nasal polyps from ECRS patients than in those from NECRS patients. Consistent with our findings in murine $CD4^+$ T cells, we demonstrated that IL-33 induced augmentation of *IL5* and *IL1RL1* expression in memory $CD4^+$ T cells from ECRS patients. IL-33 induced expression of not only *IL5* but also *IL1RL1*, suggesting that a positive-feedback mechanism resulted in a greater responsiveness to IL-33 in memory $CD4^+$ T cells from ECRS patients. In addition, IL-33-induced IL-5 augmentation also depended on the activation of p38. Our study clearly demonstrates the function and pathophysiological role of IL-33 in ECRS, a chronic inflammatory human disease.

In summary, our study has identified memory Th2 cells as an important target of IL-33 in the pathogenesis of airway inflammation. The p38-mediated signaling pathway is critical for TCR-independent IL-33-induced IL-5 expression in both murine and human memory Th2 cells. Further detailed studies focused on the IL-33-ST2-p38-axis in pathogenic memory Th2 cells might lead to the discovery of potential therapeutic targets for the treatment of chronic allergic diseases.

EXPERIMENTAL PROCEDURES

Mice

The animals used in this study were backcrossed to BALB/c or C57BL/6 mice ten times. Anti-OVA-specific TCR-αβ (DO11.10) transgenic (Tg) mice were provided by Dr. D. Loh (Washington University School of Medicine, St. Louis) (Murphy et al., 1990). *Il33*$^{-/-}$ mice were generated as previously described (Oboki et al., 2010). *Il1rl1*$^{-/-}$ mice were kindly provided by Dr. Andrew N.J. McKenzie (Medical Research Council, Cambridge) (Townsend et al., 2000). Ly5.1 mice were purchased from Sankyo Laboratory. All mice were used at 6–8 weeks old and were maintained under specific-pathogen-free conditions. BALB/c, BALB/c *nu/nu*, and *Rag2*$^{-/-}$ mice were purchased from CLEA Japan. Animal care was conducted in accordance with the guidelines of Chiba University.

The Generation and Culture of Effector and Memory Th2 Cells

Splenic $CD62L^+CD44^-KJ1^+CD4^+$ T cells from DO11.10 OVA-specific TCR Tg mice were stimulated with an OVA peptide (Loh15, 1 μM) plus antigen-presenting cells (irradiated splenocytes) under Th2-cell-culture conditions (25 U/ml IL-2, 10 U/ml IL-4, anti-IL-12 monoclonal antibody [mAb], and anti-IFN-γ mAb) for 6 days in vitro. The effector Th2 cells (3×10^6) were transferred intravenously into BALB/c *nu/nu* or BALB/c recipient mice. Five weeks after cell transfer, $KJ1^+CD4^+$ T cells in the spleen were purified by autoMACS (Miltenyi Biotec) and cell sorting (BD Aria II) and were then used as memory Th2 cells.

Assessment of Memory Th2 Cell Function In Vivo

Memory Th2 cells were purified by fluorescence-activated cell sorting and transferred (3×10^6/mouse) again into *Il33*$^{+/+}$ or *Il33*$^{-/-}$ mice. The mice were exposed to aerosolized 1% OVA four times on days 1, 3, 9, and 11. For depletion of ILC2s, *Rag2*$^{-/-}$ mice were injected intraperitoneally with anti-CD90.2 (BioX Cell) antibody at a dose of 200 μg per day on days 2, 5, and 9. BAL fluid for the analysis of cytokine production by ELISA was collected 12 hr after the last challenge, and BAL fluid for the assessment of inflammatory cell infiltration was collected on day 13. Intracellular-staining analysis was performed 12 hr after the last inhalation. Lung histology was assessed on day 13. AHR was assessed on day 12.

Quantitative Real-Time PCR

Total RNA was isolated with the TRIzol reagent (Invitrogen). cDNA was synthesized with an oligo (dT) primer and Superscript II RT (Invitrogen). Quantitative real-time PCR was performed with the ABI PRISM 7500 Sequence Detection System as described previously (Endo et al., 2011). Primers and TaqMan probes were purchased from Applied Biosystems. Primers and Roche Universal probes were purchased from Sigma and Roche, respectively. Gene expression was normalized with the *Hprt* mRNA signal or the *18S* ribosomal RNA signal.

siRNA Analysis of Gene Targeting

siRNA was introduced into memory Th2 cells by electroporation with a mouse T cell Nucleofector Kit and Nucleofector I (Amaxa). Memory Th2 cells were transfected with 675 pmol of control random siRNA or siRNA for *p38* (Applied Biosystems) and cultured for 5 days with IL-33.

Nasal Polyp Mononuclear Cells and Homogenate Preparation

Nasal polyp mononuclear cells (NPMCs) were obtained as previously described (Yamamoto et al., 2007). In brief, freshly obtained nasal polyps were immediately minced and incubated in RPMI 1640 medium containing 1 mg/ml collagenase, 0.5 mg/ml hyaluronidase, and 0.2 mg/ml DNase I (Sigma-Aldrich). After incubation, NPMCs were obtained by the Ficoll-Hypaque technique. A volume of 1 ml of PBS was added for every 100 mg of tissue and was supplemented with aprotinin and leupeptin (Roche).

Statistical Analysis

Data were analyzed with GraphPad Prism software (version 6). Comparisons of two groups were calculated with non-parametric Mann-Whitney U tests. Differences with p values below 0.05 or 0.01 were considered significant.

SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2015.01.016.

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Activation of invariant natural killer T cells in regional lymph nodes as new antigen-specific immunotherapy via induction of interleukin-21 and interferon-γ

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Summary

Invariant natural killer T (iNKT) cells play important immunoregulatory functions in allergen-induced airway hyperresponsiveness and inflammation. To clarify the role of iNKT cells in allergic rhinitis (AR), we generated bone marrow-derived dendritic cells (BMDCs), which were pulsed by ovalbumin (OVA) and α-galactosylceramide (OVA/α-GalCer-BMDCs) and administered into the oral submucosa of OVA-sensitized mice before nasal challenge. Nasal symptoms, level of OVA-specific immunoglobulin (IgE), and T helper type 2 (Th2) cytokine production in cervical lymph nodes (CLNs) were significantly ameliorated in wild-type (WT) mice treated with OVA/α-GalCer-BMDCs, but not in WT mice treated with OVA-BMDCs. These anti-allergic effects were not observed in Jα18$^{-/-}$ recipients that lack iNKT cells, even after similar treatment with OVA/α-GalCer-BMDCs in an adoptive transfer study with CD4$^+$ T cells and B cells from OVA-sensitized WT mice. In WT recipients of OVA/α-GalCer-BMDCs, the number of interleukin (IL)-21-producing iNKT cells increased significantly and the Th1/Th2 balance shifted towards the Th1 dominant state. Treatment with anti-IL-21 and anti-interferon (IFN)-γ antibodies abrogated these anti-allergic effects in mice treated with α-GalCer/OVA-BMDCs. These results suggest that activation of iNKT cells in regional lymph nodes induces anti-allergic effects through production of IL-21 or IFN-γ, and that these effects are enhanced by simultaneous stimulation with antigen. Thus, iNKT cells might be a useful target in development of new treatment strategies for AR.

Keywords: α-galactosylceramide, bone marrow-derived dendritic cells, invariant natural killer T cell, IFN-γ, IL-21

Introduction

During the past several decades, the prevalence of allergic rhinitis (AR) has increased globally [1,2] and AR now affects 400 million people worldwide as a common allergic inflammatory disease that causes medical and socioeconomic problems [3]. Significant improvement of AR symptoms can be achieved using readily available drugs such as H1-anti-histamines and topical corticosteroids, but these drugs do not treat the underlying disease [4]. Antigen-specific immunotherapy may potentially alter the natural course of AR [4–6]; however, conventional immunotherapies, including subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT), are not convenient, because several years are required to establish a stable and adequate response. In addition, some patients do not show significant improvement of symptoms even with long-term therapy [7–9]. These burdens on patients would be reduced by a new method with enhanced therapeutic efficacy and a shortened duration of treatment without serious adverse events.

Invariant natural killer T (iNKT) cells, a major subset of NK T cells, express a unique semi-invariant T cell receptor (TCR) with a Vα14-Jα18 chain in mice and a Vα24-Jα18 chain in humans [10,11]. These cells produce many T helper type 1 (Th1)- and Th2-type proinflammatory cytokines, including interferon (IFN)-γ and interleukin (IL)-4, resulting in immune modulation of autoimmune diseases and responses to tumour and infectious agents [12–16]. TCRs of iNKT cells recognize monomorphic

major histocompatibility complex (MHC) class I-like CD1d molecules on antigen-presenting cells (APCs), and glycolipid antigens such as α-galactosylceramide (α-GalCer) presented on CD1d preferentially activate iNKT cells [17]. iNKT cells have been suggested to be tolerogenic in allergic airway inflammation [18–20], but it is unclear whether iNKT cells regulate development of AR.

In this study, we administered ovalbumin (OVA)- and α-GalCer-pulsed bone marrow-derived dendritic cells (BMDCs) into the oral submucosa of OVA-sensitized mice and examined the role of activated iNKT cells in an AR mouse model. Administration of OVA/α-GalCer-pulsed BMDCs suppressed antigen-specific responses, whereas OVA-pulsed BMDCs did not do so. These results show that activation of iNKT cells in draining lymph nodes ameliorated nasal allergic responses in an AR mouse model, and that this anti-allergic effect is associated with IL-21 and IFN-γ production through activated iNKT cells.

Materials and methods

Mice

Female BALB/c mice (8 weeks old) were purchased from SLC Inc. (Hamamatsu, Japan). Jα18-deficient ($J\alpha18^{-/-}$) mice were established by specific deletion of the Jα18 gene segment [15] and back-crossing 10 times to the BALB/c background. These mice were also used at 8 weeks of age. Mice were maintained under specific pathogen-free conditions. Use of the mice was approved by the Chiba University Institutional Animal Care and Use Committee and the experiments were conducted in conformity with the guidelines of the committee.

Reagents

α-GalCer (KRN7000) was obtained from Kirin Brewery (Gunma, Japan). OVA (grade 5) was purchased from Sigma-Aldrich (St Louis, MO, USA) and dissolved in endotoxin-free D-phosphate-buffered saline (PBS) (Wako Pure Chemical Industries, Osaka, Japan). RPMI-1640 medium (Sigma-Aldrich) supplemented with 10% fetal calf serum (FCS), l-glutamine (2 μM), penicillin (100 U/ml), streptomycin (100 μg/ml), HEPES (10 mM), 2-mercaptoethanol (55 μM), 1% non-essential amino acids and 1 mM sodium pyruvate (all from Gibco BRL, Grand Island, NY, USA) was used in cell culture experiments. Anti-FcγRII/III monoclonal antibodies (mAbs) (2·4G2) (BD Biosciences, San Jose, CA, USA) were used for Fc blocking. Allophycocyanin-conjugated α-GalCer-loaded CD1d tetramer was purchased from Proimmune (Oxford, UK). Fluorescein isothiocyanate (FITC)-anti-CD11c (N418) (eBiosciences, San Diego, CA, USA), phycoerythrin (PE)-conjugated mAbs including anti-CD4 (GK1·5), anti-CD19 (6D5), anti-CD40 (3/23), anti-CD86 (GL-1) (BioLegend, San Diego, CA, USA), anti-MHC class II IA + IE (M5/114·15) or anti-CD80 (16-10A-1) (eBiosciences) were used for fluorescence activated cell sorter (FACS) analysis. Anitbodies including anti-IL-4 (11B11), anti-IL-5 (TRFK5; Mabtech Ab, Nacka, Sweden), anti-IFN-γ (AN18; BioLegend) or anti-immunoglobulin (Ig)E (RME-1; BD Pharmingen, San Jose, CA, USA), and biotin-conjugated antibodies including anti-IL-4 mAb (BVD6-24G2), anti-IL-5 (TRFK4) (Mabtech antibody), anti-IFN-γ (R4-6A2; BioLegend) or anti-IgE (R35-72; BD Pharmingen) were used in enzyme-linked immunosorbent assays (ELISAs) [21]. A mouse IL-13 ELISA Ready-SET-Go! Kit (eBiosciences) and a mouse anti-OVA IgE antibody assay kit (Chondrex, Redmond, WA, USA) were also used in the study.

Generation of BMDCs

Bone marrow cells obtained from the femurs of naive BALB/c mice were cultured with 20 ng/ml murine granulocyte–macrophage colony-stimulating factor (GM-CSF) (PeproTech, Rocky Hill, NJ, USA) for 8 days. Non-adherent cells were harvested and pulsed with or without 100 ng/ml α-GalCer for 3 h, followed by a 6-h incubation with or without 1 mg/ml OVA in 24-well plates at 1×10^6 cells/well. These cells were stimulated with 10 μg/ml lipopolysaccharide (LPS) (O111:B4; Sigma-Aldrich) for 3 h and then washed three times with PBS. DCs were analysed based on surface markers using FACS analysis (FACSCalibur; Becton Dickinson, Franklin Lakes, NJ, USA).

Administration of BMDCs in OVA-sensitized mice

BALB/c mice were sensitized intraperitoneally with 100 μg of OVA and 2 mg of alum (Pierce, Rockford, IL, USA) once a week for 3 weeks. One week after the last sensitization, the mice ($n = 5$–6 in each group) received 5×10^6 cells of BMDCs in 100 μl of PBS to the sublingual submucosa by injection and were treated intranasally with 1 mg of OVA for 7 consecutive days (challenge group) or with PBS for 6 consecutive days followed by OVA on the seventh day (control group). After the last treatment, the behaviour of the mice was documented for 5 min using a videorecorder. Sneezing and nasal-rubbing events were counted by an investigator who was blinded to the treatment. The mice were then killed and the serum, cervical lymph nodes (CLNs) and spleens were collected.

Adoptive transfer of $CD4^+$ T cells and B cells

$CD4^+$ T cells and B cells were sorted from OVA-sensitized wild-type (WT) mice by negative selection using a magnetic affinity cell sorting (MACS) system (Miltenyi Biotec, Bergisch Gladbach, Germany). A single-cell suspension was prepared from spleens [22]. After Fc blocking, splenic cells

Table 1. Polymerase chain reaction (PCR) primers used in the study.

Primer set	Sense primer, 5′–3′	Anti-sense primer, 5′–3′
Vα14	CACTGCCACCTACATCTGTGT	AGTCCCAGCTCCAAAATGCA
IL-21	GCCAGATCGCCTCCTGATTA	CATGCTCACAGTGCCCCTTT
Bcl6	CCGGCTCAATAATCTCGTGAA	GGTGCATGTAGATGTGTGAGTGA
IL-17RA	AGTGTTTCCTCTACCCAGCAC	GAAAACCGCCACCGCTTAC
RORγt	CTTTCAATACCTCATTGTAT	AGGTCCTTCTGGGGGCTTGC
Beta-actin	CCAGCCTTCCTTCTTGGGTAT	TGGCATAGAGGTCTTTACGGATGT

IL = interleukin; RORγt = RAR-related orphan receptor gamma t.

were incubated with a mixture of biotinylated antibodies, including anti-IgM (MA-69), anti-B220 (RA3-6B2), anti-CD11b (M1/70), anti-CD11c (N418), anti-TER-119 (TER-119), anti-Gr-1 (RB6-MC5) (BioLegend) or allophycocyanin-conjugated α-GalCer-loaded CD1d tetramer to collect $CD4^+$ T cells, or with a mixture of biotinylated antibodies including anti-CD3 (145-2C11; BioLegend), anti-CD11b, anti-CD11c, anti-TER-119, anti-Gr-1 or allophycocyanin-conjugated α-GalCer-loaded CD1d tetramer to collect B cells. After washing, these cells were incubated with anti-biotin beads and anti-allophycocyanin-beads (Miltenyi Biotec) and then subjected to MACS analysis. The purity of the cells was analysed using FACSCalibur (BD Biosciences) and CellQuest software (Becton Dickinson). Data were analysed with FlowJo software (TreeStar, Ashland, OR, USA). The isolated $CD4^+$ T cells (1×10^7 cells) and B cells ($1{\cdot}5 \times 10^7$ cells) were then transferred intravenously to WT or $J\alpha18^{-/-}$ mice. One day later, injection of BMDCs and nasal challenge were performed as described above.

Neutralization assay

Anti-mouse IL-21 antibody (TY25), rat IgG2a antibody (54447) (R&D Systems), anti-mouse IFN-γ antibody (R4-6A2) or rat IgG2a antibody (RTK2758) (BioLegend) (250 μg) was injected intravenously in OVA-sensitized mice 1 day before BMDC administration and on day 3 of nasal challenge.

Proliferation assay

$CD4^+$ T cells isolated from CLNs were cultured with OVA and irradiated splenic feeder cells for 48 h, with tritium-labelled thymidine (37 kBq/well) added for the last 8 h. The cells were then harvested with a cell harvester (Perkin Elmer, Waltham, MA, USA) onto a β plate and the radioactivity was measured using a liquid scintillation counter (Perkin Elmer).

Restimulation of $CD4^+$ T cells

Single-cell suspensions were prepared from CLNs and $CD4^+$ T cells were sorted by the MACS technique using a biotinylated anti-CD4 antibody (GK1·5; BioLegend) and anti-biotin beads (Miltenyi Biotec). The cells were cultured for 48 h at a density of $1{\cdot}5 \times 10^5$ cells/well in round-bottomed 96-well microculture plates in the presence of 1 mg/ml OVA with $CD4^+$ T cell-depleted and irradiated splenic feeder cells (5×10^5 cells) obtained from naive mice. The concentration of cytokines in the supernatant was measured by ELISA.

Detection of IL-21-producing iNKT cells

IL-21-producing iNKT cells were detected by an enzyme-linked immunospot (ELISPOT) assay [21] with anti-mouse IL-21 antibody and biotinylated anti-mouse IL-21 antibody (mouse IL-21 DuoSet; R&D Systems, Minneapolis, MN, USA). A single-cell suspension was prepared from CLNs and spleens, as described above. Splenocytes were incubated with anti-FcγRII/III mAbs and depleted with biotinylated antibodies, including anti-IgM, anti-B220, anti-CD11b, anti-CD11c, anti-TER-119, anti-Gr-1 and anti-biotin beads, using the MACS technique. The enriched spleen cells were incubated with allophycocyanin-conjugated α-GalCer-loaded CD1d tetramer and splenic iNKT cells were sorted using FACS ARIA II (BD Biosciences). CLN cells (2×10^5 cells/well) were cultured with α-GalCer (100 ng/well) and splenic iNKT cells (5×10^4 cells/well) were co-cultured with BMDCs (5×10^4 cells per well) in 96-well filtration plates (Multiscreen; Millipore Corp., Bedford, MA, USA) for 3 days.

Real-time reverse transcription–polymerase chain reaction (RT–PCR)

Total RNA was extracted from $CD4^+$ cells in CLNs using TRIzol reagent (Life Technologies, Gaithersburg, MD, USA) and reverse transcribed using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). Relative gene expression was calculated by a sequence detection system (StepOnePlus™; Applied Biosystems) and the amount of cDNA was normalized using the *beta-actin* housekeeping gene. Primer sets (Table 1) were purchased from Eurofins Operon MWG (Ebersberg, Germany).

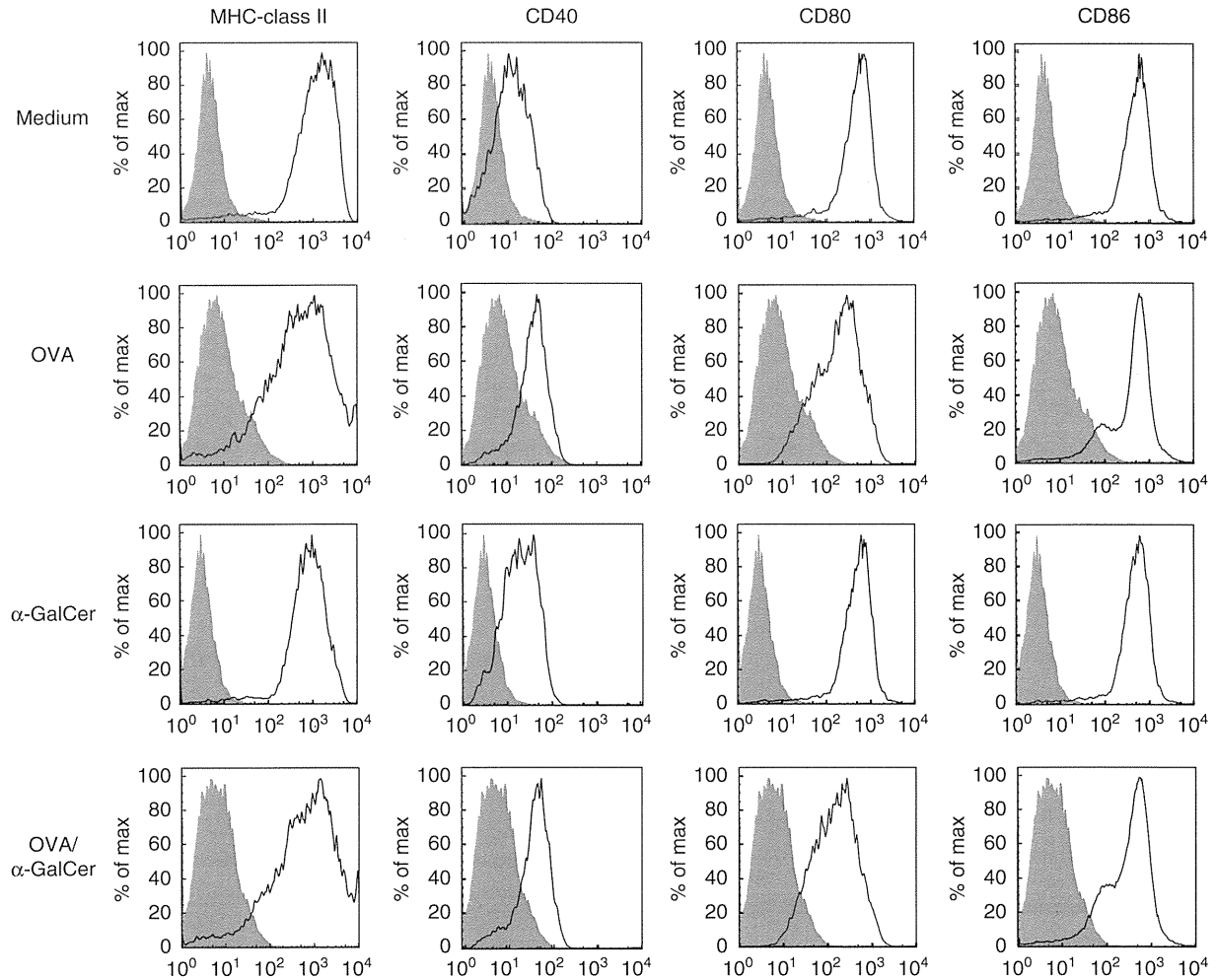

Fig. 1. Similar expression levels of surface markers in each group of bone marrow-derived dendritic cells (BMDCs). BMDCs were incubated with medium only, ovalbumin (OVA), α-galactosylceramide (α-GalCer) or OVA/α-GalCer, followed by addition of lipopolysaccharide (LPS), and surface markers were analysed by fluorescence activated cell sorter (FACS). BMDCs were gated on $CD11c^+$. Shaded profiles in the histograms show background staining with rat immunoglobulin (Ig)G2a. Data are representative of three independent experiments.

Statistical analysis

Statistical analysis was performed using a two-tailed Student's *t*-test with $P < 0{\cdot}05$ considered to be significant. Data are shown as the mean ± standard deviation.

Results

Characteristics of BMDCs

Surface marker expression levels on stimulated BMDCs were evaluated by FACS analysis prior to administration into AR mice. The percentage of $CD11c^+$ cells in the generated BMDCs was approximately 95%. Based on the MHC class II levels, there were few differences among BMDCs cultured with medium, OVA, α-GalCer and OVA plus α-GalCer. Similar patterns were observed for the expression levels of CD40, CD80 and CD86 (Fig. 1).

Oral submucosal administration of BMDCs in OVA-sensitized mice

On the 7th day of nasal challenge with OVA, mice administered OVA/α-GalCer-BMDCs showed significant decreases in the number of sneezing and nasal rubbing attacks, and in the levels of both OVA-specific and total IgE, compared with mice administered BMDCs. There were no significant differences in nasal symptoms and IgE levels among mice that received BMDCs, OVA-BMDCs or α-GalCer-BMDCs (Fig. 2a,b).

Analysis of $CD4^+$ T cells isolated from CLNs

Cytokine production by $CD4^+$ T cells in CLNs is shown in Fig. 2c. Of the Th2 cytokines examined, IL-4, IL-5 and IL-13 levels were significantly lower in $CD4^+$ T cells from mice that received OVA/α-GalCer-BMDCs compared with

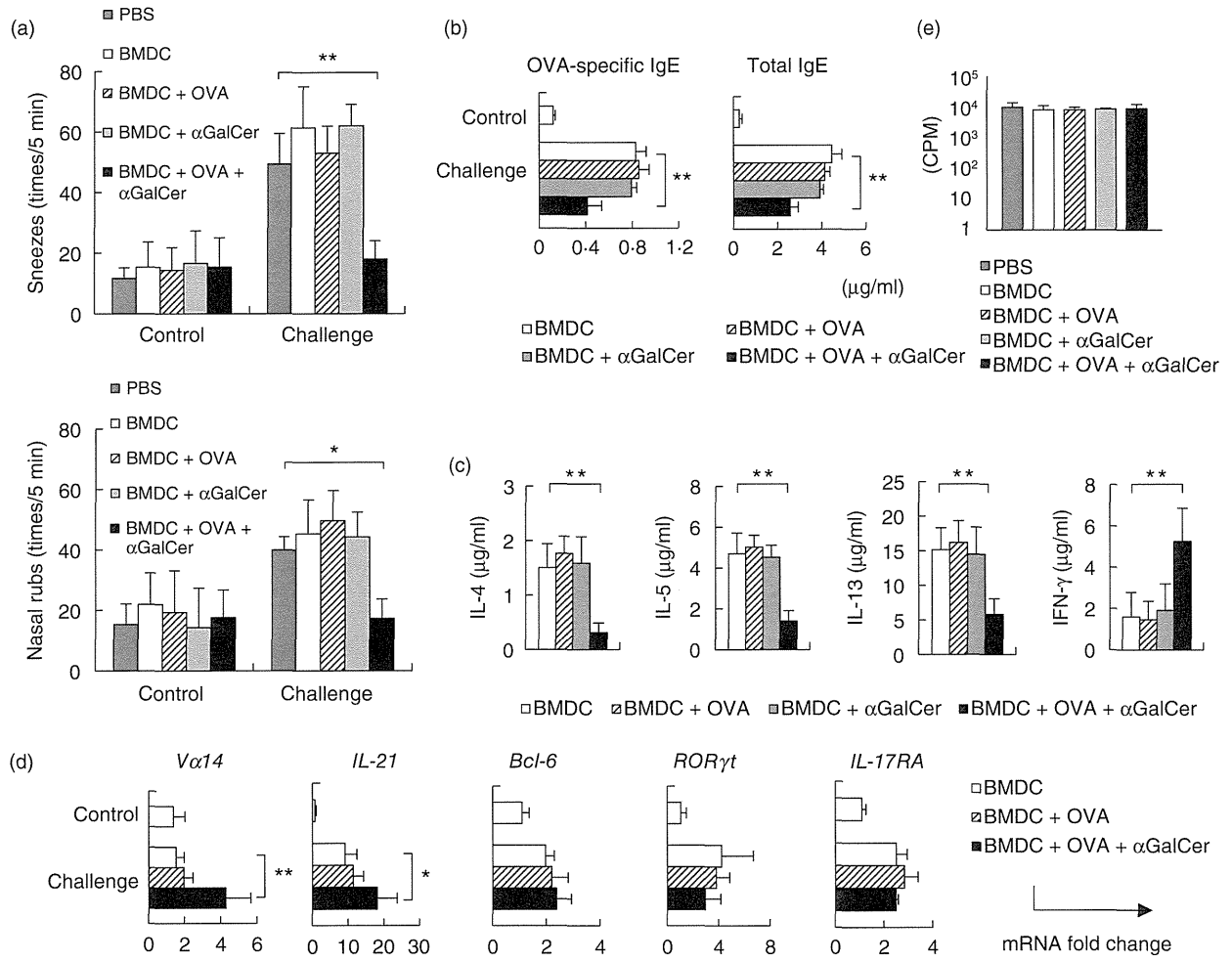

Fig. 2. Prevention of development of nasal allergic symptoms by administration of ovalbumin, α-galactosylceramide-bone marrow-derived dendritic cells (OVA, α-GalCer-BMDCs). (a) Number of sneezes and nasal rubs. (b) OVA-specific and total immunoglobulin (Ig)E levels in serum. (c,e) Cytokine production and proliferation of $CD4^+$ T cells obtained from cervical lymph nodes (CLNs). Counts per minute, cpm. (d) Relative gene expression of $CD4^+$ cells obtained from CLNs. Data are representative of three independent experiments. $^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$.

those from mice that received BMDCs or OVA-BMDCs. Enhanced IFN-γ production occurred in mice that received OVA/α-GalCer-BMDCs. Gene expression profiles from quantitative RT–PCR analysis (Fig. 2d) showed higher *Vα14* and *IL-21* expression in the CLNs of OVA/α-GalCer-BMDC-treated mice compared with other groups. However, expression of *Bcl-6*, a Tfh cell-related transcript, and Th17 cell-related transcripts such as *IL-17RA* and *RORγt*, did not differ among the groups. Proliferation of $CD4^+$ T cells also showed no differences among the groups of mice (Fig. 2e).

Adoptive transfer of $CD4^+$ T cells and B cells into $J\alpha18^{-/-}$ mice

Following adoptive cell transfer of $CD4^+$ T cells (excluding iNKT cells) and B cells from spleen of OVA-sensitized WT mice, nasal symptoms after OVA challenge in WT mice were significantly suppressed by oral submucosal administration of OVA/α-GalCer-BMDCs compared with mice administered other BMDCs. However, similar suppression was not observed in $J\alpha18^{-/-}$ [iNKT knock-out (KO)] mice that received OVA/α-GalCer-BMDCs (Fig. 3).

IL-21-producing iNKT cells in CLNs

After stimulation with α-GalCer, IL-21-producing cells increased significantly in CLN cells of mice treated with OVA/α-GalCer BMDCs (Fig. 4a), whereas IL-21 was not detected in culture supernatants of $CD4^+$ T cells (data not shown). To determine whether iNKT cells produce IL-21 in response to α-GalCer presented on BMDCs, splenic iNKT cells of naive mice were co-cultured with BMDCs plus α-GalCer. The results showed that OVA/α-GalCer BMDCs stimulated IL-21 production in iNKT cells (Fig. 4b).

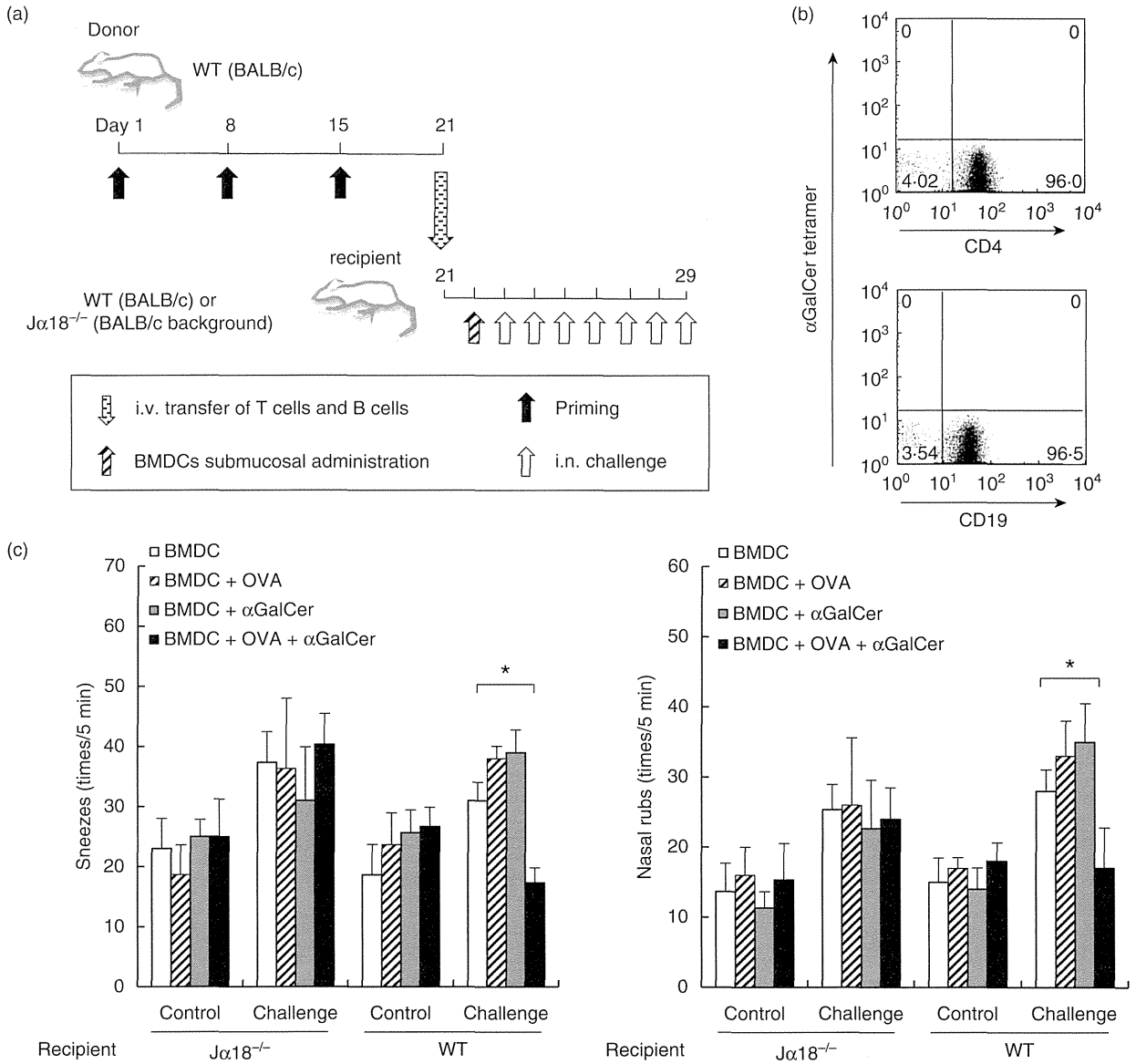

Fig. 3. Suppression of nasal symptoms by ovalbumin, α-galactosylceramide-bone marrow-derived dendritic cells (OVA, α-GalCer-BMDCs) in an invariant natural killer T (iNKT) cell-dependent manner. (a) Protocol of adoptive transfer. (b) Splenic $CD4^+$ T cells and B cells of OVA-sensitized mice were transferred after removal of iNKT cells. (c) Nasal symptoms after the final nasal challenge. Data are representative of three independent experiments. $^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$.

Treatment with anti-IL-21 or anti-IFN-γ neutralizing antibody

Treatment with anti-IL-21 mAb or anti-IFN-γ mAb and nasal challenge in OVA/α-GalCer BMDC-treated mice increased the number of sneezes and nasal rubs, compared with control mAb-treated mice (Figs 5a and 6a). OVA-specific and total IgE levels were increased by anti-IL-21 mAb, whereas only OVA-specific IgE was increased by anti-IFN-γ mAb (Figs 5b and 6b).

Discussion

The goal of this study was to assess the anti-allergic effects of activated iNKT cells in CLNs, which are regional draining lymph nodes, in an AR mouse model. Single administration of OVA/α-GalCer-BMDCs into the oral submucosa of OVA-sensitized mice suppressed nasal symptoms and the level of OVA-specific IgE in association with IL-21 and IFN-γ in an iNKT cell-dependent manner. Other BMDCs failed to alleviate the Th2 responses and, therefore, the

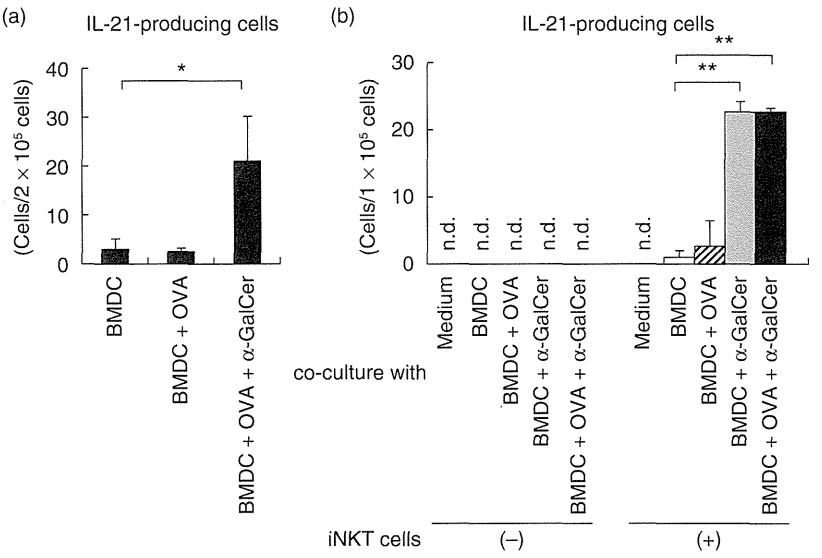

Fig. 4. Increase of invariant natural killer T (iNKT) cells producing interleukin (IL)-21 in cervical lymph nodes (CLNs). (a) CLN cells were cultured with α-galactosylceramide (α-GalCer) and IL-21-secreting cells were detected using an enzyme-linked immunospot (ELISPOT) assay. (b) Splenic iNKT cells and bone marrow-derived dendritic cells (BMDCs) were co-cultured, and IL-21-secreting cells were detected by ELISPOT assay. Data are representative of three independent experiments. n.d. = not detected; $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$.

production of both OVA-specific and total IgE was up-regulated. These findings indicate that, if antigen stimulation is provided simultaneously, activated iNKT cells in CLNs can suppress a nasal allergic reaction by producing IL-21 and IFN-γ.

IL-21, a type I cytokine, prevents B cell proliferation and correspondingly augments B cell death under certain conditions [23–26]. This cytokine is produced preferentially by activated iNKT cells and $CD4^{+}$ T cells, including Tfh cells and Th17 cells [27–29]. Expression of *Bcl6*, a Tfh cell-related transcript, and Th17 cell-related transcripts such as *IL-17RA* and *RORγt*, did not increase in this study; but *Vα14*, an iNKT cell-related transcript, was markedly up-regulated and IL-21-producing iNKT cells increased significantly in CLNs of mice treated with OVA/α-GalCer-BMDCs in the oral submucosa. In addition, a neutralization assay revealed that IL-21 plays a critical role in suppressing OVA-specific IgE production. These results are congruent with those reported by Hiromura *et al.* showing that intranasal administration of recombinant mouse IL-21 reduces nasal symptoms and the serum level of OVA-specific IgE [30].

The Th1/Th2 balance in CLNs changed towards a Th1-skewed phenotype after administration of OVA/α-GalCer-BMDCs. An IFN-γ neutralization indicated that this Th1 cytokine can play a pivotal role in suppressing the level of OVA-specific IgE. In type I allergic diseases, allergens trigger a Th2-dominant immune response that generates

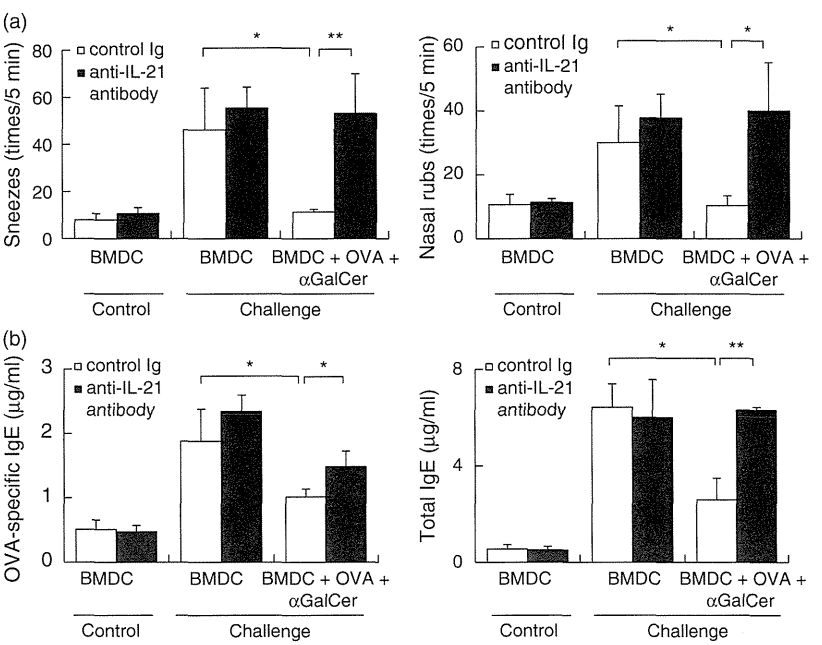

Fig. 5. Failure of ovalbumin, α-galactosylceramide-bone marrow-derived dendritic cells (OVA, α-GalCer-BMDCs) to suppress nasal symptoms by neutralization of interleukin (IL)-21. (a) Nasal symptoms were observed for 5 min after the final nasal challenge. (b) OVA-specific and total immunoglobulin (Ig)E levels in serum. Data are representative of three independent experiments. $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$.

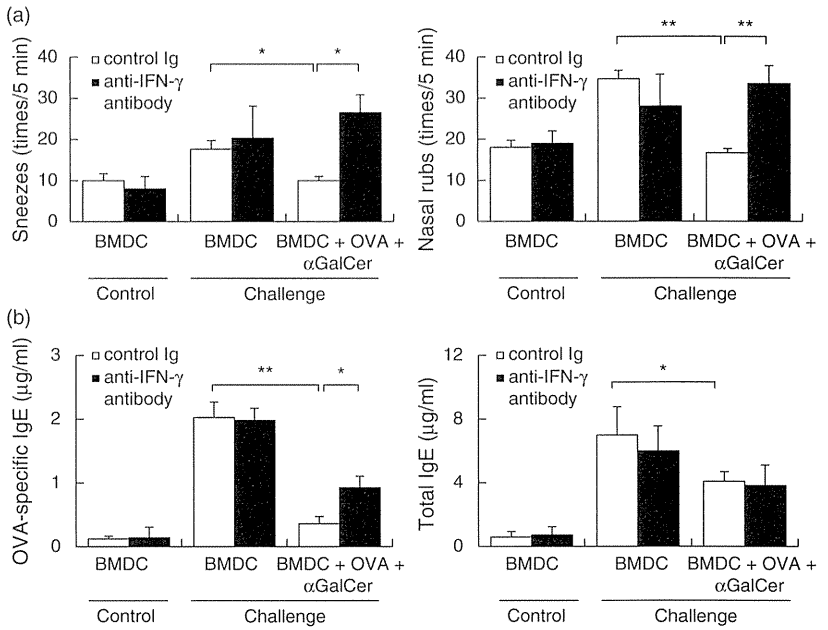

Fig. 6. Failure of ovalbumin, α-galactosylceramide-bone marrow-derived dendritic cells (OVA, α-GalCer-BMDCs) to suppress nasal symptoms by neutralization of interferon (IFN)-γ. (a) Nasal symptoms were observed for 5 min after the final nasal challenge. (b) OVA-specific and total IgE levels in serum. Data are representative of three independent experiments. $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$.

antigen-specific IgE-producing memory B cells, but the role of IFN-γ in IgE production remains unclear [31,32]. Antigen-specific IgE is produced mainly in draining lymph nodes [21,33] and CLNs are regional lymph nodes from the oral cavity, as well as the nasal cavity [33,34]. In the present study, treatment with anti-IFN-γ antibody exacerbated nasal symptoms and the OVA-specific IgE titre in mice treated with OVA/α-GalCer-BMDCs. These results suggest that IFN-γ exerts a potent inhibitory effect on IgE production in AR.

The role of iNKT cells in allergic reactions is unclear. These cells have been suggested to have a suppressive effect on allergic disease [19,20,31,35]; however, other reports show that iNKT cells have an essential role in development of airway hyperreactivity [18,36,37]. This plasticity of iNKT cells may arise partially from differences in systemic *versus* topical administration of α-GalCer and the diversity of APCs. In the present study, OVA/α-GalCer-BMDCs led to suppress OVA-induced nasal allergic symptoms and OVA-specific IgE production. These findings share some features with the previous report demonstrating that mice administered OVA/α-GalCer-BMDCs intratracheally prior to OVA challenge failed to develop airway hyperresponsiveness [38].

Brimnes *et al.* showed that repeated sublingual administration of OVA for 5 days each week for 9 weeks resulted in relief from nasal allergic symptoms in an AR mouse model [39]. Direct administration of OVA and α-GalCer to the oral mucosa failed to have this effect because α-GalCer is not a water-soluble antigen and is not readily phagocytosed by oral dendritic cells. In the present study, α-GalCer-BMDCs did not exacerbate nasal allergic symptoms and simultaneous administration of OVA and α-GalCer using BMDCs led to efficient suppression of OVA-induced allergic reactions.

We have reported previously that DCs isolated from PBMCs of patients with head and neck cancer migrated to CLNs after oral submucosal administration [34], and we showed that this treatment was safe [40]. Simultaneous administration of an antigen with α-GalCer-DCs is thus an accessible way to activate iNKT cells in regional lymph nodes; however, further studies are needed to clarify the role of activated iNKT cells in regional lymph nodes in treatment of AR.

In conclusion, oral submucosal administration of OVA/α-GalCer-pulsed BMDCs activated iNKT cells in CLNs and suppressed Th2 responses in OVA-sensitized mice. In the present study, simultaneous stimulation with antigen and α-GalCer were considered essential to exert anti-allergic effects and led to relief of nasal allergic symptoms. This finding indicates that the activated iNKT cells have the potential to alleviate nasal allergic symptoms in the presence of antigen. Thus, activation of iNKT cells in regional lymph nodes might be an important target in new treatment strategies for AR.

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Disclosure

The authors have no conflicts of interest to declare.

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MAIN ARTICLE

Pulmonary function in patients with chronic rhinosinusitis and allergic rhinitis

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Abstract

Background: A close relationship between upper and lower respiratory tract diseases has been reported. However, little is known about pulmonary function in patients with upper respiratory tract diseases.

Methods: Pulmonary function was measured in: 68 patients with chronic rhinosinusitis without nasal polyps, 135 patients with chronic rhinosinusitis with nasal polyps, 89 patients with allergic rhinitis and 100 normal control subjects. The relationships between pulmonary function and clinical parameters were assessed. These parameters included radiographic severity of chronic rhinosinusitis, serum total immunoglobulin E levels, concentrations of cytokines in nasal secretions and exhaled nitric oxide levels.

Results: The pulmonary function of patients with chronic rhinosinusitis was significantly affected. The level of interleukin-5 in nasal secretions was significantly correlated with pulmonary function in patients with chronic rhinosinusitis.

Conclusion: The findings indicated latent obstructive lung function changes in chronic rhinosinusitis patients. The cytokines in nasal secretions might be related to obstructive lung function changes in chronic rhinosinusitis.

Key words: Sinusitis; Rhinitis; Asthma; Chronic Obstructive Pulmonary Disease; COPD; Lung Function Tests

Introduction

Chronic rhinosinusitis is defined as a persistent inflammatory response involving the mucous membranes of the nasal cavity and paranasal sinuses. It has recently been divided into two subgroups: chronic rhinosinusitis with nasal polyps, and chronic rhinosinusitis without nasal polyps.[1] Allergic rhinitis is characterised by a number of symptoms, including sneezing, nasal congestion, nasal itching and rhinorrhoea.[2] Chronic rhinosinusitis and allergic rhinitis are common upper respiratory tract diseases.[3–5] The presence of allergic rhinitis is one of the risk factors for the development of asthma; the association between allergic rhinitis and asthma is explained by the 'united airway disease' hypothesis.[2,6] It has been suggested that chronic obstructive pulmonary disease (COPD) is also associated with upper airway diseases including chronic rhinosinusitis.[7–9]

Although numerous studies have described a relationship between upper and lower respiratory tract diseases, pulmonary function in patients with upper respiratory tract diseases has not been fully examined. To the best of our knowledge, no study has compared pulmonary function in patients with upper respiratory tract diseases (chronic rhinosinusitis and allergic rhinitis) with that in normal controls. This study aimed to evaluate pulmonary function in patients with chronic rhinosinusitis or allergic rhinitis who had not been diagnosed with lower respiratory tract diseases.

Materials and methods

This study was approved by the Institutional Review Board of Okayama University (approval number, RINRI-877), and was conducted in compliance with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all enrolled subjects.

Subjects

Four groups of participants were enrolled in this study: a chronic rhinosinusitis without nasal polyps group, a chronic rhinosinusitis with nasal polyps group, an allergic rhinitis group and a normal control group.

A total of 203 chronic rhinosinusitis patients who were scheduled to undergo functional endoscopic sinus surgery (FESS) at Okayama University were recruited and divided into two groups (chronic rhinosinusitis without nasal polyps and chronic rhinosinusitis with nasal polyps groups). The diagnosis of chronic

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rhinosinusitis with nasal polyps was based on the definition in the European Position Paper on Rhinosinusitis and Nasal Polyps 2012.[1] All chronic rhinosinusitis patients were resistant to medical treatment, including macrolide therapy.[10] Chronic rhinosinusitis patients with chronic lower lung diseases including bronchial asthma and COPD were excluded from this study. The diagnoses of asthma and COPD were based on the internationally accepted clinical guidelines.[11,12]

Eighty-nine patients with allergic rhinitis took part in this study. Allergic rhinitis was defined according to the clinical symptoms and serological results reported in the *Practical Guideline for the Management of Allergic Rhinitis in Japan* (2008).[13] The radioallergosorbent test was used for the diagnosis of immunoglobulin E (IgE) mediated allergic reactions. Computed tomography (CT) was performed to exclude the possibility of coexisting paranasal sinus abnormalities. Allergic rhinitis patients who were clinically diagnosed as having lower respiratory tract diseases were excluded from this study.

Age-matched, normal control subjects with no chronic respiratory diseases were also recruited ($n = 100$).

Because cigarette smoking could affect pulmonary function, smoking status was examined and the Brinkman Index (number of cigarettes per day × smoking years) was calculated.

Pulmonary function tests

Prior to FESS, pulmonary function testing was performed with the Chestac-9800 spirometer (Chest MI, Tokyo, Japan) according to the standardisation of lung function tests of the American Thoracic Society and European Respiratory Society.[14] The following parameters were measured or calculated: percentage of predicted vital capacity; forced expiratory volume in 1 second; percentage of predicted forced expiratory volume in 1 second; forced expiratory volume in 1 second / forced vital capacity ratio; mean forced expiratory flow between 25 and 75 per cent of the forced vital capacity; peak expiratory flow; maximal expiratory flow rate at 50 per cent of vital capacity; maximal expiratory flow rate at 25 per cent of vital capacity; and the maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio.

Rhinomanometry

In all chronic rhinosinusitis patients, nasal obstruction was examined (prior to FESS) by active anterior rhinomanometry with a nasal nozzle at air pressure 100 Pa (MPR-3100; Nihon Kohden, Tokyo, Japan), according to the manufacturer's instructions.[15]

Chronic rhinosinusitis assessment

The radiographic severity of chronic rhinosinusitis was assessed (prior to FESS) using the Lund–MacKay CT staging system.[16]

Blood tests

Blood samples were taken prior to FESS. The peripheral blood eosinophil count was determined. Serum total IgE levels were measured with the ImmunoCap 250 system (Phadia AB, Uppsala, Sweden), according to the manufacturer's protocols.

Inflammatory mediators assessment

Nasal secretion was collected (prior to FESS) from 13 randomly selected chronic rhinosinusitis patients without lung disease (mean age ± standard deviation (SD), 48.2 ± 12.5 years; i.e. 3 chronic rhinosinusitis patients without nasal polyps and 10 chronic rhinosinusitis patients with nasal polyps). A bicinchoninic acid assay was performed to quantify the total protein concentration in each sample using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Rockford, Illinois, USA). The concentrations of inflammatory mediators (tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-4, IL-5, IL-6, IL-8, IL-17 and interferon-γ) were determined by BD OptEIA enzyme-linked immunosorbent assay sets (BD, Franklin Lakes, New Jersey, USA). A zero value was assigned when the concentration of inflammatory mediators was under the detection limit of the enzyme-linked immunosorbent assay set. The concentrations of TNF-α, IL-1β, IL-4, IL-5, IL-6, IL-8, IL-17 and interferon-γ (pg/ml) were divided by the concentration of total protein of each sample (mg of total protein per ml) for standardisation. The calculated concentrations of each cytokine (pg/mg total protein) were used for statistical evaluation.

Exhaled nitric oxide concentration

The Niox Mino device (Aerocrine AB, Solna, Sweden) was used to measure the level (fraction) of exhaled nitric oxide according to the manufacturer's instructions. This was carried out (prior to FESS) in 13 randomly selected chronic rhinosinusitis patients without lung disease (mean age ± SD, 48.2 ± 12.5 years; i.e. 3 chronic rhinosinusitis patients without nasal polyps and 10 chronic rhinosinusitis patients with nasal polyps).

Statistical analysis

Values are presented as means ± SD. Differences in proportions were examined using the chi-square test. For comparisons between groups, a one-way analysis of variance was conducted to establish the significance of inter-group variability. The two-tailed unpaired *t*-test was then used for between-group comparisons for normally distributed data. A correlation analysis was performed using Spearman's rank correlation coefficient. *P* values less than 0.05 were considered significant. Statistical analyses were performed with the Statistical Package for the Social Sciences software (SPSS, Chicago, Illinois, USA).

Results

Subject characteristics

Demographic data are presented in Table I. There was a significantly higher ratio of males to females in the chronic rhinosinusitis group compared with the normal control group. There were no significant differences in age or smoking status among the groups.

Pulmonary function

Pulmonary function data for patients with chronic rhinosinusitis (without any clinically diagnosed lung disease) and normal control subjects are shown in Figure 1. There were no significant differences between chronic rhinosinusitis patients and normal controls in terms of forced expiratory volume in 1 second and the percentage of predicted vital capacity. However, pulmonary function was significantly affected in chronic rhinosinusitis patients (compared with normal controls) in the following parameters: percentage of predicted forced expiratory volume in 1 second; forced expiratory volume in 1 second / forced vital capacity ratio; peak expiratory flow; mean forced expiratory flow between 25 and 75 per cent of the forced vital capacity; maximal expiratory flow rate at 50 per cent of vital capacity; maximal expiratory flow rate at 25 per cent of vital capacity; and maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio. No significant differences were observed between the chronic rhinosinusitis without nasal polyps group and the chronic rhinosinusitis with nasal polyps group in any parameters.

In patients with allergic rhinitis, the percentage of predicted vital capacity was 114.9 ± 15.8 per cent, the forced expiratory volume in 1 second was 3.58 ± 0.75 litres per second, the percentage of predicted forced expiratory volume in 1 second was 106.0 ± 11.8 per cent, the forced expiratory volume in 1 second / forced vital capacity ratio was 84.2 ± 7.73 per cent, the peak expiratory flow was 8.76 ± 1.98 litres per second, the mean forced expiratory flow between 25 and 75 per cent of the forced vital capacity was 3.56 ± 1.20 litres per second, the maximal expiratory flow rate at 50 per cent of vital capacity was 4.21 ± 1.24 litres per second, the maximal expiratory flow rate at 25 per cent of vital capacity was 1.63 ± 0.82 litres per second, and the maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio was 3.10 ± 1.76. No significant differences in pulmonary function parameters were seen between allergic rhinitis patients and normal controls.

Nasal obstruction

The factors that might affect pulmonary function in chronic rhinosinusitis patients were investigated. Rhinomanometry was used to evaluate nasal obstruction. The mean nasal resistances at delta P (transnasal differential pressure) 100 Pa in the chronic rhinosinusitis without nasal polyps group was 0.32 ± 0.23 Pa/cm^3/s, and in the chronic rhinosinusitis with nasal polyps group it was 0.34 ± 0.24 Pa/cm^3/s. There was no significant difference in nasal resistance between the chronic rhinosinusitis groups (p = 0.772). No significant correlations were observed between nasal resistance and pulmonary function in either of the chronic rhinosinusitis groups (Tables II and III).

Computed tomography score

The Lund–Mackay CT score was used to evaluate chronic rhinosinusitis severity. The average Lund–Mackay scores on pre-operative CT scans were 6.75 ± 4.40 in the chronic rhinosinusitis without nasal polyps group and 11.71 ± 5.75 in the chronic rhinosinusitis with nasal polyps group; this difference was significant ($p < 0.001$). No significant correlations were observed between pre-operative CT score and pulmonary function in either of the chronic rhinosinusitis groups (Tables II and III).

Peripheral blood eosinophil count

The mean peripheral blood eosinophil count was 204.9 ± 162.8 in the chronic rhinosinusitis without

TABLE I
SUBJECT CHARACTERISTICS

Parameter	CRSsNP	CRSwNP	AR	Normal	p
Subjects (n)	68	135	89	100	
Male/female (n)	41/27	91/44	64/25	51/49	0.014*
Age (years)	39.5 ± 11.4	37.4 ± 11.9	37.1 ± 14.1	38.7 ± 10.8	0.531†
Smoking status					
– Ex	12/68 (17.6%)	26/135 (19.3%)	20/89 (22.5%)	25/100 (25.0%)	0.678*
– Current	20/68 (29.4%)	40/135 (29.6%)	22/89 (24.7%)	20/100 (20.0%)	
– Never	36/68 (52.9%)	69/135 (51.1%)	47/89 (52.8%)	55/100 (55.0%)	
Brinkman index					
– All smokers	438.2 ± 309.3	383.6 ± 305.3	335.7 ± 369.9	313.2 ± 289.3	0.328†
– Ex-smokers	380.2 ± 323.5	360.0 ± 291.1	335.5 ± 442.2	307.0 ± 301.5	0.919†
– Current smokers	473.0 ± 303.4	399.0 ± 316.9	335.9 ± 300.5	321.0 ± 280.9	0.364†

Data represent means ± standard deviation unless specified otherwise. *Chi-square test. †One-way analysis of variance. CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; AR = allergic rhinitis

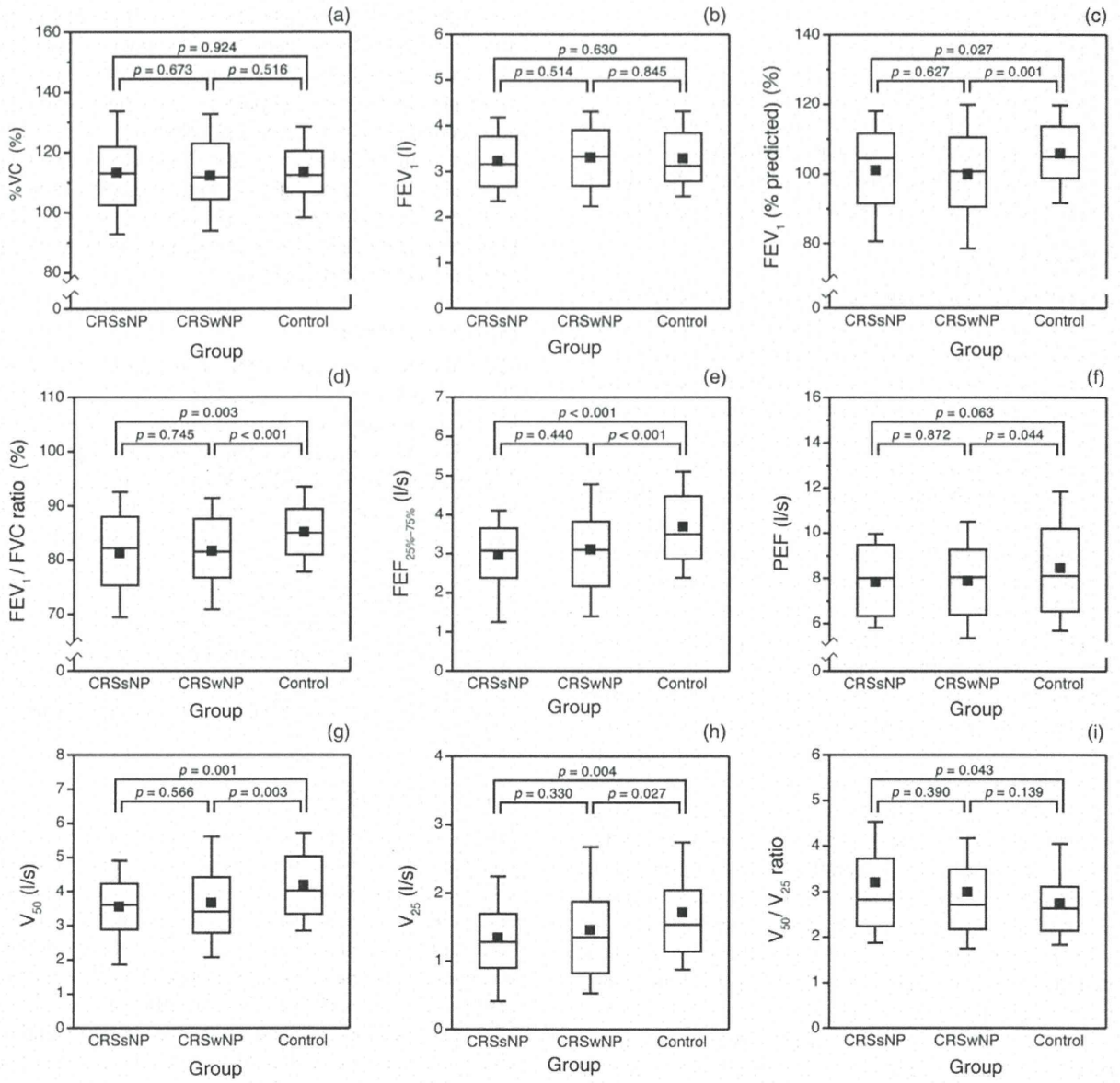

FIG. 1

Pulmonary function in patients with chronic rhinosinusitis, specifically: (a) percentage of predicted vital capacity (%VC); (b) forced expiratory volume in 1 second (FEV_1); (c) percentage of predicted forced expiratory volume in 1 second (FEV_1); (d) forced expiratory volume in 1 second / forced vital capacity (FEV_1/FVC) ratio; (e) mean forced expiratory flow between 25 and 75 per cent of the forced vital capacity ($FEF_{25\%-75\%}$); (f) peak expiratory flow (PEF); (g) maximal expiratory flow rate at 50 per cent of vital capacity (V_{50}); (h) maximal expiratory flow rate at 25 per cent of vital capacity (V_{25}); and (i) maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity (V_{50}/V_{25}) ratio. (Rectangles include range from 25th to 75th percentile, horizontal lines indicate median, vertical lines indicate range from 10th to 90th percentile and black squares represent mean value.) CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps

nasal polyps group and 343.6 ± 311.4 in the chronic rhinosinusitis with nasal polyps group; this difference was significant ($p < 0.001$). There was no significant correlation between peripheral blood eosinophil count and pulmonary function for either chronic rhinosinusitis group (Tables II and III).

Immunoglobulin E level

The mean total serum IgE level was 344.0 ± 494.7 IU/ml in the chronic rhinosinusitis without nasal polyps group and 268.6 ± 455.8 IU/ml in the chronic rhinosinusitis with nasal polyps group; this difference was not significant. There was no significant correlation between serum IgE level and pulmonary function for either chronic rhinosinusitis group (Tables II and III).

Inflammatory mediators

The mean concentrations of tumour necrosis factor-α, interleukin (IL)-1β, IL-4, IL-5, IL-6, IL-8 and interferon-γ in nasal secretions were 3.4 ± 0.6, 14.1 ± 17.7, 6.4 ± 6.3, 3.5 ± 2.2, 3.9 ± 1.9, 112.4 ± 43.3 and 2.6 ± 1.0 pg/mg total protein, respectively. Interleukin-17 was undetectable in all samples. The level of IL-5 was significantly correlated with

TABLE II
PULMONARY FUNCTION AND CLINICAL PARAMETERS CORRELATION: PATIENTS WITHOUT NASAL POLYPS

Parameter	Nasal resistance		CT score		Blood eosinophil count		Serum IgE level	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
%VC	0.276	0.339	−0.195	0.123	−0.145	0.251	−0.126	0.420
FEV_1	0.057	0.841	−0.216	0.087	−0.019	0.876	−0.004	0.978
$\%FEV_1$	0.287	0.299	−0.290	0.020	−0.228	0.065	−0.058	0.708
FEV_1:FVC	0.135	0.632	−0.121	0.342	−0.087	0.490	−0.007	0.966
PEF	−0.162	0.565	−0.169	0.180	0.097	0.441	0.237	0.122
$FEF_{25\%-75\%}$	0.089	0.754	−0.108	0.397	−0.027	0.827	−0.005	0.975
V_{50}	−0.011	0.969	−0.167	0.187	−0.108	0.387	−0.033	0.830
V_{25}	0.188	0.502	0.014	0.911	0.068	0.585	−0.048	0.756
V_{50}:V_{25}	−0.303	0.272	0.139	0.274	0.161	0.197	0.039	0.800

CT = computed tomography; IgE = immunoglobulin E; %VC = percentage of predicted vital capacity; FEV_1 = forced expiratory volume in 1 second; $\%FEV_1$ = percentage of predicted forced expiratory volume in 1 second; FEV_1:FVC = forced expiratory volume in 1 second / forced vital capacity ratio; PEF = peak expiratory flow; $FEF_{25\%-75\%}$ = mean forced expiratory flow between 25 and 75 per cent of forced vital capacity; V_{50} = maximal expiratory flow rate at 50 per cent of vital capacity; V_{25} = maximal expiratory flow rate at 25 per cent of vital capacity; V_{50}:V_{25} = maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio

pulmonary function (forced expiratory volume in 1 second / forced vital capacity ratio, $p = 0.048$; mean forced expiratory flow between 25 and 75 per cent of the forced vital capacity, $p = 0.027$; maximal expiratory flow rate at 50 per cent of vital capacity, $p = 0.043$; maximal expiratory flow rate at 25 per cent of vital capacity, $p = 0.043$; maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio, $p = 0.032$) (Table IV).

Exhaled nitric oxide

The mean level of exhaled nitric oxide was 27.8 ± 17.1 parts per billion. There were no significant correlations between levels of exhaled nitric oxide and each pulmonary function test result.

Discussion

Recent studies have shown a strong link between asthma and allergic rhinitis, and COPD may also be associated with upper airway involvement.[2,9,17–19] Patients with asthma and COPD show increased nasal symptoms and more nasal inflammation.[7]

Although numerous studies have reported an association between upper and lower airway diseases based on the concept of the 'united airway disease' hypothesis, pulmonary function in patients with upper airway diseases has not been fully examined.[20] One study reported spirometric abnormalities in patients with allergic rhinitis, but there was no normal control group in that study.[21] Furthermore, no previous study has investigated pulmonary function in chronic rhinosinusitis patients without lower respiratory tract disease. The present study showed, for the first time, that patients with chronic rhinosinusitis had latent obstruction of the small airway.

The effects of allergic rhinitis and chronic rhinosinusitis on lung function in patients with lower lung disease remain controversial. A recent report noted that

TABLE III
PULMONARY FUNCTION AND CLINICAL PARAMETERS CORRELATION: PATIENTS WITH NASAL POLYPS

Parameter	Nasal resistance		CT score		Blood eosinophil count		Serum IgE level	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
%VC	0.050	0.681	−0.036	0.689	−0.012	0.889	−0.105	0.262
FEV_1	−0.191	0.109	0.029	0.739	−0.024	0.787	0.055	0.551
$\%FEV_1$	0.039	0.749	0.021	0.813	−0.104	0.241	−0.158	0.086
FEV_1:FVC	−0.083	0.491	−0.084	0.342	−0.075	0.395	0.020	0.832
PEF	−0.090	0.457	−0.028	0.755	−0.068	0.444	0.009	0.926
$FEF_{25\%-75\%}$	−0.141	0.239	−0.034	0.699	−0.075	0.397	0.031	0.742
V_{50}	−0.111	0.357	−0.044	0.620	−0.047	0.599	0.001	0.988
V_{25}	−0.140	0.243	−0.028	0.754	−0.104	0.241	0.050	0.590
V_{50}:V_{25}	−0.018	0.879	0.018	0.842	0.207	0.018	0.017	0.857

CT = computed tomography; IgE = immunoglobulin E; %VC = percentage of predicted vital capacity; FEV_1 = forced expiratory volume in 1 second; $\%FEV_1$ = percentage of predicted forced expiratory volume in 1 second; FEV_1:FVC = forced expiratory volume in 1 second / forced vital capacity ratio; PEF = peak expiratory flow; $FEF_{25\%-75\%}$ = mean forced expiratory flow between 25 and 75 per cent of forced vital capacity; V_{50} = maximal expiratory flow rate at 50 per cent of vital capacity; V_{25} = maximal expiratory flow rate at 25 per cent of vital capacity; V_{50}:V_{25} = maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio

TABLE IV
PULMONARY FUNCTION AND NASAL SECRETION CYTOKINE LEVELS CORRELATION

Parameter	TNF-α		IL-1β		IL-4		IL-5		IL-6		IL-8		IFN-γ	
	r	*p*	*r*	*p*	*r*	*p*	*R*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
%VC	0.467	0.108	−0.111	0.718	−0.103	0.737	−0.258	0.394	0.120	0.696	0.369	0.214	−0.490	0.089
FEV_1	0.312	0.299	−0.373	0.209	−0.377	0.204	−0.531	0.062	−0.162	0.597	−0.031	0.919	−0.470	0.105
$\%FEV_1$	0.448	0.124	−0.018	0.953	−0.030	0.921	−0.037	0.904	0.004	0.988	0.236	0.437	−0.147	0.632
FEV_1:FVC	−0.177	0.562	−0.564	0.044	−0.523	0.066	−0.557	0.048	−0.434	0.138	−0.442	0.130	−0.058	0.850
PEF	0.328	0.274	−0.126	0.681	−0.187	0.541	−0.263	0.385	−0.154	0.621	−0.140	0.649	−0.129	0.676
$FEF_{25\%-75\%}$	−0.017	0.955	−0.543	0.055	−0.502	0.080	−0.609	0.027	−0.441	0.131	−0.373	0.209	−0.219	0.471
V_{50}	0.323	0.282	−0.512	0.073	−0.509	0.075	−0.568	0.043	−0.407	0.167	−0.382	0.197	−0.157	0.609
V_{25}	−0.288	0.340	−0.498	0.082	−0.438	0.133	−0.567	0.043	−0.430	0.143	−0.360	0.227	−0.219	0.472
V_{50}:V_{25}	0.647	0.017	0.580	0.037	0.496	0.084	0.594	0.032	0.269	0.375	0.268	0.377	0.347	0.246

TNF = tumour necrosis factor; IL = interleukin; IFN = interferon; %VC = percentage of predicted vital capacity; FEV_1 = forced expiratory volume in 1 second; $\%FEV_1$ = percentage of predicted forced expiratory volume in 1 second; FEV_1:FVC = forced expiratory volume in 1 second / forced vital capacity ratio; PEF = peak expiratory flow; $FEF_{25\%-75\%}$ = mean forced expiratory flow between 25 and 75 per cent of forced vital capacity; V_{50} = maximal expiratory flow rate at 50 per cent of vital capacity; V_{25} = maximal expiratory flow rate at 25 per cent of vital capacity; V_{50}:V_{25} = maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio

asthmatics without rhinitis tend to have poorer lung function than asthmatic patients with rhinitis.[6,22] In the present study, it was clear that patients with chronic rhinosinusitis had a normal percentage of predicted vital capacity. However, compared with normal control subjects, the following parameters were affected: percentage of predicted forced expiratory volume in 1 second; forced expiratory volume in 1 second / forced vital capacity ratio; peak expiratory flow; mean forced expiratory flow between 25 and 75 per cent of the forced vital capacity; maximal expiratory flow rate at 50 per cent of vital capacity; maximal expiratory flow rate at 25 per cent of vital capacity; and maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio. These findings suggest that chronic rhinosinusitis patients who are not clinically diagnosed as having lung disease do show evidence of obstructive lung function changes, even if these changes are asymptomatic. In contrast, there were no significant differences between allergic rhinitis patients and control subjects in spirometric parameters.

The present study investigated the factors that might influence obstructive lung function in chronic rhinosinusitis patients. Rhinomanometry is a sensitive and specific technique for the measurement of nasal obstruction.[23] The upper respiratory tract has important roles, including acting as a physical filter, resonator, heat exchanger and humidifier of inhaled air.[24] The conditions leading to nasal obstruction may trigger lower airway dysfunction.[7] The CT score based on the Lund–Mackay staging system is commonly used to assess the extent and severity of inflammatory changes in chronic rhinosinusitis.[1,25] Deal and Kountakis reported that the CT score was greater in chronic rhinosinusitis with nasal polyps patients than in chronic rhinosinusitis without nasal polyps patients.[26] Although the presence of polyps in the nasal area causes blocked nose, nasal resistance to airflow (measured by rhinomanometry and CT score) was not significantly correlated with lung function in the present study.

Peripheral blood eosinophil count and serum IgE level are widely used to evaluate patients with various allergic diseases, including asthma and allergic rhinitis.[27,28] In the present study, no relationship was found between pulmonary function and these inflammatory mediators.

Various explanations for the upper and lower airway association have been presented. These hypotheses include: systemic reactions; nasobronchial reflex; pharyngobronchial reflex; post-nasal drainage of inflammatory mediators from the upper to lower airways; and inhalation of dry, cold air and environmental pollutants.[24,29–31] In an animal study by Kogahara *et al.*, it was evident that a viscous post-nasal drip could flow into the lower respiratory organs when the host was asleep.[32] Cytokines and chemokines are important factors in the pathogenesis of upper respiratory

diseases, and they play a key role in asthma and COPD.[33,34] The present study showed that patients with increased nasal interleukin-5 levels had asymptomatic lung lesions. Although the number of samples was limited, the present study findings suggest that post-nasal drip containing cytokines might be associated with obstructive lung injury in patients with chronic rhinosinusitis.

- **A close relationship has been reported between upper and lower respiratory disease**
- **Spirometry indicated obstructive lung function in chronic rhinosinusitis patients without lower respiratory tract disease**
- **Cytokines in nasal secretions might be related to lung function**

Exhaled nitric oxide is a marker of airway inflammation, and the concentration of exhaled nitric oxide is elevated in patients with bronchial asthma, COPD, and chronic rhinosinusitis with nasal polyps.[35–38] In the present study, no significant correlation was found between exhaled nitric oxide level and pulmonary function test parameters.

Conclusion

Chronic rhinosinusitis patients without clinically diagnosed lung disease had latent lung obstruction. The chronic rhinosinusitis patients with decreased lung function may be in danger of developing lower respiratory disease. Our findings suggest that the patients with upper respiratory disease should be followed carefully in order to detect lung disease. Several factors in the upper respiratory tract are considered as potential explanations for the effects on lung function. Among these factors, the present findings suggest that cytokines in nasal secretions might be related to lung obstruction.

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