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Characterization of allergic inflammation in chronic uterine cervicitis

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Abstract

Female genital tract chronic inflammation is common in clinics; the pathogenesis is not fully understood yet. House dust mite (HDM) involves the pathogenesis of many chronic diseases in human. This study aims to identify HDM-specific allergic response in the cervix of patients with cervical inflammation. Patients (n = 80) with chronic cervicitis (CC) and non-CC control (NC) subjects (n = 80) were recruited into this study. Vaginal lavage fluids (VLF) were collected from CC patients and NC subjects. Cellular components and fluid part of VLF were separated by centrifugation, and analyzed by flow cytometry and enzyme-linked immunosorbent assay. We found that a portion (52 out of 80) of CC patients responded to HDM, manifesting positive skin prick test, and HDM-specific IgE and IgG was detected in the VLF (designated CCp patients). VLF of CCp patients showed a Th2-dominant profile. HDM-specific Th2 cells were detected in VLF in CCp patients. Exposure to HDM in the culture induced proinflammatory cytokine release from CCp VLF CD4$^+$ T cells. Exposure to CCp VLF CD4$^+$ T cell-conditioned medium induced *de novo* Th2 response. Direct exposure to HDM induced allergic response in the cervix of CCp patients. In summary, a portion of CC patients respond to HDM challenge in the cervix. Exposure to HDM induces an allergy-like response in the cervix of CCp patients.

Keywords: cervicitis, inflammation, house dust mite, immune response, Th2 polarization

Abbreviations: HDM, House dust mite; CC, chronic cervicitis; NC, non-CC control; VLF, vaginal lavage fluids; SPT, skin prick test; sIgG, specific IgG; sIgE, specific IgE; FACS, flow cytometry; PBMC, peripheral blood mononuclear cell; DC, dendritic cell; CM, conditioned medium; DME, dust mite extracts; PMA, phorbol myristate acetate

Introduction

Chronic uterine cervicitis (CC) is an inflammatory disease of the uterine cervix mucosa. Infection is the common causative factor of CC, including *Chlamydia trachomatis* or *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma genitalium*, and herpes simplex virus [1, 2]. However, it is estimated about half of CC with unknown etiology [3]. Clinical symptoms of CC include profound purulent exudate in the cervix, a large number of neutrophilic leukocytes in the cervical secretions, and cervical bleeding [4, 5]. It is reported that upper genital tract inflammation is associated with CC, that often interfere with pregnancy [6, 7]. To date, the CC pathogenesis is not fully understood yet; therapeutic effects on CC are not satisfactory currently [5]. Thus, it is necessary to further investigate the pathogenesis of CC, and invent novel, safe, and more effective remedies for CC treatment.

Published data indicate that house dust mite (HDM) is associated with the pathogenesis of several human diseases, e.g. allergic asthma, allergic rhinitis, allergic dermatitis, through the IgE-mediated pathway [8, 9]. Recent reports show that HDM induces cross autoantibodies that are associated with chronic airway disorders and heart diseases [10, 11]. Besides inducing airway allergy, HDM can contaminate the food to be swallowed into the digestive tract to disturb the homeostasis of epithelial barrier [12, 13]. The vagina opens to the extrinsic environment and can be easily contaminated by HDM such as during sexual activities. Yet, whether HDM is associated with the CC pathogenesis remains un-investigated. In the clinical practice, we noticed a portion of CC patients were accompanied with HDM-sensitized allergic diseases, including asthma, rhinitis, and dermatitis. We therefore screened the HDM-sensitization status in a cohort of subjects with or without CC. We found that a portion of CC patients were sensitized to HDM; Th2-dominant response was identified in vaginal lavage fluid; direct exposure to HDM extracts induced allergy-like response in the cervix.

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Materials and methods

Reagents

Antibodies of CD3 (clone#: HIT3a, AF405), CD4 (18–46, AF488), CD25 (C-9, AF546), IL-4 (8D4-8, AF594), IL-13 (F6, AF680), IFN-γ (G-23, AF790), and Ki67 (Ki-67, AF594) were purchased from Santa Cruz Biotech (Santa Cruz, CA). ELISA kits of IL-4, IL-5, IL-13, IFN-γ, IL-17, PGD2, LTC4, and eotaxin were purchased from R&D Systems (Minneapolis, MN). ELISA kits of MBP, ECP, histamine, and tryptase were purchased from Dakewe BioMart (Shenzhen, China). IL-5 (APC) antibody was purchased from eBioscience (San Diego, CA).

Human subjects

As a clinical study, female subjects with or without CC were recruited at Shenzhen Maternal & the Child Health Care

Table 1. General data of human subjects

Items	CCp	CCn	NC
Subject number	52	28	80
Age (years)	35.5 (18, 46)	34.8 (17, 45)	35.6 (17, 48)
CC history (years)	3.3 (1.2, 4.3)	3.5 (1.4, 4.5)	-
Cervical erosion (*n*, %)	42 (80.7)	22 (78.6)	
Simple erosion (*n*, %)	30 (71.4)	16 (72.7)	
Mild (*n*, %)	25 (83.3)	13 (81.3)	
Moderate (*n*, %)	3 (10)	2 (12.5)	
Severe (*n*, %)	2 (6.7)	1 (6.3)	
Granular erosion (*n*, %)	8 (19.0)	4 (18.2)	
Mild (*n*, %)	6 (75)	2 (50)	
Moderate (*n*, %)	1 (12.5)	1 (25)	
Severe (*n*, %)	1 (12.5)	1 (25)	
Papillary erosion (*n*, %)	4 (9.5)	2 (9.1)	
Mild (*n*, %)	2 (50)	2 (100)	
Moderate (*n*, %)	1 (25)	0	
Severe (*n*, %)	1 (25)	0	
Cervical hypertrophy (*n*, %)	11 (21.2)	5 (17.9)	
Cervical polyp (*n*, %)	1 (1.9)	0	

CCp: SPT (skin prick test) positive cervicitis (CC) cases. CCn: SPT negative CC cases. NC: Non-CC control subjects. Cervical erosion: including simple erosion, granular erosion, papillary erosion of three types. According to the size of the area of erosion, erosion can be divided into three degrees: mild, erosion surface is less than 1/3 of the total cervical area; Moderate, erosion surface accounted for 1/3 to 2/3 of the total cervical area; Severe, erosion area accounts for more than 2/3 of the total cervical area.

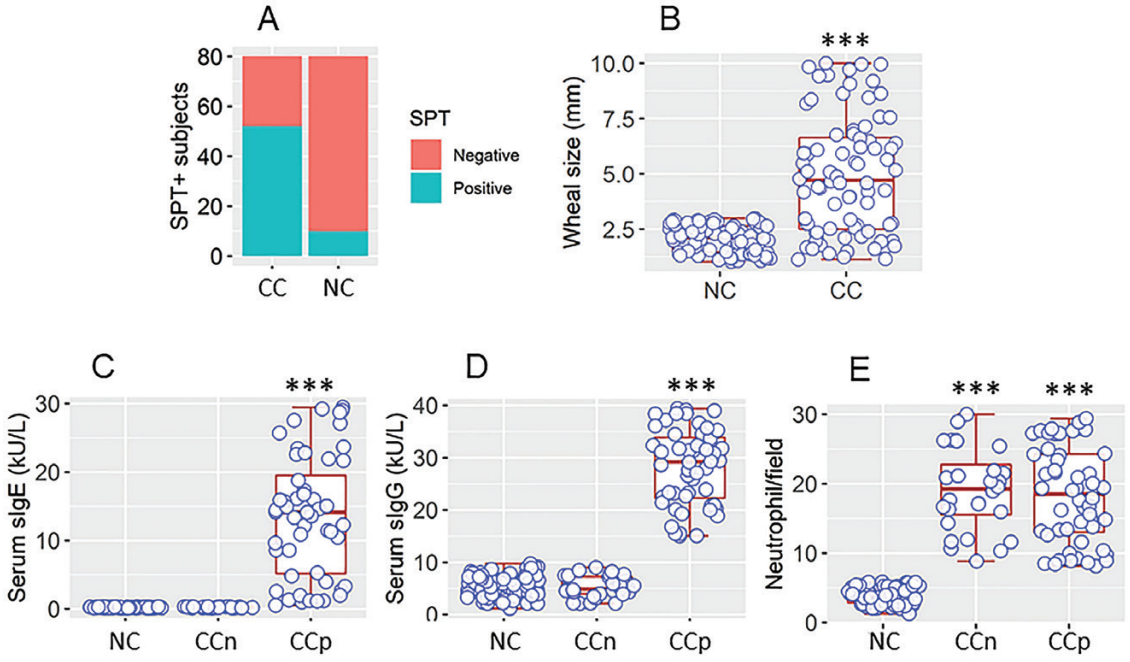

Fig. 1. Assessment of HDM-specific immune response in CC patients. (A) Stack bars show HDM skin prick test (SPT) results in 80 CC patients and 80 non-CC (NC) subjects. (B) boxplots show SPT wheal size. (C, D), boxplots show serum HDM-specific IgE (C) and sIgG (D) levels. (E) boxplots show neutrophil counts in cervix secretion smear (average of 10 high power fields per sample). Each bubble presents data obtained from one subject. ***$P <$ 0.001 (Mann–Whitney test for A and B; ANOVA + Dunnett's test for C, D, and E), compared with the NC group.

Hospital and Longgang Maternal & Child Health Care Hospital from June 2018 to September 2020. The diagnosis and management of CC were carried out by our physicians following the routine procedures [14]. The recruiting criteria of the present study included clinical diagnosis of CC and laboratory tests. The main clinical manifestations of non-infectious cervicitis are increased leucorrhea, milky white or slightly yellow, or viscous purulent, sometimes bloody or mixed with blood. Gynecological examination found cervical hypertrophy, cervical polyps, or cervical columnar epithelial ectopic, most patients have no clinical symptoms, through the speculum examination can be seen cervical secretion of purulent mucinous leucorrhea, followed by cyto-smear result confirmation; the sample collection was avoided from subjects during menstruation. The HDM sensitization status, including serum-specific IgE and specific IgG, and skin prick test, was screened in each subject (including both CC and NC subjects). Subjects with severe organ diseases, autoimmune diseases, HIV infection, pregnancy, or using immune suppressive agents were excluded. Additionally, non-CC control (NC) subjects were also recruited into this study, which did not have CC history, and gynecological examination did not find CC signs, nor were pregnant. The demographic data are given in Table 1. The experimental procedures were approved by the Human Ethical Committee at Shenzhen Maternal & the Child Health Care Hospital and Longgang Maternal & Child Health Care Hospital. A written informed consent was obtained from each human subject.

Allergen SPT

All human subjects were subjected to skin prick test (SPT). Mite mix (including Dermatophagoides *farina* and Dermatophagoides *pteronyssinus*) was purchased from Allergopharma (Germany), and used for SPT. Histamine (10 mg/ml) and saline were used as positive and negative controls in SPT, respectively. The SPT results were observed 15 min after. If the mean wheal diameter was ≥3 mm larger than the negative control, SPTs were considered positive. In addition, the SPT wheal size was measured and recorded.

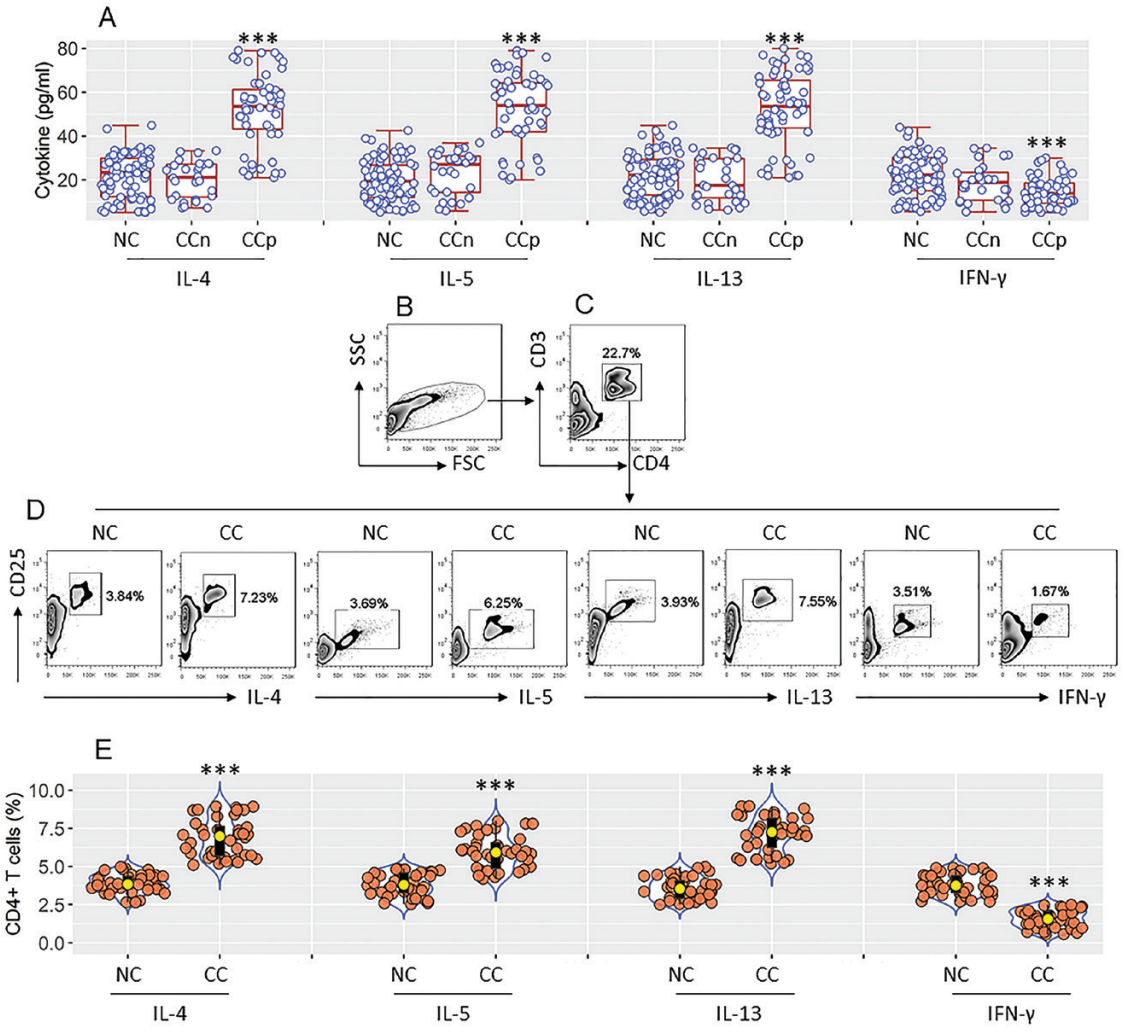

Fig. 2. Th2 cell and cytokine in VLF. VLF was obtained from 80 NC subjects and 80 CC patients, and analyzed by ELISA and FACC. (A) boxplots show $CD4^+$ T-cell-derived cytokine levels in VLF. (B) FSC/SSC plots. (C) $CD3^+$ $CD4^+$ T cells were gated. (D) Gated FACC plots show frequency of Th2 cells ($IL\text{-}4^+$, $IL\text{-}5^+$, and $IL\text{-}13^+$) and Th1 cells ($IFN\text{-}\gamma^+$). (E) violin plots show summarized cell counts of Th2 and Th1 cells in VLF. The data of boxplots are presented as median (IQR). ***$P < 0.001$ (Mann–Whitney test), compared with the NC group. Each bubble in boxplots and violin plots presents data obtained from one human subject.

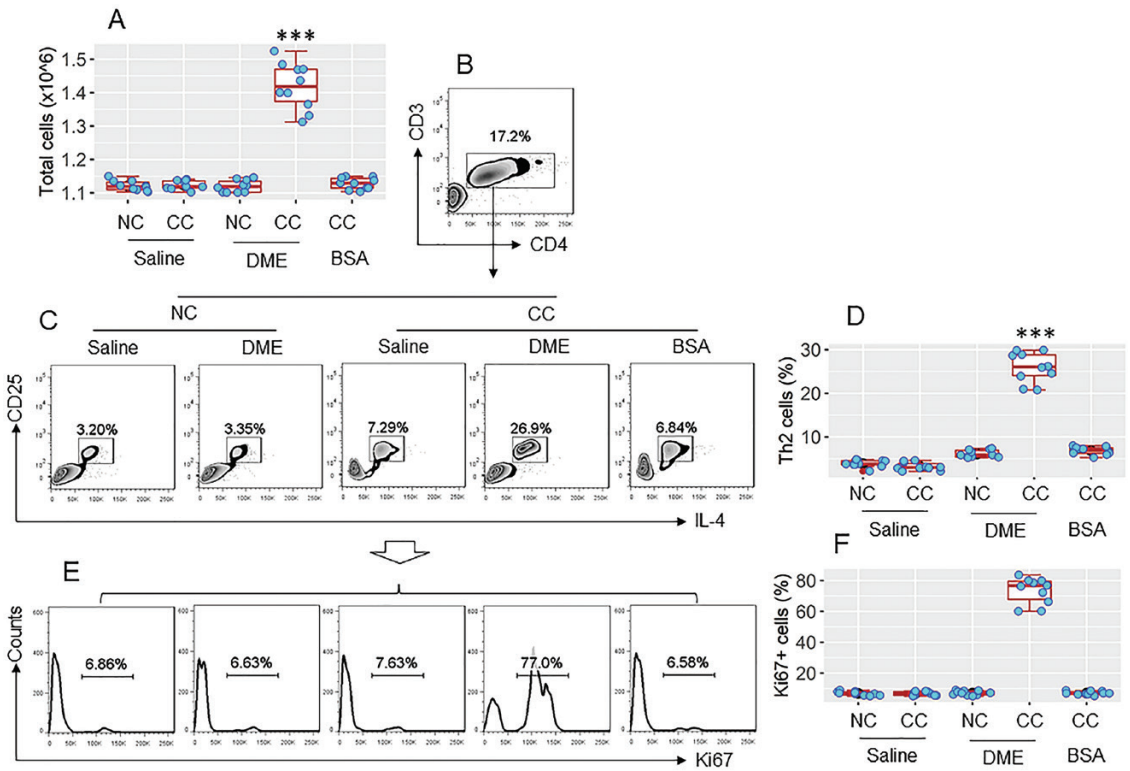

Fig. 3. Identification of HDM-specific Th2 cells in VLF. VLF was collected from 10 CC patients and 10 NC subjects. Cells were isolated from VLF, exposed to DME (or saline, or BSA) in the culture (There were DCs in VLF) for 3 days (Brefeldin A was added to the culture in the last 4 h), and analyzed by FACC. (A) Total cell counts. (B) T cells were gated first. (C) Th2 cells ($CD25^+$ $IL\text{-}4^+$) were further gated. (D) summarized Th2 cell counts. (E) gated histograms show $Ki67^+$ cell (a marker of cell activation) frequency. (F) summarized $Ki67^+$ cell counts. The data of boxplots are presented as median (IQR). $^{***}P < 0.001$ (ANOVA + Dunnett's test), compared with the NC group. Each bubble in boxplots presents data obtained from one sample.

Cervix smear and neutrophil counts

Cervical secretions were taken with a cotton swab in the aid of vaginal speculum, and smear on a glass slide. After Gimsa staining, the slide was observed under a light microscope. Neutrophils were counted in 10 randomly selected fields (×400) per sample. An average of the 10 readouts was used as one datum.

Vaginal/cervical lavage fluid (VLF) collection

The cervical surface was rinsed with 10 ml saline (warmed to 37°C); the fluids were collected immediately used as VLF; the lavage was repeated three times. VLF of three times was pooled, and passed through a filter (100 μm first, then 40 μm). Samples were then centrifuged at 1000 *g* for 5 min. Supernatant was collected, stored at –80°C until use. Pellets were resuspended in culture medium and analyzed by cellular experiments.

Enzyme-linked immunosorbent assay

The levels of cytokines in VLF were determined by enzyme-linked immunosorbent assay (ELISA) with commercial reagent kits following the manufacturer's instructions. The levels of HDM-specific IgG (sIgG) and sIgE in VLF were determined by the ImmunoCap with purchased reagent kits following the manufacturer's instructions.

Flow cytometry (FACS)

Single cells were prepared in relevant experiments. In the surface staining, cells were stained with fluorescence-labeled Abs (Abs were detailed in figures) (diluted to 1 μg/ml) or isotype IgG for 30 min at 4°C. After washing with PBS (phosphate-buffered saline) three times, cells were analyzed with a flow cytometer (FACSCanto II, BD Bioscience). In the intracellular staining, cells were fixed with 0.05% Triton x-100-containing paraformaldehyde (1%) for 1 h at room temperature. After washing with PBS three times, cells were processed with the surface staining procedures. The data were processed with a software package (FlowJo; TreeStar Inc., Ashland, OR) with the data obtained from isotype IgG staining as gating references.

Isolation of CD4+ CD62L+T cells from peripheral blood

Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples collected from healthy subjects by Percoll gradient density centrifugation. $CD4^+$ $CD62L^+$ T cells were purified from PBMCs with a magnetic antibody reagent kit (the $CD4^+$ $CD62L^+$ T cell isolation kit, purchased from Miltanyi Biotech) following the manufacturer's instruction. Purity of isolated $CD4^+$ $CD62L^+$ T cells was 96–98% as checked by FACS.

Isolation of CD4+T cells and DC from VLF and PBMCs

PBMCs and VLF single cells were prepared as described above, and subjected to $CD4^+$ T cell or dendritic cells (DC) isolation with magnetic antibody reagent kits (the $CD4^+$ T cell and DC isolation kits, purchased from Miltanyi Biotech)

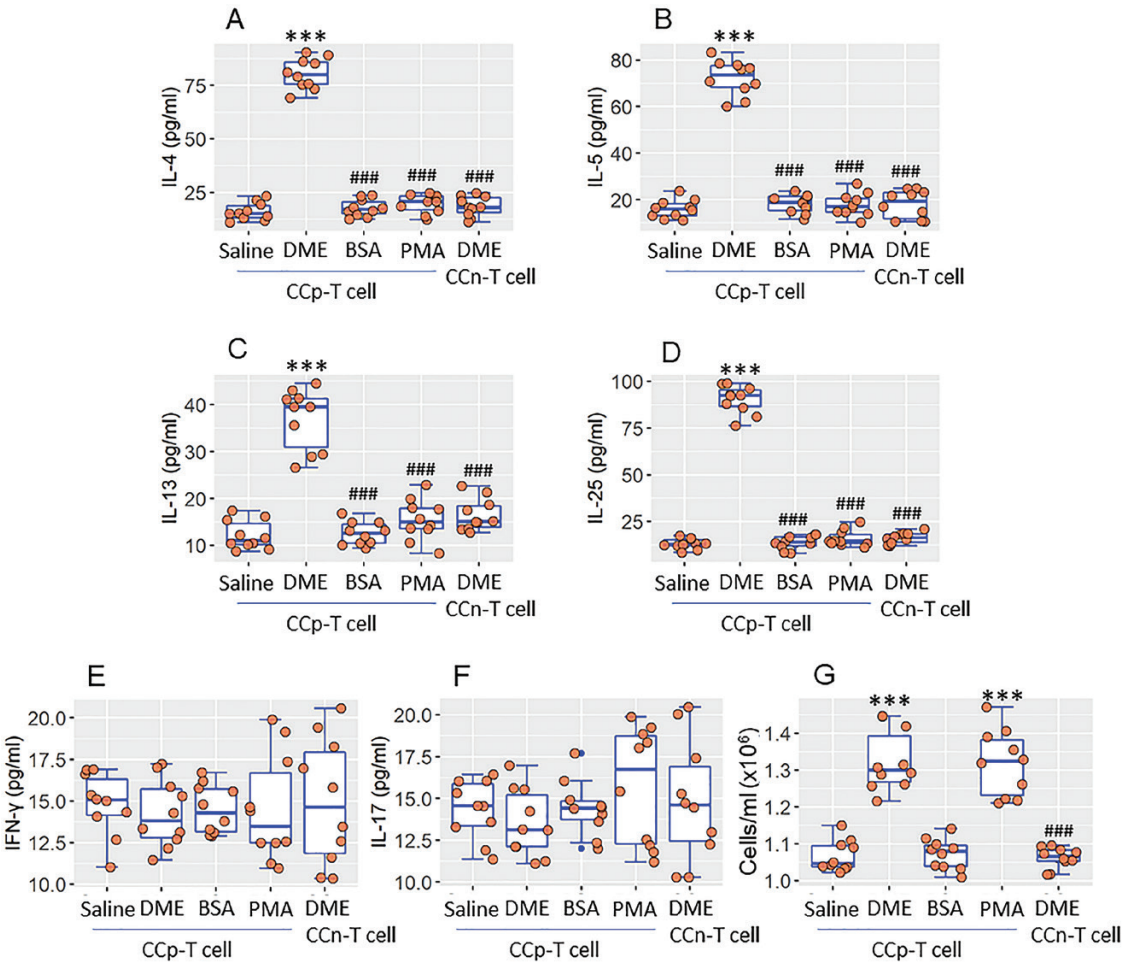

Fig. 4. Inducing VLF T cells by DME. CD4⁺ T cells were isolated from VLF samples collected from 10 CCp patients and 10 CCn patients, and respectively exposed to DME (saline or BSA were used as controls) in the culture (10^6 cells/ml) overnight in the presence of DCs. The supernatant was harvested, analyzed by ELISA. Boxplots show the levels of IL-4 (A), IL-5 (B), IL-13 (C), IL-25 (D), IFN-γ (E), and IL-17 (F) in supernatant. (G) cell counts after overnight culture. ***$P < 0.001$, compared with the saline group. ###$P < 0.001$, compared with the DME-CCp-T cell group. Statistical methods: ANOVA + Dunnett's test. Each bubble in boxplots presents data obtained from one sample. PMA (phorbol myristate acetate, 50 ng/ml).

following the manufacturer's instruction. Purity of isolated CD4⁺ T cells was 96–98% as checked by FACS.

Cell culture

Cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 2 mM glutamine. Cell viability was greater than 98% as assessed by Trypan blue exclusion assay.

Preparation of VLF CD4+ T-cell-conditioned medium

VLF CD4⁺ T cells were collected from CCp patients and CCn patients, and were prepared as described above, and cultured at 10^6 cells/ml in the presence of DME (5 μg/ml) for 3 h. Cells were centrifuged at ×500 *g* for 5 min, washed with culture medium, and cultured with fresh medium overnight. Th2 cytokines and IL-25 were detected in culture supernatant (data not shown). The medium was used as conditioned medium (CM) for further experiments. A control medium was prepared with CD4⁺ T cells isolated from NC blood-isolated PBMCs with the same procedures, and designated BM.

Induction of Th2 response using VLF CD4+ T-cell-conditioned medium

Naive CD4⁺ CD62L⁺ T cells were prepared, and cultured with the conditioned medium for 6 days. Culture supernatant was collected and analyzed by ELISA to determine the Th2 cytokine levels. Cells were collected, and analyzed by FACS to determine the frequency of Th2 cells.

Cervix challenge with DME

DME solution was prepared at 1 mg/ml, and contained in a spray bottle. The cervix was exposed with a vaginal speculum. About 0.5 ml DME solution was sprayed on the surface of the cervix. VLF was harvested from each subject 30 min after the challenge.

Statistics

The data are presented as mean ± SEM. The statistical difference between two groups was determined by the Student *t*-test or the Mann–Whitney test, or by ANOVA followed by the Dunnett's test or Bonferroni test if more than two groups. $P < 0.05$ was set as the significant criterion.

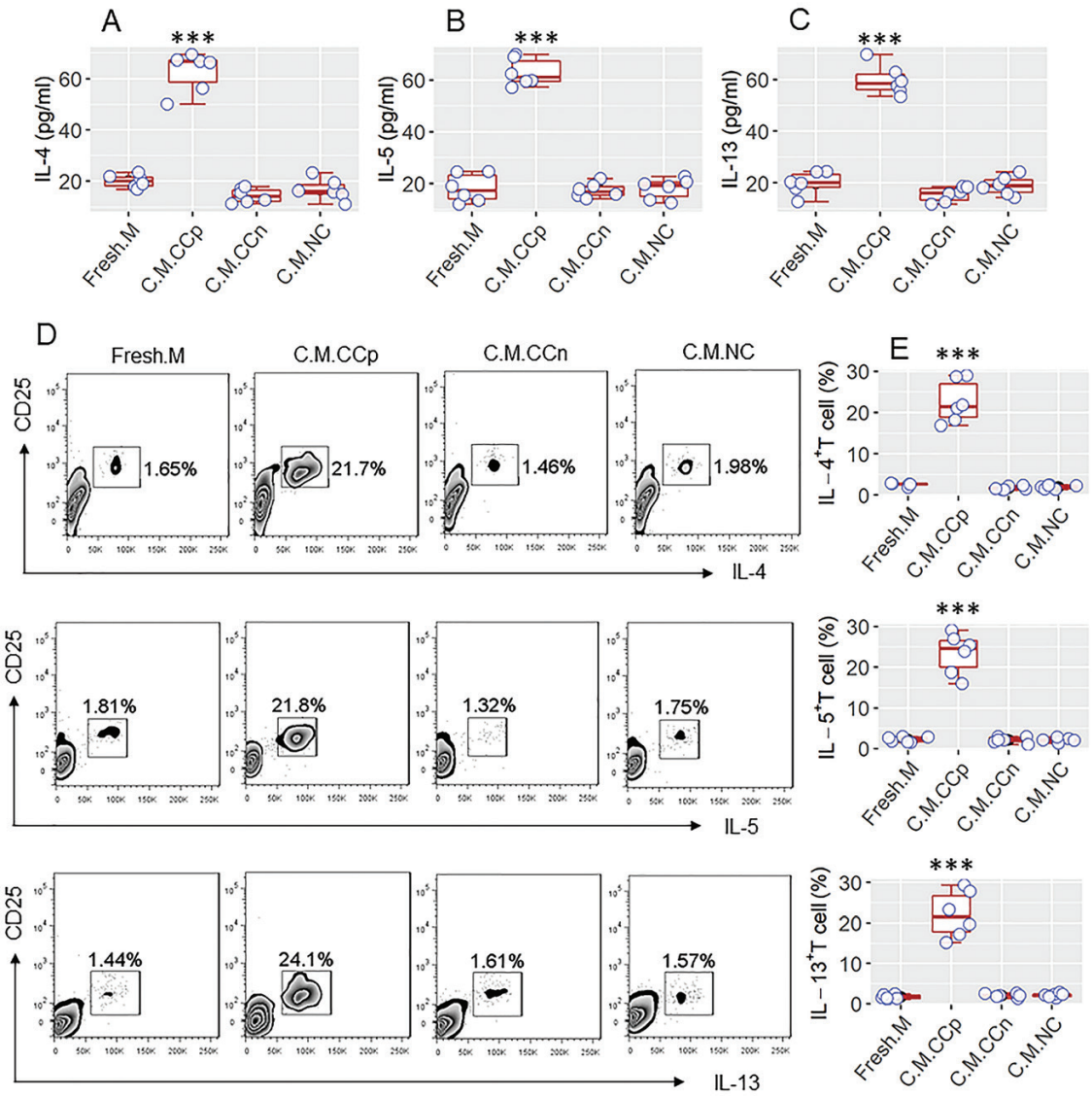

Fig. 5. VLF CD4⁺ T-cell-conditioned medium induces Th2 response. Naive CD4⁺ T cells were isolated from blood samples obtained from NC subjects, and cultured with CD4⁺ T cells (CCp: From VLT of CCp patients. CCn: From VLT of CCn patients. NC: From blood samples of NC subjects)-conditioned medium (C.M.) or fresh medium (Fresh.M) for 6 days. (A–C) Boxplots show Th2 cytokine levels in culture supernatant. (D) gated FACS plots show Th2 cell counts. (E) boxplots show Th2 cell frequency in gated FACS plots on the left side. The data of boxplots are presented as median (IQR). Each bubble in the plots presents data obtained from one sample. ***$P < 0.001$ (ANOVA followed by Dunnett's test), compared with the Fresh.M group.

Results

HDM-specific immune response is detected in a portion of CC patients

In clinical practice, we observed that a portion of CC patients also suffered HDM-sensitized asthma or allergic rhinitis (data not shown), we screened the HDM-sensitization in a cohort of subjects with or without CC. The results showed that 52/80 (65%) CC patients were sensitized to HDM manifesting positive SPT (Fig. 1A). Based on the SPT results, CC patients were divided into two groups, the SPT-positive CC group (CCp; $n = 52$) and the SPT-negative CC group (CCn; $n = 28$) (Fig. 1B). In non-CC subjects (NC group, the control group), the HDM-SPT-positive rate was 10/80 (12.5%; $P < 0.05$ vs. the CC group). The levels of HDM-specific IgE (sIgE) and sIgG were detectable in vaginal lavage fluid (VLF), that were higher in the CCp group than that in the CCn group and the NC group (Fig. 1C and D). In the 52 CCp patients, 4 of them suffered from allergic rhinitis, 2 suffered allergic asthma; the rate is 11.5% ($P > 0.05$, compared with the NC group, that was 10%). Neutrophils in cervical smear were counted, that were more in the CCp and CCn groups than that in the NC group (Fig. 1E).

Th2 profiles in VLF of CCp patients

VLF samples were collected from CCn patients, CCp patients and NC subjects. After centrifugation, the liquid part was analyzed by ELISA. The results showed higher levels of IL-4, IL-5, IL13 and lower levels of IFN-γ in CCp samples than that of CCn sample and NC samples (Fig. 2A). The cellular part of VLT sample was analyzed by flow cytometry (FACS). The results showed that both Th1 and Th2 cells were detected in the samples, in which the Th2 cell frequency was higher, the Th1 frequency was lower in the CCp group than that in the CCn group and NC group (Fig. 2B–E).

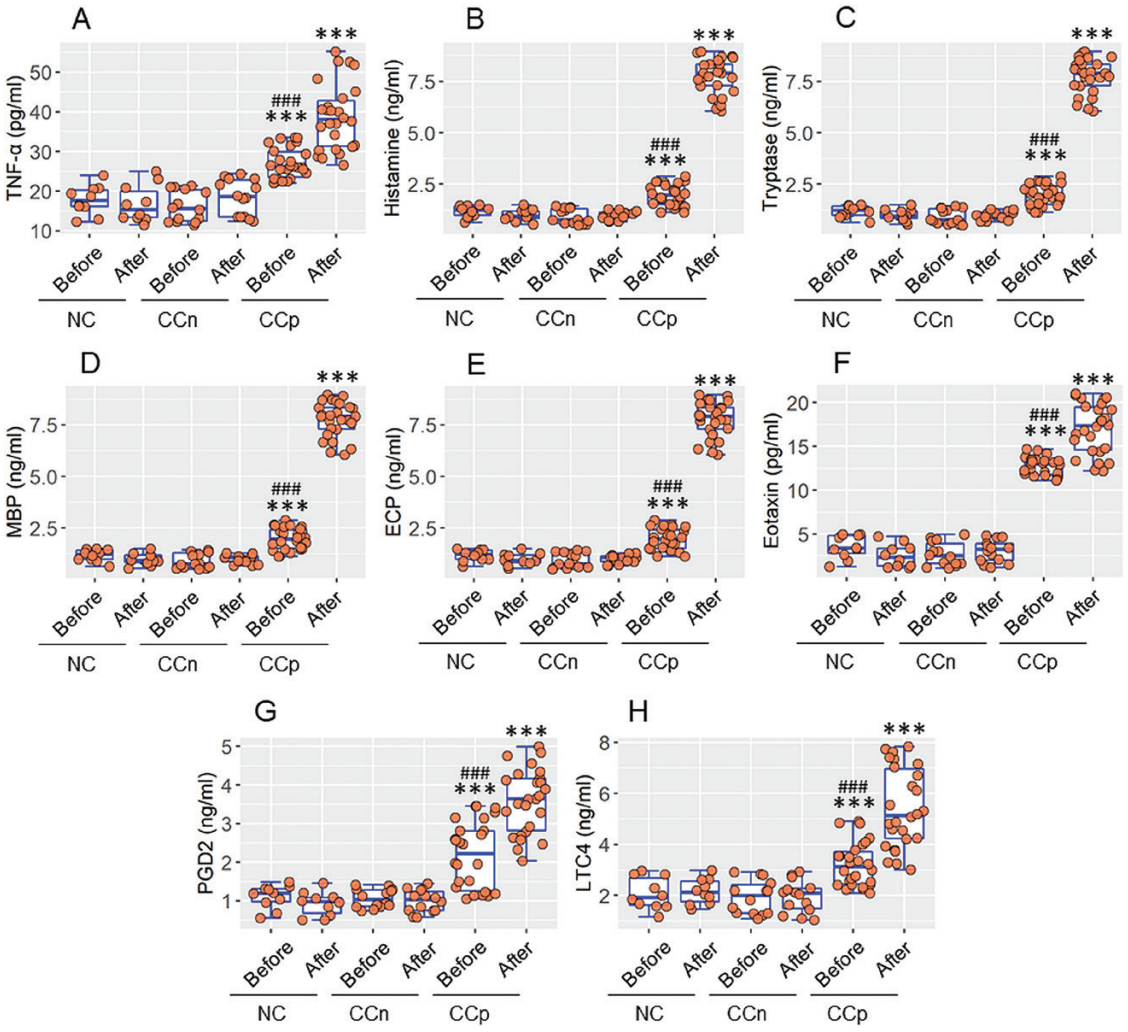

Fig. 6. Assessment of allergic response in the cervix. The cervix was challenged with DME spray. Boxplots show the levels of TNF-α (A), histamine (B), tryptase (C), MBP (D), ECP (E), eotaxin (F), PGD2 (G), and LTC4 (H) in VLF. NC: Non-CC control patients (n = 10). CCn: CC patients with HDM SPT negative (n = 14). CCp: CC patients with HDM SPT positive (n = 26). Before and After: Before or after DME challenge. The data of boxplots are presented as median (IQR). ***P < 0.001 (t test), compared with the 'Before' sub group of NC, or CCn, or CCp group. ###P < 0.001 (t test), compared with the 'after' sub group in CCp group. Each bubble presents data obtained from one sample.

HDM-specific Th2 cells are detected in VLF in CC patients

Cellular components were isolated from VLF and cultured in the presence of HDM extracts (DME; prepared in our laboratory [11, 13]) or bovine serum albumin (BSA, an irrelevant antigen, used as a control) overnight. Cells were harvested, counted, and analyzed by FACS. CCp $CD4^+$ T cells markedly proliferated upon exposure to DME, but not BSA, as compared with CCn and NC $CD4^+$ T cells (Fig. 3A). 25.5 ± 2.1% proliferated $CD3^+$ $CD4^+$ T cells showed $CD25^+$ $IL\text{-}4^+$, indicating these cells are Th2 cells (Fig. 3B–D). In the Th2 cells, 61.9 ± 11.6% cells were activated ($Ki67^+$) (Fig. 3E and F).

HDM induces proinflammatory cytokine release from CCp VLF T cells

$CD4^+$ T cells were isolated from VLF obtained from CCp group and CCn group, and respectively exposed to dust mite extracts (DME) (saline or BSA was used as a control) in the culture overnight in the presence of DCs. Culture supernatant was harvested, and analyzed by ELISA. We found that exposure to DME, but not BSA, induced a marked increase in IL-4, IL-5, IL-13, and IL-25 in supernatant (Fig. 4A–D). IL-17 and IFN-γ were also detectable, but the levels were not altered by DME (Fig. 4E and F). Exposure to DME in the culture did not induce $CD4^+$ T cells collected from CCn patients to release Th2 cytokines (Fig. 4A–D). In addition, exposure to either DME or PMA (phorbol myristate acetate, a non-specific cell activator) also markedly increased the T-cell number (Fig. 4G); however, PMA did not increase the levels of Th2 cytokines and IL-25 in the culture (Fig. 4A–D).

CCp VLF CD4+ T-cell-conditioned medium induces Th2 response

As both IL-25 and IL-4 can initiate the *de novo* Th2 response [15], we inferred that the culture supernatant of DME-primed CCp VLF T cells could induce Th2 response. To test this, the conditioned medium was prepared. Naive $CD4^+$ T cells were isolated from blood samples collected from healthy NC subjects, and cultured with the CCp-conditioned medium

(the conditioned medium was prepared with CD4+ T cells isolated from CCp patient VLF) for 6 days. The cells were harvested, washed, and cultured with fresh medium in the presence of DME overnight. We found that Th2 cells were induced, and higher Th2 cytokines were detected in culture supernatant. Naive CD4+ T cells cultured with fresh medium did not develop into Th2 cells, nor were those cultured with the medium from DME-primed naive CD4+ T cell culture or CCn-conditioned medium (CD4+ T cells were isolated from CCn patient VLF) (Fig. 5).

Exposure to HDM induces an allergic response in the cervix of patients sensitized to HDM

The data reported above suggest that HDM-specific allergic response may contribute to the pathogenesis of CC. To test this, we applied DME to the cervix (the DME challenge) of patients with positive HDM SPT results. The VLF was collected from each subject before and 30 min after the DME challenge. We found that DME challenge markedly increased the levels of TNF-α, histamine, tryptase, MBP, ECP, eotaxin, PGD2, and LTC4, in VLF (Fig. 6).

Discussion

The present study revealed a previously un-described phenomenon that a portion of CC patients responded to HDM challenge. These patients showed positive HDM SPT results; HDM-specific IgE and IgG were detected in VLF; high levels of Th2 cytokines and Th2 cells were detected in VLF. The CCp VLF T-cell-conditioned medium induced Th2 response. Exposure to HDM induced allergy-like response in the cervix of CCp patients. The data suggest that HDM sensitization may be one of the causative factors in the pathogenesis of CC.

The data show that a portion of CC patients respond to HDM. It is well documented that HDM is an important factor inducing a number of chronic diseases, such as allergic rhinitis, allergic asthma, and allergic dermatitis [8, 9]. The disease relapse is the clinical feature of these allergic diseases. CC also has such a feature of clinical relapse. Besides the well-known bacterial infection (our data also show increases in neutrophils in cervical smear), the causative factors of CC are not fully understood yet. The present data suggest that HDM sensitization may contribute to the pathogenesis of CC. Although HDM is an airborne allergen, which can be inhaled into the airways to induce allergic rhinitis or/and allergic asthma [8]. HDM also induces allergic dermatitis by direct contact [9]. HDM distributes extensively in the human living environment, including sleeping bed. The female genital tract naturally opens to the extrinsic environment; thus, it is conceivable that HDM can reach the vagina and cervix, such as during sexual activities, to contact local immune cells and induce an immune response.

Following the same concept of collecting bronchoalveolar lavage fluid, we collected VLF from CC patients. By analyzing VLF, we found the Th-dominant immune response in the female genital tract of a portion of CC patients, including high levels of Th2 cytokines, and Th2 cells. Th2 cytokines and Th2 cells are the basic components to induce an allergic response. Upon relevant stimulation, Th2 cells produce Th2 cytokines, such as IL-4, IL-5, and IL-13 [16]. Th2 cytokines, especially IL-4, can further induce Th2 response, induce B cells to differentiate into plasma cells to produce antigen specific IgE [17]. IgE can sensitize mast cells. Re-exposure to specific antigens, such as HDM, can activate sensitized mast cells, and induce an allergic response in the local tissues [18].

We found Th2 cells in VLF of CC patients. Besides expressing Th2 cytokines, such as IL-4, IL-5, and IL-13, these cells also express CD25, indicating these Th2 cells are at the mature status. In other words, these cells can respond to specific antigens. Indeed, upon exposing to a specific antigen, HDM, Ki67, the activation marker, was highly expressed by a portion of CD4+ T cells collected from VLF of CC patients, indicating that there are HDM-specific Th2 cells in VLF of CC patients.

Importantly, CD4+ T cells isolated from VLF also produce IL-25. IL-4 and IL-25 are the important Th2 response initiating cytokines; they play a critical role in regulating Th2 immunity. By eliciting and activating type 2 lymphoid cells, IL-25 acts throughout allergic inflammation [19]. By employing the culture supernatant of DME-primed CCp VLF CD4+ T cells, we induced naive CD4+ T cells to differentiate into Th2 cells. The results demonstrate that, after activating by specific antigen HDM, CCp VLF CD4+ T cells can induce Th2 cell development.

To diagnose allergic diseases, the disease history, SPT results, and serum-specific IgE are the major referencing parameters. One more direct diagnosis method is to challenge the targeted organs or tissues with specific allergens. Positive response to a specific allergen challenge can confirm the diagnosis of allergy [20]. In the present study, we applied HDM to the cervix of CC patients. The HDM challenge induced release of allergy mediators (mediators of mast cells and eosinophils were detected in VLF). This verified the cervical tissues are sensitized to HDM.

The limitation of this study is that we only observed an HDM-specific immune response in CC patients. The underlying mechanism needs to be further investigated in animal experiments; this may be carried out in the future.

In summary, the present data show that a portion of CC patients are sensitized to HDM. The HDM-specific allergic response may be associated with the pathogenesis of CC.

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Conflict of interest

None to declare.

Author contributions

F.M., J.L., X.L., and H.Z.L. performed experiments, analyzed data, and reviewed the manuscript. P.C.Y. and Y.N. designed the project, supervised experiments, and prepared the manuscript.

Data availability

All the data are included in this paper.

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