

Comparative Study of the Usefulness of the Drug-Induced Lymphocyte Stimulation Test and the Leukocyte Migration Test in Drug Allergies

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In 133 patients suspected of hypersensitivity to drugs and 102 control patients without hypersensitivity to drugs, the identification of allergenic drugs was performed by the drug-induced lymphocyte stimulation test (DLST) and the leukocyte migration test (LMT) to compare their usefulness in identifying drug allergies. In the 133 subject patients, the positive rate was 24.8% on the DLST and 60.9% on the LMT (agreement rate; 77.4%); thus, the LMT showed a significantly higher positive rate than the DLST ($p < 0.000001$, χ^2 -test). In the 102 control patients, the positive rates on the DLST and LMT were 6.9%. In addition, the LMT showed a higher positive rate than the DLST for many hypersensitivity symptoms such as skin eruptions and hepatic injury, and for many drug efficacy categories of the suspected drugs such as antibacterial drugs, etc. Furthermore, the positive rate of the DLST did not change when adjusted for the patients' serum and sex, while that of the LMT increased when adjusted for the patients' serum and was found to be higher in females than in males. Our findings indicate that the LMT may be more useful than the DLST in identifying the causative drug in drug allergies and that its interpretation is influenced by the patient's serum and sex.

Key words drug allergy; leukocyte migration test; drug-induced lymphocyte stimulation test; age and sex; hypersensitivity symptom; causative drug

The detection of the causative drug in drug allergies is essential in order to prevent secondary allergic reactions and is also extremely important so that appropriate medical treatment can be administered when such reactions occur. However, *in vivo* tests are problematic in that challenge tests are too risky, the intradermal skin test is a low-sensitivity test that has few risks but may cause penicillin-induced anaphylactic shock,¹⁾ and the patch test is also a low-sensitivity test but may induce contact dermatitis.²⁾ Therefore, more effective *in vitro* tests, which pose few risks to patients, are needed.

In vitro, specific antibodies for the allergens in patient's serum can be detected by enzyme-linked immunosorbent assay (ELISA) or the radioallergosorbent test (RAST). The specific immunoglobulin E (IgE), IgG, and IgM antibodies are measured by ELISA and specific IgE antibodies are identified by the RAST. The advantages of these methods detecting specific antibodies are that their test samples, *i.e.*, the patient's serum can be preserved for a long time. On the other hand, their disadvantages are that they show a low rate of detection and are applied to only a part of causative drugs such as penicillin antibiotics and only specific allergic symptoms such as anaphylactic shock and hemolytic anemia.^{3,4)} Furthermore, they must be performed as soon as the allergic symptoms develop because the specific antibodies disappear in about a month.

There has been a recent increase in the use of methods to prove cell-mediated immunity, that is, the presence of drug-sensitized lymphocytes. Such methods include the drug-induced lymphocyte stimulation test (DLST) and the leukocyte migration test (LMT).^{3,5–11)} The advantages of these methods detecting drug-sensitized lymphocytes are that they show a higher rate of detection than the methods detecting specific antibodies and can be applied to many causative drugs and

allergic symptoms.^{3,4)} On the other hand, their disadvantages are that their test samples, *i.e.*, the patient's mononuclear cells can't be preserved for a long time. Therefore, the methods detecting drug-sensitized lymphocytes may be more useful to detect the causative drugs in many drug allergies than the methods detecting specific antibodies if their tests are putted into practice soon after drawing bloods. However, there have been few reports to date comparing the effectiveness of these tests. Therefore, we performed both the DLST and LMT in patients suspected of drug hypersensitivity as well as in patients without drug hypersensitivity and compared the usefulness of the two tests.

MATERIALS AND METHODS

Subjects (Patients Suspected of Drug-Induced Hypersensitivity) The subjects consisted of 133 patients (60 males and 73 females; age range 1–84 years; mean age 47.8 years) who were suspected of suffering from drug-induced hypersensitivity. These patients had manifested hypersensitivity symptoms while taking drugs and were unlikely to suffer worsening of the underlying disease, and their symptoms improved when they stopped taking the drugs in question.

With respect to symptomatic categories of hypersensitivity, the subjects included 94 cases (70.7%) of skin eruption, 20 cases (15.0%) of liver damage, 11 cases (8.3%) of blood disorders, 6 cases (4.5%) of lung disorders, 5 cases (3.8%) of anaphylactic shock, 3 cases (2.3%) of fever, and 4 cases (3.0%) of other symptoms (renal damage, digestive disorders, ocular hyperemia and rhinitis). Some patients were counted in two or more categories of concomitant symptoms. Nine patients (6.8%) had a previous medical history of drug-induced hypersensitivity.

In order to perform both the DLST and LMT, we took

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25 ml of blood samples (20 ml of heparinized blood for the preparation of mononuclear cells and 5 ml of non-heparinized blood for the preparation of serum) from the peripheral veins for adults and 5–10 ml for infants.

Informed consent was obtained from all subjects. Our study was judged and approved about study ethics from the appropriate judging committee of the clinical examination in Suibarago Hospital.

Controls (Control Patients and Volunteers) The control patients were 102 patients (48 males and 54 females; age range 19–77 years; mean age 48.5 years) with no medical history of hypersensitivity to drugs. Six additional male volunteers with no medical history of hypersensitivity to drugs (age range 29–50 years; mean age 37.0 years) were tested to determine the control values of the DLST and LMT. Informed consent was obtained from all control patients and volunteers.

The Tested Drugs The tested drugs were those suspected of being causative drugs in the 133 subjects suspected of hypersensitivity to drugs. They included 184 kinds of 420 agents: antibacterial drugs, 124 agents (41 kinds); central nervous system drugs, 108 agents (52 kinds); respiratory organ drugs, 38 agents (14 kinds); digestive organ drugs, 37 agents (18 kinds); cardiovascular drugs, 33 agents (21 kinds); and other drugs, 80 agents (38 kinds).

The tested drugs caused no hypersensitivity symptoms in the 102 control patients when used for more than one week.

In addition, we used phytohemagglutinin (PHA), that is, mitogen, as a positive control in all tests. When the test showed a negative to PHA in the DLST and the LMT, we thought that there might be a trouble in the test itself and tested it again.

Antigen Preparation of the Tested Drugs The tested drugs were prepared as antigens with and without each patient's serum. In the antigens prepared with serum, we dissolved and diluted the original pharmaceutical powder in Hanks' balanced salt solution (HBSS). We then mixed 100 μ l of the drug solution with 100 μ l of the patient's serum as the antigen solution. In the antigens without serum, 100 μ l of the drug solution was mixed with 100 μ l of TC-199 culture medium (Gibco, Grand Island, NY, U.S.A.) containing 10 mM Hepes buffer and 10% inactivated horse serum. Insoluble drugs were dissolved in dimethyl sulfoxide (DMSO) and diluted with HBSS. The last concentration of DMSO was prepared at less than 0.25%.

The antigen concentrations of each tested drug were set to match the maximum blood concentration of a single dose of the drug. However, drugs that had shown an influence on the immune system were tested at concentrations of less than the maximum blood concentrations. Specifically, non-steroidal anti-inflammatory drugs (NSAIDs) were prepared at 1/2 the level of a single dose, and steroids and antineoplastics were prepared at 1/4 the level of a single dose. PHA was dissolved by HBSS and was prepared at a concentration of 1 μ g/ml. The antigen solutions of the tested drugs were created immediately prior to the test.

Preparation of Mononuclear Cells Heparinized blood was obtained from the peripheral veins of the patients, and mononuclear cells were obtained from the intermediate layer by centrifugation using Ficoll-Paque liquid (Pharmacia, Biotech Uppsala, Sweden). After the cells were washed 3

times with HBSS, they were prepared to produce a cell count of 1.25×10^6 cells/ml in the culture medium.

The Drug-Induced Lymphocyte Stimulation Test (DLST) We added 160 μ l of the patient's mononuclear cell suspension to 40 μ l of the antigen solution of the drug in a 0.5-ml microtube. The reaction solution was incubated in a 5%-CO₂ incubator at 37 °C for 48 h. Ten microliters of ³H-thymidine (18.5 kBq/tube) was then added, and the solution was incubated for an additional 24 h.

The incubated cells were washed with HBSS and lysed. Cytolysis was performed following the method described by Su.⁹⁾ Briefly, 100 μ l of 0.1-N NaOH were added to the cells, which were then maintained in warm water at 60 °C for 30 min. After cooling on ice, the sediments were obtained by centrifugation. We then added 1.25 ml of Scintisol and the cells were left in the dark at 4 °C for 1 h. The quantity of ³H-thymidine absorbed by the cells was measured using a NaI scintillation counter (2200CA; Packard Meriden, U.S.A.). All tests were carried out in triplicate.

The testing criteria were as follows. The stimulation index (SI) was calculated by the following formula:

$$SI = \frac{\text{mean value of } ^3\text{H-thymidine of the group with drug}}{\text{mean value of } ^3\text{H-thymidine of the group without drug}} \times 100.$$

The results were considered positive if the SI was higher than 200 and showed a significant difference from the SI of at least 3 volunteers ($p < 0.05$, Student's *t*-test).¹⁰⁾

The Leukocyte Migration Test (LMT) The LMT was performed following a modified version of the agarose plate method described by Clausen.¹¹⁾ Briefly, 800 μ l of the patient's mononuclear cell suspension was added to 200 μ l of the antigen solution in a 4-ml tube and incubated in a 5%-CO₂ incubator at 37 °C for 48–96 h. The supernatant fluid was separated by centrifugation, frozen, and stored at –20 °C.

The migration test was carried out as follows. Leukocyte-rich plasma was extracted from heparinized peripheral venous blood of normal donors after sitting at 37 °C for 45–60 min in the presence of physiological saline containing 5% dextran. Polymorphonuclear (PMN) leukocytes were obtained from the sediments by centrifugation using Ficoll-Paque liquid and were washed with physiological saline. The red cells in the separation were lysed by osmotic shock. The PMN leukocytes were suspended and adjusted to a density of 2.5×10^8 cells/ml in the supernatant liquid, which was obtained by the reaction of the mononuclear cell suspension and the antigen solution. Seven-microliter samples of the suspensions were placed in the wells of an agarose plate prepared from a culture medium containing 1% agarose and, after incubation in a 5%-CO₂ incubator at 37 °C for 12–24 h, the area of migration of the PMN leukocytes was measured using an immunoviewer. Migration analysis of 6 wells per test was carried out. The migration index (MI) was calculated by the following formula:

$$MI = \frac{\text{migration area in supernatant liquid with drug}}{\text{migration area in supernatant liquid without drug}} \times 100.$$

Results were considered to be positive if the MI was above 115 or below 85 and showed a significant difference from the MI of at least 3 volunteers ($p < 0.05$, Student's *t*-test).^{6–8,12,13)} MI values above the normal range (defined as the MI of vol-

unteers) was interpreted as indicating the detection of leukocyte migration activating factor (LMAF) and MI values below normal range as indicating the detection of leukocyte migration inhibitory factor (LMIF).^{12,13)}

Statistical Analysis All results were analyzed statistically using the χ^2 -test.

RESULTS

Positive Rates of the DLST and LMT in Subjects and Controls Table 1 shows the positive rates of the DLST and LMT in the 133 subjects suspected of hypersensitivity to drugs and in the 102 controls without medical history of hypersensitivity to drugs. In the present subjects, the positive rate was 24.8% for the DLST and 60.9% for the LMT, with the LMT showing a significantly higher positive rate than the DLST ($p<0.000001$, χ^2 -test). The agreement rate (=the number of testes giving agreement between the DLST and LMT/the number of total tests $\times 100$) of the DLST and LMT was 77.4% in 438 tests.

The positive rates of the DLST and LMT in the control subjects were both approximately 6.9% and there was no significant difference between the tests. The agreement rate of the DLST and LMT was 99.0% in 102 tests. Both tests showed significantly higher positive rates in test subjects than in controls (DLST, $p<0.0005$; LMT, $p<0.000001$, χ^2 -test).

Positive Rates of the DLST and LMT with and without Serum Figure 1 shows the positive rates of the DLST and LMT with and without serum in the 133 subject patients suspected of hypersensitivity to drugs. The positive rates of the

DLST with and without serum were 14.3% and 15.8%, respectively, and those of the LMT were 45.1% and 32.3%. Thus, the LMT had a significantly higher positive rate than the DLST in both cases ($p<0.005$, χ^2 -test). The DLST showed no significant difference between the two cases, while the LMT showed a significantly higher positive rate with serum than without it ($p<0.05$, χ^2 -test).

Positive Rates of the DLST and LMT by Sex and Age Figure 2 shows the positive rates of the DLST and LMT by sex. The proportion of positive results on the DLST was 21.7% in 60 males and 27.4% in 73 females, and there was no statistically significant difference between males and females. In contrast, the proportion of positive results on the LMT was 46.7% in males and 69.8% in females, with a significantly higher positive rate in females than in males ($p<0.01$, χ^2 -test).

Figure 3 shows the positive rates of the DLST and LMT in subjects aged 64 years or less and in those aged 65 years or more. The proportion of positive results on the DLST was 27.0% in 89 young subjects and 20.5% in 44 older subjects, and there was no significant difference between the two groups. On the LMT the proportion of positive results was 61.8% in young subjects and 59.1% in older subjects, again with no significant difference between the two groups.

Positive Rates of the DLST and LMT According to Hypersensitivity Symptoms Table 2 shows the positive rates of the DLST and LMT according to hypersensitivity symptoms; note that a single subject may be counted in two or more categories in cases of concomitant symptoms. For the 94 patients suspected of drug eruption, the positive rates

Table 1. The Proportion of Positive Drug-Induced Lymphocyte Stimulation Test and Leukocyte Migration Test in 133 Subject Patients and 102 Control Patients

Patients	No. of cases	DLST ^{a)}		LMT ^{b)}		Agreement rate (%)
		Positive	Rate (%)	Positive	Rate (%)	
Subject patients	133	33	24.8	81	60.9	77.4
Control patients	102	7	6.9	7	6.9	99.0

The agreement rate (%)=the number of tests giving agreement (positive or negative) between the DLST and LMT/the number of total tests (the number of the tested drugs $\times 2$ [with or without patient's serum]) $\times 100$. a) DLST: drug-induced lymphocyte stimulation test, this test was regarded as positive if the stimulation indices of the subject patients or the control patients were higher than 200 and had a significant difference from the SI of 3 volunteers ($p<0.05$, t -test). b) LMT: leukocyte migration test, this test was regarded as positive if the migration indices of the subject patients or the control patients were above 115 or below 85 and had a significant difference from the SI of 3 volunteers ($p<0.05$, t -test). * Significantly different; χ^2 -test.

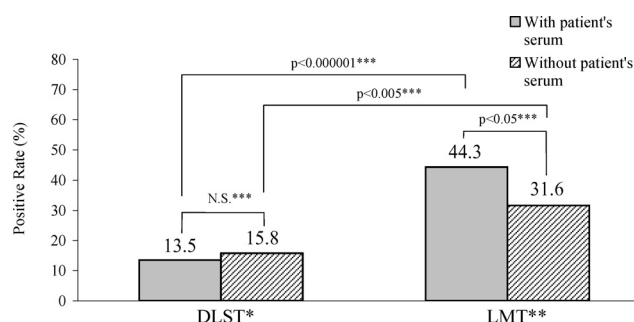


Fig. 1. The Positive Rates of Drug-Induced Lymphocyte Stimulation Test and Leukocyte Migration Test with or without Patient's Serum

*DLST: drug-induced lymphocyte stimulation test, **LMT: leukocyte migration test, ***significantly different; the Student's χ^2 -test.

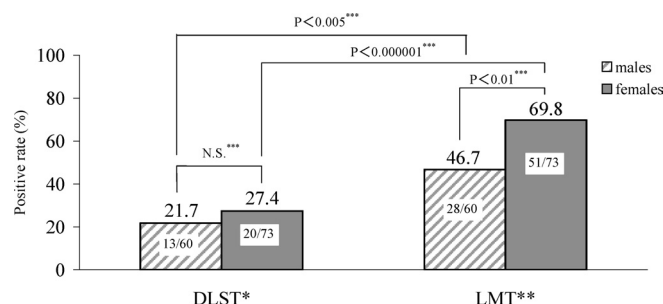


Fig. 2. The Positive Rates of Drug-Induced Lymphocyte Stimulation Test and Leukocyte Migration Test, Broken Down into Sex

*DLST: drug-induced lymphocyte stimulation test, **LMT: leukocyte migration test, ***significantly different, χ^2 -test.

Table 2. The Rate of Positive Drug-Induced Lymphocyte Stimulation Test and Leukocyte Migration Test, Broken Down into Hypersensitivity Symptomatic Categories

Hypersensitivity symptom	No. of cases	Incidence (%)	DLST		LMT	
			Positive	Rate (%)	Positive	Rate (%)
Skin eruption	94	70.7	24	25.5	56	59.6
Liver damage	20	15.0	3	15.0	12	60.0
Blood disorder	11	8.3	3	27.3	7	63.6
Pulmonary disorder	6	4.5	1	16.7	5	83.8
Anaphylactic shock	5	3.8	1	20.0	2	40.0
Fever only	3	2.3	2	66.7	2	66.7
Others	4	3.0	0	0	2	50.0

In this table, the same patients are counted in two or more categories in cases of concomitant symptoms. Significantly different; χ^2 -test.

Table 3. The Rate of Positive Drug-Induced Lymphocyte Stimulation Test and Leukocyte Migration Test, Broken Down into Drug Efficacy Categories

Drug efficacy	No. of suspected drugs	Incidence (%)	DLST		LMT	
			Positive	Rate (%)	Positive	Rate (%)
Antibacterial drugs	124	29.5	18	14.5	48	38.7
Central nerves system drugs	108	25.7	12	11.1	19	17.6
Respiratory organs drugs	38	9.0	0	0	6	15.8
Digestive organ drugs	37	8.8	2	5.2	3	7.9
Cadiovascular drugs	33	7.9	1	3.0	4	12.1
Others	80	19.0	8	10.0	12	15.0
Total	420		41	9.8	92	21.9

Significantly different: χ^2 -test a: $p<0.00005$, b: $p<0.0005$, c: $p<0.01$, d: $p<0.005$.

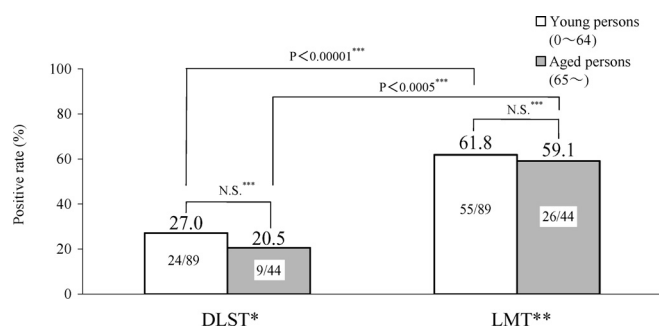


Fig. 3. The Positive Rates of Drug-Induced Lymphocyte Stimulation Test and Leukocyte Migration Test in Young Persons and Aged Persons

*DLST: drug-induced lymphocyte stimulation test, **LMT: leukocyte migration test, *** significantly different, χ^2 -test.

were 25.5% on the DLST and 59.6% on the LMT; the LMT therefore had a significantly higher positive rate than the DLST ($p<0.00001$, χ^2 -test). For the 20 patients suspected of drug-induced hepatic injury, the positive rates were 15.0% on the DLST and 60.0% on the LMT, and the LMT again had a significantly higher positive rate than the DLST ($p<0.005$, χ^2 -test). With respect to the other hypersensitivity symptoms, the LMT showed a higher positive rate than the DLST for many of the symptoms, however the difference between the two tests did not reach statistical significance. It is important to keep in mind, however, that in these cases, the total number of subjects was small.

Positive Rates of the DLST and LMT According to

Drug Efficacy Categories Table 3 shows the positive rates of the DLST and LMT for 420 suspected drugs according to drug efficacy categories. The suspected drugs were categorized as 29.5% antibacterial drugs, 25.7% central nervous system drugs, 9.0% respiratory organ drugs, 8.8% digestive organ drugs, 7.9% cardiovascular drugs and 19.0% other drugs. For the 124 suspected antibacterial drugs, the positive rates were 14.5% on the DLST and 38.7% on the LMT, with the LMT having a significantly higher positive rate than the DLST ($p<0.00005$, χ^2 -test). Although there were no significant differences between the DLST and the LMT for the other drug categories, the LMT showed higher positive rates than the DLST for many drug categories.

Comparing the results of each test individually, the DLST showed no significant differences among the drug categories, while the LMT showed a significant difference between antibacterial drugs and the other drug categories ($p<0.00005$ —0.01, χ^2 -test).

DISCUSSION

Sensitivity of the DLST and LMT with Respect to Drug Allergies Both the DLST and the LMT are bioassays used to prove the presence of drug-sensitized lymphocytes. The DLST demonstrates the growth of sensitized lymphocytes by antigen stimulation of the drug, while the LMT identifies cytokines or chemokines produced by the sensitized lymphocyte under the antigen stimulation of the drug.

At present, the DLST is the most generally used *in vitro* test for the detection of the causative drug in drug allergies and cases of drug allergies examined by means of the DLST have been extensively reported.^{14–20} Nevertheless, there are some doubts concerning the validity of the DLST.^{21–25}

In the present comparative study of the LMT and DLST for 133 patients suspected of hypersensitivity to drugs, the LMT showed positive rates twice as high as those of the DLST, indicating that the LMT is more sensitive than the DLST in identifying the causative drug in drug allergies.

Specificity of the DLST and LMT with Respect to Drug Allergies It is important to examine the possibility of false positives in order to appropriately evaluate the specificity of a test. It is undeniable that both the DLST and LMT may have false positives, and indeed, a certain number of false positives have been reported for these tests.^{26–31}

In our present study, both the DLST and the LMT showed a positive rate of 6.9% for the 102 control patients, and both tests also showed significantly higher positive rates in the test subjects than in controls. Accordingly, these results indicate that both tests may have sufficient specificity for use in patients with drug allergies. However, it is necessary to consider that they may also have a false positive rate of approximately 7%. Moreover, the DLST showed a difference of only 17.9% in the positive rate between test subjects and controls, and is therefore believed to be less reliable in identifying the causative drug in patients with drug allergies. In contrast, the LMT showed a difference of 54.0% in the positive rate between test subjects and controls, and is therefore believed to be very reliable in identifying the causative drug in patients with drug allergies.

Influence of Sex and Age on the DLST and LMT In the present study, we found no significant differences in the positive rates on the DLST between males and females, while females showed a significantly higher positive rate than males on the LMT. Thus, females may have higher reactivity than males on the LMT; that is, females may produce higher levels than males of the cytokines or chemokines from drug-sensitized lymphocytes.

With respect to the influence of age on the DLST and LMT, we found no significant differences in the positive rates between younger and older subjects on either test. Thus, the present results indicate that the reactivity of the DLST and LMT are not significantly influenced by aging. Nevertheless, other researchers have previously reported that the DLST was influenced by aging in experiments in which lymphocytes were stimulated by PHA, that is, by mitogen.³² In the present study, the DLST showed a difference of 6.5% between younger and older subjects, however this difference did not reach statistical significance. Nevertheless, we believe that this issue warrants further investigation. In the present study, the LMT had significantly higher positive rates, indicating higher reactivity, than the DLST for all subjects regardless of age.

Influence of Serum on the DLST and LMT In the present study, we examined the influence of the patient's serum on the DLST and LMT. The DLST showed no difference in the positive rate between tests with and without serum, while the LMT showed a significantly higher positive rate with serum than without it. Thus, the reactivity of the LMT is enhanced by the addition of the patient's serum. This

may be due to the possible presence of a potentiator of leukocyte migration factor in the serum. It has been reported that some cytokines are included in patient serum.^{33,34} This suggests that these cytokines may enhance the production and effect of leukocyte migration factor. In our previous studies, we have reported that the patient's serum enhances the production of LMAF in drug allergies, that the production of tumor necrosis factor- α (TNF- α) is enhanced by the addition of the patient's serum and that interleukin (IL)-1 and IL-2 also contributes to the production of LMAF.^{35,36} Moreover, we have already found that IL-1 and TNF- α have a leukocyte migration activating effect in unpublished study. Therefore, it is considered that LMAF is very likely to be IL-1 or TNF- α . Besides, there have been many reports that TNF- α plays a large role in drug allergies.^{37–42} These previous results, together with the present results, indicate that some cytokines may be present in patient serum and may enhance the production and effect of leukocyte migration factor in drug allergies.

Sensitivity of the DLST and LMT Based on Hypersensitivity Symptoms Since mononuclear cells are used as the target cells in both the DLST and in the LMT, it is thought that both tests may be useful in identifying the causative drug of many hypersensitivity symptoms, such as liver damage or pneumonia, as well as skin eruptions.^{9,12,43}

In the present study, the LMT showed a positive rate twice as high as that of the DLST for skin eruptions, and 4 times higher for liver damage. In addition, the LMT showed higher positive rates than the DLST for many other hypersensitivity symptoms. These results indicate that the LMT may be significantly more sensitive than the DLST in detecting the causative drugs of many hypersensitivity symptoms that arise in drug allergies.

Sensitivity of the DLST and LMT Based on Drug Antibacterial drugs and central nervous system drugs accounted for over half of all suspected drugs. Antibacterial drugs were the category most detected by both tests, and were detected significantly more than the other drug efficacy categories by the LMT. Thus, antibacterial drugs may have high allergenicities, possibly due to their high protein binding abilities⁴⁴ and the adjuvant effects by bacterial products.^{45,46}

Given that the LMT showed a positive rate twice as high as that of the DLST for antibacterial drugs as well as higher positive rates than the DLST for all drug efficacy categories, the LMT may be more useful than the DLST in detecting allergens.

When the LMT showed negatives, the following three reasons are considered. The first reason is that there may be no cause-effect relationship between drug and hypersensitivity symptom. The second reason is that hypersensitivity symptom may be induced by the pharmacological effect of drug itself (pseudoallergic reaction to drug). The third reason is that the cytokines and chemokines which influence on leukocyte migration may be not involved in the drug allergic reaction.

In this time, it is very difficult to find out the reason in many cases. For example, challenge test is very difficult to be performed because it is too risky. However, we think that it is necessary to analyze in detail about the LMT-negative cases from the clinical and basic viewpoint after this.

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