単行本

(和文)

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	ページ	出版年	掲載
江川形平 桃島健治	IV型アレルギーモデル	岩倉洋一郎	免疫疾患-疾患モデルの作成と利用	株式会社 エル・アイ・シー	東京	114-118	2011	
桃島健治	好酸球性膿疱性毛包炎		皮膚で見つける全身疾患	メディカルレビュー社	東京	43	2011	
桃島健治	ドライスキンと痒み	宮地良樹	全身とかゆみ	診断と治療社	東京	36-37	2011	
桃島健治	かゆみ過敏とは		今日の治療指針	診断と治療社	東京	38-39	2011	
松村由美	足趾壊疽（糖尿病）		皮膚で見つける全身疾患	メディカルレビュー社	東京	97	2011	
松村由美	紅皮症 （悪性リンパ腫）		皮膚で見つける全身疾患	メディカルレビュー社	東京	1150	2011	
宮地良樹	高齢者のスキンケアのポイントは	宮地良樹 北　徹	高齢者の皮膚トラブルQ&A	診断と治療社	東京	58-60	2011	
宮地良樹	掻破の功罪	宮地良樹	全身とかゆみ	診断と治療社	東京	41-42	2011	
宮地良樹	皮膚・粘膜分野編集	井村裕夫	症候群ハンドブック	中山書店	東京		2011	
宮地良樹	皮膚で見つける全身疾患	宮地良樹		メディカルレビュー社	東京		2011	
宮地良樹	外来皮膚科ER最前線	宮地良樹		メディカルレビュー社	東京		2011	
宮地良樹	全身とかゆみ	宮地良樹		診断と治療社	東京		2011	

(欧文)

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	ページ	出版年	掲載
Yoshiki Miyachi	Eosinophilic Pustular Folliculitis.	Eun HC, Kim SC, Lee WS,	Asian skin and skin diseases	Medrang	Seoul		2011	文献7

【V】

研究成果の刊行物・別刷り

Journal of Dermatological Science 63 (2011) 75–82

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.elsevier.com/jds

Invited review article

Regulatory T cells in cutaneous immune responses

Tetsuya Honda *, Yoshiki Miyachi, Kenji Kabashima *

Department of Dermatology, Kyoto University Graduate School of Medicine, 54 Shogoin-Kawara, Sakyo, Kyoto 606-8507, Japan

ARTICLE INFO

Article history:
Received 11 February 2011
Received in revised form 25 May 2011
Accepted 14 June 2011

ABSTRACT

Regulatory T cells (Treg) are a subset of T cells with strong immunosuppressive activity. In the skin, it has recently been revealed that Treg play important roles not only in the maintenance of skin homeostasis but also in the regulation of the immune responses, such as contact hypersensitivity and atopic dermatitis. Furthermore, the skin plays important roles in the induction of Treg in the periphery. In this review, we will provide an overview of the mechanism of Treg-mediated immunosuppression and discuss the role of Treg in the skin.

Contents

1. Introduction

Regulatory T cells (Treg) are a subset of T cells with strong immunosuppressive activity. Treg were originally identified as $CD4^+CD25^+$ T cells [1,2]. When mice were depleted of $CD4^+CD25^+$ cells, they spontaneously developed autoimmune diseases and allergies, indicating that $CD4^+CD25^+$ T cells are essential for the maintenance of self-tolerance. Later on, the forkhead box p3 (Foxp3) gene was identified as the master transcriptional factor of Treg [3].

There are at least two kinds of $Foxp3^+$ Treg: naturally occurring Treg (nTreg) and inducible Treg (iTreg) [4]. nTreg develop in the thymus, and play an important role in the maintenance of self-tolerance and immune homeostasis. Scurfy mice, which possess a defective *Foxp3* gene, exhibit hyperactivation of $CD4^+$ T cells and overproduction of proinflammatory cytokines, and typically die within a month after birth [5]. Patients with IPEX syndrome (immune dysregulation polyendocrinopathy, enteropathy, X-linked syndrome) have a mutation in the human *FOXP3* gene, and are therefore regarded as the human counterpart of scurfy mice [6]. iTreg, on the other hand, are induced from naïve T cells in the presence of transforming growth factor (TGF)-β, and develop in the periphery. Retinoic acid facilitates the differentiation of naïve T cells to $Foxp3^+$ Treg [7,8] and may be related to the establishment of oral tolerance, although it remains to be determined whether iTreg are functionally stable and to what extent they contribute under physiological conditions.

In addition to $Foxp3^+$ Treg, there are other types of Treg, such as Tr1 and Th3 cells; these are induced in the periphery [4,9,10]. Tr1 cells can be induced through the antigenic stimulation of naïve T cells in the presence of IL-10 *in vitro*, and exert a suppressive effect *in vitro* by inducing large amounts of IL-10 and TGF-β. Th3 cells

* Corresponding authors. Tel.: +81 75 751 3310; fax: +81 75 761 3002.
E-mail addresses: hontetsu@kuhp.kyoto-u.ac.jp (T. Honda), kaba@kuhp.kyoto-u.ac.jp (K. Kabashima).

0923-1811/$36.00 © 2011 Published by Elsevier Ireland Ltd on behalf of Japanese Society for Investigative Dermatology.
doi:10.1016/j.jdermsci.2011.06.004

produce TGF-β in an antigen-specific manner, and exert a suppressive effect. Intriguingly, however, both are Foxp3 and CD25 negative. No further details of this population are discussed in this manuscript.

Evidence has accumulated regarding the regulatory roles of Treg not only in self-tolerance, but also in a variety of pathophysiological immune responses, such as gastritis [11], arthritis, encephalomyelitis [12], inflammatory bowel disease (IBD) [13], insulin-dependent diabetes [14] and various allergic skin diseases such as contact hypersensitivity or atopic dermatitis.

In this review, we will provide an overview of the mechanism of Treg-mediated immunosuppression, mainly focusing on $Foxp3^+$ Treg, and discuss the role of Treg in the skin immune responses, focusing on contact hypersensitivity and atopic dermatitis.

1.1. Mechanism of suppression by Treg

Treg potently suppress the proliferation of T cells when Treg are co-cultured with responder cells that have been stimulated with a specific antigen or a polyclonal T cell receptor stimulator *in vitro*. Multiple suppression mechanisms have been proposed based on *in vitro* assays; for example, IL-10 [13], TGF-β [15], and IL-35 [16] have been considered as possible soluble suppressive factors of T cell proliferation. Absorption of IL-2 by Treg may also be involved in inhibiting T cell proliferation [17]. It has also been reported that Treg exert their regulatory functions by cell–cell contact-dependent factors, such as CD39/CD73 [18] and granzyme/perforin [19]. In addition to these direct suppressive effects, Treg indirectly suppress T cell proliferation by affecting the function of APCs. It has been reported that Treg inhibited the T cell stimulatory capacity of APCs by down-regulating CD80 and CD86 expression through cytotoxic T-lymphocyte antigen (CTLA)-4 and lymphocyte function-associated antigen (LFA)-1 [20]. Using two-photon microscopic analysis, Tadokoro et al. [21] and Tang et al. [14] have revealed that Treg inhibit stable contact and interaction between APCs and effector T cells. Treg also stimulate DCs to express the enzyme indoleamine 2,3-dioxygenase (IDO), which catabolizes the conversion of tryptophan to kynurenine, a toxic factor to T cells [22]. In addition to their effect on APCs, it has also been reported that Treg down-regulate mast cell function by suppressing mast cell degranulation and anaphylactic response through OX40–OX40L interaction [23]. The mechanisms by which suppression is achieved may vary depending on context, however, and it has not yet been determined how these *in vitro* findings correlate with *in vivo* suppression.

1.2. Characterization of Treg in the skin

Treg exist in all non-lymphoid tissues; the skin has a particularly high proportion of Treg in the steady state [24–26]. Treg in the skin are $CD44^+$ and $CD103^{high}$ [24–26], and express the chemokine receptors CCR4, CCR5, CCR6 and CCR7. $CCR5^+$ Treg preferentially migrate to cutaneous lesions of *Leishmania major* infection [27]. Mice with a complete loss of CCR4 on Treg develop spontaneous lymphocytic infiltration and severe inflammation in the skin and lungs, accompanied by peripheral lymphadenopathy and increased differentiation of skin tropic $CD4^+Foxp3^-$ T cells. Using α-1,3-fucosyltransferase VII (Fut7) deficient mice, Dudda et al. [26] have reported the importance of E- and P-selectin ligand for Treg migration to the skin. Loss of these selectin bindings caused skin-specific inflammation, indicating the essential role of skin-resident Treg for maintaining immune homeostasis locally.

2. Treg induction and expansion in the skin

Ultraviolet (UV) radiation to the skin is well known to cause immunosuppression, and is accordingly applied as a treatment for a wide variety of skin diseases. Recently, it has been revealed that one of the immunosuppressive mechanisms involved in this effect is mediated by Treg, which are induced by UV irradiation [28]. It has been proposed that the cells responsible for this induction of Treg are epidermal Langerhans cells (LCs), an important group of skin-resident dendritic cells. Loser et al. [29] have reported that the receptor activator of NF-kappaB ligand (RANKL) was induced in keratinocytes by UV exposure, and RANKL-activated LCs were responsible for the development of UV-induced Treg. It has also been reported that the induction of Treg by UV irradiation was completely abolished by the depletion of LCs using Langerin-DTR mice or steroid mometasone [30,31]. In addition, it has recently been reported that IL-10-producing and OX40 ligand-expressing mature LCs are responsible for the induction of Treg upon UV exposure [31], suggesting the importance of LCs for Treg induction. In addition to UV-induced immunosuppression, similar findings were observed concerning the mechanisms involved in immunosuppression during skin grafting. Yoshiki et al. [32] have reported that the development of contact hypersensitivity (CHS) was suppressed when mice were sensitized with a hapten through full-thickness grafted skin. In this model, $CD4^+CD25^+$ but not $CD4^+CD25^-$ T cells in draining lymph nodes (LNs) were responsible for this suppression. In addition, a high expression of RANKL was observed in the grafted skin, and recombinant RANKL stimulated LCs to produce IL-10. These findings suggest that the LCs play important roles in the peripheral induction of Treg. Recently, it has been reported that glucocorticoids modify LCs to produce TGF-β and expand regulatory T cells in humans [33] (Fig. 2), implying that glucocorticosteroids may exert their anti-inflammatory functions by inducing Treg.

The phenotypes and suppression mechanisms of UV-induced Treg are different from those of nTreg. Schwartz et al. [34,35] have reported that the administration of $CD4^+CD25^+$ cells from UV-irradiated DNFB-sensitized mice impaired sensitization of CHS. These UV-induced Treg did not suppress the CHS response when administered before elicitation, though natural $CD4^+CD25^+$ Treg did. Direct injection of UV-induced Treg into the elicitation sites did suppress the CHS response, however. They accordingly concluded that UV-induced Treg did not express skin-homing receptors for E- and P-selectins, and so failed to suppress elicitation. In addition, they reported that UV-induced Treg changed APCs in LNs from a stimulatory to a regulatory phenotype by modulating the co-stimulatory molecules on APCs, which, in turn, further induce Treg [36].

Although the importance of LCs has been suggested as mentioned above, other groups have reported the importance of dermal DCs in UV-induced immunosuppression and peripheral Treg induction. Wang et al. [37] reported the UV-induced immunosuppression was abolished by selective depletion of Langerin-positive dermal DCs, suggesting the importance of Langerin-positive dermal DCs in Treg induction. It has also been reported that retinoic-acid producing CD103-negative dermal dendritic cells have the ability to induce Treg in draining LNs [38], in contrast to the equivalent phenomenon in the gut, where CD103-positive DCs are responsible for the induction of Treg [39].

3. Treg in CHS

CHS, a frequently used mouse model of contact dermatitis, is a prototype of skin immune response, and the role of Treg in CHS has been gradually revealed.

The development of CHS is divided into two phases: sensitization and elicitation [40]. In the sensitization phase, low molecular weight compounds called haptens are cross-linked to epidermal proteins and taken up by resident DCs such as LCs and dermal DCs. Subsequently, these cells are matured by proinflammatory

cytokines such as TNF-α, IL-1β, and prostaglandin E_2, and migrate to the draining LNs to present antigens in a CCR7- and CXCR4-dependent manner [41,42]. After antigen presentation, naive T cells are activated and differentiated into antigen-specific Th1 and Tc1 cells under the influence of polarizing signals such as IL-12 and other chemical mediators [43]. Th17 cells are also involved in the pathogenesis of CHS [44]. When the skin is re-exposed to the same hapten after establishment of the sensitization, an antigen-specific T cell-mediated inflammation that is known as elicitation phase is provoked. Upon re-exposure to the same hapten, keratinocytes and mast cells produce chemokines and pro-inflammatory cytokines such as TNF-α and IL-1β, which activate endothelial cells and induce the expression of E- or P-selectins [45–47]. Then, neutrophils and antigen-specific T cells enter the dermis and release IFN-γ, which further stimulates keratinocytes to induce massive leukocyte infiltration [48].

3.1. Treg in the CHS response – elicitation phase

The effect of Treg on CHS has mainly been investigated in the elicitation phase. Ring et al. have purified $CD4^+CD25^+$ Treg from naïve mice and administered them into TNCB-sensitized recipient mice intravenously one day before elicitation [49]. Administration of Treg significantly suppressed the ear swelling response and inflammatory cell infiltration into the skin compared to those of vehicle-treated mice. Ring et al. have reported that these suppressive effects are mediated by soluble factors, especially IL-10. Administration of a culture supernatant of Treg suppressed the CHS response, and this suppression was reversed by an anti-IL-10 Ab. Furthermore, Treg from IL-10-deficient mice failed to suppress the CHS response by inhibiting the leukocyte influx into the inflamed skin.

The same group has recently reported that the adenosine produced by Treg is involved in blocking the influx of leukocytes into the skin by downregulating E- and P- selectins on endothelial cells [50]. Adenosine triphosphate (ATP) is first degraded by CD39 to adenosine diphosphate (ADP) and then to adenosine monophosphate (AMP). The AMP is serially dephosphorylated by CD73 to adenosine. Treg are strongly positive for both CD39 and CD73 expression; therefore, Treg convert ATP to adenosine and suppress the CHS response. On the other hand, conventional T cells exhibit only a low basal expression level of CD39. Accordingly, injection of adenosine or Treg abrogated the ear-swelling response in CHS, which was not seen using Treg from CD39-deficient mice [50]. Moreover, Treg further upregulate CD39 expression after activation; this activation is a prerequisite for Treg to acquire their suppressive capacity.

3.2. Treg in the CHS response – sensitization phase

While reports on the role of Treg in the sensitization phase have been rather limited compared to those discussing the elicitation phase, some interesting reports have recently been published. Dubois et al. [51], for example, have reported the involvement of Treg in the induction of oral tolerance and inhibition of DNFB-induced CHS. Oral tolerance was induced by feeding DNFB orally prior to DNFB sensitization. Although no such tolerance induction was seen in $CD4^+$ T cell-deficient mice, transfer of naïve $CD4^+CD25^+$ T cells restores oral tolerance in those mice, in a manner independent of IL-10 [51]. The same authors also showed that administration of neutralizing anti-CD25 monoclonal antibody (mAb) impairs oral tolerance in WT mice. Intriguingly, administration of anti-CD25 mAb before sensitization had no significant affect on the ear swelling response, suggesting that $CD4^+CD25^+$ T cells are responsible for oral tolerance induction, while the role of Treg in the sensitization phase remained unclear. Ring et al. have recently reported that the administration of Treg suppressed the extent of sensitization in CHS by inhibiting DCs and CD8 T cells in the draining LNs [52]. In their report, Treg and DCs established gap junctions, which caused a reduction in the capacity of DCs to stimulate CD8 T cells. In their next report, the same authors stated that Treg activation in draining LNs was mediated by ATP, because Treg acquired an activated phenotype upon ATP treatment *in vitro*, while blockage of ATP receptors on Treg abrogated ATP-mediated activation and suppressive function of Treg *in vivo* [53].

3.3. The role of endogenous Treg in CHS

As described above, exogenous administration of Treg suppresses CHS both in the sensitization phase and in the elicitation phase. It remains unclear, however, whether endogenous Treg play the same suppressive role under physiological conditions. To this end, specific depletion of Treg *in vivo* is required. Although $CD4^+CD25^+$ has been used as a marker for Treg, CD25 is expressed in activated CD4 cells as well as in Treg. Therefore, Foxp3 is a more definitive marker of Treg, but because Foxp3 is a transcriptional factor that exists intracellularly, the purification of live Treg or depletion by means of neutralizing mAb has been technically difficult.

To solve these problems, Foxp3 reporter mice expressing human CD2 and human CD52 chimeric protein have been generated and designated as $Foxp3^{hCD2/hCD52}$ mice. Since $Foxp3^+$ cells co-express hCD2 on the cellular surface, live $Foxp3^+$ Treg are sorted with anti-hCD2 mAb and depleted with neutralizing anti-hCD52 Ab [25]. The mice have been used in the investigations into the role of endogenous Treg in CHS. Depletion of Treg in the elicitation phase caused the ear swelling response to be enhanced and prolonged compared with that seen in the control, indicating that Treg is responsible for terminating skin inflammation in CHS [25].

In addition, the role and mobility of Treg in the skin during CHS was investigated. Kaede-transgenic mice are genetically engineered to ubiquitously express Kaede protein, a photoconvertible protein that changes its fluorescence from green to red under exposure to violet light. Therefore, mobility of cells from the skin under physiological conditions can be analyzed. Treg were found to localize abundantly in the inflamed skin seen in CHS, and these skin Treg were found to migrate further back to draining LNs. Treg from the skin showed significantly higher mRNA expression of T cell suppression-associated molecules such as IL-10, TGF-β and CTLA4. Consistently, Treg from the skin exhibited significantly stronger suppressive activity both *in vivo* and *in vitro* (Fig. 1). These results suggest that Treg in the skin also play important roles in the termination of dermatitis and possibly in the control of systemic immune responses.

It has been suggested that Treg in the skin contribute to its homeostasis, since chronic depletion of skin Treg leads to the development of spontaneous dermatitis [24,26]. Schneider et al. have reported that CCR7-deficient mice showed a reduced number of Treg in draining LNs and an enhanced inflammatory response in CHS after repeated hapten application [54], which suggests the homing of Treg to draining LNs through CCR7 plays an important role in eliciting the function of Treg.

Endogenous Treg regulate the extent of sensitization as well as that of challenge in CHS. Depletion of Treg during the sensitization phase leads to enhanced skin inflammation [55]. Mice depleted with Treg population showed increased numbers of memory T cells and higher expression levels of costimulatory molecules in DCs in draining LNs compared with control mice, suggesting that endogenous Treg modulate DC function and thus regulate the extent of sensitization [55]. Recent findings on the role of Treg in

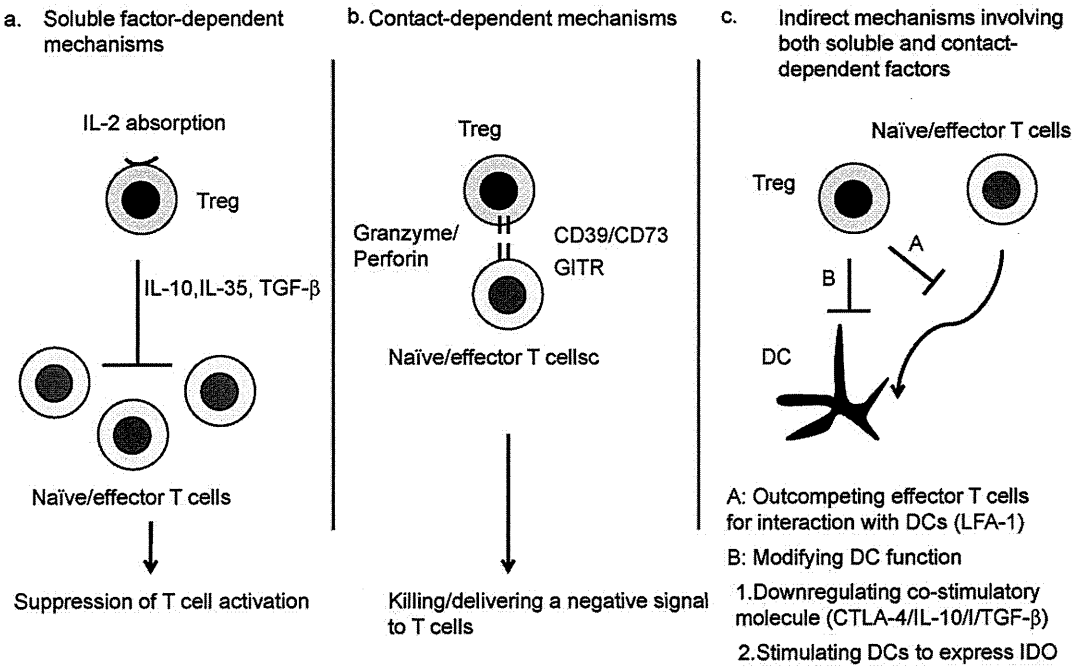


Fig. 1. Possible mechanisms involved in suppression by Treg. (a) Soluble factor-dependent mechanisms. Treg produce large amounts of IL-10, IL-35, and TGF-beta, all of which suppress naive/effector T cell activation. Treg also absorb IL-2, which causes cytokine deprivation-induced apoptosis among effector T cells. (b) Contact-dependent mechanisms. CTLA-4 on Treg deliver negative signals to T cells. CD39/CD73 on Treg catalyze ATP and generate pericellular adenosine, exerting an anti-inflammatory effect. Treg also may kill responder T cells by a granzyme or perforin-dependent mechanisms. (c) Indirect mechanisms. Treg inhibit the interaction between DCs and effector T cells. Treg also downregulate DC activation and thus cause immunosuppression.

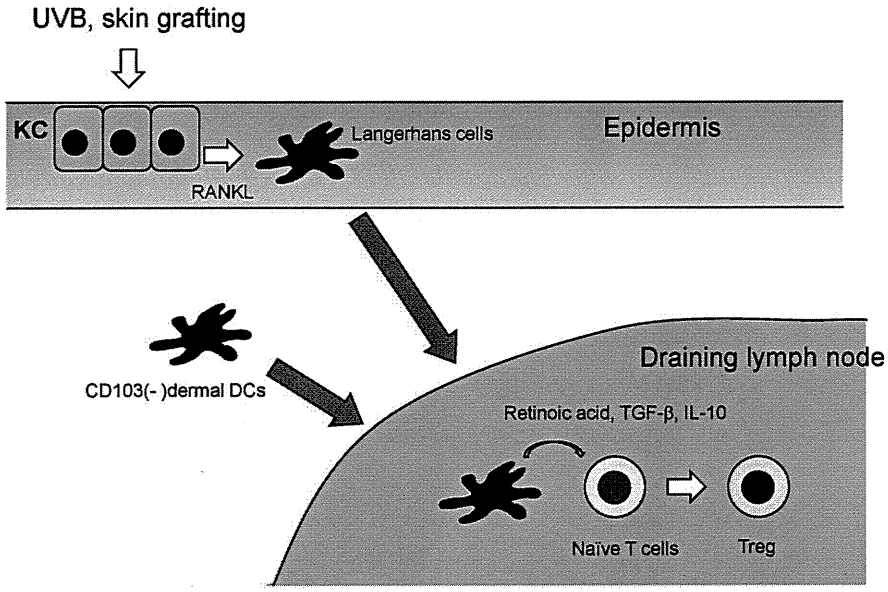


Fig. 2. Proposed mechanism of Treg induction by skin DCs. UV exposure or skin grafting induces RANKL expression on keratinocytes, which stimulate LCs. RANKL-stimulated LCs then induce Treg in draining LNs. Under conditions of UV exposure, it has also been proposed that the UV-induced Treg affect DCs and modify their functions from a stimulatory phenotype to a regulatory phenotype, which further induces Treg. In addition to LCs, CD103-negative dermal DCs can induce Treg in draining LNs.

CHS are summarized in Table 1, and schematic views of those findings are illustrated in Figs. 3 and 4.

4. Atopic dermatitis (AD) and Treg

Atopic dermatitis is one of the most common skin inflammatory disorders. New insights point to an important role of structural abnormalities in the epidermis combined with immune dysregulation [56]. Although studies on the role of Th2 cells have focused on the pathophysiology of AD, recent reports have indicated the importance of other T cell subsets such as Th17 [57] and Treg.

Ou et al. [58] have compared the numbers and functionality of peripheral blood mononuclear cells (PBMC) between healthy controls and AD patients, and reported that AD patients have higher numbers of Treg, each with a suppressive activity comparable to that of Treg in healthy controls, in the peripheral blood. Others have also reported that increased numbers of Treg in the PBMC of AD patients [59] and expansion of Treg were positively associated with disease activity in AD [60]. On the other hand, it

Table 1
An overview of recently published papers about Treg and CHS.

	Major findings	Reference
Sensitization	Attenuated sensitization by Treg induced by RANKL-activated LC in a UV-immunosuppression model	[29]
	Attenuated sensitization by Treg induced by IL-10 from RANKL-activated LC in a skin graft immunosuppression model	[32]
	Attenuated sensitization by Treg induced by orally administered antigen in an oral tolerance model	[51]
	Treg attenuate sensitization by modifying DC function through gap junction formation	[52]
	Treg acquire an activated phenotype by means of ATP in draining LNs	[53]
	Enhanced ear swelling response resulting from the depletion of endogenous Treg	[55]
Elicitation	Reduced ear swelling response resulting from the inhibition of the leukocyte influx through IL-10 from Treg	[49]
	Reduced ear swelling response resulting from the inhibition of the leukocyte influx through adenosine from Treg via CD39/CD73 (inhibition of E- and P-selectin expression in endothelial cells)	[50]
	Treg acquire activated phenotype by means of ATP in blood.	[53]
	Enhanced and prolonged ear swelling response resulting from depletion of endogenous Treg	[25]

has also been reported that the numbers of Treg among the PBMC are similar between AD and healthy controls [61]. In AD skin lesions, it was initially reported that Treg were absent, while Tr1 were detected [62]. Later on, however, several groups reported the existence of Treg in AD skin lesions [63,64]. Because AD is a chronic inflammatory disease with multiple disease stages and multiple factors, and because some treatments for AD such as cyclosporine [59,61,65], glucocoriticoids [33] and UV radiation [28], can alter the number of Treg in the PBMC, the interpretation and comparison of these studies will require careful attention.

Based on observations of IPEX syndrome patients, who show atopic-like dermatitis and high IgE levels, however, it seems probable that the number of Treg is related to the development of AD lesions [6]. As for the function of Treg in AD, it has been reported that their suppressive activity is similar to that of Treg in healthy controls [58]. Reefer et al., however, have reported that a new subtype of Treg with Th2-promoting ability has been observed in AD and that its functions depend on the expression of CCR6 [66]. In this report, CCR6-negative CD25-high positive Treg produced Th2 cytokines, and co-culture with effector T cells selectively enhanced IL-5 production, suggesting the heterogeneity of Treg in AD.

5. Psoriasis and Treg

We will discuss the recent findings about the role of Treg in psoriasis, another common chronic inflammatory skin disease. Although its pathological mechanism is still not completely clear, studies of immune-targeted therapies established it as a primarily immune-mediated disease, such as by Th17, Th1 and Th22 cells, which eventually causes epidermal abnormality [67]. Besides such effector T cells, a substantial number of Treg are detected in lesional skin of psoriasis, and the number of Treg in the psoriatic skin lesion is higher than that in healthy or uninvolved skin [68–70]. The number of Treg in peripheral blood of psoriasis patients is also increased [71], and this increase is positively correlated with the disease activity [68,71]. An anti-TNF alpha antibody infliximab, which has significant therapeutic effects on psoriasis, affects the number of Treg in peripheral blood [72]. It is also suggested that vitamin D metabolite $1,25(OH)_2D_3$ analogs, a successfully used topical treatment for psoriasis, can increase the number of Treg by modulating the function of LCs [73].

Recently, dysfunction of Treg has been reported in psoriasis patients [74]. Treg in both lesional skin and blood from psoriasis

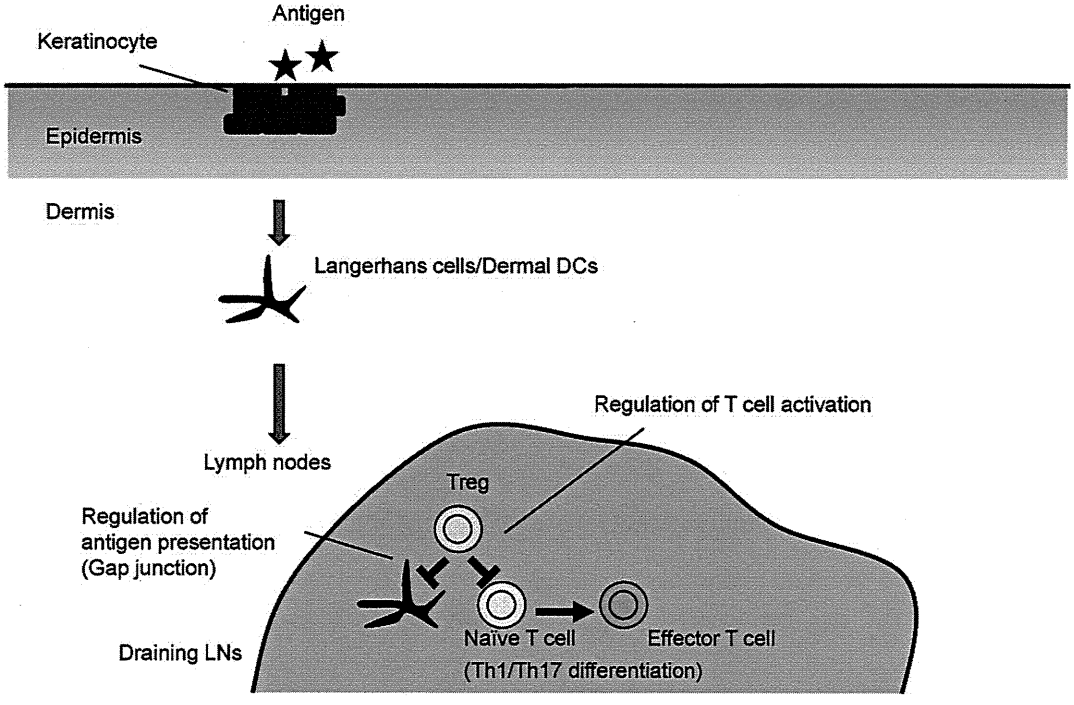


Fig. 3. Possible mechanism of suppression by Treg in sensitization phase of CHS. Treg are activated in draining LNs by ATP. They down-regulate DC activation through gap junction formation and subsequent T cell proliferation, which controls the extent of sensitization.

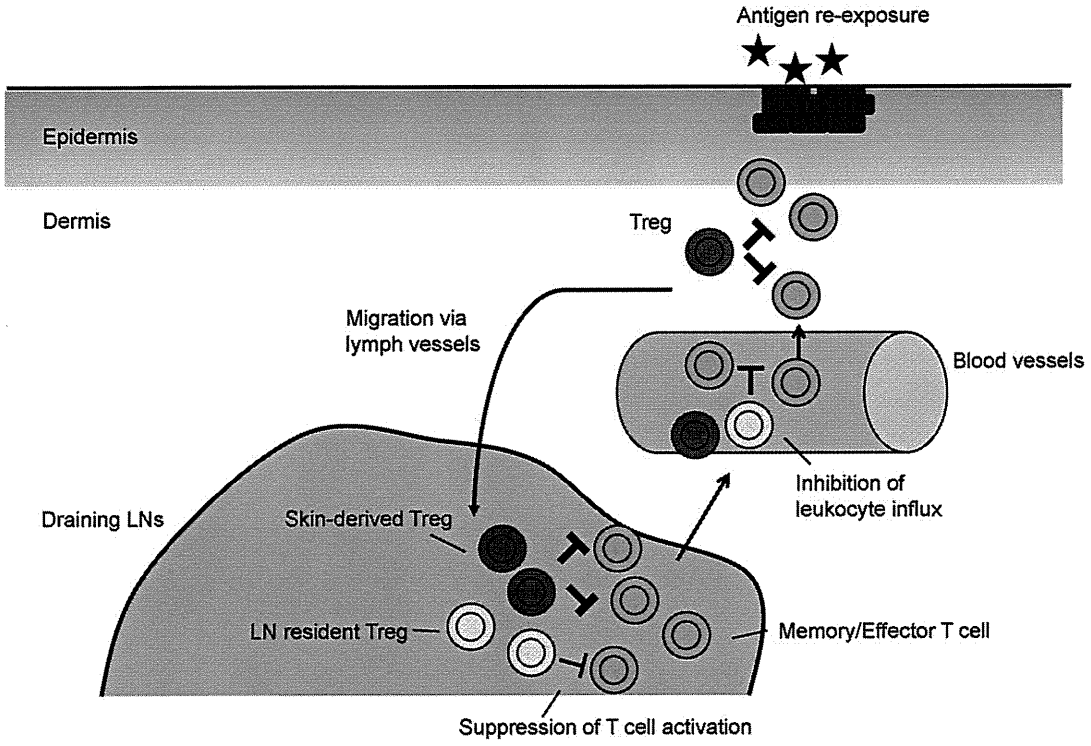


Fig. 4. Possible mechanism of suppression by Treg in elicitation phase of CHS. Treg suppress effector T cells in the LNs and inhibit leukocyte influx into the periphery through IL-10 or CD39-dependent mechanisms. In addition, Treg migrating into the skin could suppress the effector T cell functions in the skin. Furthermore, a fraction of Treg in the skin migrate back to the draining LNs through afferent lymphatic vessels, and can return from there to the skin. These skin-derived Treg possess higher suppression activity than LN-resident Treg, and contribute to the termination of skin inflammation.

patients showed reduced suppressive activity compared with those from healthy donors [74]. Later on, it has been reported that such a Treg dysfunction is caused by IL-6 signaling, which is abundantly produced in psoriasis lesion [75], and that IL-6 enables effector T cells to escape from Treg-mediated suppression both in mice and in humans [74,76,77]. Therefore, the local cytokine milieu may further lead to subsequent hyperproliferation of pathogenic T cells in psoriasis skin and enhancement of disease activities.

6. Conclusion

We have reviewed the roles of Treg in cutaneous immune responses. A considerable amount of knowledge on Treg has been accumulated, and multiple mechanisms and various molecules are reported to be involved in Treg-mediated immunosuppression. It is likely that the suppressive mechanisms of Treg may differ depending on disease stage and the skin immune response type. Analysis using Foxp3-diphtheria toxin receptor knockin mice or $Foxp3^{hCD2/hCD52}$ mice, which enable us to deplete Treg conditionally and specifically, will further reveal the molecular mechanisms and physiological functions of Treg in cutaneous immune responses.

It is crucially important to clarify how and to what extent those molecules are involved in Treg function in humans. From a clinical perspective, the precise mechanism by which Treg function in the elicitation phase is an important issue to be addressed, since most patients with cutaneous immune disease have already been sensitized. We expect that further effort in the investigation of Treg will give us important clues supporting the development of innovative therapeutic approaches for various skin diseases.

Acknowledgements

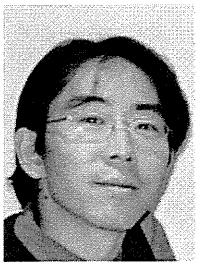
This study was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and the Ministry of Health, Labor, and Welfare of Japan.

References

[1] Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995;155:1151–64.
[2] Sakaguchi S, Toda M, Asano M, Itoh M, Morse SS, Sakaguchi N. T cell-mediated maintenance of natural self-tolerance: its breakdown as a possible cause of various autoimmune diseases. J Autoimmun 1996;9:211–20.
[3] Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003;299:1057–61.
[4] Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell 2008;133:775–87.
[5] Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet 2001;27:68–73.
[6] Ochs HD, Ziegler SF, Torgerson TR. FOXP3 acts as a rheostat of the immune response. Immunol Rev 2005;203:156–64.
[7] Benson MJ, Pino-Lagos K, Rosemblatt M, Noelle RJ. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. J Exp Med 2007;204:1765–74.
[8] Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science 2007;317:256–60.
[9] Vieira PL, Christensen JR, Minaee S, O'Neill EJ, Barrat FJ, Boonstra A, et al. IL-10-secreting regulatory T cells do not express Foxp3 but have comparable regulatory function to naturally occurring CD4+CD25+ regulatory T cells. J Immunol 2004;172:5986–93.
[10] Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, et al. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 1997;389:737–42.
[11] Suri-Payer E, Cantor H. Differential cytokine requirements for regulation of autoimmune gastritis and colitis by CD4(+)CD25(+) T cells. J Autoimmun 2001;16:115–23.
[12] Furtado GC, Olivares-Villagomez D, Curotto de Lafaille MA, Wensky AK, Latkowski JA, Lafaille JJ. Regulatory T cells in spontaneous autoimmune encephalomyelitis. Immunol Rev 2001;182:122–34.
[13] Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J Exp Med 1999;190:995–1004.
[14] Tang Q, Adams JY, Tooley AJ, Bi M, Fife BT, Serra P, et al. Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice. Nat Immunol 2006;7:83–92.

[15] Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. J Exp Med 2001;194:629–44.
[16] Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, et al. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature 2007;450:566–9.
[17] Pandiyan P, Zheng L, Ishihara S, Reed J, Lenardo MJ. CD4+CD25+Foxp3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells. Nat Immunol 2007;8:1353–62.
[18] Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med 2007;204:1257–65.
[19] Gondek DC, Lu LF, Quezada SA, Sakaguchi S, Noelle RJ. Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. J Immunol 2005;174:1783–6.
[20] Onishi Y, Fehervari Z, Yamaguchi T, Sakaguchi S. Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation. Proc Natl Acad Sci USA 2008;105:10113–8.
[21] Tadokoro CE, Shakhar G, Shen S, Ding Y, Lino AC, Maraver A, et al. Regulatory cells inhibit stable contacts between CD4+ T cells and dendritic cells in vivo. J Exp Med 2006;203:505–11.
[22] Grohmann U, Orabona C, Fallarino F, Vacca C, Calcinaro F, Falorni A, et al. CTLA-4-Ig regulates tryptophan catabolism in vivo. Nat Immunol 2002;3:1097–101.
[23] Gri G, Piconese S, Frossi B, Manfroi V, Merluzzi S, Tripodo C, et al. CD4+CD25+ regulatory T cells suppress mast cell degranulation and allergic responses through OX40-OX40L interaction. Immunity 2008;29:771–81.
[24] Sather BD, Treuting P, Perdue N, Miazgowicz M, Fontenot JD, Rudensky AY, et al. Altering the distribution of Foxp3(+) regulatory T cells results in tissue-specific inflammatory disease. J Exp Med 2007;204:1335–47.
[25] Tomura M, Honda T, Tanizaki H, Otsuka A, Egawa G, Tokura Y, et al. Activated regulatory T cells are the major T cell type emigrating from the skin during a cutaneous immune response in mice. J Clin Invest 2010;120:883–93.
[26] Dudda JC, Perdue N, Bachtanian E, Campbell DJ. Foxp3+ regulatory T cells maintain immune homeostasis in the skin. J Exp Med 2008;205:1559–65.
[27] Yurchenko E, Tritt M, Hay V, Shevach EM, Belkaid Y, Piccirillo CA. CCR5-dependent homing of naturally occurring CD4+ regulatory T cells to sites of Leishmania major infection favors pathogen persistence. J Exp Med 2006;203:2451–60.
[28] Loser K, Beissert S. Regulation of cutaneous immunity by the environment: an important role for UV irradiation and vitamin D. Int Immunopharmacol 2009;9:587–9.
[29] Loser K, Mehling A, Loeser S, Apelt J, Kuhn A, Grabbe S, et al. Epidermal RANKL controls regulatory T-cell numbers via activation of dendritic cells. Nat Med 2006;12:1372–9.
[30] Schwarz A, Noordegraaf M, Maeda A, Torii K, Clausen BE, Schwarz T. Langerhans cells are required for UVR-induced immunosuppression. J Invest Dermatol 2010;130:1419–27.
[31] Yoshiki R, Kabashima K, Sakabe J, Sugita K, Bito T, Nakamura M, et al. The mandatory role of IL-10-producing and OX40 ligand-expressing mature Langerhans cells in local UVB-induced immunosuppression. J Immunol 2010;184:5670–7.
[32] Yoshiki R, Kabashima K, Sugita K, Atarashi K, Shimauchi T, Tokura Y. IL-10-producing Langerhans cells and regulatory T cells are responsible for depressed contact hypersensitivity in grafted skin. J Invest Dermatol 2009;129:705–13.
[33] Stary G, Klein I, Bauer W, Koszik F, Reininger B, Kohlhofer S, et al. Glucocorticosteroids modify Langerhans cells to produce TGF-beta and expand regulatory T cells. J Immunol 2011;186:103–12.
[34] Schwarz A, Maeda A, Wild MK, Kernebeck K, Gross N, Aragane Y, et al. Ultraviolet radiation-induced regulatory T cells not only inhibit the induction but can suppress the effector phase of contact hypersensitivity. J Immunol 2004;172:1036–43.
[35] Schwarz A, Maeda A, Schwarz T. Alteration of the migratory behavior of UV-induced regulatory T cells by tissue-specific dendritic cells. J Immunol 2007;178:877–86.
[36] Schwarz A, Schwarz T. UVR-induced regulatory T cells switch antigen-presenting cells from a stimulatory to a regulatory phenotype. J Invest Dermatol 2010;130:1914–21.
[37] Wang L, Jameson SC, Hogquist KA. Epidermal Langerhans cells are not required for UV-induced immunosuppression. J Immunol 2009;183:5548–53.
[38] Guilliams M, Crozat K, Henri S, Tamoutounour S, Grenot P, Devilard E, et al. Skin-draining lymph nodes contain dermis-derived CD103– dendritic cells that constitutively produce retinoic acid and induce Foxp3+ regulatory T cells. Blood 2010;115:1958–68.
[39] Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. J Exp Med 2007;204:1775–85.
[40] Grabbe S, Schwarz T. Immunoregulatory mechanisms involved in elicitation of allergic contact hypersensitivity. Immunol Today 1998;19:37–44.
[41] Randolph GJ, Ochando J, Partida-Sanchez S. Migration of dendritic cell subsets and their precursors. Annu Rev Immunol 2008;26:293–316.
[42] Kabashima K, Shiraishi N, Sugita K, Mori T, Onoue A, Kobayashi M, et al. CXCL12–CXCR4 engagement is required for migration of cutaneous dendritic cells. Am J Pathol 2007;171:1249–57.
[43] Nagamachi M, Sakata D, Kabashima K, Furuyashiki T, Murata T, Segi-Nishida E, et al. Facilitation of Th1-mediated immune response by prostaglandin E receptor EP1. J Exp Med 2007;204:2865–74.
[44] Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, et al. Antigen-specific T. cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. Immunity 2002;17:375–87.
[45] McHale JF, Harari OA, Marshall D, Haskard DO. Vascular endothelial cell expression of ICAM-1 and VCAM-1 at the onset of eliciting contact hypersensitivity in mice: evidence for a dominant role of TNF-alpha. J Immunol 1999;162:1648–55.
[46] Tietz W, Allemand Y, Borges E, von Laer D, Hallmann R, Vestweber D, et al. CD4+ T cells migrate into inflamed skin only if they express ligands for E- and P-selectin. J Immunol 1998;161:963–70.
[47] Hirata T, Merrill-Skoloff G, Aab M, Yang J, Furie BC, Furie B. P-Selectin glycoprotein ligand 1 (PSGL-1) is a physiological ligand for E-selectin in mediating T helper 1 lymphocyte migration. J Exp Med 2000;192:1669–76.
[48] Mori T, Kabashima K, Yoshiki R, Sugita K, Shiraishi N, Onoue A, et al. Cutaneous hypersensitivities to hapten are controlled by IFN-gamma-upregulated keratinocyte Th1 chemokines and IFN-gamma-downregulated Langerhans cell Th2 chemokines. J Invest Dermatol 2008;128:1719–27.
[49] Ring S, Schafer SC, Mahnke K, Lehr HA, Enk AH. CD4+ CD25+ regulatory T cells suppress contact hypersensitivity reactions by blocking influx of effector T cells into inflamed tissue. Eur J Immunol 2006;36:2981–92.
[50] Ring S, Oliver SJ, Cronstein BN, Enk AH, Mahnke K. CD4+CD25+ regulatory T cells suppress contact hypersensitivity reactions through a CD39, adenosine-dependent mechanism. J Allergy Clin Immunol 2009;123:1287–96. e1282.
[51] Dubois B, Chapat L, Goubier A, Papiernik M, Nicolas JF, Kaiserlian D. Innate CD4+CD25+ regulatory T cells are required for oral tolerance and inhibition of CD8+ T cells mediating skin inflammation. Blood 2003;102:3295–301.
[52] Ring S, Karakhanova S, Johnson T, Enk AH, Mahnke K. Gap junctions between regulatory T cells and dendritic cells prevent sensitization of CD8(+) T cells. J Allergy Clin Immunol 2010;125:237–46. e231–e237.
[53] Ring S, Enk AH, Mahnke K. ATP activates regulatory T cells *in vivo* during contact hypersensitivity reactions. J Immunol 2010;184:3408–16.
[54] Schneider MA, Meingassner JG, Lipp M, Moore HD, Rot A. CCR7 is required for the in vivo function of CD4+ CD25+ regulatory T cells. J Exp Med 2007;204:735–45.
[55] Honda T, Otsuka A, Tanizaki H, Minegaki Y, Nagao K, Waldmann H, et al. Enhanced murine contact hypersensitivity by depletion of endogenous regulatory T cells in the sensitization phase. J Dermatol Sci 2011;61:144–7.
[56] Boguniewicz M, Leung DY. Recent insights into atopic dermatitis and implications for management of infectious complications. J Allergy Clin Immunol 2010;125:4–13. quiz 14–15.
[57] Koga C, Kabashima K, Shiraishi N, Kobayashi M, Tokura Y. Possible pathogenic role of Th17 cells for atopic dermatitis. J Invest Dermatol 2008;128:2625–30.
[58] Ou LS, Goleva E, Hall C, Leung DY. T regulatory cells in atopic dermatitis and subversion of their activity by superantigens. J Allergy Clin Immunol 2004;113:756–63.
[59] Hijnen D, Haeck I, van Kraats AA, Nijhuis E, de Bruin-Weller MS, Bruijnzeel-Koomen CA, et al. Cyclosporin A reduces CD4(+)CD25(+) regulatory T-cell numbers in patients with atopic dermatitis. J Allergy Clin Immunol 2009;124:856–8.
[60] Ito Y, Adachi Y, Makino T, Higashiyama H, Fuchizawa T, Shimizu T, et al. Expansion of FOXP3-positive CD4+CD25+ T cells associated with disease activity in atopic dermatitis. Ann Allergy Asthma Immunol 2009;103:160–5.
[61] Brandt C, Pavlovic V, Radbruch A, Worm M, Baumgrass R. Low-dose cyclosporine A therapy increases the regulatory T cell population in patients with atopic dermatitis. Allergy 2009;64:1588–96.
[62] Verhagen J, Akdis M, Traidl-Hoffmann C, Schmid-Grendelmeier P, Hijnen D, Knol EF, et al. Absence of T-regulatory cell expression and function in atopic dermatitis skin. J Allergy Clin Immunol 2006;117:176–83.
[63] Schnopp C, Rad R, Weidinger A, Weidinger S, Ring J, Eberlein B, et al. Fox-P3-positive regulatory T cells are present in the skin of generalized atopic eczema patients and are not particularly affected by medium-dose UVA1 therapy. Photodermatol Photoimmunol Photomed 2007;23:81–5.
[64] Caproni M, Torchia D, Antiga E, Volpi W, del Bianco E, Fabbri P. The effects of tacrolimus ointment on regulatory T lymphocytes in atopic dermatitis. J Clin Immunol 2006;26:370–5.
[65] Baumgrass R, Brandt C, Wegner F, Abdollahnia M, Worm M. Low-dose, but not high-dose, cyclosporin A promotes regulatory T-cell induction, expansion, or both. J Allergy Clin Immunol 2010;126:183–4. author reply 184.
[66] Reefer AJ, Satinover SM, Solga MD, Lannigan JA, Nguyen JT, Wilson BB, et al. Analysis of CD25hiCD4+ "regulatory" T-cell subtypes in atopic dermatitis reveals a novel T(H)2-like population. J Allergy Clin Immunol 2008;121:415–22. e413.
[67] Guttman-Yassky E, Nograles KE, Krueger JG. Contrasting pathogenesis of atopic dermatitis and psoriasis—Part I: clinical and pathologic concepts. J Allergy Clin Immunol 2011;127:1110–8.
[68] Zhang L, Yang XQ, Cheng J, Hui RS, Gao TW. Increased Th17 cells are accompanied by FoxP3(+) Treg cell accumulation and correlated with psoriasis disease severity. Clin Immunol 2010;135:108–17.
[69] Yun WJ, Lee DW, Chang SE, Yoon GS, Huh JR, Won CH, et al. Role of CD4CD25FOXP3 regulatory T cells in psoriasis. Ann Dermatol 2010;22:397–403.

[70] Fujimura T, Okuyama R, Ito Y, Aiba S. Profiles of Foxp3+ regulatory T cells in eczematous dermatitis, psoriasis vulgaris and mycosis fungoides. Br J Dermatol 2008;158:1256–63.
[71] Yan KX, Fang X, Han L, Zhang ZH, Kang KF, Zheng ZZ, et al. Foxp3+ regulatory T cells and related cytokines differentially expressed in plaque vs. guttate psoriasis vulgaris. Br J Dermatol 2010;163:48–56.
[72] Quaglino P, Ortoncelli M, Comessatti A, Ponti R, Novelli M, Bergallo M, et al. Circulating CD4+CD25 bright FOXP3+ T cells are up-regulated by biological therapies and correlate with the clinical response in psoriasis patients. Dermatology 2009;219:250–8.
[73] van der Aar AM, Sibiryak DS, Bakdash G, van Capel TM, van der Kleij HP, Opstelten DJ, et al. Vitamin D3 targets epidermal and dermal dendritic cells for induction of distinct regulatory T cells. J Allergy Clin Immunol 2011;127:1532–1540.e7.
[74] Sugiyama H, Gyulai R, Toichi E, Garaczi E, Shimada S, Stevens SR, et al. Dysfunctional blood and target tissue CD4+CD25high regulatory T cells in psoriasis: mechanism underlying unrestrained pathogenic effector T cell proliferation. J Immunol 2005;174:164–73.
[75] Goodman WA, Levine AD, Massari JV, Sugiyama H, McCormick TS, Cooper KD. IL-6 signaling in psoriasis prevents immune suppression by regulatory T cells. J Immunol 2009;183:3170–6.
[76] Pasare C, Medzhitov R. Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. Science 2003;299:1033–6.
[77] Goodman WA, Young AB, McCormick TS, Cooper KD, Levine AD. Stat3 phosphorylation mediates resistance of primary human T cells to regulatory T cell suppression. J Immunol 2011;186:3336–45.



Dr. Tetsuya Honda graduated from Kyoto University and received his MD degree in 2000. After three years of clinical training as a dermatologist, he entered graduate school of medicine at Kyoto University and received his PhD in 2007. There, he studied the role of lipid mediators in autoimmunity and allergy (Department of Pharmacology, Dr. Shuh Narumiya). After the graduation, he worked as an assistant professor in Department of Dermatology at Kyoto University, and studied the regulatory T cells and dendritic cells in skin, under the supervision of Dr. Yoshiki Miyachi and Dr. Kenji Kabashima. Since 2010, he has been working as a post doctoral fellow in Lymphocyte Biology Section, Laboratory of Immunology, National Institute of Health (Dr. Ronald N. Germain), and studying the immune systems using live imaging technique.

Original Paper

Dermatology

Dermatology 2011;223:174–181
DOI: 10.1159/000332847

Received: May 6, 2011
Accepted after revision: September 3, 2011
Published online: October 21, 2011

Acne Management in Japan: Study of Patient Adherence

Yoshiki Miyachi[a] Nobukazu Hayashi[b] Fukumi Furukawa[e] Hirohiko Akamatsu[f]
Kayoko Matsunaga[g] Shinichi Watanabe[d] Makoto Kawashima[c]

[a]Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto; [b]Department of Dermatology, Toranomon Hospital, [c]Department of Dermatology, Tokyo Women's Medical University, and [d]Department of Dermatology, Teikyo University School of Medicine, Tokyo; [e]Department of Dermatology, Wakayama Medical University, Wakayama; Departments of [f]Applied Cell and Regenerative Medicine and [g]Dermatology, Fujita Health University School of Medicine, Toyoake, Japan

Key Words
Acne · Adherence · Japan · Risk factors

Abstract
Obtaining good adherence to acne therapy is a challenge for all dermatologists. We studied 428 acne patients in Japan to determine the likelihood of good adherence and factors associated with medication-taking. This study utilized a simple validated questionnaire to assess risk of poor adherence; information about patient and treatment characteristics was also collected. There was an overall rate of poor adherence in 76% of subjects. Adherence to topical medication was poor in 52% of those treated with a topical agent only (n = 123). Among those taking combination therapies (n = 275), adherence to the topical portion of therapy was poor in 49% of subjects. The likelihood of poor adherence to oral medication was higher, both when administered alone (n = 30, 93% poor adherence) and when given as part of a combination regimen (n = 275, 86%). Factors with an impact on adherence included satisfaction with treatment (p = 0.023) and the experience of side effects (p = 0.027). Patients who felt they had a good understanding of acne and its treatment were more likely to have good adherence. These data suggest that there is significant room for improvement in acne adherence in Japan, as in other areas of the world, and that improved education may enhance adherence.

Introduction

Medication adherence is very important to the success of acne management [1]; however, many factors conspire against this. Acne improvements often occur relatively slowly and it is typically a long-lasting disease that requires prolonged treatment. It is a condition that commonly affects adolescents, who may quickly become frustrated with treatment and have difficulty fitting treatment regimens into their daily routine. Costs of acne therapy and side effects can also negatively impact adherence to acne medications. Failure of over-the-counter products can lead patients to incorrectly believe that effective treatment is not possible. Yet acne is known to be associated with negative psychological effects and it can and should be treated. Thus, clinicians face the challenge of understanding acne adherence and continually working to devise strategies to improve it.

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–8665/11/2232–0174$38.00/0

Accessible online at:
www.karger.com/drm

Yoshiki Miyachi, MD, PhD
Department of Dermatology, Kyoto University Graduate School of Medicine
54 Kawahara-cho, Shogoin, Sakyo-ku
Kyoto 606-8507 (Japan)
Tel. +81 75 751 3310, E-Mail ymiyachi@kuhp.kyoto-u.ac.jp

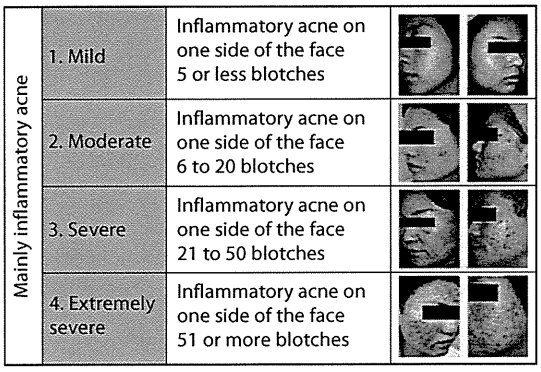


Fig. 1. Criteria to determine the severity of acne vulgaris. Reproduced with permission from [11].

Some studies have evaluated adherence to acne medications in various parts of the world [2–8]; however, there are few data specific to Japan. This study was designed to assess adherence among acne patients in Japan, to determine if there are differences in adherence based on type of treatment (topical or oral), and to evaluate the factors that can affect adherence to acne treatment.

Acne experts and guidelines emphasize that most patients with acne should be managed with a topical retinoid, often in combination with an antimicrobial agent [9, 10]. Expert recommendations stress the importance of topical retinoids in acne therapy [10]; adapalene recently became the first topical retinoid introduced into the market in Japan. Because acne therapy is rapidly evolving in Japan, we considered the timing good for a study of medication adherence. This information may help to guide physicians in treating their patients and, ultimately, to improve patient outcomes and satisfaction.

Methods

This multicenter observational study utilized self-completed and dermatologist-completed questionnaires to assess medication adherence among individuals visiting 59 dermatology clinics throughout Japan between January and March 2010. The dermatology clinics were members of a panel established by the healthcare marketing research agency conducting the study. A secondary objective was to determine factors that had an impact on acne treatment.

Subjects included acne patients undergoing treatment who had a medical consultation for acne ≥1 month to ≤3 months prior to the study consultation. Consecutive patients meeting these criteria had the study explained to them, and were offered the opportunity to participate if they completed an informed consent form. All patients had acne therapy prescriptions, which could have been initiated, changed, or re-instituted at the previous visit. Tolerability of treatment was assessed by the investigating dermatologist and a question on the patient-completed questionnaire. Acne severity at the beginning of the study period was assessed using the Severity Criteria of Acne Vulgaris rating used by the Japanese Dermatological Association [11] (fig. 1).

The likelihood of adherence was assessed using the ECOB Adherence Questionnaire created and validated by Pawin et al. [3]. ECOB assesses the patient's ability to remember 4 key aspects of acne therapy with similar but separate questions for topical and oral therapy; inability to name/describe treatment or 1 response suggesting the patient had not used treatment as directed indicates a likelihood of poor adherence. The ECOB tool was validated against dermatologist prescriptions by Pawin et al. [3]. Quality of life was assessed by the Japanese version of the Dermatology Life Quality Index (DLQI) [12].

Questionnaires were tabulated and analyzed using simple descriptive statistics. Also, the relationship between the objective variable (ECOB adherence assessment) and explanatory variables was examined by the χ^2 test. Explanatory variables included age, age at onset of acne, parents with experience of acne, DLQI overall score (degree of impact on life), consultation with other physicians prior to treatment at current facility, knowledge about acne, degree of satisfaction with current treatment, prescription of acne medications (stratified by which type of medication), period of treatment, experience of side effects, change in acne severity (comparison of severity at consultation prior to the current visit and the current visit), guidance other than drug therapy (such as private preparations and cosmetics), acne scarring seen at the current consultation, and improvement in acne at current consultation in the investigator's opinion. The odds ratio and 95% CI for each explanatory variable was calculated by multiple logistic regression analysis and the results were examined by Wald χ^2 test. The level of statistical significance is 5%.

Results

Study Population

A total of 59 dermatologists participated and recruited 428 acne patients (64% female, 36% male). The mean age of the patients was 24.4 years and females were older than males (mean 25.5 vs. 22.2 years, respectively). Among females, 49% were aged 25 years or older; in contrast, 25% of males were 26 years or older. Most males were aged 16–20 years (39%). A total of 81% of subjects reported not smoking and 62% indicated they consumed alcohol never or rarely. A summary of acne characteristics is shown in tables 1 and 2. Almost all patients (97%) had acne on the face, 11% on the neck, 8% on the chest, 5% on the back and 1% on another body location. Involvement on non-facial areas was more likely with increasing acne severity.

Notably, 42% of subjects had acne scarring. Scars were more likely with increasing disease severity, and were

Table 1. Acne characteristics of the study population (n = 428)

	Overall (n = 428)	Male (n = 153)	Female (n = 275)
Age at onset of acne (mean), years	14.9	15.2	14.8
Age at first acne consultation (mean), years	19.3	18.3	19.9
Parents with acne, %	41	36	44
Acne severity, %			
Mild	20.3 (n = 87)	15	23
Moderate	55.1 (n = 236)	58	54
Severe	20.8 (n = 89)	22	20

Table 2. Chronic vs. relapsing acne in the subgroup aged 25 or older (n = 189)

	Overall (n = 189)	Male (n = 45)	Female (n = 144)
Had no period of time without acne since onset, %	40	47	38
Had a period of time without acne since onset, %	58	51	60

present in 24% of those with mild acne, 42% of those with moderate acne, and 63% of those with severe acne.

Most patients (75%) had not consulted a medical professional for acne prior to the study period. Among those who had consulted a medical professional (n = 109), the length of time between that consultation and the dermatologist consultation was estimated to be greater than 1 year in 38% of cases.

Medications Used for Acne

Information was collected about treatment during the relevant period (at least 1 month and within 3 months after prior consultation for acne). The majority of subjects were prescribed a combination of topical and oral medications (n = 275, 64%), 29% (n = 123) had topical only and 7% (n = 30) had oral only. The mean duration of current treatment was 8.0 weeks. A large majority of subjects (83% topical and 81% oral) had some out-of-pocket medical costs for acne medications and a minority (16% topical and 13% oral) had no out-of-pocket costs.

Topical acne medications were prescribed for 93% (398/428) of the subjects. Topical retinoid therapy was prescribed in 47% of cases, with a higher likelihood of use in males (54 vs. 44%, respectively). Topical antibacterial agents were prescribed in 81% of cases, with a lower likelihood of use in males (75 vs. 84%). The use of topical retinoids increased with increasing severity of acne (from 40% in those with mild acne to 52% in those with severe acne).

Oral medications were prescribed for 71.3% (305/428) of subjects, with oral antibacterial agents being the most frequent systemic medication (79%) followed by vitamins (47%), traditional Chinese medicine (14%), and 'other' (10%). Males were more likely to be treated with an oral antibacterial than females (85 vs. 76%, respectively). The frequency of oral antibacterial use increased with increasing acne severity (from 65% in those with mild acne to 84% in those with severe acne).

In general, subjects indicated that acne medications were well tolerated, with no particular side effects reported among 77% of those using topical medications and 97% of those using oral medications. Procedures were used very infrequently, with the most common being physical removal of comedo (8%) and chemical exfoliation, light therapy, other cosmetics, laser therapy, prescription of private preparation, or other were used in <4% of the group.

Change in Acne

The severity of acne at the study consultation was compared to the severity at beginning of treatment. Overall, acne improved in 46% of patients, did not change in 50%, worsened in 1%, and was unknown in 4%. Improvement was more likely with increasing acne severity (11%

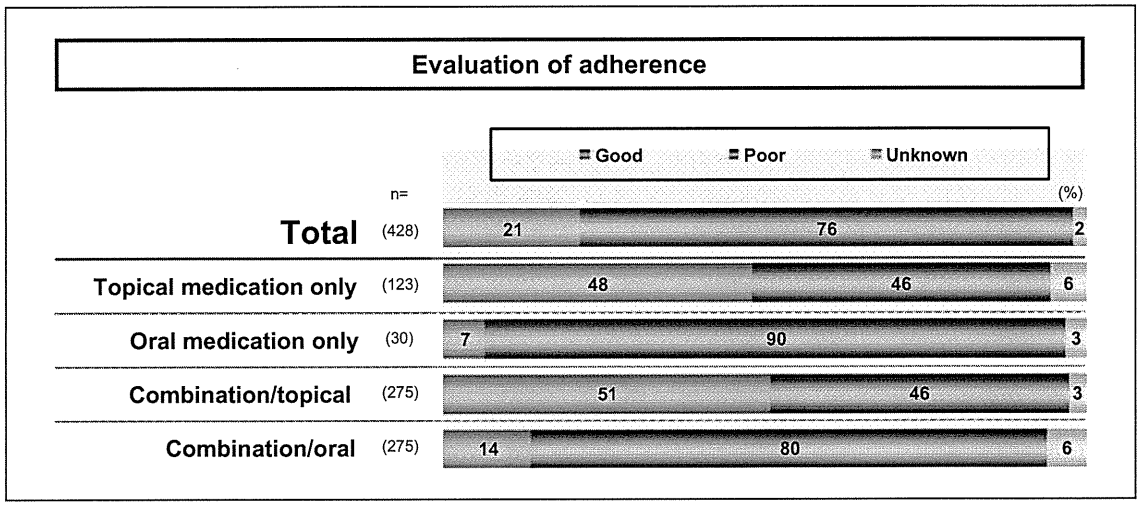


Fig. 2. Overview of adherence. Combination/topical = Use of a topical medication in any combination regimen; combination/oral = use of an oral medication in any combination regimen.

in those with mild acne, 48% in those with moderate acne, and 78% with severe acne). Additionally, patients who were treated with a combination of topical and oral medications were more likely to improve compared to those who were treated with either topical medication only or oral medication only (56 vs. 26 and 33%, respectively).

Change in Treatment at Study Visit

Treatment was changed in 17% of the cases, and the rate at which treatment was changed was similar regardless of acne severity. A total of 21% of subjects treated with oral and topical medications had a change in treatment, as did 10% of those treated with topical medications only and 3% of those treated with oral medications only. Usually, the change was to add or switch medication. The reasons given for changing therapy were insufficient efficacy (28%), an expectation of improvement in efficacy with change (24%), poor tolerability (10%), poor adherence (6%), and other (23%).

Dermatology Life Quality Index Scores

The acne sufferers in this study had a mean score on the DLQI of 3.5, indicating acne had a small impact on life. Mean scores were higher in females (3.7 vs. 3.1 in males) and those with more severe disease (4.3 for those with severe acne, 3.6 with moderate acne, and 2.4 with mild acne). Bothersome symptoms (rated as 'fairly' or 'very' bothersome) reported by subjects included itching/pain (11%) and embarrassment (23%).

Adherence Rates

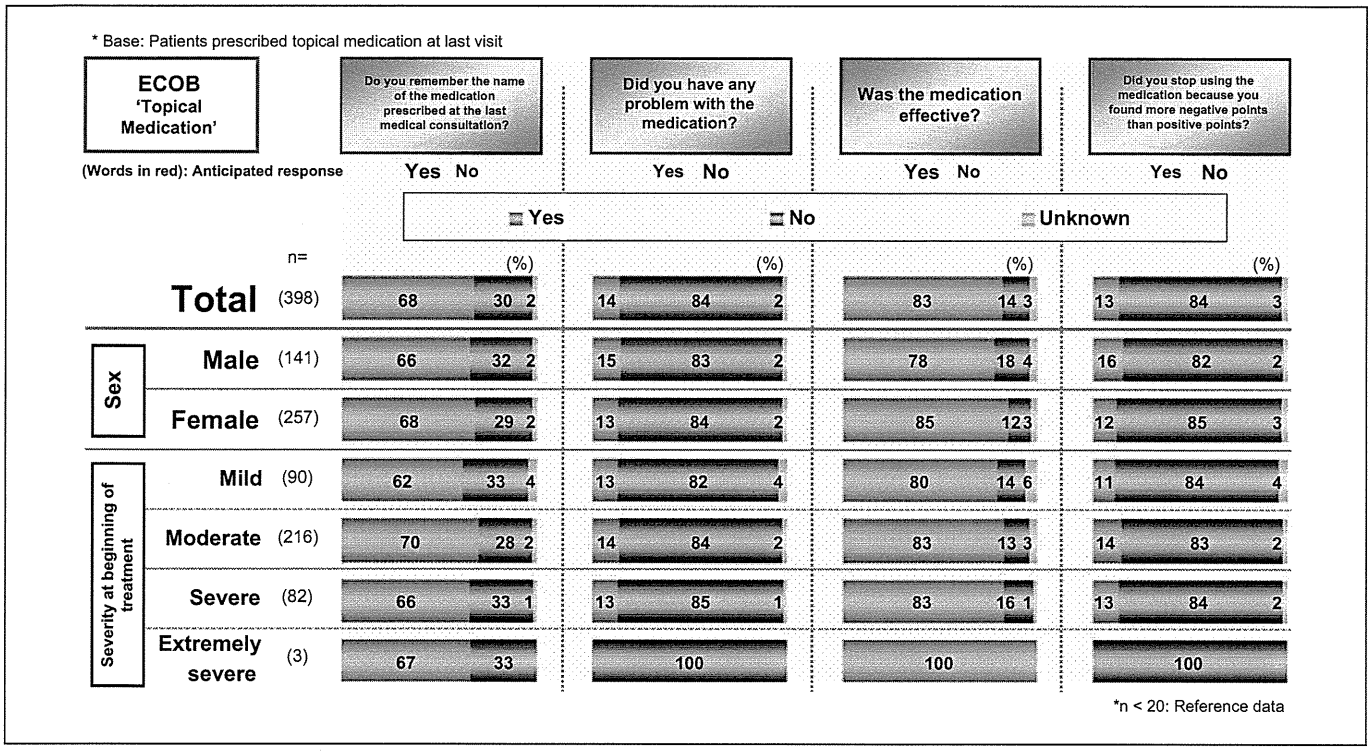
Figure 2 gives an overview of adherence in this study; the ECOB questionnaire is in two parts, one which assesses topical therapy and the other oral therapy. There was an overall rate of potentially poor adherence in 76% of subjects. Adherence to topical medication was judged likely to be good in 48% of those treated with a topical agent only (n = 123). Among those taking combination therapies (n = 275), adherence to the topical portion of therapy was good in 51% of subjects. The likelihood of good adherence to oral medication was lower, both when it was administered alone (n = 30, 7% good adherence) and when given as part of a combination regimen (n = 275, 14%). Figure 3 shows the responses to individual questions on the ECOB questionnaire.

Factors That Impacted Adherence

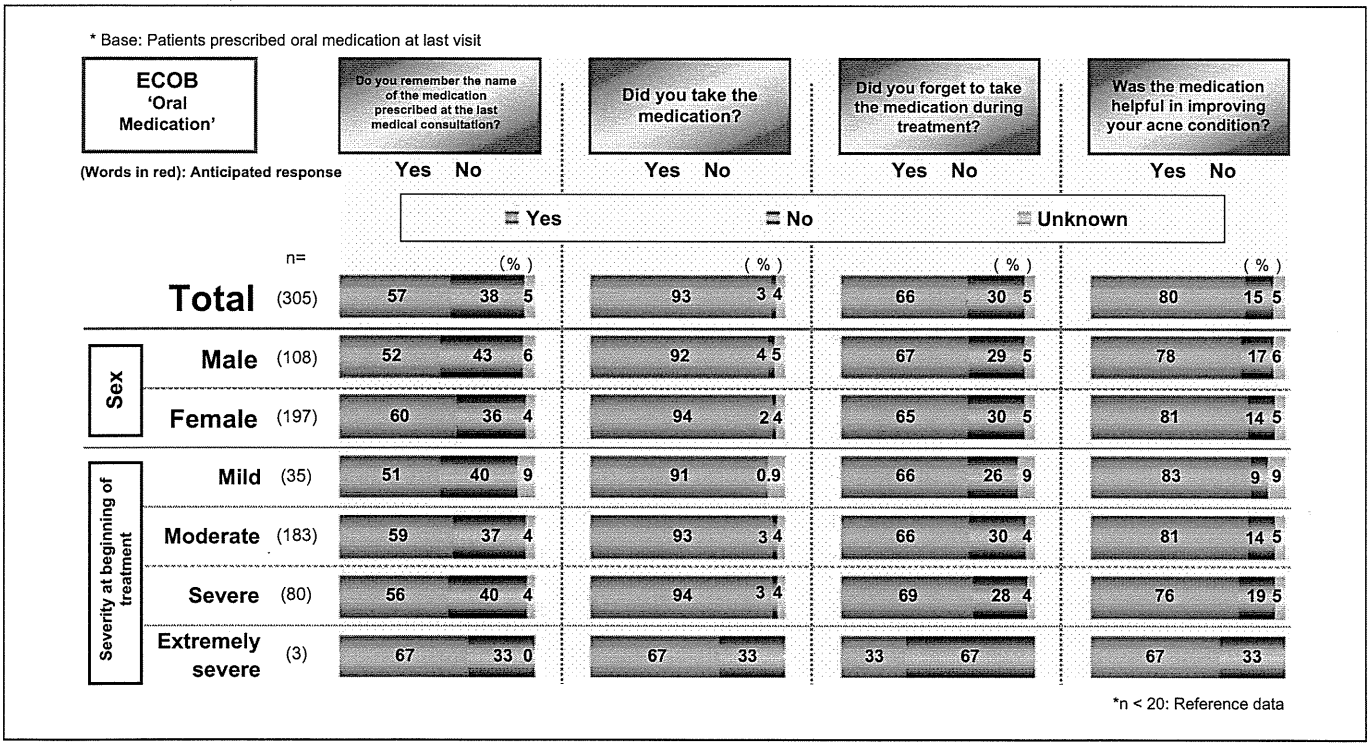
Factors associated with poor adherence included lack of satisfaction with treatment (OR 3.59) and to a lesser degree DLQI score of 6–10 (OR = 1.89), use of an over-the-counter topical antibacterial (OR = 1.75), and experience of a side effect (OR = 1.71).

Characteristics of Acne Consultation and Educational Efforts

The study investigators estimated their average length of consultation for acne as 9.6 min for a first visit and 4.7 min for a follow-up visit. Somewhat less than half (44%) of investigators indicated they distributed written materials about inflammatory acne and its treatment, 32% said

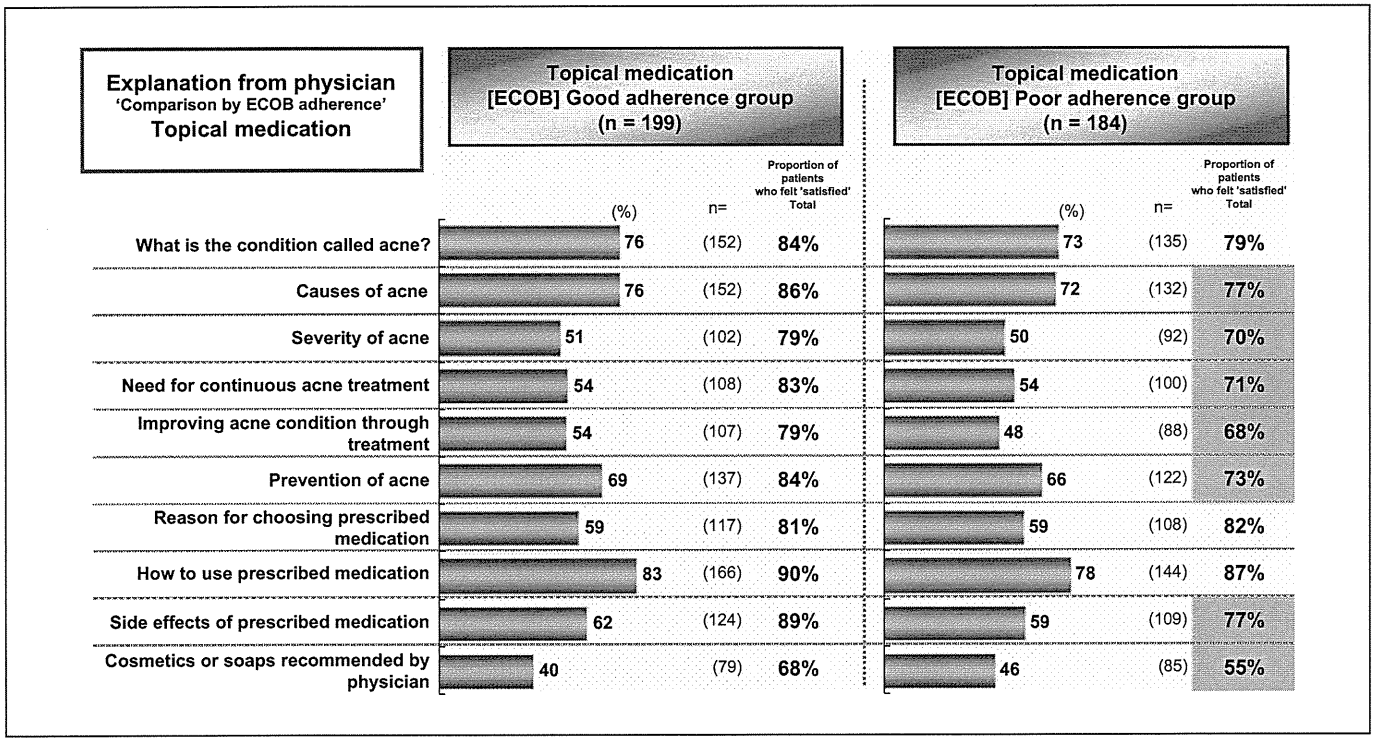


a

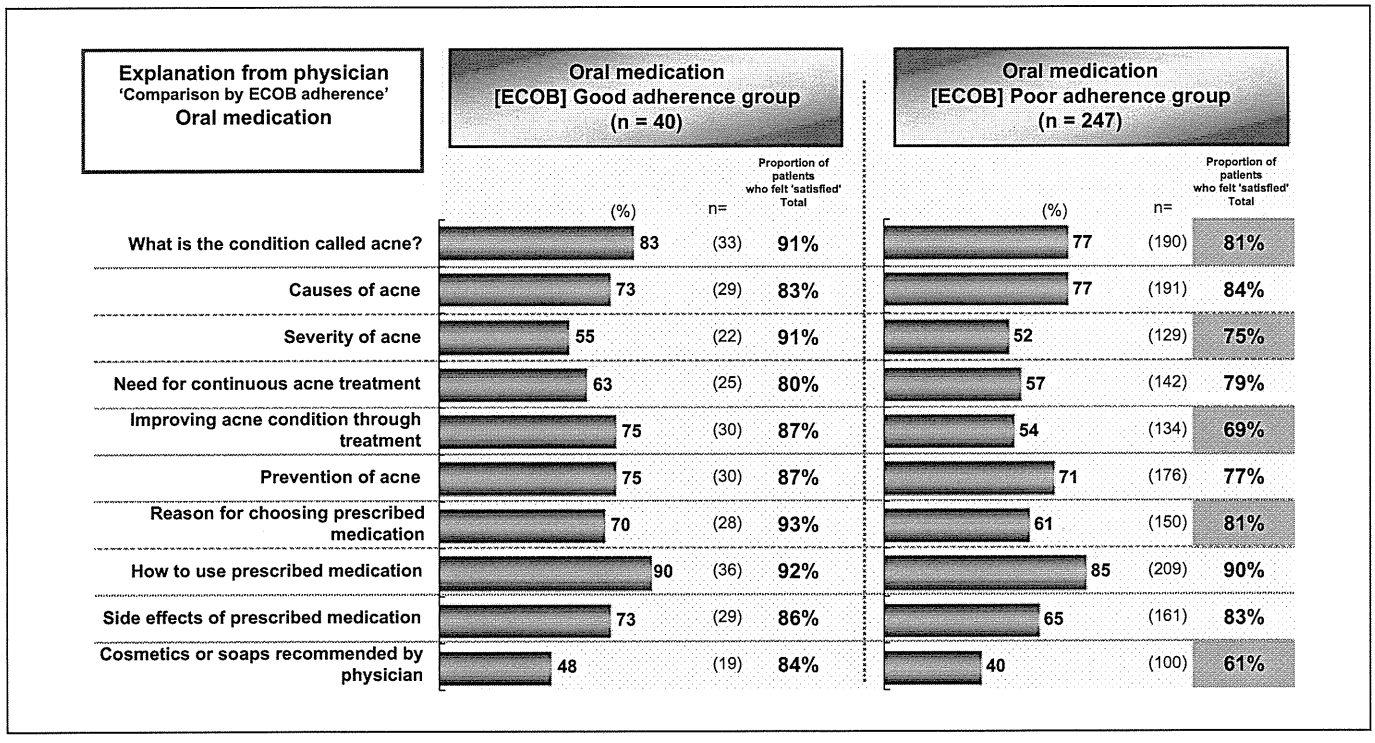


b

Fig. 3. Responses to individual questions on ECOB questionnaire. **a** Topical medication. **b** Oral medication.



a



b

Fig. 4. Relationship of good adherence to knowledge. **a** Topical medication. **b** Oral medication. Concerning the total proportion of patients who felt 'satisfied', highlighted numbers indicate proportions of the poor adherence group that were at least 10 points lower compared to those of the good adherence group.

they did not give materials, and 24% said they did some of the time. Investigators were asked what guidance they provided to acne patients; the most frequent response was lifestyle guidance (70%) followed by recommendations for hypoallergenic soap/cleansing products (14%), and recommendations or prescription for a moisturizing agent (10%).

When asked about their perception of treatment need, 83% of subjects said they were satisfied with their dermatologists' explanation of acne therapy and 73% indicated that the conversation with their physician had a positive impact on their motivation to use acne treatments. Further, subjects with good adherence were more likely to report that physician interactions had an impact on their behavior compared with those who had poor adherence (87 vs. 77%, respectively felt they had enough explanation; 77 vs. 70% felt the physician motivated them, 93 vs. 82% reported they were using medication according to instructions, and 92 vs. 90% indicated willingness to following treatment instructions). Patients who were dissatisfied with the dermatologists' explanations were most likely to report being unsure of why acne needed continuous treatment, what to expect in acne improvement during treatment, and how to prevent acne lesions.

Understanding of Acne and What to Expect from Treatment

Subjects were asked about knowledge of acne and its treatment; 50% felt they knew nothing or a little about acne and 62% knew little about its treatment. Women were more likely to report knowing 'something' or 'a lot' about acne compared with men (51 vs. 42%, respectively) as were those with severe acne compared with milder acne (54% severe, 50% moderate, and 46% mild acne). Women also felt more knowledgeable about acne treatment (42% knew something or a lot vs. 32% of men). Subjects who felt they had poor knowledge were more likely to be poorly adherent (fig. 4).

While many subjects reported being satisfied or very satisfied, 23% of subjects treated with topical medications and 24% of those treated with oral medications were only somewhat satisfied or not satisfied with therapy. Males were less likely than females to be satisfied with topical treatment (27 vs. 20% low satisfaction, respectively).

Discussion

This group had some characteristics that differed from other populations in adherence studies. First, our group had a relatively high mean age (24.4 years) and 21% were classified as having severe acne, which is interesting given the perception that Japanese have primarily mild acne; 42% had acne scarring. In an international study that included 1,191 Asian patients (from Hong Kong, India, the Philippines, and Singapore) with acne, the mean age was 23.1 ± 6.4 years and 3.9% of patients had severe acne, while 62% had scarring (pers. commun.) [4]. Kubota et al. [13] studied 1,443 Japanese adolescents, and reported that 59.5% had acne; of these, 52.9% said their acne had been present for at least 1 year but did not report the rate of scarring or severe acne. However, severe acne was present in 17% of subjects in a recent study of acne treatment in Japan by Kubota et al. [14]. Our study also included a relatively high proportion of adults (40%) who reported suffering from continuous acne since its onset.

Females consulted physician for acne at mean age of 19.9 vs. 18.3 years for males. It is interesting that females consulted physicians later than males, because in both our study and the epidemiological study of Kubota et al. [13], there were more females than males with acne. Most of our subjects had insurance with a co-pay (83%) or no out-of pocket cost (16%), suggesting that financial considerations may not have been the reason for a delay in seeking treatment and may or may not have impacted adherence. Patients had been treated on average for 8 weeks prior to study visit; in our study 17% had a change in therapy, usually addition of another acne treatment.

There was an overall rate of potentially poor adherence in 76% of subjects. Adherence to topical medication was judged likely to be good in 48% of those treated with a topical agent only (n = 123). Among those taking combination therapies (n = 275), adherence to the topical portion of therapy was good in 51% of subjects. The likelihood of good adherence to oral medication was lower, both when it was administered alone (n = 30, 7% good adherence) and when given as part of a combination regimen (n = 275, 14%). The high rate of non-adherence with oral medications is somewhat surprising. In an international study using the same tool to assess adherence (n = 3,339), Dreno et al. [4] reported an overall rate of poor adherence of 50% worldwide, but a rate of 48% in Asian patients (n = 1,191). Dreno et al. [4] also found a risk of poor adherence of 60% among patients taking a combination of both systemic and topical therapy, and a higher likelihood of poor adherence to the systemic arm of treatment compared to the topical arm (54 vs. 44%, respectively). Other studies utilizing questionnaires and/or single questions to assess adherence have found rates of poor adherence ranging from 76 to 0% [2, 3, 5, 15–17]. Studies using objective measures such as not keeping appoint-

ments, pill counts, weighing medication, number of medication refills, and electronic caps have reported adherence rates ranging from 28 to 88.3% [6–8, 18]. Clearly, more study is needed to fully understand adherence rates; however, it seems reasonable for clinicians to expect that approximately half of their patients may fail to follow prescribing instructions.

Topical retinoids were used by 47% of patients. Retinoids are new in Japan and strategies to optimize their use are evolving. In 2011, Kubota et al. [14] reported excellent success with initial treatment with clindamycin phosphate 1% gel twice daily plus adapalene 0.1% gel once daily, both in resolving acne lesions and in tolerability. Further, they found that maintenance therapy involving adapalene 0.1% gel once daily or twice weekly maintained improvements and allowed continued control of acne [14].

We found that satisfaction with treatment and side effects had an impact on adherence with acne therapy. Other studies have found that these factors impact treatment adherence as well, and also quality of life, older age, female gender, and employment [1]. Yentzer et al. [19] reported better adherence with once-daily combination products. We feel strongly that improving education will also enhance adherence.

Conclusions

There is a very high rate of non-adherence among acne patients. This probably contributes to poor outcomes in at least some cases. Selecting medications that are well tolerated and have a simple dosing regimen is likely to optimize adherence and, in turn, clinical outcomes.

Acknowledgments

Supported by an unrestricted educational grant from Galderma.

References

▶1 Lott R, Taylor SL, O'Neill JL, Krowchuk DP, Feldman SR: Medication adherence among acne patients: A review. J Cosmet Dermatol 2010;9:160–166.

▶2 Tan JK, Balagurusamy M, Fung K, Gupta AK, Thomas DR, Sapra S, Lynde C, Poulin Y, Gulliver W, Sebaldt RJ: Effect of quality of life impact and clinical severity on adherence to topical acne treatment. J Cutan Med Surg 2009;13:204–208.

▶3 Pawin H, Beylot C, Chivot M, Faure M, Poli F, Revuz J, Dreno B: Creation of a tool to assess adherence to treatments for acne. Dermatology 2009;218:26–32.

▶4 Dreno B, Thiboutot D, Gollnick H, Finlay AY, Layton A, et al: Large-scale worldwide observational study of adherence with acne therapy. Int J Dermatol 2010;49:448–456.

▶5 Jones-Caballero M, Pedrosa E, Penas PF: Self-reported adherence to treatment and quality of life in mild to moderate acne. Dermatology 2008;217:309–314.

▶6 McEvoy B, Nydegger R, Williams G: Factors related to patient compliance in the treatment of acne vulgaris. Int J Dermatol 2003; 42:274–280.

▶7 Zaghloul SS, Cunliffe WJ, Goodfield MJ: Objective assessment of compliance with treatments in acne. Br J Dermatol 2005;152: 1015–1021.

▶8 Yentzer BA, Alikhan A, Teuschler H, Williams LL, Tusa M, Fleischer AB Jr, Kaur M, Balkrishnan R, Feldman SR: An exploratory study of adherence to topical benzoyl peroxide in patients with acne vulgaris. J Am Acad Dermatol 2009;60:879–880.

▶9 Gollnick H, Cunliffe W, Berson D, Dreno B, Finlay A, Leyden JJ, Shalita AR, Thiboutot D: Management of acne: a report from a global alliance to improve outcomes in acne. J Am Acad Dermatol 2003;49:S1–S37.

▶10 Thiboutot D: New insights in the management of acne: an update from the global alliance to improve outcomes in acne group. J Am Acad Dermatol 2009;60:S1–S50.

▶11 Hayashi N, Akamatsu H, Kawashima M, Acne Study Group: Establishment of grading criteria for acne severity. J Dermatol 2008; 35:255–260.

▶12 Takahashi N, Suzukamo Y, Nakamura M, Miyachi Y, Green J, Ohya Y, Finlay AY, Fukuhara S: Japanese version of the dermatology life quality index: validity and reliability in patients with acne. Health Qual Life Outcomes 2006;4:46.

▶13 Kubota Y, Shirahige Y, Nakai K, Katsuura J, Moriue T, Yoneda K: Community-based epidemiological study of psychosocial effects of acne in Japanese adolescents. J Dermatol 2010;37:617–622.

▶14 Kubota Y, Munehiro A, Shirahige Y, Nakai K, Katsuura J, Moriue T, Murakami Y, Matsunaka H, Yoneda K: Effect of sequential application of topical adapalene and clindamycin phosphate in the treatment of Japanese patients with acne vulgaris. J Dermatolog Treat 2011, E-pub ahead of print.

▶15 Eichenfield LF, Nighland M, Rossi AB, Cook-Bolden F, Grimes P, Fried R, Levy S: Phase 4 study to assess tretinoin pump for the treatment of facial acne. J Drugs Dermatol 2008;7:1129–1136.

▶16 Rapp DA, Brenes GA, Feldman SR, Fleischer AB, Jr., Graham GF, Dailey M, Rapp SR: Anger and acne: implications for quality of life, patient satisfaction and clinical care. Br J Dermatol 2004;151:183–189.

▶17 Marazzi P, Boorman GC, Donald AE, Davies HD: Clinical evaluation of double strength isotrexin versus benzamycin in the topical treatment of mild to moderate acne vulgaris. J Dermatolog Treat 2002;13:111–117.

▶18 Cook-Bolden F: Subject preferences for acne treatments containing adapalene gel 0.1%: results of the MORE trial. Cutis 2006;78:26–33.

▶19 Yentzer BA, Hick J, Reese EL, Uhas A, Feldman SR, Balkrishnan R: Acne vulgaris in the United States: a descriptive epidemiology. Cutis 2010;86:94–99.

PGD_2 induces eotaxin-3 via PPARγ from sebocytes: A possible pathogenesis of eosinophilic pustular folliculitis

Kyoko Nakahigashi, MD,[a] Hiromi Doi, MS,[a] Atsushi Otsuka, MD, PhD,[a] Tetsuya Hirabayashi, PhD,[b] Makoto Murakami, PhD,[b] Yoshihiro Urade, PhD,[c] Hideaki Tanizaki, MD, PhD,[a] Gyohei Egawa, MD, PhD,[a] Yoshiki Miyachi, MD, PhD,[a] and Kenji Kabashima, MD, PhD[a] *Kyoto, Tokyo, and Osaka, Japan*

Background: Eosinophilic pustular folliculitis (EPF) is a chronic intractable pruritic dermatosis characterized by massive eosinophil infiltrates involving the pilosebaceous units. Recently, EPF has been regarded as an important clinical marker of HIV infection, and its prevalence is increasing in number. The precise mechanism by which eosinophils infiltrate into the pilosebaceous units remains largely unknown. Given that indomethacin, a COX inhibitor, can be successfully used to treat patients with EPF, we can assume that COX metabolites such as prostaglandins (PGs) are involved in the etiology of EPF.
Objective: To determine the involvement of PGs in the pathogenesis of EPF.
Methods: We performed immunostaining for PG synthases in EPF skin lesions. We examined the effect of PGD_2 on induction of eotaxin, a chemoattractant for eosinophils, in human keratinocytes, fibroblasts, and sebocytes and sought to identify its responsible receptor.
Results: Hematopoietic PGD synthase was detected mainly in infiltrating inflammatory cells in EPF lesions, implying that PGD_2 was produced in the lesions. In addition, PGD_2 and its immediate metabolite 15-deoxy-Δ 12,14-PGJ_2 (15d-PGJ_2) induced sebocytes to produce eotaxin-3 via peroxisome proliferator–activated receptor gamma. Consistent with the above findings, eotaxin-3 expression was immunohistochemically intensified in sebaceous glands of the EPF lesions.
Conclusion: The PGD_2/PGJ_2-peroxisome proliferator–activated receptor gamma pathway induces eotaxin production from sebocytes, which may explain the massive eosinophil infiltrates observed around pilosebaceous units in EPF. (J Allergy Clin Immunol 2012;129:536-43.)

Key words: Prostaglandin D_2, hematopoietic prostaglandin D synthase, eotaxin-3/CCL26, sebocyte, peroxisome proliferator–activated receptor gamma

Abbreviations used
CRT_H2: Chemoattractant receptor–homologous molecule expressed on T_H2 cells
EPF: Eosinophilic pustular folliculitis
GAPDH: Glyceraldehyde 3-phospate dehydrogenase
H-PGDS: Hematopoietic prostaglandin D synthase
L-PGDS: Lipocalin-type prostaglandin D synthase
PG: Prostaglandin
PPARγ: Peroxisome proliferator–activated receptor gamma
siRNA: Small-interfering RNA

Eosinophilic pustular folliculitis (EPF) is a chronic intractable pruritic dermatosis characterized by massive eosinophil infiltrates involving the pilosebaceous units.[1] The evidence accumulated to date indicates that T_H2-mediated immunologic mechanisms are involved in the pathogenesis of EPF.[2,3] Recently, EPF has been regarded as an important clinical marker of HIV infection, and its prevalence is increasing in number.[4] An immunohistochemical study has demonstrated the expression of intercellular adhesion molecules for inflammatory cells including eosinophils around hair follicles.[5] Other studies have reported that IL-5 level, which induces proliferation and differentiation of eosinophils, is elevated in the blood and skin lesions of patients with EPF, but it can be decreased by treatment with IFN-γ.[6,7] Three members of the eotaxin family—eotaxin-1/CCL11, eotaxin-2/CCL24, and eotaxin-3/CCL26—are known to promote the growth and recruitment of eosinophils and skin inflammation.[8] T_H2 cytokines, such as IL-4, -5, and -13, enhance the production of eotaxins by skin component cells, such as lymphocytes, macrophages, endothelial cells, fibroblasts, and keratinocytes.[9-11] These findings suggest that the pathogenesis of EPF consists of a T_H2-type immune response; intriguingly, however, EPF is usually resistant to topical or systemic corticosteroids that suppress the functions of T cells. Therefore, the pathogenesis of EPF might not be explained solely by T_H2 immunity. Since EPF can be successfully treated with indomethacin, a COX inhibitor,[12] we hypothesize that the prostaglandin (PG) family known as the prostanoids, which occur downstream of COX, might be involved in the etiology of EPF.

Prostanoids are released from cells immediately after their formation. Because they are chemically and metabolically unstable, they usually function only locally through membrane receptors on target cells.[13] Recently, individual prostanoid receptor gene–deficient mice have been used as models to dissect the respective roles of each receptor in combination with the use of compounds that selectively bind to prostanoid receptors as agonists or antagonists.[14,15] The prostanoids PGD_2 and PGE_2 are 2 of the major COX metabolites in the skin. PGE_2 has been reported to have an inhibitory effect on eosinophil trafficking and

From [a]the Department of Dermatology, Graduate School of Medicine, Kyoto University, Kyoto; [b]the Lipid Metabolism Project, Tokyo Metropolitan Institute of Medical Science, Tokyo; and [c]the Department of Molecular Behavioural Biology, Osaka Bioscience Institute, Osaka.

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, and Health and from the Ministry of Health, Labour and Welfare of Japan.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication August 5, 2011; revised October 21, 2011; accepted for publication November 23, 2011.

Available online December 28, 2011.

Corresponding author: Kenji Kabashima, MD, PhD, Department of Dermatology, Graduate School of Medicine, Kyoto University, 54 Shogoin-Kawara, Sakyo, Kyoto 606-8507, Japan. E-mail: kaba@kuhp.kyoto-u.ac.jp.

0091-6749/$36.00

doi:10.1016/j.jaci.2011.11.034

activation.[16] PGD_2, on the other hand, is known to be involved in chronic allergic inflammation.[17] Two types of PGD synthase (PGDS), which catalyzes the isomerization of PGH_2, a common precursor of various prostanoids that catalyze PGD_2, have been identified: one is the lipocalin-type PGDS (L-PGDS), and the other is the hematopoietic PGDS (H-PGDS).[18] L-PGDS is localized in the central nervous system, the male genital organs, the heart, and melanocytes in skin.[18,19] H-PGDS is widely distributed in the peripheral tissues and localized in antigen-presenting cells, mast cells, megakaryocytes, T_H2 lymphocytes, and dendritic cells.[18,20-22]

The aim of this study was to verify the hypothesis that prostanoids are involved in the development of eosinophil infiltration in the pilosebaceous units of the EPF skin lesions. We found that inflammatory cells in EPF lesions were positively immunostained for H-PGDS, suggesting that PGD_2 production was increased in EPF lesions. Moreover, we found that PGD_2 increased eotaxin-3 mRNA expression in sebocytes via peroxisome proliferator–activated receptor gamma (PPARγ) and that eotaxin-3 was detected around sebaceous glands in EPF lesions. Our data suggest that PGD_2 is involved in the pathogenesis of EPF lesions by inducing eotaxin-3 from sebocytes via PPARγ.

METHODS

Human subjects

We obtained biopsy specimens from 5 patients with EPF, 6 patients with folliculitis, and 4 healthy subjects. Informed consent was obtained from all subjects involved in this study. The Ethics Committee of Kyoto University approved the study.

Histologic examination

Paraffin-embedded sections were stained with hematoxilin-eosin and immunostained with H-PGDS, a monoclonal mouse antihuman antibody (dilution 1:500), L-PGDS, a polyclonal rabbit antihuman antibody (dilution 1:1000) (both were established at the Osaka Bioscience Institute, Osaka, Japan), and eotaxin-3/CCL26, a polyclonal goat antihuman antibody (dilution 1:100, R&D Systems, Minneapolis, Minn). As negative controls for H-PGDS and L-PGDS antibodies, we used isotype-matched control antibody and rabbit serum, respectively. Antigen retrieval was achieved by pepsin treatment for L-PGDS and preincubation with proteinase K for eotaxin-3. Nonspecific binding was blocked by addition of 10% goat serum for 30 minutes at room temperature. Afterward, sections were incubated for 1 hour at room temperature with the primary antibody followed by incubation with a species-specific biotinylated immunoglobulin (Vector, Burlingame, Calif) for 30 minutes at room temperature. Thereafter, they were incubated for 30 minutes with the avidine-biotin-peroxidase complex kit (Vector) and visualized with 3,3′-diaminobenzidine. They were lightly counterstained with hematoxylin. The number of immunoreactive cells per high power field was enumerated at 3 locations (original magnification ×200) per sample, and data were expressed as the number of H-PGDS– and L-PGDS–positive cells per high power field.

Preparation of human eosinophils and flow cytometry

Peripheral blood was obtained from 3 patients with EPF and 3 healthy donors. Polynuclear cells were separated by centrifugation of whole blood over Mono-Poly Resolving Medium (DS Pharma Biomedical, Osaka, Japan), followed by removal of remaining red cells by ACK lysing buffer (Lonza Walkersville, Inc, Walkersville, Md). They were stained with the antibodies against surface markers of eosinophils: antihuman CCR3-phycoerythrin (dilution 1:100, R&D Systems) and antihuman CD16-fluorescein isothiocyanate (dilution 1:100, Becton Drive Biosciences, Franklin Lakes, NJ). Eosinophils were identified with CCR3 positive and CD16 negative by flow cytometric analysis. With the use of an IntraStain kit (Becton Drive Biosciences), intracellular H-PGDS was detected by staining with polyclonal rabbit antihuman H-PGDS antibody (dilution 1:50, Cayman Biochemical) followed by antirabbit Alexa Fluor 647 (dilution 1:200, Life Technologies, Tokyo, Japan). The expression of H-PGDS was analyzed for mean fluorescence intensity.

For purification of eosinophils, the peripheral blood of patients with mild allergic rhinitis was collected by negative selection by using Eosinophil Isolation Kit (Miltenyl Biotec, Bergisch Gladbach, Germany). Both the purity and the viability of eosinophils were confirmed to exceed 95%.

Cell culture

Normal human epidermal keratinocytes (Kurabo, Osaka, Japan) were grown in Humedia-KG2 medium (Kurabo) with human epidermal growth factor (0.1 ng/mL), insulin (10 μg/mL), hydrocortisone (0.5 μg/mL), gentamicin (50 μg/mL), amphotericin B (50 ng/mL), and bovine brain pituitary extract (0.4%, v/v). Primary skin fibroblasts were isolated by standard methods[23] from healthy human skin and were cultured grown in Dulbecco modified Eagle medium (Gibco, Karlsrude, Germany) with 10% FBS (Gibco).

The immortalized human sebaceous gland cell lines SZ95 (a kind gift from Dr Christos C. Zouboulis) were cultured in sebomed basal medium (Biochrom AG, Berlin, Germany) with 10% FBS and recombinant human epidermal growth factor (Sigma Chemical, St Louis, Mo).

As for normal human epidermal keratinocytes and fibroblasts, the cells grew to 80% to 90% confluent and were starved for 3 hours, followed by treatment with PGD_2 (10 μM) (Cayman Biochemical) for 24 hours at 37°C in 5% CO_2.

Agonists used were the DP agonist BW245c (Cayman Biochemical), the chemoattractant–homologous receptor expressed on T_H2 cells (CRT_H2) agonist 15-keto-PGD_2 (DK-PGD_2) (Cayman Biochemical), and the PPARγ agonist 15-deoxy-Δ 12,14-PGJ_2 (15d-PGJ_2) (Cayman Biochemical). Antagonists used were the DP antagonist BWA868c (Cayman Biochemical), the CRT_H2 antagonist CAY10471 (Cayman Biochemical), and the PPARγ antagonist GW9662 (Cayman Biochemical). Sebocytes were starved for 3 hours and treated with PGD_2 (1-20 μM), BW245c (1-10 μM), DK-PGD_2 (1-10 μM), and 15d-PGJ_2 (1-7 μM) for 21 hours at the confluency of 30% to 40%. For treatment with antagonists, BWA868c (1-10 μM), CAY10471 (1-10 μM), and GW9662 (1-3 μM) (Cayman Biochemical) were preadded at 30 minutes.

SZ95 cells were transfected with PPARγ small-interfering RNA (siRNA) or nontargeting siRNA (Dharmacon, Lafayette, Colo) at 20% confluence by using Lipofectamine 2000 (Life Technologies). At 48 hours after transfection, the cells were starved for 3 hours and treated with or without PGD_2 (7.5 μM) for an additional 21 hours.

For detection of PGD_2, purified eosinophils (1×10^6 cells per well) were incubated in 50 μL of RPMI 1640 with 10% FBS in the presence and absence of 10^{-6} mol/L phorbol 12-myristate 13-acetate (Sigma-Aldrich, St Louis, Mo) and 10^{-5} mol/L calcium ionophore A23187 (Sigma-Aldrich). The concentration of PGD_2 in the supernatant was detected by the use of PGD_2-MOX Enzyme Immunoassay Kit (Cayman Biochemical).

Quantitative RT-PCR

Total RNA was isolated with RNeasy kits and digested with DNase I (Qiagen, Hilden, Germany). The cDNA was reverse transcribed from total RNA samples by using the Prime Script RT reagent kit (Takara Bio, Otsu, Japan). Quantitative RT-PCR was performed by using Light Cycler 480 SYBR Green I Master (Roche, Mannheim, Germany) and the Light Cycler real-time PCR apparatus (Roche) according to the manufacturer's instructions. The primers used for PCR had the following sequences: eotaxin-1, 5′-CTC CGCAGCACTTCTGTGGC-3′ (forward) and 5′- GGTCGGCACAGA-TATCCTTG-3′ (reverse); eotaxin-2, 5′-GCCTTCTGTTCCTGGGTGTC-3′ (forward) and 5′- CCTCCTGAGTCTCCACCTTG-3′ (reverse); eotaxin-3, 5′-CCTCCTGAGTCTCCACCTTG-3′ (forward) and 5′-AAGGGGCTTGT