ORIGINAL RESEARCH

Association of Nonpuerperal Mastitis with Cytokines Related to Helper T Cells TH1/TH2 and TH17/Treg

Jin Zhao, MD; Haoyang Ji, MD; Xiuhong Wang, MD; Yueqi Wang, MD; Zhongyuan Xia, MD

ABSTRACT

Objective • To investigate the association of nonpuerperal mastitis with cytokines related to the helper T cells T_H1/T_H2 and T_H17/Treg and associated immune balance.

Methods • From 2016 to 2021, we included 40 patients with non-puerperal mastitis who underwent surgery at China-Japan Friendship Hospital and compared them with 40 control patients with benign non-infectious breast disease. Hematoxylin-eosin staining detects inflammatory infiltrates of breast tissue. The expression of interferon γ and interleukin 4 in breast tissue was detected by immunofluorescence imaging, and the relative protein expression of T_H1/T_H2 and T_H17/Treg cell-associated cytokines in $CD4^+$ T cells was detected by western blotting. $CD4^+$ T cells were isolated by fluorescence-activated cell sorting for detection of the relative protein expression of interferon γ and interleukin 4 in $CD4^+$ T cells.

Results • Hematoxylin-eosin staining showed that the nonpuerperal mastitis group had significantly greater inflammatory infiltration than the control group. Immunofluorescence images showed the relative fluorescence intensity of interferon γ was significantly higher in the nonpuerperal mastitis group than in the control group ($P < .001$), but the relative fluorescence intensity of interleukin 4 did not significantly differ between the 2 groups ($P = .0686$). Western blotting revealed that the relative protein expression of interferon γ, interleukin 2, and interleukin 17 was significantly higher in the nonpuerperal mastitis group than in the control group ($P < .001$), but the relative protein expression of interleukin 4 ($P = .0512$), interleukin 10 ($P = .3088$), and transforming growth factor β ($P = .0653$) did not significantly differ between the 2 groups. Flow cytometry of isolated $CD4^+$ T cells showed the relative protein expression of interferon γ was significantly higher in the nonpuerperal mastitis group than in the control group ($P < .001$), but the relative protein expression of interleukin 4 did not significantly differ between the 2 groups ($P = .0680$).

Conclusion • The expression of the T_H1 cytokines interferon γ and interleukin 2 and the T_H17 cytokine interleukin 17 was significantly higher in patients with nonpuerperal mastitis, while the T_H2 cytokine interleukin 4 and the Treg cytokines interleukin 10 and transforming growth factor β were expressed at lower levels. This study provides new research ideas for the treatment of mastitis. (*Altern Ther Health Med.* 2023;29(8):150-155).

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INTRODUCTION

Nonpuerperal mastitis (NPM) is a group of nonspecific chronic inflammatory diseases of the breast, the most common of which are granulomatous mastitis and plasma cell mastitis (PCM). Although NPM accounts for only 1.4% to 5% of benign breast diseases,[1] its incidence rate has been recently and steadily increasing. NPM typically affects women of reproductive age, especially those between 30 to 40 years; NPM is a refractory benign breast disease, and the major clinical manifestations are difficult elimination of breast masses, and long-lasting sinus tracts, fistulas, and ulcers.[2] Although there are different opinions on the pathogenesis of NPM, most scholars agree that NPM is an autoimmune disease and that PCM is caused by aseptic inflammation of the ductal wall as a result of dilatation of the inner wall of the mammary duct and obstruction of the lumen by lipid secretions; granulomatous mastitis also results in similar histological changes to other autoimmune diseases.[3]

CD4+ T lymphocytes are central players in immune system defense. There are 4 subsets of CD4+ T lymphocytes: the helper T cells T_H1, T_H2, T_H17, and Treg; each has a specific biological function. Specifically, T_H1 cells are mainly associated with cellular immunity,[4] T_H2 cells help B cells to produce antibodies,[5] T_H17 cells promote inflammatory responses,[6] and Treg cells suppress inflammatory responses.[7] Interferon (IFN) γ (IFN-γ) is a cytokine characteristic of T_H1 cells, and interleukin (IL) 4 (IL-4) is a cytokine characteristic of T_H2 cells. T_H17 cells mainly secrete cytokines such as IL-17A and IL-17F, while Treg cells secrete inhibitory cytokines such as transforming growth factor (TGF) β (TGF-β) and IL-10.

By secreting the appropriate cytokines, these 4 subsets of CD4+ T lymphocytes communicate with and restrain one another, forming an immunoregulatory network of CD4+ T cells.[8-10] Disrupted balance between proinflammatory T_H17 cells and inhibitory Treg cells is a major factor in the pathogenesis of many inflammatory and autoimmune diseases.

It is important to study the etiology of NPM, especially its association with autoimmune disorders. Only by exploring the mechanism of occurrence and development of NPM can clinical drug selection be better guided, thereby laying a foundation for further improving clinical treatment efficacy. The changes in the expression of a single cytokine in PCM or granulomatous mastitis have been studied,[11] but there are few studies on the correlation of NPM with cytokines produced by T_H1/T_H2 and T_H17/Treg cells that control immune balance.

Therefore, exploring the relationship between NPM and immune balance can offer fresh research ideas for the clinical treatment of NPM.

METHODS

Subjects

From 2016 to 2021, we included 40 patients with non-puerperal mastitis who underwent surgery at China-Japan Friendship Hospital, and another 40 patients who were pathologically diagnosed with benign noninfectious breast diseases at the China-Japan Friendship Hospital during the same period were enrolled as the control group. Fresh breast tissue biopsies were taken to make paraffin sections of breast tissue for experimental comparison. Information about the patients' general condition and their clinical data were collected. This study was reviewed and approved by the Ethics Committee of the China-Japan Friendship Hospital (approval No. Zryhyy61-22-06-04).

Hematoxylin-eosin staining

Paraffin sections were rehydrated and then stained with hematoxylin solution (C0107, Beyotime Biotechnology) for 5 minutes, washed 3 times in 1% hydrochloric acid in 70% ethanol, rinsed with distilled water for 1 minute, and then stained with eosin solution for 3 minutes. Then, the sections were dehydrated in an ethanol gradient and deparaffinized with xylene.[12] The hematoxylin-eosin stained sections were imaged with Motic DSAssistant software.

Immunofluorescence assay

The paraffin sections were put into xylene I.15min-xylene II.15min-absolute ethanol I.5min-absolute ethanol II.5min-85% alcohol 5min-75% alcohol 5min-distilled water in turn. The sections were placed in 0.1-mol/L citric acid (pH 6) to extract the antigens, washed in phosphate-buffered saline (PBS), and blocked with blocking serum for 1 hour in a humid chamber at room temperature. After the blocking buffer was wiped off with filter paper, the sections were washed twice with PBS, incubated with the rabbit monoclonal primary antibodies anti-IFN-γ receptor β (1:500, ab224197, Abcam) or anti-IL-4 (1:500, ab62351, Abcam) in a humid chamber overnight at 4 °C, washed with PBS, and incubated with the fluorescent secondary antibody goat anti-rabbit immunoglobulin G H&L conjugated to Alexa Fluor 555 (1:200, ab150078, Abcam) in the dark for 1 hour at room temperature. Then, the sections were washed 3 times with PBS (3 minutes each time), incubated in PBS for 15 minutes, and washed with PBS. Add one drop of sealant, seal. The samples were imaged by fluorescence microscopy. The results were analyzed using image J.

Isolation of CD4+ T cells by fluorescence-activated cell sorting flow cytometry

The breast tissues were aseptically isolated, washed with Dulbecco's PBS, and cut into 1×3-mm tissue blocks with sterile scissors. The tissue blocks were digested with 0.2% collagenase IV (17104019, Thermo Fisher Scientific) and 0.25% trypsin (25200056, Thermo Fisher Scientific) at 37 °C and stirred for 5 minutes. After 1700 rpm centrifugal enrichment, termination of digestion with complete medium, the supernatant was collected and filtered through a 200-μm mesh, and the cells were centrifuged and resuspended in 1× PBS containing 1% bovine serum albumin and refrigerated at 4 °C. After 1700 rpm centrifugal enrichment, the cell density was adjusted to 1×10^6/100 μL, and the cells were incubated with an anti-CD4 monoclonal antibody (RPA-T4) conjugated to fluorescein isothiocyanate (11-0049-42, Invitrogen) in the dark for 30 minutes. The stained cells were analyzed and sorted using a BD FACSAria III Cell Sorter flow cytometer (Becton Dickinson).[13,14] Sorted CD4+ T cells are used in flow cytometry and western blotting experiments.

Western blotting

The CD4+ T cells were ground in liquid nitrogen and treated with protease and phosphatase inhibitors. The protein concentration of each sample was measured using a BCA Protein Concentration Assay Kit (P0012S, Beyotime Biotechnology). Then, the proteins were denatured by heating for 10 minutes, separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (20 μg/well), and transferred onto polyvinylidene difluoride membranes (300 mA). The membranes were incubated in 5% skim milk powder (P0216-300g, Beyotime Biotechnology) and 5% fatty acid-free bovine serum albumin (A8850, Solarbio) for 2 hours at room temperature.[15,16] The membranes were

Table. Clinical Characteristics of Enrolled Patients and Breast Lesions

Characteristics	Patients with NPM (NPM group), n = 40	Patients with benign noninfectious breast diseases (control group), n = 40
Age, mean (SD), y	38.2 (1.8)	38.4 (2.1)
Lumps only	17	9
Mass with sinus tract	23	4
Lumps with inverted nipples	0	18
Mass with sinus tract and nipple invagination	0	9
Lesion site within 2 cm of the areola	7	40
Lesion site >2 cm outside the areola	33	0

incubated with the primary antibodies anti-IFN-γ (1:1000, ab171081, Abcam), anti-IL-2 (1:1000, ab207325, Abcam), anti-IL-4 (1:1000, ab62351, Abcam), anti-IL-10 (1:5000, ab52909, Abcam), anti-IL-17A (1:2000, ab79056, Abcam), anti-TGF-β1 (1:1000, ab215715, Abcam), and anti-glyceraldehyde 3-phosphate dehydrogenase (1:1000, ab8235, Abcam) overnight at 4 °C. After washing, the membranes were incubated with Goat anti-rabbit IgG H&L (HRP) (1:2000, ab6721, Abcam) for 1 hour at room temperature and developed with an enhanced chemiluminescence kit. The results were analyzed using the ECL method.

Data analysis

Data and statistical analyses were performed using GraphPad Prism version 9 software (GraphPad Software). Measurement data are presented as mean ± standard deviation. One-way analysis of variance was used for comparisons among groups, and *t* tests were used for comparisons between 2 groups. $P < .05$ was considered statistically significant.

RESULTS

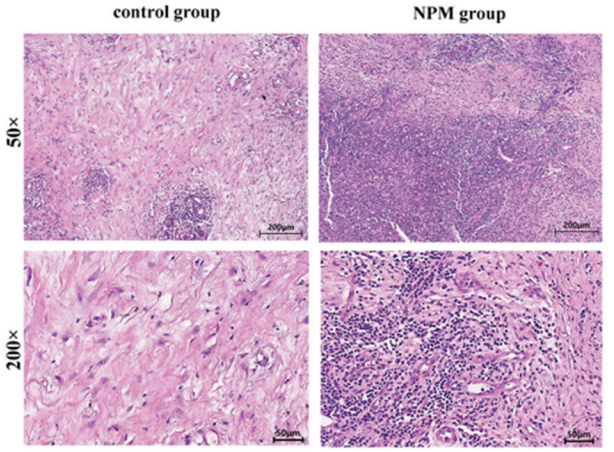
In breast tissue samples from patients with NPM, inflammatory cell infiltration increases

The clinical characteristics of the patients with NPM and the patients with benign noninfectious breast diseases are given in the Table. Hematoxylin-eosin staining of breast tissue sections showed that the mammary gland cells were intact and arranged in an orderly manner without inflammatory cell infiltration in samples from the control group, while there was severe inflammatory cell infiltration in samples from the NPM group (Figure 1).

Increased fluorescence intensity of IFN-γ but not IL-4 in patients with NPM

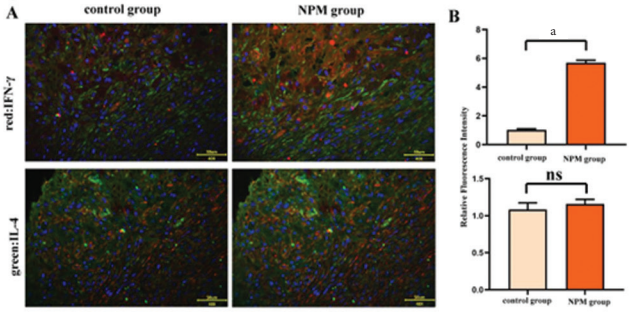
Quantification of immunofluorescence images showed that the relative fluorescence intensity of IFN-γ in breast tissue was significantly higher in the NPM group than in the control group ($P < .001$). The relative fluorescence intensity of IL-4 in breast tissue was higher in the NPM group than in the control group, but this difference was not statistically significant ($P = .0686$) (Figure 2).

Figure 1. Inflammatory Infiltration in Breast Tissue. Representative images of breast tissue samples with hematoxylin-eosin staining from patients in the control and NPM groups. Magnification: 50×, 200×; Scale: 200 μm, 50 μm



Abbreviation: NPM, nonpuerperal mastitis.

Figure 2. IFN-γ and IL-4 Relative Fluorescence Intensity **A,** Representative immunofluorescence images of IFN-γ (red) and IL-4 (green) from samples from the control and NPM groups. Compared with the control group, the red fluorescence brightness was significantly enhanced in the NPM group, and there was no difference in green fluorescence brightness. **B**. Comparison of the relative fluorescence intensity of IFN-γ (control group SD:1.026; NPM group SD:5.694) and IL-4 (control group SD:1.083; NPM group SD:1.159) between the control and NPM groups.



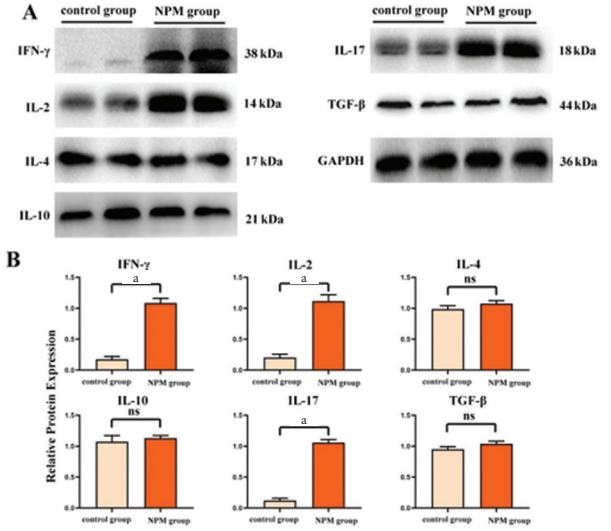
[a]$P < .001$;
ns, $P > .05$ (n = 8/group).

Abbreviations: IFN, interferon; IL, interleukin; NPM, nonpuerperal mastitis; ns, not significant.

Increased protein expression levels of IFN-γ, IL-2, and IL-17 but not IL-4, IL-10, and TGF-β in patients with NPM

Quantitative analysis of CD4+ T cells by western blotting, revealed that the relative protein expression levels of IFN-γ, IL-2, and IL-17 were significantly higher in the NPM group than in the control group ($P < .001$). The relative protein expression levels of IL-4 ($P = 0.0512$), IL-10 ($P = .3088$), and TGF-β ($P = .0653$) were higher in the NPM group than in the

Figure 3. Expression of T_H1/T_H2- and T_H17/Treg-Related Cytokines **A**, Western blot results of protein expression bands of IFN-γ, IL-2, IL-4, IL-10, IL-17, TGF-β, and GAPDH in protein samples from CD4+ T cells of the control and NPM groups. **B**. Quantification of the Western blot results in (A) shows protein expression levels of IFN-γ (control group SD:0.175; NPM group SD:1.088), IL-2 (control group SD:0.205; NPM group SD:1.115), IL-4 (control group SD:0.9875; NPM group SD:1.075), IL-10 (control group SD:1.073; NPM group SD:1.133), IL-17 (control group SD:0.1225; NPM group SD:1.058) and TGF-β (control group SD:0.95; NPM group SD:1.020) in the control and NPM groups relative to the expression of GAPDH.



$^{**}P < .001$;
ns, $P > .05$ (n = 4/group).

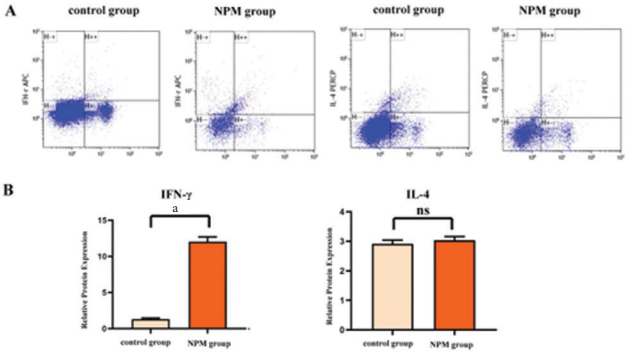
Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IFN, interferon; IL, interleukin; NPM, nonpuerperal mastitis; ns, not significant; TGF, transforming growth factor.

control group, but this difference was not statistically significant. Thus, the proportions of IFN-γ/IL-4, IL-17/IL-10, and IL-17/TGF-β and the corresponding T_H1/T_H2 and T_H17/Treg immune balance were disrupted in the NPM group (Figure 3).

Increased IFN-γ but not IL-4 in flow-sorted CD4+ T cells disrupts the T_H1/T_H2 and T_H17/Treg immune balance

$CD4^+$ T cells were isolated by fluorescence-activated cell sorting flow cytometry in breast tissue from both groups, and the relative protein expression of IFN-γ and IL-4 was quantified detected using flow cytometry. The relative protein expression of IFN-γ in $CD4^+$ T cells was significantly higher in the NPM group than in the control group ($P < .001$). The relative protein expression of IL-4 was higher in the NPM group than in the control group, but this difference was not statistically significant ($P = .0680$) (Figure 4). Thus, we

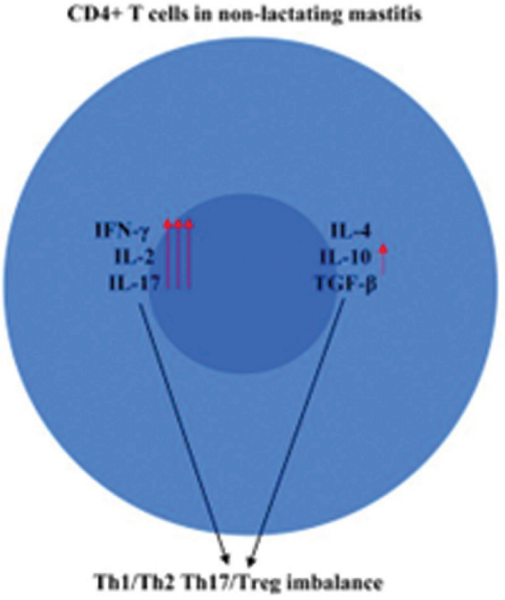
Figure 4. Expression of IFN-γ and IL-4 in Flow-Sorted $CD4^+$ T Cells **A**, Flow cytometry data of IFN-γ and IL-4 in $CD4^+$ T cells isolated from breast tissue from the control and NPM groups. H++ is the first quadrant, H-+ is the second quadrant, H- is the third quadrant, and H+- is the fourth quadrant. **B**. Quantification of the expression of IFN-γ (control group SD:1.328;NPM group SD:12.04) and IL-4 (control group SD:2.916;NPM group SD:3.038) in $CD4^+$ T cells in the control and NPM groups.



$^{**}P < .001$
ns, $P > .05$ (n = 8/group).

Abbreviations: IFN, interferon; IL, interleukin; NPM, nonpuerperal mastitis; ns, not significant.

Figure 5. Association of Nonpuerperal Mastitis With Cytokines Related to T_H1/T_H2 and T_H17/Treg Immune Balance



Abbreviations: IFN, interferon; IL, interleukin; TGF, transforming growth factor.

conclude that the T_H1/T_H2 and T_H17/Treg immune balance was disrupted in patients with NPM (Figure 5).

DISCUSSION

The incidence rate of puerperal mastitis has decreased, while that of NPM has increased, with the recent rise in knowledge of the importance of hygiene during pregnancy and puerperium.[1,4] So-called NPM should include mastitis during infancy, adolescence, menopause, and senectitude. Mammary inflammation can occur at any physiological stage, and mastitis in infancy and adolescence is frequently caused by a hormone imbalance in the body.[17,18] NPM is characterized by breast swelling, dull pain, or nodules, which are nonbacterial, self-limiting, and self-healing inflammatory manifestations.

NPM manifests clinically as an acute breast abscess, a breast mass, or a chronic fistula. For the acute breast abscess type, patients suddenly develop redness, swelling, heat, pain, and abscess formation in the breast. [19-23] In severe cases, multiple fistulas and breast deformity occur, and there may be inflammatory masses in the breast or around the fistula and repeated discharge of pus.[24-27] Exploring the mechanism of occurrence and development of NPM can help in clinical drug selection and generate new ideas for further improving clinical treatment efficacy.

T lymphocyte subsets are important for maintaining immune homeostasis.[28-30] Until now, studies have reported changes in the expression of only single cytokines in PCM or granulomatous mastitis, and there have been few studies on the correlation of NPM with cytokines that regulate T_H1/T_H2 and T_H17/Treg immune balance. The normal immune function of T_H1/T_H2 cells is in dynamic balance; once this balance is upset, the normal immune function cannot be maintained, resulting in infectious disease, autoimmune disease, tumor development, and risk to pregnancy progression. T_H1-type responses are generally considered to enhance a host's immune defense against viral and intracellular pathogen infections, while T_H2-type responses are associated with the progression, persistence, and chronicity of infections. The cytokine TGF-β plays an important role in the differentiation of Treg cells and T_H17 cells. There are complex interactions between T_H17 and Treg cells, despite the fact that they are currently thought to play very different roles in the body's immune and disease processes.[31]

T_H17 cells primarily promote inflammatory responses by producing cytokines, while Treg cells exert an immunosuppressive effect. T_H17 and Treg cells inhibit each other, and the T_H17/Treg balance is similar to the T_H1/T_H2 balance. TGF-β can convert mature T lymphocytes into Treg cells to prevent the occurrence of autoimmune diseases. However, in the presence of large amounts of IL-6, TGF-β promotes the differentiation of mature T lymphocytes into T_H17 cells, which promote inflammation and the development of autoimmune diseases. Disruption of the T_H17/Treg balance is likely to be a key factor in many inflammatory and autoimmune diseases.[32] Research has shown that the expression of IL-2 in tissue samples from patients with PCM in the acute phase is higher from that in the subacute and chronic phases and from samples from control patients.[33] IL-2 is a T_H1 cytokine that is dominant in PCM, while IL-4, a T_H2 cytokine, so the T_H1/T_H2 balance in PCM tissues may shift to favor T_H1 cells. In addition, expression of the T_H1 cytokines IFN-γ and IL-12A is significantly higher in granulomatous mastitis tissue than in normal breast tissue, whereas the expression of T_H2- and T_H17-related cytokines IL-4, IL-10 and TGF-β are not significantly different.[34] Rainard and colleagues reported that the plasma IL-17 concentration is high but the concentrations of plasma IL-10 and TGF-β1 are low in PCM, indicating a T_H17/Treg imbalance.[35] This study explores the pathological mechanisms in mastitis, as well as the ways in which they are treated. As lipopolysaccharides, dexamethasone, thyroglobulin, thyroid-stimulating hormone receptor, and T cells migration inhibitory factor may all be used to treat nonlactating mastitis, thus restoring the balance of T_H1/T^H2 and T_H17/Treg in NPM.[36]

CONCLUSION

NPM has an underlying association with cytokines that regulate the T_H1/T_H2 and T_H17/Treg immune balance. The balance of T_H1/T_H2 and T_H17/Treg in NPM is disrupted. Exploring the mechanism of occurrence and development of NPM can better guide the selection of clinical drugs and provide new ideas for further improving clinical treatment efficacy.

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Not applicable.

CONFLICT OF INTEREST

All authors declare that they have no competing interests.

REFERENCES

1. Shi L, Wu J, Hu Y, et al. Biomedical indicators of patients with non-puerperal mastitis: a retrospective study. *Nutrients*. 2022;14(22):4816. doi:10.3390/nu14224816
2. Patey DH, Thackray AC. Pathology and treatment of mammary-duct fistula. *Lancet*. 1958;2(7052):871-873. doi:10.1016/S0140-6736(58)92304-3
3. Kessler E, Wolloch Y. Granulomatous mastitis: a lesion clinically simulating carcinoma. *Am J Clin Pathol*. 1972;58(6):642-646. doi:10.1093/ajcp/58.6.642
4. Al-Khaffaf B, Knox F, Bundred NJ. Idiopathic granulomatous mastitis: a 25-year experience. *J Am Coll Surg*. 2008;206(2):269-273. doi:10.1016/j.jamcollsurg.2007.07.041
5. Reiner SL. Development in motion: helper T cells at work. *Cell*. 2007;129(1):33-36. doi:10.1016/j.cell.2007.03.019
6. Kim BS, Park YJ, Chung Y. Targeting IL-17 in autoimmunity and inflammation. *Arch Pharm Res*. 2016;39(11):1537-1547. doi:10.1007/s12272-016-0823-8
7. Rodrigues FMD, Coelho Neto GT, Menezes JGPB, et al. Expression of Foxp3, TGF-β and IL-10 in American cutaneous leishmaniasis lesions. *Arch Dermatol Res*. 2014;306(2):163-171. doi:10.1007/s00403-013-1396-8
8. Wei D, Ma P, Fan Q, Yu H, Peng Y, Li X. Yanning Syrup ameliorates the lipopolysaccharide-induced inflammation: adjusting the gut microbiota, short-chain fatty acids, and the $CD4^+$ T cell balance. *J Ethnopharmacol*. 2022;283:114729. doi:10.1016/j.jep.2021.114729
9. Zheng B, Song J, Lu M, Chen C, Sun S. Current research describing the role of $CD4^+$ T lymphocyte subsets in the pathogenesis of granulomatous lobular mastitis. *J Invest Surg*. 2022;35(10):1790-1795. doi:10.1080/08941939.2022.2090035
10. Zhou Y, Feng BJ, Yue WW, et al. Differentiating non-lactating mastitis and malignant breast tumors by deep-learning based AI automatic classification system: A preliminary study. *Front Oncol*. 2022;12:997306. doi:10.3389/fonc.2022.997306
11. Liu C, Yu H, Chen G, et al. An herbal drug combination identified by knowledge graph alleviates the clinical symptoms of plasma cell mastitis patients: A nonrandomized controlled trial. *eLife*. 2023;12:e84414. doi:10.7554/eLife.84414
12. Liu Y, Zhang J, Zhou YH, et al. IL-6/STAT3 signaling pathway is activated in plasma cell mastitis. *Int J Clin Exp Pathol*. 2015;8(10):12541-12548.
13. Liu Y, Zhang J, Zhou YH, et al. Activation of the IL-6/JAK2/STAT3 pathway induces plasma cell mastitis in mice. *Cytokine*. 2018;110:150-158. doi:10.1016/j.cyto.2018.05.002
14. Liu Y, Sun Y, Zhou Y, et al. Sinomenine hydrochloride inhibits the progression of plasma cell mastitis by regulating IL-6/JAK2/STAT3 pathway. *Int Immunopharmacol*. 2020;81:106025. doi:10.1016/j.intimp.2019.106025

15. Flynn J, Gorry P. Flow cytometry analysis to identify human CD4$^+$ T cell subsets. *Methods Mol Biol*. 2019;2048:15-25. doi:10.1007/978-1-4939-9728-2_2
16. Kestens L, Mandy F. Thirty-five years of CD4 T-cell counting in HIV infection: from flow cytometry in the lab to point-of-care testing in the field. *Cytometry B Clin Cytom*. 2017;92(6):437-444. doi:10.1002/cyto.b.21400
17. Wang X, Han Y, Liu J, et al. Exosomes play an important role in the progression of plasma cell mastitis via the PI3K-Akt-mTOR signaling pathway. *Mediators Inflamm*. 2019;2019:4312016. doi:10.1155/2019/4312016
18. Liu D, Lin J, He W, Huang K. Selenium and taurine combination is better than alone in protecting lipopolysaccharide-induced mammary inflammatory lesions via activating PI3K/Akt/mTOR signaling pathway by scavenging intracellular ROS. *Oxid Med Cell Longev*. 2021;2021:5048375. doi:10.1155/2021/5048375
19. Kornfeld HW, Mitchell KB. Management of idiopathic granulomatous mastitis in lactation: case report and review of the literature. *Int Breastfeed J*. 2021;16(1):23. doi:10.1186/s13006-021-00370-8
20. Lipatov KV, Komarova EA, Nasibov BS. [Peculiarities of the course, diagnosis and surgical treatment of non-lactating mastitis]. *Khirurgiia (Mosk)*. 2016;12(12):25-30. doi:10.17116/hirurgia20161225-30
21. Gopalakrishnan Nair C, Hiran, Jacob P, Menon RR, Misha. Inflammatory diseases of the non-lactating female breasts. *Int J Surg*. 2015;13:8-11. doi:10.1016/j.ijsu.2014.11.022
22. Enger BD, Crutchfield CE, Yohe TT, et al. *Staphylococcus aureus* intramammary challenge in non-lactating mammary glands stimulated to rapidly grow and develop with estradiol and progesterone. *Vet Res*. 2018;49(1):47. doi:10.1186/s13567-018-0542-x
23. Bagenal J, Bodhinayake J, Williams KE. Acute painful breast in a non-lactating woman. *BMJ*. 2016;353:i2646. doi:10.1136/bmj.i2646
24. Xing M, Zhang S, Zha X, Zhang J. Current understanding and management of plasma cell mastitis: can we benefit from what we know? *Breast Care (Basel)*. 2022;17(3):321-329. doi:10.1159/000517572
25. Wolfrum A, Kümmel S, Theuerkauf I, Pelz E, Reinisch M. Granulomatous mastitis: a therapeutic and diagnostic challenge. *Breast Care (Basel)*. 2018;13(6):413-418. doi:10.1159/000495146
26. Zhang J, Xu J, Zhang J, Ren Y. Chinese herbal compound combined with western medicine therapy in the treatment of plasma cell mastitis: A protocol for systematic review and meta-analysis. *Medicine (Baltimore)*. 2020;99(44):e22858. doi:10.1097/MD.0000000000022858
27. Lam E, Chan T, Wiseman SM. Breast abscess: evidence based management recommendations. *Expert Rev Anti Infect Ther*. 2014;12(7):753-762. doi:10.1586/14787210.2014.913982
28. Kulkarni D. Clinical presentations of breast disorders in pregnancy and lactation. *Adv Exp Med Biol*. 2020;1252:33-39. doi:10.1007/978-3-030-41596-9_5
29. Yanagisawa W, Sedaghat N, Gordon-Thomson D. Endovascular management of iatrogenic arteriovenous fistula of the breast. *Breast J*. 2021;27(1):52-55. doi:10.1111/tbj.14131
30. Xiao M, Xie L, Cao G, et al. CD4$^+$ T-cell epitope-based heterologous prime-boost vaccination potentiates anti-tumor immunity and PD-1/PD-L1 immunotherapy. *J Immunother Cancer*. 2022;10(5):e004022. doi:10.1136/jitc-2021-004022
31. Halle S, Halle O, Förster R. Mechanisms and dynamics of T cell-mediated cytotoxicity in vivo. *Trends Immunol*. 2017;38(6):432-443. doi:10.1016/j.it.2017.04.002
32. Laidlaw BJ, Craft JE, Kaech SM. The multifaceted role of CD4(+) T cells in CD8(+) T cell memory. *Nat Rev Immunol*. 2016;16(2):102-111. doi:10.1038/nri.2015.10
33. Wang W, Sung N, Gilman-Sachs A, Kwak-Kim J. T helper (Th) cell profiles in pregnancy and recurrent pregnancy losses: Th1/Th2/Th9/Th17/Th22/Tfh cells. *Front Immunol*. 2020;11:2025. doi:10.3389/fimmu.2020.02025
34. Li Q, Liu Y, Wang X, et al. Regulation of Th1/Th2 and Th17/Treg by pDC/mDC imbalance in primary immune thrombocytopenia. *Exp Biol Med (Maywood)*. 2021;246(15):1688-1697. doi:10.1177/15353702211009787
35. Sifnaios E, Mastorakos G, Psarra K, et al. Gestational Diabetes and T-cell (Th1/Th2/Th17/Treg) Immune Profile. *In Vivo*. 2019;33(1):31-40. doi:10.21873/invivo.11435
36. Rainard P, Foucras G, Martins RP. Adaptive cell-mediated immunity in the mammary gland of dairy ruminants. *Front Vet Sci*. 2022;9:854890. doi:10.3389/fvets.2022.854890