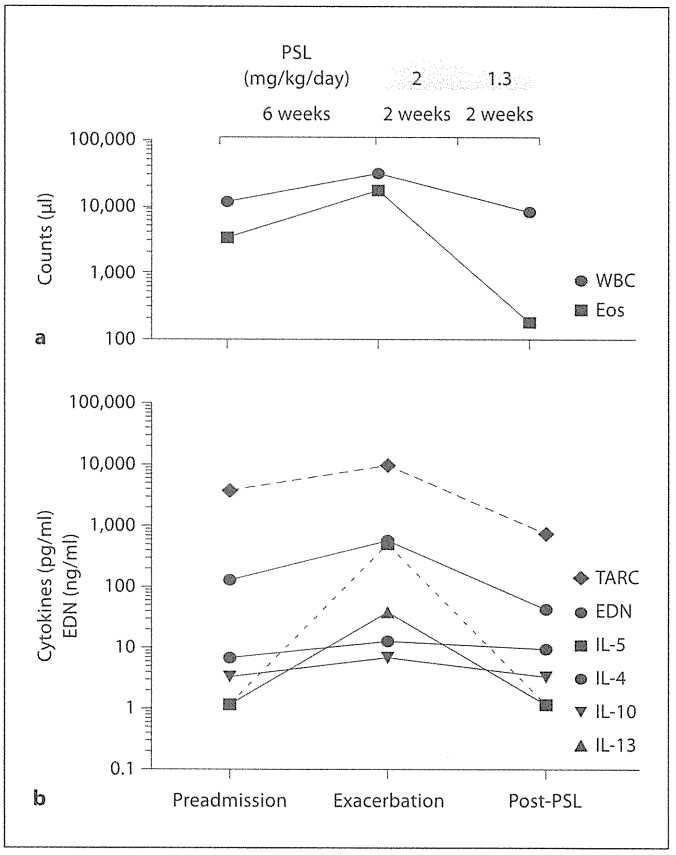

Fig. 3. Course of laboratory data. Peripheral white blood cell, eosinophil, serum cytokine and EDN levels were elevated remarkably at the time of exacerbation. PSL inhibited the white blood cell and eosinophil counts (**a**) and EDN, TARC, IL-5, and IL-13 (**b**). Eos = Eosinophils.

phil-associated pathology [1]. In addition, since the 4th revision of the WHO classification of myeloid neoplasms and acute leukemia, the diagnosis of HES requires exclusion of other acute or chronic myeloid neoplasms, no evidence of phenotypically abnormal and/or clonal T lymphocytes, and absence of both cytogenetic abnormality including rearrangements of *PDGFRA*, *PDGFRB* or *FGFR1* and >2% peripheral blasts or >5% bone marrow blasts [2, 3].

Since HES is extraordinarily rare in children, we first suspected food-induced eosinophilic gastrointestinal disorders or *H. pylori* infection [4–6] as possible causes for the gastrointestinal symptoms and profound eosinophilia. However food elimination and eradication of *H. pylori* did not have any effect. Although lung function was normal, abnormal lung CT findings (fig. 1b, c) suggested significant organ damage associated with eosinophilia. In addition, the bone marrow findings did not show any evidence of secondary eosinophilia. We then diagnosed HES.

HES is predominantly a disease of men (male:female ratio 9:1) and is usually diagnosed between the ages of 20 and 50 years [7]. Katz et al. [8] reported that pediatric HES has a slight male dominance (55.3% male vs. 44.7% female), and the mean age at diagnosis was 8.2 years. The common presenting symptoms in children with HES are fever, arthralgia, and skin rash. Diarrhea and abdominal pain, which were major presenting symptom in this case, are less common in children [8]. Various organs can be involved in HES. Hematologic, cardiovascular, dermatologic, and neurologic manifestations are common [9]. Despite the fact that the patient complained only of gastrointestinal symptoms, radiologic examination strongly suggested that the lungs and bladder were also involved, indicating that systemic screening procedures are necessary in the diagnosis and evaluation of HES.

The lymphocytic variant of HES results from overproduction of eosinophilopoietic cytokines, mainly IL-5, by clonally aberrant T cells. Detection of the aberrant T cell phenotype in peripheral blood by flow cytometry and the presence of T cell receptor clonal rearrangement are required for diagnosis [10, 11]. We could not detect aberrant phenotype T cells by flow cytometry and tried to examine for rearrangements of T cell receptors. However, we could not proceed to the diagnostic step because of poor cooperation of the patient's family. Lymphocytic variant HES are characterized by resistance to imatinib mesylate, normal serum levels of tryptase and vitamin B12, and increasing serum IgE, TARC, and IL-5; our case is consistent with this feature [12].

Regarding the treatment of HES, first-line drugs are oral corticosteroids. Ogbogu et al. [12] reported that 85% of patients experienced a complete or partial response after 1 month of corticosteroid treatment in HES. Parrillo et al. [13] demonstrated an overall corticosteroid response rate of nearly 70% in HES. However, poor therapeutic responses to PSL in children have been reported [8]. Serum TARC levels were reported to be significantly elevated in patients who responded to corticosteroids compared with nonresponders [12]. The present case showed good responses to PSL, and elevated TARC may have been a marker for the therapeutic response to steroids. Hydroxyurea, IFN-α, vincristine, anti-IL-5 antibody, and bone marrow transplantation have been reported to show some efficacy for HES, especially when PSL alone did not control eosinophilia and symptoms [12, 14, 15]. Imatinib mesylate, a tyrosine kinase inhibitor,

given to patients with eosinophilia associated with rearrangements involving PDGFRA produced a response rate of 100% [16, 17]. The overall response rate to imatinib in patients HES without abnormalities of PDGFRA was about 20–40% [12, 18]. Therefore, we administered imatinib mesylate, although the rearrangement of PDGFRA was negative in our case. However, imatinib mesylate did not decrease peripheral eosinophil levels and could not reduce the dose of PSL. Three of 5 (60%) HES patients who received cyclosporine monotherapy achieved a complete or partial response [12]. Cyclosporine could reduce the PSL dose in our patient, so cyclosporine might be effective against HES in spite of only a few reports about cyclosporine against HES.

In summary, we presented a rare case of childhood HES. Despite improvements in medical management, HES remains a serious condition with a poor prognosis for the majority of patients [14]. Moreover, the long-term prognosis in pediatric HES is not well known. Comprehensive diagnostic procedures are vital for the early detection and management of complications in pediatric HES.

Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the contents of this article.

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Differential Effects of Corticosteroids on Serum Eosinophil Cationic Protein and Cytokine Production in Rhinovirus- and Respiratory Syncytial Virus-Induced Acute Exacerbation of Childhood Asthma

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Key Words

Eosinophil cationic protein · IL-5 · Asthma, childhood · Rhinovirus · Respiratory syncytial virus · Corticosteroids

Abstract

Background: Little information is available on eosinophil activation and the cytokine profile in virus-induced acute exacerbation of bronchial asthma; therefore, we examined the effects of treatments that included systemic corticosteroids on serum eosinophil cationic protein (ECP) and 17 cytokines/chemokines in rhinovirus- and respiratory syncytial (RS) virus-induced acute exacerbation of childhood asthma. ***Methods:*** We measured the peripheral eosinophil count, as well as the serum levels of ECP and 17 types of cytokines/chemokines (IL-1β, 2, 4, 5, 6, 7, 8, 10, 12, 13, and 17 and IFN-γ, TNF-α, GM-CSF, G-CSF, MCP-1, and MIP-1β), using a multiplex bead-based assay in 21 cases of rhinovirus- and 12 cases of RS virus-induced acute exacerbation of childhood asthma and 13 controls. We also compared the clinical data and the effects of systemic corticosteroids on these responses between rhinovirus and RS virus groups. ***Results:*** The serum levels of ECP, IL-5, and IL-6 were significantly elevated in patients with rhinovirus-induced acute exacerbation of asthma compared with controls, while serum IL-1β and IFN-γ were significantly lower in patients with rhinovirus-induced acute exacerbation of asthma than in controls. On the other hand, in RS virus-induced acute exacerbation of asthma, only the peripheral eosinophil count was significantly decreased compared with that in rhinovirus-induced acute exacerbation of asthma and controls. Furthermore, the serum levels of ECP, IL-5, and IL-6 in rhinovirus-induced acute exacerbation of asthma and levels of G-CSF in RS virus-induced acute exacerbation of asthma were significantly reduced after treatments that included systemic corticosteroids, respectively. ***Conclusion:*** These results suggest that the effects of systemic corticosteroids on serum ECP and the cytokine profile are different between rhinovirus- and RS virus-induced acute exacerbation of childhood asthma.

Introduction

Viral infection induces both the development and exacerbations of bronchial asthma [1]. In infants, respiratory syncytial (RS) virus is a leading cause of serious lower respiratory tract infections, including acute bronchi-

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olitis. RS virus infection also exacerbates recurrent wheezing attacks in patients with established asthma [2]. A number of case-control studies appear to have established at least a statistical connection between RS virus infection in infancy and the development of recurrent wheezing and asthma in young children [3–8]. In later life, it appears unlikely to be a cause of atopic asthma [3, 5, 8]. On the other hand, rhinovirus infection is a major cause of acute exacerbation of asthma in both adults [9] and children [10]. A recent report showed that the most significant risk factor for the development of preschool childhood wheezing is the occurrence of symptomatic rhinovirus illnesses during infancy [11]. The COAST (Childhood Origins of Asthma) study group also reported that wheezing attacks during childhood (2–16 years of age) can be linked to infection with rhinovirus together with evidence of atopy or eosinophilic airway inflammation [12, 13].

The purpose of this study was to investigate changes in the clinical data and serum levels of eosinophil cationic protein (ECP) and several cytokines and chemokines in rhinovirus- and RS virus-induced acute exacerbation of childhood asthma and to evaluate the effects of corticosteroids treatment on these parameters in 2 groups.

Patients and Methods

Patients

The 62 subjects who were hospitalized with acute respiratory symptoms (acute exacerbation of asthma: 43 males and 19 females; mean/median age 3.5/2.8 years) at Gunma Children's Medical Center between November 1, 2003, and October 31, 2006, were enrolled into this study. All recruited patients had a history of 3 or more separate episodes of recurrent wheezing and documented evidence of wheezing by auscultation. The diagnosis of asthma and its severity in subjects with acute exacerbation of asthma were defined according to the guidelines of the Japanese Society of Pediatric Allergy and Clinical Immunology [14]. The diagnosis of asthma was defined as patients with 3 or more independent episodes of wheezing. The symptom severity score was defined as follows: mild attack = 1, moderate attack = 2, and severe attack = 3. Acute exacerbation of asthma was diagnosed by the emergency department physician based on the presence of wheezing and increased difficulty breathing. Briefly, a mild attack was defined as mild wheezing with stability and no dyspnea and an S_pO_2 of ≥96%; a moderate attack was defined as wheezing with dyspnea, apparent retraction, and an S_pO_2 of 92–95%, and a severe attack was defined as more severe wheezing and dyspnea and an S_pO_2 of ≤91%. Patients with mild and moderate attacks were treated with intravenous infusion, salbutamol and disodium cromoglycate inhalation (3 times/day), and/or intravenous theophylline and prednisolone. Patients who experienced a severe attack were treated with isoproterenol inhalation instead of salbutamol. When the S_pO_2 was ≤95%, oxygen therapy was started. Patients with acute exacerbation of asthma had a history of a cold prior to exacerbation. We excluded children with obvious bacterial infections, congenital heart diseases, and chronic lung diseases, as well as those who showed the presence of a foreign body or had signs of severe infection and those who were immunosuppressed, as these complications could interfere with the assessment of asthma-related outcome measures. The control group included 13 healthy children (8 males and 5 females; mean/median age 3.7/4.2 years) with no symptoms of wheezing at the time of examination. Exclusion criteria for the controls included immunosuppression, the presence of other respiratory tract symptoms, or a history of wheezing and asthma. Goals for matching among asthma and control patients included age and sex. This study was approved by the Ethics Committee of Gunma Children's Medical Center. Informed consent was obtained from parents, and assent was obtained from children when they were old enough (usually over 9 years old).

Virus Detection

Nasal aspirates were obtained from patients during acute exacerbation of asthma. Nasal secretions were aspirated into a mucus trap (attached to wall suction) by inserting the tip of a flexible 5F Argyle suction catheter (Nippon Sherwood, Tokyo, Japan) into the anterior nares. Nasal samples were analyzed for RS virus using antigen detection kits (Becton Dickinson, Franklin Lakes, N.J., USA). The remaining secretions were frozen at −80°C until examination by further reverse transcription polymerase chain reaction (RT-PCR) and then by direct DNA sequencing analysis. The RT-PCR method used for rhinoviruses and RS virus was as previously described [15]. The DNA fragments were purified using a QIAquick PCR Purification kit (QIAGEN), and the nucleotide sequence was determined with an automated DNA sequencer ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems, Foster City, Calif., USA) using a Big Dye Terminator v1.1 cycle sequencing kit (Applied Biosystems) [15]. For identification of the virus, newly determined sequences were compared with those available in the sequences using GenBank DNA databases (http://www.ncbi.nlm.nih.gov) and the standard nucleotide-nucleotide BLAST algorithm. The identities of the sequences were determined on the basis of the highest percentage of total nucleotide match in GenBank.

Serum Eosinophil Cationic Protein and Cytokines/Chemokines

We measured the peripheral eosinophil count and the serum levels of ECP and 17 types of cytokines/chemokines [interleukin (IL)-1β, 2, 4, 5, 6, 7, 8, 10, 12, 13, and 17 and interferon (IFN)-γ, tumor necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), monocyte chemoattractant protein (MCP)-1, and macrophage inflammatory protein (MIP)-1β] from 33 acute asthma patients who had not used systemic corticosteroids at the time of examination and from 13 control subjects. We analyzed the effects of the treatments including systemic corticosteroids on these parameters by measuring them both at the time of admission and at the time when wheezing disappeared (mean days after admission 6.8) and then compared each factor before and after treatments in patients with acute exacerbation of asthma. The ECP

Table 1. Characteristics of subjects

	n	Mean/median age (range), years	Male gender %	>1 positive aeroallergen CAP-RAST (positive), %
Rhinovirus-induced acute asthma	21	2.5/2.6 (0.3–5.1)	76.2	68.4
RS virus-induced acute asthma	12	3.4/3.0 (0.8–8.1)	66.7	72.7
Control	13	3.7/4.2 (1.3–6.4)	61.5	0.0

CAP-RAST = Capsulated hydrophilic carrier polymer-radioallergosorbent test.

Table 2. Comparison of clinical data between rhinovirus- and RS virus-induced acute asthma

	Severity score	Admission period days	Wheeze period days	Systemic corticosteroid use, days
Rhinovirus-induced acute asthma	2.0 ± 0.4*	9.0 ± 4.5	6.5 ± 4.1	4.7 ± 2.4
RS virus-induced acute asthma	1.8 ± 0.5	7.7 ± 2.3	4.8 ± 1.8	4.3 ± 2.9

* Mean ± SD.

content in serum was measured with a fluoroenzyme immunoassay kit (Pharmacia, Uppsala, Sweden). Serum cytokines/chemokines were determined using a multi-cytokine detection system (Bio-Rad, Hercules, Calif., USA) following the manufacturer's instructions, measured using a Luminex System (Austin, Tex., USA), and then quantified using Bio-Plex software (Bio-Rad) [15].

Statistical Analysis

Patient characteristic data were evaluated using Pearson's χ^2 test and Fisher's exact test for categorical variables. Multivariate analyses were conducted using multivariate linear regression or multivariate logistic regression analysis, allowing simultaneous assessment of each factor from patients in the acute wheezing group or controls. Age, sex, and atopic status were potential confounders and adjustments were made for the multivariate analyses. Paired data or unpaired data were analyzed using the Wilcoxon or Mann-Whitney U test, respectively. Correlation coefficients for the parameters were calculated using Spearman's rank correlation coefficient analysis. $p < 0.05$ (2-sided) was considered statistically significant. All analyses were performed using a statistical software package (SPSS for Windows, version 18.0; SPSS Japan, Inc., Tokyo, Japan).

Results

Patient Characteristics and Clinical Data

We detected 21 rhinoviruses, 12 RS viruses, 8 enteroviruses including 7 enterovirus type 68 and 1 coxsackievirus, and 7 other viruses including 3 rhinovirus plus RS virus, 3 RS virus plus coxsackievirus, and RS virus plus parainfluenza-3 virus; no viruses were detected in 10 samples, and 4 were not examined in the investigation in a total of 62 patients with acute exacerbations of wheezing. We next compared several clinical data such as the symptom severity score, admission and wheeze period, and systemic corticosteroid use in the 2 major virus groups, i.e. rhinovirus and RS virus. Patient characteristics are shown in table 1. No significant differences for age, sex, or atopic status between each group were found. Twenty-eight patients with acute exacerbation of asthma were treated with intravenous prednisolone for 1 mg/kg/day (total mean dose; 5.3 ± 1.8 mg/kg, mean ± SD, total median dose; 5.5 mg/kg, range 2–9 mg/kg). The admission or wheeze period in patients with rhinovirus-induced acute exacerbation of asthma seemed to be longer than in patients with RS-induced acute exacerbation of asthma although there was no significant difference between the 2 groups (table 2).

Serum Eosinophil Cationic Protein and Cytokines/Chemokines

The serum levels of ECP, IL-5, and IL-6 were significantly elevated in patients with rhinovirus-induced acute exacerbation of asthma compared with controls (fig. 1). In contrast, serum IL-1β and IFN-γ were significantly decreased in patients with rhinovirus-induced acute ex-

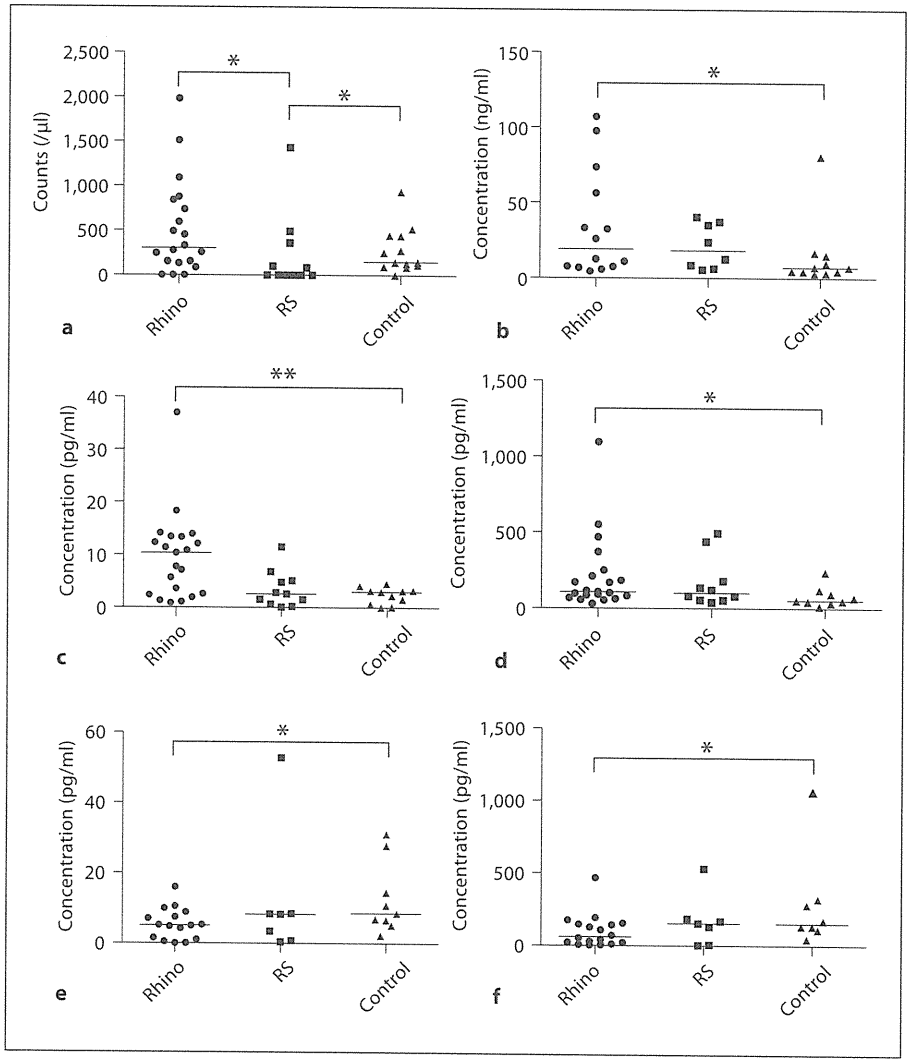

Fig. 1. Comparison of peripheral blood eosinophil counts, serum levels of ECP, and cytokines between rhinovirus- or RS virus-induced acute exacerbation of asthma and controls. **a** Eosinophils. **b** ECP. **c** IL-5. **d** IL-6. **e** IL-1β. **f** IFN-γ. Values are shown for each subject. The median is represented by the horizontal bars. Data were analyzed using the Mann-Whitney U test. * $p < 0.05$; ** $p < 0.01$.

acerbation of asthma compared with controls. On the other hand, in patients with RS virus-induced acute exacerbation of asthma, only the peripheral eosinophil count was significantly decreased compared with that of patients with rhinovirus-induced acute exacerbation of asthma and controls. Other cytokines/chemokines including IL-2, 4, 7, 8, 10, 12, 13, and 17 and TNF-α, GM-CSF, MCP-1, and MIP-1β were not significantly changed in patients with rhinovirus- or RS virus-induced acute exacerbation of asthma or in controls.

Next, we investigated the effects of systemic corticosteroids on these parameters. In virus-induced acute exacerbation of asthma, serum IL-5, IL-6, and G-CSF were significantly reduced after the treatment that included systemic corticosteroids (n = 28) while serum IL-8 was significantly decreased after the treatment that did not included systemic corticosteroids (n = 5) (data not shown). We further examined the effects of systemic corticosteroids on each parameter in the group with rhinovirus- or RS virus-induced acute exacerbation of asthma. The treatments that included systemic corticosteroids significantly reduced the elevated serum ECP, IL-5, and IL-6 levels in rhinovirus-induced acute exacerbation of asthma (fig. 2). Conversely, lowered serum levels of IL-1β and IFN-γ were recovered after the treatments that included systemic corticosteroids (fig. 2). Furthermore, in RS virus-induced acute exacerbation of asthma, the same treatment significantly decreased serum G-CSF although this was not significantly elevated compared with that in controls (fig. 2).

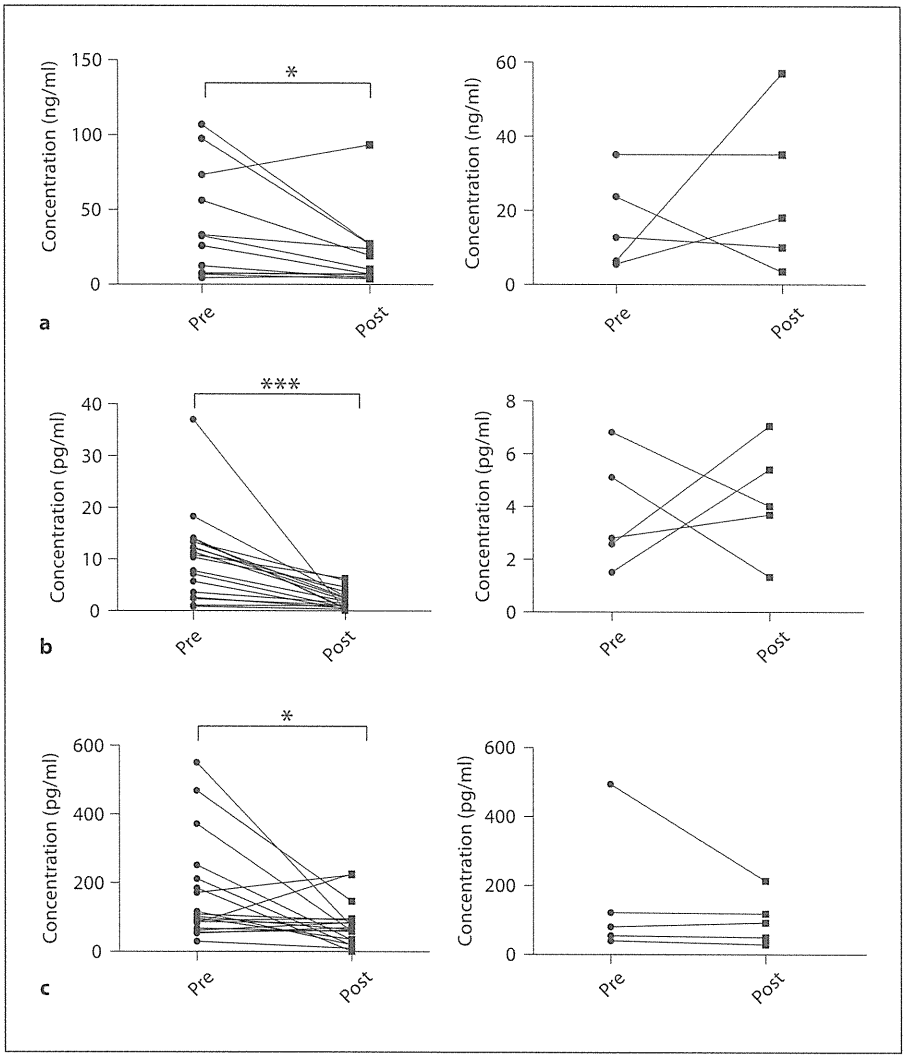

Fig. 2. Effects of treatments that included systemic corticosteroids on serum levels of ECP and cytokines in rhinovirus- (left side in figure) or RS virus- (right side in figure) induced acute exacerbation of asthma. **a** ECP. **b** IL-5. **c** IL-6. Values are shown for each subject. Data were analyzed using the Wilcoxon test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, pretreatment vs. posttreatment.

Discussion

In this study, we found that the serum levels of ECP, IL-5, and IL-6 in rhinovirus-induced acute exacerbation of childhood asthma were significantly reduced after treatments that included systemic corticosteroids, while those of G-CSF in RS virus-induced acute exacerbation of asthma were significantly reduced after the same treatments. These results suggest that the pathogeneses of rhinovirus- and RS virus-induced acute exacerbation of childhood asthma might be different and that eosinophil activation is involved in rhinovirus-induced acute exacerbation of childhood asthma.

However, at the present time, we do not know the exact mechanism by which rhinoviruses might induce acute exacerbation of asthma and enhance eosinophil activation. A recent report found that, with the sensitive indirect in situ RT-PCR method, rhinoviruses were detected in the mucosal biopsies of 73% of patients with asthma and 22% of nonasthmatic control subjects. Subjects positive for rhinovirus had lower pulmonary function, higher numbers of blood eosinophils and leukocytes, and eosinophilic infiltration in bronchial mucosa [16]. Further evidence suggests a role of deficient IFN-γ [17], IFN-β [18], and type III IFN-λ [19] production in rhinovirus-induced asthma exacerbation and indicates novel mechanisms for the increased susceptibility of subjects with asthma to rhinovirus infection [19, 20]. Our results of lower serum IFN-γ production in rhinovirus-induced acute exacerbation of asthma also confirm these observations. In our study, serum IL-1β was also decreased in patients with rhinovirus-induced acute exacerbation of

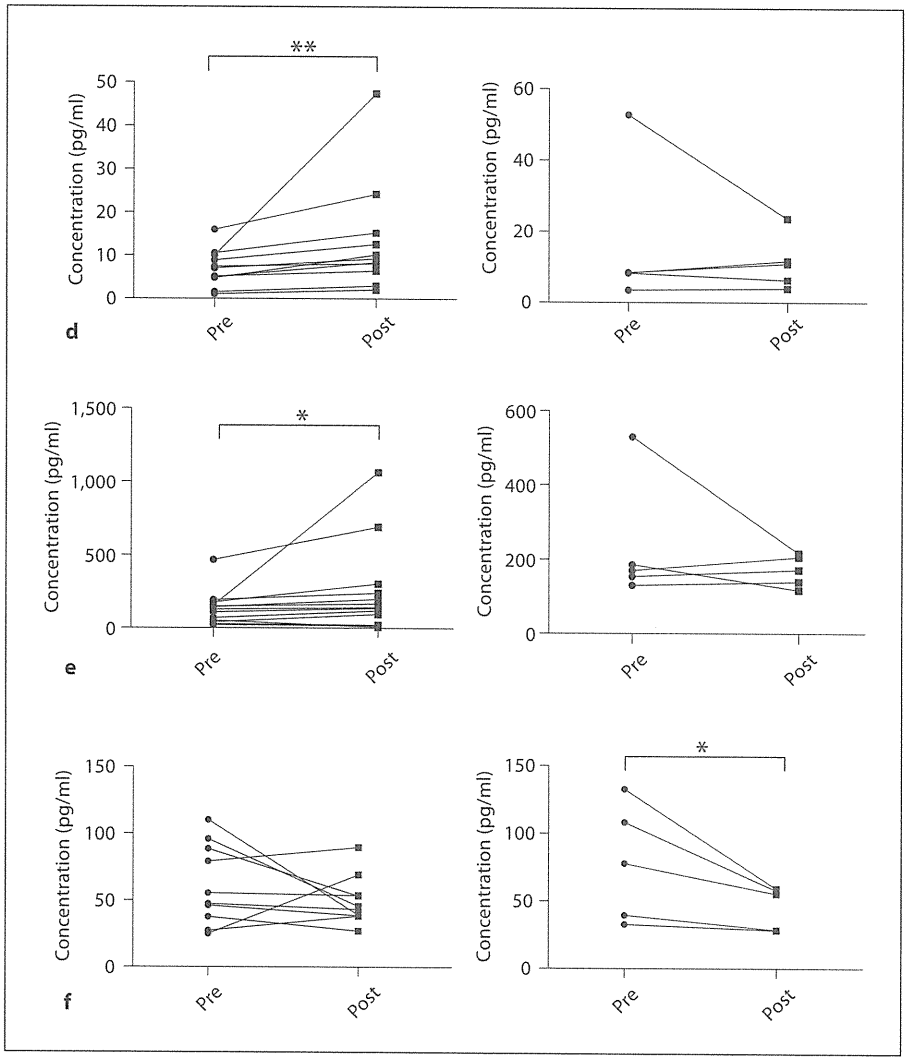

Fig. 2. Effects of treatments that included systemic corticosteroids on serum levels of ECP and cytokines in rhinovirus- (left side in figure) or RS virus- (right side in figure) induced acute exacerbation of asthma. **d** IL-1β. **e** IFN-γ. **f** G-CSF. Values are shown for each subject. Data were analyzed using the Wilcoxon test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, pretreatment vs. posttreatment.

asthma compared with controls. IL-1β is known to be released from human alveolar macrophages [21], monocytes [22], and tracheal epithelial cells [23]. Although we did not find a report of serum IL-1β in patients with rhinovirus-induced acute exacerbation of asthma, an in vitro study showed that rhinovirus infection induced IL-1β production in supernatants of human tracheal epithelial cells [23]. On the other hand, another report found that IL-1β-derived human monocytes were not detected in experimental rhinovirus infection [22]. We do not know the exact mechanism by which serum IL-1β is decreased in rhinovirus-induced acute exacerbation of asthma. However, various factors including multiple cytokine networks and different sources of IL-β may affect our results for the serum samples in contrast to the in vitro studies.

There are a number of papers relating to experimental rhinovirus infection and the induction of a variety of cellular responses. Human rhinoviruses induce an increase in airway reactivity and epithelial [24] or sputum [25] eosinophils in asthmatic patients. Rhinovirus infection upregulates the expression of intracellular adhesion molecule-1 (ICAM-1) mRNA, the major rhinovirus receptor, and the increased production of IL-1α, IL-1β, IL-6, IL-8, TNF-α, and GM-CSF in supernatants of human tracheal epithelial cells [21]. Similarly, rhinovirus infection upregulates GM-CSF [26], eotaxin, and eotaxin-2 expression in bronchial epithelial BEAS-2B cells [27]. In human airway submucosal glands, eosinophil chemotaxis was augmented during rhinovirus infection [28]. A very recent paper found that rhinovirus infection enhances airway epithelial remodeling through VEGF production [29]. Collec-

tively, evidence suggests that rhinoviruses could induce eosinophil activation, particularly through eosinophil-active cytokines/chemokines such as IL-5, GM-CSF, and eotaxin, as well as an increase in the number of eosinophils.

At present, there is no specific treatment for virus-induced acute exacerbation of asthma. Corticosteroid therapy is one of the most effective treatments for asthma. However, treatment with inhaled corticosteroids does not improve airway inflammation induced by rhinovirus infection [30]. On the other hand, oral glucocorticoids improve lung function and decrease the elevation of serum IL-6, soluble ICAM-1, and ECP [31]. Recently, we reported that treatment that included systemic corticosteroids could decrease serum levels of ECP, IL-5, IL-6, IL-1ra, and IP-10 in acute exacerbation of childhood asthma [32]. In this study, we further found that treatment with systemic corticosteroids decreased serum ECP and IL-5 in rhinovirus-induced acute exacerbation of asthma but not in RS virus-induced acute exacerbation of asthma. In experimental data, Wark et al. [33] reported that rhinovirus infection induced IP-10 and RANTES in the greatest quantities, followed by IL-6 and IL-8, and that dexamethasone partially suppressed IP-10 and TNF-α but was more effective at suppressing RANTES, IL-6, and IL-8 production. Another paper showed that dexamethasone inhibits rhinovirus infection by reducing the surface expression of ICAM-1 and the production of IL-1β, IL-6, and IL-8 in cultured human tracheal epithelial cells [21]. In addition to these reports, our results indicate that systemic corticosteroids might decrease eosinophil activation through IL-5 in rhinovirus-induced acute exacerbation of asthma. Finally, we do not know the mechanism by which serum IL-1β and IFN-γ are increased after treatment that includes systemic corticosteroids in rhinovirus-induced acute exacerbation of asthma. In addition to the in vivo study, the time lag (mean 36 h) between the final day of steroid treatment (mean 5.3 days after admission) and the time samples are taken for post-treatment (mean 6.8 days after admission) may affect the recovery of their values.

Further work is needed to better explore the mechanisms behind the association between asthma exacerbations and rhinovirus or RS virus infections. These studies might ultimately lead to specific treatment to prevent and/or treat the significant burden of acute exacerbation of asthma caused by different virus infections.

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Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the contents of this article.

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Review

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FIP1L1/PDGFRα-Associated Systemic Mastocytosis

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Chronic eosinophilic leukemia · Eosinophils · FIP1L1/PDGFRα · Hypereosinophilic syndromes · Interleukin-5 · Mast cells · Stem cell factor · Systemic mastocytosis

Abstract
Since the identification of the *FIP1L1/PDGFRA* fusion gene as a pathogenic cause of the hypereosinophilic syndrome (HES), the importance of the molecular classification of HES leading to the diagnosis of chronic eosinophilic leukemia (CEL) has been recognized. As a result, a new category, 'myeloid and lymphoid neoplasm with eosinophilia and abnormalities in PDGFRA, PDGFRB or FGFR1', has recently been added to the new WHO criteria for myeloid neoplasms. FIP1L1/PDGFRα-positive disorders are characterized by clonal hypereosinophilia, multiple organ dysfunctions due to eosinophil infiltration, systemic mastocytosis (SM) and a dramatic response to treatment with imatinib mesylate. A murine HES/CEL model by the introduction of FIP1L1/PDGFRα and IL-5 overexpression also shows SM, representing patients with FIP1L1/PDGFRα-positive HES/CEL/SM. The murine model and the in vitro development system of FIP1L1/PDGFRα-positive mast cells revealed the interaction between FIP1L1/PDGFRα, IL-5 and stem cell factor in the development of HES/CEL/SM. Current findings of FIP1L1/PDGFRα-positive HES/CEL are reviewed focusing on aberrant mast cell development leading to SM.

Introduction

The importance of the molecular classification of the hypereosinophilic syndrome (HES) has been increasingly recognized. The fusion gene *FIP1L1/PDGFRA* was identified in a large number of patients initially diagnosed as having a myeloproliferative variant of HES or chronic eosinophilic leukemia (CEL) [1]. Subsequently, other variant PDGFRA fusion genes as well as those involving PDGFRB or FGFR1 have also been described in myeloproliferative neoplasms with eosinophilia in the last years [2–4]. As a result, a new category of myeloid neoplasms, 'myeloid and lymphoid neoplasm with eosinophilia and abnormalities of PDGFRA, PDGFRB or FGFR1', has recently been added to the new WHO criteria [5]. FIP1L1/PDGFRα fusion-positive disorders are characterized by clonal myeloproliferation resulting in hypereosinophilia, multiple organ dysfunctions due to eosinophil infiltration, a dramatic response to treatment with

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imatinib mesylate and systemic mastocytosis (SM) [6]. Murine models of FIP1L1/PDGFRα-induced diseases have been reported recently [7, 8]. Interestingly, these models demonstrated severe SM representing patients with FIP1L1/PDGFRα fusion-positive diseases [9]. In this review, the clinical manifestation of FIP1L1/PDGFRα fusion-associated disorders are summarized, focusing on mastocytosis induced by FIP1L1/PDGFRα expression, and the mechanisms of mastocytosis in FIP1L1/PDGFRα-positive HES/CEL are discussed.

Eosinophilia in SM Patients

Peripheral blood eosinophilia has been reported in 15–28% of SM patients [10–12]. This is no big surprise since crosstalk between eosinophils and mast cells is well known, especially in allergic inflammation. For instance, mast cell activation by major basic protein, an eosinophil granule protein, elicits the generation of lipid mediators and cytokines. Eosinophils also produce cytokines associated with mast cell activation such as stem cell factor (SCF), granulocyte/macrophage colony-stimulating factor and nerve growth factor [13]. The D816V mutation in the *KIT* gene resulting in constitutive activation of the receptor tyrosine kinase has been shown in the majority of patients as a cause of SM [14]. D816V-kit mutation-positive SM with eosinophilia has been clinically distinguished from that without eosinophilia. D816V-kit mutation-positive SM patients with eosinophilia present hepatosplenomegaly, lymphadenopathy, anemia and monocytosis more frequently as well as higher levels of circulating tryptase, whereas anaphylaxis is seen with a low frequency in these patients, in comparison to patients without eosinophilia [10].

Clinical Manifestations of Mastocytosis Associated with FIP1L1/PDGFRα

A recent report has shown that FIP1L1/PDGFRα-associated SM is a clinically distinguishable disease from D816V mutation SM with eosinophilia [10]. FIP1L1/PDGFRα-associated SM shows lower tryptase levels in the circulation, less aggregation of bone marrow mast cells, more severe eosinophilia, higher serum vitamin B12 levels and more frequent pulmonary and cardiac involvement than D816V SM with associated eosinophilia. The clinical difference is important since it would justify the differential diagnosis between these two entities, based on the analysis of the expression of the FIP1L1/*PDGFRA* fusion gene. More recently, Klion [15] proposed a scoring system to approach FIP1L1/PDGFRα fusion-positive HES/CEL therapy appropriately.

SM in Murine Models of FIP1L1/PDGFRα-Positive Disorders

First, Cools et al. [7] reported that the introduction of the *FIP1L1/PDGFRA* fusion gene into bone marrow hematopoietic stem cells and progenitors (HSC/P) induces a murine model of a myeloproliferative disorder (MPD) similar to that found in p210-BCR/ABL-induced chronic myelogenous leukemia-like disease (F/P-MPD). Subsequently, an HES/CEL murine model was developed by the introduction of the *FIP1L1/PDGFRA* fusion gene into bone marrow HSC/Ps in the presence of T-cell overexpression of IL-5 (F/P-HES/CEL) [8]. More recently, these two murine models were also shown to develop tissue mast cell infiltration and increased circulating mast cell protease 1 (MMCP-1) levels, which is a systemic assay of mast cell content and degranulation in the mouse resembling serum tryptase determination in SM patients [9]. Similar to the patients with *FIP1L1/PDGFRA* fusion gene, tissue mast cell infiltration of hematopoietic organs, skin and intestine, where mast cell morphology is aberrant, is present in F/P-HES/CEL mice. Tissue mast cell shape is irregular with frequent cytoplasmic extensions reminiscent of the 'spindle shape' found in clinical SM. In addition, serum levels of MMCP-1 are extremely elevated in F/P-HES/CEL mice.

A possible interaction of IL-5 with the SM phenotype was analyzed in F/P-HES/CEL mice. F/P-HES/CEL and F/P-MPD mice showed significantly greater mast cell infiltration in their skin and intestine, and higher levels of MMCP-1 compared to both controls with and without IL-5 overexpression. Interestingly, intestinal mast cell infiltration and serum MMCP-1 levels in F/P-HES/CEL mice were significantly higher compared to F/P-MPD, suggesting that FIP1L1/PDGFRα in conjunction with IL-5 exacerbates mastocytosis in murine F/P-HES/CEL [9].

Mechanism of FIP1L1/PDGFRα-Promoted Mast Cell Development

Since the c-kit signaling pathway is pivotal for normal mast cell development and function, the question of whether FIP1L1/PDGFRα-associated SM is still c-kit de-

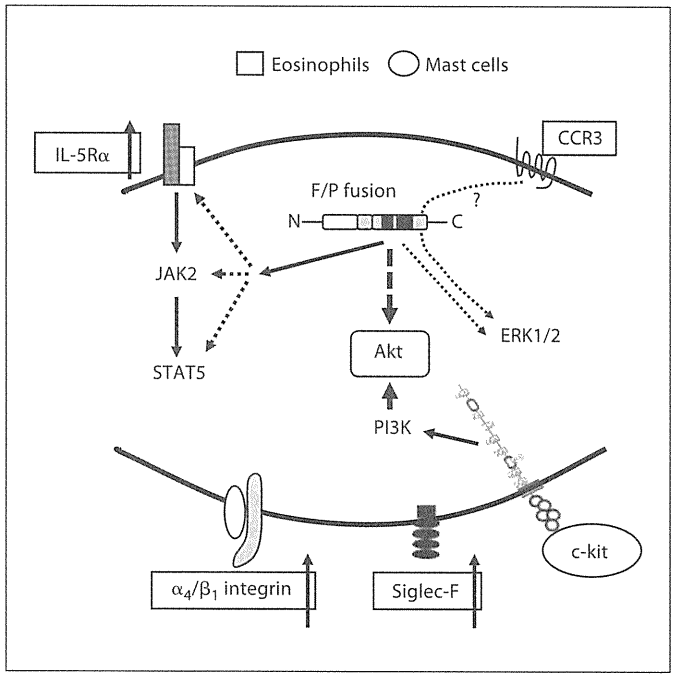

Fig. 1. Intracellular signaling of FIP1L1/PDGFRα+ (F/P) cells. FIP1L1/PDGFRα+ (F/P) primary mouse eosinophils express up-regulated IL-5Rα and FIP1L1/PDGFRα activates the JAK2/STAT5 pathway. The CCR3/ERK1/2 signaling pathway may be amplified by FIP1L1/PDGFRα expression [25]. Up-regulated expressions of α_4 integrin and Siglec-F were observed in FIP1L1/PDGFRα+ murine eosinophils [8]. FIP1L1/PDGFRα synergizes with SCF stimulation via c-kit to activate Akt signaling in mouse mast cells. Eosinophils and mast cells also express c-kit and IL-5Rα, respectively.

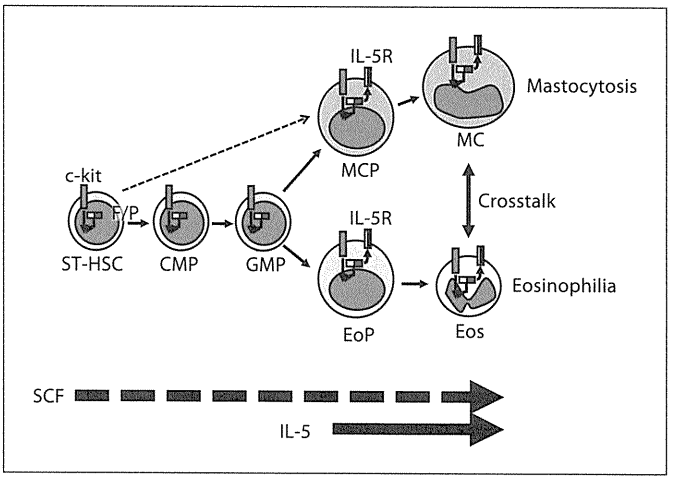

Fig. 2. FIP1L1/PDGFRα in conjunction with SCF and IL-5 promote leukemic hematopoiesis and eosinophil and mast cell (MC) development. FIP1L1/PDGFRα may occur in hematopoietic stem cells or early progenitor cells resulting in the expression of FIP1L1/PDGFRα in most hematopoietic cells. Progenitors including earlier and mature eosinophils and MCs express c-kit. In contrast, IL-5Rα expression has been observed on eosinophil progenitor (EoP), MC progenitor (MCP) and mature eosinophils and MCs. FIP1L1/PDGFRα enhances SCF/c-kit signaling by sharing downstream signaling and up-regulates IL-5Rα expression facilitating its intracellular signaling. There is significant crosstalk between eosinophils and MCs. These findings imply that FIP1L1/PDGFRα in collaboration with SCF may affect leukemic myeloproliferation, and synergistically with IL-5 expand and activate MC and eosinophil lineages. ST-HSC = Short-term HSC; CMP = common myeloid progenitor; GMP = granulocyte-macrophage progenitor.

pendent arises. In fact, following blockade of the the c-kit signaling pathway using anti-c-kit antibody, tissue mast cells and circulating levels of MMCP-1 were significantly decreased, suggesting that tissue infiltration of FIP1L1/PDGFRα fusion-positive mast cells are associated with SCF/c-kit signaling. The ex vivo cytokine-dependent mast cell differentiation of FIP1L1/PDGFRα-expressing HSC/Ps was largely dependent on the use of SCF in the culture conditions. Moreover, FIP1L1/PDGFRα fusion-positive mast cells showed prolonged survival and enhanced migration toward SCF. Specific synergistic stimulation of the Akt signaling pathway by FIP1L1/PDGFRα and SCF indicated collaboration of two tyrosine kinase activities in their downstream signaling pathways. Taken together, FIP1L1/PDGFRα synergizes with the SCF/c-kit pathway to promote mast cell development, activation and survival both in vivo and in vitro [9].

Pathogenesis of FIP1L1/PDGFRα-Positive HES/CEL/SM Associated with SCF and IL-5

Previously, we reported that the induction of murine HES/CEL by FIP1L1/PDGFRα requires a second event that is associated with IL-5 overexpression [8]. In addition, the level of expression of IL-5Rα was exclusively up-regulated in FIP1L1/PDGFRα-positive splenocytes and FIP1L1/PDGFRα fusion protein shares the downstream JAK2/STAT5 pathway with IL-5 signaling (fig. 1) [16]. Interestingly, polymorphisms of the human *IL-5RA* gene have been found linked to the constitutional *IL-5RA* genotype and the severity of FIP1L1/PDGFRα-positive CEL [17]. These findings suggest that amplification of IL-5 signaling by FIP1L1/PDGFRα triggers a CEL-like disease. Interestingly, IL-5Rα is expressed on eosinophil and mast cell progenitors [18, 19] as well as mature eosinophils [20]

and mast cells [21], whereas c-kit expression is not only found on progenitors but also on mature eosinophils and mast cells [14, 18, 19]. Importantly, expression of the *FIP1L1/PDGFRA* fusion gene or deletion of the surrogate marker CHIC2 have been detected in non-eosinophilic cells, including neutrophils, monocytes, mast cells, lymphoid lineage cells and bone marrow CD34-positive cells in part of the patients, suggesting that the fusion of the *FIP1L1/PDGFRA* genes may occur in HSCs or early progenitors [22–24]. Taken together, these findings imply that FIP1L1/PDGFRα in collaboration with SCF may affect leukemic myeloproliferation and synergistically with IL-5 expand and activate mast cell and eosinophil lineages (fig. 2).

Conclusion

HES/CEL has attracted a lot of attention since the patients were successfully treated with imatinib mesylate, and subsequently the target, FIP1L1/PDGFRα, was discovered in a large number of patients initially diagnosed as myeloproliferative variant of HES. To our knowledge, there is little doubt that FIP1L1/PDGFRα preferentially affects eosinophil and mast cell proliferation, survival, differentiation and tissue infiltration, and leukemogenesis is induced combined with systemic or local extrinsic factors, as demonstrated by crucial roles of IL-5 and SCF in the pathogenesis of FIP1L1/PDGFRα-initiated HES/CEL/SM. This disease, an example of the crosstalk between oncogenesis and inflammation, represents an excellent model to study cellular integration of biochemical signals in cancer, being responsible for crucial aspects of cancer biology, e.g. cell proliferation, survival, tissue invasion and communication with the specific tissue microenvironment.

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TABLE I. Sensitivity and specificity* for Ara h 2 and whole peanut extract

Test	Cutoff point (kU_A/L)	Sensitivity (%)	Specificity (%)	Correctly classified (%)
Ara h 2	0.30	100.00	90.20	93.75
	0.32	100.00	94.12	95.00
	0.35	100.00	96.08	97.50
	0.38	96.55	96.08	96.25
	0.40	93.10	98.04	96.25
	0.55	93.10	100.00	97.50
	0.87	89.66	100.00	96.25
Whole extract	0.35	96.55	26.92	51.85
	3.91	79.31	84.62	82.72
	5.00	75.86	90.38	85.19
	5.30	75.86	94.23	87.65
	5.96	72.41	94.23	86.42
	7.81	72.41	96.15	87.65
	15.00	55.17	96.15	81.48
	43.86	34.85	98.08	75.31

Analysis included all children with available data (81 for sIgE to whole peanut extract and 80 for sIgE to Ara h 2).
*Sensitivity refers to the proportion of subjects who have peanut allergy and give positive test results. Specificity refers to the proportion of subjects without the target condition and a negative test result for peanut allergy.

peanut allergy and 50 are peanut-tolerant. By using sIgE to component Ara h 2 with a cutoff point of 0.35 kU_A/L, all children with peanut allergy would be correctly classified. The specificity of this test is given as 96.1% (Table I). In this example we expect 2 children who are not allergic to peanuts to be misclassified as having peanut allergy and the other 48 children to have a negative result. By using this cutoff point, 97.5% of the population is correctly classified. A similar proportion of children would be correctly classified by using a cutoff point of 0.55 kU_A/L; however, in this case 3 children with peanut allergy would be misclassified as tolerant. This cutoff point corresponds to a gain in specificity (100%) but a loss in sensitivity (93.1%). Given the importance of not misdiagnosing children with peanut allergy as being tolerant, we propose that the optimal cutoff point in our population is 0.35 kU_A/L.

The cutoff for whole peanut sIgE of 5.30 kU_A/L provides the maximum proportion of correctly classified subjects (87.6%), with a sensitivity of 75.9% and a specificity of 94.2%. However, approximately 24% of children with peanut allergy would be inappropriately classified as peanut-tolerant. The cutoff of 15 kU_A/L has excellent specificity, with 96.2% of children at greater than this level being correctly classified as allergic; however, this decision point has relatively poor sensitivity, with almost half of the subjects with peanut allergy being classified as tolerant. These data suggest that in our population the quantification of whole peanut sIgE has lower accuracy in discriminating peanut allergy from tolerance compared with quantification of sIgE to Ara h 2.

In conclusion, having identified sIgE to Ara h 2 as an important predictor of clinical reactivity to peanut using microarray technology,[5] we have now demonstrated the value of its quantification using a routinely available laboratory test. Among school-aged children in the United Kingdom, a cutoff of 0.35 kU_A/L Ara h 2 IgE confers 100% sensitivity and 96.1% specificity. By using this cutoff point, 97.5% of the subjects in our study population were correctly classified, with all children with peanut allergy given the correct classification. The importance of Ara h 2 has been suggested in studies from other Central and Northern European countries[7,8]; however, in other populations and geographic areas, IgE to other components might be relevant (eg, Ara h 9 in the Mediterranean[9]). Our findings need to be replicated in other populations and age groups before general application.

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Four distinct subtypes of non–IgE-mediated gastrointestinal food allergies in neonates and infants, distinguished by their initial symptoms

To the Editor:

Although most food allergies are IgE-mediated, there are a number of non–IgE-mediated gastrointestinal food allergies that affect mainly infants and young children.[1,2] Because most such

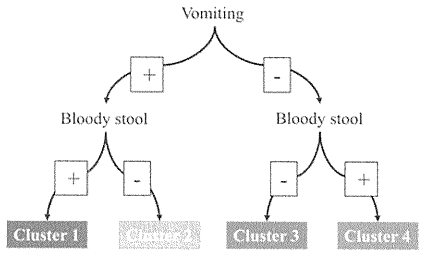

FIG 1. Tree analysis using 2 variables (vomiting and bloody stool at initial presentation) enables assignment of patients into 4 clusters.

patients develop the allergy more than 2 hours after ingestion of the offending food and show negative skin prick tests and/or absence of serum specific IgE against the offending food, these allergies are thought to be cell-mediated. However, the precise pathogenetic mechanisms of these disorders remain poorly understood. Several investigators have described different subtypes of non–IgE-mediated gastrointestinal food allergies: food protein-induced enterocolitis syndrome (FPIES),[3] food protein-induced proctocolitis syndrome (hereafter referred to as "proctocolitis"),[4] food protein-induced enteropathy syndrome (hereafter referred to as "enteropathy"),[5] celiac disease, and allergic eosinophilic gastroenteropathies.

Presumably because the main target organ of these syndromes is the gastrointestinal tract, patients with non–IgE-mediated gastrointestinal food allergies often exhibit similar symptoms, such as vomiting and diarrhea. However, it remains unclear whether these syndromes have the same pathogenesis and merely differ in severity, or whether the pathogenesis of each is distinct, meaning that they should be classified as separate clinical entities.

We applied cluster analysis to the clinical and laboratory findings to characterize these non–IgE-mediated food allergies and determine whether they are made up of distinct clinical entities. A total of 176 patients with detailed clinical records who had been registered in the database of the Japanese Research Group for Neonatal, Infantile Allergic Disorders from 2007 to 2010 were enrolled. Among them, 136 patients fulfilled 3 of the Powell[6] criteria: (1) a switch to therapeutic milk led to resolution of symptoms, (2) differential diagnosis from other disorders was possible, and (3) there was verified body weight gain. Definitive diagnosis was possible for 46 patients by oral food challenge tests that were performed after complete resolution of the initial symptoms (see this article's Fig E1 in the Online Repository at www.jacionline.org). These 46 patients were subjected to further analysis. Details of food challenge test are available in this article's Food challenge test, method section in the Online Repository at www.jacionline.org. Our total cohort included 15 patients who developed the most severe reactions, including ileus, shock, and developmental retardation. The clinical characteristics of those patients are summarized in this article's Table E1 in the Online Repository at www.jacionline.org. Because of the medical and ethical justification, even though these patients fulfilled 3 elements of the Powell[6] criteria, oral challenge tests were not performed. Thus, these patients were excluded from this cluster analysis of 46 patients. This study was approved by the Ethics Committee of the National Center for Child Health and Development.

We omitted clinical and laboratory findings found only in a few patients and finally selected 5 variables: birth weight, age at first presentation (days after birth), severity of vomiting (ranked as 0, none; 1, 1-2 times a day; 2, 3-5 times a day; and 3, more than 5 times a day or bilious vomiting) and severity of bloody stool (0, none; 1, spotty; 2, intermediate; and 3, massive) at first presentation, and milk-specific IgE antibody titer (class 0-6). Unsupervised cluster analysis and discriminant analysis were performed by using SPSS version 18 software (SPSS, Inc, Chicago, Ill). The Wald minimum-variance hierarchic clustering method was performed by using an agglomerative (bottom-up) approach and Ward's linkage. The squared Euclidean distance was used as a proximity measure. Values were transformed by a maximum magnitude of 1. ANOVA, the Tukey-Kramer test, and the χ^2 test were used for parametric continuous, nonparametric continuous, and categoric variables. As a result, the 46 definitively diagnosed patients were classified into 4 distinct clusters, and a dendrogram was generated (see this article's Fig E2 in the Online Repository at www.jacionline.org).

Stepwise discriminate analysis identified the 2 strongest discriminatory variables for cluster assignment: vomiting and bloody stool (Fig 1). Cluster 1 was the patient group with vomiting and bloody stool at initial presentation. Cluster 2 had vomiting but not bloody stool. Cluster 3 had neither vomiting nor bloody stool. Cluster 4 had bloody stool but not vomiting. One patient initially assigned to cluster 3 in fact had clear bloody stool, and was thus reassigned to cluster 4 in accordance with Fig 1. As a result, clusters 1 through 4 consisted of 14, 16, 5, and 11 patients, respectively.

Table I presents the demographic data for each cluster. Cluster 3 showed a significantly lower birth weight and later onset of disease. Clusters 1 and 4 both had bloody stool, but they had normal birth weight and a somewhat earlier onset (median of 7 days after birth).

The laboratory data generated within the initial several days after onset showed that the peripheral blood eosinophil ratio was high in all clusters, with no significant differences among them. In contrast, eosinophils were found in the stool mainly of patients in clusters 1 and 4, in which all patients, by definition (Fig 1), had bloody stool. The presence of eosinophilia suggests that patients with non–IgE-mediated gastrointestinal food allergies tend to have a T_H2-prone immune deviation at baseline, but some additional factors such as overproduction of eosinophil-attracting chemokines are probably necessary to induce immune responses involving eosinophils in the gut (see this article's Fig E3 in the Online Repository at www.jacionline.org).

A positive milk-specific IgE antibody titer was observed in 37% of the patients, with no statistically significant differences among any of the clusters. In addition, almost all symptoms at initial presentation as well as in oral food challenge tests began to manifest at more than 2 hours after ingestion of the offending food, whereas no patients developed typical IgE-mediated symptoms such as urticaria or wheeze. These results strongly suggest that the presence of milk-specific IgE antibody neither causes the gastrointestinal symptoms nor rules out a diagnosis of non–IgE-mediated gastrointestinal food allergy.

One of the most notable findings of this study was the remarkably high reproducibility of symptoms provoked in the oral food challenge tests and those found at the initial presentation in all 4 clusters, even though the oral challenge tests were performed several months after the initial presentation (Table I). This observation suggests that the upper or lower gastrointestinal tract–specific hypersensitivity and perhaps the responsible

TABLE I. Demographic data of the patients (total = 46) whose diagnosis was confirmed by oral food challenge tests

Clinical characteristics	Cluster 1 (n = 14)		Cluster 2 (n = 16)		Cluster 3 (n= 5)		Cluster 4 (n = 11)		*P* value
Birth weight (g)		2642 (2410-3030)		2745 (2223-3079)		1008 (907-2491)		2678 (2512-3170)	.03*
Male/female (n)		6/8		9/7		2/3		5/6	.95
Initial presentation									
Day of onset		7.5 (3-23)		16.5 (9.5-33.5)		37 (8.5-132)		7 (2-56)	.17
Vomiting (%)		100		100		0		0	.000*
Bloody stool (%)		100		0		0		100	.000*
Fever (%)		7.1		18.8		20.0		0	.45
(Laboratory data)†	n		n		n		n		
Blood eosinophil ratio (%)‡	13	15 (3.0-23)	14	7 (3.9-19.3)	5	27 (3.2-39.3)	11	14 (4.5-25)	.63
WBC ($\times 10^3$/mL)§	13	18.4 (13.7-22.7)	14	15.7 (11.4-21.9)	5	21.8 (11.0-27.7)	11	13.1 (8.2-18.3)	.64
Total IgE (IU/mL)‖	14	5.2 (4.8-28.3)	16	11.4 (5.0-80.8)	5	7.4 (5.5-653.5)	10	5.0 (2.0-5.8)	.36
Positive for milk-specific IgE (class ≥1) (%)	14	57	16	37.5	5	40	11	9	.28
C-reactive protein (% positive, ≥0.5)	13	46	14	50	5	40	10	30	.47
Stool eosinophil (% positive)	8	50	6	33	3	0	7	100	.01*
Diet (reaction to each milk, %)									
Cow's milk	14	100	16	100	5	100	10	100	1.00
Breast milk	8	38	7	0	2	50	7	27	.40
Hydrolyzed formula	9	0	10	20	2	0	8	63	.02*
Oral food challenge test									
Onset of reaction (h)		6 (1.8-12)		10 (2-24)		48 (24-60)		24 (24-48)	.17
Vomiting (%)		85.7		81.3		0		9.1	.000*
Bloody stool (%)		28.6		6.3		0		72.7	.001*
Diarrhea (%)		21.4		31.3		60.0		18.2	.33

WBC, White blood cell count.
Data are shown as the median and the interquartile range.
*$P < .05$.
†*n,* Number with medical records.
‡Normal range of blood eosinophils is 0% to 4%. However, it is known to rise to some degree in the neonatal period, especially in low-birth-weight infants.[10]
§Normal range of WBC in neonatal period is 7.0 to 25.0 $\times$ $10^3/\mu L$.
‖Normal range of total IgE in infantile period is less than 20 IU/mL.

immune cells remain in the same part of the gastrointestinal tract even after several months' remission.

Because the patients in clusters 1 and 2 had vomiting that was provoked at relatively early time points, they are likely to be diagnosed as having FPIES, although the bloody stool and eosinophilia seen mainly in cluster 1 patients were not emphasized in earlier reports.[7,8] The nearly simultaneous manifestation of vomiting and bloody stool suggests that FPIES may affect both the upper and lower gastrointestinal tracts.

The main symptoms of the patients in cluster 3 were poor weight gain and diarrhea and were similar to those found in patients with enteropathy. The significantly lower birth weight and marked eosinophilia characteristically found in cluster 3 patients imply the involvement of immature gastrointestinal function in the pathogenesis of this syndrome.

Bloody stool was the main symptom of the patients in cluster 4. Some patients in this cluster had no systemic manifestation other than bloody stool, whereas others also had diarrhea and/or poor weight gain. Therefore, these patients may be diagnosed as having proctocolitis or early onset of allergic eosinophilic gastroenteropathies, respectively. However, the pathogenetic similarity and/or disparity of proctocolitis and allergic eosinophilic gastroenteropathies need to be studied further.

In our cohort, 3 children with exclusive breast-feeding have developed FPIES. This information is available in this article's Breast-feeding and FPIED section in the Online Repository at www.jacionline.org.

Elevated serum C-reactive protein levels were found in 30% to 50% of patients with non–IgE-mediated gastrointestinal food allergies. In addition, some patients developed a fever during oral food challenge tests, suggesting that TNF-α and other proinflammatory cytokines may be involved in the pathogenesis of these syndromes.[9]

To confirm the results of cluster analysis, we performed the same analysis for the aforementioned 136 patients who fulfilled 3 of the Powell[6] criteria (consisting of the 46 patients definitively diagnosed by oral food challenge and 90 patients not subjected to oral food challenge; Fig E1). We obtained exactly the same results: the patients were assigned to 4 clusters in accordance with the tree analysis shown in Fig 1. The patients' demographics (see this article's Table E2 in the Online Repository at www.jacionline.org), birth weight (see this article's Fig E4 in the Online Repository at www.jacionline.org) and peripheral blood eosinophils (see this article's Fig E5 in the Online Repository at www.jacionline.org) confirmed the earlier cluster analysis findings.

In our ongoing cohort, 52% of the patients acquired tolerance to the offending food by 1 year of age, 88% by 2 years, and 94% by 3 years. Therefore, assuming that identification and elimination of the offending food had been done properly, it can be assumed that most patients outgrew their allergy by the age of 2 to 3 years. On the other hand, just like patients with severe IgE-mediated food allergy, patients with non–IgE-mediated gastrointestinal food allergies may develop severe reactions

(Table E1). Thus, early diagnosis is very important, and refinement of the diagnostic method is truly necessary.

Our findings clearly demonstrated that patients with these non–IgE-mediated gastrointestinal food allergies showed similar T_h2-prone laboratory data (eosinophilia and presence of specific IgE antibody), but the disease entities of each cluster had distinct clinical features and may have different pathogenetic mechanisms.

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FOOD CHALLENGE TEST, METHOD

Generally, oral challenge tests were performed at 4 to 6 months of age. First, 4 mL milk/kg body weight was administered. If no reaction occurred, the dose of milk was increased daily until symptoms manifested. If the reaction had been evoked by a very small volume of milk in the initial presentation, the test was started using a lesser volume to avoid a serious reaction. Because of the medical and ethical justification for oral food challenge tests, patients with the most severe reactions were excluded from the initial cluster analysis. Their clinical characteristics are summarized in Table E1.

BREAST-FEEDING AND FPIES

Six of the 46 patients were exclusively breast-fed. Three of them were included in cluster 1 and can be diagnosed as FPIES. Those 3 patients showed a positive reaction to cow's milk as well as breast milk even after their mothers stopped consuming milk products. These patients also developed symptoms when orally challenged with rice and/or soy. Therefore, these findings indicate that not only proctocolitis but also FPIES can develop even in children who are exclusively breast-fed. A recent case report supports our findings.[E1]

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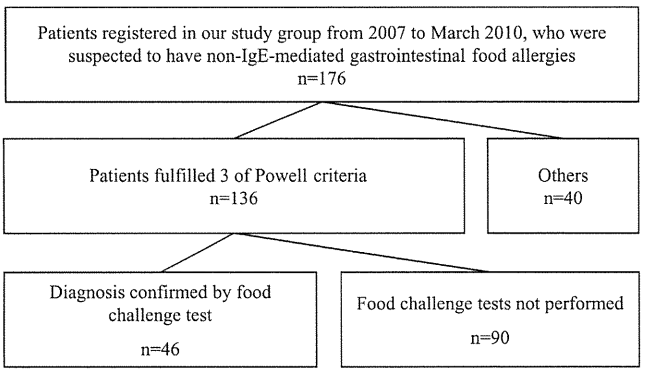

FIG E1. A total of 176 patients with gastrointestinal symptoms who were suspected of having non–IgE-mediated allergy from 1999 to 2009 were registered by doctors of the Japanese Research Group for Neonatal, Infantile Allergic Disorders. Of them, 136 patients fulfilled elements 1 through 3 of the Powell criteria. Forty-six patients underwent food challenge tests and had a positive result, whereas the remaining 90 patients were not tested. Seventeen patients showed no reaction in the oral challenge tests. However, it was unclear whether this was because the patients had outgrown their allergy or because of misdiagnosis. Those 17 patients were excluded from further analysis in this study.