Expression and existence forms of mast cell activating molecules and their antibodies in systemic lupus erythematosus

Yuping Wang1 | Tengkai Wang2 | Meijuan Cai1 | Shuzhen Zhu1 | Lijun Song3 | Qian Wang1,4

1Department of Laboratory Medicine, Qilu Hospital (Qingdao), Cheeloo College of Medicine, Shandong University, Qingdao, Shandong, China
2Department of Internal Medicine, Qilu Hospital of Shandong University, Jinan, Shandong, China
3Department of Rheumatology, Qilu Hospital of Shandong University, Jinan, Shandong, China
4Department of Laboratory Medicine, Qilu Hospital of Shandong University, Jinan, Shandong, China

Correspondence
Qian Wang, Department of Laboratory Medicine, Qilu Hospital (Qingdao), Cheeloo College of Medicine, Shandong University, 758 Hefei Rd, Qingdao, Shandong 266035, China.
Email: sd.wangqian@163.com

Abstract
Introduction: Mast cells are regarded as a kind of classical anaphylaxis cells. However autoimmune diseases and allergic reactions have many similarities or overlaps. A large number of papers have proved that mast cells play a significant role in the pathogenesis of systemic lupus erythematosus (SLE). It is speculated that IgE, anti-IgE antibodies, FcεRI, and anti-FcεRI antibodies activate mast cells through autoimmunity pathways and participate in the disease process of SLE. Naturally occurring protein molecules not only exist in monomer form, but also in polymer of protein molecules. Therefore, whether IgE, FcεRIα, anti-IgE antibodies, and anti-FcεRI antibodies also exist in polymeric forms in the natural state is worthy of further investigation.

Methods: The serum samples and clinical data of 131 patients with SLE were collected from Qilu Hospital (Qingdao). Sixty healthy individuals were collected as the control group. Serum FcεRIα, anti-IgE, and anti-FcεRI were detected by enzyme-linked immunosorbent assay. Serum IgE was detected by rate scatter nephelometry. A Chinese hamster ovarian cancer cell line CHO3D10 transfected with human FcεRIα was cultured and the cell protein extract was prepared. The existence forms of FcεRIα in the cell protein extract were detected by the native-page method.

Results: The serum FcεRIα in SLE patients was significantly higher than that in control group (3.52 [2.18, 4.71] µg/ml and 1.87 [1.52, 2.33] µg/ml, respectively; p < .05). Anti-IgE was significantly lower than that in the control group (0.85 [0.55, 1.21] µg/ml and 1.23 [0.95, 1.58] µg/ml, respectively; p < .05). The CHO3D10 cell line expressed the FcεRIα, which had one kind of monomer (mFcεRIα) and two kinds of polymers (pFcεRIα) in the degeneration conditions.

Conclusion: In patients with SLE, the expression of FcεRIα was increased and the level of anti-IgE was decreased. FcεRIα had one kind of monomer and two...
1 | INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease involving multiple systems and organs and has multiple autoantibodies which are caused by the combined influence of genetics, hormones, and environmental factors. The pathogenesis is resulted from the breakdown of the homeostasis of apoptotic bodies, leading to the proliferation of self-reactive T and B cells, and the production of autoantibodies against dsDNA, Sm, SS-A, and SS-B.1,2

However, in recent years, researchers have paid more and more attention to other inflammatory cells except for T and B cells. For example, a large number of papers have proved that mast cells play a significant role in the pathogenesis of SLE.3 Mast cells and their activation-related antibodies are involved in the occurrence and development of various autoimmune diseases such as rheumatoid arthritis and multiple sclerosis.4 The previous research also found that the levels of FcεRIα, anti-IgE antibodies, and anti-FcεRI antibodies related to the activation of mast cells in Graves’ disease were higher than those in the healthy control group.5 It is speculated that IgE, anti-IgE antibodies, FcεRI, and anti-FcεRI antibodies activate mast cells through autoimmune pathways and participate in the disease process of SLE.

Naturally occurring protein molecules not only exist in monomer form, but also in polymer of protein molecules.6 Studies have reported that CRP not only exists in the form of monomer, but there are also multiple polymers of CRP in serum in the natural state, and perform different functions.7 Using the native-page method to detect dog recombinant antigens Can f 1 and Can f 2 also showed the existence of dimer structure.8 Therefore, whether IgE, FcεRIα, anti-IgE antibodies, and anti-FcεRI antibodies also exist in polymeric forms in the natural state is worthy of further investigation.

Based on the above research foundation, this study systematically analyzed the expression levels of IgE, FcεRIα, anti-IgE antibodies, and anti-FcεRI antibodies in the serum of SLE patients, and explored their existence forms in the serum of SLE patients.

2 | OBJECT AND METHODS

2.1 | Object

2.1.1 | SLE patient group

Choosing 131 SLE patients treated between January and December 2019 in Qilu Hospital (Qingdao), Cheelu College of Medicine, Shandong University, including 17 males and 114 females, aged 14.00–75.00 (38.50 ± 13.58) years old. These patients were all newly diagnosed according to the 1997 ACR revised criteria without allergic history. The autoantibody test results of SLE patients were different. The degree of abnormality and the degree of disease activity in SLE patients were scored (Table 1).

2.1.2 | Healthy control group

Sixty cases of the healthy control group, including 20 males and 40 females, aged 31.00–57.00 (45.64 ± 6.47) years old, excluded SLE, allergies, and other autoimmune diseases, and autoantibody testing, liver and kidney function, and routine laboratory tests of hematuria were normal.

| TABLE 1 | Autoantibody levels in patients with SLE |
|----------|------------------------------------------|
| Male/female | 17/114 |
| ANA       | 90.07 (118/131) |
| anti-dsDNA | 36.64 (48/131) |
| anti-Sm   | 16.79 (22/131) |
| anti-SSA  | 32.06 (42/131) |
| anti-SSB  | 19.85 (26/131) |
| anti-RNP  | 28.24 (37/131) |
| SLEDAI    | 11.00 (7.00, 14.00) |

Abbreviations: ANA, anti-nuclear antibody; anti-dsDNA, anti-double-stranded DNA antibody; anti-Sm, anti-nucleoprotein antibody; anti-SSA, anti-SSA antibody; anti-SSB, anti-SSB antibody; anti-RNP, anti-ribonucleoprotein antibody; SLEDAI, SLE disease activity score.
2.1.3 | CHO3D10 cells

CHO3D10 cell line is a Chinese hamster ovarian cancer cell line. Our research group transfected human FceRIα gene clone into CHO3D10 cells through plasmid transfection, and obtained CHO3D10 cells with over-expression of human FceRIα.

This study was approved by the ethics committee of Qilu Hospital (Qingdao), Cheeloo College of Medicine, Shandong University.

2.2 | Methods

2.2.1 | Serum antinuclear antibody detection

Indirect immunofluorescence method (EUROIMMUN Antinuclear Antibody IgG Detection Kit).

2.2.2 | Serum anti-dsDNA antibody detection

Indirect enzyme-linked immunosorbent assay (ELISA) method (Shanghai Kexin Bio Anti-double-stranded DNA (dsDNA) Antibody Detection Kit).

2.2.3 | Serum IgE detection

Rate scatter turbidimetry (Siemens BNII instrument and its supporting reagents).

2.2.4 | Serum FcεRIα, anti-IgE antibody, and anti-FcεRI antibody detection

Competition ELISA method (BlueGene Biotech ELISA Kit).

2.2.5 | CHO3D10 cell protein extract

CHO3D10 cells transfected with human FcεRIα were resuscitated and cultured, washed twice with pre-cooled phosphate-buffered saline (PBS), when it was 80%–90% of the 9 cm diameter petri dish. Then we added 1 ml PBS to the petri dish and scraped the cells with the cell scraping, and collected the cell suspension in an EP tube. The whole process was operated on an icebox. The cell suspension was centrifuged at 1500g*10 min, and washed two times with pre-cooled PBS, 2500g*5 min. Remove the supernatant, add the cell lysate according to the ratio of 10^6 mouse-derived cells corresponding to 20 µl lysate (1 mol/L Tris–HCl pH = 7.5 3 ml, 10% SDS 10 ml, distilled water to 50 ml) to CHO3D10 cells. Metal bath 100°C, 10 min, until making the protein into fluid and transparent.

2.2.6 | CHO3D10 cell protein extract concentration detection

Refer to Biyuntian BCA Protein Concentration Determination Kit (enhanced).

2.2.7 | Native-page method to detect the existence forms of FcεRIα expressed by CHO3D10 cells

CHO3D10 cell protein extract was subjected to native-page electrophoresis and transferred to membrane, fixed in acetic acid fixative (8 ml of acetic acid, distilled water to 100 ml) for 15 min, and then the membrane was washed, closed. Mouse anti-human FcεRIα (Ebioscience; 1:1000) was used as the primary antibody, and antimouse IgG (Dako) conjugated with HRP (1:2000) was used as the secondary antibody. The reaction signal was chemiluminescent by ECL substrate (Amersham) detection. Native-page electrophoresis instrument and its supporting reagents (Life).

2.3 | Statistical analysis

All statistical analyses were performed using SPSS 21.0 analysis software. Mast cell activation-related antibodies and molecules IgE, FcεRIα, anti-IgE, and anti-FcεRI quantitative detection data showed a skewed distribution, represented by M (P25, P75), and the Mann–Whitney U rank-sum test was used for comparison between groups. The difference was statistically significant with p < .05.

3 | RESULTS

3.1 | Results of serum IgE, FcεRIα, anti-IgE antibody, and anti-FcεRI antibody levels in SLE patient group and healthy control group

There was no significant difference in the detection results of serum IgE and anti-FcεRI antibodies between the SLE patient group and the healthy control group.
(p > .05). Serum FcεRIα: 3.52 (2.18, 4.71) µg/ml in SLE patient group was significantly higher than that in healthy control group: 1.87 (1.52, 2.33) µg/ml; serum anti-IgE antibody level in SLE patient group: 0.85 (0.55, 1.21) µg/ml was significantly lower than that in healthy control group: 1.23 (0.95, 1.58) µg/ml (p = .000; Figure 1).

3.2 | Morphological observation of CHO3D10 cells

Under the inverted microscope, it can be seen that the cells adhere to the wall, evenly distributed, the cell size was relatively uniform, fusiform or polygonal, and good refractive index (Figure 2).

3.3 | Native-page method to detect the existence forms of FcεRIα expressed by CHO3D10 cells

The protein extract concentration of the extracted CHO3D10 cells was determined by the BCA method to be 0.86 µg/µl. Native-page method was used to detect the existence forms of FcεRIα. The results showed that FcεRIα existed in three forms under non-denaturing conditions: one monomer (mFcεRIα) and two polymers (pFcεRIα) forms, and mFcεRIα had the most content (Figure 3).

4 | DISCUSSION

Mast cells are regarded as a kind of classical anaphylaxis cells.11,12 However autoimmune diseases and allergic reactions have many similarities or overlaps. Studies have also paid attention to the increase of mast cells in diseases such as Graves’ ophthalmopathy, allergic rhinitis, SLE, and Sjogren’s syndrome.13,14 The results of this study showed that the level of FcεRIα in the serum of patients with SLE was significantly higher than that in healthy controls (p = .000), however, FcεRIα is the result of over-transcription of DNA encoding FcεRIα during cell activation induced by FcεRI cross-linking on the surface of mast cells.15 This study speculated that the cross-linking FcεRI has activated the mast cells and contributed to the injury of chronic tissue damage during the pathogenesis of SLE.

Anti-IgE antibodies and anti-FcεRI antibodies are the results of the body’s autoimmunity against excess IgE and FcεRIα, leading to complex and diverse forms of IgE, FcεRIα, anti-IgE antibodies, and anti-FcεRI antibodies. IgE and anti-IgE antibodies, FcεRIα and anti FcεRI antibodies can form immune complexes.16-18 Miescher et al.19 reported that only when FcεRIα is not bound by IgE, anti-FcεRI antibodies can bind to the cell surface FcεRIα, activate peripheral blood basophils, and cause inflammation. The results of this study also verified that there was no significant difference in serum IgE levels between the SLE group and the healthy control group. As a result, the autoimmunity of IgE and anti-IgE antibodies...
caused the anti-IgE antibody level in the SLE patient group to be significantly lower than that of the healthy control group, while the autoimmune result of FcεRI and anti-FcεRI antibody triggers tissue inflammatory damage caused by mast cell activation, which is worthy of further study and clarification.

As patients have different disease phenotypes and antibody detection results, I performed SLEDAI scores for SLE activity. The correlation between serum IgE, FcεRIα, anti-IgE antibody, and anti-FcεRI antibody levels in SLE patients and SLEDAI was analyzed. The results showed that there was no correlation between these four substances and SLEDAI, so this part was not included in the article.

As protein macromolecules, IgE, FcεRIα, anti-IgE antibodies, and anti-FcεRI antibodies must have a variety of complex forms in nature, such as BCR, CRP, recombinant antigens, and other biological macromolecules that all have monomers, dimers, and multiple polymers. However, when we detected the existence forms of FcεRIα in serum, we did not get good detection results. The reason may be that IgE, anti-IgE, and anti-FcεRI antibodies in serum can form immune complexes with FcεRIα and affect the detection result. In this study, the CHO3D10 cells transfected with human FcεRIα was used as the research object, and the interference of IgE, anti-IgE, FcεRIα, and anti-FcεRI antibodies in the formation of immune complexes in the serum was excluded, and the native-page method was used to detect FcεRIα protein molecule in its natural state. The results showed that the FcεRIα expressed by CHO3D10 cells existed in one monomer and two polymers under non-denaturing conditions, but mFcεRIα had the most content. It implicates that although the protein macromolecules exist polymer under the natural state, monomer is still the main form of existence, which also explains why the deviation caused by the evaluation of traditional detection methods is still within the acceptable range. However, whether the existence of this polymer is a form of storage of protein molecules in the body, or whether it is a potential cause of disease in the body's environmental disorder still needs to be further explored.

In summary, IgE, FcεRIα, anti-IgE antibodies, and anti-FcεRI antibodies are the key factors in activating mast cells. An in-depth study of the relationship between...
the existence of IgE, anti-IgE, FcεRIz, anti-FcεRI antibodies, and SLE provides a new reference basis for the understanding and further discussion of the inflammatory mechanism of the disease.

ACKNOWLEDGMENTS

This study was supported by the 973 Program (No. 2015CB755402); Wu Jie Ping Medical Foundation (No. 320.6750.17181); and the Science and Technology Development Program of Qingdao (19-6-1-20-nsh).

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Conception and design of the study: Yuping Wang and Qian Wang. Acquisition of data: Tengkai Wang and Meijuan Cai. Analysis and interpretation of data: Shuzhen Zhu and Lijun Song. Drafting the article: Yuping Wang and Tengkai Wang. Revising it critically for important intellectual content: Qian Wang. Final approval of the version to be submitted: Yuping Wang, Tengkai Wang, Meijuan Cai, Shuzhen Zhu, Lijun Song, and Qian Wang.

ORCID

Yuping Wang https://orcid.org/0000-0002-8522-9597

REFERENCES

1. Kiriakidou M, Ching CL. Systemic lupus erythematosus. *Ann Intern Med*. 2020;172(11):ITC81–ITC96.
2. Wieliczko M, Matuszkiewicz-Rowińska J. Systemic lupus erythematosus – news 2017. *Wiad Lek*. 2017;70(6 pt 2): 1201-1204.
3. Caraffa AI, Gallenga CE, Kritas SK, Ronconi G, Conti P. Impact of mast cells in systemic lupus erythematosus: can inflammation be inhibited? *J Biol Regul Homeost Agents*. 2019;33(3):669-673.
4. Li X, Kimberly RP. Targeting the Fc receptor in autoimmune disease. *Expert Opin Ther Targets*. 2014;18(3):335-350.
5. Wang Y, Cui Z, Lin L, et al. Analysis on the expressions of serum IgE, FcεRI and their antibodies in patients with benign thyroid disease. *Lab Med*. 2015;30(5):450-453.
6. Yang YI, Holmberg AL, Olsen BD. Artificially engineered protein polymers. *Annu Rev Chem Biomol Eng*. 2017;8:549-575.
7. Asztalos BF, Horan MS, Horvath KV, McDermott AY, Chalasani NP, Schaefer EJ. Obesity associated molecular forms of C-reactive protein in human. *PLOS One*. 2014;9(10):e109238.
8. Kamata Y, Miyanomae A, Nakayama E, et al. Characterization of dog allergens Can f 1 and Can f 2. 2. A comparison of Can f 1 with Can f 2 regarding their biochemical and immunological properties. *Int Arch Allergy Immunol*. 2007;142(4):301-308.
9. Li F, Wang Y, Lin L, et al. Mast cell-derived exosomes promote Th2 cell differentiation via OX40L-OX40 ligation. *J Immunol Res*. 2016;2016:3623898.
10. Subhadarshanee B, Mohanty A, Jagdev MK, Vasudevan D, Behera RK. Surface charge dependent separation of modified and hybrid ferritin in native PAGE: impact of lysine 104. *Biochim Biophys Acta Proteins Proteom*. 2017;1865(10):1267-1273.
11. Bayar Muluk N, Bafaqeeh SA, Cingi C. Anti-IgE treatment in allergic rhinitis. *Int J Pediatr Otorhinolaryngol*. 2019;127:109674.
12. Bonadonna P, Scaffidi L. Hymenoptera anaphylaxis as a clonal mast cell disorder. *Immunol Allergy Clin N Am*. 2018;38(3):455-468.
13. Marone G, Spadaro G, Palumbo C, Condorelli G. The anti-IgE/anti-FcεRI autoantibody network in allergic and autoimmune diseases. *Clin Exp Allergy*. 1999;29(1):17-27.
14. Shuang D, Hongjiang W, Yue Y. Expression and mechanism of mast cell activating molecules and their antibodies in allergic rhinitis and nasal polyps. *J Clin Otorhinolaryngol Head Neck Surg*. 2019;33(10):975-978.
15. Platzer B, Ruitter F, Mee J, Fiebig E. Soluble IgE receptors—elements of the IgE network. *Immunol Lett*. 2011;141(1):36-44.
16. Khodoun MV, Morris SC, Shao WH, et al. Suppression of IgE-mediated anaphylaxis and food allergy with monoclonal anti-FcεRIz mAbs. *J Allergy Clin Immunol*. 2021;147(5):1838-1854.
17. Lommatzsch M, Geißler K, Bergmann K-C, Christian Virchow J. IgE and Anti-IgE in asthma: a chequered history. *Pneumologie*. 2017;71(6):398-405.
18. Khodoun MV, Morris SC, Angerman E, et al. Rapid desensitization of humanized mice with anti-human FcεRIz monoclonal antibodies. *J Allergy Clin Immunol*. 2020;145(3):907-921.
19. Miescher SM, Horn MP, Pachlopnik JM, Baldi L, Vogel M, Stadler BM. Natural anti-FcεRIz alpha autoantibodies isolated from healthy donors and chronic idiopathic urticaria patients reveal a restricted repertoire and autoreactivity on human basophils. *Hum Antibodies*. 2001;10(3-4):119-126.
20. Ansell RJ. Characterization of the binding properties of molecularly imprinted polymers. *Adv Biochem Eng Biotechnol*. 2015;150:51-93.
21. Ji W, Chen L, Ma X, et al. Molecularly imprinted polymers with novel functional monomer for selective solid-phase extraction of gastrodin from the aqueous extract of *Gastrodia elata*. *J Chromatogr A*. 2014;1342:1-7.

How to cite this article: Wang Y, Wang T, Cai M, Zhu S, Song L, Wang Q. Expression and existence forms of mast cell activating molecules and their antibodies in systemic lupus erythematosus. *Immun Inflamm Dis*. 2022;10:235-240. doi:10.1002/iid3.567