International Archives of Allergy and Immunology

Experimental Allergy – Research Article

Int Arch Allergy Immunol
DOI: 10.1159/000507289

Received: January 11, 2020
Accepted: March 16, 2020
Published online: December 7, 2020

SELE Downregulation Suppresses Mast Cell Accumulation to Protect against Inflammatory Response in Chronic Idiopathic Urticaria

Hong Wang[a] Yangchun Xu[b] Meishan Jin[c] Wen Yuan[a]

[a]Department of Dermatology and Venereology, The First Hospital of Jilin University, Changchun, PR China;
[b]Department of Dermatology and Venereology, The Second Hospital of Jilin University, Changchun, PR China;
[c]Department of Pathology, The First Hospital of Jilin University, Changchun, PR China

Keywords
Chronic idiopathic urticaria · E-selectin · Mast cells · Histamine · Tumor necrosis factor-α · Homologous restriction factor · Interleukin-6

Abstract
Background: Chronic idiopathic urticaria (CIU) represents a common skin disorder often characterized by mast cell activation and secretion of histamine and other proinflammatory factors. E-selectin (SELE) has been implicated in the pathogenesis of common inflammatory cutaneous disorders, while the role of SELE in CIU is yet to be fully understood. Thus, we aimed to investigate the mechanism by which SELE influences CIU in connection with the involvement of mast cells. ***Methods:*** SELE expression was measured in blood samples obtained from CIU patients and normal individuals. A CIU mouse model was subsequently established by intradermally injecting a normal saline solution with ovalbumin IgE antiserum into the mice. Loss- and gain-of-function investigations were conducted on the mouse models. The number of degranulated mast cells and the amount of histamine release in vitro were determined. The levels of SELE, tumor necrosis factor (TNF)-α, homologous restriction factor (HRF), and interleukin (IL)-6 levels were determined. ***Results:*** The CIU clinical samples exhibited upregulated SELE, while the CIU mice showed increased mast cell degranulation and an increased rate of histamine directional release, as well as an elevated expression of SELE, TNF-α, HRF, and IL-6. SELE silencing was found to decrease the number of degranulated mast cells and reduce the rate of histamine directional release, along with suppressed TNF-α, HRF, and IL-6 expression, in the serum of CIU mice. Ketotifen was observed to rescue the increased expression of TNF-α, HRF, and IL-6 caused by SELE overexpression. ***Conclusions:*** This study highlights the potential of SELE downregulation to repress inflammatory factor secretion caused by the accumulation of mast cells, which ultimately inhibits the development of CIU.
© 2020 S. Karger AG, Basel

Introduction

As a chronic and often debilitating skin disease, chronic idiopathic urticaria (CIU) is characterized by the recurrent eruption of urticarial wheals often due to an unknown cause for a period greater than 6 weeks [1]. The incidence of CIU has been reported to be lower among children compared to adults, whereas previous studies

Edited by: H.-U. Simon, Bern.

karger@karger.com
www.karger.com/iaa
© 2020 S. Karger AG, Basel

Hong Wang
Department of Dermatology and Venereology, The First Hospital of Jilin University
No. 71, Xinmin St.
Changchun, Jilin Province 130021 (PR China)
w_hong@jlu.edu.cn

Downloaded by: C. Alessandri - 87218
84.220.48.124 - 12/18/2020 7:27:58 AM

have indicated that there is no statistical difference in CIU incidence between children and adolescents [2]. Owing to the notable similarity between urticaria lesions induced by allergens on the skin and those caused by injection of histamine, reports have highlighted mast cells as key factors in the generation of localized urticarial lesions [3]. The existing literature indicates that activated mast cells express transglutaminase 2, which has been linked to elevated levels of inflammatory mediators such as histamine, tumor necrosis factor (TNF)-α and interleukin (IL)-6, highlighting that mast cells and varied inflammatory cells play crucial roles in the pathogenesis of chronic spontaneous urticaria (CSU) [4].

E-selectin (SELE) is expressed on postcapillary vessel linings and it has been shown to regulate low-affinity binding interactions with ligands on leukocytes and inflammation [5]. Previous research has indicated that mast cells release various receptors and ligands on the cell surface and molecules activating the cells of the immune system, with these mediators released by mast cells shown to exacerbate skin inflammation [6]. Activation of human mast cells has been shown to release a variety of mediators such as histamines and many cytokines comprising TNF, IL-33, and IL-1, which results in the generation of nuclear factor (NF)-kB and AP1 [7]. A close correlation between chronic urticaria (CU) and inflammatory responses has been documented, with markers of inflammation such as the mean platelet volume and C-reactive protein abnormally elevated in patients with CU [8, 9]. Evidence has been presented previously confirming the notable role of selectins in allergic diseases, with studies suggesting that blockade of selectins might ameliorate several diseases such as chronic allergic inflammation, airway hyperresponsiveness, and allergic late-phrase reactions [10]. Hence, we asserted the hypothesis that SELE might regulate mast cells to produce inflammatory factors, which leads to CIU. Thus, the central objective of the current study was to explore the correlation between SELE, mast cells, and CIU in an attempt to analyze the effect of SELE on mast cells in mice with CIU.

Materials and Methods

Patient Enrollment

Sixty-five patients with CIU treated at the Department of Dermatology of The First Hospital of Jilin University and the Department of Dermatology of The Second Hospital of Jilin University from June 2017 to December 2018, in addition to 33 healthy individuals, were selected for the current study. The CIU patient inclusion criterion was based on whether they met a previously published international standard [11]. The patients (33 males and 32 females) were aged 13–60 years, with a mean age of 36.55 ± 6.90 years. The disease duration was between 3 and 60 months, with mean disease duration of 28.18 ± 11.82 months, and the incidence frequency was 7.63 ± 2.44 times/month. The healthy individuals (16 males and 17 females) were aged 14–61 years, with a mean age of 35.06 ± 7.54 years. There were no statistically significant differences in relation to the general clinical data of gender and age between CIU patients and healthy individuals ($p > 0.05$). The expression of SELE in the mast cells of the patients with CIU and the healthy individuals was measured by means of reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

Establishment of a Mouse Model with CIU

Seventy-eight specific pathogen-free healthy male C57BL/6 mice (aged 8 weeks and weighing 18–22 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Among the mice, 15 were randomly selected as the normal group while the remaining 63 were used to establish the CIU mouse model (CIU group). The mice were placed on a regular granular diet with sterilized tap water. Additionally, the mice were stored in a controlled environment with conditions as follows: temperature, 18–22 °C; relative humidity, 40–70%; noise, <50 dB; and 12-h light/dark cycle. After 7 days of adaptive feeding, the CIU mice were injected intradermally with 0.1 mL/point of normal saline solution containing ovalbumin immunoglobulin E antiserum (1:1). The antigen challenge was performed 1 h after the final dose administration; 1 mL of 0.5% Evans blue solution (containing 30 mg ovalbumin) was injected into the mice via the tail vein. The CIU mouse model was considered to be successfully established if the mice exhibited the following changes: (1) physiological state: itchy skin with bright red or pale wheals of varying sizes, and (2) pathological examination: hematoxylin-eosin (HE) staining and optical density (OD) value measurement in blue plaque [12]. The successfully modeled mice were used for the subsequent experiments.

HE Staining

Three mice in the normal group and 3 mice in the CIU group were anesthetized with 3% sodium pentobarbital (50 mg/kg) and subsequently euthanized via decapitation, with the hind skin region separated. The skin tissue was washed using 4 °C normal saline to wash the bloodstains on the surface. After repeated rinsing with phosphate-buffered saline (PBS), the tissues were fixed with 10% neutral formaldehyde for 24 h, dehydrated with gradient alcohol (ethanol concentrations of 70, 80, 90, 95, and 100%, respectively, for 5 min/time), and permeabilized with xylene twice (10 min/time). After immersion and embedding in paraffin, the tissues were cut into serial sections (4 μm). The sections were then dried at 60 °C for 1 h and deparaffinized with xylene, followed by xylene removal with gradient ethanol. The sections were subsequently stained with hematoxylin for 10 min, and then the sections were differentiated with 1% hydrochloric acid alcohol for 20 s. The sections returned to blue via exposure to 1% ammonia water for 30 s, followed by staining with eosin solution for 3 min. The sections were then dehydrated with gradient ethanol, permeabilized twice with xylene, and mounted with neutral balsam. Finally, the sections were observed under a 100-fold ordinary optical microscope (Olympus Optical Co., Ltd., Tokyo, Japan). The experiment was repeated 3 times.

Downloaded by: C. Alessandri - 87218
84.220.48.124 - 12/18/2020 7:27:58 AM

Measurement of OD Values in Blue Plaque

Six mice in the normal group and 6 mice in the CIU group were taken. The blue patches on the back skin of the mice were cut into pieces. The pieces were added to 4 mL of acetone-a normal saline mixture (7:3), soaked for 48 h, and centrifuged at 3,000 rpm for 10 min. The supernatant was then placed on a 721-type spectrophotometer to detect the OD value at a wavelength of 610 nm via chromatometry, with the average OD values of each group recorded.

Animal Treatment and Grouping

The successfully established mouse models were subjected to the following procedures: 14.5 μg of nucleic acid was diluted to 0.5 μg/μL with endotoxin-free pure water and fully mixed with 25 μg 10% glucose solution (w/v) until the final glucose concentration was confirmed to be 5% with a final volume of 50 μL. A total of 25 μL of Entranster TM in vivo reagent (Engreen Biosystem Co. Ltd., Beijing, China) was diluted and mixed with 25 μL of 10% glucose solution until the final glucose concentration was confirmed to be 5%, with a final volume of 50 μL. The diluted transfection reagent was immediately added to the diluted nucleic acid solution (Invitrogen, USA), shaken well in a prompt manner, and left to sit at room temperature for 15 min. After the mice had been anesthetized with 2% sodium pentobarbital (50 mg/kg), 200 μL of plasmids were injected into the bilateral femur. The successfully modeled mice were then injected with siRNA-(NC) plasmid, siRNA-SELE plasmid, pcDNA3 (vector-NC) plasmid, pcDNA3-SELE (vector-SELE) plasmid vector-SELE + PBS, or vector-SELE + 0.1% Ketotifen ($n = 6$) [13].

Toluidine Blue Staining

Based on a previous report, the toluidine blue staining method was employed to calculate the degranulation rate of mast cells [14]. The tissue sections were routinely deparaffinized, stained with 1% toluidine blue, and differentiated using 0.5% glacial acetic acid. The section staining was observed under a microscope. The sections were permeabilized and mounted accordingly. For each section, 3 visual fields with a relatively broad distribution of mast cells were selected. The number of degranulated cells in 100 mast cells was tallied under a high-power microscope to calculate the percentage of degranulation. The mast cell degranulation rate (%) was calculated as follows: number of degranulated cells/total number of mast cells × 100%.

Immunohistochemistry

The paraffin-embedded tissues were sliced into 4-μm sections. The sections were dried at 60 °C for 3–4 h, deparaffinized 3 times with xylene solution (15 min/time), and hydrated with gradient alcohol (100, 95, 90, 80, and 70%), followed by high-pressure repair using citrate buffer (pH = 6.0) for 2 min. The sections were then blocked with 3% hydrogen peroxide methanol solution for 10 min. Following the addition of nonimmune goat serum (FK-MB593J; Shanghai Fanke Biotechnology, Shanghai China), the sections were then incubated with polyclonal rabbit antibody against TNF-α (1:1,000, ab6671, Abcam Inc., Cambridge, UK) and goat anti-rabbit IgG (ab6721; Abcam Inc., Cambridge, UK) marked with horseradish peroxidase for 30 min under room temperature conditions. The sections were then stained with diaminobenzidine and observed under a microscope. The sections were subsequently stained with hematoxylin and alkalized with ammonia. The sections were dehydrated with gradient alcohol (100, 95, 90, 80, and 70%) (3 min for each), washed 3 times with xylene (3 min/time), air-dried, and mounted with neutral balsam. The sections that were not subjected to primary antibody addition were regarded as the negative control. Five visual fields were randomly selected under a 200-fold optical microscope, with the staining results expressed as the percentage of positive cells.

Enzyme-Linked Immunosorbent Assay Method

Six mice in the normal group and 6 mice in the CIU group were anesthetized with 2% sodium pentobarbital (50 mg/kg) with 2 mL of blood collected from the posterior arteries of the mice. After the blood had been anticoagulated with heparin, the serum was separated for detection in a timely fashion or preserved at –20 °C. ELISA kits (Cusabio Biotech Co., Ltd. Wuhan, Hubei, China) were taken out of the refrigerator 20 min in advance and equilibrated to room temperature prior to use. The concentrations of the known antigens were diluted with a carbonate coating buffer (pH = 9.6) to 180, 90, 45, 23, 11, and 6 pg/mL. Each standard well was added to 50 μL of diluted antigen, respectively. The blank well and the sample well were set accordingly. A total of 50 μL of the sample dilution was added to the blank well and 10 μL of the sample to be tested and 40 μL of the sample dilution were added to the sample well. All of the samples were incubated at 37 °C for 1 h and dried following liquid removal. In addition to the blank wells, 50 μL of the enzyme-labeled reagent was added to the remaining wells, respectively, and they were incubated at 37 °C for 1 h. Next, 100 μL of the temporarily prepared tetramethylbenzidine substrate solution (EL0001; InnoReagents Co., Ltd., Huzhou, Zhejiang, China) was added to each reaction well. The solution was incubated at 37 °C for 15 min under conditions void of light. The OD value of each well was measured at a wavelength of 450 nm within 15 min. The concentration of the standard solution and the OD value were regarded as the horizontal and vertical coordinates, respectively. The regression equation of the standard curve was calculated using a computer, and the OD value of the sample was substituted into the equation to calculate the sample concentration, which was the actual concentration of the sample. The levels of SELE, TNF-α, homologous restriction factor (HRF), and IL-6 were detected by ELISA.

Measurement of Histamine Release in vitro

Three randomly selected mice from each group were anesthetized with 3% sodium pentobarbital (50 mg/kg) and killed by decapitation. In accordance with the methods described in previous literature [12], the peritoneal mast cells were promptly collected for the measurement of histamine release in vitro. Histamine measurement was used to classify the samples into a directional release group and a total histamine group. A total of 0.5 mL of cell suspension from each group was mixed at an equal volume of 5 mg/mL allergen protein, respectively, and incubated at 37 °C for 1 h. The suspension of the directional release group was centrifuged at 1,500 rpm for 10 min; the suspension of the total histamine group was boiled for 1 min and then centrifuged. Following the addition of 0.5 mL NaOH (0.4 mol/L), the supernatant was added with 0.1 mL 0.1% phthalaldehyde and left to sit at room temperature for 10 min, followed by the addition of 0.5 mL 0.1 mol/L HCl for reaction termination. A fluorescence spectrophotometer was employed using the colorimetric method (excitation wavelength of 365 nm; emission wavelength of 422 nm). The results are expressed at a

Downloaded by: C. Alessandri - 87218
84.220.48.124 - 12/18/2020 7:27:58 AM

Table 1. Primes for RT-qPCR

Gene	Primer sequences
SELE	F: 5′-GATCCAACGCCAGAACAACA-3′ R: 5′-CCCTTCCACACAGTCAAACG-3′
TNF-α	F: 5′-CATGTACGTTGCTATCCAGGC-3′ R: 5′-CTCCTTAATGTCACGCACGAT-3′
IL-6	F: 5′-ACAGCCACTCACCTCTTCAG-3′ R: 5′-CCATCTTTTTCAGCCATCTTT-3′
GAPDH	F: 5′-TTCACCACCATGGAGAAGGC-3′ R: 5′-GGCATGGACTGTGGTCATGA-3′

F, forward; R, reverse.

directional release rate as follows: directional release of the directional release group/directional release of the total histamine group × 100%.

Reverse Transcription-Quantitative Polymerase Chain Reaction

Total RNA was extracted from each group in accordance with the instructions of the Trizol reagent (AM1931; Invitrogen). RNA was dissolved using diethylpyrocarbonate-treated ultrapure water. OD values at 260 and 280 nm were measured using an ND-1000 UV/Vis spectrophotometer (NanoDrop Technologies Inc., Wilmington, NC, USA), with the concentration and quality of the total RNA subsequently determined. Complementary DNA (cDNA) was synthesized using a reverse transcription kit (M1701; Promega Corporation, Madison, WI, USA) with the cDNA sample subjected to warm bathing at 80 °C for 5 min to inactivate the reverse transcriptase process. The sample was used for subsequent PCR detection. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was employed as the internal control for mRNA. The primer sequences used are depicted in Table 1. Each sample was evaluated 3 times, with the mean value recorded. The reliability of the PCR results was evaluated using a melting curve. The relative expression levels of each gene were calculated using the $2^{-\Delta\Delta Ct}$ method.

Western Blot Analysis

After 48 h of transduction, the mast cells were trypsinized and added to cell lysate to extract total protein. The protein concentration was measured using a bicinchoninic acid protein assay kit. Protein was boiled and denatured, followed by separation using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis. A total of 25 μg of protein samples were added to each well. Following electrophoresis, the protein was transferred to a polyvinylidene fluoride membrane via the wet turning method. The membrane was blocked with 5% skimmed milk powder for 2 h at room temperature and then rinsed with Tris-buffered saline (TBS) buffer 3 times (10 min/time). The membrane was then incubated at 4 °C overnight with the TBST-diluted primary polyclonal rabbit antibody against TNF-α (1:1,000, ab6671; Abcam), histamine (ab154063; Abcam), and IL-6 (1:20, ab7737; Abcam). After that, the membrane was incubated with horseradish peroxidase-labeled goat anti-rabbit IgG (1:5,000, ab205718; Abcam) for 2 h at room temperature. The membrane was allowed to react with the enhanced chemiluminescence solution for 1 min at room temperature for development. GAPDH (ab181602; Abcam) was employed as the internal reference for the genes. The relative expression of the proteins was expressed as the gray value ratio of the target band to the internal control band.

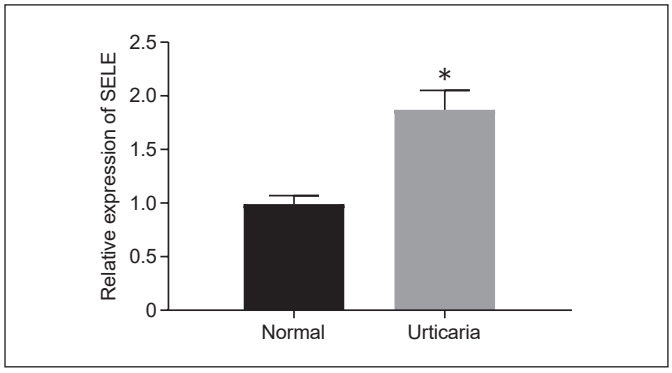


Fig. 1. Upregulation of SELE expression is observed in the blood samples of CIU patients. * $p < 0.05$ vs. the healthy individuals. Measurement data are expressed as means ± SD. Comparisons between 2 groups were analyzed using a *t* test. Healthy individuals: $n = 33$. Urticarial group: $n = 65$.

Statistical Analysis

All data were evaluated using SPSS 21.0 statistical software (IBM Corp. Armonk, NY, USA). Measurement data are expressed as means ± SD. Comparisons between 2 groups were analyzed using a *t* test. For multiple-group comparisons, one-way analysis of variance (ANOVA) was used, followed by the Tukey post hoc test. When a significant difference in the analysis of variance was identified, a Q test was subsequently performed for pairwise comparisons. In the event of heterogeneity of variance, a nonparametric rank-sum test was performed. Enumeration data are expressed as a percentage with comparisons, performed using a χ^2 test. $p < 0.05$ was considered statistically significant.

Results

SELE Is Highly Expressed in the Blood Samples of Patients with CIU

In order to assess the effects of SELE on CIU, RT-qPCR was performed to detect the expression of SELE in the CIU clinical samples. The results demonstrated that, compared to the healthy individuals, the expression of SELE was significantly higher in the blood samples of the CIU patients ($p < 0.05$; Fig. 1). Based on the aforementioned results, the expression of SELE was confirmed to be upregulated in CIU.

Downloaded by: C. Alessandri - 87218
84.220.48.124 - 12/18/2020 7:27:58 AM

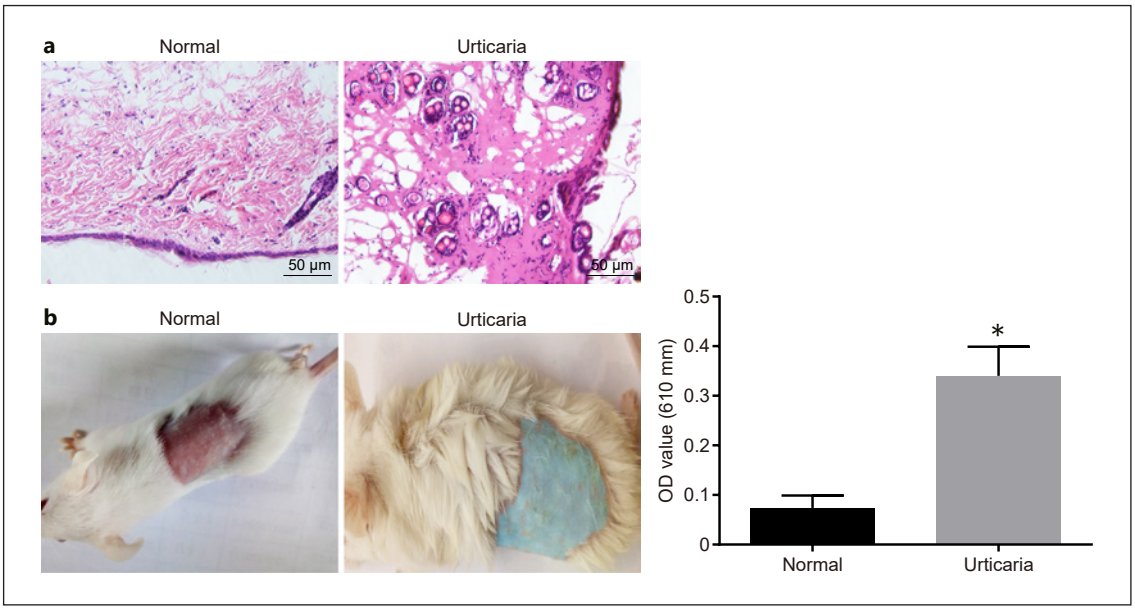


Fig. 2. Successful CIU model establishment. **a** Representative images of the skin tissue condition of normal and CIU mice, as detected by HE staining (×200). **b** Representative images of blue plaque and OD values of blue plaque of normal and CIU mice. * $p < 0.05$ vs. normal mice. Measurement data are expressed as means ± SD. Comparisons between 2 groups were analyzed using a *t* test ($n = 6$).

Successful Establishment of an Animal Model of CIU

To ascertain whether the mouse model of CIU had been successfully established, HE staining was performed; thereafter, the OD value of blue plaque was determined. HE staining results demonstrated that CIU mice exhibited dermal edema, skin telangiectasia, lymphangiectasis, and mild infiltration of inflammatory cells compared to the normal mice; edema was most evident in the upper part of the dermis (Fig. 2a). As illustrated in Figure 2b, compared to the normal mice, the OD value of blue plague on the skin tissues of CIU mice was significantly increased ($p < 0.05$). The results demonstrated that the CIU mouse model was successfully established.

Mast Cells Participate in Development of CIU by Releasing TNF-α, HRF, IL-6, and Histamine

Following collection of the results showcasing upregulation of SELE in the CIU blood samples, we shifted our focus to determine the relationship between mast cells and CIU. The number of degranulated mast cells demonstrated that the mast cells were structurally intact, round, and blue with smooth edges, and the cytoplasm was filled with coarse purple-red granules compared to the normal mice. In the CIU mice, the mast cells were identified to be swollen and deformed, with irregular edges and broken membranes, and those granules were found to be detached. Compared to the normal mice, the CIU mice exhibited an elevated number of degranulated mast cells ($p < 0.05$; Fig. 3a), which indicated that the number of degranulated mast cells was significantly increased after CIU. Immunohistochemistry demonstrated that TNF protein was expressed in both the cytoplasm and the nuclear membrane of skin cells of normal and CIU mice, and brown-yellow granules were positive. The positive rate of TNF protein was (23.88% ± 4.58) in normal mice and (53.17% ± 8.90) in CIU mice, which was highly suggestive of an elevated TNF protein expression in mice with CIU (Fig. 3b). The ELISA findings revealed that, in contrast to the values in normal mice, SELE, TNF-α, HRF, and IL-6 expression in the serum of CIU mice were markedly elevated (all $p < 0.05$; Fig. 3c). In each group the mice exhibited the highest serum immunoglobulin (sIgE) titer on the 14th day after provocation; the directional histamine release from abdominal mast cells was determined on the 14th day after provocation (Fig. 3d). The directional release rate was found to be 9.05% in normal mice but 62.80% in CIU mice. These results provide evidence that mast cells are involved in CIU progression through the production of TNF-α, HRF, IL-6, and histamine.

Downloaded by: C. Alessandri - 87218
84.220.48.124 - 12/18/2020 7:27:58 AM

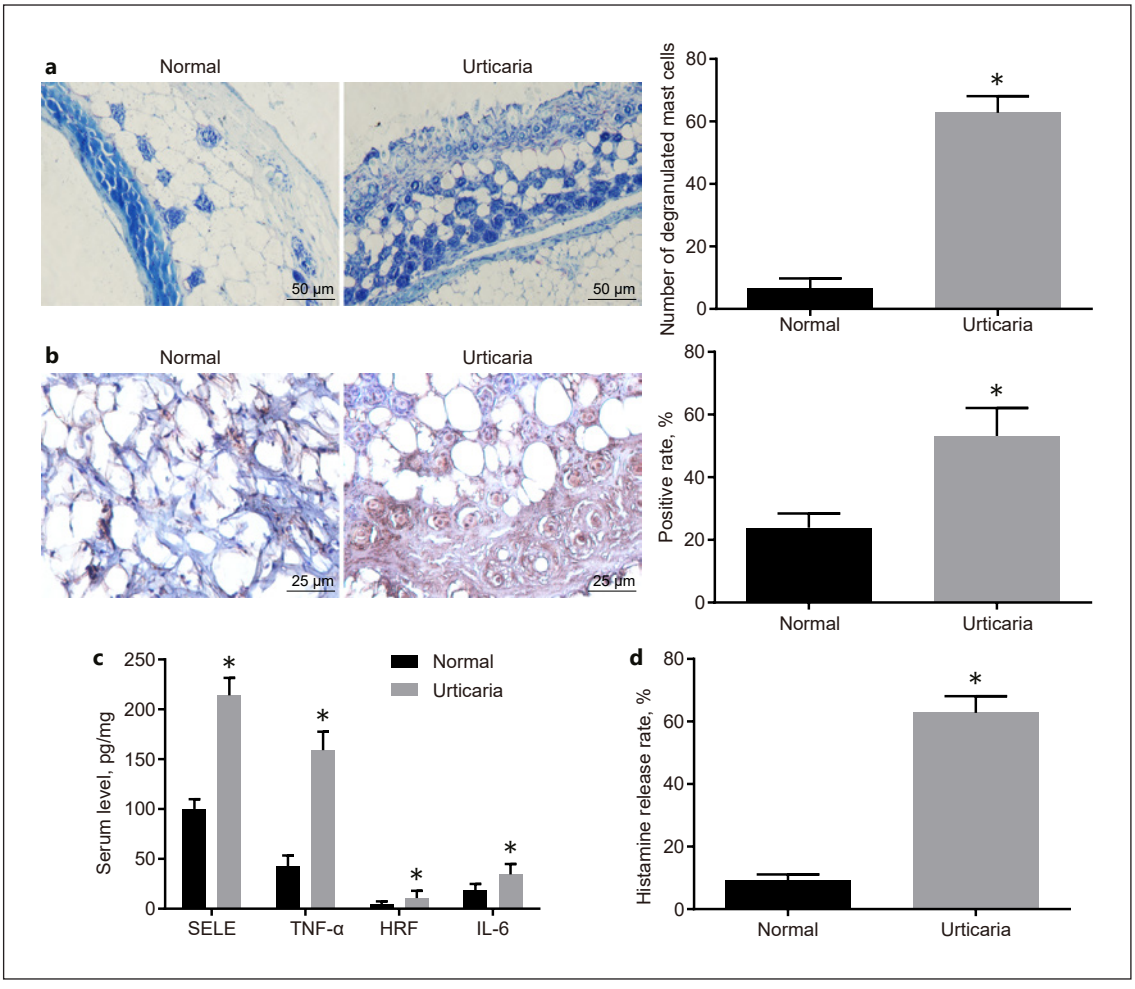


Fig. 3. Mast cells are correlated with the biological processes of CIU via the release of TNF-α, HRF, IL-6, and histamine. **a** Mast cell degranulation of skin tissues in normal and CIU mice detected by toluidine blue staining (×200). **b** Representative image of immunohistochemistry and quantification of the TNF protein positive rate in normal and CIU mice detected by immunohistochemistry (×400). **c** Expression of SELE, TNF-α, HRF, and IL-6 in the serum of normal and CIU mice, as measured by ELISA. **d** Histamine release rate of mast cells in normal and CIU mice. * $p < 0.05$ vs. normal mice. Measurement data are expressed as means ± SD. Comparisons between 2 groups were analyzed using a *t* test ($n = 6$).

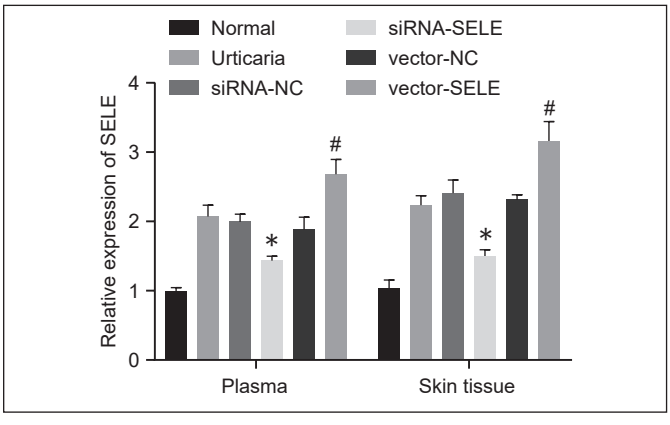
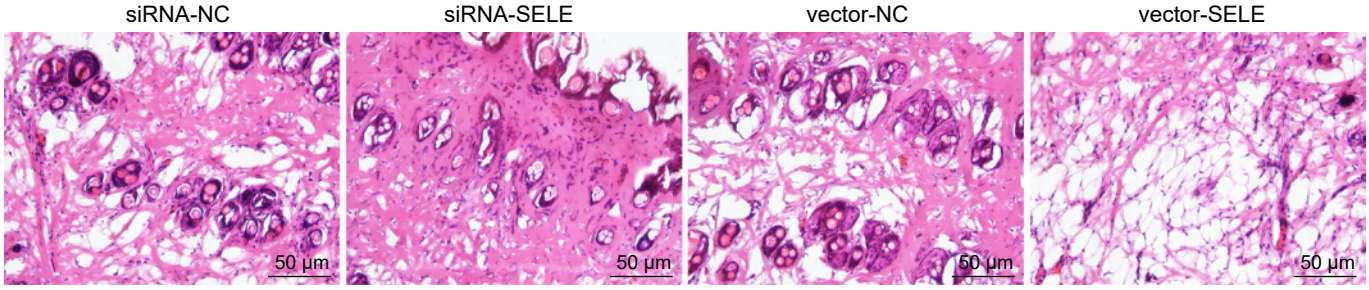


Fig. 4. SELE expression in the blood and tissue samples of mice with different injections as detected by RT-qPCR. * $p < 0.05$ vs. CIU mice treated with siRNA-NC. # $p < 0.05$ vs. CIU mice treated with vector-NC. Measurement data are expressed as means ± SD. One-way ANOVA was used for comparison of data among multiple groups, followed by the Tukey post hoc test.

Downloaded by: C. Alessandri - 87218
84.220.48.124 - 12/18/2020 7:27:58 AM



Color version available online

Fig. 5. Histopathological changes in skin tissues in CIU are ameliorated with silencing of SELE (×200).

Expression of SELE in the Blood and Tissue Samples of Mice with Different Injections

Following the administration of different plasmids, RT-qPCR assay was employed to determine the expression of SELE in mouse blood samples and skin tissue samples. As illustrated in Figure 4, the expression of SELE was lowest in the normal mice. Moreover, the CIU mice injected with siRNA-NC or with vector-NC exhibited no notable difference in SELE expression. Compared to these mice regarded as NC, CIU mice with siRNA-SELE treatment exhibited a reduced SELE expression, while those with vector-SELE treatment showed elevated SELE expression.

SELE Silencing Alleviates the Histopathological Changes of Skin Tissues in Mice with CIU

HE staining was performed to examine the specific mechanism by which SELE influences CIU when the expression of SELE is altered. The results exhibited that, compared to the siRNA-NC-treated mice, mice treated with siRNA-SELE had dermal edema, mitigative skin telangiectasia, lymphatic vessel expansion, and alleviated inflammatory cell infiltration. CIU mice exhibited exacerbated dermal edema, skin telangiectasia, lymphangiectasis, and severe inflammatory cell infiltration and edema, which was most evident in the upper part of the dermis after injection with vector-SELE (Fig. 5). Taken together, these results indicate that silencing of SELE mitigates the histopathological changes of skin tissues in mice with CIU.

Silencing of SELE Downregulates the Expression of TNF-α, HRF, IL-6, and Suppresses Histamine Release from Mast Cells in Mice with CIU

Next, to evaluate the effects of SELE on mast cells, SELE expression was upregulated or downregulated in mice with CIU and treated with ketotifen. As depicted in Figure 6a, CIU mice treated with siRNA-SELE exhibited alleviated edema as well as distorted mast cells with irregular edges and broken membranes. In addition, the degranulation of granules was mitigated with a significantly diminished number of degranulated mast cells. In contrast, among the CIU mice treated with vector-SELE, the mast cells exhibited evidence of swelling and deformity, with irregular edges and broken membranes. The degranulation of the granules was exacerbated, with the number of degranulated mast cells evidently increased. Immunohistochemistry results demonstrated that the expression of TNF in CIU mice was markedly decreased following SELE silencing, with an opposite trend observed following SELE overexpression (Fig. 6b). The ELISA results indicated that TNF-α, HRF, and IL-6 expression in the serum of CIU mice was notably downregulated following treatment with siRNA-SELE, which was reversed after treatment with vector-SELE (Fig. 6c). The result of the directional histamine release revealed that treatment with siRNA-SELE led to a reduction in the directional release rate of CIU, while the treatment of vector-SELE exhibited a contrasting trend (Fig. 6d). RT-qPCR documented that TNF-α and IL-6 mRNA expression in CIU mice treated with vector-SELE + ketotifen was decreased versus values in CIU mice treated with vector-SELE + PBS ($p < 0.05$; Fig. 6e). Western blot analysis (Fig. 6f) revealed that TNF-α, HRF, and IL-6 protein expression was decreased in the CIU mice treated with vector-SELE + ketotifen compared to the CIU mice treated with vector-SELE + PBS ($p < 0.05$). Altogether, silencing of SELE was determined to inhibit the expression of TNF-α, HRF, and IL-6 as well as a reduce histamine release from mast cells in mice with CIU.

Downloaded by: C. Alessandri - 87218
84.220.48.124 - 12/18/2020 7:27:58 AM

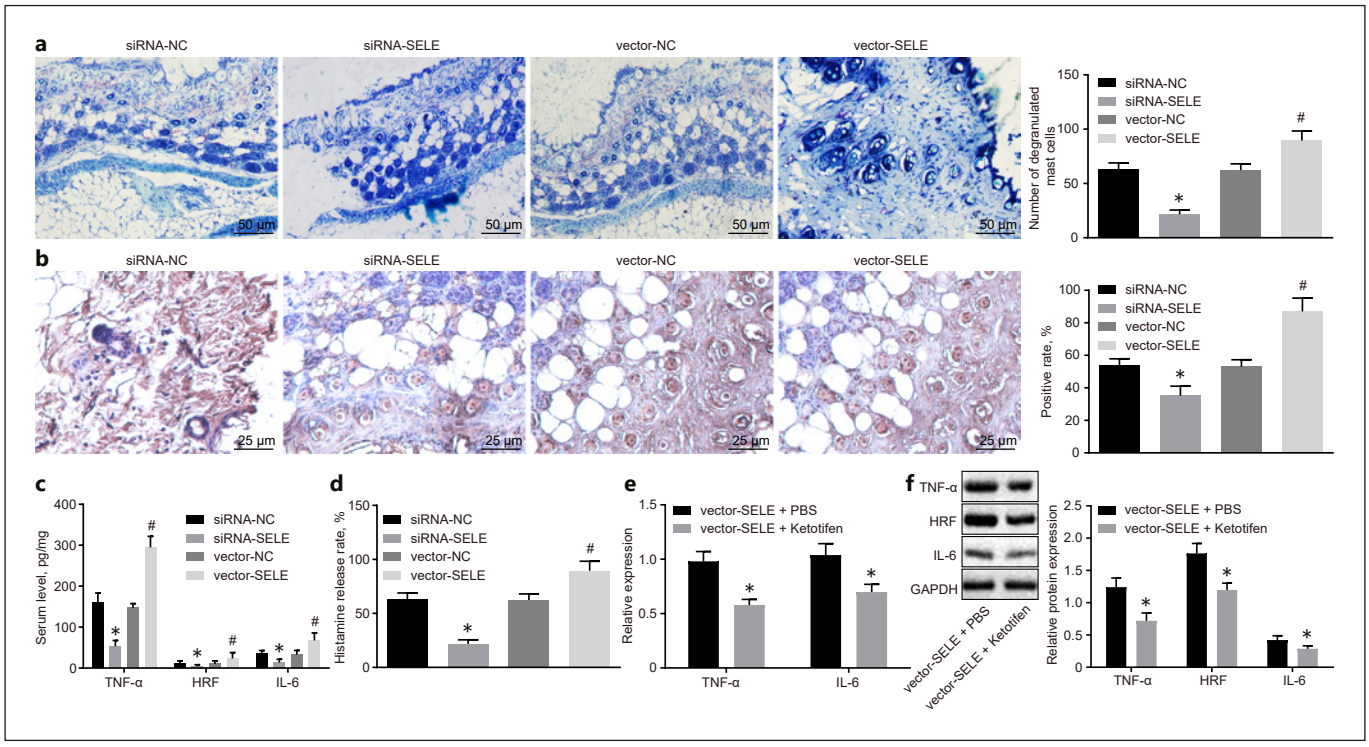


Fig. 6. The expression of TNF-α, HRF, and IL-6 is decreased in addition to repressed histamine release from mast cells in CIU mice following SELE silencing. **a** Toluidine blue staining of mast cell degranulation in skin tissues after alteration of SELE (×200). **b** Representative images of immunohistochemistry and quantification of the positive rate of TNF protein after alteration of SELE (×400). **c** Content of TNF-α, HRF, and IL-6 in the serum of mice after alteration of SELE detected by ELISA. **d**, Histamine release rate after alteration of SELE. * $p < 0.05$ vs. CIU mice treated with siRNA-NC. # $p < 0.05$ vs. CIU mice treated with vector-NC. **e** mRNA expression of TNF-α and IL-6 after treatment of vector-SELE and Ketotifen measured using RT-qPCR. **f** Protein expression of TNF-α, HRF, and IL-6 after treatment with vector-SELE and ketotifen as determined by Western blot analysis. * $p < 0.05$ vs. CIU mice treated with vector-SELE + PBS. Measurement data are expressed as means ± SD. Comparisons between the 2 groups were analyzed using a *t* test. One-way ANOVA was used for comparison of data among multiple groups, followed by the Tukey post hoc test ($n = 6$).

Discussion

A complex disorder that often bewilders many clinicians, CIU is a condition characterized by the presence of unremitting sensations of pruritus as well as eruption of afflictive wheals for a period longer than 6 weeks. CIU has been well documented to be a condition detrimental to patients' physical and psychological well-being [15]. CIU is at times referred to as a refractory disorder among some patients with serious clinical symptoms owing to the fact that the standard treatment provides only partial curative effects [16]. The molecular mechanism of CIU requires urgent analysis in order to improve CIU treatment. This study aimed to verify the correlation between SELE and CIU via the activation mechanism of mast cells. Consequently, we uncovered evidence suggesting that the upregulation of SELE might potentially promote mast cell production of inflammatory factors such as TNF-α, HRF, IL-6, and histamines to boost the progression of CIU.

Our initial observations revealed that SELE was highly expressed in clinical samples of CIU. SELE forms part of a family of cell adhesion receptors involved in the initial interactions between leukocytes and vascular endothelia, which serves as a prelude to their solid attachment and extravasation into tissues [17]. SELE has also been shown to engage in the process of chronic and acute inflammation covering postischemic, brain, lung, heart, and skin inflammatory diseases [18]. Ismayilov et al. [19] concluded that P-selectin and SELE are upregulated in Legg-Calve-Perthes disease, which may serve as a clinical indicator of activated platelets and possibly endothelial activation. Consistent with our results, significant levels of

Downloaded by: C. Alessandri - 87218
84.220.48.124 - 12/18/2020 7:27:58 AM

SELE expression have been reported in the skin tissue samples of CIU patients compared to normal controls [20].

Our results further revealed that mast cells participate in the development of CIU by releasing TNF-α, HRF, IL-6, and histamine. The pathogenesis of CIU is comprised predominately of histamine and other mediators, including platelet-activating factor and TNF-α [21]. Mast cells are widely considered to be the primary effector cells of urticaria [22]. CIU patients have been shown to exhibit a markedly elevated expression of mast cell proteases (chymase and tryptase) and proinflammatory cytokines (IL-1β, IL-3, IL-6, and TNF-α) [20]. Hidvégi et al. [23] concluded that T-cell activation and mast cell degranulation can be viewed as characteristics of CU, with mast cell activation suggested to be an indicator of the severity of autoimmune CU. Previous studies have revealed that mast cells are particularly important in initiating the release of inflammatory mediators such as histamine and proinflammatory cytokines (TNF-α and IL-6), which plays a significant role in allergy and inflammation [24]. Greater serum levels of TNF-α have been confirmed among patients with CIU and these have been shown to be a significant indicator of pathogenesis in CIU [25]. The plasma concentration of IL-6 was found to be higher in CIU patients, suggesting that a low-grade inflammatory process takes place in the skin of patients with CIU [26].

The potential therapeutic value of SELE in inflammatory diseases and cancers has been highlighted in the literature owing to its unique temporal and spatial expression profile [5]. A previous study revealed that significantly decreased SELE serum levels may reflect the inhibitory activity on neutrophil rolling and extravasation towards inflamed skin [27]. SELE silencing has been shown to potentially inhibit the development of immunologic contact urticaria by inhibiting the cell adhesion ability of vascular endothelial cells [28]. In the present study, we detected that SELE silencing could prevent the production of TNF-α, HRF, IL-6, and histamine from mast cells and consequently ameliorate the progression of CIU. A prior study concluded that mast cells were closely connected with chronic skin inflammatory diseases in that mast cells could latently gather the cells of the immune system by secreting some soluble cytokines involved in chronic skin inflammation [6]. The inhibited secretion of IL-6 and TNF-α from mast cells may contribute to the treatment of allergic inflammation [29]. A previous study highlighted elevated levels of histamine as well as an increased positive expression rate of SELE as indicators of the successful establishment of mouse models of immunologic contact urticaria [28], suggesting a positive correlation between histamine and SELE. Besides, an increased endothelial surface expression of SELE has been reported to exert a synergistic effect on histamine and TNF-α [30]. Proinflammatory cytokines such as TNF-α, IFN-γ, and IL-6 from mast cells have been shown to be capable of inducing the expression of adhesion molecules (such as P-selectin and SELE) in murine heart endothelial cells, contributing to the pathogenesis of vascular inflammatory diseases [31]. SELE has been shown to be strongly associated with inflammation severity [18]. Jiao et al. [10] confirmed that SELE plays a crucial role in the process of allergic inflammation of the lung and skin based on the notion that the types of initiating inflammatory stimuli or the vascular beds engaged in the inflammatory response might affect the SELE-mediated leukocyte migration. SELE is regarded as the vital part of the pathogenesis of common inflammatory skin diseases due to the fact that SELE mediates the process of leukocyte recruitment during inflammatory responses [32]. The aforementioned findings are all indicative of the involvement of SELE in CIU via the regulation of mast cell-dependent inflammatory factor release.

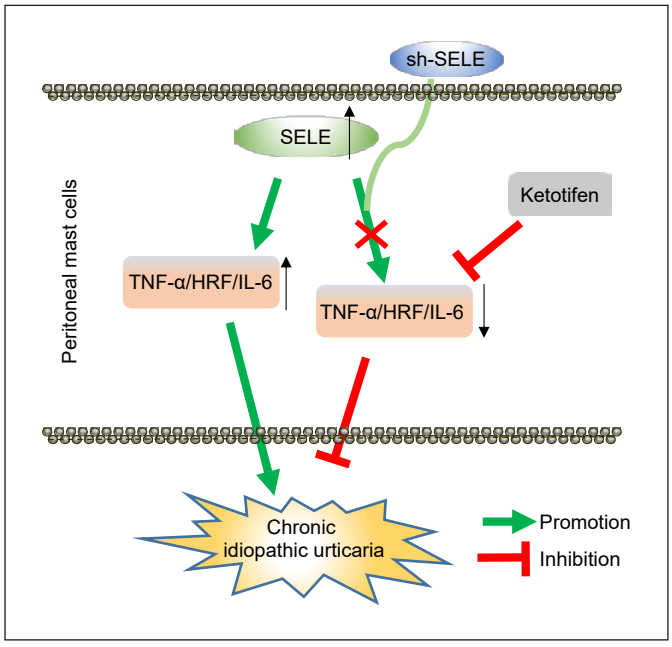


Fig. 7. SELE silencing alleviates the development of CIU by inhibiting the release of inflammatory factors from mast cells. Silencing of SELE or ketotifen treatment may suppress the release of TNF-α, HRF, IL-6, and histamine from mast cells, thus ameliorating the progression of CIU.

Downloaded by: C. Alessandri - 87218
84.220.48.124 - 12/18/2020 7:27:58 AM

In conclusion, the findings of the current study provide evidence suggesting that a high SELE expression may promote disease progression by inducing mast cells to produce inflammatory factors such as TNF-α and histamine in CIU. Consist with this conclusion, SELE may help shed light on future novel treatment approaches for CIU, which may be realized by regulating the expression of SELE to inhibit mast cells producing inflammatory factors (Fig. 7). One of the limitations of this study that should be acknowledged is the absence of literature highlighting the correlation between inflammatory factors and CIU in this report. Hence more detailed experiments are required to address this limitation.

Acknowledgement

We acknowledge and appreciate our colleagues for their valuable suggestions for and technical assistance with this study.

Statement of Ethics

The study protocols were approved by the Ethics Committee and the Experimental Animal Ethics Committee of The First Hospital of Jilin University. All of the patients signed informed consent forms prior to participating in this study. The animal experiments were performed in strict accordance with the principle of using the least number of animals and minimizing the pain of the experimental animals.

Conflict of Interest Statement

The authors have no conflict of interests to disclose.

Funding Sources

This study was supported by the Natural Science Foundation of Jilin Province (No. 20150101167JC) and Health and Family Planning Research Projects of Jilin Province (No. 2015Z025).

Author Contributions

Y.X. and M.J. designed this study. H.W. collected the data, performed the data analyses, and produced the initial draft of this paper. W.Y. contributed to the drafting this paper. All of the authors read and approved the final version of this work.

References

1 Kang MJ, Kim HS, Kim HO, Park YM. The impact of chronic idiopathic urticaria on quality of life in korean patients. Ann Dermatol. 2009 Aug;21(3):226–9.
2 Goldstein S, Gabriel S, Kianifard F, Ortiz B, Skoner DP. Clinical features of adolescents with chronic idiopathic or spontaneous urticaria: review of omalizumab clinical trials. Ann Allergy Asthma Immunol. 2017 Apr;118(4):500–4.
3 Vonakis BM, Saini SS. Basophils and mast cells in chronic idiopathic urticaria. Curr Allergy Asthma Rep. 2005 Jul;5(4):270–6.
4 Hong GU, Ro JY, Bae Y, Kwon IH, Park GH, Choi YH, et al. Association of TG2 from mast cells and chronic spontaneous urticaria pathogenesis. Ann Allergy Asthma Immunol. 2016 Sep;117(3):290–7.
5 Barthel SR, Gavino JD, Descheny L, Dimitroff CJ. Targeting selectins and selectin ligands in inflammation and cancer. Expert Opin Ther Targets. 2007 Nov;11(11):1473–91.
6 Harvima IT, Nilsson G. Mast cells as regulators of skin inflammation and immunity. Acta Derm Venereol. 2011 Oct;91(6):644–50.
7 Gu Y, Yang DK, Spinas E, Kritas SK, Saggini A, Caraffa A, et al. Role of TNF in mast cell neuroinflammation and pain. J Biol Regul Homeost Agents. 2015 Oct-Dec;29(4):787–91.
8 Confino-Cohen R, Chodick G, Shalev V, Leshno M, Kimhi O, Goldberg A. Chronic urticaria and autoimmunity: associations found in a large population study. J Allergy Clin Immunol. 2012 May;129(5):1307–13.
9 Takahagi S, Mihara S, Iwamoto K, Morioke S, Okabe T, Kameyoshi Y, et al. Coagulation/fibrinolysis and inflammation markers are associated with disease activity in patients with chronic urticaria. Allergy. 2010 May;65(5):649–56.
10 Jiao A, Fish SC, Mason LE, Schelling SH, Goldman SJ, Williams CM. A role for endothelial selectins in allergic and nonallergic inflammatory disease. Ann Allergy Asthma Immunol. 2007 Jan;98(1):83–8.
11 Williams P, Kavati A, Pilon D, Xiao Y, Zhdanava M, Balp MM, et al. Health care burden and treatment patterns in commercially insured children with chronic idiopathic/spontaneous urticaria: A real-world study in the United States. Allergy Asthma Proc. 2018 May;39(3):201–11.
12 Gao Y, Xu B, Zhang P, He Y, Liang X, Liu J, et al. TNF-α Regulates Mast Cell Functions by Inhibiting Cell Degranulation. Cell Physiol Biochem. 2017;44(2):751–62.
13 Patel D, Sarala N, Datti NP. Topical Olopatadine Hydrochloride versus Ketotifen Fumarate for Allergic Conjunctivitis. J Ophthalmic Vis Res. 2018 Apr-Jun;13(2):119–23.
14 Petrosino S, Schiano Moriello A, Verde R, Allarà M, Imperatore R, Ligresti A, et al. Palmitoylethanolamide counteracts substance P-induced mast cell activation in vitro by stimulating diacylglycerol lipase activity. J Neuroinflammation. 2019 Dec;16(1):274.
15 Greaves MW. Chronic idiopathic urticaria. Curr Opin Allergy Clin Immunol. 2003 Oct;3(5):363–8.
16 Erbagci Z. The leukotriene receptor antagonist montelukast in the treatment of chronic idiopathic urticaria: a single-blind, placebo-controlled, crossover clinical study. J Allergy Clin Immunol. 2002 Sep;110(3):484–8.
17 Somers WS, Tang J, Shaw GD, Camphausen RT. Insights into the molecular basis of leukocyte tethering and rolling revealed by structures of P- and E-selectin bound to SLe(X) and PSGL-1. Cell. 2000 Oct;103(3):467–79.
18 Impellizzeri D, Cuzzocrea S. Targeting selectins for the treatment of inflammatory diseases. Expert Opin Ther Targets. 2014 Jan;18(1):55–67.

Downloaded by: C. Alessandri - 87218
84.220.48.124 - 12/18/2020 7:27:58 AM

19 Ismayilov V, Aksoy DY, Sayinalp N, Haznedaroğlu IC, Aksoy MC. Increased soluble selectins as a reflection of activated platelets and endothelium in Legg-Calve-Perthes disease. J Pediatr Hematol Oncol. 2014 Oct; 36(7):e410–1.
20 Vena GA, Cassano N, Filieri M, Filotico R, D'Argento V, Coviello C. Fexofenadine in chronic idiopathic urticaria: a clinical and immunohistochemical evaluation. Int J Immunopathol Pharmacol. 2002 Sep-Dec;15(3): 217–24.
21 Johnson M, Kwatra G, Badyal DK, Thomas EA. Levocetirizine and rupatadine in chronic idiopathic urticaria. Int J Dermatol. 2015 Oct; 54(10):1199–204.
22 Barlow RJ, Ross EL, MacDonald DM, Kobza Black A, Greaves MW. Mast cells and T lymphocytes in chronic urticaria. Clin Exp Allergy. 1995 Apr;25(4):317–22.
23 Hidvégi B, Nagy E, Szabó T, Temesvári E, Marschalkó M, Kárpáti S, et al. Correlation between T-cell and mast cell activity in patients with chronic urticaria. Int Arch Allergy Immunol. 2003 Oct;132(2):177–82.
24 Park HH, Lee S, Son HY, Park SB, Kim MS, Choi EJ, et al. Flavonoids inhibit histamine release and expression of proinflammatory cytokines in mast cells. Arch Pharm Res. 2008 Oct;31(10):1303–11.
25 Serhat Inaloz H, Ozturk S, Akcali C, Kirtak N, Tarakcioglu M. Low-dose and short-term cyclosporine treatment in patients with chronic idiopathic urticaria: a clinical and immunological evaluation. J Dermatol. 2008 May; 35(5):276–82.
26 Kasperska-Zajac A, Brzoza Z, Rogala B. Plasma concentration of interleukin 6 (IL-6), and its relationship with circulating concentration of dehydroepiandrosterone sulfate (DHEA-S) in patients with chronic idiopathic urticaria. Cytokine. 2007 Aug;39(2):142–6.
27 Jurakić Toncić R, Lipozencić J, Marinović B. Treatment of chronic urticaria. Acta Dermatovenerol Croat. 2009;17(4):305–22.
28 Meng ZD, Wang XL, Du TP, Wang Y, Qin GF, Zhao HB, et al. Effects of RNA interference-mediated E-selectin gene silencing on cell adhesion molecule expression and cell-cell adhesion in vascular endothelial cells in mice with immunologic contact urticaria. J Cell Biochem. 2019 Jan;120(1):115–25.
29 Kempuraj D, Huang M, Kandere-Grzybowska K, Basu S, Boucher W, Letourneau R, et al. Azelastine inhibits secretion of IL-6, TNF-alpha and IL-8 as well as NF-kappaB activation and intracellular calcium ion levels in normal human mast cells. Int Arch Allergy Immunol. 2003 Nov;132(3):231–9.
30 Chen C, Khismatullin DB. Synergistic effect of histamine and TNF-α on monocyte adhesion to vascular endothelial cells. Inflammation. 2013 Apr;36(2):309–19.
31 Zhang J, Alcaide P, Liu L, Sun J, He A, Luscinskas FW, et al. Regulation of endothelial cell adhesion molecule expression by mast cells, macrophages, and neutrophils. PLoS One. 2011 Jan;6(1):e14525.
32 Schön MP. Inhibitors of selectin functions in the treatment of inflammatory skin disorders. Ther Clin Risk Manag. 2005 Sep;1(3):201–8.

Downloaded by: C. Alessandri - 87218
84.220.48.124 - 12/18/2020 7:27:58 AM