



Basic Study

Inflammatory responses in esophageal mucosa before and after laparoscopic antireflux surgery

Pelin Ergun, Sezgi Kipcak, Nur Selvi Gunel, Eser Yildirim Sozmen, Serhat Bor

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Bordin DS, Russia

Received: December 5, 2023

Peer-review started: December 5, 2023

First decision: January 4, 2024

Revised: January 12, 2024

Accepted: February 28, 2024

Article in press: February 28, 2024

Published online: March 27, 2024



Pelin Ergun, Department of Otolaryngology, Medical College of Wisconsin, Milwaukee, WI 53226, United States

Pelin Ergun, Sezgi Kipcak, Serhat Bor, Division of Gastroenterology, Ege University, Faculty of Medicine, Ege Reflux Group, Izmir 35100, Turkiye

Sezgi Kipcak, Nur Selvi Gunel, Department of Medical Biology, Ege University, Faculty of Medicine, Izmir 35100, Turkiye

Eser Yildirim Sozmen, Department of Medical Biochemistry, Ege University, Faculty of Medicine, Izmir 35100, Turkiye

Corresponding author: Pelin Ergun, PhD, Postdoctoral Fellow, Department of Otolaryngology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, United States. pelinergun@yahoo.com

Abstract

BACKGROUND

Currently, the primary treatment for gastroesophageal reflux is acid suppression with proton pump inhibitors, but they are not a cure, and some patients don't respond well or refuse long-term use. Therefore, alternative therapies are needed to understand the disease and develop better treatments. Laparoscopic anti-reflux surgery (LARS) can resolve symptoms of these patients and plays a significant role in evaluating esophageal healing after preventing harmful effects. Successful LARS improves typical gastroesophageal reflux symptoms in most patients, mainly by reducing the exposure time to gastric contents in the esophagus. Amelioration of the inflammatory response and a recovery response in the esophageal epithelium is expected following the cessation of the noxious attack.

AIM

To explore the role of inflammatory biomolecules in LARS and assess the time required for esophageal epithelial recovery.

METHODS

Of 22 patients with LARS (pre- and post/5.8 ± 3.8 months after LARS) and 25 healthy controls (HCs) were included. All subjects underwent 24-h multichannel intraluminal impedance-pH monitoring and upper gastrointestinal endoscopy, during which esophageal biopsy samples were collected using endoscopic tech-

niques. Inflammatory molecules in esophageal biopsies were investigated by reverse transcription-polymerase chain reaction and multiplex-enzyme-linked immunosorbent assay.

RESULTS

Post-LARS samples showed significant increases in proinflammatory cytokines [interleukin (IL)-1 β , interferon- γ , C-X-C chemokine ligand 2 (CXCL2)], anti-inflammatory cytokines [CC chemokine ligand (CCL) 11, CCL13, CCL17, CCL26, CCL1, CCL7, CCL8, CCL24, IL-4, IL-10], and homeostatic cytokines (CCL27, CCL20, CCL19, CCL23, CCL25, CXCL12, migration inhibitory factor) compared to both HCs and pre-LARS samples. CCL17 and CCL21 levels were higher in pre-LARS than in HCs ($P < 0.05$). The mRNA expression levels of AKT1, fibroblast growth factor 2, HRAS, and mitogen-activated protein kinase 4 were significantly decreased post-LARS *vs* pre-LARS. CCL2 and epidermal growth factor gene levels were significantly increased in the pre-LARS compared to the HCs ($P < 0.05$).

CONCLUSION

The presence of proinflammatory proteins post-LARS suggests ongoing inflammation in the epithelium. Elevated homeostatic cytokine levels indicate cell balance is maintained for about 6 months after LARS. The anti-inflammatory response post-LARS shows suppression of inflammatory damage and ongoing postoperative recovery.

Key Words: Anti-reflux surgery; Gastroesophageal reflux disease; Cytokine; Inflammatory response; Esophagus

©The Author(s) 2024. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Even six months after laparoscopic anti-reflux surgery, specific pro-inflammatory cytokines continue to exhibit activity. Elevated levels of anti-inflammatory and regulatory cytokines suggest their involvement in preserving cellular homeostasis and regulating inflammation. As a precaution, we recommend that patients who have undergone laparoscopic anti-reflux surgery avoid refluxogenic foods to prevent short-term gastroesophageal reflux disease symptoms.

Citation: Ergun P, Kipcak S, Selvi Gunel N, Yildirim Sozmen E, Bor S. Inflammatory responses in esophageal mucosa before and after laparoscopic antireflux surgery. *World J Gastrointest Surg* 2024; 16(3): 871-881

URL: <https://www.wjgnet.com/1948-9366/full/v16/i3/871.htm>

DOI: <https://dx.doi.org/10.4240/wjgs.v16.i3.871>

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a chronic public health problem characterized by typical symptoms of heart-burn and/or regurgitation. It is a common worldwide condition and ranks among the most prevalent diseases in adults [1]. Although GERD is widespread, the factors initiating the pathogenesis of the disease are not fully understood. Two theories exist on the disease's pathogenesis. The first of these is the direct effect of gastric contents on the surface epithelium, where the inflammatory process begins in the lumen and advances with the assistance of dilated intercellular spaces [2]. However, this theory is insufficient to explain patients without erosion. Except for erosive esophagitis, no difference has been shown in other phenotypes of GERD according to the level of dilated intercellular spaces [1]. The other theory is cytokine-mediated damage. According to this theory, proinflammatory cytokines recruit immune cells, and tissue damage occurs as a result of the inflammatory response mediated by incoming immune cells starting from the basolateral cell layers [3]. Substances like acid and pepsin in the reflux content contribute to this damage [4].

The primary treatment modality currently is inhibiting gastric acid secretion with proton pump inhibitors (PPIs). However, some patients resist long-term drug usage. While erosive reflux patients generally respond well to PPIs, others require continuous medical treatment due to the absence of a cure through drug use [5]. In addition, some phenotypes of the disease do not totally or even partially respond to PPIs. Drug therapy provides temporary relief but is not a definitive treatment method. For treatment of the disease, exploring alternative therapeutic approaches becomes imperative. Understanding the disease pathogenesis is crucial to identifying target molecules for the development of preventive or therapeutic medications.

Since symptom resolution can be achieved in up to 93.1% of patients following laparoscopic anti-reflux surgery (LARS) [6], this modality is crucial for assessing the healing process of the esophageal epithelium after preventing the effects of noxious agents. The aim of this study was to investigate the role of inflammatory and recovery biomolecules after LARS by exploring the inflammatory pathways that may contribute to the pathogenesis of the disease. Additionally, we aimed to determine healing time frame to ascertain whether a meaningful period for healing allows the esophageal epithelium to fully recover.

MATERIALS AND METHODS

Subjects

In total, 35 patients with GERD who had been approved for LARS by the Ege University GERD Study Group, and 25 healthy controls (HCs) were included in the study. However, the follow-up upper gastrointestinal (GI) endoscopy continued with 23 patients, as 12 patients did not attend their post-LARS upper GI endoscopy appointments. The interval between the two upper GI endoscopies ranged from 2 to 18 months (mean 5.8 ± 3.8 months). All patients had pyrosis and/or regurgitation at least once a week or more frequently and completed the GERDQ (Validated Mayo Clinic) and QoLRAD (quality of life) questionnaires. Patients were ceased proton-pump inhibitors, H2 blockers, and antacids at least 10 d pre-procedure.

Esophageal motility tests were done before placing the multichannel intraluminal impedance-pH (MII-pH) catheter at the upper lower esophageal sphincter (LES) boundary. Data were analyzed using MMS software version 8.1 (MMS - Laborie, the Netherlands). An eight-channel motility catheter with four radial and four circumferential openings was used for motility measurements. After an 8-h fast, the catheter was placed 50-55 cm deep *via* the nasal passage. LES location was identified using intragastric pressure. For 24-h MII-pH monitoring, a calibrated MII-pH catheter (MMS - Laborie, the Netherlands) was positioned 5 cm above the LES, connected to a recording device (MMS - Laborie, the Netherlands). All HCs had normal intraesophageal 24-h MII-pH and high-resolution manometry and a negative history of upper GI disease or surgery. The patients with GERD who were treated with LARS already had a pathological reflux burden according to MII-pH monitoring and/or endoscopically observed esophageal erosions. Surgical indications were determined by the entire GERD team, which included specialists in gastroenterology, surgery, ENT, pulmonary medicine, and psychiatry.

The exclusion criteria for both patients and HCs included primary esophageal motility disorders, Barrett's esophagus, previous upper GI surgery, chronic renal failure, severe coronary artery disease, severe chronic obstructive pulmonary disease, uncontrolled diabetes mellitus, pregnancy, lactation, and other disorders that may affect the study, with the exception of cancer (except non-melanoma skin cancer).

Biopsy specimens

Upper GI endoscopy was conducted by one gastroenterologist (Bor S), and the biopsy samples were taken by one technician. Esophageal biopsy specimens ($n = 4$) were endoscopically taken from normal mucosa 3-5 cm above the Z-line without erosion using biopsy forceps (Radial Jaw 4, opening diameter 2.8 mm, Boston Scientific, United States). Two biopsies were preserved in RNazol[®] (GeneCopoeia, Rockville, MD) for subsequent mRNA studies at -80°C , while the remaining samples were immediately frozen at -80°C for later protein measurements (Figure 1).

Gene expression

The biopsy samples were homogenized using a Bioprep-6 Homogenizer (Hangzhou Allsheng Instruments Inc., Zhejiang, China), and total RNA was isolated with an Aurum[™] Total RNA Mini Kit (Bio-Rad Laboratories, Inc., Hercules, CA) following to the manufacturer's instructions. The absorbance, indicating the concentration and purity of the total RNA, was measured at 260/280 nm with a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE) using 2 μL of each homogenized and isolated sample.

cDNA was synthesized from total RNA in each sample using qPCR and an iScript cDNA Synthesis Kit with a reverse transcriptase enzyme (Bio-Rad Laboratories, Inc., Hercules, CA) following to the manufacturer's instructions. Real-time polymerase chain reaction was conducted using a LightCycler[®] 480 (Roche Diagnostics Inc., Basel, CH). iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA) and two different primer libraries - (Human JAK/STAT Signaling Primer Library and Human NF κ B Primer Library) Real Time Primers (LLC) - were employed according to the manufacturer's specifications. The housekeeping genes selected were actin-beta, beta-2-microglobulin, and ribosomal protein L13a.

Multiplex protein measurements

The biopsy samples were homogenized using a Bioprep-6 Homogenizer (Hangzhou Allsheng Instruments Inc., Zhejiang, China), and total protein was extracted with a Bio-Plex[™] Cell Lysis Kit (Bio-Rad Laboratories, Inc., Hercules, CA) according to the manufacturer's instructions. After centrifugation (4500 rpm for 10 min)[7], the isolated proteins were divided into aliquots, and protein amounts were determined using the Lowry method[7]. The protein levels of chemokines and phospho-cell signaling proteins were measured using Bio-Plex Multiplex Immunoassays (Human Chemokine 40-Plex panel, Pro Cell Signaling Phospho 7-plex panel, Pro Cell Signaling Phospho NF κ B p65 ve Pro Cell Signaling Phospho p38 MAPK, Bio-Rad Laboratories, Inc., Hercules, CA) according to the manufacturer's instructions.

Statistical analysis

The $2^{-\Delta\Delta\text{Ct}}$ method was used for the quantitation analysis of gene expression. The corresponding gene expression levels in each group were compared. Gene expression levels in each group were compared, and genes with a fold change ≥ 1.5 were included in the evaluation. Statistical analyses were performed using ANOVA, Student's *t* test (for parametric data) and the Mann-Whitney *U* test (for nonparametric data) with IBM[®] SPSS[®] Statistics 25.0. A *P* value of < 0.05 was considered statistically significant in all comparisons. A paired samples *t*-test was applied for pre-LARS and post-LARS comparisons. Parametric values were presented as mean \pm SD, while nonparametric tests used median and variance values.

Table 1 Demographics

Group	n	Gender	Age	BMI
GERD	22	9 females/13 males	42.9 ± 11.5	25.1 ± 2.5
HC	20	13 females/7 males	41.9 ± 10.8	24.5 ± 1.8

There is no significant relationship between gender, age and body mass index. GERD: Gastroesophageal reflux disease; HC: Healthy control; BMI: Body mass index.

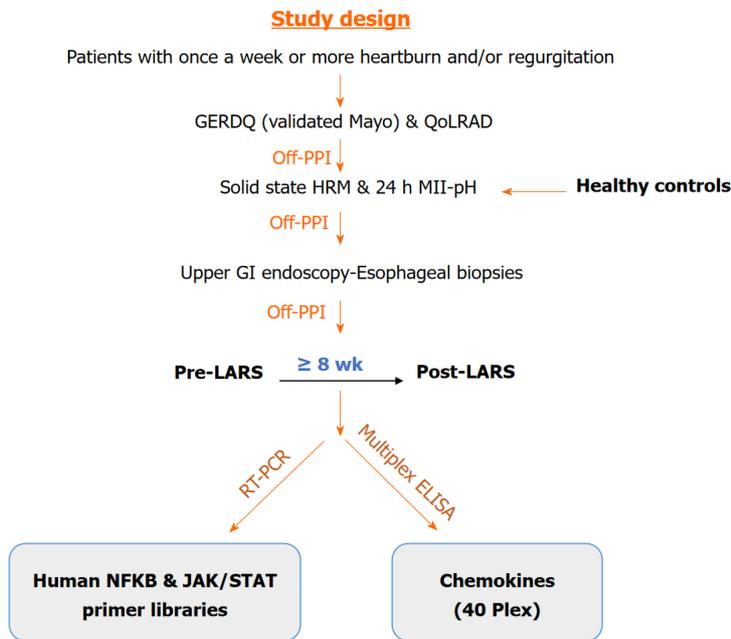


Figure 1 Study design. Pre-LARS: Pre-laparoscopic anti-reflux surgery; Post-LARS: Post-laparoscopic anti-reflux surgery; HRM: High-resolution manometry; MII-pH: Multichannel intraluminal impedance-pH; PPI: Proton pump inhibitor; RT-PCR: Real-time polymerase chain reaction; ELISA: Enzyme-linked immunosorbent assay; GI: Gastrointestinal.

RESULTS

Study group

One patient with erosive reflux disease (ERD) C/D out of 23 patients and 5 out of 25 HCs were excluded for various reasons: The presence of multiple polyps observed during upper GI endoscopy, excessive bleeding during biopsies, desaturation, and other related issues with the sedation procedure, as well as the relapse of erosions and/or symptoms after LARS. Ultimately, a total of 22 patients [10 ERD A/B, 6 ERD C/D, 6 non-ERD (NERD)] and 20 HCs were included in the study (Table 1).

Gene expression

CC chemokine ligand (CCL)2 (-2.3-fold) and epidermal growth factor (EGF) (-2.2-fold) gene expression levels were lower in the pre-LARS group compared to the HC group (Supplementary Table 1). On the other hand, mRNA expression levels of JUN (1.7-fold) and RAF1 (1.6-fold) were increased, while those of fibroblast growth factor 2 (FGF2) (-5.2-fold), mitogen-activated protein kinase 4 (MAP2K4) (-3.1-fold), EP300 (-2.8-fold), MCM5 (-2.8-fold), AKT1 (-2.4-fold), IRF9 (-2.3-fold), PIK3R2 (-2.3-fold), MYC (-2.2-fold), B-cell CLL/lymphoma 3 (BCL3) (-2.0-fold), PIAS4 (-2.0-fold), interferon (IFN) (alpha, beta and omega) receptor 1 (-2.0-fold), HRAS (-1.8-fold), IKBKE (-1.8-fold), RELA (-1.8-fold), TICAM1 (-1.5-fold), and PTPN11 (-1.5-fold) were decreased in the post-LARS group compared to levels in HCs (Figure 2A and Supplementary Table 2).

The fold changes in the post-LARS group compared to the pre-LARS group depicted in Figure 2B and Supplementary Table 3. While EGF (1.5-fold) and BCL3 (2.7-fold) expression increased after LARS, FGF2 (-5.4-fold), RIPK1 (-4.6-fold), tumor necrosis factor receptor superfamily, member 1 (-4.0-fold), MAP2K4 (-2.4-fold), HRAS (-2.2-fold) and AKT1 (-1.7 fold) decreased compared to pre-LARS measurements.

Protein levels

In the pre-LARS group, c-Jun levels were significantly lower compared to those in HCs ($P < 0.05$). Nuclear factor kappa-

Table 2 Cell signaling proteins

Protein	mean ± SD (FI)	Median	Variance
MEK1			
Post-LARS	344.9 ± 328.5	288.5 ^a	102992.8
Pre-LARS	416.3 ± 697.9	230	464957.9
HC	161.9 ± 151.4	102.5	22920.5
c-Jun			
Post-LARS	48.9 ± 17.4 ^a	49	287.8
Pre-LARS	43.8 ± 10.9	42	113.6
HC	50.9 ± 11	50.5	121.1
p38-MAPK			
Post-LARS	16.3 ± 15.2	10.0 ^a	219.9
Pre-LARS	12.1 ± 10.5	8	106
HC	7.3 ± 1.2	7	1.3
NF-κB			
Post-LARS	10.5 ± 1.9 ^{a,b}	10.5	3.4
Pre-LARS	8.8 ± 3	8	8.4
HC	8.2 ± 1.6	8.5	2.6

^a $P < 0.05$ vs healthy control.

^b $P < 0.05$ vs pre-laparoscopic anti-reflux surgery.

mean ± SD values are given for Student's *t* test, Median and Variance values are given for Mann-Whitney *U* test. LARS: Laparoscopic anti-reflux surgery; HC: Healthy control; NF-κB: Nuclear factor kappa-beta.

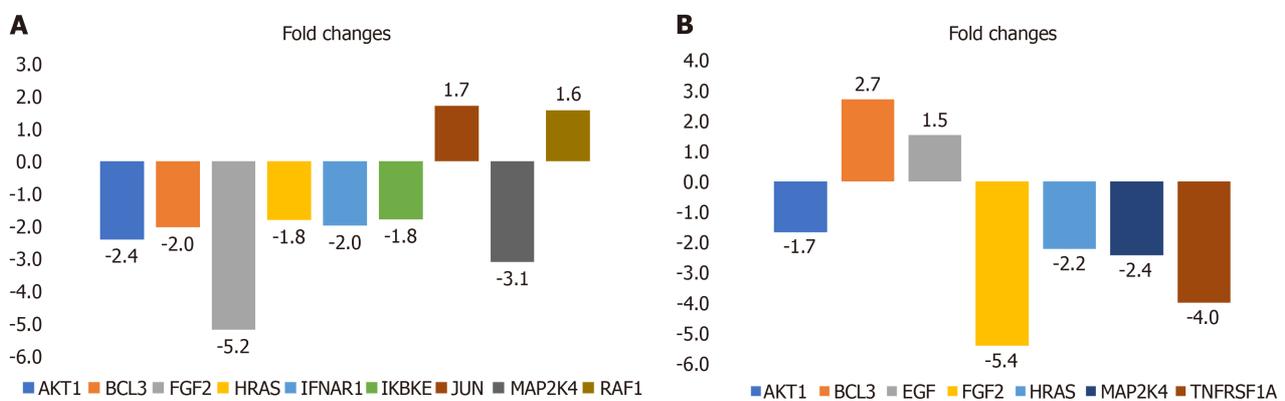


Figure 2 Significant gene expression. A: Significant gene expression in the post-laparoscopic anti-reflux surgery group compared to healthy controls; B: Significant gene expression in the post-laparoscopic anti-reflux surgery group compared to pre-laparoscopic anti-reflux surgery group. All comparisons are given as fold changes. BCL3: B-cell CLL/lymphoma 3; FGF2: Fibroblast growth factor 2; IFNAR1: Interferon (alpha, beta and omega) receptor 1; IKBKE: Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon; JUN: Jun proto-oncogene; MAP2K4: Mitogen-activated protein kinase 4; EGF: Epidermal growth factor; TNFRSF1A: Tumor necrosis factor receptor superfamily, member 1A.

beta (NFκB) ($P < 0.01$), MEK1, and p38-MAPK levels were significantly higher than those in HCs ($P < 0.05$) (Table 2). The levels of the proinflammatory cytokines interleukin (IL)-1β, IFNγ and C-X-C chemokine ligand 2 (CXCL2) were significantly higher in the post-LARS compared to both the pre-LARS and HC groups ($P < 0.05$) (Figure 3A, Supplementary Table 4).

Anti-inflammatory cytokines, including CCL11, CCL13, CCL17, CCL26, CCL1, CCL7, CCL8, CCL24, IL-4 and IL-10, showed a significant increase compared to levels in the HC and/or pre-LARS groups ($P < 0.05$) (Figure 3B-D, Supplementary Table 4). Specifically, CCL17 levels were higher in the pre-LARS group than in the HC group ($P < 0.05$).

The levels of homeostatic cytokines, including CCL27, CCL20, CCL19, CCL23, CCL25, CXCL12 and migration inhibitory factor (MIF), were higher in the post-LARS group than in the HC and/or pre-LARS groups (Table 3, Supplementary Table 4). Specifically, CCL21 levels were higher in the pre-LARS group than in the HC group ($P < 0.05$).

Table 3 Cytokine results indicating the homeostatic response

		mean ± SD (pg/mL)	Median	Variance
CCL21	Post-LARS	66.8 ± 75.5	46.5	5442.8
	Pre-LARS	123 ± 142.1	81.4 ^a	19274.4
	HC	48.9 ± 67.7	22.4	4588.6
CCL27	Post-LARS	8.5 ± 5.4 ^b	6.8 ^a	27.5
	Pre-LARS	4.4 ± 4.6	2.1	20.5
	HC	3.5 ± 2.1	3.2	4.5
CCL20	Post-LARS	13.4 ± 17.1 ^a	8	278.8
	Pre-LARS	9.4 ± 12.3	4.7	144.3
	HC	5.5 ± 4.8	3.1	22.8
CCL19	Post-LARS	62.2 ± 33.1 ^a	56.2	1048.1
	Pre-LARS	52.9 ± 77.1	29.5	5620.6
	HC	31.8 ± 23.3	23.4	544.5
CCL23	Post-LARS	14.0 ± 11.6 ^a	10.7	128.5
	Pre-LARS	10.8 ± 10.6	8.9	107.5
	HC	7.8 ± 4.9	7.7	24.1
CCL25	Post-LARS	629.7 ± 104 ^{a,b}	631.9	10318.7
	Pre-LARS	391.2 ± 170	315	27576.1
	HC	365.7 ± 121	379.7	14629.2
CXCL12	Post-LARS	76.2 ± 71.8	52.8 ^a	4912
	Pre-LARS	83.2 ± 147.1	48.6	20568.1
	HC	40.4 ± 20.2	33.8	408.6
MIF	Post-LARS	167465.0 ± 61928.5 ^{a,b}	160339	106780256
	Pre-LARS	95257.5 ± 61594.5	63357.2	362142872
	HC	89235.6 ± 51629.5	82917.8	266560193

^a*P* < 0.05 vs healthy control.

^b*P* < 0.05 vs pre-laparoscopic anti-reflux surgery.

CCL: CC chemokine ligand; CXCL: C-X-C chemokine ligand; LARS: Laparoscopic anti-reflux surgery; HC: Healthy control; MIF: Migration inhibitory factor.

DISCUSSION

GERD is typically treated with PPIs, aimed at suppressing gastric acid secretion. However, long-term drug use can pose challenges, and some patients may exhibit a low response to PPI treatment, necessitating a more permanent solution. Laparoscopic antireflux surgery is offered as an alternative method to alleviate reflux symptoms, with a success rate of approximately 90% [8,9] in experienced centers. Following LARS, the contact of the esophagus with gastric contents and noxious agents is significantly reduced, leading to a drastic alleviation of symptoms and observable healing of the epithelium during endoscopy, as seen in our patients.

In this study, CCL21 levels were found to be higher in pre-LARS patients compared to controls. These intriguing findings may be explained by the role of the CCL21/CCR7 axis in the regulation of T-cell immunity. Unsoeld *et al* [10] observed that transgenic mice with high expression of CCL21 failed in the CD4 T-cell response against local skin infections. They suggested that a high concentration of CCL21 downregulated CCR-7, which is responsible for mediating the T-cell adaptive immune response and peripheral tolerance [10,11]. It could be speculated that reflux disease is associated with an imbalance between CCL21 and CCR7 expression, characterized by an increase in favor of CCL21.

Chemotactic response of antireflux surgery

We investigated cellular-level changes before and after surgical treatment to comprehend the pathophysiological mechanism underlying GERD. Upon comparing data from patients' post-LARS to those from HCs, we observed an increase in IL1 β , MEK-1, p38 MAP kinase, and certain chemokine levels (CCL1, CCL19, CCL20, CCL21, CCL23, and CCL24) in the

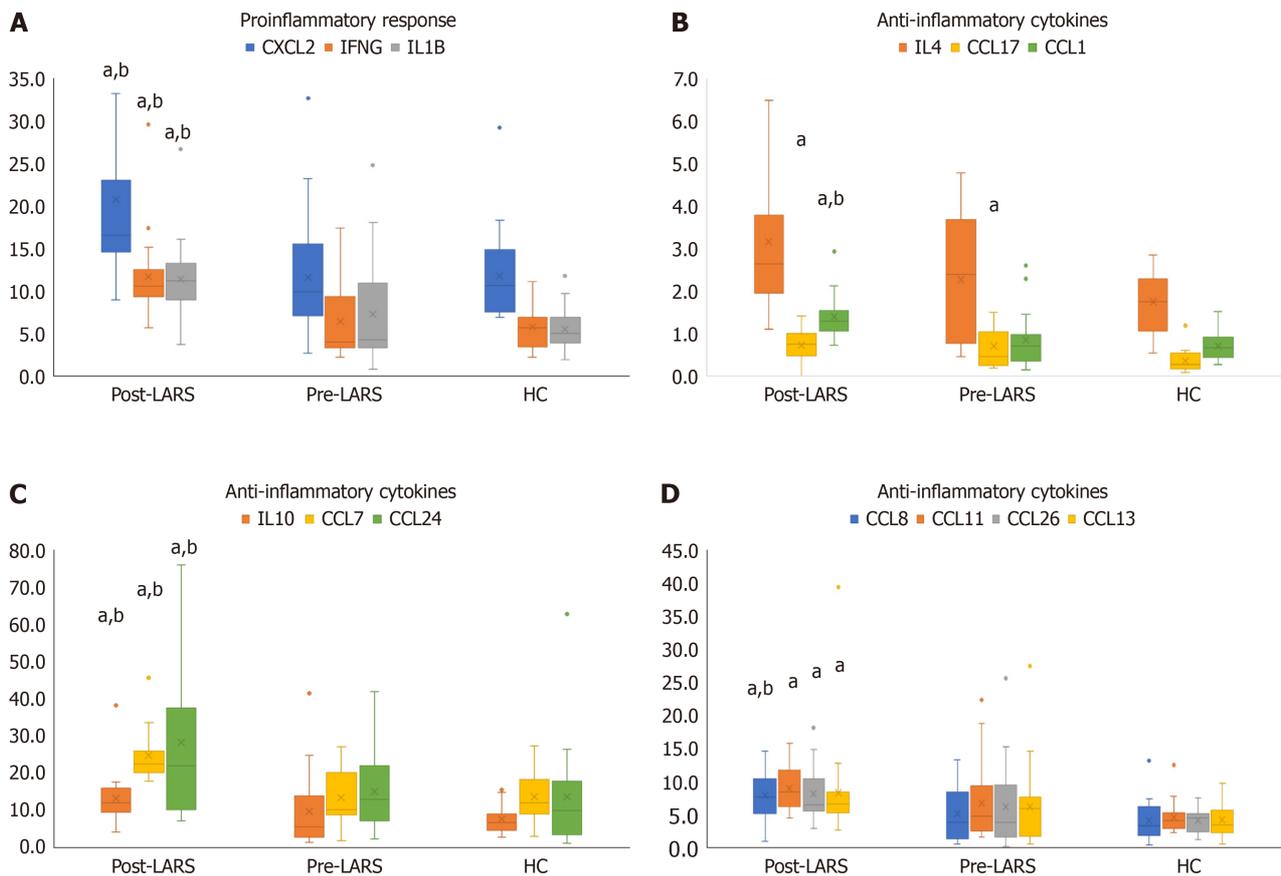


Figure 3 Cytokine results. A: Cytokine results indicating the proinflammatory response; B-D: Cytokine results indicating the anti-inflammatory response. ^a*P* < 0.05 vs healthy control; ^b*P* < 0.05 vs pre-laparoscopic anti-reflux surgery. CXCL: CXC motif chemokine ligand; IFN: Interferon gamma; IL: Interleukin; LARS: Laparoscopic anti-reflux surgery; HC: Healthy control.

post-LARS group. These findings suggest an elevation in the inflammatory process in reflux disease through the toll-like receptors (TLR) signaling pathway and MEK/ERK pathway. While the MEK/ERK pathway is primarily activated by growth factors, osmotic stress, and cytokines[12], p38-MAPK is predominantly triggered by oxidative stress, UV radiation, hypoxia, ischemia, and specific proinflammatory cytokines like IL-1 and tumor necrosis factor-alpha (TNF α)[13].

Additionally, the expression level of RAF1, an activator of the MEK/ERK pathway that transmits chemical signals outside the cell to the cell nucleus, was significantly increased in post-LARS patients. Overactivity of these pathways results in NF κ B activation and subsequently increased levels of pro-inflammatory cytokines, especially IL1 β and IFN γ . In our study, the elevated IL1 β levels may have been regulated by these two pathways and NF κ B[14,15].

When we evaluated our data concerning the type of reflux, we observed varied responses in protein levels after surgery among different reflux phenotypes (Supplementary Table 5). Interestingly, there was no significant change in notable chemokine and protein levels in reflux patients with ERD C/D after surgery. This might be explained by the limited number of patients (*n* = 6) in this group or the time of control endoscopy after surgery (approximately 5.8 months after LARS).

While MAPK4, TNF receptor, HRAs and AKT1 gene expression decreased, the expression of proinflammatory molecules (IL1 β , IFN γ), chemotactic molecules (MIF, CCL1, CCL7, CCL11, CCL25, CCL27, CXCL2), and macrophage activation-related proteins (IL10, CCL19) increased after surgery in patients with ERD A/B and NERD compared to presurgical levels.

IL1 β , a potent proinflammatory regulator, is secreted from many immune cells and triggers the production of acute phase proteins, proinflammatory cytokines, and adhesion molecules. It also activates T and B lymphocytes[16]. Together, IFN γ and TNF α are precursors of the inflammatory response. IFN γ , predominantly secreted from activated T lymphocytes, is a crucial cytokine with pleiotropic immunological functions. Although elevated mostly in pathogenic infections, it has many functions, including promoting macrophage growth, antigen production, activation of the innate immune system, fostering lymphocyte-endothelial interaction, regulating type 1 T helper (Th1)/Th2 balance, and controlling cellular proliferation and apoptosis[17]. IFN γ can also trigger IL1 β synthesis[18].

The elevation of CXCL2 levels in post-LARS provides evidence of the presence of neutrophils in the tissue[19]. IL1 β also induces the production of macrophage MIF, a regulator of innate immunity. MIF mostly causes macrophage accumulation in hypersensitivity regions[20]. MIF contributes to the activation of NF κ B by inhibiting the MEK/ERK signaling pathway and IKBA, an inhibitor of NF κ B[21]. It might be suggested that the TLR signaling pathway through MAP kinase and the MEK/ERK pathway was suppressed after surgery, likely due to depletion of stimulants in the lumen. On the other hand, the proinflammatory status, demonstrated by increases in IL1 β , NF κ B, and IFN γ levels, remained active in

tissues after surgery.

This could be explained by two theories: Oxidative stress that might be elevated due to ischemia-reperfusion after surgery stimulates NF κ B activation by increasing nuclear factor E2-related factor 2 and heme oxygenase levels. The second explanation involves chloride sensing regulation of NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasome activation[22,23]. Recent studies have shown that the chloride concentration in cells is a critical control point for NLRP3 inflammasome activation. Mayes-Hopfinger *et al*[22] revealed that decreased intracellular Cl activates the NLRP3 inflammasome, promoting an immune response by switching the proinflammatory status of a phagocyte. Although their study was conducted in macrophages, we can speculate that depletion of intracellular chloride concentration due to a decrease in extracellular chloride concentration, achieved by blocking acid flux to the esophagus with surgical intervention, might activate the NLRP3 inflammasome in the esophageal epithelium. These theories warrant further study.

TECK/CCL25 and CTACK/CCL27 levels were also increased in the post-LARS group compared to both the pre-LARS and HC groups. T memory and effector lymphocytes activated by IL1 β rapidly migrate to the inflammatory epithelium *via* CCL25 and CCL27. However, it is known that these two chemokines primarily act *via* memory T cells[19]. CCL25 and CCL27 have more homeostatic effects on memory cells[19,24].

Additionally, I309/CCL1 and monocyte chemoattractant protein (MCP)2/CCL8, which have a homeostatic effect on memory T cells and have anti-inflammatory effects on Th2 and regulatory T cells during inflammation, were significantly increased after surgery. Moreover, there was an increase in MCP3/CCL7[25] and IL-10 levels, which can block the Th1 response that mediates monocyte motility, supported the anti-inflammatory activation post-LARS.

Our study showed that EOTAXIN-2/CCL24, MIP1d/CCL15, and MIP3b/CCL19 levels increased in NERD patients after surgery. EOTAXIN2/CCL24, responsible for the recruitment of basophils and eosinophils, promotes cell migration and regulates inflammatory and fibrotic activities. It is secreted from various cells, especially activated fibroblasts, leading to fibroblast proliferation and collagen synthesis[26]. The increase in CCL24 levels indicates that the collagen deposition and reorganization process was active, with the effect of anti-inflammatory regulation in post-LARS tissues. Increased EGF expression in this group also supports the healing process and proliferation[27] and provides information about the presence of eosinophils or basophils in tissues.

CC chemokines are well-known chemoattractants for monocytes (RANTES, MCP 1-5), eosinophils (eotaxins 1-3), basophils (MCP 4-5), and lymphocytes [macrophage inflammatory protein (MIP)-1 α and β]. Our study demonstrated an increase in CC chemokines and many chemoattractants (MIP1, MIP3, EOTAXIN-2) after surgery in NERD patients. An increase in TNF α and IL1 β might suggest that surgical treatment in NERD patients induced inflammatory processes, likely in response to surgical intervention, even after approximately 6 months.

Anti-inflammatory process following antireflux surgery

On the other hand, elevated levels of CCL1, CCL11 and CCL24 indicate that the Th2 response is activated, and that the resolution of the inflammatory response is increased post-LARS. CCL11 mediates the Th2 response as well as eosinophil and basophil migration[19]. In addition to its anti-inflammatory properties, CCL17 also helps maintain homeostatic balance by mediating the transition of effector memory T cells to the inflammatory region.

MPIF1/CCL23 levels increased after surgery in patients with ERD A/B. CCL23 secreted by neutrophils *via* CXCL2, supports the inflammatory response by activating lymphocytes, monocytes and macrophages[28]. However, homeostatic chemokines, such as MIP3-b/CCL19 and SDF1-a+b/CXCL12 were also increased[24] along with CCL25 and CCL27. CCL19 helps stabilize the inflammatory response by inducing naive T cells and central memory T cells to return to lymph nodes[25]. Similarly, neutrophils, monocytes and B cells return to the bone marrow and mediate the suppression of the inflammatory response[19].

IL-4 levels increased after surgery in NERD patients compared to HCs. IL-4 has potent cytoprotective properties[29]. We thought that it may have a major role in preserving the mucosal integrity after surgery. IL-4 also exerts anti-inflammatory effects by inducing the production of CCL7 and CCL11 from peripheral cells in the inflammatory region[25]. These two elevated chemokines may be secreted *via* IL-4. IL-4 can also suppress important cytokines in the proinflammatory process, such as IL1 β and TNF α [30,31]. The significant increase in important anti-inflammatory cytokines such as IL-4 and IL-10 in the NERD and ERD A/B groups may have caused the suppression of important proinflammatory markers in the postoperative group[32].

These findings suggest that the postoperative recovery process is ongoing after successful surgery. In addition, the proinflammatory effect is still ongoing, and it is possible that the anti-inflammatory response overwhelms the ongoing proinflammatory process. After the operation, patients were rescored for symptoms, improvements noted in the control endoscopy, and relapsed patients were excluded. But there may be patients whose mucosal damage had healed but who still had insensible acid attacks. Therefore, reflux symptoms that may occur after LARS may not always indicate failure of the surgery. A limitation of our study is the inability to perform a 24-h pH-impedance test in the post-LARS group, preventing the collection of rational data on acid attacks. In addition, only three patients (11, 14, and 18 months) visited our clinic for control endoscopy after LARS (the other 19 patients were observed for < 6 months). Although no significant change was observed in pro-inflammatory and chemotactic cytokines when these three patients were excluded, it was noted that levels returned to the preoperative level (data not shown) according to the inflammatory cytokine levels in these three patients. However, a statistical calculation could not be made because we only had three patients in the long term. More patients are needed to examine the long-term effects.

CONCLUSION

In conclusion, inflammatory processes, especially involving the TLR signaling pathway, may play a significant role in the pathophysiology of reflux disease. Surgical treatment of reflux disease yields varied responses in cells: The MEK/ERK pathway is suppressed, while inflammatory molecule levels, particularly NF κ B and IL1 β , increase through different mechanisms, including TLR signaling. Surgical treatment induces chemotactic cytokines and inflammatory responses in NERD patients. Elevated levels of macrophage activation markers after surgery in patients with NERD and erosive A/B promote macrophage differentiation into the M2a and M2b phenotypes, crucial for the tissue healing process.

The post-LARS group was included in the study approximately 6 months (2-18 months) after the operations. Proinflammatory proteins like IL1 β , IFN γ , and CXCL2 persist, indicating the status of the ongoing inflammatory response. Additionally, high levels of CCL25, CCL27, CXCL12, CCL17, CCL1, CCL20, and MIF homeostatic cytokines/chemokines are present about 6 months post-operation, aiming to preserve cell homeostasis. Anti-inflammatory response proteins, including IL-10, CCL1, CCL7, CCL8, MCP4/CCL13, EOTAXIN3/CCL26, EOTAXIN/CCL11, TARC/CCL17, and IL-4, are also observed, working to suppress inflammatory damage responses in patients with GERD in the post-LARS group. These persistent cytokine levels suggest that, even at 6 months post-operation, complete recovery has not been achieved. Therefore, it is advisable to continue avoiding refluxogenic foods to prevent symptom recurrence.

ARTICLE HIGHLIGHTS

Research background

Laparoscopic anti-reflux surgery (LARS) is the preferred therapeutic approach for gastroesophageal reflux disease (GERD), as it effectively prevents the reflux of gastric contents into the esophagus. While there is existing knowledge about the recovery period of LARS (typically reported as 8-10 wk in the literature), limited data is available regarding the healing process within the esophageal mucosa following this procedure. This study aims to illuminate the recovery process of patients with GERD who have undergone LARS, with a specific focus on the inflammatory pathways within the esophageal mucosa.

Research motivation

Patients who have undergone LARS often report the eventual healing of symptoms such as heartburn and regurgitation after the surgery. However, a small percentage continues to experience GERD symptoms even post-LARS. The available data on LARS is primarily derived from patients' responses.

Research objectives

We aim to focus on the inflammatory and recovery processes within the esophageal mucosa before and after the surgery.

Research methods

Twenty-two patients with GERD (the same patients before and after LARS) and 25 healthy controls (HCs) were enrolled in the study. Esophageal biopsies were homogenized, and the expressions of inflammatory and cell signaling genes were measured using real-time polymerase chain reaction. Protein levels were assessed using the multiplex enzyme-linked immunosorbent assay method.

Research results

The approximate period between pre- and post-LARS was 6 months (5.8 ± 3.8 months). We demonstrated that proinflammatory cytokines remained activated in post-LARS patients. However, we also observed a significant increase in homeostatic and anti-inflammatory cytokines in the post-LARS group compared to both pre-LARS and HCs.

Research conclusions

We conclude that the toll-like receptor signal is involved in the activation of inflammatory cytokines, while the MEK/ERK pathway is suppressed after LARS. Despite the higher levels of inflammatory cytokines, regulatory and anti-inflammatory markers were also activated in these patients. The persistence of cytokine levels suggests that recovery may not be complete even at 6 months. Patients who have undergone LARS should avoid refluxogenic foods to prevent short-term GERD symptoms.

Research perspectives

We plan a follow-up study with esophageal biopsies and 24-h multichannel intraluminal impedance-pH impedance monitoring in the long term for those patients.

ACKNOWLEDGEMENTS

The results of this study were presented and awarded with a travel grant at IUBMB-FEBS-PABMB 2022 Congress between 9 to 14 July 2022 at Lisbon, Portugal.

FOOTNOTES

Author contributions: Ergun P, Yildirim Sozmen E, and Bor S contributed to the conceptualization, writing-review and editing; Ergun P and Bor S were involved in the investigation; Ergun P, Kipcak S, Selvi Gunel N, Yildirim Sozmen E, and Bor S participated in the methodology and project administration; Ergun P, Kipcak S, Selvi Gunel N, and Yildirim Sozmen E contributed to the software; Ergun P was involved in the writing-original draft; and all authors have read and agreed to the published version of the manuscript.

Supported by the Scientific and Technological Research Council of Turkiye/TUBİTAK, No. 118S260; and Turkish Society of Gastroenterology, No. 797-TGD-2021.

Institutional review board statement: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Ege University, Izmir, Turkiye (18-2.1/36, 20/02/2018).

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <https://creativecommons.org/licenses/by-nc/4.0/>

Country/Territory of origin: United States

ORCID number: Pelin Ergun 0000-0002-3155-0633; Sezgi Kipcak 0000-0003-0615-3844; Nur Selvi Gunel 0000-0003-0612-2263; Eser Yildirim Sozmen 0000-0002-6383-6724; Serhat Bor 0000-0001-5766-9598.

Corresponding Author's Membership in Professional Societies: Turkish Society of Gastroenterology; Turkish Biochemical Society.

S-Editor: Wang JJ

L-Editor: A

P-Editor: Xu ZH

REFERENCES

- Gorgulu V, Ergun P, Kipcak S, Doganavsargil B, Sifrim D, Bor S. Revisiting the Role of Esophageal Mucosal Dilated Intercellular Spaces in the Diagnosis and Pathophysiology of Heartburn. *J Neurogastroenterol Motil* 2023; **29**: 436-445 [PMID: 37814434 DOI: 10.5056/jnm22142]
- Ergun P, Kipcak S, Gunel NS, Bor S, Sozmen EY. Roles of Cytokines in Pathological and Physiological Gastroesophageal Reflux Exposure. *J Neurogastroenterol Motil* 2023 [PMID: 37957115 DOI: 10.5056/jnm22186]
- Souza RF, Huo X, Mittal V, Schuler CM, Carmack SW, Zhang HY, Zhang X, Yu C, Hormi-Carver K, Genta RM, Spechler SJ. Gastroesophageal reflux might cause esophagitis through a cytokine-mediated mechanism rather than caustic acid injury. *Gastroenterology* 2009; **137**: 1776-1784 [PMID: 19660463 DOI: 10.1053/j.gastro.2009.07.055]
- Ergun P, Kipcak S, Bor S. Epigenetic Alterations from Barrett's Esophagus to Esophageal Adenocarcinoma. *Int J Mol Sci* 2023; **24** [PMID: 37175524 DOI: 10.3390/ijms24097817]
- Dean BB, Gano AD Jr, Knight K, Ofman JJ, Fass R. Effectiveness of proton pump inhibitors in nonerosive reflux disease. *Clin Gastroenterol Hepatol* 2004; **2**: 656-664 [PMID: 15290657 DOI: 10.1016/s1542-3565(04)00288-5]
- Pessaux P, Arnaud JP, Delattre JF, Meyer C, Baulieux J, Mosnier H. Laparoscopic antireflux surgery: five-year results and beyond in 1340 patients. *Arch Surg* 2005; **140**: 946-951 [PMID: 16230543 DOI: 10.1001/archsurg.140.10.946]
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265-275 [PMID: 14907713]
- Palmer K. Review article: indications for anti-reflux surgery and endoscopic anti-reflux procedures. *Aliment Pharmacol Ther* 2004; **20** Suppl 8: 32-35 [PMID: 15575870 DOI: 10.1111/j.1365-2036.2004.02226.x]
- Roark R, Sydor M, Chatila AT, Umar S, Guerra R, Bilal M, Guturu P. Management of gastroesophageal reflux disease. *Dis Mon* 2020; **66**: 100849 [PMID: 30798984 DOI: 10.1016/j.disamonth.2019.02.002]
- Unsoeld H, Mueller K, Schleicher U, Bogdan C, Zwirner J, Voehringer D, Pircher H. Abrogation of CCL21 chemokine function by transgenic over-expression impairs T cell immunity to local infections. *Int Immunol* 2007; **19**: 1281-1289 [PMID: 17914120 DOI: 10.1093/intimm/dxm098]
- Hughes CE, Nibbs RJB. A guide to chemokines and their receptors. *FEBS J* 2018; **285**: 2944-2971 [PMID: 29637711 DOI: 10.1111/febs.14466]
- Bahar ME, Kim HJ, Kim DR. Targeting the RAS/RAF/MAPK pathway for cancer therapy: from mechanism to clinical studies. *Signal Transduct Target Ther* 2023; **8**: 455 [PMID: 38105263 DOI: 10.1038/s41392-023-01705-z]
- Ahmadi A, Ahrari S, Salimian J, Salehi Z, Karimi M, Emamvirdizadeh A, Jamalkandi SA, Ghanei M. p38 MAPK signaling in chronic obstructive pulmonary disease pathogenesis and inhibitor therapeutics. *Cell Commun Signal* 2023; **21**: 314 [PMID: 37919729 DOI: 10.1186/s12964-023-01337-4]
- Wang P, Qian H, Xiao M, Lv J. Role of signal transduction pathways in IL-1 β -induced apoptosis: Pathological and therapeutic aspects. *Immun Inflamm Dis* 2023; **11**: e762 [PMID: 36705417 DOI: 10.1002/iid3.762]
- Laurindo LF, Santos AROD, Carvalho ACA, Bechara MD, Guiguer EL, Goulart RA, Vargas Sinatora R, Araújo AC, Barbalho SM.

- Phytochemicals and Regulation of NF- κ B in Inflammatory Bowel Diseases: An Overview of In Vitro and In Vivo Effects. *Metabolites* 2023; **13** [PMID: 36677021 DOI: 10.3390/metabo13010096]
- 16 **Germolec DR**, Shipkowski KA, Frawley RP, Evans E. Markers of Inflammation. *Methods Mol Biol* 2018; **1803**: 57-79 [PMID: 29882133 DOI: 10.1007/978-1-4939-8549-4_5]
- 17 **Tau G**, Rothman P. Biologic functions of the IFN-gamma receptors. *Allergy* 1999; **54**: 1233-1251 [PMID: 10688427 DOI: 10.1034/j.1398-9995.1999.00099.x]
- 18 **Masters SL**, Mielke LA, Cornish AL, Sutton CE, O'Donnell J, Cengia LH, Roberts AW, Wicks IP, Mills KH, Croker BA. Regulation of interleukin-1beta by interferon-gamma is species specific, limited by suppressor of cytokine signalling 1 and influences interleukin-17 production. *EMBO Rep* 2010; **11**: 640-646 [PMID: 20596075 DOI: 10.1038/embor.2010.93]
- 19 **Griffith JW**, Sokol CL, Luster AD. Chemokines and chemokine receptors: positioning cells for host defense and immunity. *Annu Rev Immunol* 2014; **32**: 659-702 [PMID: 24655300 DOI: 10.1146/annurev-immunol-032713-120145]
- 20 **Indreu L**, Sasse S, Karlberg AT, Karlsson I. Haptention of Macrophage Migration Inhibitory Factor: A Potential Biomarker for Contact Hypersensitivity. *Front Toxicol* 2022; **4**: 856614 [PMID: 35465102 DOI: 10.3389/ftox.2022.856614]
- 21 **He J**, Zheng L, Li X, Huang F, Hu S, Chen L, Jiang M, Lin X, Jiang H, Zeng Y, Ye T, Lin D, Liu Q, Xu J, Chen K. Obacunone targets macrophage migration inhibitory factor (MIF) to impede osteoclastogenesis and alleviate ovariectomy-induced bone loss. *J Adv Res* 2023; **53**: 235-248 [PMID: 36657717 DOI: 10.1016/j.jare.2023.01.003]
- 22 **Mayer-Hopfinger L**, Enache A, Xie J, Huang CL, Köchl R, Tybulewicz VLJ, Fernandes-Alnemri T, Alnemri ES. Chloride sensing by WNK1 regulates NLRP3 inflammasome activation and pyroptosis. *Nat Commun* 2021; **12**: 4546 [PMID: 34315884 DOI: 10.1038/s41467-021-24784-4]
- 23 **Perry JSA**, Morioka S, Medina CB, Iker Etchegaray J, Barron B, Raymond MH, Lucas CD, Onengut-Gumuscu S, Delpire E, Ravichandran KS. Interpreting an apoptotic corpse as anti-inflammatory involves a chloride sensing pathway. *Nat Cell Biol* 2019; **21**: 1532-1543 [PMID: 31792382 DOI: 10.1038/s41556-019-0431-1]
- 24 **Palomino DC**, Marti LC. Chemokines and immunity. *Einstein (Sao Paulo)* 2015; **13**: 469-473 [PMID: 26466066 DOI: 10.1590/S1679-45082015RB3438]
- 25 **Murphy PM**, Rich RR, Fleisher TA, Shearer WT, Schroeder HW, Frew AJ, Weyand CM. *Clinical Immunology*. United States: Elsevier, 2018
- 26 **Segal-Salto M**, Barashi N, Katav A, Edelshtein V, Aharon A, Hashmueli S, George J, Maor Y, Pinzani M, Haberman D, Hall A, Friedman S, Mor A. A blocking monoclonal antibody to CCL24 alleviates liver fibrosis and inflammation in experimental models of liver damage. *JHEP Rep* 2020; **2**: 100064 [PMID: 32039405 DOI: 10.1016/j.jhepr.2019.100064]
- 27 **Villarreal-Ponce A**, Tiruneh MW, Lee J, Guerrero-Juarez CF, Kuhn J, David JA, Dammeyer K, Mc Kell R, Kwong J, Rabbani PS, Nie Q, Ceradini DJ. Keratinocyte-Macrophage Crosstalk by the Nrf2/Ccl2/EGF Signaling Axis Orchestrates Tissue Repair. *Cell Rep* 2020; **33**: 108417 [PMID: 33238115 DOI: 10.1016/j.celrep.2020.108417]
- 28 **Bonaventura A**, Montecucco F. CCL23 is a promising biomarker of injury in patients with ischaemic stroke. *J Intern Med* 2018; **283**: 476-478 [PMID: 29443424 DOI: 10.1111/joim.12742]
- 29 **Fitzgerald RC**, Onwuegbusi BA, Bajaj-Elliott M, Saeed IT, Burnham WR, Farthing MJ. Diversity in the oesophageal phenotypic response to gastro-oesophageal reflux: immunological determinants. *Gut* 2002; **50**: 451-459 [PMID: 11889061 DOI: 10.1136/gut.50.4.451]
- 30 **Chatterjee P**, Chiasson VL, Bounds KR, Mitchell BM. Regulation of the Anti-Inflammatory Cytokines Interleukin-4 and Interleukin-10 during Pregnancy. *Front Immunol* 2014; **5**: 253 [PMID: 24904596 DOI: 10.3389/fimmu.2014.00253]
- 31 **Dunleavy C**, Elsworth RJ, Upthegrove R, Wood SJ, Aldred S. Inflammation in first-episode psychosis: The contribution of inflammatory biomarkers to the emergence of negative symptoms, a systematic review and meta-analysis. *Acta Psychiatr Scand* 2022; **146**: 6-20 [PMID: 35202480 DOI: 10.1111/acps.13416]
- 32 **Arthur JS**, Ley SC. Mitogen-activated protein kinases in innate immunity. *Nat Rev Immunol* 2013; **13**: 679-692 [PMID: 23954936 DOI: 10.1038/nri3495]



Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: office@baishideng.com
Help Desk: <https://www.f6publishing.com/helpdesk>
<https://www.wjgnet.com>

