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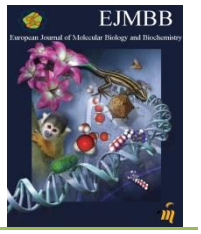

EXPLORING THE ROLE OF HMGB1 PROTEIN IN CHRONIC RHINOSINUSITIS WITH NASAL POLYPS: A COMPREHENSIVE IMMUNOHISTOLOGICAL STUDY

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ABSTRACT

Chronic rhinosinusitis with nasal polyposis can arise from various factors, including mechanical forces, viral attacks, bacterial infections, fungal exposure, immune disorders, and environmental pollutants. These stimuli can lead to epithelial damage and mucosal inflammation in the nasal cavity and paranasal sinuses, resulting in symptoms like nasal congestion, secretion, postnasal drip, and facial pain/headache. The release of cytokines, subepithelial edema, and infiltration of inflammatory cells, including eosinophils, neutrophils, mast cells, macrophages, and lymphocytes, characterize this condition. The HMGB-1 protein, implicated in inflammatory diseases, is released from damaged or necrotic cells, leading to the activation of endothelial function and enhanced survival of inflammatory cells by inducing pro-inflammatory mediators. This study aimed to investigate the expression of HMGB1 in chronic rhinosinusitis with nasal polyps, examining its correlation with eosinophil production, IL5 and IL8 levels typical of nasal and paranasal sinus inflammation, and its potential contribution to the pathogenesis of nasal polyposis. Immunohistochemistry was employed on nasal mucosa samples from 42 patients with nasal polyps, assessing HMGB1 protein presence in various tissue sections, including nuclear and cytoplasmic staining, focal extracellular infiltration, and inflammatory staining. The results indicated enhanced nuclear expression of HMGB1 in epithelial cells from patients compared to controls, while cytoplasmic staining was notably reduced. Inflammatory cells demonstrated significantly higher HMGB1 production in patients, whereas subepithelial focal areas expressed lower levels compared to controls. These findings, combined with existing studies, strongly suggest that HMGB1 plays a role in eosinophil infiltration in chronic rhinosinusitis with nasal polyposis.

INTRODUCTION

Various diseases may result in chronic rhinosinusitis, including polyps in the nose. Rhinosinusitis (viral, bacterial, fungal) may occur as a result of cystic fibrosis and primary ciliary dyskinesia [1]. Allergies, intolerances to medications, gastroesophageal reflux disease, pollution, and adverse reactions to medications can cause a dysreactive immune system.

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These cases present with subepithelial swelling, epithallium damage, eosinophil infiltration, mast cells, macrophages, and neutrophils infiltrations (mostly eosinophils), and inflammatory cell infiltrations [2].

The release of mediators and cytokines by inflammatory cells is a physiological response to infections and injuries of any kind, therefore inflammation should be considered a physiological defense against these infections and injuries. Amplification of the inflammatory process results from

recruitment and retention of inflammatory cells. Numerous inflammatory diseases have been linked to HMGB1 [3]. Specific membrane receptors 6 activate necrotic/damaged tissues 8 and immune-activated tissues 10, which release inflammatory mediators. Eosinophils, mainly, are protected by these mechanisms thanks to their ability to activate the endothelium and survive [4]. Among leukocytes and epithelia, this ancient evolution protein functions as an "alarmin" in the granules, cytoplasm, and nucleus. HMGB1, a late mediator of inflammation, is actively released by macrophages and monocytes in the extracellular medium. When exogenous pro-inflammatory products, such as endotoxins, are released from macrophages and monocytes, HMGB1 is actively released. Necrotic and damaged cells, including epithelia, can also passively release HMGB1. Endothelial cells are diffusely activated when HMGB1 is released into the environment [5]. Recent research shows that NP nasal mucosa expresses higher levels of NF-kB than NP. NF-kB is capable of promoting cytokine, chemokine, and adhesion molecule transcription through the NF-kB pathway, which has both pathogenic and therapeutic implications. In addition to NP treatment, transcription factors are also involved in the effects of corticosteroids. A HMGB1 protein may cause allergic or non-allergic nasal mucosal inflammation. A chronic inflammatory process resulting from chronic HMGB1 in the sinuses and nasal cavity may be triggered by HMG1, TNF, and IL.

MATERIALS AND METHODS

This collaborative study involving multiple countries and universities focused on analyzing a nuclear protein implicated in inflammation pathogenesis. The initial phase examined a common ENT pathology, with an ongoing study on inflammatory processes in the ENT district. 42 nasal polyp tissue specimens from patients with chronic rhinosinusitis with nasal polyps (CRSwNP) were collected at Beijing PLA General Hospital, including healthy controls, asthmatics, and allergic rhinitis patients. Diagnoses were confirmed through medical history, nasal endoscopy, and computed tomography scans following the European Position Paper on rhinosinusitis and nasal polyps 3 criteria. Atopic status was determined using a skin prick test for aeroallergens. Symptoms were assessed on a 0-10 scale, and Lund-Mackay classification was applied to CT scans. Exclusion criteria included specific medical conditions. Pre-surgery biopsy specimens were graded according to the Lund Kennedy system and subjected to immunohistochemistry for HMGB1, IL-5, and IL-16. Tissue freezing and subsequent analysis provided insights into the clinical characteristics of patients and controls [6].

As determined by hematoxylin staining 16 controls, 20 patients with eosinophilia, and 22 patients without eosinophilia with CRSwNP. In 20 HP fields (x400 magnification), the degree of infiltration of eosinophils was measured. An eosinophil count of more than 20 per HP field defined a patient as Eos CRSwNP. CRSwNP results for eosinophils and non-eosinophils were reported in Table II.

Immunohistochemical staining

A cryostat was used to section biopsy samples (7m) after fixation and dehydration. After air drying for 10 minutes, sections were permeabilized in PBS and placed on chromegelatin-coated slides. Endogenous peroxidases were blocked by applying 3% H2O2 to the skin for 10 minutes at room temperature in the dark. To prepare the slides for blocking, the slides were rinsed in PBS three times for 2 minutes each before being blocked for 20 minutes in PBS containing 10% normal horse serum. Incubation was carried out overnight at 4° with rabbit polyclonal antibodies (ab-18256, dilution 1:300), mouse monoclonal antibodies (ab25034, dilution 1:200), mouse monoclonal antibodies (ab1793, dilution 1:200) and goat polyclonal antibodies (ab10769, dilution 1:200). A five-minute rinse in PBS was followed by three rinses in PBS [7]. As an alternative, secondary antibodies were prepared using PV-9003 or PV-9000 kits. ZSJQ kits provided two minutes of diaminobenzidine development. Mayer's haematoxylin was used to counterstain the slides and neutral resin was used to mount them. Using normal rabbit serum as the positive control, PBS was used as the negative control.

SPSS13.0 software was used to perform all statistical analyses. Except where otherwise noted, all using a repeated measures ANOVA with LDS posthoc multiple comparisons. In cases where there were differences between two groups, Mann-Whitney U tests were applied. Spearman rank correlation and Pearson correlation are both used to evaluate correlations. An analysis of multivariate regression was conducted to determine whether the expression of HMGB1 protein correlates with eosinophils, IL-5, IL-8, and TNF-□ positive cells. The significance of a p value in statistics was defined as less than 0.05.

Table 1: Describe the clinical characteristics of the study participants

	Control subjects	CRSw	NP	p value
No. of subjects	16	20	22	–
Sex (male/female)	5/3	8/2	8/3	NS
Mean age(y), median range	37(22-70)	42 (30-54)	38 (17-56)	NS
Nasal congestion	2	18	22	< 0.001

Rhinorrhoea	0			18			18	0.001	
Headache	2			12			6	NS	
Facial pain/pressure	0			2			2	NS	
Hyposmia	0			16			12	0.014	
No. of bilateral polyps	0			20			8	NS	
Recurrent history	0			4			6	NS	
Atopy	Y	N	U	Y	N	U	Y N	U	NS
	0	8	8	4	10	6	0 10	12	
Asthma	0	16	0	2	18	0	0 22	0	
Increased eosinophils*		0			16		6		0.005
Increased lymphocytes*		0			4		2		NS

Table 2: By comparing CT scans and nasal endoscopic examinations, differences in symptoms between individuals with Eos CRSwNP and those without Eos CRSwNP were determined.

MS	3.30 ± 0.60	3.00 ± 0.68	NS
AES	4.10 ± 1.03	1.00 ± 1.61	NS
PES	3.50 ± 0.87	2.36 ± 1.29	0.041
SS	3.30 ± 2.03	0.61 ± 1.04	0.020
FS	3.50 ± 2.17	2.54 ± 1.43	NS0
OMCS	4.50 ± 0.60	3.55 ± 3.24	NS
ES	6.70 ± 2.75	4.37 ± 1.75	0.041
TS	26.50 ± 5.17	20.45 ± 6.84	0.010
Endoscopy	4.50 ± 2.26	4.55 ± 1.56	NS
Nasal congestion	8.5 ± 1.67	4.56 ± 1.58	NS
Rhinorrhoea	6.70 ± 1.53	6.81 ± 2.14	NS
Headache	4.50 ± 1.80	2.36 ± 2.21	0.041
Hyposmia	6.00 ± 3.16	4.35 ± 5.36	0.010

RESULTS

A nasal polyp from chronic rhinosinusitis eosinophilic patients, a chronic rhinosinusitis unaffected patient, and a nasal polyp from chronic rhinosinusitis naive patients, The immunohistochemistry staining of sections from patients with eosinophil chronic rhinosinusitis and nasal polyps, and patients without, CRSwNP patients and controls both showed immunohistochemical recognition of HMGB1. The staining was divided into four sections based on HMGB1 protein expression level: epithelial cytoplasm, epithelial nucleus, focal extracellular infiltration, and inflammation. CRSwNP patients with non-eosinophilic CRS expressed HMGB1 at higher levels than controls. The epithelial cytoplasm staining of HMGB1 was significantly reduced in patients with CRSwNP who were eosinophilic and non-eosinophilic. Subepithelial HMGB1 protein infiltration is lower in chronic rhinosinusitis with nasal polyps caused by eosinophils. There was an increase in HMGB1 expression in inflammatory cells associated with eosinophilic chronic rhinosinusitis with nasal polyps as well as non-eosinophilic chronic rhinosinusitis with nasal polyps.

DISCUSSION

It is clinically known as chronic rhinosinusitis if there is inflammation of the nasal mucosa associated with nasal polyps. According to a recently proposed hypothesis, CRS is caused by a defect or excessive immune response to foreign agents, which results in persistent influxes of inflammatory cells, since nasal anatomical variations do not seem to correlate with CRS incidence [8]. Since inflammation is primarily observed at the interface with the external environment, the incidence of CRS has not been correlated with nasal anatomical variations. It was widely believed that CRSwNP was a multifactorial disease in the past few decades [9], due to a lack of understanding of its aetiology and pathogenic mechanisms. CRS with or without nasal polyposis appears to be less determined by one microbial factor or environmental factor than by host susceptibility [10]. Cells with cilia, goblet cells, and respiratory epithelium form tight junctions to protect the sinus mucosa. Microbes and foreign substances are protected by mucociliary goblet cells. As a result of foreign proteins stimulating the immune system, the epithelial barrier is mechanically broken down, stimulating microbial colonization. Sinonasal epithelial cells 15 are responsible for adaptive immune responses and the physical barrier. Pattern recognition receptors (PRRs) on airway epithelial cells recognize molecular patterns associated with pathogens [11]. Epithelial cells attract innate defenses by releasing chemokines, cytokines, and innate protective agents. DAMPs, as well as PAMPs, can also help cells detect cellular damage [12]. A well-established DAMP, HMGB1, causes inflammation in the lower airways. Patients with CRSwNP have greater HMGB1 protein levels in their epithelial nuclei, or in their subepithelial

areas and have smaller HMGB1 proteins in their epithelial cytoplasm [13]. Chronic rhinosinusitis associated with nasal polyps containing Eos did not differ significantly in HMGB1 expression, indicating that the protein plays a role in the pathogenesis of chronic rhinosinusitis regardless of the underlying cause. Positive cells expressing IL-5, IL-8, or TNF-5 can affect the regulation of HMGB1. CRSwNP pathogenesis should be investigated through research on HMGB1.

CONCLUSIONS

This study explored the nuclear protein HMGB1 in chronic rhinosinusitis with nasal polyps (CRSwNP) and provided valuable insight into inflammatory processes within the ENT field. HMGB1 expression was found to differ in various cellular components between CRSwNP patients and controls, particularly in eosinophil infiltration. Clinical assessments, diagnostic criteria, and immunohistochemical analysis were combined in the study to understand CRSwNP pathogenesis. Eosinophilic involvement is particularly important in nasal and paranasal sinus mucosa, where HMGB1 plays a crucial role. In addition to shedlighting the link between HMGB1 and CRSwNP, the study also paves the way for future studies. We are deepening our understanding of ENT pathologies and exploring potential therapeutic targets through collaboration among several countries and universities. Continuing this research and exploring inflammatory processes in the ENT district will provide more nuanced insights that could ultimately result in better diagnosis and treatment strategies for chronic rhinosinusitis with nasal polyps. In order to advance ENT research, the authors continually discuss the results to ensure a robust and comprehensive interpretation.

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