radicals [2], eventually resulting cellular cytotoxicity, while photoallergic reaction is induced and elicited by immunological consequences involving various immunocompetent cells and molecules [3,4].

Photosensitive chemicals have a haptenic moiety upon irradiation with UVA. Two hypotheses have been put forward to explain the formation of photoallergen. One is that the photosensitizer is a prohapten, which is converted to a complete hapten by UV irradiation, and the resultant hapten can bind to protein. In another theory, the photosensitizer is a photohapten, which needs to coexist with protein upon UV irradiation, and a covalent bond takes place *via* the formation of free radicals. In the case of photohapten, therefore, UVA-preirradiated photosensitive chemicals are incapable of binding to protein. Our studies [1,4,5] show that the vast majority of clinically photoallergic chemicals are photohaptens rather than prohaptens. In this concept, when the skin is painted with a photohaptenic chemical and exposed to UVA, skin dendritic cells bear photohaptenic determinant(s) on the surface. Simultaneously, they express major histocompatibility complex (MHC) class II molecules and costimulatory molecules [6], which represents the key event in the sensitization phase of photocontact sensitivity.

The *in vitro* screening methods for photoallergic substances are not established. THP-1 cells, derived from human monocytic leukemia cells, are used for screening of ordinary allergic chemicals or haptens and called human cell line activation test (h-CLAT) [7,8]. When cultured in the presence of allergic chemicals or metals, THP-1 cells express costimulatory molecule CD86 and CD54 as well as MHC class II molecule. Thus, we can evaluate their allergic potencies with the augmented expression of these surface markers. In this study, we modified this assessment method for detection of the photoallergic potential of chemicals by incubation of THP-1 cells with a test chemical and subsequent irradiation with UVA.

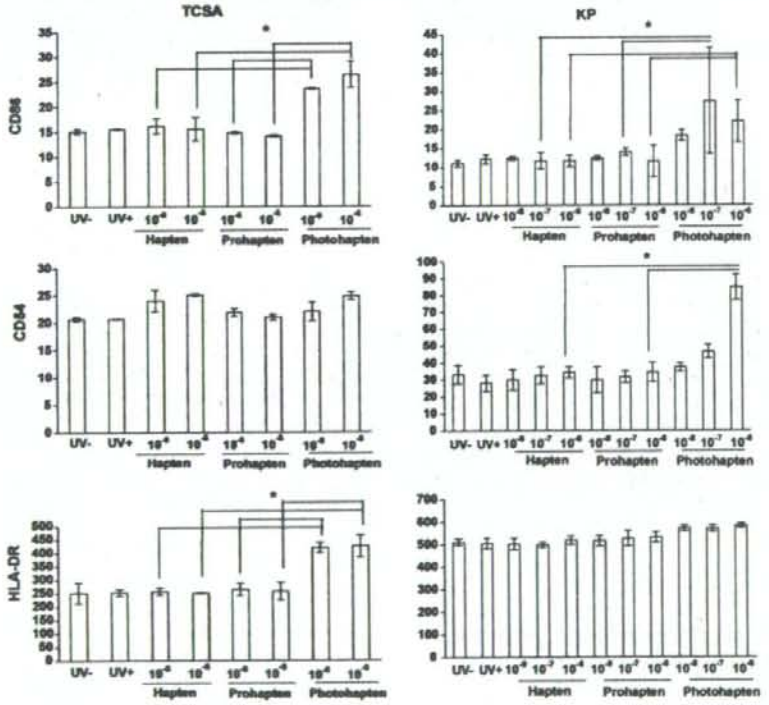

Fig. 1 Effects of photocontactants plus UVA on the expression of CD86, CD54, and HLA-DR. Photohapten: THP-1 cells suspended in PBS containing TCSA or KP at the indicated doses were irradiated with UVA at 1 J/cm^2. Prohapten: THP-1 cells were incubated with UVA-preirradiated TCSA or KP. Hapten: THP-1 cells were incubated with non-irradiated TCSA or KP. After 24-h culture in complete RPMI, they were subjected to the flow-cytometric analysis. *$P < 0.05$, between the means.

As chemicals to be tested, we used 3,3',4',5-tetrachlorosalicylanilide (TCSA) [6] and ketoprofen (KP) [9], which are causative agents for photoallergic contact dermatitis, and sparfloxacin (SPFX) [2,3] and afloqualone (AQ) [5], which are representative drugs for drug photosensitivity. Black light emitting UVA ranging from 320 to 400 nm (Toshiba Electric Co., Tokyo, Japan) was used for light source. THP-1 cells were maintained in complete RPMI (Gibco-BRL Inc., Grand Island, NY). To examine the photohaptenic ability, THP-1 cells were incubated in phosphate-buffered saline (PBS, pH 7.4) containing varying concentrations of chemicals for 1 h in 24-well plates (1×10^6 cells/mL) and subsequently irradiated with UVA at 1 J/cm^2 as described previously [10]. Cells were then cultured for 24 h in complete RPMI. To see the prohaptenic ability, the chemicals were preirradiated with UVA (1 J/cm^2) in PBS and added to THP-1 cells, and the culture was maintained for 24 h in complete RPMI. To see the ordinary haptenic ability, the non-irradiated chemicals were added to THP-1 cells, and the culture was maintained for 24 h in complete RPMI without UVA exposure. In all the groups, cells were stained with fluorescein isothiocyanate (FITC)-conjugated anti-CD86 monoclonal antibody (mAb) (BD-PharMingen, San Jose, CA, USA), phycoerythrin (PE)-conjugated anti-CD54 mAb (BD-PharMingen) and PerCP-conjugated anti-HLA-DR mAb (BD-PharMingen). The fluorescence intensities of these surface markers were analyzed by flow cytometry (FACS Canto, Becton Dickinson, San Jose, CA, USA).

TCSA and KP, causative chemicals for photocontact dermatitis, augmented the expression of the surface molecules on THP-1 cells only when they were exposed to UVA (Fig. 1, photohapten), indicating their photohaptenic properties. On the other hand, the UVA-preirradiated chemicals (prohapten) did not enhance the expression, suggesting the absence of prohaptenic properties. Simple incubation of cells with the non-irradiated chemicals (hapten) unaffected the expression, indicating lack of ordinary haptenic moieties. Thus, both TCSA and KP are photohaptens but not prohaptens or ordinary haptens, and the photohaptenic potential can be evaluated by this THP-1 system. There were some differences in the

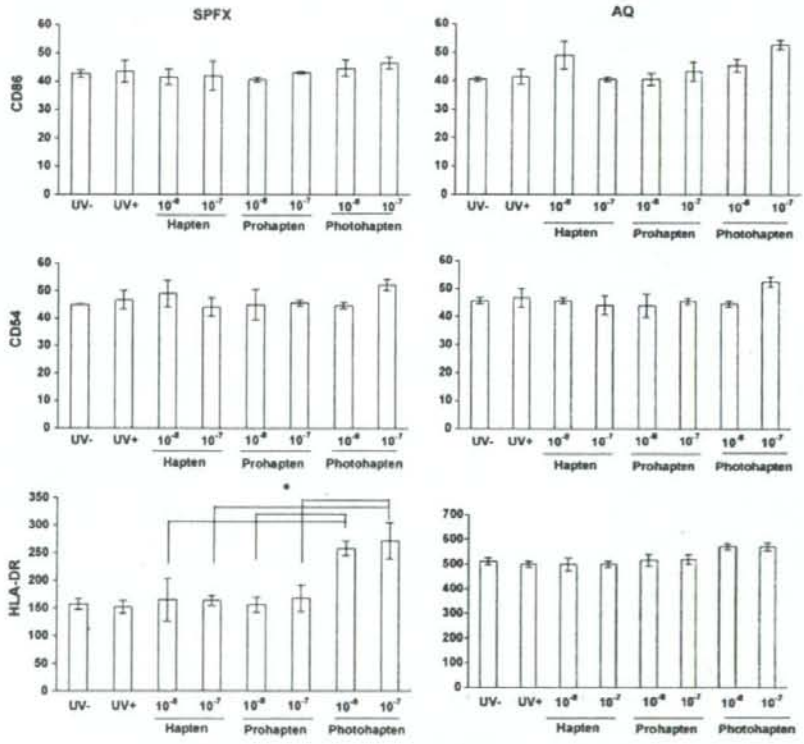

Fig. 2 Effects of photosensitive drugs plus UVA on the expression of CD86, CD54, and HLA-DR; see footnote of Fig. 1. SPFX and AQ were used instead of TCSA and KP. *$P < 0.05$, between the means.

photohaptenic abilities between TCSA and KP. TCSA at 10^{-6} and 10^{-7} M augmented the expression of CD86 and HLA-DR but not CD54, while KP at 10^{-6} and/or 10^{-7} M increased the expression of CD86 and CD54 but not HLA-DR. TCSA at 10^{-4} M or more and KP at 10^{-5} M or more were phototoxic as assessed by trypan blue dye exclusion test.

SPFX and AQ, causative oral drugs for photosensitivity, had lower activities to enhance the surface molecules than did TCSA and KP (Fig. 2). The treatment of THP-1 cells simultaneously with SPFX and UVA augmented the expression of HLA-DR, whereas UVA irradiated or non-irradiated SPFX did not enhance the expression, suggesting the photohaptenic property of SPFX. However, the expression of CD86 or CD54 was not elevated by SPFX plus UVA. AQ plus UVA did not significantly increase the expression of any molecules. Both SPFX and AQ were phototoxic at 10^{-6} M or more as assessed trypan blue dye exclusion test.

TCSA [6], KP [9], and AQ [5] belong to photohaptens, and SPFX has a weak photohaptenic moiety [4]. This study showed that the two photohaptenic contactants, TCSA and KP, were able to augment the expression of costimulatory molecules, and TCSA further increased HLA-DR expression. It is noted, however, that all photoallergic chemicals usually possess a various degrees of phototoxic property, because the photoallergic reaction needs the phototoxic step where photosensitizers bind to protein *via* the formation of free radicals [1]. Presumably because of the strong phototoxicity [2,5], SPFX and AQ could not increase the expression of costimulatory molecules despite having the prohaptenic potentials. It is suggested that the THP-1 system is useful for detection of photoallergenicity of chemicals, but it should be careful that the phototoxicity might conceal the photoallergenicity depending on the chemicals and their concentration. When a given chemical does not have strong phototoxicity, the results obtained from our *in vitro* system are clinically relevant to the occurrence of photosensitivity.

References

[1] Tokura Y. Immune responses to photohaptens: implications for the mechanisms of photosensitivity to exogenous agents. J Dermatol Sci 2000;23(Suppl. 6–9).

[2] Tokura Y, Iwamoto T, Mizutani K, Takigawa M. Sparfloxacin phototoxicity: potential photoaugmentation by ultraviolet A and B sources. Arch Dermatol Res 1996;288:45–50.

[3] Tokura Y, Seo N, Yagi H, Furkawa F, Takigawa M. Cross-reactivity in murine fluoroquinolone photoallergy: exclusive usage of TCR Vbeta 13 by immune T cells that recognize fluoroquinolone-photomodified cells. J Immunol 1998;160: 3719–28.

[4] Tokura Y, Nishijima T, Yagi H, Furukawa F, Takigawa M. Photohaptenic properties of fluoloquinolones. Photochem Photobiol 1996;64:838–44.

[5] Tokura Y, Ogai M, Yagi H, Takigawa M. Afloqualone photosensitivity: immunogenicity of afloqualone-photomodified epidermal cells. Photochem Photobiol 1994;60: 262–7.

[6] Nishijima T, Tokura Y, Imokawa G, Takigawa M. Photohapten TCSA painting plus UVA irradiation of murine skin augments the expression of MHC class II molecules and CD86 on Langerhans cells. J Dermatol Sci 1999;19: 371–6.

[7] Ashikaga T, Yoshida Y, Hirota M, Yoneyama K, Itagaki H, Sakaguchi H, et al. Development of an in vitro skin sensitization test using human cell lines; human cell line activation test (h-CLAT). Part I. Optimization of the h-CLAT protocol. Toxicol In Vitro 2006;20:767–73.

[8] Sakaguchi H, Ashikaga T, Miyazawa M, Yoshida Y, Ito Y, Yoneyama K, et al. Development of an in vitro skin sensitization test using human cell lines; human cell line activation test (h-CLAT). Part II. An inter-laboratory study of the h-CLAT. Toxicol In Vitro 2006;20:774–84.

[9] Atarashi K, Kabashima K, Akiyama K, Tokura Y. Stimulation of Langerhans cells with ketoprofen plus UVA in murine photocontact dermatitis to ketoprofen. J Dermatol Sci 2007;47: 151–9.

[10] Kurita M, Shimauchi T, Kobayashi M, Atarashi K, Mori K, Tokura Y. Induction of keratinocyte apoptosis by photosensitizing chemicals plus UVA. J Dermatol Sci 2007;45: 105–12.

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CONTACT DERMATITIS

Percutaneous penetration *via* hand eczema is the major accelerating factor for systemic absorption of toluene and xylene during car spray painting

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Background: For absorption of organic solvents, the respiratory tract is well known as the major site, but percutaneous absorption might be critical in some workplaces.

Objectives: The aim of the study is to determine whether the skin, if disordered, is 1 of the major routes of organic solvent absorption.

Patients/Methods: 72 male workers who painted the car body in the booth of a Japanese car company were participated in this study. The severity of hand eczema, urinary metabolites of organic solvents and the concentration of airborne organic solvents were measured.

Results: The correlation coefficiency between the skin severity index and the urinary concentration of hippuric acid or methylhippuric acid was statistically significant. There was no significant correlation between their urinary values and the air concentration of mixed organic solvents.

Conclusions: The skin is a more critical absorption route for organic solvents than the respiratory tract in some occupational settings. Hand eczema is a common disease and has a possibility to be a critical absorption route of organic solvents.

Key words: hand eczema; hippuric acid; methylhippuric acid; toluene; xylene.

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It is well known that exposure to a low or moderate concentration of organic solvents may cause various symptoms such as headache and dizziness (1, 2). The health risk is assessed by biological monitoring of exposure through the evaluation of the internal dose. The threshold limit values (TLVs) are guidelines designed for use by industrial hygienists in making decisions regarding safe levels of exposure to various chemical substances and physical agents found in the workplace. Toluene and xylene are used in various workplaces, in particular industrial settings. When toluene and xylene are absorbed into the body, they are discharged in urine after several steps of chemical changes (3, 4). Urinary hippuric acid and methylhippuric acid are the most frequently used biomarkers in the biological monitoring of occupational exposure to toluene and xylene (5). There have been data of urinary hippuric acid in 3,947 workers and of methylhippuric acid in 1,523 workers in Japan (5). The results can be classified into 3 levels, and the category 3 represents the urinary levels of metabolites of organic solvents higher than the classified American Conference of Governmental Industrial Hygienists (ACGIH) recommendations of TLVs and the biological exposure indices issued in 1988–1989.

In addition to the respiratory intake, the percutaneous absorption is expected to be an important factor of the biological exposure to organic solvents, but the skin has not been estimated as a critical route to determine the TLVs. Only a couple of chemicals are known to be absorbed through the skin, as represented by *N,N*-dimethylformamide (6). While normal skin may be resistant to absorption of organic solvents (7), it is possible that pathological or injured skin allows the solvents to penetrate the skin. In this respect, hand eczema

is thought to be the most critical skin disorder, because its incidence is very high (8) and the hand is easily exposed to the solvents (9). Moreover, hand eczema often occurs in patients with atopic dermatitis or atopic backgrounds (10, 11). A recent finding has suggested that a high incidence of mutation of filaggrin is seen in atopic patients (12). Because filaggrin deeply participates in the skin barrier function, individuals with the disordered barrier are prone to have atopic dermatitis as a result of feasible skin penetration of exogenous substances.

To investigate the influence of hand eczema on the absorption of organic solvents, we monitored the levels of urinary hippuric acid and methylhippuric acid in relation to the severity of hand eczema in spray painters of an automobile plant.

Materials and Methods

72 male workers who painted car body for 8 hrs a day in the painting booth of a Japanese car company located in Kosai City, Japan, were enrolled in this study. They worked in 7 different, 65 × 6 m-wide booths equipped with general ventilation system. All the workers wore Tyvek dustproof overalls, gas masks that are suitable for Japanese Industrial Standards, and nylon electrostatic gloves. The concentrations of organic solvents in air were lower than their respective TLVs of ACGIH recommendation in all painting booths. For biological monitoring of toluene and xylene, urine hippuric acid and methylhippuric acid were measured, respectively. Urine samples were collected on the last day of the working week. To avoid the influence of benzoic acid, aspirins or food preservatives are prohibited for 1 week. The concentrations of hippuric acid and methylhippuric acid were measured by high performance liquid chromatography (Enshu Medical Laboratory, Hamamatsu, Japan). The lowest detection levels of these metabolites were 0.01 g/l. Urine hippuric acid (mean ± SD = 0.3375 ± 0.3283 g/l) and methylhippuric acid (mean ± SD = 0.0436 ± 0.0346 g/l) levels belonged to the category 1 or 2 in all the workers, and thus satisfied the ACGIH issued recommendations. Samples of air were collected with a 1 l vacuumed glass sampling tube. The levels of toluene and xylene were quantified by gas chromatography and mass spectrometry (Department of Experiment and Analysis, Suzuki Motor Company, Hamamatsu, Japan).

The severity of hand eczema was evaluated by a dermatologist in dryness, erythema, scaling, dyshidrosis, fissuring, and lichenification using simple visual scoring: 0 = none, 1 = mild, 2 = moderate, and 3 = severe. The skin severity index was represented by the sum of these values (13). To explore the association of the urinary metabolites of organic solvents with the skin severity index, Spearman's correlation coefficients were used. All statistical analyses were performed with JMP V5.0.1™.

Results

As shown in Fig. 1, the urine hippuric acid level of 72 spray painters (mean ± SD = 0.3375 ± 0.283 g/l) was significantly higher than that of healthy 41 male workers who had not used organic solvents (mean ± SD = 0.1671 ± 0.2243 g/l) ($P = 0.0038$). The urine methylhippuric acid level of 41 normal subjects was under the lowest detection level. The skin severity index in the workers ranged from 0 to 6 points. The correlation coefficiency between the skin severity index and the urinary concentration of hippuric acid or methylhippuric acid was statistically significant (Fig. 2). In particular, a high correlation coefficiency (0.6099) was observed in hippuric acid. It was noted that workers without any hand eczema (the skin severity index, 0) exhibited extremely low levels of urinary hippuric acid, except for 1 worker. All the workers wore a gas mask that was regularly maintained, and the

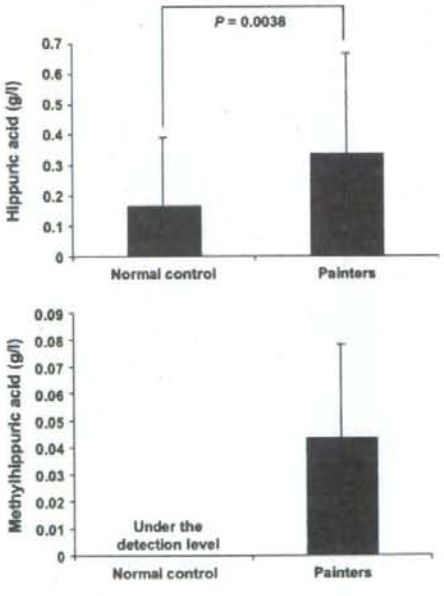

Fig. 1. Urinary metabolites of toluene and xylene of 72 spray painters are significantly higher than those of 41 normal subjects who have not been used organic solvents.

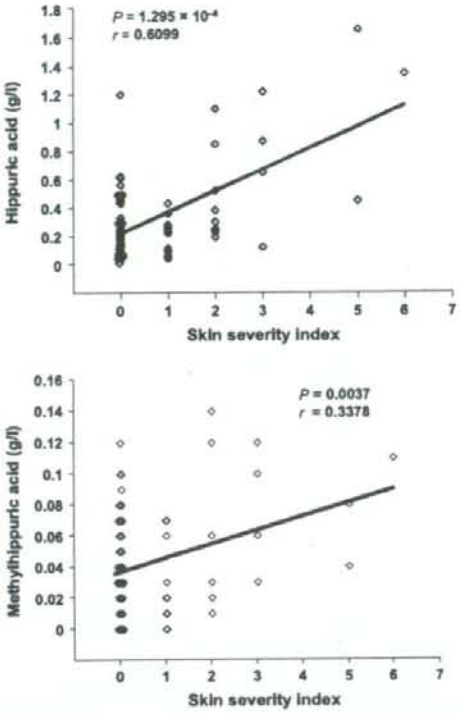

Fig. 2. Positive correlation between urinary hippuric acid (top) or methylhippuric acid (bottom) and skin severity index of hand eczema.

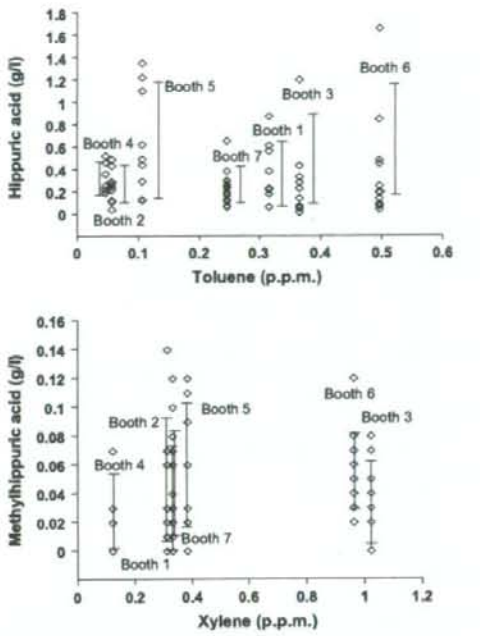

Fig. 3. Absence of correlation between urinary hippuric acid and toluene concentration in air (top) or methylhippuric acid and xylene concentration in air (bottom). The concentrations of toluene and xylene in air were measured in the 7 different booths and correlated with the concentrations of urinary hippuric and methylhippuric acids of the workers in each booths. Error bar represents the mean ± SD of urinary hippuric acid or methylhippuric acid levels of painters in each booths.

air exchange was efficiently performed in the 7 booths. To further exclude the possible absorption through the respiratory tract, we also examined the relationship between the urinary concentration of hippuric acid or methylhippuric acid, and the air concentration of toluene and xylene, respectively. Figure 3 shows these correlations in 7 different booths. There was no significant correlation between the air levels of toluene and xylene and urine concentrations of hippuric acid and methylhippuric acid, respectively, confirming that the respiratory absorption of organic solvents did not contribute to the urinary levels in this workplace. These data indicate that the concentrations of urinary hippuric acid and methylhippuric acid depend on the severity of hand eczema.

Discussion

Our study clearly showed that workers with hand eczema have high urinary levels of hippuric acid and methylhippuric acid when occupationally exposed to organic solvents. Although the respiratory tract has been estimated as the major absorption system of the solvents (3, 4), we found that the urinary concentration of hippuric acid or methylhippuric acid did not depend on the air concentration of mixed organic solvents in our occupational settings. Thus, the skin is 1 of the crucial absorption routes for organic solvents.

Skin protection items such as protective gloves and barrier creams are necessary for prevention from the irritation by or penetration of organic solvents (13). The workers in our study wore nylon electrostatic gloves, but they could not protect the penetration of organic solvents. The workers also used emollients for skin care with insufficient protection. Our findings indicate that the skin protection from organic solvents is difficult. The actual advantage of barrier creams over emollients for skin protection is still debated (13).

Hand eczema is a common disease and induced by organic solvents themselves as well as atopic background (10, 11). Given that organic solvents can disrupt the skin barrier by removing ceramide of the stratum corneum (14), the induced acute

barrier disruption may further exaggerate the percutaneous absorption of the solvents. It should be kept in mind that the absorption *via* eczematous skin is the important mechanism underlying systemic absorption of organic solvents when workers deal with the solvents by hands.

References

1. Elofsson S, Gambarale F, Hindmarsh T et al. Exposure to organic solvents. *Scand J Work Environ Health* 1980: 6: 239–273.
2. Husman K. Symptoms of car painters with long exposure to mixture of organic solvents. *Scand J Work Environ Health* 1980: 6: 19–32.
3. Pierce C H, Dills R L, Morgan M S et al. Biological monitoring of controlled toluene exposure. *Int Arch Occup Environ Health* 1998: 71: 433–44.
4. Jacobson G A, McLean S. Biological monitoring of low level occupational xylene exposure and the role of recent exposure. *Ann Occup Hyg* 2003: 47: 331–336.
5. Ogata M, Numano T, Hosokawa M, Michitsui H. Large-scale biological monitoring in Japan. *Sci Total Environ* 1997: 199: 197–204.
6. Boman A, Maibach H I. Percutaneous absorption of organic solvents. *Int J Occup Environ Health* 2000: 6: 93–95.
7. Scott R C, Dugard P H. A model for quantifying absorption through abnormal skin. *J Invest Dermatol* 1986: 86: 208–212.
8. Coenraads P J, Diepgen T L. Problems with trials and intervention studies on barrier creams and emollients at the workplace. *Int Arch Occup Environ Health* 2003: 76: 362–366.
9. Funke U, Diepen T L, Fartasch M. Risk-group-related prevention of hand eczema at the workplace. *Curr Probl Dermatol* 1996: 25: 123–132.
10. Meding B. Prevention of hand eczema in atopics. *Curr Probl Dermatol* 1996: 25: 116–122.
11. Coenraads P J, Diepgen T L. Risk for hand eczema in employees with past or present atopic dermatitis. *Int Arch Occup Environ Health* 1998: 71: 7–13.
12. Palmer C N, Irvine A D, Terron-Kwiatkowski A et al. Common loss-of-function variants of the epidermal barrier protein fillagrin are a major predisposing factor of atopic dermatitis. *Nat Genet* 2006: 38: 441–446.
13. Berndt U, Wigger-Alberti W, Gabard B, Elsner P. Efficacy of a barrier cream and its vehicle as protective measures against occupational irritant contact dermatitis. *Contact Dermatitis* 2000: 42: 77–80.
14. Nishijima T, Tokura Y, Imokawa G, Seo N, Furukawa F, Takigawa M. Altered permeability and disordered cutaneous immunoregulatory function in mice with acute barrier disruption. *J Invest Dermatol* 1997: 109: 175–182.

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The Potential of Selected Prostanoid Receptors as Targets for New Therapeutic Strategy for Allergy and Immune Diseases

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Abstract: Allergy and immune diseases including asthma, atopic dermatitis, rhinitis, and autoimmune diseases have been treated mainly with steroid based therapy that has multiple side effects. Even then, these allergic diseases are sometimes still not easy to control. Therefore, new effective treatments with fewer side effects are expected to be developed. Prostanoids consisting of prostaglandins and thromboxane are cyclooxygenase metabolites of arachidonic acid. They exert a range of actions mediated through their respective receptors expressed in target cells. In the skin, a wide variety of prostanoids and their receptors are highly expressed under pathophysiological conditions. However, their roles have not been well clarified. Recent developments in molecular biology have enabled us to investigate the physiological roles for each receptor *via* the disruption of the respective gene in combination with receptor selective compounds. It has been demonstrated that each prostanoid receptor has multiple functions whose expression is regulated in a context dependent manner, sometimes resulting in opposite, excitatory and inhibitory, outcomes. The balance of prostanoid production and receptor expression is important for homeostasis of the human body. Here, we review new findings on the roles of prostanoids in allergy and immune diseases, focusing on skin disorders as a representative, and discuss the clinical potentials of receptor-selective drugs.

Keywords: Prostanoid, atopic dermatitis, contact dermatitis, NSAID, prostaglandin, asthma.

1. INTRODUCTION

The number of allergy and immune diseases including asthma, atopic dermatitis, rhinitis, and autoimmune diseases are related to each other as 'atopic march', and increasing in number [1]. They have been treated mainly with steroid-based therapy that has multiple side effects, such as obesity, hyperglysemia, osteoporosis, etc, focusing on suppressing inflammation. However, allergic reactions are related not only to inflammation but also include other factors [2]. Recently, some advanced therapy for these allergic and immune diseases [3, 4] have been developed, but are also very expensive. Therefore, new effective inexpensive treatments with less side effects are strongly in demand.

When tissues are exposed to diverse pathophysiological stimuli, arachidonic acid (AA) is liberated from membrane phospholipids, and converted to lipid mediators, such as prostanoids, leukotrienes (LTs) and hydroxy-eicosatetraenoic acids (HETEs). Prostanoids are formed by cyclooxygenase (COX) pathway, whereas LTs and HETEs are formed by the 5-, 12- and 15-lipoxygenase (LO) pathways. The COX has two isoforms, COX-1 and COX-2, and this reaction results in the formation of an unstable endoperoxide intermediate, prostaglandin (PG) H_2, which, in turn, is metabolized to PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane (TX) A_2 by specific synthases.

Prostanoids are released from cells immediately after formation. Because they are chemically or metabolically unstable, they usually function only locally through membrane receptors on target cells [5]. Nine types and subtypes of membrane prostanoid receptors are conserved in mammals from mouse to human: two subtypes of the PGD receptor (DP, and chemoattractant receptor homologous-molecule expressed on Th2 cells; CRTH2), four subtypes of the PGE receptor (EP1, EP2, EP3, and EP4), the PGF receptor (FP), the PGI receptor (IP), and the TXA receptor (TP) (Fig. 1). All are G protein–coupled rhodopsin-type receptors with seven transmembrane domains (Fig. 1).

Recently, mice in which individual prostanoid receptor genes have been inactivated have been used as models to dissect out the relative roles of each receptor in combination with the use of compounds that selectively bind to prostanoid receptors as agonists or antagonists [6]. These genetic and pharmacological approaches have revealed new roles for prostanoids and their receptors on allergy and immune diseases. In this review, we describe the current investigative status of prostanoids on cutaneous allergy and immune diseases and discuss the clinical potentials of receptor-selective drugs.

2. PROSTANOID FORMATION IN THE SKIN

The skin is an organ important for self-defense to foreign antigen exposure. Therefore, the skin consists of many immune cells, such as keratinocytes (KCs), T cells, B cells, mast cells, eosinophils, fibroblasts, and two types of dendritic cells (DCs), epidermal Langerhans cells (LCs) and dermal dendritic cells (dDCs). Most prostanoids are detected in the skin (Table 1). Metabolism of AA to prostanoids is *via* the COX pathway. The COX-1 is constitutively expressed in cells whereas the COX-2 requires specific stimulation, by substances such as acetone and phorbol ester, TPA [7]. In the normal human skin, immunohistochemical examination revealed that COX-1 is observed throughout the epidermis,

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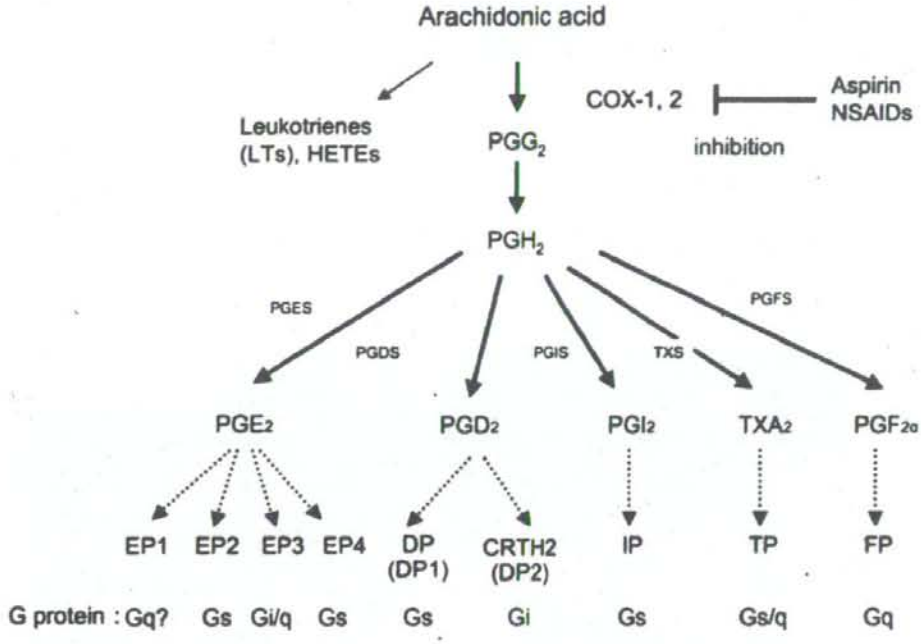

Fig. (1). Biosynthetic pathways of prostanoids.

The formation of PGD_2, PGE_2, $PGF_{2\alpha}$, PGG_2, PGH_2, and PGI_2, and TXA_2, from arachidonic acid is shown. The first two steps of pathway, i.e., conversion of arachidonic acid to PGG_2 and then to PGH_2, are catalyzed by cyclooxygenase (COX), and subsequent conversion of PGH_2 to each PG is catalyzed by the respective synthase as shown. All are G protein-coupled rhodopsin-type receptors.

whereas COX-2 exists in more differentiated, suprabasilar KCs and outer root sheath cells of hair follicles [8, 9]. Among prostanoids, PGE_2 is the main COX product in human epidermal homogenates [10]. PGD_2 has been detected in human skin [10], and PGD synthase is present predominantly in LCs, dDCs, dermal macrophages and mast cells, but not in KCs [11, 12]. Mast cells are one of the major cellular sources of PGD_2. Only very low TX synthase activity has been found in the skin, however, TXB_2, as a metabolites of TXA_2, was highly detected from the cultured supernatant of LCs and DCs [13]. The above findings on the synthesis of prostanoids are summarized in Table **1**.

3. PROSTANOID RECEPTOR EXPRESSION IN THE SKIN

Adult human KCs express mRNA for all subtypes of PGE_2 receptors [14, 15]. Mouse LCs and DCs express DP [16], EP1, 2, 3, 4 [17], and IP [18]. T cells express EP1, 2, 3,

Table 1. Expression of Prostanoid Synthases and Prostanoid Receptors in the Skin

	PGDS	PGES	PGFS	PGIS	TXS	DP	CRTH2	EP1	EP2	EP3	EP4	FP	IP	TP
Keratinocytes	m	m, h						h	h	h	h			
Langerhans cells	h	m, h			m	m, h		m	m	m	m			
Dendritic cells	h	m, h			m, h				m, h		m, h			
T cells							m, h(Th2)	m	m	m	m, h		m	m
B cells								m, h	m, h	m, h	m, h		m	
Macrophages	h	m, h		m	m, h		m	m	m	m	m, h			
Eosinophils	h	h	h		h	m	m		m, h		h			h
Mast cells, basophils	m, h				h	m, h	m, h	m	m	m	m			
Neutrophils		h			h	h	m		m, h		m			
Blood vessels		h	h	h				m, h	m, h	m, h	m, h		m, h	m, h

PG; prostaglandin, S; synthase, m; mouse, h; human.
Modified from the reference by Tilley *et al.*

4 [19], IP [20] and TP [13]. PGE_2 suppresses T cell proliferation, T cell differentiation in the thymus, and IL-1 production by acting at EP2 and EP4 [21]. B cells express EP1, 2, 3, and 4 [19], and PGE_2 facilitates IgE class switching through EP2 and EP4 [22]. Mast cells express EP1, 2, 3, 4, DP, and IP [19, 23], and PGE_2 acts at EP3 to suppress degranulation [24]. Human eosinophils express EP2, EP4, and TP [23], and PGE_2 seems to prolong eosinophil survival [25, 26]. PGE_2 suppresses TNF-α production and enhances IL-6 production from neutrophils stimulated by lipopolysaccharide (LPS) through EP2 and EP4 [23, 27]. These findings are summarized in Table **1**.

4. NSAIDs AND THE SKIN

The roles of prostanoids on allergy and immune diseases have been suggested by clinically monitoring the effects of non-steroidal anti-inflammatory drugs (NSAIDs), a COX inhibitor. It is well known that cutaneous immune responses are associated with an increase in prostanoid formation, however, the roles of prostanoids have been less well defined. This is presumably because the effects of NSAIDs are far less marked compared with steroids [28]. There are some skin diseases which are effectively treated with NSAIDs [4, 28, 29]. For example, ibuprofen piconol has an anti-inflammatory effect on acne vulgaris, by inhibiting leukocyte migration induced by *Propionibacterium acnes* [30]. However, NSAIDs are generally not useful for inflammatory skin diseases, such as contact dermatitis and atopic dermatitis (AD) [4, 28]. Nevertheless, NSAIDs occasionally induces contact dermatitis to NSAIDs themselves. Therefore, NSAIDs have not been used for inflammatory skin diseases, and the role of prostanoids has not been evaluated.

5. NSAIDs, AND ALLERGY AND IMMUNE DISEASES

Since NSAID is a COX inhibitor, formation of all prostanoids is blocked. Therefore, observations obtained from NSAIDs neither clearly indicate which type of prostanoid or which class of prostanoid receptor is involved in a given process, nor suggest how critical the actions of prostanoids might be. However, recent genetic and pharmacological approaches have revealed some unexpected findings on each prostanoid receptor. It is high time to reconsider the significance of each prostanoid receptor on allergy and immune diseases.

Ingestion of aspirin induces severe bronchoconstriction in a proportion of subjects with asthma. The patients have asthma, recurrent rhinosinusitis, and/or nasal polyposis [31]. Therefore, the syndrome is named as aspirin intolerant asthma (AIA). AIA is triggered by a specific COX inhibition in the respiratory tract, which is followed by a reduction of PGE_2 and overproduction of cysLTs. There is a series of evidence supporting the hypothesis that the COX enzyme has a pivotal role in the initiation of the intolerance reaction [31]. However, a COX-2 selective inhibitor did not induce AIA. Following marked inhibition of COX-1 dependent PGE_2, there is marked anti-inflammatory action which leads to rebound activation of mast cells due to dramatic removal of isoforms of COX-1 dependent PGE_2. Recently, using a mouse model, EP3 signaling suppressed allergic asthma through down-regulating activation of mast cells [24], suggesting that AIA can be avoided by a selective prostanoid receptor antagonist instead of using NSAIDs to retain the EP3 signaling cascade.

6. CONTACT HYPERSENSITIVITY AND SKIN INFLAMMATION, AND PROSTANOIDS

In order to evaluate physiological roles of prostanoids in immune responses of the skin, contact hypersensitivity (CHS) model (in other words, allergic contact dermatitis) is a useful tool [32, 33]. Migration of cutaneous DCs to the lymph nodes is a crucial step in the initiation of CHS. This activation of cutaneous DCs is initiated by KCs that secrete pro-inflammatory cytokines upon antigen application. Thus, by virtue of their specific cytokine secretion pattern, KCs determine the microenvironment for LC maturation and migration (Fig. **2**).

PGE_2 produced by KCs upon antigen exposure acts at EP4 on cutaneous DCs to facilitate initiation of cutaneous immune responses by promoting the migration and maturation of cutaneous DCs [17] (Fig. **2**). Interestingly, the opposite activity of prostanoids has been documented. PGD_2 produced by percutaneous infection with helminth parasite *Schistosoma mansoni* specifically impedes the migration of LCs through DP receptor [16]. And PGI_2 receptor IP inhibits the proinflammatory cytokine production and T cell stimulatory function of DCs [34]. These modulatory activities of lipid mediators are not only limited to prostanoids. LC migration from the skin to the lymph nodes utilizes the LTC_4 transporter multidrug resistance-associated protein 1 [35]. The relative of these prostanoid receptors in physiological conditions remained to be elucidated, but modulation of the signaling of these receptors may lead a possible candidate of immune reaction.

Once cutaneous DCs migrate to regional lymph nodes, cutaneous DCs present antigens to naïve T cells to prime them. This DC-T cell interaction is essential for inducing T cell maturation and differentiation *in vivo*. It was demonstrated that cutaneous DCs produce abundant TXA_2, which acts on naïve T cells to impair the DC-T cell interaction [13]. Consistently, TP-deficient mice or wild-type mice treated with TP antagonist, S-145, during the sensitization period showed enhanced CHS response [13] (Fig. **2**). It has also been known that DCs produce a high amount of PGE_2. A variety of prostanoid receptors, including EP subtypes, IP, and TP, are expressed in T cells of mice, and PGE_2 has been implicated in T cell development, inhibition of T cell proliferation and IL-1 production *in vitro* through EP2 and EP4 [21, 36-38]. At present, physiological roles of PGE_2 in this context remain unknown.

After establishment of memory T cells after antigen presentation by DCs, antigen re-challenge onto the skin stimulates KCs to produce memory T cell attracting cytokines and chemokines, such as CCL27, known to be induced by PGE_2 [33, 39].

On the other hand, TPA is known to induce skin inflammation. After TPA application, acute edema is induced and followed by inflammatory cell infiltration induced by TNF-α and PGE_2 [40]. In fact, intradermal injections of PGE_2 into human skin causes erythema with vascular permeability changes [41-43]. Similar skin inflammation is induced by ultraviolet B, which is mediated through EP2 and EP4 receptors [44]. Such direct effects of prostanoids on

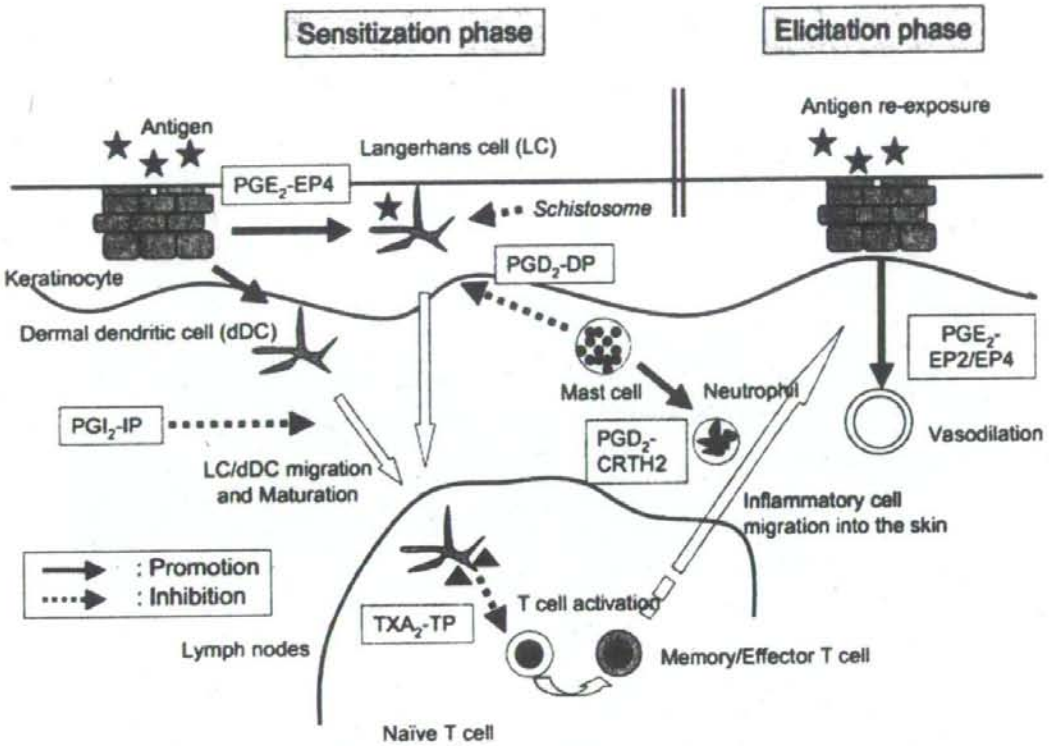

Fig. (2). Hypothesis on the role of prostanoids in contact hypersensitivity response.

During the sensitization period, antigen induces pro-inflammatory cytokine secretion by keratinocytes, which enhances cutaneous DCs (LCs and dDCs) activation and migration to regional lymph nodes. In the lymph nodes, cutaneous DCs activate naïve T cells into mature memory T cells. During antigen exposure to the skin, KCs produce PGE_2, and mast cells produce PGD_2. Moreover, *schistosomes* produce PGD_2 during helminthic infection. The PGE_2-EP4 pathway promotes but PGD_2-DP and PGI_2-IP pathways inhibit cutaneous DC migration and maturation. TXA_2 produced by activated cutaneous DCs seems to act on naïve T cells to disrupt DC-T cell interaction.

During the elicitation phase of CHS response, secondary antigen exposure to the skin stimulates keratinocytes to secrete pro-inflammatory cytokines, chemokines and other mediators, which activate the endothelial activation of blood vessels. This activation attracts memory T cell infiltration into the skin. Then, antigen-loaded antigen-presenting cells activate memory T cells to induce mediator release. PGE_2 dilates blood vessels possibly through EP2 and EP4.

blood vessels might affect the elicitation phase of CHS, but the physiological role in this context is still unknown.

7. ATOPIC DERMATITIS AND PROSTANOIDS

Atopic dermatitis (AD) is a common pruritic and chronic inflammatory skin disease that is regarded as one of the T helper type 2 (Th2) diseases. In the dermis, a cellular infiltrate is present consisting of lymphocytes, monocytes and mast cells. Histamine is one of the mediators suspected to play an important role in Th2 disease processes, but its role in AD is uncertain because antihistamines improve the disease only partially, not dramatically [45, 46]. Therefore, in the search for potential mediators involved in the inflammatory processes of AD, other mediators than histamine have to be considered. In this respect, it would be of great relevance to examine the potential role of prostanoids in the molecular pathology of AD. In biopsies from patients with AD, PGE_2 has been determined in biologically active amounts in both lesional and perilesional skin [47]. In contrast, normal levels of eicosanoids were found in the uninvolved skin of these patients [47]. Using an ovalbumin-induced mouse AD model, COX-2 inhibition induced enhanced eosinophil infiltration, elevated IL-4 expression in the skin lesion with elevated serum IgE and IgG1, suggesting that COX-2-derived prostanoids have protective roles in the development of AD.

PGE_2 has the capacities to induce wheal and flare when injected into human skin [48] and to modulate the inflammatory responses elicited by other mediators [43, 49]. In contrast, one of the characteristics of AD is the elevation of IgE in the sera of patients, which is related to pruritis [50]. PGE_2 drives Ig class switching to IgE by acting at EP2 and EP4 on B cells under LPS and IL-4 stimulation *in vitro* [22]. The physiological role of PGE_2 on class switching in AD patients should be pursued in future. On the other hand, in a repeated

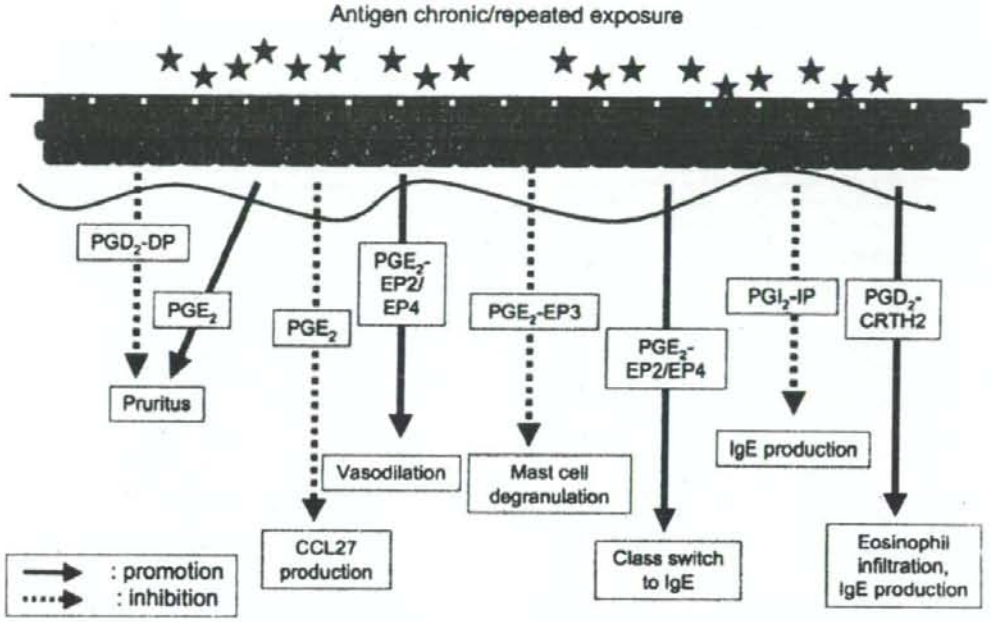

Fig. (3). Possible roles of prostanoids in atopic dermatitis.

A considerable number of recent data have suggested that prostanoid receptors play some critical roles in the pathogenesis of atopic dermatitis in a context dependent manner. Possible roles are summarized.

hapten application-induced murine AD model, IP-deficient mice showed a suppressed immune response with a decreased serum level of IgE, and TP-deficient mice exhibited an enhanced immune response with an increased serum IgE level.

PGD_2 is the major prostanoid produced by activated mast cells. PGD_2 has two types of receptors, DP and CRTH2. Involvement of DP in the formation of allergic asthma through inducing chemokines from epithelial cells and airway hyperreactivity has been demonstrated with DP-deficient mice [51]. Since AD and asthma are categorized similarly into a Th2 disease group, this study attracts interest of DP in the pathogenesis of AD. However, PGD2-DP signaling has another mode of action, *via* suppressing DC function and pruritus (Figs. **2**,**3**). On the other hand, CRTH2 induces chemotaxis in Th2 cells, eosinophils and basophils with enhanced degranulation [52, 53]. In response to PGD_2, CRTH2 also induces Th2 cell and neutrophil migration into inflammatory skin sites [54]. Virtually all $CRTH2^+$ $CD4^+$ lymphocytes have a pure Th2 phenotype and occupy not all but a large proportion of circulating Th2 cells in both normal and AD subjects. In AD patients, a preferential increase of $CRTH2^+$ cells was noted within the disease-related cutaneous lymphocyte-associated antigen-positive $CD4^+$ T cell compartment [55]. There remains a need to clarify the respective role for DP and CRTH2 in pathophysiological conditions.

Pruritus is also an important hallmark of AD. PGE_2 is known to evoke pruritus in AD patients [56]. And PGD_2, but not CRTH2 agonist, 13, 14-dihydro-15-keto-PGD_2, reduced scratching behavior in NC/Nga atopic dermatitis model mice, suggesting that DP suppresses pruritic activity [57].

The above findings indicate that each prostanoid receptor has its own role in a context dependent manner (Fig. **3**). AD might be controlled by antagonists for DP, CRTH2, EP2, EP4, and/or IP, and agonists for EP4 and/or TP. And this field has recently investigated vigorously by pharmaceutical companies.

8. CONCLUSIONS

In this review, we have summarized current findings on the actions of prostanoids and their receptors in allergy and immune diseases of the skin. It is worthwhile to mention the role of prostanoid receptors on cutaneous DC function, where EP4 and DP receptors mediate in the opposite direction. Therefore, NSAID treatment may mask the complex effects of prostanoids in the disease pathway. Clarification of each prostanoid pathway should widen our understanding not only of the actions of prostanoids but also of the delicate regulation of cutaneous immune reactions. At present, the studies on the role of allergy and immune diseases at the prostanoid receptor level were mostly performed using experimental animals. The next question is how much this pathway contributes to initiation and progression of human diseases, and how effective the therapy directed to this signaling is. There are several ongoing efforts to develop better prostanoid receptor agonists and antagonists. Clinical trials using those drugs may clarify these interesting issues. Selective manipulation of the actions mediated by each receptor

may be a novel therapeutic strategy for cutaneous allergic or inflammatory disorders.

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REFERENCES

[1] Spergel JM. Atopic march: link to upper airways. Curr Opin Allergy Clin Immunol 2005; 5: 17-21.

[2] Marenholz I, Nickel R, Ruschendorf F, *et al.* Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. J Allergy Clin Immunol 2006; 118: 866-871.

[3] Sicherer SH, Leung DY. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects. J Allergy Clin Immunol 2007;

[4] Abramovits W, Perlmutter A. Steroids versus other immune modulators in the management of allergic dermatoses. Curr Opin Allergy Clin Immunol 2006; 6: 345-354.

[5] Narumiya S, Sugimoto Y, Ushikubi F. Prostanoid receptors: structures, properties, and functions. Physiol Rev 1999; 79: 1193-1226.

[6] Narumiya S, FitzGerald GA. Genetic and pharmacological analysis of prostanoid receptor function. J Clin Invest 2001; 108: 25-30.

[7] Scholz K, Furstenberger G, Muller-Decker K, Marks F. Differential expression of prostaglandin-H synthase isoenzymes in normal and activated keratinocytes *in vivo* and *in vitro*. Biochem J 1995; 309 (Pt 1): 263-269.

[8] Leong J, Hughes-Fulford M, Rakhlin N, *et al.* Cyclooxygenases in human and mouse skin and cultured human keratinocytes: association of COX-2 expression with human keratinocyte differentiation. Exp Cell Res 1996; 224: 79-87.

[9] Torii E, Segi E, Sugimoto Y, *et al.* Expression of prostaglandin E(2) receptor subtypes in mouse hair follicles. Biochem Biophys Res Commun 2002; 290: 696-700.

[10] Hammarstrom S, Lindgren JA, Marcelo C, *et al.* Arachidonic acid transformations in normal and psoriatic skin. J Invest Dermatol 1979; 73: 180-183.

[11] Ujihara M, Horiguchi Y, Ikai K, Urade Y. Characterization and distribution of prostaglandin D synthetase in rat skin. J Invest Dermatol 1988; 90: 448-451.

[12] Ruzicka T, Aubock J. Arachidonic acid metabolism in guinea pig Langerhans cells: studies on cyclooxygenase and lipoxygenase pathways. J Immunol 1987; 138: 539-543.

[13] Kabashima K, Murata T, Tanaka H, *et al.* Thromboxane A2 modulates interaction of dendritic cells and T cells and regulates acquired immunity. Nat Immunol 2003; 4: 694-701.

[14] Konger RL, Malaviya R, Pentland AP. Growth regulation of primary human keratinocytes by prostaglandin E receptor EP2 and EP3 subtypes. Biochim Biophys Acta 1998; 1401: 221-234.

[15] Tober KL, Wilgus TA, Kusewitt DF, *et al.* Importance of the EP(1) receptor in cutaneous UVB-induced inflammation and tumor development. J Invest Dermatol 2006; 126: 205-211.

[16] Angeli V, Faveeuw C, Roye O, *et al.* Role of the parasite-derived prostaglandin D2 in the inhibition of epidermal Langerhans cell migration during schistosomiasis infection. J Exp Med 2001; 193: 1135-1147.

[17] Kabashima K, Sakata D, Nagamachi M, *et al.* Prostaglandin E2-EP4 signaling initiates skin immune responses by promoting migration and maturation of Langerhans cells. Nat Med 2003; 9: 744-749.

[18] Huang Q, Liu D, Majewski P, *et al.* The plasticity of dendritic cell responses to pathogens and their components. Science 2001; 294: 870-875.

[19] Tilley SL, Coffman TM, Koller BH. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. J Clin Invest 2001; 108: 15-23.

[20] Takahashi Y, Tokuoka S, Masuda T, *et al.* Augmentation of allergic inflammation in prostanoid IP receptor deficient mice. Br J Pharmacol 2002; 137: 315-322.

[21] Nataraj C, Thomas DW, Tilley SL, *et al.* Receptors for prostaglandin E(2) that regulate cellular immune responses in the mouse. J Clin Invest 2001; 108: 1229-1235.

[22] Fedyk ER, Phipps RP. Prostaglandin E2 receptors of the EP2 and EP4 subtypes regulate activation and differentiation of mouse B lymphocytes to IgE-secreting cells. Proc Natl Acad Sci U S A 1996; 93: 10978-10983.

[23] Nguyen M, Solle M, Audoly LP, *et al.* Receptors and signaling mechanisms required for prostaglandin E2-mediated regulation of mast cell degranulation and IL-6 production. J Immunol 2002; 169: 4586-4593.

[24] Kunikata T, Yamane H, Segi E, *et al.* Suppression of allergic inflammation by the prostaglandin E receptor subtype EP3. Nat Immunol 2005; 6: 524-531.

[25] Mita H, Hasegawa M, Higashi N, Akiyama K. Characterization of PGE2 receptor subtypes in human eosinophils. J Allergy Clin Immunol 2002; 110: 457-459.

[26] Peacock CD, Misso NL, Watkins DN, Thompson PJ. PGE 2 and dibutyryl cyclic adenosine monophosphate prolong eosinophil survival *in vitro*. J Allergy Clin Immunol 1999; 104: 153-162.

[27] Yamane H, Sugimoto Y, Tanaka S, Ichikawa A. Prostaglandin E(2) receptors, EP2 and EP4, differentially modulate TNF-alpha and IL-6 production induced by lipopolysaccharide in mouse peritoneal neutrophils. Biochem Biophys Res Commun 2000; 278: 224-228.

[28] Kabashima K, Miyachi, Y. Prostanoids in the cutaneous immune response. J Dermatol Sci 2004; 34: 177-184.

[29] Youn CS, Cho KH. Eosinophilic pustular folliculitis treated with naproxen. Br J Dermatol 2001; 145: 514-515.

[30] Wong RC, Kang S, Heezen JL, Voorhees JJ, Ellis CN. Oral ibuprofen and tetracycline for the treatment of acne vulgaris. J Am Acad Dermatol 1984; 11: 1076-1081.

[31] Szczeklik A, Stevenson, DD. Aspirin-induced asthma: advances in pathogenesis and management. J Allergy Clin Immunol 1999; 104: 5-13.

[32] Becker D, Knop J. Mechanism in allergic contact dermatitis. Exp Dermatol 1993; 2: 63-69.

[33] Grabbe S, Schwarz T. Immunoregulatory mechanisms involved in elicitation of allergic contact hypersensitivity. Immunol Today 1998; 19: 37-44.

[34] Zhou W, Hashimoto K, Goleniewska K, *et al.* Prostaglandin i2 analogs inhibit proinflammatory cytokine production and T cell stimulatory function of dendritic cells. J Immunol 2007; 178: 702-710.

[35] Robbiani DF, Finch RA, Jager D, *et al.* The leukotriene C(4) transporter MRP1 regulates CCL19 (MIP-3beta, ELC)-dependent mobilization of dendritic cells to lymph nodes. Cell 2000; 103: 757-768.

[36] Rocca B, Spain LM, Pure E, *et al.* Distinct roles of prostaglandin H synthases 1 and 2 in T-cell development. J Clin Invest 1999; 103: 1469-1477.

[37] Goodwin, JS, Webb, DR. Regulation of the immune response by prostaglandins. Clin Immunol Immunopathol 1980; 15: 106-122.

[38] Kunkel SL, Chensue SW, Phan SH. Prostaglandins as endogenous mediators of interleukin 1 production. J Immunol 1986; 136: 186-192.

[39] Homey B, Alenius H, Muller A, *et al.* CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. Nat Med 2002; 8: 157-165.

[40] Murakawa M, Yamaoka K, Tanaka Y, FukudaY. Involvement of tumor necrosis factor (TNF)-alpha in phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced skin edema in mice. Biochem Pharmacol 2006; 71: 1331-1336.

[41] Flower RJ, Harvey EA, Kingston WP. Inflammatory effects of prostaglandin D2 in rat and human skin. Br J Pharmacol 1976; 56: 229-233.

[42] Crunkhorn P, Willis AL. Cutaneous reactions to intradermal prostaglandins. Br J Pharmacol 1971; 41: 49-56.

[43] Basran GS, Morley J, Paul W, Turner-Warwick M. Evidence in man of synergistic interaction between putative mediators of acute inflammation and asthma. Lancet 1982; 1: 935-937.

[44] Kabashima K, Nagamachi M, Honda T, *et al.* Prostaglandin E(2) is required for ultraviolet B-induced skin inflammation *via* EP2 and EP4 receptors. Lab Invest 2007; 87: 49-55.

[45] Kawashima M, Harada S. Effect of standard medication on quality of life of patients with atopic dermatitis. J Dermatol 2007; 34: 9-16.

[46] Kawashima M, Tango T, Noguchi T, *et al.* Addition of fexofenadine to a topical corticosteroid reduces the pruritus associ-

ated with atopic dermatitis in a 1-week randomized, multicentre, double-blind, placebo-controlled, parallel-group study. Br J Dermatol 2003; 148: 1212-1221.

[47] Fogh K, Herlin T, Kragballe K. Eicosanoids in skin of patients with atopic dermatitis: prostaglandin E2 and leukotriene B4 are present in biologically active concentrations. J Allergy Clin Immunol 1989; 83: 450-455.

[48] Archer CB, Page CP, Juhlin L, Morley J, MacDonald DM. Delayed-onset synergism between leukotriene B4 and prostaglandin E2 in human skin. Prostaglandins 1987; 33: 799-805.

[49] Archer CB, FrohlichW, Page CW, *et al*. Synergistic interaction between prostaglandins and PAF-acether in experimental animals and man. Prostaglandins 1984; 27: 495-501.

[50] Lee CH, Chuang HY, Shih CC, *et al*. Transepidermal water loss, serum IgE and beta-endorphin as important and independent biological markers for development of itch intensity in atopic dermatitis. Br J Dermatol 2006; 154: 1100-1107.

[51] Matsuoka T, Hirata M, Tanaka H, *et al*. Prostaglandin D2 as a mediator of allergic asthma. Science 2000; 287: 2013-2017.

[52] Hirai H, Tanaka K, Yoshie O, *et al*. Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils *via* seven-transmembrane receptor CRTH2. J Exp Med 2001; 193: 255-261.

[53] Yoshimura-Uchiyama C, Iikura M, Yamaguchi M, *et al*. Differential modulation of human basophil functions through prostaglandin D2 receptors DP and chemoattractant receptor-homologous molecule expressed on Th2 cells/DP2. Clin Exp Allergy 2004; 34: 1283-1290.

[54] Takeshita K, Yamasaki T, Nagao K, *et al*. CRTH2 is a prominent effector in contact hypersensitivity-induced neutrophil inflammation. Int Immunol 2004; 16: 947-959.

[55] Iwasaki M, Nagata K, Takano S, *et al*. Association of a new-type prostaglandin D2 receptor CRTH2 with circulating T helper 2 cells in patients with atopic dermatitis. J Invest Dermatol 2002; 119: 609-616.

[56] Neisius U, Olsson R, Rukwied R, Lischetzki G, Schmelz M. Prostaglandin E2 induces vasodilation and pruritus, but no protein extravasation in atopic dermatitis and controls. J Am Acad Dermatol 2002; 47: 28-32.

[57] Arai I, Takano N, Hashimoto Y, *et al*. Prostanoid DP1 receptor agonist inhibits the pruritic activity in NC/Nga mice with atopic dermatitis. Eur J Pharmacol 2004; 505: 229-235.

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2. 皮膚の樹状細胞サブセットと機能

椛島健治

皮膚は生体における最大の免疫臓器であり外的刺激に対して多彩な免疫応答を引き起こす．皮膚は表皮と真皮に分かれ，表皮にはランゲルハンス細胞，真皮には真皮樹状細胞が存在する．これらの皮膚樹状細胞サブセットは，皮膚における局在が異なるのみならず，抗原暴露後のリンパ節への到達時間，リンパ節における局在，さらには機能も異にしており，多彩な免疫反応の方向付けに重要な役割を果たしていることが明らかにされつつある．

はじめに

皮膚は生体を外界と隔てる最大の臓器であり，大きく表皮と真皮より構成されるが，表皮は表皮角化細胞，ランゲルハンス細胞[*1]，メラノサイトなどからなり，真皮は真皮樹状細胞，マクロファージ，線維芽細胞，血管内皮細胞，リンパ球，肥満細胞などより構成される．皮膚という場では，Th1，Th2，Th17型免疫反応のみならず，免疫寛容を誘導することも可能である．ランゲリン-EGFPマウスの表皮シートでは，表皮1 mm^2あたり約1,000個ものランゲルハンス細胞が観察され（図1），S100抗体により皮膚を免疫染色すると，さらに真皮にも真皮樹状細胞やマクロファージが密に存在していることから（図2），皮膚の免疫臓器としての重要性を物語っている．

また，皮膚は外敵から生体を保護するのみならず，過剰な反応が炎症性皮膚疾患を引き起こすため，生体における皮膚免疫の功罪は表裏一体である．近年filaggrinの遺伝子異常による皮膚バリアの破壊が気管支喘息の発症につながることが報告され，皮膚を介した外来抗原による感作が他臓器のアレルギー疾患の発症にも関与することが明らかにされた[1]．本稿では，多彩な免疫応答を制御する司令塔的な役割を果たす皮膚に存在する樹状細胞サブセットと各機能について最

[キーワード&略語]
ランゲルハンス細胞，皮膚樹状細胞，ランゲリン，免疫寛容，接触過敏反応

CGRP：calcitonin gene-related protein（カルシトニン遺伝子関連ペプチド）
HIV：human immunodeficiency virus（ヒト免疫不全ウイルス）
IDEC：inflammatory dendritic epidermal cell
IL：interleukin（インターロイキン）
MHC：major histocompatibility complex（主要組織適合遺伝子複合体）
MMP：matrix metalloproteinase
pDC：plasmacytoid dendritic cell（形質細胞様樹状細胞）
RANKL：receptor activator of NF-κB ligand
TGF-β：transforming growth factor-β（トランスフォーミング成長因子-β）
TSLP：thymic stromal lymphopoietin（胸腺間質性リンホポイエチン）

Subsets and functions of cutaneous dendritic cells
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近の知見を交えて述べたい．

■1 皮膚の樹状細胞サブセット

1）表皮の樹状細胞

表皮に存在する樹状細胞であるランゲルハンス細胞は，表皮を構成する細胞の約1～3％をなし，Langerin（CD207）※2やmajor histocompatibility complex（MHC）クラスⅡの他，CD54，CD80，CD86などの共刺激分子，FcγRⅡ，補体受容体の1つであるC3bi受容体，E-cadherin，を発現し，ATPaseやS-100も有している（表）．電子顕微鏡ではテニスラケット様のバーベック顆粒を有することが特徴で，他の樹状細胞はこの顆粒をもたない．

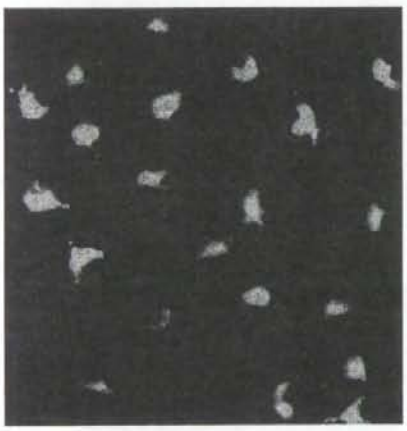

図1　表皮シートにおけるランゲルハンス細胞
（巻頭カラー図7参照）
ランゲリン-EGFPマウスを用いてマウスランゲルハンス細胞を同定した．1 mm²あたり約1,000個のランゲルハンス細胞が存在している

※1　ランゲルハンス細胞

ドイツの病理学者Paul Langerhansにより1868年に表皮有棘層で発見同定された樹状細胞の1つで，皮膚のみならず膣粘膜などにも存在する．樹枝状に進展した細胞質突起を周囲の細胞間に張り巡らせているが，細胞間橋を形成せず細胞質内にテニスラケット状のバーベック（Birbeck）顆粒を有する．

※2　ランゲリン（Langerin）

ランゲルハンス細胞に発現するⅡ型C型レクチンの1つであり，近年真皮樹状細胞の一部にも発現することが明らかにされた．Langerinの機能の詳細は不明であるが，HIV感染からの防御，デキストランなどの取り込み受容体としての働き，*Candida albicans*との結合などの機能が報告されている．

ランゲルハンス細胞は，表皮内で自身，あるいはその前駆細胞が細胞増殖を行うため，定常状態や小さな皮膚傷害程度では，骨髄由来細胞の血液を介しての補充の必要がない．また，transforming growth factor（TGF）-β欠損マウスやId2欠損マウスではランゲルハンス細胞も欠失する．さらに，CD14陽性細胞が，真皮において前駆細胞として存在し，TGF-βとともに培養するのみでランゲルハンス細胞へ分化する[2]．

以上より，表皮角化細胞のみならず，ランゲルハンス細胞自身が産生するTGF-βがランゲルハンス前駆細胞にId2を誘導し，ランゲルハンス細胞への最終分化に重要な役割を果たしていることが示唆される[3]．

2）真皮の樹状細胞

真皮における主な樹状細胞は，Langerin⁻真皮樹状細胞であるが，抗原提示細胞としては真皮マクロファージも存在する．真皮樹状細胞は，ヒト組織切片では，CD-1c（BDCA-1）などの染色により同定ができ，またランゲルハンス細胞と異なり，C型レクチンのDC-SIGNやマンノース受容体を発現している（表）．以前有用とされていたfactor XIIIaは真皮樹状細胞でなく，真皮マクロファージのマーカーであることも近年明らかにされている．

真皮樹状細胞はランゲルハンス細胞と異なり，血液を介して骨髄由来前駆細胞から常に供給を受けてホメオスタシスを保っている．従来Langerinはランゲルハンス細胞のみに存在しており，真皮に存在するLangerin陽性細胞はランゲルハンス細胞がリンパ管に入る途中の状態であると考えられていたが，ランゲルハンス細胞とは異なるLangerin陽性真皮樹状細胞が，主に真皮上層の毛包周囲に存在することがマウスで示された[4)～6)]．この樹状細胞は血液から真皮に移動してきてから，リンパ節へ移行し，真皮自己抗原に対する免疫寛容に関与することを示唆する．

定常状態の真皮では，Ⅰ型interferon（IFN）を産生するヒトCD123⁺BDCA-2⁺BDCA-4⁺あるいはマウスCD11c^{int+}B220^{int+}形質細胞様樹状細胞（plasmacytoid dendritic cell：pDC）や，inflammatory dendritic epidermal cell（IDEC）はほとんど見られないが，これらの樹状細胞サブセットは，尋常性乾癬や接触皮膚炎局所でともに増加している．さらに，SLE病変部ではpDCのみが増加し，アトピー性皮膚炎ではIDECのみが増加することが報告された[7]．このIDECがIL-12

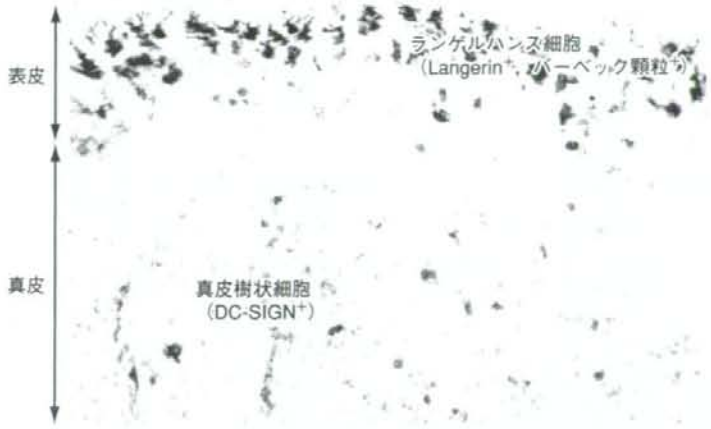

図2　免疫染色を用いた皮膚の樹状細胞の分布（巻頭カラー図8参照）
表皮にはランゲルハンス細胞，真皮には真皮樹状細胞が存在する．近年真皮にはLangerin陰性樹状細胞が存在することが報告された

表　皮膚樹状細胞のマーカー

	ランゲルハンス細胞	真皮樹状細胞
バーベック顆粒	+	−
CD1a	+ (h)	−
CD1b	−	+ (h)
CD1c	(?)	+ (h)
CD1d	−	+ (h)
CD36	−	+ (h)
ATPase/CD39	+	−
Langerin/CD207	+	一部+ (m)
DC-SIGN/CD209	−	+ (h)
マンノース受容体CD206	−	+
E-cadherin	+	−
factor XIIIa	−	−

h：ヒトにおいてのみ，m：マウスにおいてのみ発現している

やIL-18を産生してTh1反応を誘導することが推測されている．pDCは尋常性乾癬の発症に重要な役割を果たすことが示されているが，本稿ではpDCとIDECについては誌面の都合上詳細を割愛する．

2 皮膚樹状細胞の遊走と局在

皮膚樹状細胞は抗原を取り込んだ後，所属リンパ節へ遊走し，ナイーブT細胞をプライミングする．ランゲルハンス細胞は，活性化するとE-cadherinの発現を低下させて表皮角化細胞との接着を減弱させる．さらに表皮と真皮の境界に存在する基底膜を通過する際にmatrix metalloproteinase（MMP）-2やMMP-9を産生して基底膜コラーゲンを破壊する．

ランゲルハンス細胞の遊走や活性化を促進するサイトカインとしては，表皮角化細胞の産生するTNF-α，GM-CSF，IL-1，PGE2，さらにはランゲルハンス細

胞自身の産生するIL-1βがあげられる．

定常状態ではCCR1，CCR2，CCR5，CCR6，CXCR1，CXCR2，CXCR4などを発現しているが，活性化した皮膚樹状細胞はCCR7やCXCR4の発現を上昇させてそのリガンドであるCCL21やCXCL12が存在するリンパ管を介し，さらにCCL19，CCL21が存在するリンパ節内のT細胞領域に遊走する．

蛍光色素であるTRITCの皮膚塗布後，所属リンパ節では，Langerin⁻樹状細胞は，24～48時間後をピークに傍皮質（paracortex）のB細胞領域に近いところに存在し，Langerin⁺樹状細胞は4日後をピークに傍皮質のより内側かつ深部のT細胞領域に存在する[8]．各樹状細胞サブセットの所属リンパ節に到達するまでの時間やリンパ節における局在が異なることより，両者の役割分担の違いが示唆されるが，その詳細はまだ解明されていない．

また，定常状態でも少数ではあるが，ランゲルハンス細胞は所属リンパ節に遊走しており，それらはセンチネルランゲルハンス細胞と呼ばれ，定常状態と活性化した樹状細胞の中間レベルの共刺激分子を発現している．TGF-β欠損マウスではランゲルハンス細胞が欠失するが，このマウスにおいて所属リンパ節へのメラノソーム輸送が認められなかったこと[9]より，ランゲルハンス細胞が表皮の自己抗原を定常状態でもリンパ節に輸送し，末梢の免疫寛容を保っている可能性がある．

一方，紫外線照射を受けると皮膚はビタミンDを合成する．このビタミンDの存在下で分化成熟した樹状細胞は，プライミングしたT細胞に皮膚へのホーミングに重要なCCR10を発現させ，より皮膚にホーミングしやすい形質を付与する[10]．すなわち，皮膚で感作された抗原に対するメモリーT細胞は，他臓器に比べて皮膚にホーミングしやすくなるわけであり，これは生体にとって好都合となる巧妙なメカニズムである．

3 皮膚樹状細胞の免疫応答制御における役割

皮膚樹状細胞の生理的機能を考えるモデルの1つとして，接触過敏反応があげられる．皮膚はハプテンに暴露されると表皮角化細胞が前炎症性メディエーターを産生し，皮膚樹状細胞を活性化させる．皮膚樹状細胞は抗原を取り込み，成熟しながら所属リンパ節へ遊走し，ナイーブT細胞のメモリーT細胞への分化・成熟を誘導させ，感作相を成立させる[11) 12)]．この感作成立後，同一抗原に暴露されるとメモリーT細胞は局所に集積し炎症を惹起する．

この接触過敏反応における皮膚樹状細胞の役割について，以前よりランゲルハンス細胞の重要性が指摘されていた[13)]．実際*in vitro*においてランゲルハンス細胞が強力な抗原提示能を示すことは周知の事実である．ところがLangerin遺伝子にジフテリア毒素の受容体をノックインしたり，ヒトLangerinプロモーターにジフテリア毒素を導入するなどして，Langerin陽性樹状細胞を特異的に除去したところ，3つのグループにより，接触過敏反応が，亢進，不変，減弱という相異なる結果が得られた[8) 14) 15)]．原因の詳細は不明であるが，Langerin陽性樹状細胞が接触過敏反応において，時に負の作用を有していることを示唆する[16) 17)]．さらには，真皮に存在するLangerin陽性樹状細胞が接触過敏反応の開始に重要であることが近年示された．一方，惹起相における抗原提示細胞の詳細な同定はなされていない．

また，皮膚の単純ヘルペスウイルス感染においてはランゲルハンス細胞ではなく真皮樹状細胞がウイルス特異的エフェクターT細胞の誘導に重要であること[18)]，皮膚リーシュマニア感染の際のT細胞への抗原提示にランゲルハンス細胞は直接関与しないこと[19)]などが示された．一方，human immunodeficiency virus（HIV）-1感染においては，皮膚や粘膜に存在するランゲルハンス細胞が，ケモカイン受容体を介してHIVに感染し，リンパ節においてT細胞に伝達する．以上のように皮膚は外的刺激に応じて，皮膚の各樹状細胞サブセットを使い分けている．

一方，Th1，Th2，Th17などの多彩な皮膚免疫応答を制御しているのもおそらく樹状細胞である．近年，アトピー性皮膚炎などのTh2型病において，表皮角化細胞からthymic stromal lymphopoietin（TSLP）が産生され，樹状細胞に作用しTh2反応を促進すること[20)]，さらにTSLPがOX40Lを樹状細胞上に誘導し，Th2型反応を誘導することが示された[21)]．また，皮膚樹状細胞が産生するIL-12はTh1細胞の分化を誘導し，IL-4はTh2型，IL-6，TGF-βはTh17型免疫反応を誘導する．これらがどのような外的刺激による樹状細胞の分化成熟に繋がっているのかを解明することは今後の課題であろう．

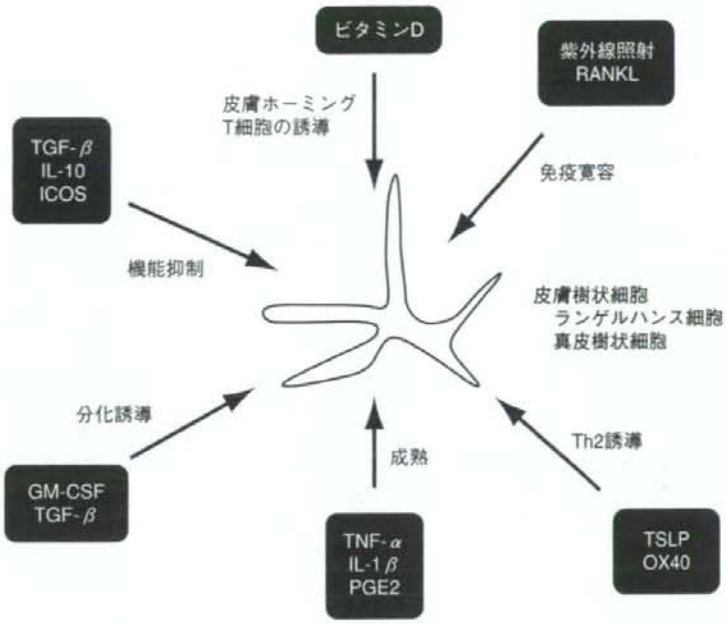

図3　皮膚樹状細胞の機能調節
図に示すような刺激などに伴い皮膚樹状細胞は多彩な機能を発揮する

4 皮膚における樹状細胞の免疫抑制細胞としての役割

前述したように皮膚は免疫寛容や免疫抑制状態を呈することも可能であり，紫外線照射，抗原の大量暴露，植皮後などが例としてあげられる．紫外線照射後には，表皮角化細胞上のRANKL（receptor activator of NF-κB ligand）が皮膚樹状細胞のRANKに作用し，制御性T細胞を誘導することにより免疫寛容が起こると考えられている[22]．刺激のない定常状態でも，樹状細胞は表皮などに存在する自己抗原をT細胞に提示し，免疫寛容に関与しているとされる．

また，骨髄細胞をTGF-βとIL-10とともに培養すると樹状細胞が制御機能を獲得する[23]．TGF-βとIL-10はともに表皮に多く存在しているサイトカインであり，また，TGF-βはランゲルハンス細胞の最終分化に必要な因子であることから，制御性樹状細胞とランゲルハンス細胞との関係が今後注目される．

一般に，皮膚樹状細胞の分化や機能を抑制する因子として，IL-10，TGF-β，calcitonin gene-related protein（CGRP）などがあげられる．共刺激分子の1つである樹状細胞上のICOS-Lを介したシグナルが制御性T細胞を誘導するが，皮膚樹状細胞との関連の詳細は明らかではない[24]．

おわりに

皮膚樹状細胞は皮膚という生体の外界との最前線において，微細な調節を行いホメオスタシスの維持，感染や外来物からの防御，炎症性皮膚疾患の発症など，さまざまな役割を担っている（図3）[25]．今後は皮膚樹状細胞の分化成熟に対する表皮角化細胞の役割の解明や，皮膚樹状細胞の他の皮膚構成細胞との相互作用を介した機能修飾などが大きな課題となってくるであろう．今後，皮膚樹状細胞の機能制御を介する炎症性皮膚疾患や腫瘍免疫の制御などへの応用が期待される．

文献

1）Palmer, C. N. et al.：Nat. Genet., 38：441-446, 2006
2）Larregina, A. T. et al.：Nat. Immunol., 2：1151-1158, 2006
3）Hacker, C. et al.：Nat. Immunol., 4：380-386, 2003
4）Bursch, L. S. et al.：J. Exp. Med., 204：3147-3156, 2007