Drug-Induced Lymphocyte Stimulation Test is not Useful for the Diagnosis of Drug-Induced Pneumonia

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MATSUNO, O., OKUBO, T., HIROSHIGE, S., TAKENAKA, R., ONO, E., UENO, T., NUREKI, S., ANDO, M., MIYAZAKI, E. and KUMAMOTO, T. Drug-Induced Lymphocyte Stimulation Test is not Useful for the Diagnosis of Drug-Induced Pneumonia. Tohoku J. Exp. Med., 2007, 212 (1), 49-53 —— Diagnosis of drug-induced pneumonia, which represents pulmonary toxicity caused by certain drugs, is difficult, as a large number of different drugs can elicit various immune-mediated diseases with distinct pathomechanisms. The drug-induced lymphocyte stimulation test (DLST) is widely used for diagnosing drug-induced pneumonia in Japan. Recent reports, however, indicate that DLST is not reliable for diagnosis of drug-induced pneumonia. To diagnose drug-induced pneumonia, a provocation test with the suspected drug is the most reliable method of assessing the relationship between the drug and pneumonia. We examined the correlation between the DLST and the provocation test in 6 cases of suspected drug-induced pneumonia. DLST was performed in all of the patients. The causes of pneumonia in all patients were confirmed by a provocation test. The DLST was positive in 3 of 6 cases of suspected drug-induced pneumonia, but the suspected drugs were ruled out by the provocation test. If we had relied solely on the DLST, these 3 cases would have been labeled as false allergy. The results of the DLST did not coincide with the results of the provocation test in any of the cases. Our results suggest that the DLST is not useful for the diagnosis of drug-induced pneumonia. Following provocation with the causative drug, reappearance of pulmonary infiltration was not observed in any of the cases. These findings indicate that a carefully performed provocation test is the safe and most reliable method. ——— drug-induced lymphocyte stimulation test (DLST); drug-induced pneumonia; provocation test; challenge test; patch test

The drug-induced lymphocyte stimulation test (DLST) is widely used for the diagnosis of drug-induced pneumonia and liver injury in Japan, whereas in Western countries, DLST for specific drug hypersensitivity is considered to be unreliable or experimental (Beers and Berkow 1999; Mantani et al. 2003). DLST is used for the diagnosis of drug-induced pneumonia only in Japan (Kunichika et al. 2002). DLST for drug-induced pneumonia is based on Tamura’s criteria in Japan, but it is not usually confirmed by a re-challenge test (Tamura 1983). Tamura’s crite-
Provocation tests can cause severe lung injury. It has been reported that the DLST is not reliable, and instead a drug provocation test is proposed as a safe means for diagnosis of drug-induced pneumonia (Yasui and Fujimura 2003). There are several single case reports of drug-induced pneumonia in which both a provocation test and DLST were used, but a summary of these cases has not been reported in the English literature. Here, we describe for the first time the relationship between the results of the DLST and provocation test for the diagnosis of drug-induced pneumonia. We examined the correlation between DLST and provocation test in 6 cases of suspected drug-induced pneumonia.

**Methods**

**Patients**

Patient backgrounds and data are summarized in Table 1. The 5 patients with drug-induced pneumonia (4 patients with antibiotic-induced pneumonia and 1 patient with bucillamine-induced pneumonia) and one patient

<table>
<thead>
<tr>
<th>case</th>
<th>age (years)</th>
<th>sex</th>
<th>atopy</th>
<th>underlying disease</th>
<th>IgE (IU/ml)</th>
<th>WBC (/μl)</th>
<th>Eo (%)</th>
<th>TC (×10³/ml)</th>
<th>AM (%)</th>
<th>Lym (%)</th>
<th>Neut (%)</th>
<th>Eo (%)</th>
<th>CD4/8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55</td>
<td>F</td>
<td>(−)</td>
<td>None</td>
<td>&lt;18</td>
<td>34,060</td>
<td>0.4</td>
<td>6.3</td>
<td>68.4</td>
<td>5.2</td>
<td>0</td>
<td>26.4</td>
<td>1.89</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>F</td>
<td>(−)</td>
<td>RA</td>
<td>507</td>
<td>7,100</td>
<td>1.4</td>
<td>1.8</td>
<td>32.2</td>
<td>27.7</td>
<td>11.4</td>
<td>28.7</td>
<td>0.62</td>
</tr>
<tr>
<td>3</td>
<td>82</td>
<td>M</td>
<td>(−)</td>
<td>GC</td>
<td>1,376</td>
<td>12,000</td>
<td>8.1</td>
<td>3</td>
<td>54</td>
<td>0</td>
<td>1</td>
<td>45</td>
<td>5.1</td>
</tr>
<tr>
<td>4</td>
<td>59</td>
<td>M</td>
<td>(−)</td>
<td>None</td>
<td>816</td>
<td>7,900</td>
<td>8.4</td>
<td>6.04</td>
<td>39.3</td>
<td>27.4</td>
<td>0</td>
<td>33.3</td>
<td>0.88</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>M</td>
<td>(−)</td>
<td>None</td>
<td>1,170</td>
<td>14,100</td>
<td>0.9</td>
<td>4.13</td>
<td>49.6</td>
<td>45.6</td>
<td>4</td>
<td>0.8</td>
<td>0.42</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>M</td>
<td>(−)</td>
<td>AEP</td>
<td>468</td>
<td>6,900</td>
<td>6.7</td>
<td>7.98</td>
<td>26.6</td>
<td>5.9</td>
<td>2.6</td>
<td>65.5</td>
<td>2.84</td>
</tr>
</tbody>
</table>

RA, rheumatoid arthritis; GC, gastric cancer (post operation); AEP, acute eosinophilic pneumonia; TC, total cell counts; AM, alveolar macrophages; Lym, lymphocytes; Neut, neutrophils; Eo, eosinophils.
with acute eosinophilic pneumonia were diagnosed at the Oita University Faculty of Medicine Hospital and related hospitals from 2003 to 2006. A presumptive diagnosis of drug-induced pneumonia was made based on the following diagnostic criteria: fever or some respiratory symptoms, diffuse pulmonary infiltrates on chest x-ray films, prompt improvement after cessation of the causative drug, or absence of other possible causes.

**Bronchoalveolar lavage (BAL)**

BAL was performed in an affected lung segment using three 50-ml aliquots of saline solution. The differential cell count was determined by cytocentrifugation followed by Wright staining. Fluid samples were processed immediately for cytologic and microbiologic analysis. No organisms were cultured from the BAL culture. Cytology was negative for malignant cells.

**Determination of DLST**

Routine laboratory tests were performed in all cases at the diagnosis. DLST was performed in all of the patients within 1 week of admission. None of the patients was treated with glucocorticoids before the DLST was completed. The DLST was performed by SRL, Inc., which is a company that provides comprehensive laboratory testing service in Japan. In the DLST, mitogenic activity was quantified by $[^3]H$ thymidine incorporation. The SI is defined as the counts per minute obtained with the allergen divided by the counts per minute of the negative control. The DLST was considered positive if the SI was 180% or greater.

**Provocation test**

According to Yasui’s criteria (Yasui and Fujimura 2003), provocation tests were performed using the same drug and route of administration as in the initial case. A drug provocation test was performed with careful observation and under informed consent. These tests were started with the smallest dosage that could be achieved by separating a preparation of the suspected drug, increasing the dose step by step (not gradually) at daily intervals until reaching a normal daily dose or until symptoms occurred, (e.g., 10% of single dose of medicine, then single dose of medicine, up to a normal daily dose).

The causes of pneumonia in all patients were confirmed by the provocation test, and withdrawal of the causative drugs led to a favorable outcome without specific treatment.

**RESULTS**

Patient background is summarized in Table1. None of the patients had a history of atopic disease. Immunoglobulin E levels were increased, however, in 5 cases. Whether atopic patients are more prone to the development of drug-induced pneumonia remains unknown. Based on our experience, however, the incidence of drug-induced pneumonia is not higher in atopic patients than in non-atopic patients. In all but 1 case, the drug-induced pneumonia was eosinophilic pneumonia. Intralobular septal thickening and ground-glass opacity were frequent on CT scans examination in our drug-induced pneumonia patients. Drug-induced fever was observed in all patients. The DLST was positive in 3 cases, but the suspected drugs were ruled out by the provocation test (Table 2). The provocation test was performed 3 to 6 weeks after initial symptom. If we had relied solely on the DLST, these 3 cases would have been labeled as a false allergy. Especially in case 6, the pneumonia was not drug-induced. The patch test was negative in all cases.

**DISCUSSION**

DLST aims to detect circulating drug-specific memory T cells, which proliferate following drug stimulation. The usefulness of DLST is established in patients with different types of drug eruptions (Luque et al. 2001; Pichler and Tilch 2004). The DLST has a general sensitivity of 60% to 70%, whereby this specificity is mainly based on the analysis of β-lactam hypersensitivity (Luque et al. 2001). The DLST is not associated with the severity of clinical symptoms, as it only reflects a high precursor frequency of drug-specific T-cells, which is not necessarily associated with severe clinical symptoms. In contrast to drug-induced eruption, the usefulness of DLST in drug-induced pneumonia has not been established (Pichler and Tilch 2004).

DLST is used for the diagnosis of drug-induced pneumonia and drug-induced hepatitis only in Japan (Kunichika et al. 2002; Mantani et al. 2003). Recent reports indicated that the DLST is unreliable for the diagnosis of drug-induced...
liver injury (Mantani et al. 2003). DLST for drug-induced pneumonia is based on Tamura’s criteria in Japan (Tamura 1983). There are, however, certain problems; Tamura’s criteria have not been confirmed by re-challenge test and patch test; DLST was used only in 4 of 10 cases of suspected drug-induced pneumonia.

The results of the DLST did not correlate with the results of the provocation test in any of the cases. The positive results of provocation test were observed within 6 weeks in our cases. The problem of false positives and false negatives in the DLST in drug-induced pneumonia has not been reported in the English literature. Our results suggest that the DLST is not useful for the diagnosis of drug-induced pneumonia especially for drug-induced eosinophilic pneumonia, although our study is limited due to the small number of subjects and therefore the findings are only preliminary.

Drug-induced skin eruptions were observed in 3 cases. These patients have eruption on admission. Following provocation with the causative drug in these cases, dermatologic manifestations reappeared without fever or pulmonary symptoms. Wengrower et al. (1986) also reported

<table>
<thead>
<tr>
<th>case</th>
<th>Days until onset</th>
<th>Patch test</th>
<th>DLST (positive; SI)</th>
<th>DLST (negative; SI)</th>
<th>provocation test</th>
<th>cause</th>
<th>timing at this test</th>
<th>dosage</th>
<th>symptom</th>
<th>interval (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>acetaminophen1 (441%)</td>
<td>theophylline (144%)</td>
<td>minocycline</td>
<td></td>
<td>4 weeks after initial symptom</td>
<td>single medication dose</td>
<td>F, WBC, CRP, AaDo2</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>1w</td>
<td>negative</td>
<td>(-)</td>
<td>minocycline</td>
<td></td>
<td>buclillamine1 (132%)</td>
<td>4 weeks after initial symptom</td>
<td>single medication dose</td>
<td>E, CRP</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>1m</td>
<td>negative</td>
<td>(-)</td>
<td>minocycline</td>
<td></td>
<td>moofezolac2 (112%)</td>
<td>6 weeks after initial symptom</td>
<td>10% of the single medication dose</td>
<td>F, WBC, AaDO2, CRP</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>1m</td>
<td>negative</td>
<td>(-)</td>
<td>minocycline</td>
<td></td>
<td>amoxicillin1 (123%)</td>
<td>3 weeks after initial symptom</td>
<td>10% of the single medication dose</td>
<td>E</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>9d</td>
<td>N.D</td>
<td>acetaminophen2 (209%)</td>
<td>clindamycine2</td>
<td></td>
<td>tosufloxacine (143%)</td>
<td>6 weeks after initial symptom</td>
<td>10% of the single medication dose</td>
<td>E</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>1w</td>
<td>negative</td>
<td>(-)</td>
<td>clindamycine1</td>
<td></td>
<td>Diclofenac sodium3 (111%)</td>
<td>6 weeks after initial symptom</td>
<td>10% of the single medication dose</td>
<td>E</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>(-)</td>
<td>N.D</td>
<td>ranitidine1 (342%)</td>
<td>non-drug</td>
<td></td>
<td>magnesium aluminium silicate2 (146%)</td>
<td>3 weeks after initial symptom</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

F, fever; L, lung infiltration; E, eruption; DLST, Drug-induced lymphocyte stimulation test; AaDo2, alveolar-arterial difference for oxygen partial pressure.

Superscript indicates the provocation test order.
that antibiotic challenges with penicillin caused
drug-induced skin eruptions without reactivation
of pneumonia. These results suggest that the
threshold for causing drug-induced skin eruptions
is different from that for drug-induced fever and
drug-induced pneumonia.

Following provocation with the causative
drug, the reappearance of pulmonary infiltration
was not observed in any of the cases. These find-
ings indicate that a carefully performed perfor-
ance of provocation test can be safe. In addi-
tion, there are no reports of severe or fatal lung
injury induced by a provocation test. Caution
must be used, however, in interpreting the DLST
for the diagnosis of drug-induced pneumonia.

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