Peripheral Dendritic Cells in Asthma

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Key words: Asthma. Lymphocyte. Dendritic cell. Regulatory T cell.

Dendritic cells (DCs) affect helper T cell (Th) cell commitment [1]. The two major DC subsets are myeloid DCs (mDCs), which shift the T-cell response in the direction of Th2 cells [2], and plasmacytoid DCs (pDCs), which induce regulatory T cells (Tregs) and which are major inhibitors of adaptive immunity [3]. We studied the association between prevalence of DC subsets and specific target cells (Tregs, Th1 CD4+, and Th2 CD4+ cells) and asthma symptoms.

We enrolled 28 patients (15 men, 13 women) with persistent asthma of differing severity (values expressed as median [interquartile range]). The Asthma Control Test (ACT) [4] score was 20 (16-22), median age was 32 (29-39) years, and the body mass index was 23.37 (21.04-26.92). Patients were taking inhaled corticosteroids (beclomethasone or equivalent) at 800 (500-1000) µg/d and long-acting β-agonists at 2 (0-4) puffs/d. Forced vital capacity was 93% (80%-104%) of predicted, peak expiratory volume in 1 second was 82% (72%-103%) of predicted, peak expiratory flow was 74% (67%-93%) of predicted, airway resistance was 0.31 (0.20-0.39) kPa/s/L, and peripheral oxygen saturation was 98% (96%-99%). The inclusion criteria were acute exacerbation of asthma, severe comorbidity, medication other than inhaled corticosteroids or long-acting β-agonists, infection during the previous 6 weeks, and smoking.

Blood samples were taken after informed consent was obtained. Peripheral blood mononuclear cells (PBMCs) were isolated and stained with surface markers (CD4, CD11c, CD123, CCR4, CXCR3, HLA-DR, Lin1, CD3, CD14, CD16, CD19, CD20, and CD56) (BD Biosciences Pharmingen, San Diego, California, USA) and FoxP3 (eBioscience, San Diego, California, USA) [5]. Fifty-four healthy controls were also enrolled. The Mann-Whitney test, Spearman test, and multiple regressions were applied using Statistica 8 (Statsoft, Tulsa, Oklahoma, USA).

Total DC prevalence (Lin1-CD11c+/PBMCs) was comparable in both asthma patients and controls (1.19 [0.77-1.54] vs 1.16 [0.60-2.60]). The prevalence values for mDC (CD11c+/CD123+/DCs) and pDC (CD123+/CD11c+/DCs) were also comparable (59.64 [52.07-65.19] vs 63.65 [56.02-72.31] and 31.36 [18.26-36.22] vs 25.57 [22.25-32.34], respectively). The mDC/pDC ratio was lower in asthma patients than in controls (1.95 [1.48-2.92] vs 2.53 [1.93-3.51], P=.038). The prevalence of Th1 and Th2 cells (CD4+CXCR3+CCR4-/CD4+ and CD4+CCR4+CXCR3-/CD4+, respectively) was also comparable (25.56 [19.74-31.97] vs 35.64 [29.46-45.64], and 3.01 [1.50-4.40] vs 2.10 [1.79-3.58], respectively), while the Th1/Th2 ratio was lower in patients with asthma (7.85 [4.40-10.12] vs 9.34 [6.39-11.47], P=.039). Treg (CD4+FoxP3+/CD4+) prevalence was also similar in both groups (2.70 [1.45-4.40] vs 3.02 [2.26-5.80]).

The ACT score correlated inversely with the prevalence of mDC and positively with the prevalence of pDC (r=-0.607, P=0.013 and r=0.607, P=0.007, respectively) (Figure). The ACT score correlated with the prevalence of Th1 and inversely with the prevalence of Th2 (r=0.491, P=0.017; r=-0.417, P=0.048). The correlations remained significant after adjustment for age, body mass index, and gender, but ceased after adjustment for the antiasthmatic drugs used.

Recent experiments demonstrated that the elimination of mDCs abrogates Th2 response [6]. pDCs are regarded as modulators of the immune response by inducing Treg cells [7]. Our results were consistent with those of other authors, namely, we also found that pDCs predominated (ie, low mDC/pDC ratio), although we could not confirm previous findings on high pDC prevalence [8]. The lack of higher pDC prevalence in our study may be due to specific patient characteristics, as we enrolled patients with poorer asthma control (ACT≤19) and, therefore, lower pDC prevalence (Figure).

Asthma control is determined by drug use. In addition, recent studies demonstrated that inhaled corticosteroids and long-acting β-agonists influence DC subsets [9,10]. In our study, there was no direct impact of antiasthmatic drugs on peripheral DC prevalence. However, these drugs may still modulate cell prevalence values, as the correlation between asthma control and cell prevalence ceased to be observed after adjustment for drug use [8-10].

Our data suggest that DC phenotype is strongly associated with general well-being, as reflected by the total ACT score, namely, lower mDC and higher pDC values are associated with better asthma control. This finding reinforces the potential role of DCs as biomarkers for the assessment of asthma control.
Figure. Correlations between total asthma control test scores and the prevalence of (A) myeloid dendritic cells ($r=-0.607$, $P=.013$), (B) plasmacytoid dendritic cells ($r=0.650$, $P=.007$), (C) T$_{h}$1 committed lymphocytes ($r=0.491$, $P=.017$), (D) T$_{h}$2 committed lymphocytes ($r=-0.417$, $P=.048$), and (E) regulatory T cells (nonsignificant).
Acknowledgments

This work was supported by grants TÁMOP-4.2.2-08/1/KMR-2008-0004 and OTKA K-68758.

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Manuscript received January 22, 2010; accepted for publication May 18, 2010.

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Comparison of Basophil Activation Test Results in Blood Preserved in Acid Citrate Dextrose and EDTA

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Key words: ACD. Aeroallergens. Basophil activation test. Blood storage. EDTA.

Palabras clave: ACD. Aeroalérgenos. Test de activación de basófilos. Preservación de sangre. EDTA.

The basophil activation test (BAT) complements skin tests and specific immunoglobulin (Ig) E determination in analyzing immediate-type reactions to allergens such as aeroallergens [1], hymenoptera venom [2], latex [3], foodstuffs, and drugs [4]. This technique enables the number of provocation tests, which are often dangerous, to be substantially lowered. However, no single universal protocol is available, and results vary considerably. Sturm et al [5] recently compared blood from venom-allergic patients that was collected in EDTA tubes with the same blood after 18 hours’ storage. They reported a significant drop in the percentage of basophil activation leading to a very high rate of negative BAT results. The results of our analysis were not consistent with these observations.

The objective of this study, therefore, was to compare basophil activation between blood preserved in acid-citrate dextrose (ACD) tubes (BD Vacutainer Systems, Plymouth, UK) and blood preserved in K3EDTA (EDTA) tubes (BD Vacutainer Systems) at 0, 24, and 48 hours’ storage at 4ºC in patients allergic to Dermatophagoides pteronyssinus or Phleum pratense. These allergens were chosen because BAT has been thoroughly validated for both and the positivity criteria are universally accepted [1].

Three patients with allergic rhinitis, asthma, or both, and proven allergy to D pteronyssinus or P pratense were randomly enrolled from our clinic. Allergy was confirmed by a suggestive clinical history with positive results for skin tests, specific IgE, or both. After informed consent detailing the purpose of this study was obtained, peripheral blood was collected using ACD and EDTA tubes. BAT with either preservative free of D pteronyssinus extract (final concentrations 1.411 and 0.353 mg/mL) or preservative free of P pratense extract (final concentrations 0.148 and 0.037 mg/mL) (Bial Aristeuroll, Bilbao, Spain) was performed on a blood sample from each patient (ACD and EDTA) immediately after collection and again at 24 and 48 hours. The blood was stored at 4ºC. BAT was performed on the buffy coat fraction using CD63 as the activation marker, as reported elsewhere [1]. Data were analyzed using a FACScan flow cytometer (Becton Dickinson, New Jersey, USA).

After data analysis, an individual threshold for basophil activation was defined for each patient. The threshold used
to evaluate both the ACD and EDTA samples at the different timepoints was applied to ensure an accurate comparison. The percentage of activated basophils to monoclonal anti-IgE receptor and both concentrations of allergens used are presented in the Figure.

After stimulation with monoclonal anti-IgE receptor and both aeroallergens, we found that basophil activation was similar in fresh blood and blood stored for 24 hours. Even after 48 hours, basophil activation was still substantial. The differences between ACD and EDTA tubes were negligible. These results contradict those of Sturm et al [5], which revealed a substantial reduction in the number of activated basophils to anti-IgE and to hymenoptera venom in venom-allergic patients. Although our patients were allergic to aeroallergens and not to hymenoptera venom, basophil reactivity has been shown to be very high in both [1,2].

In conclusion, our study showed that the results of the BAT using fresh blood were similar to those for blood stored for 24 hours at 4°C. There was slight drop in activation after 48 hours, although the results remained clearly positive. A similar study should be performed using drug allergens.

References


Figure. Percentage of activated basophils in response to monoclonal anti-IgE receptor and both allergen concentrations (Dermatophagoides pteronyssinus and Phleum pratense) after 0, 24, and 48 hours’ storage at 4°C. A, ACD tubes; B, EDTA tubes. ACD indicates acid-citrate dextrose.
Monoclonal antibodies became available for clinical use in the early 1980s, after Köhler and Milstein [1] described the hybridoma technique, which made it possible to use biological agents to treat numerous conditions. Since then, several immune system mediators have been cloned and modified and are now used to treat inflammatory diseases. One of the most important groups is that of the tumor necrosis factor (TNF-α) antagonists, namely, infliximab, adalimumab, etanercept, and certolizumab, the latter having recently been approved for human use by the United States Food and Drug Administration. Several adverse reactions to TNF-α antagonists have been reported, although very few have an immunoglobulin E–mediated mechanism [8]. Such a mechanism had been postulated in allergic reactions to TNF-α antagonists, yet no clear evidence has come to light [9]. To our knowledge, this is the first report of BAT being used to analyze the reaction to a TNF-α antagonist.

In conclusion, we report the case of a patient who suffered a systemic hypersensitivity reaction after administration of the TNF-α antagonist infliximab. The patient tolerated administration of adalimumab, as previously described [10]. The results of BAT suggest that an IgE-mediated mechanism is not involved.

Acknowledgments

These results were presented as an oral communication in the Oral Communication Session “Various I” at the International Symposium on Drug Hypersensitivity (Logroño, Spain, 2009), organized by SEAIIC.

Editorial assistance was kindly provided by Oliver Shaw.

References

The basic treatment for adverse reactions to food has traditionally been to avoid the offending food [1]. Specific oral tolerance induction (SOTI) achieved by oral exposure to increasing doses of the specific food allergen seems to be a promising therapeutic approach. No standard protocol for SOTI has been designed and there is wide variation among hospitals.

The study population comprised 10 patients (aged 4-14 y) referred to Gifu University Hospital (Gifu, Japan) with allergy to cow’s milk. Diagnosis was based on serum CAP system fluorescent enzyme immunoassay (cow’s milk–specific immunoglobulin [Ig] E, 0.92 IU/mL to >100 IU/mL; mean 18.2 IU/mL) and a positive allergic reaction to cow’s milk during the 3 months before the beginning of the study (7 patients) or a positive allergic reaction to a double-blind placebo-controlled food challenge (3 patients) (Table).

The initial dose of our slow dose-up method was 1 daily drop of cow’s milk (approximately 0.025 mL) in 20 mL of water. The dose was increased every 2 weeks. The challenge was performed at Gifu University Hospital under medical supervision. When symptoms appeared, the daily dose was not increased and the previous dose was repeated. The children were then carefully assessed. A reaction was considered to be positive in the presence of at least one of the following symptoms: urticaria/angioedema or erythema with pruritus; rhinitis and/or conjunctivitis; bronchial asthma; vomiting and/or diarrhea with abdominal pain; and general malaise or loss of consciousness.

Eight patients completed the protocol and were able to tolerate 100 mL of cow’s milk. The 2 patients who did not complete the protocol experienced symptoms, one with urticaria following a 5-mL dose and the other with perioral exanthema following a 20-mL dose. In these patients, the daily dose of cow’s milk was not increased and the last dose was repeated after 2 weeks. The same symptoms reappeared with each repeated dose. Therefore, the patients could not continue the protocol. Three patients experienced mild side effects, including perioral exanthema, which did not require medication or dose reduction.

The protocol of Staden et al [2] required 2 months to reach the maximum dose (250 mL). The adverse reactions observed were exacerbation of eczema in 13 of 25 patients and urticaria in 11. Seven of the 20 patients (35%) tolerated milk and were considered the control group. The protocol of Longo et al [3] consisted of a rush-phase SOTI and a slow increasing-phase SOTI. Five of 30 patients who underwent SOTI required epinephrine for the treatment of adverse reactions. Eleven of 30 patients who underwent SOTI (36%) achieved complete tolerance to cow’s milk. In our slow dose-up method, the severity and frequency of adverse reactions were almost the same as those of the protocols mentioned above.

Protocols are divided into 2 groups on the basis of the time required to reach the maintenance doses. Rush immunotherapy is performed in the hospital [3-5] and consists of doubling the doses of cow’s milk every 1 or 2 hours. It takes approximately 1 week to achieve the maintenance dose. The other is performed at home. The time to reach the maintenance doses varies from 65 to 200 days [2,6-9].

We previously observed transiently elevated lymphocyte stimulation [10] and interferon γ production with β-lactoglobulin, and the CD4/CD25 ratio increased after SOTI (data not shown). These immunological changes may be associated with milk-allergen–specific regulatory T cells.

SOTI is a promising method for the treatment of food allergy. Although we consider it to be safe and effective, it should be performed by trained staff and following a standard protocol.
Table. Patient Characteristics and Serum IgE Levels Before SOTI

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age, y</th>
<th>Symptoms Presented</th>
<th>With Cow’s Milk Before SOTI</th>
<th>Specific IgE (kU/mL)</th>
<th>E. Lactoglobin</th>
<th>Cow’s Milk</th>
<th>Casein</th>
<th>β-Lactoglobulin</th>
<th>Symptoms During SOTI</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>5</td>
<td>Cough, erythema with pruritus</td>
<td>Yes</td>
<td>86.7</td>
<td>1.6</td>
<td>2</td>
<td>No symptoms</td>
<td>No symptoms</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>6</td>
<td>Perioral exanthema</td>
<td>Yes</td>
<td>250</td>
<td>9.5</td>
<td>12</td>
<td>No symptoms</td>
<td>No symptoms</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>7</td>
<td>Conjunctivitis, bronchial asthma, erythema with pruritus</td>
<td>Yes</td>
<td>1200</td>
<td>42</td>
<td>42</td>
<td>No symptoms</td>
<td>No symptoms</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>8</td>
<td>Cough, urticaria, perioral exanthema</td>
<td>Yes</td>
<td>500</td>
<td>50</td>
<td>50</td>
<td>No symptoms</td>
<td>No symptoms</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>8</td>
<td>Cough, erythema with pruritus</td>
<td>Yes</td>
<td>20</td>
<td>0.02</td>
<td>0.02</td>
<td>No symptoms</td>
<td>No symptoms</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>9</td>
<td>Erythema with pruritus</td>
<td>Yes</td>
<td>100</td>
<td>1.6</td>
<td>1.6</td>
<td>No symptoms</td>
<td>No symptoms</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>10</td>
<td>Cough, urticaria, perioral exanthema</td>
<td>No</td>
<td>2</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Acknowledgments**

This study was partially supported by a grant from the Ministry of Health, Labor and Welfare of Japan.

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**Manuscript received March 6, 2010; accepted for publication June 28, 2010.**

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**Metrorrhagia as an Uncommon Symptom of Anaphylaxis**

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**Keywords:** Anaphylaxis. Hypersensitivity. Metrorrhagia. Vaginal bleeding.

**Palabras clave:** Anafilaxia. Hipersensibilidad. Metrorragia. Hemorragia vaginal.

Anaphylaxis is a severe allergic reaction that usually presents with mucocutaneous, gastrointestinal, respiratory, cardiovascular, or neurologic symptoms and signs. The diagnosis is based primarily on clinical criteria. Involvement of the skin is reported in 80% to 90% of episodes, the respiratory tract in up to 70%, the gastrointestinal tract in up to 45%, the cardiovascular system in up to 45%, and the central nervous system in up to 15% [1,2]. The objective of this work was to assess the presence of metrorrhagia during anaphylaxis, as vaginal bleeding is an unusual clinical manifestation of this condition.

We diagnosed 65 cases of anaphylaxis (0.95% of all patients attending the clinic for the first time) over a 10-year period (2000-2009). Thirty-six cases occurred in

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**Table Clinical Characteristics and Causative Agents**

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Age, y</th>
<th>Symptoms</th>
<th>Onseta</th>
<th>Agent</th>
<th>Treatment</th>
<th>Resolution of Anaphylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52</td>
<td>Dizziness, hypotension, rhinitis, wheezing, generalized erythema, urticaria, nausea, diarrhea, abdominal pain, metrorrhagia</td>
<td>12 h</td>
<td>Trimethoprim-sulfamethoxazole</td>
<td>Corticosteroids and antihistamines (oral and IV)</td>
<td>4 d</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>Headache, dizziness, hypotension, pharyngeal itching, generalized rash, lower abdominal pain, nausea, vomiting, diarrhea, metrorrhagia</td>
<td>4 h</td>
<td>Amoxicillin-clavulanic acid</td>
<td>Corticosteroids and antihistamines (IV), adrenaline (SC), saline infusion</td>
<td>6 h</td>
</tr>
<tr>
<td>3</td>
<td>39</td>
<td>Dizziness, generalized itching and erythema, urticaria, dyspnea, wheezing, abdominal cramping, vomiting, diarrhea, metrorrhagia</td>
<td>6 h</td>
<td>Amoxicillin-clavulanic acid</td>
<td>Corticosteroids and antihistamines (IM)</td>
<td>4 h</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>Dizziness, warmth, cutaneous itching, abdominal cramping, metrorrhagia</td>
<td>1 h</td>
<td>Hymenoptera venom</td>
<td>Unknown (IM)</td>
<td>2 h</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>Dizziness, palmar and plantar itching followed by generalized itching with erythema and urticaria, conjunctival hyperemia, hydrorrhrea, dyspnea, lower abdominal pain, metrorrhagia</td>
<td>1 h</td>
<td>Pollen immunotherapy</td>
<td>Corticosteroids and antihistamines (IM), adrenaline (SC)</td>
<td>3-4 h</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
<td>Dizziness, hypotension, itching (palmar, plantar, otic, and pharyngeal), lower abdominal pain, metrorrhagia</td>
<td>1 h</td>
<td>Amoxicillin</td>
<td>Corticosteroids and antihistamines (IM), adrenaline (SC)</td>
<td>2 h</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>Dizziness, hypotension, palmar and plantar itching, urticaria, dyspnea, dysphagia, diarrhea, lower abdominal pain, metrorrhagia</td>
<td>6 h</td>
<td>Unknown</td>
<td>Corticosteroids and antihistamines (IM), oxygen, inhaled bronchodilators</td>
<td>8 h</td>
</tr>
</tbody>
</table>

Abbreviations: IM, intramuscular; IV, intravenous; SC, subcutaneous

a Time between onset of anaphylaxis and onset of metrorrhagia
women (55.4%) and 29 in men (44.6%). Only 7 women (19%) experienced vaginal bleeding in association with the anaphylactic episode. The clinical characteristics and causative agents of these 7 cases are shown in the Table. Mean age was 40 years. Onset of anaphylaxis was always acute, ranging from 15 to 30 minutes after the trigger, and metrorrhagia appeared from 1 to 12 hours (mean, 4.4 h) after onset. All the patients complained of abdominal pain and underwent additional examinations (abdominal X-ray, ultrasound scan, and—in some cases—gynecological evaluation).

Anaphylaxis has been defined as a serious allergic reaction that is rapid in onset and can be fatal [1]. Prevalence is estimated at 0.05% to 2%, and the rate of occurrence appears to be increasing, mainly in young people [2]. The pathogenesis of anaphylaxis involves immunological mechanisms (immunoglobulin [Ig] E–mediated or non–IgE-mediated) and nonimmunological mechanisms (eg, physical factors, alcohol, opioids). Idiopathic anaphylaxis, currently a diagnosis of exclusion, makes it possible to identify unrecognized triggers. Regardless of the trigger and mechanism, activation of mast cells and basophils results in the rapid release of immediate mediators (preformed and lipid) and delayed mediators (cytokine secretion). Severity and mortality are affected by age, concomitant diseases (eg, asthma, cardiovascular disorders, or mastocytosis), and concurrent medication [1,2]. The most common cause of anaphylaxis in childhood is food allergy, whereas drugs and hymenoptera venom are more common in adults [3]. In any case, anaphylaxis is unpredictable, underrecognized by patients, and underdiagnosed by health care professionals [1].

According to previous reports [4-6], metrorrhagia is a rare symptom of anaphylaxis and is not usually included in the list of possible symptoms of this condition. However, the most recent expert consensus adds “uterine contractions in postpuberal female patients” to the possible manifestations of anaphylaxis [2]. The pathogenic mechanism of this symptom has not been established, but the effects of mediators on smooth muscle secondary to histamine and other agents might explain this finding. In our series, only 7 patients (19%) experienced this symptom, and the most common trigger was drugs (mainly antibiotics). Alcoceba et al [4] described a series with 5 patients (12% of all women with anaphylaxis) in which the most common trigger of anaphylaxis was hymenoptera venom (4 patients). There has been 1 report of metrorrhagia secondary to cold allergy [5] and another following immunotherapy [6]. In our series, metrorrhagia was not associated with anaphylaxis when the patients were treated in the emergency room. Awareness of metrorrhagia in the context of anaphylaxis could help avoid unnecessary complementary examinations.

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II Manuscript received June 17, 2010; accepted for publication June 29, 2010.

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