected group and one control group. The animals were maintained in a controlled environment with unrestricted access to standard food and water. Prior to the study, all procedures received approval from the Animal Ethics Committee at Golestan University of Medical Sciences under the ethical code IR.GOUMS.REC.1397.341. The work was subsequently carried out in the university's animal housing facility, adhering strictly to established guidelines for research involving animals.

Mice were allocated into two experimental groups. Animals in the RSV-infected cohort received an intranasal inoculation, under anesthesia, with the RSV-A2 strain. This viral strain was provided as a gift by Dr. Salimi, and the challenge dose was standardized to 3 × 106 plaque-forming units (PFU). All infected subjects were humanely euthanized on the fifth day following infection, which corresponds to the documented peak of viral replication. Euthanasia was performed via ketamine injection (Ketamine 10%, Alfasan, Woerden, Netherlands), after which lung tissues were harvested for subsequent RNA isolation. Prior to inoculation, the RSV virus was cultured and amplified in HEp-2 cells (ATCC® CCL-23™). The titer of the infectious viral stock was quantified using a standard plaque assay performed on Vero cell monolayers (ATCC® CCL-81™), as reported in earlier work (6).

To evaluate gene expression, total RNA was first isolated from lung tissue samples. RNA extraction was performed using RNX-Plus solution (CinnaGen, Iran). The purified RNA was then converted into complementary DNA (cDNA) using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). Subsequent quantitative polymerase chain reaction (qPCR) analysis was carried out on an ABI PRISM 7900 sequence detection system (Applied Biosystems, USA). The reactions utilized SYBR Premix Ex Taq II (Takara Bio, Japan). The qPCR protocol included an initial denaturation step at 95°C for 10 seconds, followed by 40 amplification cycles consisting of denaturation at 95°C for 5 seconds and annealing/extension at 60°C for 40 seconds.

Specific primer sets were designed and used for the target gene, A20, and the reference gene, β-actin. Relative gene expression levels were calculated using the 2-ΔΔCt method. All target gene expression data were normalized to the endogenous β-actin control prior to final analysis (7).

Statistical analysis

Statistical analysis was conducted using GraphPad Prism software version 6.0 (GraphPad Software, San Diego, California). All data are presented as the mean ± standard error of the mean (SEM). Differences between experimental groups were assessed using unpaired, two-tailed Student's t-tests. A probability (P) value of less than 0.05 was considered statistically significant.

Results and Discussion

The core aim of this research was to determine whether RSV infection alters A20 gene expression in mouse lungs. Using quantitative PCR, we observed a clear and statistically significant increase in A20 expression in RSV-infected mice compared to healthy controls (P = 0.0048; Figure 1). This upregulation implies that RSV infection directly modifies transcription of the A20 gene, a pivotal player in immune regulation.

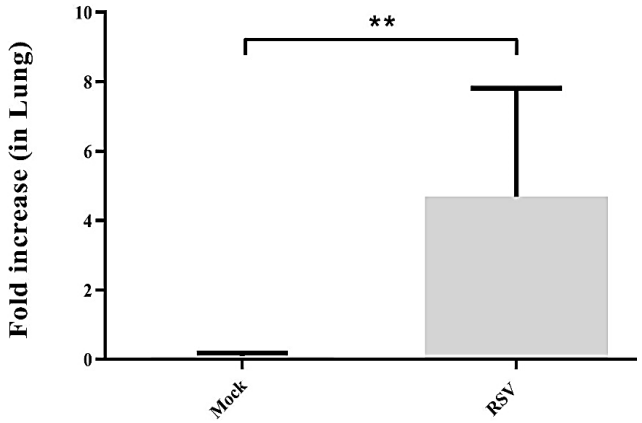

Figure 1. Effect of RSV infection on A20 expression in mouse lung tissue. Relative expression of A20 was evaluated in lung tissue of mice five days after infection using specific primers targeting the A20 gene and normalized to the housekeeping gene (β-actin). Results represent the mean ± SEM of six animals per group (** P = 0.0048).

The pathogenesis of RSV is largely driven by the host immune response, highlighting the importance of endogenous regulatory mechanisms (1). Our investigation revealed that RSV infection induces a substantial increase in the expression of A20 (TNFAIP3) messenger RNA within pulmonary tissue in a murine model. This marked upregulation was observed during the acute infectious stage, corresponding to day five post-infection, which represents the documented peak of viral load. This in vivo finding confirms and extends previous in vitro work, such as that by Martín-Vicente et al., who demonstrated that RSV induces a strong, delayed increase in A20 expression in human lung epithelial cells (4). Collectively, this evidence establishes A20 as a pivotal negative regulator of inflammatory signaling during RSV infection.

A20 functions as a molecular brake on the NF-κB and IRF3 signaling pathways. Mechanistically, it acts in complexes with partners such as TAX1BP1 and ABIN1 to deubiquitinate key kinases, including TBK1 and IKKε, thereby attenuating the RIG-I-initiated signaling cascade that drives the production of interferons, cytokines, and chemokines (8,9).

The functional consequences of A20 induction, however, appear to be dual in nature. Studies have shown that genetic ablation of A20 in epithelial cells leads to a heightened early innate immune response to RSV, characterized by elevated levels of cytokines, interferons, and interferon-stimulated genes, which correlates with a marked reduction in viral titers (10). This finding indicates that A20's anti-inflammatory role may inadvertently create a cellular environment more permissive to viral replication. Furthermore, A20 depletion enhances apoptosis in infected cells, a process that can serve as an antiviral defense (11). Thus, the upregulation reported in this study may also function to inhibit this cell death pathway, potentially prolonging the survival of infected cells.

The critical balance maintained by A20 is underscored by a genetic association study linking specific TNIP1 variants, which are associated with lower TNIP1 expression, to a reduced risk of severe RSV bronchiolitis in infants (10). This observation suggests that a less efficient A20/TNIP1 "brake" may permit a more potent yet controlled innate immune response, emphasizing that the activity level of this regulatory complex is a finely tuned determinant of disease outcome.

A notable limitation of this research is its focus on a single time point, specifically day five following infection. Although this phase represents the peak of infection, the study design does not elucidate the complete temporal expression profile of the A20 protein. While the increased A20 levels detected at this time point suggest its involvement in host immune regulation, its activity during the early or late stages of infection remains undefined. In addition, the study lacked confirmatory protein-level analyses, such as Western blotting, and did not include functional experiments to determine the consequences of altered A20 activity. Future studies incorporating longitudinal analyses and mechanistic evaluations will be essential to clarify the precise role of A20 in RSV disease progression.

Conclusion

Our data validate A20 as a key endogenous modulator of inflammation in a physiologically relevant model of RSV infection. Its role appears to be complex, limiting harmful inflammation while potentially facilitating viral persistence, thereby positioning it as a promising candidate for therapeutic intervention. Future investigations employing kinetic analyses and functional studies in conditional knockout models are necessary to define the therapeutic window for modulating A20 in order to reduce RSV-related lung damage without compromising viral clearance.

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Ethical statement

The experimental protocol for this investigation received formal ethical clearance from the institutional review board of Golestan University of Medical Sciences (Ethics approval code: IR.GOUMS.REC.1397.341).

Conflicts of interest

The authors report no potential conflicts of interest, including financial interests, related to this work.

Author contributions

Bahman Aghcheli was responsible for the study design and data collection and prepared the initial manuscript draft. Romina Yavarinamini contributed to the study design and investigation and also assisted in drafting the first version of the manuscript. Alireza Tahamtan developed the core concept, oversaw the methodology, and performed validation, formal analysis, and investigation. He also provided resources, supervised the project, secured funding, and conducted the review and editing of the manuscript.

Data availability statement

The data underpinning the primary conclusions of this study are available from the corresponding author upon receipt of a justified request.

References

1. Asseri AA. Respiratory Syncytial Virus: A Narrative Review of Updates and Recent Advances in Epidemiology, Pathogenesis, Diagnosis, Management and Prevention. J Clin Med. 2025;14(11):3880. [View at Publisher] [DOI] [PMID] [Google Scholar]

2. Chaumont A, Martin A, Flamaing J, Wiseman DJ, Vandermeulen C, Jongert E, et al. Host immune response to respiratory syncytial virus infection and its contribution to protection and susceptibility in adults: a systematic literature review. Expert Rev Clin Immunol. 2025;21(6):745-60. [View at Publisher] [DOI] [PMID] [Google Scholar]
3. Feldman C, Anderson R. RSV: an overview of infection in adults. Pneumonia. 2025;17(1):15. [View at Publisher] [DOI] [PMID] [Google Scholar]
4. Martín-Vicente M, González-Sanz R, Cuesta I, Monzón S, Resino S, Martínez I. Downregulation of A20 Expression Increases the Immune Response and Apoptosis and Reduces Virus Production in Cells Infected by the Human Respiratory Syncytial Virus. Vaccines (Basel). 2020;8(1):100. [View at Publisher] [DOI] [PMID] [Google Scholar]
5. Wu Y, He L, Li R, Li J, Zhao Q, Shao B. A20 as a Potential Therapeutic Target for COVID-19. Immun Inflamm Dis. 2025;13(1):e70127. [View at Publisher] [DOI] [PMID] [Google Scholar]
6. Sun Y, López CB. Preparation of Respiratory Syncytial Virus with High or Low Content of Defective Viral Particles and Their Purification from Viral Stocks. Bio Protoc. 2016;6(10):e1820. [View at Publisher] [DOI] [PMID] [Google Scholar]
7. Jiang X, Tian H, Fan Y, Chen J, Song Y, Wang S, et al. Expression of tumor necrosis factor alpha-induced protein 3 mRNA in peripheral blood mononuclear cells negatively correlates with disease severity in psoriasis vulgaris. Clin Vaccine Immunol. 2012;19(12):1938-42. [View at Publisher] [DOI] [PMID] [Google Scholar]
8. Catrysse L, Vereecke L, Beyaert R, van Loo G. A20 in inflammation and autoimmunity. Trends Immunol. 2014;35(1):22-31. [View at Publisher] [DOI] [PMID] [Google Scholar]
9. Maelfait J, Roose K, Bogaert P, Sze M, Saelens X, Pasparakis M, et al. A20 (Tnfaip3) Deficiency in Myeloid Cells Protects against Influenza A Virus Infection. PLoS Pathog. 2012;8(3):e1002570. [View at Publisher] [DOI] [PMID] [Google Scholar]
10. Martin-Vicente M, Mthiyane H, Jiménez-Sousa MA, Subramoney K, Hellferscee O, Wolter N, et al. TNFAIP3-interacting protein 1 polymorphisms and their association with symptomatic human respiratory syncytial virus infection and bronchiolitis in infants younger than one year from South Africa: A case-control study. Int J Infect Dis. 2023;136:107-10. [View at Publisher] [DOI] [PMID] [Google Scholar]
11. Priem D, Devos M, Druwé S, Martens A, Slowicka K, Ting AT, et al. A20 protects cells from TNF-induced apoptosis through linear ubiquitin-dependent and -independent mechanisms. Cell Death Dis. 2019;10(10):692. [View at Publisher] [DOI] [PMID] [Google Scholar]

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