


RESEARCH NOTE OPEN ACCESS

Mediation of LPS-Induced Inflammation With Pro-Resolving Treatment in Human Nasal Polyps: A Pilot Study

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Summary

1. Chronic inflammation occurring in nasal polyp tissues also contains some degree of active tissue inflammatory resolution in epithelial and immune cell populations.
2. Specialized pro-resolving mediators, such as resolvin D2, may mitigate inflammatory responses initiated by lipopolysaccharide exposure.
3. Active inflammation resolution is a novel approach to chronic rhinosinusitis (CRS) management, and more research is required to examine cell-specific temporal effects of specialized-pro-resolving mediators in CRS tissues.

contribution to etiopathogenesis or sustenance of inflammation in CRS. Nuclear factor-kappa B (NF- κ B) signaling is one of several important contributors in the modulation of important inflammatory responses. It is recognized as an important transcription factor in the expression of various pro-inflammatory genes in chronic inflammatory diseases including CRS [3].

Lipid-derived molecules known as specialized-pro-resolving mediators (SPMs) [4] have recently been described as active components in temporal modulation of acute airway inflammatory responses and may play some role in the CRS disease process [5–7]. SPMs influence development, recruitment, and function of several inflammatory cells, including macrophages, dendritic cells, neutrophils, and lymphocytes [8].

In this study, we seek to understand SPM regulatory effects on NF- κ B-associated pro-inflammatory genes using a fresh sinus tissue explant model. We hypothesize that SPM, resolvin D2 (RvD2), will mitigate lipopolysaccharide (LPS)-induced inflammation.

1 | Introduction

Chronic rhinosinusitis (CRS) shares a common pathophysiology with other chronic inflammatory diseases, including periodic acute exacerbations and altered wound-healing processes [1]. A healthy sinonasal mucosal barrier relies on immune function to appropriately respond to airborne insults while choreographing a temporally regulated resolution of the acute physiological inflammatory response [2]. When this spatiotemporal response is disrupted, chronic inflammation may result as a potential

2 | Methods

2.1 | Tissue Procurement

Nasal polyp tissue was obtained via endoscopic sinus surgery at Indiana University School of Medicine (IRB #14784). Nasal

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polyp tissues were obtained from three subjects. All of these patients had clinical features of type 2 inflammatory disease, with either allergic rhinitis, asthma, or aspirin-exacerbated airway disease (Table S1). Tissues from the middle meatus and ethmoid sinus cavities were collected from patients. Upon harvest, specimens were immediately taken to the laboratory, serially rinsed with phosphate-buffered saline (PBS), and gently centrifuged to remove excess blood and mucus. Tissues were then preserved in 1:1 PBS and 10% dimethyl sulfoxide in liquid nitrogen. When preparing for experimental exposures, tissue was thawed and weighed. Plates were filled with 0.04 g tissue in 1 mL culture medium. Each sample was exposed to 10 µg/mL LPS, 50 nM RvD2, and 10 µg/mL LPS + 50 nM RvD2, or exclusively culture medium for 24 h ± 1 h.

2.2 | RNA Isolation and Analysis

Tissue RNA was extracted and isolated, then cDNA was synthesized using a reverse transcription kit according to standard manufacturer protocol (see Supporting Information for additional details). A microarray NF-κB panel was used to amplify cDNA by real-time PCR. Fold increase was compared to control via $\Delta\Delta\text{CT}$ method, and those with the most significant differences were selected as candidate genes for further validation. To validate the effects of RvD2 on gene expression, qPCR for selected gene products was applied for CXCL1, G-CSF, and MYD88 in triplicate. qPCR was utilized to quantitatively assess the production of RNA in each of these genes with the same tissue samples used in PCR microarray.

Statistical analysis was performed using GraphPad Prism. A paired *t*-test was performed to test the effects of SPM treatment with RvD2 for each inflammatory mediator. Multiple comparisons correction was applied between groups, and an alpha <0.05 was set for statistical significance.

3 | Results

In the NF-κB microarray, the physiologically relevant genes with the highest fold-changes after LPS exposure were MYD88, CXCL1, and G-CSF. RvD2 treatment was also found to significantly alter expression of these genes (Figure 1). QPCR validation illustrated that G-CSF expression increased in response to LPS stimulation and was abolished with RvD2 treatment ($p < 0.01$; Figure 2). MYD88 and CXCL-1 were inconsistent in their expression response patterns. CXCL-1 and MYD88 exhibited no significant increase in expression at 24 h with LPS exposure compared to control, limiting experimental potential for beneficial effects of RvD2 treatment on these target genes.

4 | Discussion

CRS remains enigmatic in its pathophysiology, time course, and idiosyncratic responses to various treatments. Prior studies show that defects in the production of pro-resolving mediators, such as RvD2, have been implicated in the pathophysiology of airway disease [9]. Actions of pro-resolving mediators may offer a novel approach to treating the inflammatory processes that facilitate

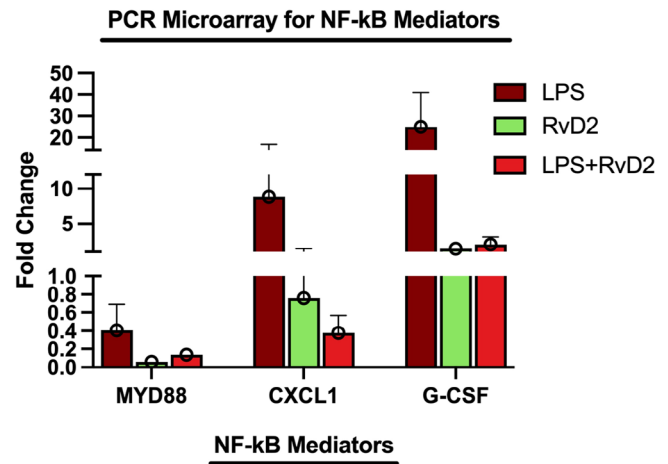


FIGURE 1 | PCR microarray for NF-κB mediators. Decreased expression of MYD88, CXCL1, and G-CSF genes with LPS + RvD2 groups with fold change compared to control.

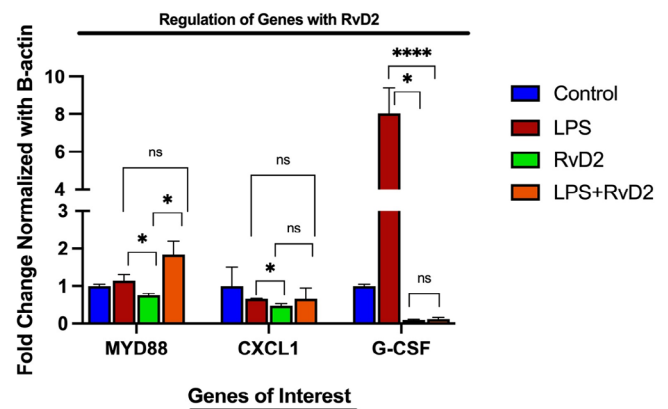


FIGURE 2 | Quantitative PCR validation of RvD2 effects with select gene targets. Following normalization with housekeeping gene, B-actin, G-CSF was found to be significantly downregulated in the LPS + RvD2 group compared to LPS alone. CXCL1 was found to be significantly upregulated in the RvD2 group when compared to LPS. MYD88 was found to be significantly downregulated in the RvD2 group when compared to LPS and upregulated in the LPS + RvD2 when compared to RvD2.

disease chronicity via attenuation of NF-κB effects in numerous cell types. In a PCR microarray, ex vivo polyp tissue treated with LPS for 24 h demonstrated a significant inflammatory response in G-CSF, MYD88, and CXCL1 expression, mitigated with concomitant RvD2 treatment. We attempted to validate the microarray results, and while MYD88 and CXCL1 showed an inconsistent response to LPS and RvD2, G-CSF expression pattern was convincing in its increase with LPS exposure and significant reduction with RvD2 treatment. G-CSF plays a vital role in innate immunity and inflammation by increasing the production and chemotaxis of neutrophils [10] and could be a biologically important target for SPMs in CRS.

While this pilot investigation of RvD2 effects on inflammation in CRS tissues showed some interesting findings, further work is required. The small study size ($n = 3$) could be expanded to include subjects with more neutrophilic or mixed inflammatory disease, such as nonpolyp CRS or select CRSwNP cases. More

cellular and molecular targets could be included, along with specific assessment of NF- κ B activity. The inflammatory response is dependent on temporal regulation, and additional exposure time courses to pro-inflammatory stimuli, RvD2, and other SPMs could be tested. Lastly, we utilized a novel ex vivo tissue culture approach to include the numerous cell types involved in CRS, and further work is required to understand nuances of this experimental model, including a detailed understanding of the ex vivo maintenance and function of the various cell populations.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

5 | Conclusion

This study establishes a potential effect of the specialized pro-resolving mediator, RvD2, in a CRS polyp tissue explant model. SPMs may elicit changes in gene expression that resolve active inflammation, including gene products that influence immune cell development and recruitment, and cytokines that direct acute phase responses.

Disclosure

VRR has served as a consultant for Medtronic, Inc., and 3D-Matrix, which are unaffiliated with the current study.

References

1. R. R. Orlandi, T. T. Kingdom, T. L. Smith, et al., "International Consensus Statement on Allergy and Rhinology: Rhinosinusitis," *International Forum of Allergy & Rhinology* 6, no. Suppl (1) (2016): S22–209.
2. A. Kato, R. P. Schleimer, and B. S. Bleier, "Mechanisms and Pathogenesis of Chronic Rhinosinusitis," *Journal of Allergy and Clinical Immunology* 149, no. 5 (2022): 1491–1503.
3. T. Liu, L. Zhang, D. Joo, and S.-C. Sun, "NF- κ B Signaling in Inflammation," *Signal Transduction and Targeted Therapy* 2, no. 1 (2017): 17023.
4. C. N. Serhan, "Pro-Resolving Lipid Mediators are Leads for Resolution Physiology," *Nature* 510, no. 7503 (2014): 92–101.
5. J. M. Owens, K. R. Shroyer, and T. T. Kingdom, "Expression of Cyclooxygenase and Lipoxygenase Enzymes in Nasal Polyps of Aspirin-Sensitive and Aspirin-Tolerant Patients," *Archives of Otolaryngology—Head and Neck Surgery* 132, no. 6 (2006): 579–587.
6. P. Z. Robinson, D. N. Frank, and V. R. Ramakrishnan, "Inflammation Resolution and Specialized Pro-Resolving Lipid Mediators in Chronic Rhinosinusitis," *Expert Review of Clinical Immunology* 19, no. 8 (2023): 969–979.
7. T. W. Vickery, M. Armstrong, J. M. Kofonow, et al., "Altered Tissue Specialized Pro-Resolving Mediators in Chronic Rhinosinusitis," *Prostaglandins Leukotrienes and Essential Fatty Acids* 164 (2021): 102218.
8. N. Chiang and C. N. Serhan, "Specialized Pro-Resolving Mediator Network: An Update on Production and Actions," *Essays in Biochemistry* 64, no. 3 (2020): 443–462.
9. B. D. Levy, I. Vachier, and C. N. Serhan, "Resolution of Inflammation in Asthma," *Clinics in Chest Medicine* 33, no. 3 (2012): 559–570.
10. S. Castro-Alcaraz, V. Miskolci, B. Kalasapudi, et al., "NF-Kappa B Regulation in Human Neutrophils by Nuclear I Kappa B Alpha: Correlation to Apoptosis," *Journal of Immunology* 169, no. 7 (2002): 3947–3953.