A pharmacological and immunopharmacological study on allergic reaction induced by rosin

TAKAFUMI KATUTA and TOMOKO OHSUMI

Abstract: Dental drugs and dental materials induce several allergic reactions, but our knowledge about the causal substances and the mechanism of allergic reactions is still limited. In this study, we conducted pharmacological and immunopharmacological examinations of rosin, which is included in many preparations, such as pulp capping agents and root canal filling agents. First, we used the adjuvant and patch test in guinea pigs. Twenty-four hours after challenge, erythema was noted in all 3 animals to which we applied 25, 12.5, and 5% rosin and edema was also noted in one of the animals to which we applied 25% rosin, second, to examine places where rosin causes type IV allergic reactions, the ear swelling test in mice was conducted. At 5% rosin, the difference was significant; at 25% rosin, the swelling rate was as high as 55%. From the above two examinations, the results indicated that contact dermatitis, which is type IV allergy, may be induced by dental drugs and dental materials containing rosin, and the induction of allergy depended on the concentration of rosin. Third, the kinetics of immunocytes was examined in mice to which we applied 25% rosin in the ear swelling test. The distribution of immunocytes in the ears and lymph nodes was immunohistochemically examined by the indirect enzyme-labeled antibody method. Thy-1.2, CD4, interleukin-2 receptor (IL-2R), IL-4R and CD8-positive cells were detected by light microscopy. The number of IL-4R and CD8-positive cells was increased in the effector phase. It is suggested that Th-2 rather than Th-1 was involved in allergy induced by rosin, and clarified that CD8 positive cells were involved.

Key words: Rosin, Contact dermatitis, Ear swelling, Helper T cell, Cytotoxic T cell

Introduction

Various allergic diseases such as bronchial asthma, pollinosis and atopic dermatitis have been receiving social attention. There have been a number of reports on allergies in dental treatment, not only in patients who were administered drugs for endodontics and periodontics or received treatments using dental materials such as metals used as prosthesis but also in those who are engaged in dental treatment by exposure to dental materials such as resin and latex gloves1-3). Although there have been a number of reports on metal allergy caused by metals in a prosthesis4-7) and latex allergy which has drawn attention recently8-10), only a small number of studies have been made on allergy against drugs used for endodontics and periodontics, and our knowledge about the causal substances and the mechanism of allergic reactions is still limited11).

We have been interested in allergy against dental drugs and dental materials and have conducted pharmacological and immunopharmacological examinations of various components of dental drugs and dental materials that are suspected to cause allergic reactions. In the course of the study, rosin, which is included in many dental drugs and dental materials, came to draw our attention. Rosin, which is described in the Thirteenth Revision Japanese Pharmacopoeia, is a mixture of resin acids with abietic acid as the main component12-14). In the dental field, this drug is included in many preparations such as pulp capping agents and root canal filling agents at a rate of 15–
30%. This is also widely used as an adhesive agent of tape.

Most of allergic reactions caused by dental drugs and dental materials can be classified into contact dermatitis. To examine whether rosin elicits contact dermatitis, skin sensitization test was conducted. From many different methods for skin sensitization test, we adopted the adjuvant and patch test, which is one of the methods described in the guideline for non-clinical tests of medicines by the Ministry of Health and Welfare. Allergic reactions are generally classified into type I to type IV. Contact dermatitis belongs to type IV allergic reactions due to cellular immunity in which T cells play the central role. To examine whether rosin causes type IV allergic reactions, the ear swelling test was conducted, and kinetics of various kinds of T cells were investigated by immunohistochemical methods.

With the above 3 methods, we attempted to elucidate the occurrence and the mechanism of allergic reactions by rosin.

Materials and Methods

I. Skin sensitization test by the adjuvant and patch test

1. Materials

1) Experimental animals

Specific pathogen-free (SPF) Hartley guinea pigs (male), weighing 300-350g, (Kyudo Inc.) were used after a week of preliminary rearing.

2) Chemicals

Rosin (reagent grade, Nacalai Tesque Inc.), Sudan I (1st grade, Wako Pure Pharmaceuticals Inc.), and acetone (infinity pure reagent grade, Wako Pure Pharmaceuticals Inc.) were used. Sudan I was used as a positive control, and acetone as a solvent and a negative control.

2. Methods

Many different methods are available for skin sensitization test. Considering the safety assessment methods for dental materials and for biomaterials used in medical/dental fields, we adopted the adjuvant and patch test according to the method of Sato et al. (Fig. 1). It is based on the guideline for non-clinical tests of medicines by the Ministry of Health and Welfare. Applying adjuvant makes it possible to detect even the weak allergens and to evaluate their allergenicity. In addition the guinea pig is found to be an excellent laboratory model for examining the skin test response.

![Fig. 1 Schedule of adjuvant and patch test](image)

1) Induction

On day 1, the dorsal cervical region of the animals was shaved with an electric clipper and a razor, and the animals were injected with an emulsion made by 1:1 mixture of Freund's complete adjuvant (FCA, Difco Lab.) and distilled water as an immunopotentiator, 0.1mL each at 4 corners of a 2×4 cm rectangle. Shallow wounds in the shape of grid (#) were made at the injection sites, and a piece of tape for the patch test (Torii Pharmaceuticals Inc.) with 0.1g of ointment made by mixing a test drug at a concentration of 50% for rosin and acetone, and 0.1% for Sudan I into yellow petrolatum (reagent grade, Wako Pure Pharmaceuticals Inc.) was applied onto each wound, and covered with a surgical drape (Steri-Drape, 3M) for 24 hours. On days 2 and 3, the drug was applied to the same sites by the same method, and on day 4, application of the drug was terminated.

On day 8, an appropriate amount of yellow petrolatum mixed with sodium lauryl sulfate (1st grade, Wako Pure Pharmaceuticals Inc.) at a final concentration of 10% was applied. On day 9, the above ointment was removed, and a piece of filter paper (2.5×4 cm, Advantec. co.) on which 0.2g of the same ointment as used on days 1-3 was applied to the injection site with a piece of surgical tape (Blenderm, 3M) and left for 48 hours.

2) Challenge

On day 22, after removing hair and shaving
Allergic reaction induced by rosin

1. Materials

1) Experimental animals

SPF BALB/cAnN mice (male, 5-week-old) (Charles River Japan Inc.) were used after a week of acclimation.

2) Chemicals

Rosin and acetone were the same in quality as the ones used in experiment I. Oxazolone (Sigma) and olive oil (specially prepared reagent, Nacalai Tesque, Inc.) were used. Oxazolone was used as a positive control, and a 1:4 mixture of acetone:olive oil as a solvent and a negative control.

2. Methods

Type IV allergic reaction, which is called delayed type hypersensitivity, is usually tested with a model in which oxazolone is applied to the ear of mice and induction of swelling is judged\textsuperscript{21-23}. The type of allergic reaction induced by rosin was examined using the ear swelling test\textsuperscript{22-24} (Fig. 2).

1) Induction

On day 1, after shaving the abdomen of the mice with a razor, 0.1mL emulsion of FCA was injected into the peritoneum, and 20μL of the test solution was applied onto the abdominal skin. The concentrations of the test substances were 25, 12.5, 5, and 1% for rosin and 3% for oxazolone. The undiluted mixture of acetone and olive oil was used in the same dose. On day 2, the same amount of the test substances were applied onto the abdomen.

2) Challenge

On day 7, the thickness of the ear of the animals was measured with a dial thickness gauge (Mitutoyo), and then 10μL of the test solution was applied onto the ear. The concent-

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**Table 1 Evaluation of skin reaction**

<table>
<thead>
<tr>
<th>(1) Erythema formation</th>
<th>None</th>
<th>0</th>
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<tbody>
<tr>
<td></td>
<td>Very slight</td>
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</tr>
<tr>
<td></td>
<td>Well defined</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Moderate to severe</td>
<td>3</td>
</tr>
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<td></td>
<td>Severe erythema to slight eschar formation</td>
<td>4</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>(2) Edema formation</th>
<th>None</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slight</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>3</td>
</tr>
</tbody>
</table>

Mean response = \( \frac{\sum [(1)+(2)]}{n} \)

Total number of animals

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**Fig. 2 Schedule of ear swelling test**
trations of the test substances were 25, 12.5, 5, and 1% for rosin and 1% for oxazolone. The undiluted mixture of acetone and olive oil was also used in the same dose.

3) Judgment
On day 8, which was 24 hours after the application of the test solution, the thickness of the ear of the animals was measured and the swelling rate was obtained using the formula shown in Table 2.

Table 2 Calculation of ear swelling

\[ \text{Ear swelling (\%)} = \frac{E_2 - E_1}{E_1} \times 100 \]

\[ E_1: \text{Ear thickness before challenge} \]
\[ E_2: \text{Ear thickness after challenge} \]

3. Statistical analysis
The measurements were shown as the means \pm standard error. Statistical differences were tested by Student's t-test, and a value of \( p < 0.01 \) was regarded as significant.

III. Examination of the kinetics of immunocytes
1. Materials
The mice applied with 25% rosin in the ear swelling test (experiment II) were used.
2. Methods
1) Sampling and preparation of sections
In experiment II, mice were sacrificed by an excessive dose of ether 30 minutes after the application of the drug on day 1 (induction phase) or on day 8 (effector phase), and inguinal lymph nodes and ears were dissected and frozen in liquid nitrogen (Fig. 3). Six \( \mu m \) frozen sections of the lymph nodes and ears were made using a cryostat (Jung Frigocut-N, Leica).

2) Immunohistochemical staining
The distribution of immunocytes in the above samples was immunohistochemically examined by the indirect enzyme-labeled antibody method\(^{25,26}\) (Fig. 4). The markers used were Thy-1.2 for T cells\(^{27}\), CD4 for helper T cells (Th)\(^{28}\), Interleukin-2 receptor (IL-2R) for Th-1\(^{29}\), IL-4R for Th-2\(^{30}\), and CD8 for suppressor T cells (Ts)/cytotoxic T cells (Tc)\(^{31}\). The primary antibodies used were the anti-Thy-1.2 monoclonal antibody (Cedarlane), anti-mouse CD4

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**Fig. 3** Observation of immunocyte kinetics in ear swelling test

**Fig. 4** Indirect enzyme-labeled antibody technique
monoclonal antibody (Cedarlane), anti-IL-2R monoclonal antibody (Cedarlane), anti-IL-4R monoclonal antibody (genzyme), anti-mouse CD8 monoclonal antibody (Pharmingen). The secondary antibodies used were peroxidase-conjugated rabbit anti-mouse IgG (Daco) and peroxidase-conjugated goat anti-rat IgG (Cedarlane). For blocking, rabbit serum (Japan Biotest Laboratory) and goat serum (Cedarlane) were used. For enzyme-histochemical staining of horseradish peroxidase used as a label, 3,3'-diaminobenzidine tetrahydrochloride (DAB, Dojindo Laboratories) was used. The sections were then subjected to nuclear staining with methyl green (Sigma), dehydrated, mounted in Entellan neu (Merck), and examined for positive cells by light microscopy. The kinetics of immunocytes was examined by detecting the distribution of positive cells in lymph nodes and ears both in the induction phase and the effector phase.

Results

1. Adjuvant and patch test for skin sensitization

The reaction of the skin 24 and 48 hours after challenge is summarized in Table 3. Twenty-four hours after challenge, erythema was noted in all 3 animals applied with 25, 12.5, and 5% rosin and edema was also noted in one of the animals with 25% rosin. Forty-eight hours after challenge, erythema was noted in all 3 animals applied with 25, 12.5, and 5% rosin, but no animals showed edema. No animals applied with acetone showed erythema and edema.

With regard to MR scores, which indicate the degree of reaction, the animals that received 25, 12.5, and 5% rosin showed high scores of 1.8-2.5 24 hours after challenge, though not as high as 3.7 of the animals with the positive control (Sudan I 0.1%). Forty-eight hours after challenge, MR for the rosin-applied animals was

Table 3 The allergenicity after challenge

<table>
<thead>
<tr>
<th>Sample</th>
<th>Challenge concentration (%)</th>
<th>24hr after challenge</th>
<th>48hr after challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FR</td>
<td>MR</td>
<td>FR</td>
</tr>
<tr>
<td></td>
<td>Erythema</td>
<td>Edema</td>
<td></td>
</tr>
<tr>
<td>Rosin</td>
<td>25</td>
<td>3/3</td>
<td>1/3</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>3/3</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3/3</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Sudan I</td>
<td>1×10^{-1}</td>
<td>3/3</td>
<td>2/3</td>
</tr>
<tr>
<td></td>
<td>1×10^{-2}</td>
<td>3/3</td>
<td>1/3</td>
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<tr>
<td></td>
<td>1×10^{-3}</td>
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</tr>
<tr>
<td></td>
<td>1×10^{-4}</td>
<td>1/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

FR : Fractional response (=positive/total)
MR : Mean response
II. Allergic reactions in ear swelling test

The swelling rates determined 24 hours after application of the drugs onto the ear are shown in Fig. 5. The swelling rate of a negative control (vehicle) was about 1%. With 1% rosin, there was no significant difference between the experimental and a negative control (vehicle). At 5% rosin, the difference was significant, and at 25% rosin, the swelling rate was as high as 55%.

III. Kinetics of immunocytes

1. Immunocytes distribution in lymph nodes

Thy-1.2-positive cells were detected in the follicles and more prominently in the paracortical areas in the induction phase (photo. 1A), while in the effector phase, more positive cells were noted in the follicles (Photo. 1B).

CD4-positive cells were absent in the follicles and very scarce in the paracortical areas in the induction phase (Photo. 2A). In the effector phase, many positive cells were noted in the paracortical areas and a few positive cells were observed in the follicles (Photo. 2B).

No IL-2R-positive cells were found in the induction phase (Photo. 3A), and there were only a small number of positive cells in the paracortical areas in the effector phase (Photo. 3B).

IL-4R-positive cells were present in a small number in the follicles and in a large number in the paracortical areas in the induction phase (Photo. 4A). More positive cells were noted in the follicles in the effector phase (Photo. 4B).

There were no CD8-positive cells in the follicles in both phases. The number of positive cells in the paracortical areas was very small in the induction phase (Photo. 5A) but increased in the effector phase (Photo. 5B).

2. Immunocytes distribution in the ear

Thy-1.2-positive cells in the dermis and muscular layers were equally abundant in both induction and effector phases (Photo. 1C and 1D).

CD4-positive cells were observed in the dermis and muscular layers in both induction and effecter phases (Photo. 2C and 2D), and no difference in the number was noted between the two phases.

There was a larger number of IL-2R-positive cells in the muscular layers than in the dermis in the induction phase (Photo. 3C), while in the effector phase, few positive cells were observed in the muscular layers (Photo. 3D).

IL-4R-positive cells (Photo. 4C) and CD8-positive cells (Photo. 5C) were both present in the dermis and muscular layers in the induction phase. Both cells were more abundant in the muscular layers in the effector phase (Photo. 4D and 5D).

Discussion

Rosin is a natural resin, mainly consisting of resin acids such as abietic acid and neoabiectic acid although there are small differences in the components between regions of production. Rosin, which is described in the Japanese Pharmacopoeia, is usually used as an adhesive agent of tape. In the dental field, this drug is included in many preparations and materials. For example, zinc oxide eugenol powders as an indirect pulp capping agent contain rosin at a rate of about 30% to obtain smooth malaxation, high adherence, suppression of reaction speed, and drug stability. As allergy reaction during dental treatment, administration of drugs for local anesthesia and latex gloves cause immediate allergy such as contact urticaria and anaphylactic shock. These reactions are classified into type I allergy due to humoral immunity. Metals contained in prosthesis cause contact dermatitis, which is delayed type allergy. This is classified into type IV allergy due to cellular immunity. In this study, we performed various experiments to examine whether dental drugs and materials containing rosin cause contact dermatitis.

To examine the occurrence of contact dermatitis, we performed the adjuvant and patch test in guinea pigs. Twenty-four hours after challenge, neither erythema nor edema was observed in the animals applied with 1% rosin, but erythema was observed in all 3 animals even with 5% rosin, and the MR score was 1.8. This level was close to the MR score (2.3) in the animals applied with 0.01% Sudan I as the positive control, indicating a strong positive reaction. Edema was also noted in one of the animals with 25% rosin. Edema is a reaction caused by strong allergy, and the MR score was 2.5 in the animals with 25% rosin. Forty-eight hours after challenge, the reactions with rosin and Sudan I became weak, compared to those 24 hours after challenge, but erythema was observed in all 3 animals applied with 25, 12.5, and 5% rosin. The MR score increased
Photo. 1  Thy-1.2-positive cells on frozen sections of lymph nodes (A and B) and ears (C and D)
A and C; the induction phase, B and D; the effector phase

Photo. 2  CD4-positive cells on frozen sections of lymph nodes (A and B) and ears (C and D)
A and C; the induction phase, B and D; the effector phase

Photo. 3  IL-2R-positive cells on frozen sections of lymph nodes (A and B) and ears (C and D)
A and C; the induction phase, B and D; the effector phase

Photo. 4  IL-4R-positive cells on frozen sections of lymph nodes (A and B) and ears (C and D)
A and C; the induction phase, B and D; the effector phase

Photo. 5  CD8-positive cells on frozen sections of lymph nodes (A and B) and ears (C and D)
A and C; the induction phase, B and D; the effector phase
with increases of the concentration of rosin, and the area with erythema also increased, indicating that the allergy reaction caused by rosin depended on the concentration.

Ester gums, which are a compound similar to rosin, consisting of glyceryl, methyl and ethyl ester of rosin\(^3\)\(^3\), are contained in root canal filling agents. The MR score of 25% ester gums was 2.5, and it did not induce edema, although the MR score was the same as that with 25% rosin. The MR score of 50% ester gums was 2.8, but edema was not noted\(^3\)\(^4\).

Thymol, eucalyptol and eugenol are also used as root canal filling agents. Thymol and eucalyptol showed no positive reaction at a concentration of 50%, while the MR score of eugenol was 2.0 at 50% and 1.4 at 25% but the reaction was weaker than that with rosin\(^3\)\(^5\). These findings indicated that the allergic reaction induced by rosin was stronger than that by the other components contained in root canal filling agents.

Kawahara et al.\(^3\)\(^5\) reported that the irritation index by the patch test using 20% rosin in humans was 12.5%. The concentration of rosin contained in clinically used dental drugs is 30-50% in periodontal dressing powders and 15-25% in preparations after malaxation. In pulp capping agent powders, rosin is contained at a concentration of about 30%, while it is contained in preparations at a concentration of about 15%. Therefore, if preparations containing rosin at a concentration of 15-25% are clinically used, allergy, i.e., contact dermatitis, is likely to occur.

We also performed the ear swelling test, which is used for examination of the occurrence of type IV allergy, in mice, and evaluated the allergic type induced by rosin. There was no significant difference in the swelling rate of the ear between 1% rosin and the negative control solvent; however, the rate (32%) was significantly different with 5% rosin. The swelling rate of the ear with 25% rosin was 55%, showing marked swelling, although it was lower than that with 1% oxazolone as the positive control (94%). These results indicated that rosin induced type IV allergy and that swelling of the ear induced by rosin was concentration-dependent.

We previously reported that the swelling rate of the ear induced by 100% eugenol was 18%\(^3\)\(^6\). Eugenol, which is the main component of clove oil, is contained in food and cosmetics in large amounts, and it is known that eugenol causes allergy. We suggested that eugenol contained in dental drugs also causes contact dermatitis\(^3\)\(^7\). The swelling rate of the ear induced by 100% eugenol was 18%, while that with 25% rosin was 55%, indicating that rosin induced very strong swelling of the ear, compared to eugenol. As described above, because rosin is contained in clinically used periodontal dressing and pulp capping agents at a concentration of 15-25%, type IV allergy is likely to occur by dental drugs containing rosin.

The adjuvant and patch test and ear swelling test demonstrated that rosin caused contact dermatitis as type IV allergy. This type allergy is due to cellular immunity in which T cells are involved\(^3\)\(^8\). Functionally, T cells are classified into effector cells such as Th, Ts and Tc. Th is further divided into Th-1 and Th-2\(^3\)\(^9\), and Th-1 is considered to be involved in the occurrence of type IV allergy such as contact dermatitis induced by rosin in the present study\(^4\)\(^0,4\)\(^1\). We examined the changes in the distribution of various types of T cells in inguinal lymph nodes and the ear in the mice with ear swelling induced by rosin.

Thy-1.2 antigens are used as T cell markers in a broad sense. In the lymph nodes, Thy-1.2-positive cells were detected in the follicles and more prominently in the paracortical areas in the induction phase, while in the effector phase, more positive cells were noted in the follicles. Generally, in the lymph nodes, B cells are predominant in the follicles and T cells in the paracortical areas. In this study, Thy-1.2-positive cells in the follicles increased in the effector phase, indicating that T cells increased. These results indicated that T cells in each lymph node were involved in the occurrence of allergy induced by rosin.

Th is essential for the occurrence of type IV allergy, and CD4 is used as the Th marker. CD4-positive cells were very scarce in the lymph nodes in the induction phase, while in the effector phase, many CD4-positive cells were noted in the paracortical areas. In this study, CD4-positive cells in the follicles increased in the effector phase, indicating that T cells increased. These results indicated that T cells in each lymph node were involved in the occurrence of allergy induced by rosin.
in the distribution between the two phases. The increase in Th indicated that it was closely involved in the occurrence of allergy induced by rosin.

Mosmann et al. divided Th into Th-1 and Th-2, and indicated that Th-1 produces IL-2 and Th-2 produces IL-4. We used IL-2R as the Th-1 marker in the present study. In the lymph nodes, IL-2R-positive cells were not observed in the induction phase, and in the effector phase, only a few IL-2R-positive cells were observed in the paracortical areas. In the ear, a large number of IL-2R-positive cells was observed in the muscular layers, compared to the dermis, in the induction phase, while in the effector phase, few positive cells were observed in the muscular layers. Upon the occurrence of allergy, the number of IL-2R-positive cells slightly increased in the lymph nodes but markedly decreased in the regions where allergy was induced.

On the other hand, IL-4R was used in this study as the Th-2 marker. In the lymph nodes, only a few IL-4R-positive cells were observed in the follicles and a large number in the paracortical areas in the induction phase, while in the effector phase, more positive cells were observed in the follicles. In the ear, IL-4R-positive cells were present in the dermis and muscular layers in the induction phase, while IL-4R-positive cells were more abundant in the muscular layers in the effector phase. Upon the occurrence of allergy, the number of IL-4R-positive cells increased in the lymph nodes and markedly increased in the regions where allergy was induced.

The authors performed similar experiments using eugenol, and observed that IL-2R-positive cells increased in the paracortical areas of lymph nodes and in the muscular layers of the ear in the induction phase. IL-4R-positive cells were not observed in the lymph nodes in both induction and effector phases, while in the ear, IL-4R-positive cells were more abundant in the muscular layers in the effector phase. These results indicated that kinetics of Th-1 and Th-2 induced by rosin and eugenol were different.

In allergy induced by rosin, slight involvement of Th-1 in the lymph nodes and strong involvement of Th-2 in the lymph nodes and regions with allergy were suggested. On the other hand, in allergy induced by eugenol, involvement of Th-2 in the regions with allergy was suggested, but no involvement of Th-2 in the lymph nodes was observed, suggesting that Th-1 was involved in the lymph nodes and regions with allergy.

Th-1 is considered to be involved in type IV allergy. However, it was reported that hapten made Th-1 or Th-2 predominant, and it was also reported that Th-1 predominance was shifted to Th-2 predominance by repeated administration of hapten. Atopic dermatitis, which is classified into type I allergy, consists of early and late phase reactions. The late phase reaction is similar to the reaction in contact dermatitis. Kay et al. clarified that Th-2 is involved in the late phase reaction in atopic dermatitis. These findings indicated that there will be differences in the mechanism of allergy between dental drugs. Unlike eugenol, rosin was considered to have Th-2-predominant reaction but not Th-1-predominant reaction.

CD8 is used as the Ts and Tc markers. In the lymph nodes, the number of CD8-positive cells was very small in the paracortical areas in the induction phase but increased in the effector phase. In the ear, CD8-positive cells were observed in the dermis and muscular layers in the induction phase, while in the effector phase, CD8-positive cells were more abundant in the muscular layers, where swelling occurred, in the effector phase, suggesting that CD8-positive cells were Tc.

A type of T cells, which mediates delayed type allergy such as contact dermatitis considered to be involved in type IV allergy, is called T cell mediating delayed type hypersensitivity (TDTH). It is considered that activated TDTH secretes various lymphokines and induces delayed type allergy, but TDTH itself has not been clarified: whether TDTH is entirely different from Th and Tc and whether there are T cells with some of TDTH function. In clone cells, it has been clarified that there are T cells with TDTH function. In the present study, CD8-positive cells increased in the lymph nodes and in the muscular layers where allergy occurred, indicating that CD8-positive cells were T cells...
which play the important role in the occurrence of allergy and could be T<sub>0</sub>DTH itself.

**Conclusions**

The occurrence of allergy by rosin contained in dental drugs and materials was examined by the adjuvant and patch test and ear swelling test. The results indicated that contact dermatitis, which is type IV allergy, may be induced by dental drugs and materials containing rosin, and the induction of allergy depended on the concentration of rosin. The immunohistochemical evaluation of kinetics of T cells in the lymph nodes and in the ear where allergy was induced suggested that Th-2 rather than Th-1 was involved in allergy induced by rosin, and clarified that CD8-positive cells were involved.

**Acknowledgement**

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ロジンによるアレルギー反応の薬理学的および免疫薬理学的研究

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歯科用薬剤や歯科材料によりアレルギーが発症するが、原因となる物質やそのメカニズムなどは充分解明されていない。今回、覆歯剤や根管充填剤などに配合されているロジンについて、薬理学的および免疫薬理学的検討を行った。まず、モルモットを用いた adjuvant and patch test を行った。濃度 25, 12.5, 5 % のロジンで、感作誘発 24 時間後、3 匹全てに紅斑が認められ、25% では 1 匹に浮腫も認められた。次に、ロジンがIV型アレルギーを発症するかを、マウスを用いて ear swelling test で検討した。耳介腫脹率は、ロジン濃度 5 % 以上で対照群との間に有意差が認められ、25% では 55% という高い値となった。以上の 2 種の試験結果より、ロジンを含有する歯科用薬剤や歯科材料によって、IV型アレルギーである接触性皮膚炎を発症すること、アレルギー反応の強さはロジンの濃度に依存する可能性のあることが示唆された。さらに、ear swelling test において、濃度 25% のロジンを投与したマウスの免疫担当細胞の動態を検討した。すなわち耳介とリンパ節における免疫担当細胞の分布を、間接酵素抗体法を用いて免疫組織化学的に検討した。Thy-1.2, CD4, IL-2R, IL-4R および CD8 陽性細胞を光学顕微鏡で検出したところ、IL-4R および CD8 陽性細胞の数が、惹起反応誘発期に増加した。その所見から、ロジンによるアレルギー発症には Th-1 よりも Th-2 の関与がより大きいことが示唆され、また、CD8 陽性細胞の関与も明らかになった。

キーワード：ロジン、接触性皮膚炎、耳介腫脹、ヘルパー T 細胞、細胞障害性 T 細胞