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Effect of Cnidii Rhizoma (Senkyu) on triphasic skin response in passively sensitized mice

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Abstract

Previous studies have reported that mice passively sensitized with anti-DNP (dinitrophenol) IgE antibody exhibited IgE-mediated skin reaction with an immediate phase response (IPR) at 1 h and a late phase response (LPR) at 24 h after the challenge of DNFB (dinitrofluorobenzene). We recently found that a third phase inflammatory reaction with intense and persisting infiltration of eosinophils, named very late phase response (vLPR), was induced by DNFB challenge peaking at 8 days. The development of vLPR was partly decreased in mast cell-deficient WBB6F1-W/W^{v} mice and was absent in T cell-deficient BALB/c-nu/nu mice in passive sensitization. We examined the effects of a Kampo medicine, Shimotsu-to (Si-Wu-Tang, 四物湯), and its constituent crude drugs on triphasic skin reaction in passively sensitized mice. Shimotsu-to inhibited ear swelling in LPR and vLPR after DNFB challenge in a dose-dependent manner. The inhibitory effect on LPR and vLPR was partly due to Cnidii Rhizoma (Senkyu) in Shimotsu-to formulation, especially its fraction 5 containing cnidilide. And cnidilide inhibited ear swelling in LPR and vLPR, one component of fraction 5. These findings indicate that the cnidilide is useful for the inhibition of cutaneous inflammatory diseases.

Key words vLPR, eosinophilic infiltration, Cnidii Rhizoma, cnidilide.

Abbreviations DNP, dinitrophenol; DNFB, dinitrofluorobenzene; IPR, immediate phase response; LPR, late phase response; vLPR, very late phase response; mAb, monoclonal antibody; DTH, delayed type hypersensitivity.

Introduction

A recent increase in the incidence of chronic allergic diseases including atopic dermatitis has been reported.[1,2] To search for new anti-allergic agents, we have investigated the effect of several plant materials and herbal medicines on murine IgE-mediated skin reaction. In this model, passive sensitization with a murine monoclonal IgE antibody specific for dinitrophenoyl group (anti-DNP IgE mAb) followed by the challenge of dinitrofluoro-benzene (DNFB) to the mouse ear induces a biphasic skin reaction with immediate phase response (IPR) and late phase response (LPR) at 1 and 24 h after the challenge.[3-6] In the process of our study, we recently found a third inflammatory phase response following LPR, temporarily designated "very late phase response (vLPR)".

We have previously reported that spikelets of *Miscanthus sinensis* [7] and some Kampo (Japanese herbal) medicines[8,9] inhibited the IgE-mediated biphasic skin reaction. The inhibitory effect of more than 20 Kampo formulations on skin reaction was divided into three groups, +/+, -/+ and -/- of IPR/LPR. Some formulations such as Shimotsu-to (Si-Wu-Tang, 四物湯) and Unsei-in (Wen-Qing-Yin, 温清飲) inhibited mainly LPR, but not IPR (-/+ group), whereas some anti-allergic agents including H1 receptor antagonists and mediator-release inhibitors showed the oposite effects (+/- of IPR/LPR).

In the present study, we revealed the very late

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phase response, and investigated the effect of Cnidii Rhizoma (Senkyu), a key component of Simotsu-to (a Kampo medicine), on triphasic skin reaction in passively sensitized mice.

Materials and Methods

Mice: Specific pathogen-free BALB/c mice (6 weeks old), BALB/c-nu/nu mice (6 weeks old), and WBB6F1-+/+ mice (8 weeks old) and WBB6F1-W/W^{v} mice (8 weeks old) were purchased from Japan SLC Inc., Hamamatsu, Japan, and maintained in the Laboratory for Animal Experiments, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University. This study was conducted in accordance with the standards established by the Guidelines for the Care and Use of Laboratory Animals of Toyama Medical and Pharmaceutical University.

Antigens and chemicals: DNFB was purchased from Nacalai Tesque, Kyoto, Japan, and dissolved in 100% ethanol. DNP-derivatization of ovalbumin (DNP-OVA) was performed by the method of Eisen *et al.*[10] The DNP-OVA preparation was calculated to contain 3.5 DNP groups per OVA molecule. Aluminum hydroxide gel (Alum) was prepared according to the method of Levine and Vaz [11] and used as an adjuvant for the immunization with DNP-OVA antigen. Shimotsu-to is composed of four crude drugs which were quality-controlled by Japanese Pharmacopeia XIII. To prepare the extracts of each crude drug in the Shimotsu-to formulation, Angelicae Radix (Japanese name; Toki, No. YA143020), Rehmanniae Radix (Jio, No. 061197), and Paeoniae Radix (Shakuyaku, No. 290797) were purchased from Tochimoto Pharmaceuticals, Osaka, Japan. Cnidii Rhizoma (Senkyu, No.142217) was purchased from Uchida Pharmaceuticals, Tokyo, Japan. The extracts of each crude drug were prepared by boiling in water for 50 min and freeze-dried into powder. The formulations and extracts were dissolved in distilled water before oral administration.

The procedure for extraction and fractionation of Cnidii Rhizoma (Senkyu) is summarized in Fig. 1. Briefly, the materials were extracted with hexane to give hexane extract. The hexane extract was dissolved in diethyl ether and washed with 1 N NaOH.

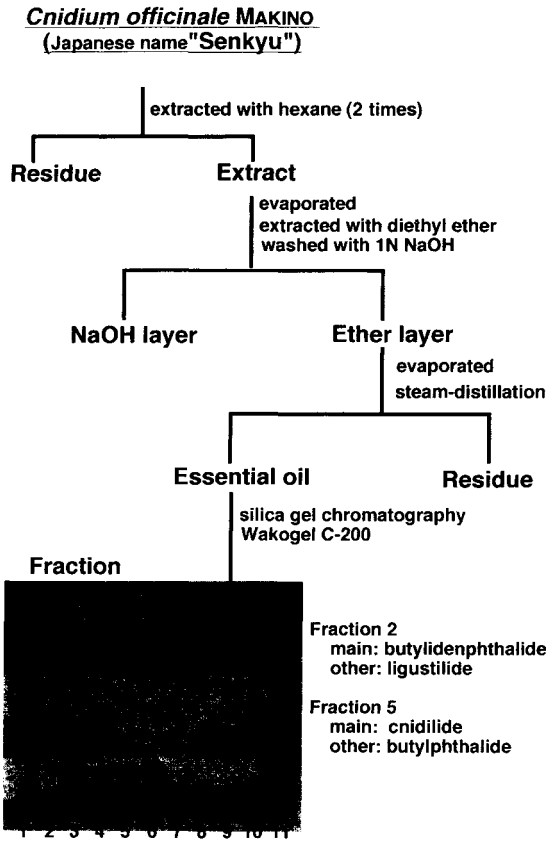

Fig. 1 Extraction and fractionation of Cnidium officinale. The materials were extracted with hexane to give hexane extract. The hexane extract was dissolved in diethyl ether and washed with 1N NaOH. Then the ether layer was concentrated and steam-distilled to give an essential oil. This was subjected to silica gel column chromatography using Wakogel C-200 to give ten fractions by thin layer chromatography. Fraction 2 is mainly composed of butylidenphthalide with a little ligustilide, and fraction 5 is mainly cnidilide with a bit of butylphthalide.

Then the ether layer was concentrated and distilled with steam to give an essential oil. This was subjected to silica gel column chromatography using Wakogel C-200 to give ten fractions, according to the reported behavior on thin layer chromatography.[12] Fraction 2 (composed of butylidenphthalide with a little ligstilide) and fraction 5 (cnidilide with a bit of butylphthalide) were dissolved in 0.5 % dimethyl sulfoxide before use in *in vivo* experiment. Cnidilide and butylphthalide were kindly donated by Tsumura & Co. Ltd. (Tokyo, Japan). Each formulation and preparation were administered orally 2 h before and 2 to 6 days after the challenge.

Anti-DNP IgE preparation : An anti-DNP mAb-producing cell line (EC1) was cultured in 10 ml of an equal volume mixture of RPMI-1640 and Dulbecco's modified Eagle minimum essential medium with high glucose supplemented with 10 % heat-inactivated fetal bovine serum (GIBCO Laboratories, Life Technologies, Inc., Grand Island, NY) and 2 mM glutamine until reaching confluence. The supernatant was harvested, centrifuged at 400×g and stored at -80°C until use.[13] The IgE antibody titer was estimated to be 1 : 1024 by heterologous passive cutaneous anaphylaxis in rats injected intravenously with DNP-bovine serum albumin as antigen.[14]

Induction of skin reaction in mouse ears : BALB/c mice were actively or passively sensitized with DNP-OVA or anti-DNP IgE mAb, respectively. In the passive sensitization model, mice were given an i.v. injection of a 1-ml aliquot of anti-DNP IgE mAb-containing fluid 24 h before the DNFB challenge. Skin reaction was elicited by applying 10 μl of 0.1 % DNFB in 100 % ethanol to each side of each ear of sensitized mice. The reaction to DNFB was evaluated by measuring ear thickness using a dial thickness gauge (G-1A type, Peacock, Ozaki MFG., Co., LTD., Osaka, Japan) immediately before the challenge and at appropriate intervals after. The results were expressed as average ear swelling (increase in ear thickness, μm) ± S.D. of 3-5 mice. For active sensitization, mice were immunized i.p. with 10 μg of DNP-OVA admixed with 1 mg of Alum 2 weeks before the DNFB challenge. All other procedures were the same way as for passive sensitization. At the time of DNFB challenge, serum IgE antibody titer against DNP was estimated to be 1: 512 by hPCA.

Histological examinations : The control and treated mice were sacrificed under anesthetized conditions at appropriate times after DNFB challenge, and the ears were removed. The tissues were fixed with 4 % paraformaldehyde solution and embedded in paraffin after dehydration with a series of ethanol. The paraffin sections were stained with hematoxylin and eosin, toluidine blue, or naphthol AS-D chloroacetate (esterase). The corresponding cells were counted under a light microscope in 5 sections of 5 mm length at a magnification of×1000.

Statistical analysis : Statistical significance of difference between the groups was determined by Mann-Whitney's U-test on ear swelling experiment or Student's t-test on histological examination.

Results

Time course of IgE-mediated skin reaction in passively or actively sensitized mice

Fig. 2 shows the time course of IgE-mediated cutaneous reaction in mice which were passively and actively sensitized with anti-DNP IgE antibody and DNP-OVA plus Alum, respectively. In passively sensitized mice, biphasic skin reaction consisting of IPR and LPR was induced within 3 days after the challenge of DNFB. The peak response of IPR was at 1 h, and that of response of LPR was at 24 h after the skin test. These results were well consistent with the previous findings.[7] Interestingly, a third intense cutaneous reaction (ear swelling) following LPR was observed during 5-10 days after the DNFB challenge, peaking at approximately 8 days. Thereafter, the ear swelling gradually decreased to a normal level beyond 1 month. The third phase reaction was temporarily designated "very late phase reaction (vLPR)". The degree of vLPR was more intense and sustaining than LPR after the skin test. Similarly, vLPR was also

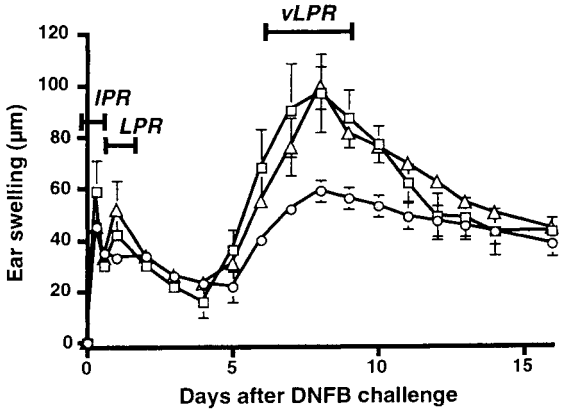

Fig. 2 Time course study of DNFB-specific skin reaction in mice. BALB/c mice were actively or passively sensitized with 10 μg DNP-OVA plus 1mg Alum or 1.0 ml anti-DNP IgE mAb preparation, 2 weeks or 24 h before antigen challenge, respectively. Skin reaction was elicited by applying 0.1 % DNFB in 100 % ethanol to the ear skin of the actively and passively sensitized, and non-sensitized mice. Each value represents mean ear swelling (μm) ± S.D. of 3 mice.

observed after the challenge of DNFB in actively sensitized mice, with a similar pattern to that in passively sensitized mice. In contrast, LPR was not induced in non-sensitized mice, and the ear swelling at 8 days after the challenge was much less than that of the passively and actively sensitized mice. Thus, the presence of a third-phase cutaneous reaction (*i.e.* vLPR) following IPR and LPR in response to DNFB in sensitized mice was clearly established.

Histopathological study of skin reaction

Fig. 3 shows that the number of esterase-positive cells (neutrophils, and macrophages) slightly increased in a close relation to the skin reaction (ear swelling) at 1 h and 24 h, but there was no discernible change of the number of toluidine blue-positive cells (mast cells) after the challenge. Eosinophils were rarely seen in the ear of passively sensitized mice before DNFB challenge, and conspicuously increased at 24 h (LPR) after the challenge. Although they then decreased at 4 days, a massive infiltration into the tested ear skin was then observed at 8 days after the challenge. Epidermal proliferation was also observed in some lesions of ears of passively sensitized mice at 8 days. In non-sensitized mice, the number of eosinophils increased at 8 days, but was much less than that of passively sensitized mice.

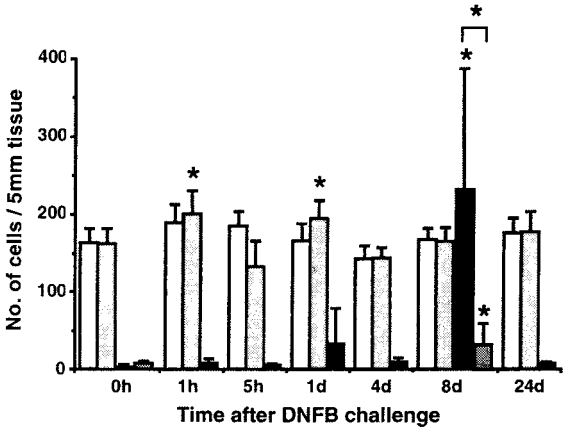

Fig. 3 Histopathological analysis of skin reaction in passively sensitized mice. Mice received intravenous injection of 1.0 ml of anti-DNP IgE mAb preparation 24 h before skin testing with 0.1 % DNFB in 100 % ethanol. After DNFB challenge, the mice were sacrificed and the ears were removed for histopathological examination. The thin sections were stained with toluidine blue, naphthol-AS-D-chloroacetate esterase, or hematoxylin and eosin. The toluidine blue-positive (□), esterase-positive (□), eosinophilic cells (■) in passively sensitized mice and eosinophilic cells in non-sensitized mice at 0 and 8 days (▩) were manually counted under a light microscope in 5 sections of 5 mm length. Each value represents mean±S.D. of 5 sections. *, $p<0.05$ by Student's t-test.

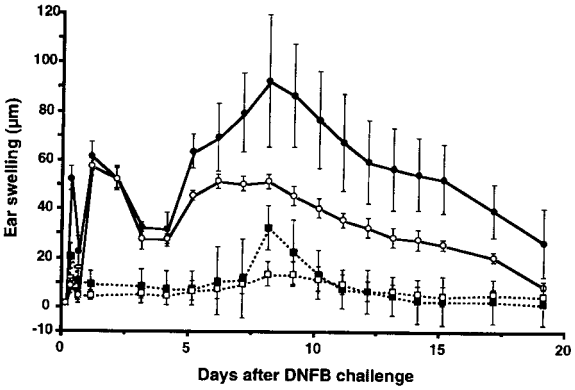

Fig. 4 Time course study of IgE-mediated skin reaction in passively sensitized WBB6F1 mice. Mast cell-deficient WBB6F1-W/W^v mice (○) and their littermates W/W++ (●) were passively sensitized with 1.0 ml anti-DNP IgE mAb preparation 24 h before skin testing with 0.1 % DNFB in 100 % ethanol, and WBB6F1-W/W^v mice (□) and W/W+/+ (■) were non-sensitized. Each value represents mean ear swelling (μm) ±S.D. of 3 mice.

Elicitation of IgE-mediated skin reaction in mast cell- or T cell-deficient mice

To examine the participation of mast cells and T cells in vLPR following epicutaneous challenge, we investigated the triphasic cutaneous reaction in

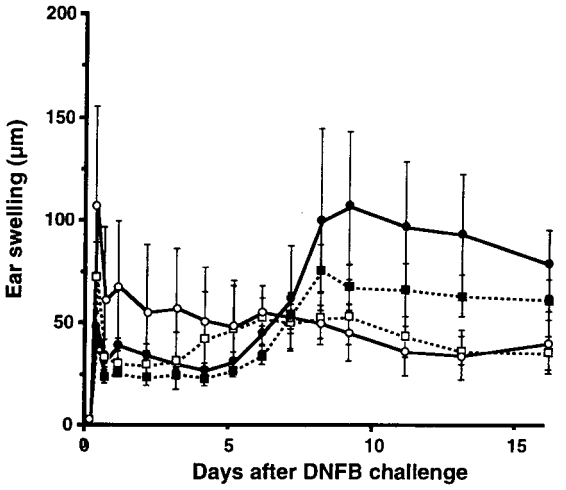

Fig. 5 Time course study of IgE-mediated skin reaction in passively sensitized BALB/c-nu/nu mice. T cell-deficient BALB/c-nu/nu mice (○) and their littermates (●) were passively sensitized with 1.0 ml anti-DNP IgE mAb preparation 24 h before skin testing with 0.1 % DNFB in 100 % ethanol, and BALB/c-nu/nu (□) and BALB/c mice (■) were non-sensitized. Each value represents mean±S.D. of 3 mice.

genetically deficient mice which were passively sensitized with anti-DNP IgE antibody. IPR was absent in mast cell-deficient WBB6F1-W/W^v mice as compared with their congenital littermates W/W++, but LPR was sufficiently observed (Fig. 4). On the other hand, vLPR was apparently attenuated in WBB6F1-W/W^v mice. In non-sensitized W/W+/+ mice diminished vLPR was elicited compared with passively sensitized W/W+/+ mice. But vLPR was not observed in non-sensitized W/W^v mice.

As shown in Fig. 5, both IPR and LPR were surveyed after the DNFB challenge in passively sensitized BALB/c nu/nu mice, and the degree of the responses was greater than in BALB/c mice. In contrast, vLPR had essentially disappeared in BALB/c nu/nu mice, but was strongly present in BALB/c mice. In non-sensitized BALB/c nu/nu mice, slight increase of ear swelling was detected conforming with

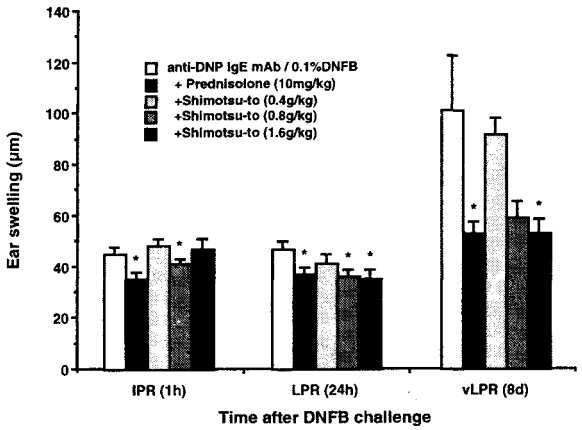

Fig. 6 Effect of Shimotsu-to constituents on triphasic skin reaction in passively sensitized mice. Mice received intravenous injection of 1.0 ml of anti-DNP IgE mAb preparation 24 h before skin testing with 0.1 % DNFB in 100 % ethanol. Each crude drug in Shimotsu-to was given orally 2 h before and 2 to 6 days after DNFB challenge. Prednisolone was given intraperitoneally 2 h before and 4 to 6 days after the challenge. Each value represents mean±S. D. of 3 mice. *, $p<0.005$ by Mann-Whitney's U-test.

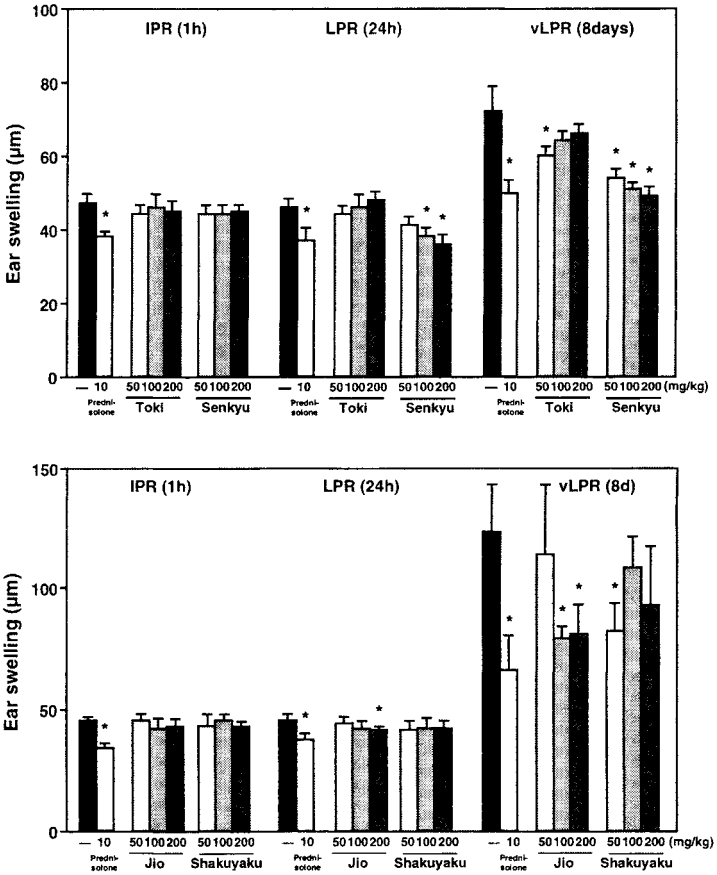

Fig. 7 Effect of Shimotsu-to constituents on triphasic skin reaction in passively sensitized mice. Mice received intravenous injection of 1.0 ml of anti-DNP IgE mAb preparation 24 h before skin testing with 0.1 % DNFB in 100 % ethanol. Each crude drug in Shimotsu-to was given orally 2 h before and 2 to 6 days after DNFB challenge. Prednisolone was given intraperitoneally 2 h before and 4 to 6 days after the challenge. Each value represents mean±S.D. of 3 mice. *, $p<0.005$ by Mann-Whitney's U-test.

vLPR.

Effect of Kampo medicines on triphasic skin reaction in passively sensitized mice

Oral administration of Shimotsu-to inhibited LPR and vLPR in a dose-dependent manner, but not or only slightly inhibited IPR (Fig. 6). Prednisolone

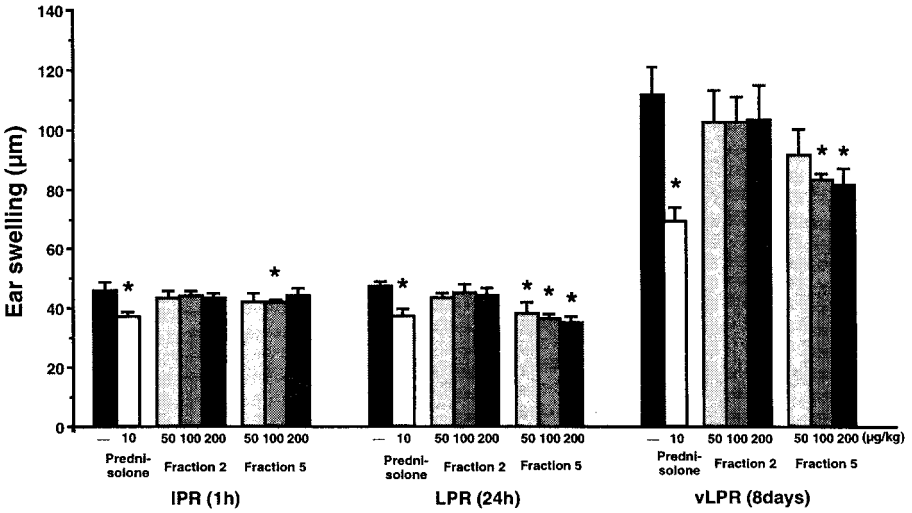

Fig. 8 Effect of fraction 2 and 5 from Cnidii Rhizoma (Senkyu) on triphasic skin reaction in passively sensitized mice. Mice received intravenous injection of 1.0 ml of anti-DNP IgE mAb preparation 24 h before skin testing with 0.1 % DNFB in 100 % ethanol. Each fraction was given orally 2 h before and 2 to 6 days after DNFB challenge. Prednisolone was given intraperitoneally 2 h before and 4 to 6 days after the challenge. Each value represents mean±S.D. of 3 mice. *, $p<0.005$ by Mann-Whitney's U-test.

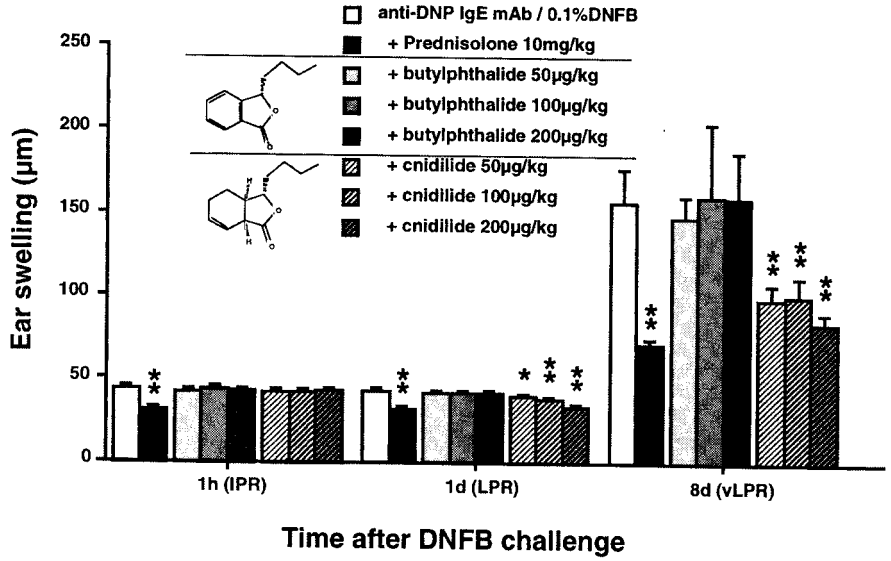

Fig. 9 Effects of butylphthalide and cnidilide on triphasic skin reaction in passively sensitized mice. Mice received intravenous injection of 1.0ml of anti-DNP IgE mAb preparation 24 hours before skin testing with 0.1 % DNFB in 100 % EtOH. Prednisolone was given intraperitoneally 2 hours before challenge and 4 to 6 days. Butylphthalide and cnidilide was given orally 2 hours before challenge and 2 to 6 days after challenge. Each value represents mean±S.D. of 3 mice and was statistically analyzed vs. each group of control (anti-DNP IgE mAb / 0.1 % DNFB) by Mann-Whitney's U-test ; *, $p<0.05$ **, $p<0.005$

significantly inhibited IPR, LPR and vLPR.

Effect of Shimotsu-to constituents on triphasic skin reaction in passively sensitized mice

We next investigated the effect of the four crude drugs in the Shimotsu-to formulation on triphasic skin reaction (Fig. 7). Cnidii Rhizoma (Senkyu) extract significantly inhibited both LPR and vLPR in a dose-dependent manner. Extracts of Angelicae Radix (Toki) or Paeoniae Radix (Shakuyaku) did not show any effect on triphasic skin reaction. Rehmanniae Radix (Jio) extract inhibited vLPR, but did not affect IPR and LPR. Prednisolone was effective at inhibiting the triphasic skin reaction.

Effect of fractions from Cnidii Rhizoma extract on triphasic skin reaction in passively sensitized mice

The above results indicated that Cnidii Rhizoma (Senkyu) extract was effective at inhibiting ear swelling in both LPR and vLPR. We investigated the effect of fractions 2 and 5 extracted from Cnidii Rhizoma (Senkyu) on triphasic skin reaction. As shown in Fig. 8, fraction 5, which is composed of cnidilide with a bit of butylphthalide, inhibited both LPR and vLPR. In contrast, fraction 2 (composed of butylidenphthalide with a little ligustilide) had no effect on triphasic skin reaction.

Effect of cnidilide on triphasic skin reaction in passively sensitized mice

To reveal the effect of cnidilide, we examined the same experiment with cnidilide. Cnidilide inhibited both LPR and vLPR, rather than butylphthalide. (Fig 9)

Discussion

Several investigations have reported that mice passively sensitized with IgE-containing solution exhibited immediate and late phase skin reactions (IPR and LPR) to the subsequent challenge of antigen.[3-6] The inflammation associated with LPR is of great clinical importance, as it accounts for the morbidity and severity of chronic allergic diseases like bronchial asthma, rhinitis and atopic dermatitis.[15] LPR has been shown to accompany polymorphic inflammatory infiltrates such as neutrophils, eosinophils, and lymphocytes.[5,6] Particularly, eosinophils are responsible for this phenomenon in IgE-mediated skin reaction as important effector cells.[4-6,16] However, many studies have reported that LPR in IgE-mediated skin reaction was observed at most 72 h after the antigen exposure.[5-8,16-18]

In the present study, we noticed that passive sensitization with anti-DNP IgE antibody followed by the challenge of DNFB to mouse ears can induce the triphasic cutaneous reactions of IPR, LPR and vLPR peaking at 1 h, 24 h and 8 days after antigen challenge, respectively (Fig. 1). The third-phase inflammatory response, named vLPR, was more intense for ear swelling than LPR, and persisted for longer periods. vLPR was markedly induced in actively sensitized mice as well as passively sensitized mice, but was only slightly observed in non-sensitized mice.

Histopathological examination revealed massive infiltration of eosinophils in vLPR at 8 days, suggesting that eosinophils are responsible for the development of this reaction. However, no marked increase of eosinophils in peripheral blood was observed at the time of vLPR in passively sensitized mice (data not shown). Although many studies have shown that eosinophilic infiltration was observed in LPR at 24 h after skin test,[5,6,17] our present results indicated that the accumulation of eosinophils at vLPR was more marked than at LPR in passively sensitized mice. This suggests that vLPR with eosinophil infiltration actually represents an important inflammatory reaction in allergic diseases. The LPR seen in bronchial disease[19-22] may be very similar to the vLPR in our study. Especially, Hutson *et al.*[21] have reported two delayed broncho-constrictor events including a peak response at 17 h after challenge and a further response at 72 h with increased eosinophils in BAL from guinea pig in an asthma model. Also, vLPR is apparently different from post late phase reaction (pLPR), meaning non-allergic hyperactivity in bronchial asthma,[23] because the third inflammation continued for very long periods and more intensely than LPR.

In genetically mast cell-deficient WBB6F1-W/W^{V} mice with mutation of the W/c-kit locus, IPR was absent but LPR was strongly manifested after DNFB challenge (Fig. 6). vLPR was also present, but to a lesser degree than in WBB6F1-+/+ mice. This finding indicates that mast cells or mediators originating from them may be prerequisite for the development of

vLPR. Since mutations in the W/c-kit locus result in the absence of the c-kit receptor or the production of abnormal receptors,[24-26] stem-cell factor, a ligand for the c-kit receptor, may be partly associated with the development of allergic response. Diminished vLPR was detected in non-sensitized W/W+/+ mice than in sensitized W/W+/+ mice, but disappeared in non-sensitized W/W^v mice. These findings suggest that the presence of IgE antibody might enhance the development of vLPR. On the other hand, both IPR and LPR were induced after DNFB challenge in T cell-deficient BALB/c-nu/nu mice passively sensitized with anti-DNP IgE antibody, but vLPR was almost completely absent, in contrast to BALB/c mice. These results clearly indicate that LPR is a T cell-independent response while vLPR is mainly mediated by T cells and factors derived from them. Since T cells and eosinophils have been reported to accumulate in the skin of a patient with atopic dermatitis,[27-29] further study will be needed to examine the close association between T cells and eosinophils in the skin reaction. A slight increase of ear swelling in non-sensitized mice coming into view during 6 to 9 days, might depend on mast cell or its mediators released by irritant chemical effect of DNFB. Allergic reaction has been considered as being divided into two separate categories, IgE mediated response and delayed type hypersensitivity (DTH). vLPR might be one of the DTH which is so-called a "flare-up" in the dermatology field, wheal and flare phenomenon observed at late phase of patch test. If vLPR would be DTH, it was not pure DTH. vLPR is increased in the presence of IgE, it might be considered to be IgE-enhanced DTH. Two separate allergic categories might be observed at the same time, in vLPR.

We have previously investigated the efficacy of Kampo medicines on IgE-mediated biphasic skin reaction in mice.[8,9] All of the Kampo formulations tested were divided into the three groups of -/-, -/+ and +/+ of IPR/LPR.[9] Interestingly, most Kampo medicines did not belong to the +/- group, which includes histamine H1 receptor antagonists (diphenhydramine and terfenadine) and mediator-release inhibitors (amlexanox).[30] The inhibitory effect of the +/+ group was similar to that of prednisolone. Shimotsu-to inhibited mainly LPR, but not IPR.[9] In the present study, Shimotsu-to significantly inhibited the third phase reaction vLPR with intense ear swelling and massive infiltration of eosinophils as well as LPR.[9]

Shimotsu-to is a key formulation which has been used in some Kampo medicines such as Unsei-in and Juzen-taiho-to (Shi-Quan-Da-Bu-Tang, 十全大補湯) and consists of four crude drugs. Among the four constituents, Cnidii Rhizoma (Senkyu) and Angelicae Radix (Toki) have been used to improve "OKETSU", a state of insufficient blood-circulation and blood stasis resulting in chronic autoimmune and allergic inflammatory, and thrombopoietic diseases as diagnosed by the system of Kampo medicine. These crude drugs are considered to stimulate the circulation of "BLOOD" (refers to blood, hormones, autonomic nervous system and other regulatory functions of the body's internal environment) and "KI" (a concept that encompasses mental nervous activity, especially the appetite for food and actual process of digesting and absorbing nutrients) in Kampo medicine.[31] As shown in Fig. 3, Shimotsu-to without Cnidii Rhizoma (Senkyu) decreased the inhibitory effect on triphasic skin reaction as compared with intact Shimotsu-to. Cnidii Rhizoma (Senkyu) extract markedly inhibited LPR and vLPR as compared with other constituents in the Shimotsu-to formulation (Fig. 4). This indicates that Cnidii Rhizoma (Senkyu) may be primarily involved in the development of Shimotsu-to-mediated inhibition of skin reaction. In addition, the inhibitory effect of Cnidii Rhizoma (Senkyu) extract was partly due to fraction 5 which contains cnidilide with a small amout of butylphthalide, but not fraction 2 containing butylidenphthalide with a little ligustilide (Fig. 7). This is clearly consistent with our data that Angelicae Radix (Toki), which abundantly contains butylidenphthalide and ligustilide,[32] did not show any inhibitory effect on triphasic skin reaction. To our knowledge, an anti-allergic effect of Cnidii Rhizoma (Senkyu) extract, especially cnidilide and other components, has not been reported yet. It is worthy of notice that a very small amount of cnidilide inhibited both LPR and vLPR rather than prednisolone.

The detailed mechanisms for the inhibition of triphasic skin reaction by the extract of Cnidii Rhizoma (Senkyu) as well as Shimotsu-to are not

clear. Since our preliminary study observed that their inhibitory effect (-/+/+ of IPR/LPR/vLPR) was similar to that of a leukotriene B4 receptor antagonist (ONO-4057), Cnidii Rhizoma (Senkyu) and Shimotsu-to may possess a similar mechanism of action and inhibit the infiltration of eosinophils into the local site at vLPR. The detailed mechanism of the inhibitory effect of Shimotsu-to and Cnidii Rhizoma (Senkyu) is now under investigation.

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和文抄録

マウスに抗DNPモノクローナルIgE抗体を静脈内投与して受動感作，またはDNP-OVA+Alumで能動感作し，DNFBをマウスの耳介に塗布して反応を惹起する系で種々の検討を行った。感作BALB/cマウスの耳介に抗原を塗布すると，2相性反応 (Immediate Phase Response ; IPR および Late Phase Response ; LPR) の後8日目をピークとする3相目の強い耳介腫脹が観察された (very Late Phase Response ; vLPR)。8日目の耳介を病理組織学的に検討したところ，著明な好酸球の浸潤を認めた。WBB6F1-W/W^{v} と BALB/c-nu/nu マウスで同様に反応を惹起した結果，WBB6F1-W/W^{v} で vLPR は減弱し，BALB/c-nu/nu マウスでは消失した。漢方エキス製剤を用いてマウスにおけるアレルギー性3相性皮膚反応に対する抑制効果を検討し，さらに四物湯，川芎において，その有効成分について検討した。各方剤，試料は抗原塗布2時間前と2-6日目に経口投与し，抑制効果を検討した。この3相性皮膚反応に対する漢方方剤の抑制効果を検討した結果，四物湯は特にLPRとvLPRを抑制した。4つの構成生薬の3相性皮膚反応に及ぼす作用について検討したところ，乾地黄と川芎がLPRとvLPRを有意に抑制した。さらに川芎の分画成分の中で，Fraction 2と5について抑制効果を検討した結果，Fraction 5にLPRとvLPRの抑制効果を認めた。Fraction 5に含まれる成分のうち，cnidilideに強い抑制活性を認めた。

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