Epigallocatechin Gallate
The Major Causative Agent of Green Tea-Induced Asthma*

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We describe three patients who worked in green tea factories and developed asthmatic and nasal symptoms after exposure to green tea dust. To clarify what component(s) of green tea leaves might be responsible for causing asthma, we prepared catechins, the major components of green tea leaves. Epigallocatechin gallate (EGCG; MW: 458 daltons), a major catechin, was purified by high-performance liquid chromatography. Subjects included three patients with green tea-induced asthma, five asthmatics with no previous exposure to tea dust, and five healthy controls. It was found that all three patients exhibited an immediate skin and bronchial response to EGCG. Prausnitz-Küstner test with EGCG was also positive. However, none of the asthmatic and healthy controls showed a positive reaction. These results indicate that EGCG is a causative agent of green tea-induced asthma and suggest that an IgE-mediated response is, at least in part, responsible for causing this type of occupational asthma.

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In 1970, Uragoda first reported a case of occupational asthma associated with tea fluff and designated tea maker's asthma.1 Subsequently, several authors have described similar cases associated with tea dust or tea fluff.2-4 However, to our knowledge, no report has yet identified which tea component(s) cause the bronchial response.

In this study, we describe three patients with green tea-induced asthma and present data indicating that epigallocatechin gallate (EGCG), the major catechin component in green tea leaves, is a causative agent.

MATERIAL AND METHODS

Subjects

Subjects consisted of three groups: nonatopic nonasthmatic volunteers (n=5), asthmatics with no previous exposure to green tea dust (asthmatic controls) (n=5), and patients with green tea-induced asthma (n=3).

The clinical characteristics for patients with green tea-induced asthma are summarized in Table 1. All patients had worked at different green tea factories in Shizuoka Prefecture. Their asthmatic symptoms (dyspnea, cough, and wheezing) and nasal symptoms (rhinorrhea and obstruction) began within 1 h after starting work and subsided on returning home. Furthermore, all three patients exhibited bronchial hyperresponsiveness as determined by an acetylcysteine or methacholine inhalation test.5,6 Only one patient (patient 3) developed an asthmatic attack after drinking green tea.

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Preparation of Green Tea Components

Powdered Green Tea Extract: A powdered green tea extract was prepared by boiling 300 g of green tea leaves in water for 5 min and then allowing the extract to air dry.

Crude Catechins and Noncatechin Components: The procedure for purifying catechins is illustrated in Figure 1. Briefly, the powdered green tea extract was dissolved in boiled water, followed by adding an equal portion of chloroform. After the aqueous layer had separated out, three equal portions of ethyl acetate were added. Crude catechins of 91% purity were obtained from the ethyl acetate layer by evaporation and lyophilization. Noncatechin components that consist of unknown substances other than catechins were also prepared from the aqueous layer by evaporation.

Catechin Components (EGCG, EGC, ECg, and EC): The crude catechins were dissolved in water, filtered (0.45 μm, Millipore, Yonezawa, Japan), and placed on a reverse-phase distribution column (Waters Co, model LC500A, cartridge column of C18) using the mixture of acetone, tetrahydrofuran, and water (12:10:78, percent by volume). The procedure produced four peaks (Fig 1). Each peak fraction was evaporated by a stream of nitrogen gas. Through the use of infrared and ultraviolet absorption and elemental analysis, resultant white residues were found to consist of (−)epigallocatechin gallate (EGCG), (−)epigallocatechin (EGC), (−)epicatechin gallate (ECg), or (−)epicatechin (EC).

Other Tea Extracts

Extracts of black tea and oolong tea were prepared by stirring 1 g of each tea in 20 mL of 0.9% saline solution for 30 min, followed by filtration (0.45 μm).

Skin Test

Each tea component was dissolved in saline solution at concentrations of 1 ng/mL to 1 mg/mL and filtered (0.45 μm). Intradermal skin tests were performed with 0.02 mL of each solu-
**Table 1—Clinical Characteristics of Patients With Green Tea-Induced Asthma**

<table>
<thead>
<tr>
<th>Patient No./Sex/ Age, yr</th>
<th>Positive Skin Reaction*</th>
<th>Duration of Exposure to Tea Dust Prior to Onset, yr</th>
<th>Duration of Symptoms, yr</th>
<th>Total IgE, IU/mL</th>
<th>PC20, † mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/52</td>
<td>+</td>
<td>11</td>
<td>3</td>
<td>995</td>
<td>5</td>
</tr>
<tr>
<td>2/F/60</td>
<td>+</td>
<td>1.7</td>
<td>0.3</td>
<td>39</td>
<td>5</td>
</tr>
<tr>
<td>3/F/47</td>
<td>−</td>
<td>7</td>
<td>7</td>
<td>40</td>
<td>0.78†</td>
</tr>
</tbody>
</table>

*Skin reactions were defined by utilizing 44 inhalant allergens.
†Provocation concentration of acetylcholine causing a 20% fall in FEV$_1$.  

Inhalation Challenge

Subjects were being treated with only intermittent $\beta_2$-agonist inhalation before the study, and they were asked to not take that medication for at least 12 h prior to the inhalation challenge. Each tea component preparation was inhaled as a mist using a nebulizer (output: 0.16 mL/min) (Nisskey, Tokyo, Japan) for 2 min. A 20% fall in FEV$_1$ compared with the baseline value was evaluated as positive. To establish the reliability of the baseline values, changes in FEV$_1$ after saline solution inhalation were evaluated hourly for 12 h. FEV$_1$ variability after saline solution inhalation was less than 5% in all subjects. Inhalation challenges with each preparation were subsequently performed. The concentration inhaled was increased in tenfold increments, from 10 ng/mL to 1 mg/mL. To detect a late asthmatic reaction (LAR), spirometry values were taken hourly for 12 h.

Open Oral Challenge

Subjects were given an oral administration of 1 mL of powdered green tea extract dissolved in water at a concentration of 10 mg/mL, and spirometry values were taken over a 12-h period.

Prausnitz-Küstner Test

With appropriate consent, serum was obtained from patients with green tea-induced asthma, and 0.1 mL of the serum was transferred intradermally into the back of a family member who previously had shown a negative skin reaction to powdered green tea extract or EGCg. Twenty-four hours later, each skin site was challenged by a 0.02 mL intradermal injection of powdered green tea extract or EGCg at a concentration of 1 mg/mL. As a control, other skin sites were sensitized with serum inactivated at 56°C for 2 h.

Results

Skin Test

All patients with green tea-induced asthma showed an immediate positive reaction to powdered green tea extract, crude catechins, EGCg, and noncatechin components, and two of three patients had a positive reaction to EGC, ECg, and EC (Tables 2 and 3). However, none of the normal and asthmatic controls reacted to these preparations (Table 2). No late intradermal reactions were detected in any group.

Inhalation Challenge

Time courses of serial FEV$_1$ measurements for the three patients with green tea-induced asthma were examined (Fig 2). In patients 1 and 2, an immediate asthmatic reaction (IAR) was induced by inhalation of powdered green tea extract, crude catechins, and EGCg at a concentration of 1 mg/mL. When patient 3 was challenged with EGCg at a concentration of 0.01 mg/mL, such a severe attack occurred that subsequent spirometric analysis could not be completed. No LARs were observed in any patient. Inhalation challenges with EGC, ECg, EC, and noncatechin components were performed in patients 1 and 2 and failed to produce a bronchial response. None of the preparations caused positive reactions in healthy and asthmatic controls (Table 4).

Oral Challenge

Patient 3 demonstrated a positive reaction to an

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**Figure 1.** Separation procedure to obtain catechins from powdered green tea extract. The final step consisted of high-performance liquid chromatography.
oral challenge with powdered green tea extract. A maximum fall in FEV₁ of 37% occurred 30 min after the exposure with no late reaction (Fig 2). Patients 1 and 2 exhibited no bronchial reaction to oral challenge.

**Effects of Premedication on Bronchial Responses to Powdered Green Tea Extract**

The effects of bronchodilators and disodium cromoglycate (DSCG) on the bronchial response to powdered green tea extract were studied in a single-blind way in patient 1. The patient received 300 mg of aminophylline orally 12 and 4 h prior to the inhalation. The blood level of aminophylline at the time of inhalation was 8.7 µg/mL. Salbutamol sulfate (1.5 mg) was inhaled 1 h before the challenge. Forty milligrams of DSCG was inhaled immediately before the challenge.

It was found that treatment with each medication completely inhibited IARs (Fig 3).

**Prausnitz-Küstner Test**

The Prausnitz-Küstner (P-K) test revealed a positive reaction to powdered green tea extract and EGCg in each family member, while an injection of either saline solution or heat-treated serum caused no reaction.

**Other Tea Extracts**

All three patients with green tea-induced asthma had an isolated immediate skin reaction to the extracts from black tea and oolong tea. We also performed inhalation challenges with these tea extracts in patient 1, and he showed an isolated IAR (Fig 4).

**DISCUSSION**

We have described three patients with green tea-induced asthma and demonstrated that EGCg is a causative agent.

Tea is classified according to the method of manufacturing: unfermented tea (green tea), semifermented tea (oolong tea), and fermented tea (black tea). All three types are derived from the same tea plant, *Camellia sinensis*, and contain EGCg: 8% (green tea), 4% (oolong tea), and 1% (black tea) by weight. Thus, it is possible that EGCg is also a causative agent in black tea-associated asthmatic cases that have been previously reported. This would...
be supported by the positive immediate skin reactions to black tea and oolong tea extracts demonstrated in our three patients and also the exhibited bronchial response to challenges with these extracts in one of the patients. These findings suggest that EGCg is a causative agent in several kinds of tea-associated asthma.

However, there are some discrepancies between our cases and those previously reported by Roberts and Thompson and Cartier and Malo. They reported that skin prick tests to black tea dust extracts were negative, and that some of their patients showed late and prolonged immediate asthmatic responses. These discrepancies may result from different subjects studied, preparations tested, or methods used for challenges. It is known that the intradermal testing method that we used provides increased sensitivity compared with prick testing. Furthermore, they performed inhalation challenges by tipping tea dust between two containers, which may have resulted in inhalation of more tea products than our inhalation challenge.

Catechins are the source of the astringent taste of green tea and are the major soluble component of green tea, occupying 15% by weight of dried Japanese green tea leaves. Catechins consist of several different polyphenols, and EGCg accounts for more than half of the total catechin. Recent studies have shown that tea catechins have hypcholesterolemic, antioxidative, and antimutagenic effects.

Occupational asthma due to small molecular weight molecules remains complicated. Both immunologic and nonimmunologic mechanisms have been proposed. For example, occupational asthma induced by trimellitic anhydride is considered to be mediated by IgE, IgG, and nonspecific irritant stimulation. In western red cedar induced asthma, air-

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Powdered Green Tea Extract</th>
<th>Crude Catechins</th>
<th>EGCg</th>
<th>EGC</th>
<th>ECg</th>
<th>EC</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>10</td>
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<td>10</td>
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<td>10</td>
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</tr>
<tr>
<td>2</td>
<td>10</td>
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<td>100</td>
<td>Neg*</td>
<td>10</td>
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</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.01</td>
<td>1,000</td>
<td>0.1</td>
<td>100</td>
</tr>
</tbody>
</table>

*Neg=negative.

Table 3—Threshold Concentration (μg/mL) That Produced an Immediate Skin Reaction

![Figure 3](image-url) **Figure 3.** The effect of premedications on the bronchial response to powdered green tea extract in patient 1. Aminophylline (300 mg, oral), salbutamol sulphate (1.5 mg, inhaled), and disodium cromoglycate (DSCG) (40 mg, inhaled) demonstrated an immediate inhibitory effect on the asthmatic reaction.

![Figure 4](image-url) **Figure 4.** The results of inhalation challenge to the extracts from black tea (diamonds) and oolong tea (triangles) in patient 1. The inhalation of both extracts provoked an immediate, but not late asthmatic reaction.

1804
way hyperreactivity may be related to a stimuli-causing reflex, β-adrenergic blockade, direct activation of the complement pathway, and a specific IgE-mediated response.13

Our patients exhibited an immediate skin and bronchial response to EGCg, and a positive reaction to EGCg was shown in a P-K test. These findings indicate that an IgE-mediated reaction is, at least in part, responsible for causing bronchial and dermal responses in green tea-induced asthma. Since EGCg is a small molecular weight substance (458 daltons) (Fig 5), one possibility is that it may act as a hapten by combining with a host protein(s).

Although coworkers of our patients in the same factories had no respiratory symptoms, it is probable that there are a certain number of workers suffering from the symptoms due to exposure to green tea dust in this area, where substantial amounts of green tea are produced and manufactured. The mechanisms and the prevalence of green tea-induced asthma should be investigated further because medications including administration of bronchodilators and DSCG enable patients to return to their workplaces when they cannot afford to change the job.

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