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Kampo formulations and allergic inflammatory diseases - Efficacy for murine IgE-mediated triphasic cutaneous reaction -

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We found that passive sensitization with anti-DNP IgE antibody followed by the challenge with DNFB to the mouse ear can induce the triphasic cutaneous reactions (ear swelling) of immediate phase response (IPR), late phase response (LPR) and very late phase response (vLPR), peaking at 1 h, 24 h and 8 days after the challenge, respectively. IPR was absent in mast cell-deficient mice but LPR was sufficiently observed, and vLPR was partly attenuated. LPR is a T cell-independent response, while vLPR is almost completely absent in T cell-deficient nude mice. Thus, the third phase response (vLPR) with massive infiltration of eosinophil actually represents an important inflammatory reaction mediated by T cells and partially mast cells. In this model, some Kampo formulations and synthetic anti-allergic agents inhibited the IgE-mediated triphasic cutaneous reaction. The inhibitory effects of the Kampo formulations on the triphasic cutaneous reaction were divided into several groups according to the efficacies for IPR/LPR/vLPR. For instance, the group consisting of formulations such as Tokaku-joki-to (Tao-He-Cheng-Qi-Tang, 桃核承気湯), Ji-zuso-ippo (Zhi-Tou-Chuang-Yi-Fan, 治頭瘡一方), Sho-sei-ryu-to (Xiao-Qing-Long-Tang, 小青竜湯) and Sho-saiko-to (Xiao-Chai-Hu-Tang, 小柴胡湯) significantly inhibited IPR, LPR and vLPR (*i.e.* +/+/+ group that showed inhibitory effects against the triphasic response), similar to the effect of prednisolone as a positive control. Oral administration of Yokukan-san (Yi-Gan-San, 抑肝散), an anti-psychosis drug in Kampo medicine, attenuated the isolation stress-exacerbated triphasic skin reactions in a dose-dependent manner, while it had almost no effect on the cutaneous reactions in the unstressed group-housed mice. On the other hand, the i.p. administration of diazepam, a classic benzodiazepine receptor agonist, suppressed the enhanced IPR and LPR in socially isolated mice, but surprisingly stimulated vLPR in both stressed and unstressed mice, differing from the efficacy of Yokukan-san.

This article focuses on the anti-allergic properties of Kampo formulations and describes the effect of some Kampo formulations on IgE-mediated triphasic skin reaction in group-housed or socially isolated mice. We also discuss the mechanism of the inhibitory action and the importance of the formulation and the constituent drugs in determining the efficacy.

Key words Kampo formulations, IgE-mediated triphasic cutaeous reaction, very late phase response, psychosocial stress, harmonization effect, SHO (証).

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

Introduction

A recent increase of patients with chronic allergic diseases, such as bronchial asthma, allergic rhinitis and atopic dermatitis, has been reported.[1,2] However, the detailed mechanism(s) of atopic dermatitis in response to environmental factors has remained unclear. Allergen-induced reaction in the skin and airway consists of two inflammatory reactions: an immediate phase response (IPR) and a late phase response (LPR) after antigen exposure (Figure 1).[3-8] IPR is primarily caused within 1 h after the antigen exposure by IgE-dependent activation of mast cells, resulting in release of proinflammatory mediators,[7] and is often followed by an intense inflammatory reaction termed LPR.[3] LPR appears 3 to 48 h after the elicitation and is considered to be an important inflammatory reaction due to its similarity to the clinical manifestation of chronic allergic diseases and the difficulty of suppressing it without side effects by anti-allergic drugs. An increase of mast cells is essentially observed in IPR, while LPR is characterized by the accumulation of inflammatory cells including neutrophils, mononuclear cells and eosinophils.[7,8]

Although glucocorticoids and immunosuppressants seem to be the most effective drugs for the improvement of the symptoms of both IPR and LPR, they cause several undesirable effects, such as the decreased resistance to microbial infections, digestive ulcers, diabetes mellitus, bone fractures, and the dysfunction of the adrenal cortex. Recently, several traditional herbal medicines have been reported to experimentally and clinically suppress the allergic reactions in bronchial asthma and atopic dermatitis without any adverse effects.[9-11] Therefore, such herbal medicines might contribute to meeting the clinical need for new therapeutic agents.

Pruritus is an unpleasant symptom of atopic dermatitis,

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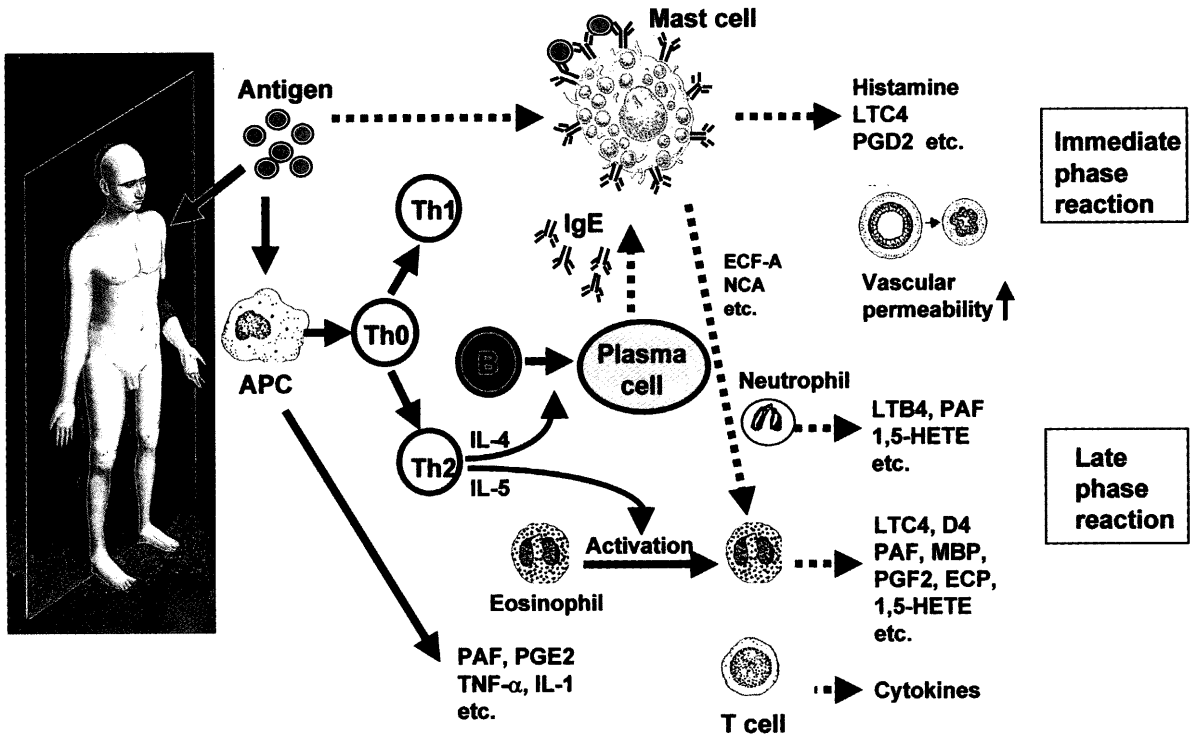

Fig. 1 Mechanism of IgE-mediated allergic reaction
Symptoms of allergic inflammation such as bronchial asthma, allergic rhinitis and atopic dermatitis involve an immediate phase reaction (IPR), which consists of acute reactions of the permeability and broncho contraction induced by degranulation of mast cells, and a late phase reaction (LPR), which is a chronic inflammatory reaction, accompanied by the infiltration of inflammatory cells to the lesional site and the induction of various chemical mediators and cytokines.

as well as other cutaneous diseases, and it accompanies several visceral disorders such as chronic renal failure. Pruritus produces scratching, worsening the condition of atopic patients.[11,12] Therefore, improvement of pruritus is also important in the therapy of atopic dermatitis. However, the physiological and pathological mechanisms of pruritus and how pruritus is related to immunological mechanisms remain unknown, partly because of the lack of a reliable animal model.[13] It has been reported that scratching with the hind paws induced by subcutaneous injections of pruritogenic agents such as compound 48/80 and substance P into the rostal back in mice was likely an itch-associated behavior.[14]

A model for IgE-mediated cutaneous dermatitis in mice has been used to evaluate the efficacies of new anti-allergic agents including plant materials and herbal drugs.[9-11] In this model (Figure 2), passive sensitization with a murine monoclonal IgE antibody specific for the dinitrophenyl group (anti-DNP IgE mAb) followed by a challenge of dinitrofluorobenzene (DNFB) to mouse ears can induce biphasic cutaneous reactions with IPR and LPR.[3-8] In the process of our study, we recently found a third inflammatory phase response following LPR, temporarily named the "very late phase response (vLPR)".[15]

We review here the effect of some Kampo formulations and their constituent crude drugs on the IgE-mediated triphasic cutaneous reaction in mice and scratching behavior that is probably an itch-associated behavior after the challenge, and the mechanism of inhibitory action of these agents.

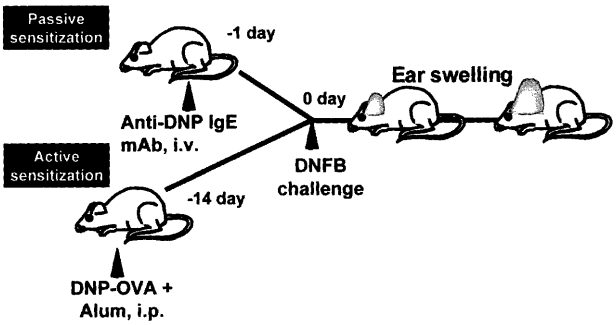

Fig. 2 Induction of skin reaction in mouse ears
BALB/c mice were actively or passively sensitized with 10 μg of DNP-OVA plus 1mg of Alum or 1.0 ml of anti-DNP IgE mAb preparation, 2 weeks or 24 h before antigen challenge, respectively. Skin reaction was elicited by applying 0.1% DNFB in 100% ethanol to the ear skin of the actively and passively sensitized mice.

Induction of IgE-mediated triphasic cutaneous reaction in mice.

BALB/c mice were actively or passively sensitized with DNP-OVA or anti-DNP IgE mAb, respectively (Figure 2). In the passive sensitization model, mice were given an i.v. injection of anti-DNP IgE mAb 24 h before the DNFB challenge. For active sensitization, mice were immunized

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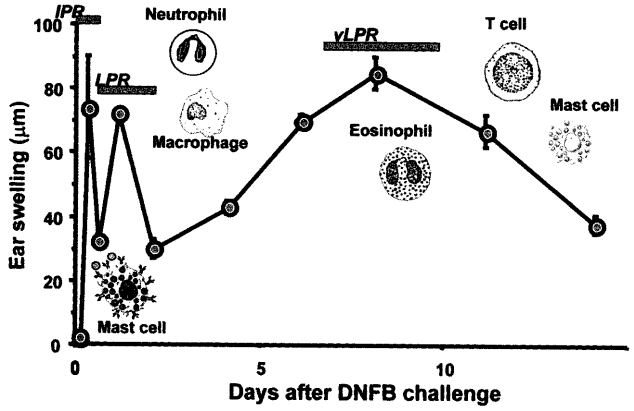

Fig. 3 IgE-mediated triphasic cutaneous reaction
vLPR in the triphasic cutaneous reaction was mainly mediated by T cells, partially dependent on mast cells and/or IgE antibody, and obviously different from LPR. It led to intense ear swelling accompanied by massive infiltration of eosinophils.

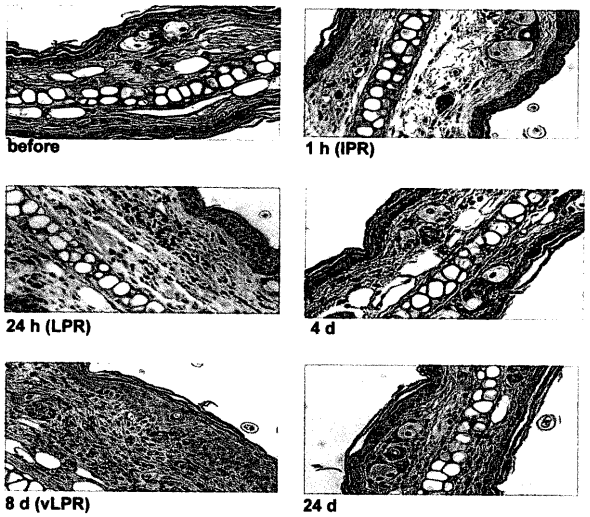

Fig. 4 Histopathological analysis of skin reaction in passively sensitized mice
One day after the challenge, infiltration of inflammatory cells was observed with edema of the dermis as compared with skin before the challenge. No significant change was seen at 4 days after the DNFB challenge, but then eosinophils infiltrated in conjunction with vLPR into the challenged ear, and particularly around hair roots. Epidermal proliferation was also observed in some lesions of ears of passively sensitized mice at 8 days.

i.p. with DNP-OVA admixed with Alum 2 weeks before the DNFB challenge. The reaction to DNFB was evaluated by measuring ear thickness immediately before the challenge and at appropriate intervals after. Figure 3 shows the time course of the IgE-mediated cutaneous reaction in passively sensitized mice. Passive sensitization with anti-DNP IgE antibody followed by a challenge of DNFB to mouse ears can induce triphasic cutaneous reactions of IPR, LPR and vLPR peaking at 1 h, 24 h and 8 days after antigen challenge, respectively.[15] 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. The triphasic cutaneous reactions appeared as a result of injection of OVA in actively sensitized mice, which indicates that vLPR may be due to an antigen (hapten)-specific response in sensitized mice, but not specific for DNFB.

As shown in Figure 4, infiltration of inflammatory cells was observed with edema of the dermis 1 day after challenge as compared with skin before the challenge. Histopathological examination revealed massive infiltration of eosinophils in vLPR at 8 days, suggesting that eosinophils are responsible for the development of this reaction. Epidermal proliferation was also observed in some lesions of ears of passively sensitized mice at 8 days. 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 tests,[7,8,16] our present results indicated that the accumulation of eosinophils in vLPR was more marked than that in LPR in passively sensitized mice. This suggests that vLPR with eosinophil infiltration actually represents an important inflammatory reaction in allergic diseases (Figure 3). The LPR seen in bronchial disease[17-20] may be very similar to the vLPR in our study. Especially, Hutson et al.[19] 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 pigs in an asthma model. Also, vLPR is apparently different from the post late phase reaction (pLPR), *i.e.*, non-allergic hyperactivity in bronchial asthma,[21] because the third inflammation continued for very long periods and more intensely than LPR.

Studies using genetically mast cell-deficient WBB6F1-W/Wv mice with mutation of the W/c-kit locus[22-24] revealed that mast cells or mediators originating from them may be required for the development of vLPR and that the presence of IgE antibody may enhance the development of vLPR (Figure 3). 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 the effects in 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 (Figure 3). Thus, vLPR might include two allergic categories: an IgE-mediated response and delayed type hypersensitivity (DTH), that is, the so-called "flare-up" in the dermatology field.

Effect of Kampo formulations and anti-allergic drugs on IgE-mediated triphasic cutaneous reaction in passively sensitized mice

We examined the efficacy of 19 Kampo formulations on a third phase response vLPR following IPR and LPR (Figure 5). These formulations were used for **HEAT**-symptom and **COLD**-symptom (熱証, 寒証) according to

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IPR	LPR	vLPR	Kampo medicines		Anti-allergic agents
+	+	+	Tokaku-joki-to	桃核承気湯	Predonisolone (steroid)
			Byakko-ka-ninjin-to	白虎加人参湯	Y-24180 (PAF receptor antagonist)
			Sho-seiryu-to	小青竜湯	Cyclosporin A, FK-506
			Ji-zuso-ippo	治頭瘡一方	
			Sho-saiko-to	小柴胡湯	
-	+	+	Shimotsu-to	四物湯	ONO-4057
			Ogi-kenchu-to	黄耆建中湯	(LTB4 receptor antagonist)
			Toki-inshi	当帰飲子	
-	-	+	Juzen-taiho-to	十全大補湯	
+	+	-	Shofu-san	消風散	Azelastine
			Hochu-ekki-to	補中益気湯	(Membrane stabilizer)
			Toki-shakuyaku-san	当帰芍薬散	ONO-1078
					(LTC4, D4, E4 receptor antagonist)
-	+	-	Unsei-in	温清飲	
+	-	-			Diphenhydramine, Terfenadine,
					(H1 receptor antagonist)
					Amlexanox (Membrane stabilizer)
-	-	-	Inchin-ko-to	茵陳蒿湯	
			Keigai-rengyo-to	荊芥連翹湯	
			Rokumi-gan	六味丸	
			Oren-gedoku-to	黄連解毒湯	
			Sho-kenchu-to	小建中湯	
			Yoku-kan-san	抑肝散	

Fig. 5 Summary of the effect of Kampo formulations and anti-allergic agents on the triphasic cutaneous reaction
The effects were categirized into several groups according to the efficacies for IPR/LPR/vLPR (for example, +/+/+ or -/-/- groups).

the pathological condition and nature of the disease, as diagnosed by the system of Kampo medicine. For instance, symptoms of the condition of **HEAT** include the feeling of heat inside the body, hyperemia and local status of heat, while symptoms of the condition of **COLD** include feeling cold in the body, insufficient blood circulation and local status of cold. Each Kampo formulation was given orally 2 h before and 2 to 6 days after DNFB challenge. As summarized in Figure 5, the inhibitory effects of the Kampo formulations on the triphasic cutaneous reaction were divided into several groups according to the efficacies for IPR/LPR/vLPR.[25]

The group consisting of Tokaku-joki-to (Tao-He-Cheng-Qi-Tang, 桃核承気湯), Ji-zuso-ippo (Zhi-Tou-Chuang-Yi-Fan, 治頭瘡一方), Sho-sei-ryu-to (Xiao-Qing-Long-Tang, (小青竜湯), Byakko-ka-ninjin-to (Bai-Hu-Jia-Ren-Sheng-Tang, 白虎加人参湯) and Sho-saiko-to (Xiao-Chai-Hu-Tang, 小柴胡湯) significantly inhibited IPR, LPR and vLPR (i.e. +/+/+ group of IPR/LPR/vLPR), similar to the effect of prednisolone as a positive control. Shofu-san (Xiao-Feng-San, 消風散), Hochu-ekki-to (Bu-Zhong-Yi-Qi-Tang, 補中益気湯) and Toki-shakuyaku-san (Dang-Gui-Shao-Yao-San, 当帰芍薬散) were included in the +/+/- group of IPR/LPR/vLPR. Oral administration of Inchinko-to (Yin-Chen-Gao-Tang, 茵陳蒿湯), Keigai-rengyo-to (Jing-Jie-Lian-Qiao-Tang, 荊芥連翹湯) and Rokumi-gan (Liu-Wei-Wan, 六味丸) did not cause any inhibition of the triphasic cutaneous reaction (-/-/- group). Thus, in this study, there was no specific relationship between the efficacy on the triphasic cutaneous reaction and the formulations determined according to Kampo diagnosis based on **HEAT**-symptom and **COLD**-symptom.[25]

We also found the differential effects of several synthetic anti-allergic drugs with different mechanisms of action on the IgE-mediated triphasic cutaneous reaction,[26] and the pattern of the effectiveness of these drugs was additionally summarized in Figure 5. Platelet activating factor (PAF) is known to be a potent chemotactic factor for eosinophils to induce allergic inflammation,[27] and have a variety of biological properties such as vasodilation[28] and broncho-constriction.[29] It has also been reported to enhance the adhesion of eosinophils to endothelial cells through the up-regulation of cell adhesion molecules,[30] degranulation of eosinophils,[31] and vascular permeability.[32,33] Leukotriene B4 (LTB4) has been reported to be produced mainly by neutrophils, pulmonary alveoli macrophages and monocytes and to have strong chemotactic activity against several types of inflammatory cells, such as eosinophils, neutrophils and macrophages.[34] Since PAF receptor antagonist (Y-24180) and LTB4 receptor antagonist (ONO-4057) were effective at inhibiting the triphasic cutaneous reaction,[26] Kampo formulations in the +/+/+ and/or -/+/+ groups may show such anti-PAF and LTB4 properties. Also, suppression of LTB4 and PAF activities may be primarily important in downregulating eosinophilic inflammation. Immunosuppressants (cyclosporin A and FK-506) have been used with beneficial effects in the

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treatment of serious allergic diseases. They have been reported to inhibit production of cytokines such as IL-4 and IL-5 by Th2-type lymphocytes.[35,36] Cyclosporin A and FK-506 inhibited the IgE-mediated triphasic skin reaction as did prednisolone (Figure 5). Since vLPR essentially disappeared in T-cell deficient nude mice with passive sensitization,[15] the effect of these immuno-suppressants on vLPR may also affect T cell-mediated inhibition. Interestingly, the +/-/- group, which inhibited IPR only, included H1 receptor antagonists (terfenadine and diphenhydramine) and a membrane stabilizer (amlexanox), whereas none of the tested Kampo formulations were included in this group (+/-/-).[25]

1) Sho-seiryu-to

Sho-seiryu-to is a representative Kampo formulation for improving an unbalanced stasis of body fluids which is referred to as **WATER IMBALANCE** (水毒), a concept that includes overall water metabolism and various functions such as the defense system, and is mainly used for respiratory diseases including bronchial asthma or rhinitis.[37,38] Figure 5 suggests that the Sho-seiryu-to (+/+/+ group) may also be effective for patients with atopic dermatitis.

2) Tokaku-joki-to

Toki-shakuyaku-san (+/+/- group) and Tokaku-joki-to (+/+/+ group) have been used to improve **OKETSU** (瘀血), 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.[37] Terasawa *et al*[39] reported four cases of atopic dermatitis successfully treated with Tokaku-joki-to. Therefore, it may be of particular interest to investigate the possible effects of other **OKETSU**-improving formulations on the IgE-mediated triphasic cutaneous reaction. Oral administration of Tokaku-joki-to resulted in a significant inhibition of the murine triphasic cutaneous reaction in a dose-dependent manner. Among the five crude drug constituents in the Tokaku-joki-to formulation, the extracts of Glycyrrhizae Radix (Kanzo, in Japanese) and Cinnamomi Cortex (Keihi) significantly inhibited IPR, LPR and vLPR. Persicae Sememn (Tonin) extract significantly inhibited vLPR, but did not affect IPR or LPR. Natrii Sulfus (Bosho) and Rubarb Rhizome (Daio) extract did not show any effect on the triphasic skin reaction. Tokaku-joki-to as well as diphenhydramine (an H1 receptor antagonist) significantly inhibited scratching behavior (pruritus) in IPR.

3) Shimotsu-to

Shimotsu-to (Si-Wu-Tang, 四物湯; -/+/+ group) is a key formulation, which has been used in some Kampo medicines such as Unsei-in (Wen-Qing-Yin, 温清飲) 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, in Japanese) and Angelicae Radix (Toki) have been used to improve **OKETSU**, and are considered to stimulate the circulation of **KETSU** (血, refers to blood, hormones, autonomic nervous system and other regulatory functions of the body's internal environment) and **KI** (気, a concept that encompasses mental nervous activity, especially the appetite for food and actual process of digesting and absorbing nutrients) in Kampo medicine.

Shimotsu-to inhibited ear swelling in LPR and vLPR after DNFB challenge in a dose-dependent manner, and slightly diminished the scratching behavior considered to be associated with pruritis in IPR (Figures 6 and 7).[40] A variant formulation, Shimotsu-to without Cnidii Rhizoma (Senkyu), decreased the inhibitory effect on triphasic skin reaction as compared with intact Shimotsu-to (Figure 7). The extract of Cnidii Rhizoma (Senkyu) markedly inhibited LPR and vLPR as compared with Angelicae Radix (Toki), Paeoniae Radix (Shakuyaku) or Rehmanniae Radix (Jio) in the Shimotsu-to formulation. Cnidii Rhizoma (Senkyu) may

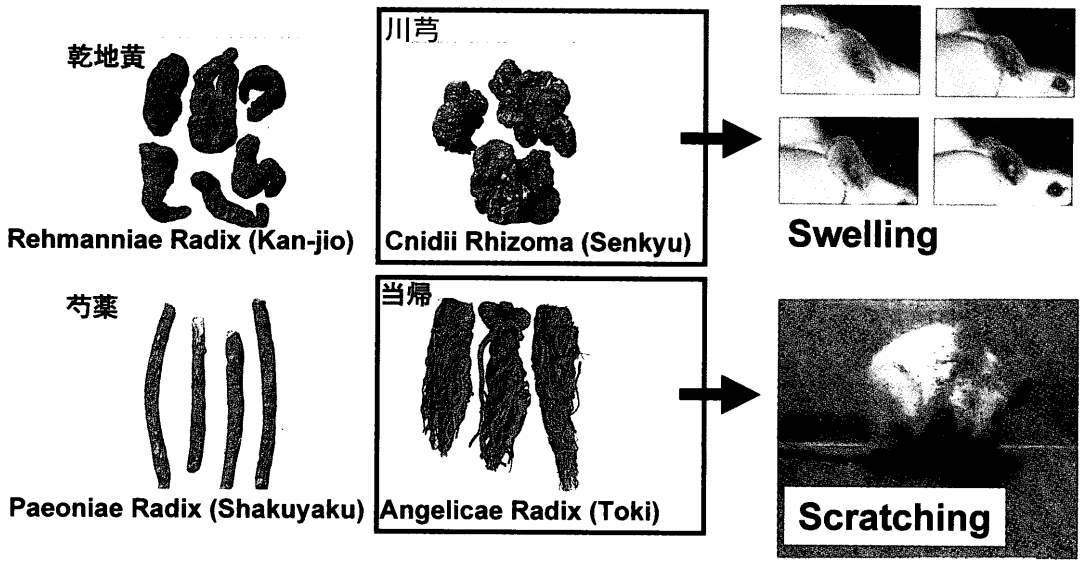

Fig. 6 Effect of Shimotsu-to constituents on triphasic skin reaction and scratching behavior (pruritis) in passively sensitized mice
The inhibitory effect on LPR and vLPR was partly due to Cnidii Rhizoma (Senkyu) in the Shimotsu-to formulation, especially cnidilide. On the other hand, Angelicae Radix (Toki) rather than Cnidii Rhizoma (Senkyu) in Shimotsu-to inhibited the scratching behavior, although it did not inhibit the ear swelling in IPR.

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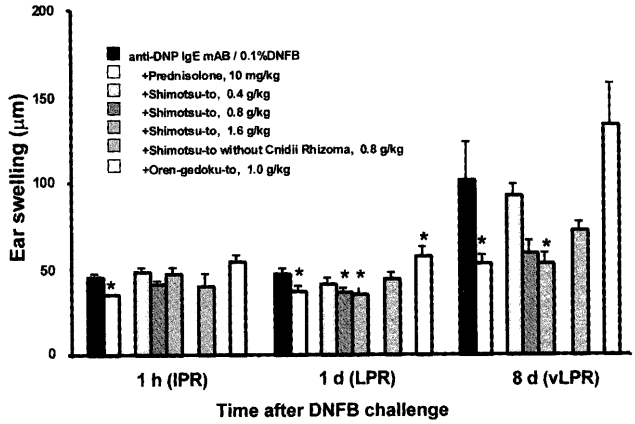

Fig. 7 Effect of Shimotsu-to and its variant formulation on IgE-mediated triphasic skin reaction in passively sensitized mice
Mice received intravenous injection of 1.0 ml of anti-DNP IgE mAb preparation 24 h before skin testing with 0.1% DNFB in 100% ethanol. Each Kampo formulation was given orally 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.

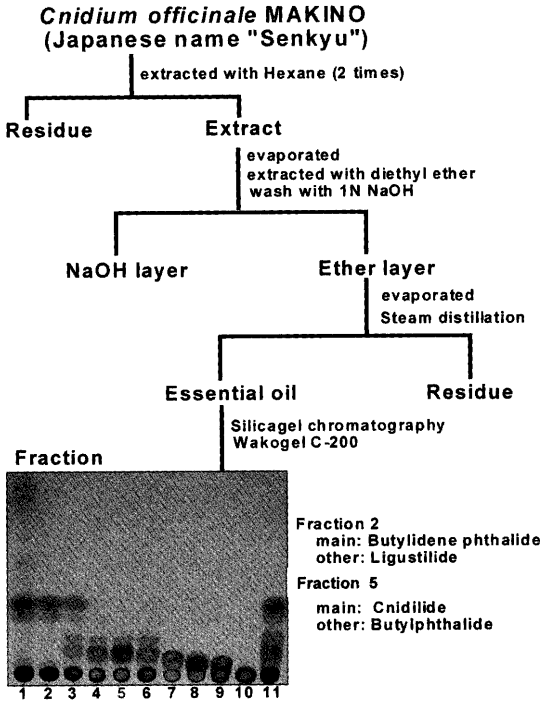

Fig. 8 Extraction and fractionation of *Cnidium officinale*
The materials were extracted with hexane to give a hexane extract. The hexane extract was dissolved in diethyl ether and washed with 1N NaOH. Then the ether layer was concentrated and steam-distilled to give an essential oil. This was subjected to silica gel column chromatography using Wakogel C-200 to give ten fractions by thin layer chromatography. Fraction 2 was mainly composed of butylidenphthalide with a little ligustilide, and fraction 5 was mainly cnidilide with a bit of butylidenphthalide.

be primarily involved in the development of the Shimotsu-to-mediated inhibition of the skin reaction. In addition, the inhibitory effect by 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 (Figure 8).[40] This is clearly consistent with our observation that Angelicae Radix (Toki), which contains abundant butylidenphthalide and ligustilide,[41] did not show any inhibitory effect on the triphasic skin reaction. Interestingly, very small amounts of cnidilide (50-200 mg/kg) in Fraction 5 of the Cnidii Rhizoma (Senkyu) extract markedly inhibited both LPR and vLPR as compared with 10 mg/kg prednisolone, but butylphthalide in Fraction 5 did not inhibit them (Figure 9).

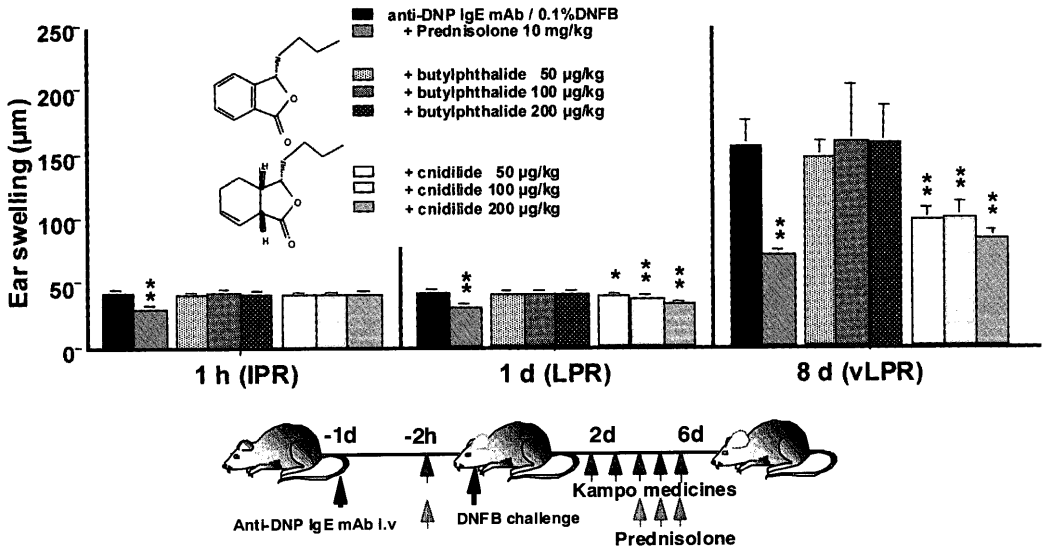

Fig. 9 Effects of butylphthalide and cnidilide on triphasic skin reaction in passively sensitized mice
Mice received intravenous injection of 1.0 ml of anti-DNP IgE mAb preparation 24 hours before skin testing with 0.1% DNFB in 100% EtOH. Prednisolone was given intraperitoneally 2 hours before challenge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

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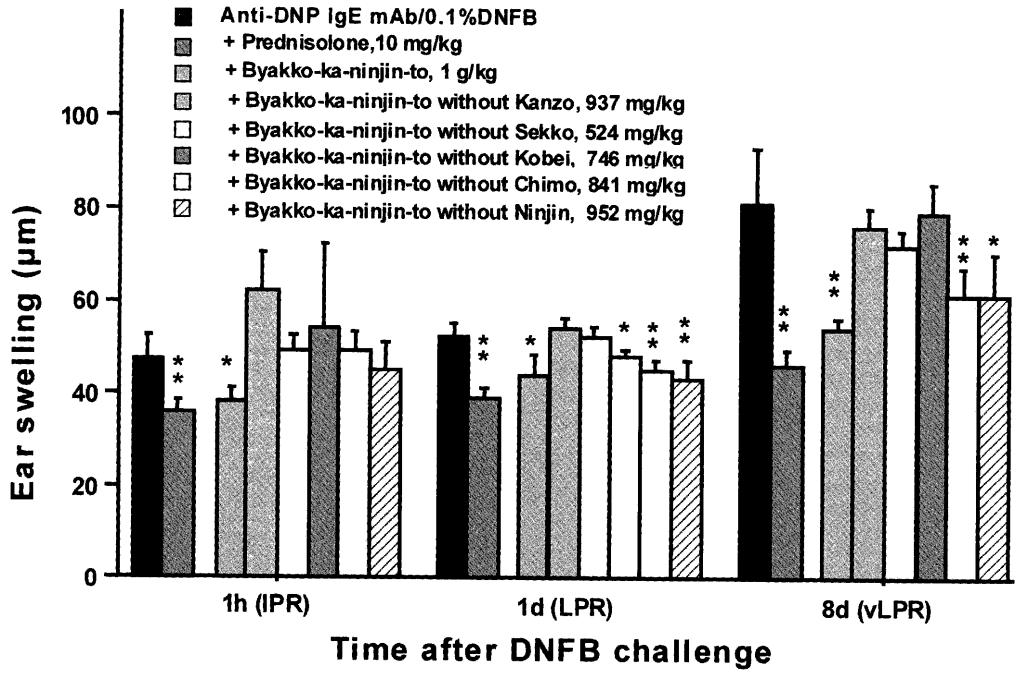

Fig. 10 Effect of variant formulations of Byakko-ka-ninjin-to on triphasic skin reaction in passively sensitized mice
Byakko-ka-ninjin-to and its variant formulations (without one crude drug) were given orally 2 h before and 2 to 6 days after DNFB challenge. Prednisolone (10 mg/kg) was given intraperitoneally 2 h before and 4 to 6 days after the challenge. Each value represents mean ± S.D. of 3 mice. *, $p<0.05$, **, $p<0.01$ by Steel's test.

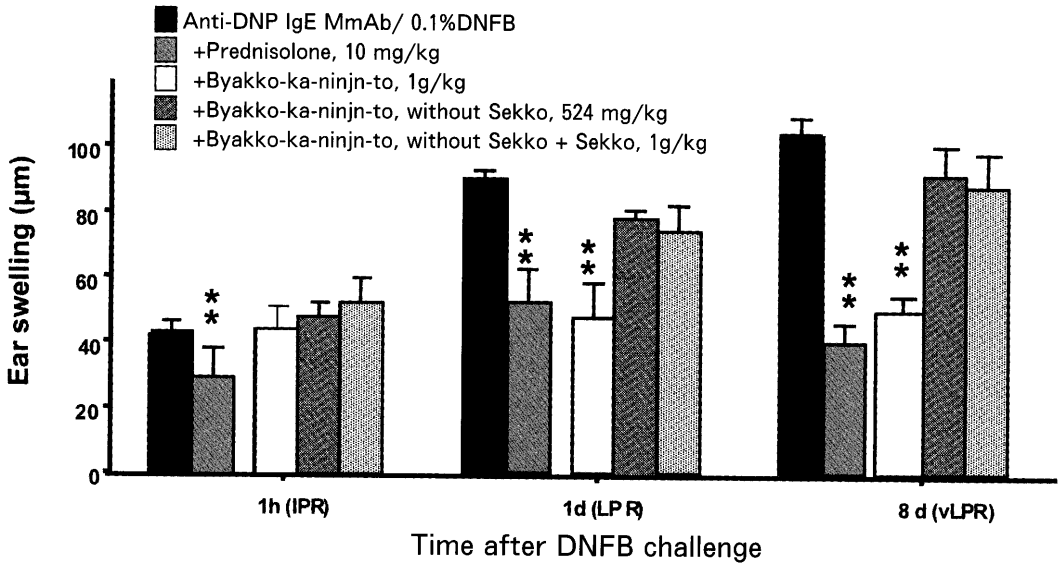

Fig. 11 Effect of a mixture of <Byakko-ka-ninjin-to without Sekko> and Sekko on triphasic skin reaction in passively sensitized mice
Byakko-ka-ninjin-to, <Byakko-ka-ninjin-to without Sekko>, or a mixture of <Byakko-ka-ninjin-to without Sekko> and Sekko were given orally 2 h before and 2 to 6 days after DNFB challenge. Prednisolone was given intraperitoneally 2 h before and 4 to 6 days after the challenge. Each value represents the mean ± S.D. of 3 mice. *,$p<0.05$, **, $p<0.01$ by Steel's test.

On the other hand, Angelicae Radix (Toki) rather than Cnidii Rhizoma (Senkyu) in Shimotsu-to, inhibited the scratching behavior, although it did not inhibit the ear swelling in IPR.[42] Shimotsu-to containing both crude drugs evidently showed both inhibitory properties.

4) Byakko-ka-ninjin-to

Oral administration of Byakko-ka-ninjin-to inhibited the IgE-mediated triphasic skin reaction in mice passively sensitized with anti-DNP IgE antibody (Figure 10).[43] Variant formulations of Byakko-ka-ninjin-to without Gypsum Fibrosum (Sekko, $CaSO_4 \cdot 2H_2O$), Glycyrrhizae Radix (Kanzo) or Oryzae Semen (Kobei) exerted an attenuated inhibitory effect as compared with intact Byakko-ka-ninjin-to (Figure 10). The decreased effect of Byakko-ka-ninjin-to without Kanzo was restored by the addition of Kanzo extract to the variant formulations before oral

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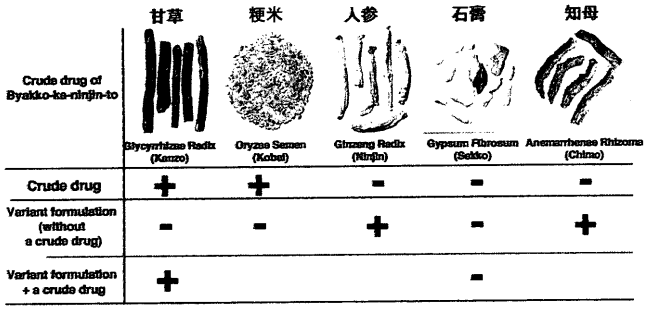

Fig. 12 Summary of the effect of Byakko-ka-ninjin-to and its variant formulations on IgE-mediated triphasic cutaneous reaction
Variant formulations of Byakko-ka-ninjin-to without Gypsum Fibrosum (Sekko), Glycyrrhizae Radix (Kanzo) or Oryzae Semen (Kobei) showed an attenuated inhibitory effect as compared with Byakko-ka-ninjin-to. The decreased effect of Byakko-ka-ninjin-to without Kanzo was restored by the addition of Kanzo to the variant formulations before oral administration, while the decreased effect of Byakko-ka-ninjin-to without Sekko could not be restored by the addition of Sekko.

administration, because the Kanzo extract itself was active. In contrast, the decreased effect of Byakko-ka-ninjin-to without Sekko could not be restored by the addition of Sekko extract, although the active Kanzo is part of this variant formulation (Figure 11). The above results using crude drug extracts and variant formulations of Byakko-ka-ninjin-to are summarized in Figure 12.

Since Kampo formulations are generally prepared from the combination of many crude drugs, they may have combined effects which differ from the sum of the effects of the individual constituent crude drugs. They must have an acceptable efficacy and quality when used as therapeutic medicines. Formulations prepared from crude drugs with different qualities would have different biological activities and efficacies. Therefore, it is necessary to control and confirm the quality of the formulations and their constituent crude drugs, because their quality may vary with the origins of crude drugs and the place of harvest, etc. HPLC pattern analysis, the so-called "fingerprint" method, could provide a useful means of identifying the crude drugs and preparing batches of constant formulation.[44] Although the many compounds which have no UV absorbance can not be detected by this method, the fingerprint similarity of the formulation may be primarily useful in assessing the homogeneity of the formulation, which should lead to constant efficacy. Figures 13 and 14 summarized the HPLC profiles of Byakko-ka-ninjin-to and its variant formulations without one crude drug and their efficacies for the IgE-mediated triphasic cutaneous reaction.

Disappearance of the peaks in the HPLC profile of Byakko-ka-ninjin-to without Kanzo may, in part, be due to the absence of Kanzo (peaks b, e, f, g, h-k, and l in Figure 13 and 14). Since elimination of Sekko from the Byakko-ka-ninjin-to constituents attenuated the efficacy although Sekko did not show any activity per se, mutual interaction of Sekko with other constituents during the preparation process may result in the production of new components. These results may be related to the clinical observations that Byakko-ka-ninjin-to containing increasing doses of Sekko actually becomes successively more effective in terms of the holistic patterns of individual pathogenic alterations, so-called **SHO** (証), of patients with atopic dermatitis.[45,46]

Byakko-ka-ninjin-to without Kobei or without Ninjin

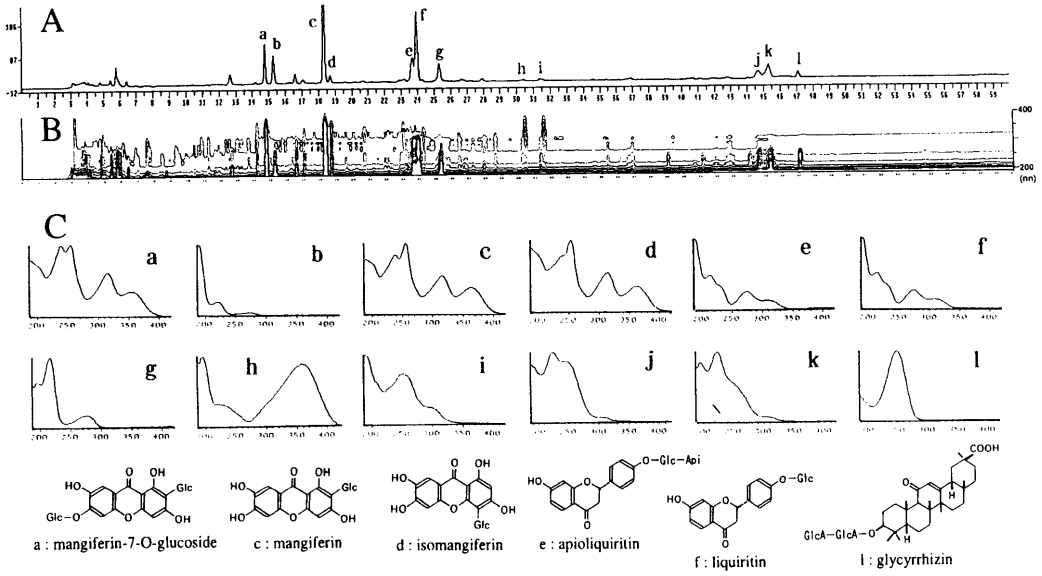

Fig. 13 HPLC profile of Byakko-ka-ninjin-to and UV spectra.
A preparation of Byakko-ka-ninjin-to was extracted with H_2O-EtOH (9:1, 200 ml), filtered and analyzed by HPLC (HP-1090, Hewlett-Packard) under the following conditions: column, TSK gel 80 Ts ODS (4.6 x 250 mm); mobile phase, 10 mM phosphoric acid: CH_3CN (linear gradient, 95:5→20:80, for 1 h); flow rate, 0.8 ml/min; oven temperature, 40°C; injection volume, 5 μl.
A: HPLC pattern analyzed by absorbance at 220 nm,
B: Contour plot of HPLC pattern by UV absorbance (190-420 nm),
C; UV spectra of main peaks. Origins of peaks [a: Anemarrhenae Rhizoma (mangiferin-7-O-glucoside), b: Glycyrrhizae Radix, c: Anemarrhenae Rhizoma (mangiferin), d: Anemarrhenae Rhizoma (isomangiferin), e: Glycyrrhizae Radix (apioliquiritin), f: Glycyrrhizae Radix (liquiritin), g: Unknown, h-k: Glycyrrhizae Radix, l: Glycyrrhizae Radix (glycyrrhizin)

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	HPLC profile (peaks) a	b	c	d	e	f	g	h	i	j	k	l	Triphasic skin reaction IPR	LPR	vLPR
Byakko-ka-ninjin-to	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Byakko-ka-ninjin-to															
without Kanzo	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-
without Kanzo + Kanzo	(not analyzed)												-	+	+
without Sekko	+	+	+	+	+	+	-	+	+	-	-	+	-	-	-
without Sekko + Sekko	(not analyzed)												-	-	-
without Kobei	+	+	+	+	+	+	-	+	+	-	-	+	-	+	-
without Chimo	-	+	-	+	+	+	-	+	+	-	-	+	-	+	+
without Ninjin	+	+	+	+	+	+	-	+	+	-	-	+	-	+	+

Peaks g, j and k (shaded) were found in the HPLC profile of Byakko-ka-ninjin-to only when the five component crude drugs were present during the decoction procedure.

Fig. 14 Summary of the efficacies of variant formulations of Byakko-ka-ninjin-to and HPLC profiles
Comparison of HPLC profiles of variant formulations lacking one crude drug with that of original Byakko-ka-ninjin-to revealed that some peaks could be detected only when the five constituent crude drugs were simultaneously present during the preparation of the Byakko-ka-ninjin-to formulation. Since elimination of Sekko from the Byakko-ka-ninjin-to constituents attenuated the efficacy although Sekko did not show any activity per se, mutual interaction of Sekko with other constituents during the preparation may result in the production of new components.

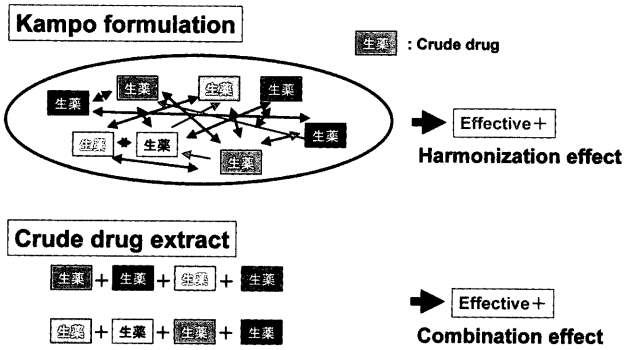

Fig. 15 "Harmonization effect" and "combination effect"
The effect of the Byakko-ka-ninjin-to formulation on cutaneous inflammatory disease may differ from the sum of the effect of the individual constituents.

showed similar HPLC patterns to Byakko-ka-ninjin-to without Sekko (Figures 13 and 14).[43] Byakko-ka-ninjin-to without Ninjin was still active in inhibiting the skin reaction, although a slight reduction of its anti-allergic effect was observed. In contrast, Byakko-ka-ninjin-to without Sekko had a markedly reduced effect, suggesting that other components in the Byakko-ka-ninjin-to without Ninjin, which can not be detected by HPLC analysis, such as polysaccharides and peptides, are responsible for the efficacy. However, since Kobei was effective at inhibiting the skin reaction (Figure 10), the marked decrease of the inhibitory effect by Byakko-ka-ninjin-to without Kobei may be due to the absence of the active components of Kobei.

Peaks g, j and k in Figures 13 and 14 could not be commonly found in the HPLC profiles of any of the variant formulations, including Byakko-ka-ninjin-to without Sekko. Therefore, these peaks may appear only when the five crude drugs are simultaneously present and interact mutually during the decoction preparation. Although further chemical studies will be needed to examine this issue in detail, the effect of Byakko-ka-ninjin-to formulation on cutaneous inflammatory disease may differ from the sum of the effect of the individual constituents (combination of the extract of individual crude drugs), which are referred to as the "harmonization effect" and "combination effect", respectively (Figure 15).

SHO (証, symptom and constitution) and HO-SHO-SO-TAI (方証相対)

Pathogenic recognition by Kampo medicine is based on the diagnosis of individual pathogenic alterations, so called **SHO**, consisting of the symptom (**SHOKO** in Japanese, 症候) and constitution (responder/non-responder, **TAISHITSU** in Japanese, 体質) of patients with different disease states (Figure 16). Therefore, Kampo medical doctors must properly diagnose the **SHO**, in order to determine the appropriate treatment modality with Kampo formulations that is suitable for improving the state of individual patients. This is referred to as **HO-SHO-SO-TAI** in Japanese (Figure 17).

There are two characteristics for the treatment modality of Kampo medicines. In the diagnosis of Kampo medicine, patients with the same disease, for instance, atopic dermatitis, can be treated with different Kampo formulations according to the pathogenic alteration **SHO** such as **KI-KYO**

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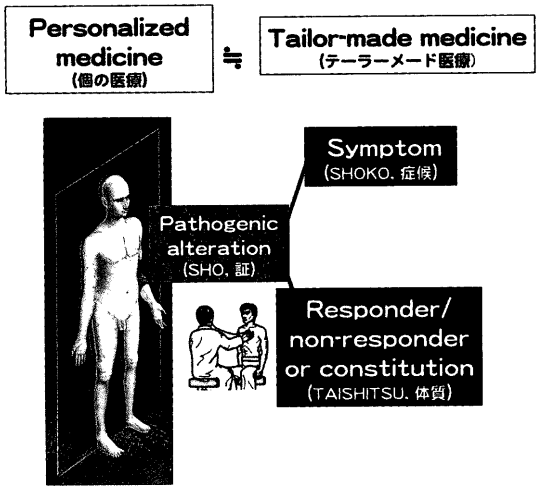

Fig. 16 SHO.
Pathogenic recognition in Kampo medicine, is based on the diagnosis of individual pathogenic alterations, so called **SHO**, consisting of symptoms (**SHOKO** in Japanese) and constitution (**TAISHITSU** in Japanese, or responder/non-responder) of patients with different disease states.

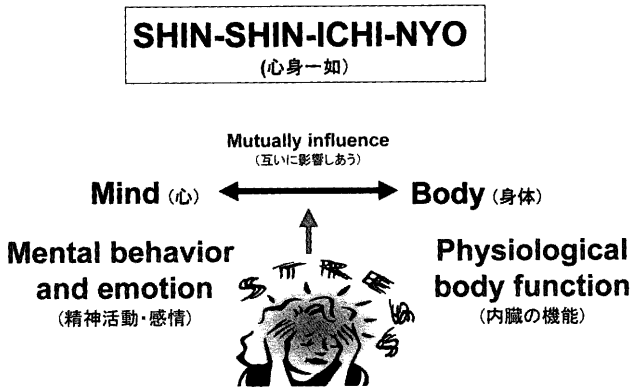

Fig. 18 Mutual and intimate interaction between mental/emotional alteration and biological/physiological organ function (**SHIN-SHIN-ICHI-NYO**)
Besides the genetic factors underlying the development of the diseases, environmental factors, including psychosocial stress, have also been suggested to provoke and exacerbate the diseases.

(気虚), **OKETSU**, **SUITAI** (水滞) illustrated by latitude in the globe (Figure 17). In other words, if patients with the same disease exhibit different **SHO**, *i.e.* different symptoms and constitutions, they are given different Kampo formulations. However, in Western medicine, these patients with the same diseases would be primarily or actually treated with similar or the same anti-allergic agents for atopic dermatitis. In contrast, as shown by longitude in the globe (Figure 17), patients with different diseases such as diabetic retinopathy, rheumatoid arthritis, atopic dermatitis, and menopausal syndrome can be treated with the same Kampo formulation based on a common diagnosis of **SHO**, such as **OKETSU**, a state of insufficient blood circulation and blood stasis. Thus, the diagnosis in Kampo medicine is markedly different from that of Western medicine with respect to the determination of the disease name.

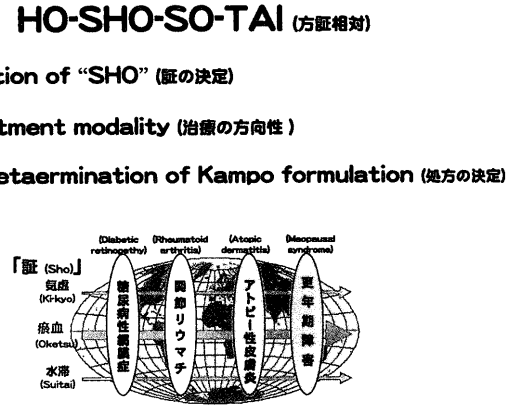

Fig. 17 HO-SHO-SO-TAI (方証相対)
Kampo medical doctors firstly examine the Sho including symptoms and constitution of patients, in order to determine the appropriate treatment modality or Kampo formulations that are suitable for improving the state of individual patients.
A summary of the difference of pathogenic recognition between Western/modern medicine and Oriental/Kampo medicine is shown. As illustrated in the globe, pathogenic recognition in Western medicine is based on the diagnosis of the disease name, represented as longitude. In contrast, pathogenic recognition in Kampo medicine, is based on the diagnosis of individual pathogenic alterations, so called **SHO**, consisting of symptoms and constitution (responder/non-responder) of patients with different disease states, represented as latitude. In Kampo medicine, **SHO** parameters such as **KI-KYO**, **OKETSU**, **SUITAI** and so on are used for the diagnosis, instead of the disease name.

Psychosocial stress enhances IgE-mediated triphasic cutaneous reaction in mice: Antagonism by Yokukan-san (Yi-Gan-San, 抑肝散)

Besides the genetic factors underlying the development of the diseases, environmental factors, including psychosocial stress, have also been suggested to provoke and exacerbate the diseases.[47-50] Thus, mental and emotional alterations can mutually and intimately influence biological and physiological organ function, which is referred to as **SHIN-SHIN-ICHI-NYO** (心身一如) in Kampo medicine (Figure 18).

Increasing evidence has suggested the important impact of psychosocial stress, such as the loss of an intimate relationship, divorce, bereavement or other adverse life events, on the subsequent onset or exacerbation of many types of human diseases, such as depression, cardiovascular diseases, cancer and atopic dermatitis.[47-50] Evidence in support of this hypothesis comes from clinical studies, including stressful life events often preceding the exacerbation of atopic dermatitis, and daily emotional stress (such as a rigid family structure or negative communication with significant others) predicting symptom severity in children and adults with atopic dermatitis.[50-55] However, to our knowledge, few experimental studies of the influence of psychosocial stress on allergic cutaneous reactions in animals have been reported.

Social isolation, *i.e.* individual housing of laboratory animals, is a model of a lack of social interactions among animals involving the anxiety emotion (Figure 19), and is considered to be relatively comparable with the situation of humans who feel isolated. In rodents, social isolation

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results in marked behavioral disturbances such as increased aggressiveness, enhanced locomotive activity and elevated morphine consumption as well as reduced pentobarbital-induced sleeping time.[56-58] There are also physiological disturbances including high levels of plasma corticosterone and catecholamine and high activity of corticotropin releasing factor (CRF) and attenuated immune functions.[59-62] Our previous studies revealed that social isolation stress could enhance liver metastasis and angiogenesis of colon carcinoma cells, and suppress immune functions such as NK cell- and macrophage-mediated cytotoxicity in mice.[63-65] Because of its inherently social nature, social isolation is viewed as a relatively natural and convenient model for constituting psychosocial stress, and should be useful for investigating the modulatory role of psychosocial stress on allergic inflammatory reactions in mice (Figure 19).

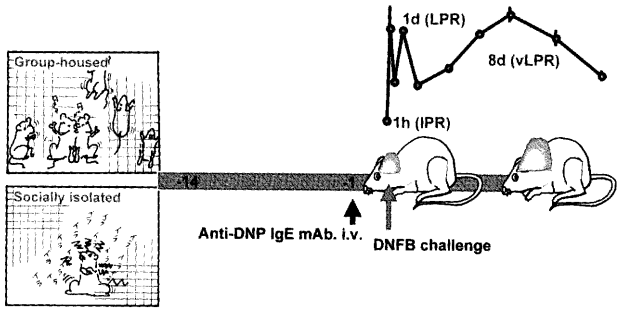

Fig. 19 Induction of IgE-mediated skin reaction in mice under socially isolated conditions
BALB/c mice were randomly assigned to be group-housed (n=3 per cage: 24x17x12cm) or individually-housed in same-sized cages (n=1 per cage: 24x17x12cm) for 2 weeks before starting skin testing. Mice were given an i.v. injection of anti-DNP IgE mAb-containing fluid 24 h before DNFB challenge. The skin reaction to DNFB was evaluated by measuring ear thickness immediately before and at appropriate times after the challenge.

As shown in Figure 20, social isolation stress could exacerbate the triphasic cutaneous reaction (IPR, LPR and vLPR) in response to 0.01-0.05% DNFB in passively sensitized mice. It is well known that complex alterations of the autonomic nervous, immune and endocrine systems are modulated by psychosocial stress. Psychological or physiological stresses can stimulate the hypothalamic-pituitary adrenal axis (HPA), sympatho-adrenomedullary system and sympathetic nervous system, but inhibit the hypothalamic-pituitary-testicular axis[59-61,66]. CRH, which can be activated by isolation stress, was observed to induce skin mast cell degranulation and increase vascular permeability.[67,68] It is known that mast cells are responsible for skin reactions, and in particular IPR and vLPR in the triphasic cutaneous reaction model.[15] Therefore, isolation stress-induced exacerbation of the skin reaction may be associated with increased production of glucocorticoid and catecholamine, a decreased testosterone level, over-activity of CRH, or a combination of these alterations.

A Kampo formulation with anti-psychotic action, Yokukan-san, has anti-convulsive, sedative and analgesic properties, including preservation of emotional balance, and is often used to improve oversensitive, quick-tempered and restless symptoms in patients.[69,70] It is currently administered to small children with crying fits during the night, patients with convulsions due to fever, twitching and jerking

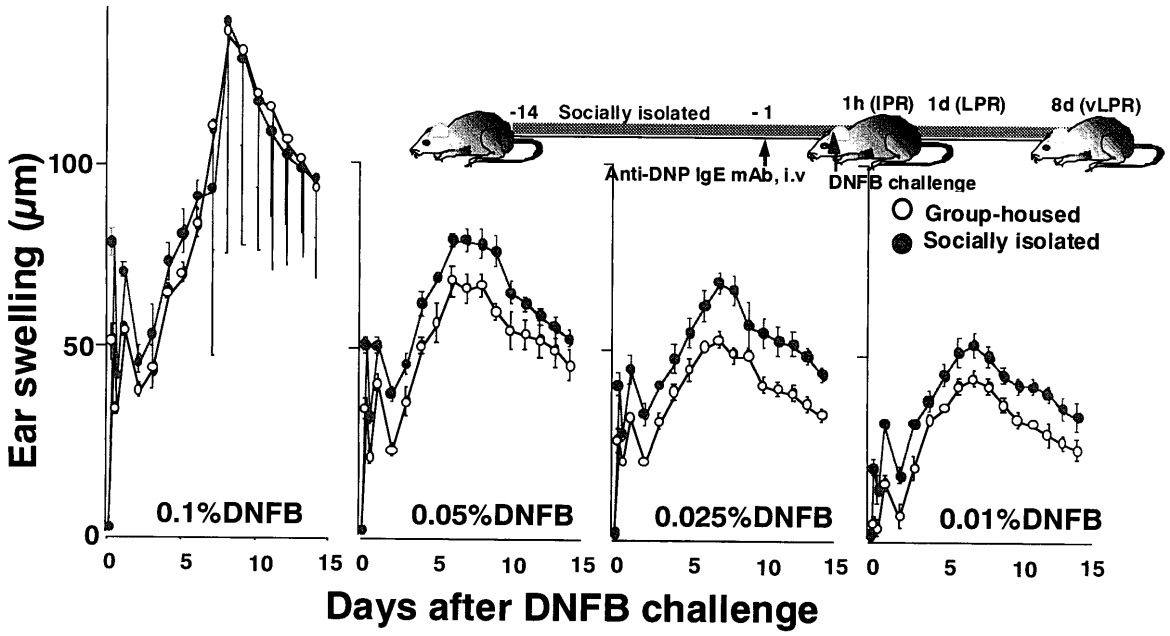

Fig. 20 Effect of social isolation stress on IgE-mediated triphasic skin reaction in passively sensitized mice
BALB/c mice (4 weeks old, male) were group-housed or socially isolated (n=3) for 2 weeks before skin testing and throughout the experiment. Mice received intravenous injection of 1.0 ml of anti-DNP IgE mAb preparation 24 h before skin testing with different doses of DNFB (0.01%, 0.025%, 0.05%, 0.1%) in 100% ethanol. Ear swelling was measured at 1 h, 24 h and 8 days following the DNFB challenge to evaluate IPR, LPR and vLPR, respectively. Group-housed (○), socially isolated (●).

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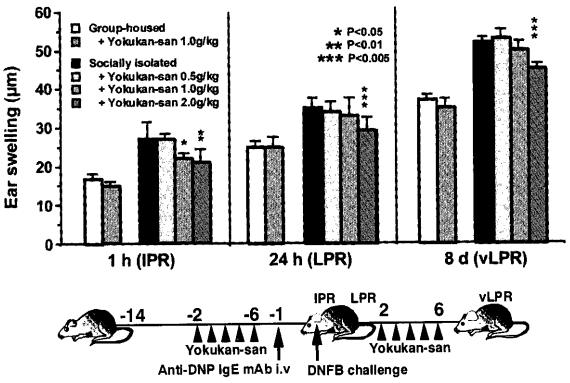

Fig. 21 Effects of Yokukan-san on IgE-mediated triphasic skin reaction in group-housed and socially isolated mice
Mice were group-housed or socially isolated for 2 weeks before skin testing and throughout the experiment. Mice received intravenous injection of 1.0 ml of anti-DNP IgE mAb 24 h before skin testing with 0.025% DNFB in 100% ethanol. Yokukan-san was given orally 6 to 2 days before and 2 to 6 days after DNFB challenge. Each value represents the mean±S.D. of 3 mice. *: $p<0.05$,**: $p<0.01$,***: $p<0.005$ vs. vehicle (control of each group), by Mann-Whitney's U-test.

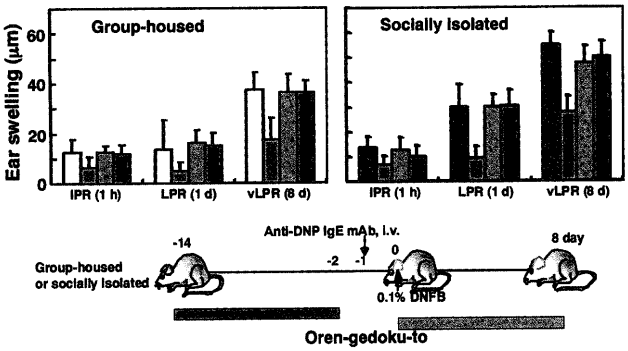

Fig. 23 Effects of Oren-gedoku-to on IgE-mediated triphasic skin reaction in group-housed and socially isolated mice
Mice were group-housed or socially isolated for 2 weeks before skin testing and throughout the experiment. Mice received intravenous injection of 1.0 ml of anti-DNP IgE mAb 24 h before skin testing with 0.025% DNFB in 100% ethanol. Oren-gedoku-to was given orally 6 to 2 days before and 2 to 6 days after DNFB challenge. Each value represents the mean±S.D. of 3 mice.

of muscles, mania, insomnia and neurological symptoms. As shown in Figure 21, oral administration of Yokukan-san dose-dependently inhibited the enhancement of IPR, LPR and vLPR in socially isolated mice, although it did not show any inhibition in the group-housed mice and belongs to the group -/-/- in Figure 5. In contrast, a typical tranquilizer (diazepam), which acts on both central and peripheral benzodiazepine receptors, reduces anxiety and inhibits the stress-induced increase in the secretion of anterior pituitary hormones, including ACTH, corticosterone, and behavior-associated epinephrine.[71-74] Intraperitoneal administration of diazepam dose-dependently inhibited the enhancement of IPR and LPR in socially isolated mice, but markedly exacerbated vLPR of both the group-housed and socially isolated mice in a dose-dependent manner (Figure 22), and its effects thus differed from the inhibitory effect of Yokukan-san on

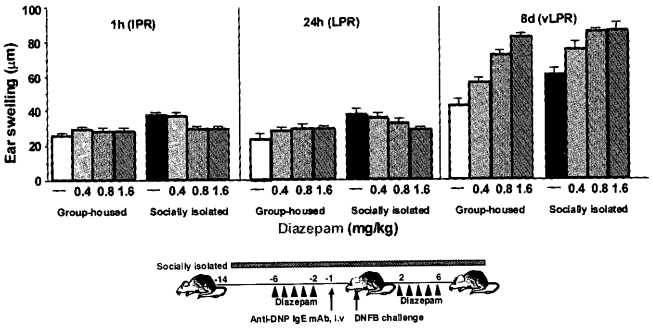

Fig. 22 Effects of diazepam on IgE-mediated triphasic skin reaction in group-housed or socially isolated mice
Mice were group-housed or socially isolated for 2 weeks before skin testing and throughout the experiment. Mice received intravenous injection of 1.0 ml of anti-DNP IgE mAb 24 h before skin testing with 0.025% DNFB in 100% ethanol. Diazepam was given intraperitoneally 6 to 2 days before and 2 to 6 days after DNFB challenge. Each value represents mean±S.D. of 3 mice.

vLPR.

In addition to the improvement of the exacerbated allergic skin reaction by Yokukan-san and diazepam in socially isolated mice, diazepam also showed an inhibitory effect on both locomotive and aggressive behavior stimulated by stress, while Yokukan-san exhibited a suppressive effect on the former but not the latter.[74] Thus, Yokukan-san and diazepam exhibited different patterns of inhibitory effect on isolation stress-enhanced triphasic cutaneous reactions and stress-evoked behavioral disturbances. These results suggest that Yokukan-san and diazepam antagonize isolation stress-provoked cutaneous functions in part through their sedative action on social isolation stress. The mechanism underlying the inhibitory effects of Yokukan-san and diazepam on triphasic cutaneous reaction in socially isolated mice will still need to be examined in detail.

On the other hand, Oren-gedoku-to (Huan-Lian-Jie-Du-Tang, 黄連解毒湯) did not affect the exacerbated triphasic skin reaction in this isolation stress model or the skin reaction in group-housed conditions (Figure 23). According to the theory of the five elements; **WOOD** (木), **FIRE** (火) , **EARTH** (土), **METAL** (金) and **WATER** (水) in Kampo medicine (**GO-GYO** theory in Japanese, 五行論, Figure 24), everything in this world is represented by five elements or symbols taken from nature, and various interactions can be interpreted according to this theory. It is also used as a guide for diagnosing pathological changes and treating diseases. The organs related to these elements show positive and/or negative influences on others, for instance, a tonifying effect of the **LIVER** (肝) on the **HEART** (心, arrow) or a sedating effect of the **LIVER** on the **SPLEEN** (脾, dotted arrow). Yokukan-san is used to improve the symptoms related to the LIVER, such as oversensitivity, quick-temperedness and restlessness, *i.e.*, **LIVER** in a state of excess of **WOOD**. On the other hand, Oren-gedoku-to is well known to improve some symptoms related to the heart such as flushed face, irritable, easily frightened, *i.e.*, **HEART** in a state of excess of **FIRE**. Yokukan-san was effective at improving the exacerbated

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skin reaction in this model but Oren-gedoku-to was not effective. Thus, social isolation may cause a change of **SHO** in mice, in particular symptom of an enhanced state of the **LIVER** in Kampo medicine, and induce psychological stress, differing from the state of mice reared under group-housing conditions.

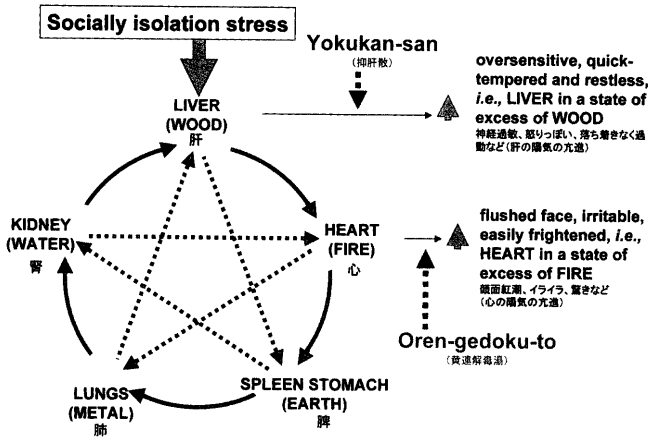

Fig. 24 Theory of five elements (**GO-GYO** theory)
In Kampo medicine, everything in this world is represented by five elements (**WOOD**, **FIRE**, **EARTH**, **METAL** and **WATER**) or symbols taken from nature, and various interactions can be interpreted by this theory. The organs related to these elements show positive and/or negative influences on others, for instance, a tonifying effect of the **LIVER** on the **HEART** (arrow) or a sedating effect of the **LIVER** on the **SPLEEN** (dotted arrow).

Strain difference in IgE-mediated triphasic cutaneous reaction in mice

Various strains of mice were actively or passively sensitized with DNP-OVA or anti-DNP IgE mAb, respectively, and then the skin reaction to DNFB was evaluated as shown in Figure 2. Various degrees of IgE-mediated triphasic cutaneous reaction were observed in the different strains of mice (Figures 25 and 26). The DBA/2 and DBA/1 strains as well as the BALB/c strain showed markedly high activities of the triphasic cutaneous reaction. In contrast, SJL, C57BL/6N and C3H/HeN strain mice exhibited lower responsiveness to DNFB, even under the same conditions of sensitization and challenge. Interestingly, C57BL/6N strain mice were lower responders than BALB/c mice, although they showed a high level of serum IgE compared with other strains of mice, including BALB/c mice. This may be partly due to the difference in other allergy-related factors such as Th1 and Th2 cytokines. Thus, markedly different responsiveness in allergic skin reaction was observed in mice with different genetic backgrounds. Strain differences in the allergic skin reaction in mice may reflect differences of **SHO**, especially of constitution (**TAISHITSU** shown in Figure 16) rather than symptoms (**SHOKO**). Further studies will be needed to examine the differential anti-allergic effects of Kampo medicines in various strains of mice.

Acknowledgments

I thank all the members in my laboratory in the

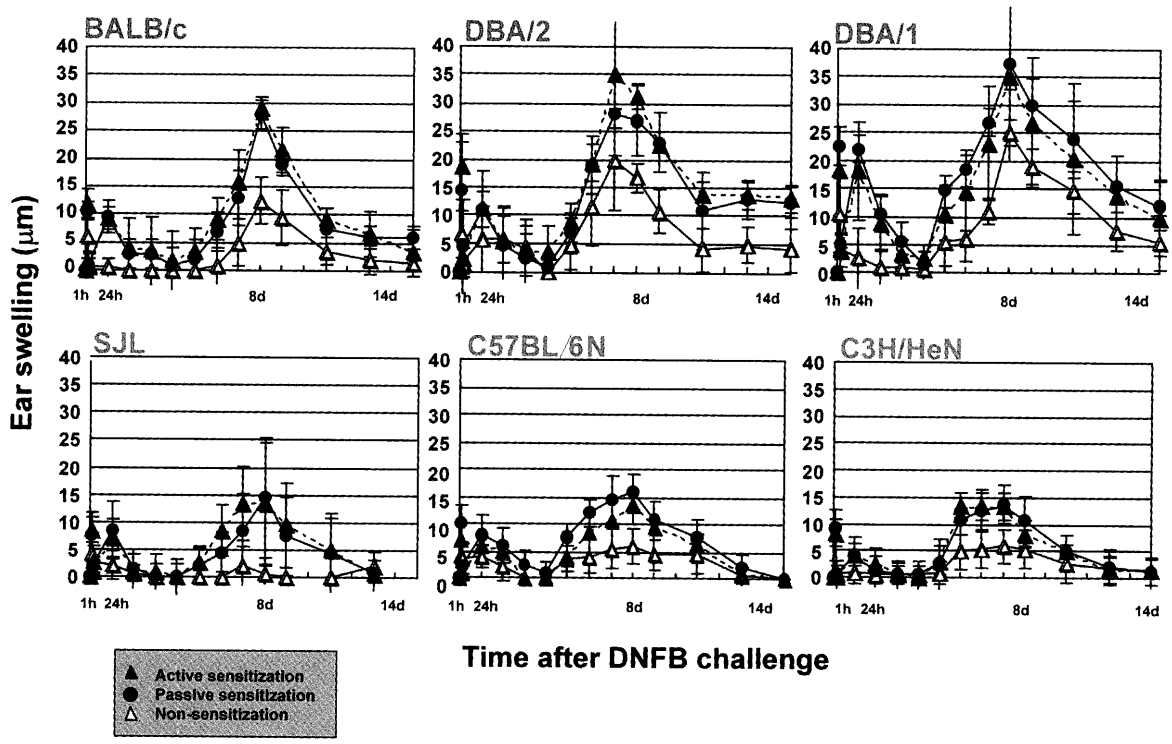

Fig. 25 Strain difference in murine IgE-mediated triphasic cutaneous reaction
Various strains of mice were actively or passively sensitized with DNP-OVA + Alum or anti-DNP IgE mAb, respectively, and then skin reaction to DNFB was evaluated as shown in Figure 2.

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Strain	IgE titer 14 d after sensitization	IPR/LPR/vLPR	Characteristics	References
BALB/c	+	++ / ++ / ++	Th2 dominant immune response, High sensitivity against radiation	J. Immunol., 136: 2348, 1986
DBA/2	+	++ / ++ / ++	Model of IgE production by feeding protein Infectivity of malaria parasites in C5-deficientDBA/2 mice	Eur. J. Immunol., 27: 3427, 1997 Infect. Immun., 63: 3702, 1995
DBA/1	+	++ / ++ / ++	High incidence of collagen-induced arthritis Susceptible to tuberculosis infection	Int. J. Immunopharmac., 17: 597, 1995
SJL	±	+ / + / +	Suppression of IgE Ab production	J. Exp. Med., 143: 833, 1976
C57BL/6N	+	+ / + / +	Th1 dominant immune response, Low rate of spontaneous carcinogenesis Antigen-induced pulmonary eosinophilia	J. Immunol., 136: 2348, 1986 Immunology, 98: 345, 1999
C3H/HeN	+	+ / + / +	High activity for complement	
BALB/c-nu	±	++ / – / –	T cell deficient athymic mice	Nature, 217: 370, 1968
CBA/J	±	+++ / + / +	Induction of immunological tolerance Mast cell dependence of DTH response Susceptible to *S. pneumonia*	J. Exp. Med., 157: 1604, 1983
MRL/lpr	+	+ / + / ++	Induction of autoimmune disease (SLE-like syndromes), Alteration of T cell components with the appearance of disease	J. Exp. Med., 149: 516, 1979

Fig. 26 Summary of IgE-mediated skin reaction and IgE production in high and low responder mice
Various strains of mice were actively or passively sensitized with DNP-OVA + Alum or anti-DNP IgE mAb, respectively, and then the skin reaction to DNFB was evaluated as shown in Figure 2. Fourteen days after active sensitization with DNP-OVA, the DNP-specific IgE titer in sera was measured by ELISA.

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Japanese abstract

抗 DNP IgE 抗体で受動感作したマウスの耳介に dinitrofluorobenzene (DNFB) を塗布することにより，1時間，24時間および8日目をピークとする即時相 (immediate phase response, IPR)，遅発相 (late phase response, LPR) および超遅発相 (very late phase response, vLPR) の三相性皮膚反応が誘導されることを見い出した。IPR は肥満細胞が欠損したマウスでは認められず，LPR は明らかに観察され，vLPR は部分的に低下した。LPR はT細胞に非依存的な反応相であるが，一方，vLPR は T 細胞欠損マウスではほぼ完全に消失した。このように，第三相目の皮膚反応 (vLPR) は多数の好酸球の浸潤を伴い，主にT細胞と部分的に肥満細胞が関与した重要な炎症性反応であることを明らかにした。このモデルを用いて検討した結果，いくつかの漢方方剤および合成抗アレルギー薬がこの IgE 介在性三相性皮膚反応を抑制することを見い出した。三相性皮膚反応に対する漢方方剤の抑制効果は，IPR, LPR, vLPR に対する効果に基づき，いくつかの群に分類された。たとえば，桃核承気湯，治頭瘡一方，小青竜湯および小柴胡湯のような方剤からなる群は，陽性対照として用いたステロイド剤 prednisolone と同様に，有意に三相性皮膚反応を抑制した (すなわち，+/+/+群)。抗不安作用を有する抑肝散を通常の群居飼育したマウスに経口投与した結果，三相性皮膚反応にほとんど効果を示さなかったが，隔離飼育によるストレス負荷マウスの増悪した三相性皮膚反応に対して，用量依存的な抑制効果を示した。一方，隔離飼育したマウスに benzodiazepine 受容体アゴニストである diazepam を腹腔内投与した結果，増悪した IPR, LPR は抑制されたが，vLPR に対する効果は隔離あるいは群居飼育にかかわらず，抑肝散の効果と明らかに異なり，さらなる増悪化が認められた。

本総説では，漢方方剤の抗アレルギー作用に焦点をあて，通常の群居飼育あるいは隔離飼育マウスを用いて，いくつかの漢方方剤の IgE 介在性三相性皮膚反応に及ぼす効果を記述する。さらに，抑制機序や漢方方剤やその構成生薬の効果発現における重要性について述べる。

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