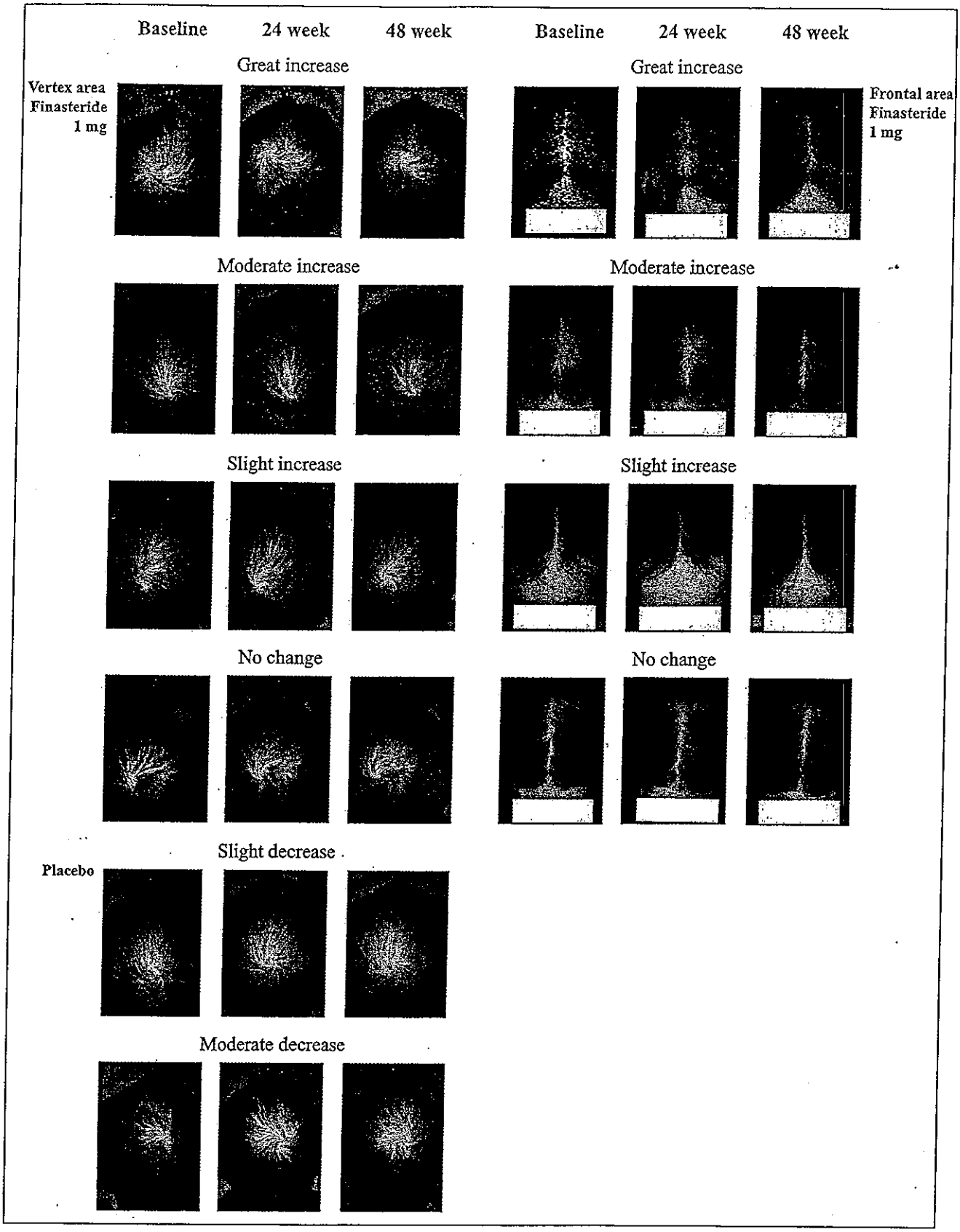

Figure 4. *Representative global photographs of patients at baseline and after 24 and 48 weeks of treatment with placebo and finasteride 1 mg. Changes in hair growth relative to baseline were rated by the expert panel.*

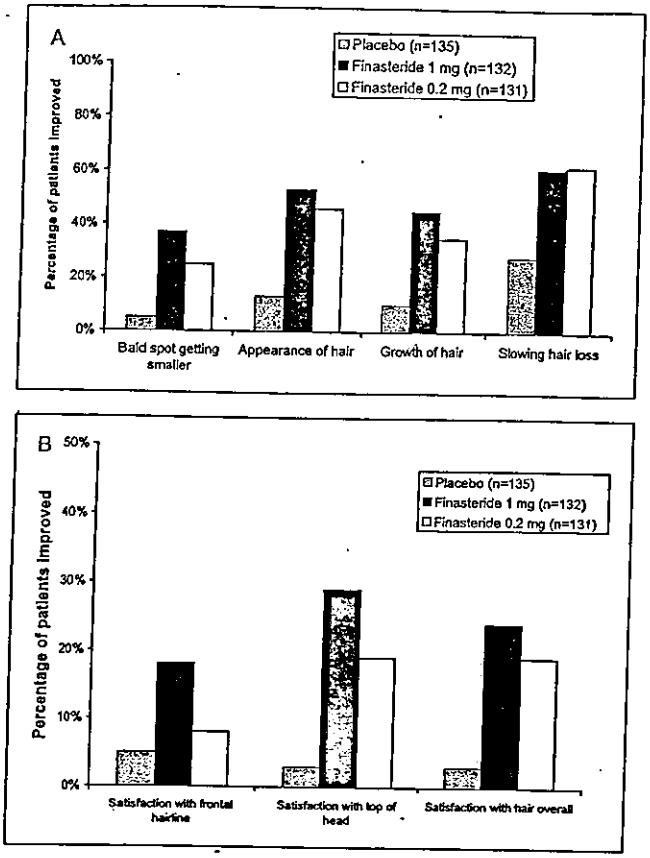

Figure 5. *Percentages of patients reporting improvement in hair growth (A) and satisfaction with appearance of hair (B) on final scoring of patient self assessment questionnaire (FAS).*

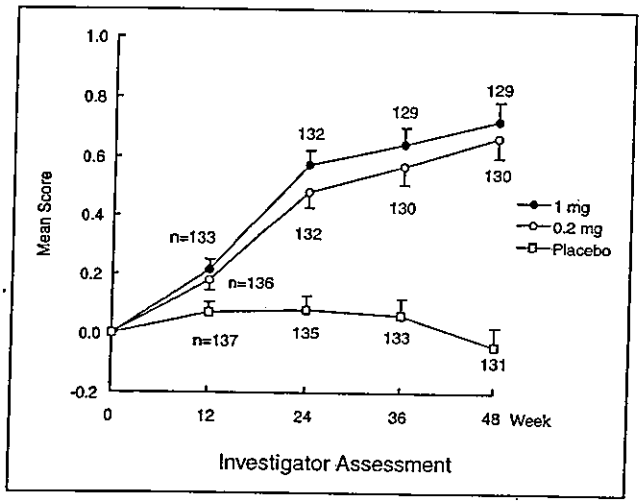

Figure 6. *Mean (± SE) investigator assessment score (FAS). P ≤ 0.024 for the comparison between each finasteride treatment group and placebo at 12, 24, 36, and 48 weeks. n in Fig means the number of statistically analyzed patients.*

men in their 30s is about one third that in similarly aged Caucasian men. At later ages, the incidence in Japanese is about the same as that in Caucasians 10 years younger [4] ; hence our decision to include patients from 20-50 years of age in this study. Ishino and coworkers concluded that the progression of male pattern hair loss, as measured by the rate of increase in percentage of vellus hairs, is slower in Japanese than Caucasians. This led them to suggest that the detection of moderate changes in hair loss may be important when assessing the efficacy of treatments for hair loss in Japanese men.

Global photographic assessment at 48 weeks was the primary efficacy endpoint in this study. This endpoint assesses change in hair growth and scalp coverage with time. This reflects changes in both the number of hairs and the overall appearance of the hair, which includes changes in hair thickness and growth rate. We found that the results of global photographic assessments, demonstrating improvements in men treated with finasteride compared to placebo, were supported by the results of patient self-assessments and investigator assessments. Global photographic assessment also demonstrated that only 2% of patients treated with finasteride, compared with 22% of patients in the placebo group were rated as worsened at Week 48, demonstrating that finasteride treatment helped men maintain their scalp hair coverage.

Finasteride was generally well tolerated in this study. The incidence of drug related adverse experiences was not significantly different between treatment groups, and no patients stopped intake of the study drug because of a drug related adverse experience. The overall profile of drug related adverse experiences in our study was similar to that in previous US studies [11- 13].

In summary, we found that treatment with finasteride slows the progression of hair loss and improves hair growth in Japanese patients with male pattern hair loss. A dose of 1 mg given once a day is the optimal dose for treatment of this condition. Finasteride treatment is generally well tolerated in Japanese men with male pattern hair loss. ■

Acknowledgements. *Authors are grateful to : Emiko Akiyoshi, MD[1], Takeo Idezuki, MD[2], Etsuko Fujita, MD[3], Michiyasu Hamada, MD[4], Yuko Higaki, MD[5], Masami Ishida, MD[6], Naoko Ishiguro, MD[7], Kenzo Kaji, MD[8], Tami Kimura, MD[9], Satomi Kobayashi, MD[10], Etsuko Komiyama, MD[11], Mariko Kume, MD[12], Yuko Miura, MD[13], Junichi Mizushima, MD[14], Chitose Morita, MD[15], Noboru Nakamichi, MD[16], Hyota Saga, MD[17], Kumi Jin, MD[18], Takenori Takahashi, MD[19], Sunao Toda, MD[20], Minoru Tsuboi, MD[21], Shu Ueda, MD[22], Hironobu Ura, MD[23], and Kyoko Yanagisawa, MD[24] as cooperative investigators, and Katsuji Oguchi[25] as a controller.*

From[1,15,24] Skin Clinic Daikanyama ; [2,3,8,12,19,23] Kanto Medical Center NTT EC ; [4,6,16,21] NS Clinic ; [14,17,22] Tokyo Women's Medical University, School of Medicine, Department of Dermatology ; [5,10] Shinjuku Minamiguchi Dermatology ; [7,18] Yotsuya 3 chome Dermatology ; [9,11,13] Juntendo University School of Medicine, Department of Dermatology ; [20] Toda Dermatology Clinic ; [25] Showa University

This study was supported by a grant from Banyu pharmaceutical Co. Ltd. Tokyo, Japan.

References

1. Hamilton JB. Patterned loss of hair in man : types and incidence. *Ann N Y Acad Sci* 1951 ; 53 : 708-28.

2. Norwood OT. Male pattern baldness : classification and incidence. *South Med J* 1975 ; 68 : 1359-65.

3. Rhodes T, Girman CJ, Savin RC, Kaufman KD, Guo S, Lilly FRW, Siervogel RM, Chumlea WC. Prevalence of male pattern hair loss in 18-49 year old men. *Dermatol Surg* 1998 ; 24 : 1330-2.

4. Takashima I, Iju M, Sudo M. Alopecia androgenetica – its incidence in Japanese and associated conditions. In : Orfanos CE, Montagna W, Stüttgen G, eds. *Hair Research : Status and Future Aspects*. Berlin Heidelberg : Springer-Verlag, 1981 : 287-93.

5. Ishino A, Uzuka M, Tsuji Y, Nakanishi J, Hanzawa N, Imamura S. Progressive decrease in hair diameter in Japanese with male pattern baldness. *J Dermatol* 1997 ; 24 : 758-64.

6. Imperato-McGinley J, Guerrero L, Gautier T, Peterson RE. Steroid 5 α-reductase deficiency in man : an inherited form of male pseudohermaphroditism. *Science* 1974 ; 186 : 1213-5.

7. Drake L, Hordinsky M, Fiedler V, *et al.* The effects of finasteride on scalp skin and serum androgen levels in men with androgenetic alopecia. *J Am Acad Dermatol* 1999 ; 41 : 550-4.

8. Price VH, Menefee E, Sanchez M, Ruane P, Kaufman KD. Changes in hair weight and hair count in men with androgenetic alopecia after treatment with finasteride, 1 mg, daily. *J Am Acad Dermatol* 2002 ; 46 : 517-23.

9. Van Neste D, Fuh V, Sanchez-Pedreno P, *et al.* Finasteride increases anagen hair in men with androgenetic alopecia. *Br J Dermatol* 2000 ; 143 : 804-10.

10. Whiting DA, Waldstreicher J, Sanchez M, Kaufman KD. Measuring reversal of hair miniaturization in androgenetic alopecia by follicular counts in horizontal sections of serial scalp biopsies : results of finasteride 1 mg treatment of men and postmenopausal women. *J Investig Dermatol Symp Proc* 1999 ; 4 : 282-4.

11. Kaufman KD, Olsen EA, Whiting D, Savin R, DeVillez R, Bergfeld W, Price VH, Van Neste D, Roberts JL, Hordinsky M, Shapiro J, Binkowitz B, Gormley GJ. Finasteride in the treatment of men with androgenetic alopecia. *J Am Acad Dermatol* 1998 ; 39 : 578-89.

12. Leyden J, Dunlap F, Miller B, *et al.* Finasteride in the treatment of men with frontal male pattern hair loss. *J Am Acad Dermatol* 1999 ; 40 (6 Pt 1) ; 930-7.

13. Kaufman K. The Finasteride Male Pattern Hair Loss Study Group. Long-term (5-year) multinational experience with finasteride 1 mg in the treatment of men with androgenetic alopecia. *Eur J Dermatol* 2002 ; 12 : 38-49.

14. Canfield D. Photographic documentation of hair growth in androgenetic alopecia. *Dermatol Clin* 1996 ; 14 : 713-21.

15. Kaufman K, Binkowitz B, Savin R, Canfield D. Reproducibility of global photographic assessments of patients with male pattern baldness in a clinical trial with finasteride. *J Invest Dermatol* 1995 ; 104 : 659. Abstract.

16. Barber BL, Kaufman KD, Kozloff RC, Girman CJ, Guess HA. A hair growth questionnaire for use in the evaluation of therapeutic effects in men. *J Dermatol Treat* 1998 ; 9 : 181-6.

17. Roberts JL, Fiedler V, Imperato-McGinley J, *et al.* Clinical dose ranging studies with finasteride, a type 2 5 α- reductase inhibitor, in men with male pattern hair loss. *J Am Acad Dermatol* 1999 ; 41 (4) : 555- 63.

18. Lin JH, Chen WC. Finasteride in the treatment of Taiwanese men with androgenetic alopecia : a 12-month open-label study. *Kaohsiung J Med Sci* 2002 ; 18 : 379-85.

19. Khandpur S, Suman M, Reddy BS. Comparative efficacy of various treatment regimens for androgenetic alopecia in men. *J Dermatol* 2002 ; 29 : 489-98.

4 King DT, Brosman S, Hirose FM *et al.* Idiopathic calcinosis of scrotum. *Urology* 1979; **14**: 92–4.
5 Song DH, Lee KH, Kang WH. Idiopathic calcinosis of the scrotum: histopathologic observations of fifty-one nodules. *J Am Acad Dermatol* 1988; **19**: 1095–101.
6 Akosa AB, Gilliland EA, Ali MH *et al.* Idiopathic scrotal calcinosis: a possible aetiology reaffirmed. *Br J Plast Surg* 1989; **42**: 624–7.
7 Demir Y, Latifoğlu O, Yenidünya S *et al.* Idiopathic calcinosis of the scrotum. *Ann Plast Surg* 2000; **45**: 223–4.
8 Wright S, Navsaria H, Leigh IM. Idiopathic scrotal calcinosis is idiopathic. *J Am Acad Dermatol* 1991; **24**: 727–30.
9 Füzesi L, Hollweg G, Lagrange W *et al.* Idiopathic calcinosis of the scrotum: scanning electron microscopic study with X-ray microanalysis. *Ultrastruct Pathol* 1991; **15**: 167–73.
10 Takayama H, Pak K, Tomoyoshi T. Electron microscopic study of mineral deposits in idiopathic calcinosis of the scrotum. *J Urol* 1982; **127**: 915–18.

Topical tacrolimus for cutaneous lupus erythematosus

SIR, In their paper on treatment of severe recalcitrant chronic discoid lupus erythematosus with topical tacrolimus, Walker *et al.*[1] state that 'Tacrolimus ointment is ... not yet commercially available in the U.K. ...'. Tacrolimus ointment has been available since April 2002, in 0·03% and 0·1% strengths and is licensed for the treatment of atopic dermatitis in adults (0·03% and 0·1%) and children over the age of 2 years (0·03% only) who are not adequately responsive to or are intolerant of conventional therapies.

Fujisawa Ltd, Medical Information, Staines, Middlesex, U.K. C.A.CHAMBERS
E-mail: colette.chambers@fujisawaco.uk

Reference

1 Walker SL, Kirby B, Chalmers RJG. The effect of topical tacrolimus on severe recalcitrant chronic discoid lupus erythematosus. *Br J Dermatol* 2002; **147**: 405–6.

Topical tacrolimus for cutaneous lupus erythematosus: reply from authors

SIR, Mrs Chambers is correct to highlight the fact that topical tacrolimus (Protopic® 0·03% and 0·1% ointment) is now available in the U.K. This was not, however, the case either when we were treating our patients or when our manuscript was accepted for publication.[1] Since then, another report of the use of topical tacrolimus for cutaneous lupus erythematosus (LE) has been published.[2] Eleven patients were treated with tacrolimus 0·1% ointment once daily for 4 weeks. None of three patients with discoid LE responded although four patients with less infiltrated, erythematous skin lesions of systemic or subacute cutaneous LE and two with dermatomyositis did show marked improvement.

We have highlighted the dramatic superiority of the combination of 0·3% tacrolimus in 0·05% clobetasol propionate used twice daily over the corticosteroid alone in two patients with recalcitrant chronic discoid LE;[1,3] we are currently undertaking a formal study to compare these treatments in a larger number of patients. The contrasting findings reported by Yoshimasu *et al.*[2] might be explained by chance but are more likely to be due either to inadequate penetration of the 0·1% tacrolimus preparation, which was applied only once daily, into the thick, infiltrated plaques of discoid LE or, alternatively or additionally, to a synergistic effect between tacrolimus and potent topical corticosteroids in our patients. It is important for the place of topical tacrolimus in cutaneous LE to be clarified in controlled clinical trials.

Dermatology Centre, Hope Hospital, University of Manchester Medical School, Salford M6 8HD, U.K. S.L.WALKER
**Department of Dermatology, Meath Adelaide Hospital, Tallaght, Dublin, Ireland* B.KIRBY*
E-mail: drstevewalker@hotmail.com R.J.G.CHALMERS

References

1 Walker SL, Kirby B, Chalmers RJG. The effect of topical tacrolimus on severe recalcitrant chronic discoid lupus erythematosus. *Br J Dermatol* 2002; **147**: 405–6.
2 Yoshimasu T, Ohtani T, Oshima A *et al.* Topical FK506 (tacrolimus) therapy for facial erythematous lesions of cutaneous lupus erythematosus and dermatomyositis. *Eur J Dermatol* 2002; **12**: 56–2.
3 Walker SL, Kirby B, Chalmers RJG. Letter to the editor. *Eur J Dermatol* 2002; **12**: 387–8.

Tumour necrosis factor-related apoptosis-inducing ligand expression in atopic dermatitis

SIR, Atopic dermatitis is a chronic relapsing inflammatory disorder that is characterized by pruritic and eczematous skin lesions often associated with elevated serum IgE levels.[1,2] Recently, apoptosis of epidermal keratinocytes was highlighted as a mechanism underlying the pathology of eczema in atopic dermatitis.[3] Activated T cells that infiltrate into the skin in atopic dermatitis upregulate the expression of Fas by keratinocytes through interferon-α and induce apoptosis through Fas ligand, which is expressed on the T-cell surface.[4] The damaged keratinocytes decrease the effectiveness of the epidermis as a barrier against allergens and infectious agents and may contribute to the development of chronic eczema. However, it remains unclear whether only the Fas/Fas ligand system is involved in the induction of keratinocyte apoptosis in atopic dermatitis.

Tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF family which regulates cell death and survival, and includes TNF-α, lymphotoxin-α and Fas ligand.[5] TRAIL induces apoptosis in tumour cells and virus-infected cells.[5] Interestingly,

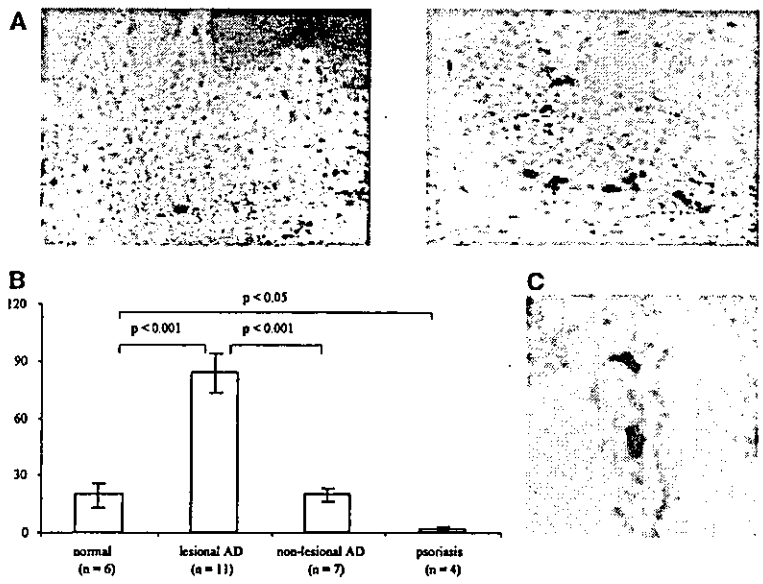

Figure 1. (A) Immunoreactivity of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) (red) detected predominantly in mononuclear cells infiltrating into the dermis, particularly in the perivascular area, in the lesions of atopic dermatitis. (B) Quantification of TRAIL-positive cells in the dermis (cells mm^{-3}) of normal, lesional atopic dermatitis (AD), nonlesional AD and psoriatic skin by using the KS400 Zeiss Image Analzser (Germany). Statistical analysis was performed using the Mann–Whitney *U*-test. (C) Double staining procedure with anti-TRAIL (red) and anti-CD68 antibodies (blue) (Pharmingen). The double-positive staining is shown in purple. Immunostaining was performed by the three-step streptavidin-biotin complex-alkaline phosphatase method.

Leverkus *et al.* recently reported that TRAIL induces apoptosis in human primary keratinocytes.[6] Thus, it is possible that TRAIL, like Fas ligand, is involved in keratinocyte apoptosis in atopic dermatis.

Accordingly, we examined expression of TRAIL in skin samples from 11 patients with atopic dermatitis. Atopic dermatitis was diagnosed based on the standard criteria.[7] Skin punch biopsies from the lesions of atopic dermatitis patients ($n = 11$) and from nonlesional skin of the same atopic dermatitis patients ($n = 7$) were obtained after written informed consent, and TRAIL expression was examined by immunohistochemistry using an antihuman TRAIL monoclonal antibody.[8] As controls, skin punch biopsies from normal subjects ($n = 6$) and psoriasis patients ($n = 4$) were examined. The biopsy samples were taken at the time of initial diagnosis before any treatment for atopic dermatitis or psoriasis.

As shown in Fig. 1(A), immunoreactivity of TRAIL was detected predominantly in mononuclear cells infiltrating into the dermis, particularly in the perivascular area, in the lesions of atopic dermatitis. Quantification of TRAIL-positive cells in the dermis (cells mm^{-3}) by using the KS400 Zeiss Image Analyzer (Germany) showed a significantly higher number of TRAIL-positive mononuclear cells in the lesions of atopic dermatitis than in nonlesional skin of atopic dermatitis, normal skin and psoriasis (Fig. 1B). A double-staining procedure revealed that TRAIL-positive dermal cells coexpressed CD68, but not CD3, CD4, CD8, CD19 or CD56 (Fig. 1C and data not shown). Thus, significant numbers of TRAIL-positive mononuclear cells that infiltrated into the lesions of atopic dermatitis were CD68-positive macrophages.

Few TRAIL-positive mononuclear cells were observed in the skin lesions of psoriasis (Fig. 1B) although significant numbers of macrophages or other leucocytes had infiltrated into the inflammatory lesions (data not shown). Thus, it appears that infiltration of TRAIL-positive macrophages may be specific to the lesions of atopic dermatitis. Staining with the anti-TRAIL antibody in biopsy samples from other skin diseases is in progress and this will confirm if TRAIL staining of infiltrating macrophages is specific to atopic dermatitis.

In conclusion, we found a significant infiltrate of TRAIL-positive macrophages in the lesions of atopic dermatitis. Taken together with the recent finding that TRAIL induces apoptosis in human primary keratinocytes,[6] the results suggest that TRAIL, like Fas ligand, may also be involved in keratinocyte apoptosis in atopic dermatitis. However, we cannot exclude the possibility that TRAIL may have other unknown functions in the lesions of atopic dermatitis.

Atopy Research Center, P.WARNNISSORN*
†Department of Dermatology and A.NAKAO*
‡Department of Immunology, H.SUTO*†
Juntendo University School of H.USHIO*
Medicine, 2-1-1 Hongo, Bunkyo-ku, N.YAMAGUCHI‡
Tokyo 113-8421, Japan H.YAGITA‡
Correspondence: Atsuhito Nakao. K.OKUMURA*‡
E-mail: anakao@med.juntendo.ac.jp H.OGAWA*†

References

1 Rudikoff D, Lebwohl M. Atopic dermatitis. *Lancet* 1998; **351**: 1715–21.
2 Leung DYM. Atopic dermatitis: new insights and opportunities for therapeutic intervention. *J Allergy Clin Immunol* 2000; **105**: 860–76.
3 Trautmann A, Akdis M, Brocker EB *et al.* New insights into the role of T cells in atopic dermatitis and allergic contact dermatitis. *Trends Immunol* 2001; **22**: 530–2.

4 Trautmann A, Akdis M, Keemann D *et al.* T cell-mediated Fas-induced keratinocyte apoptosis plays a key pathogenetic role in eczematous dermatitis. *J Clin Invest* 2000; **106**: 25–35.
5 Griffith TS, Lynch DH. TRAIL: a molecule with multiple receptors and control mechanisms. *Curr Opin Immunol* 1998; **10**: 559–63.
6 Leverkus M, Neumann M, Mengling T *et al.* Regulation of TRAIL sensitivity in primary and transformed human keratinocytes. *Cancer Res* 2000; **60**: 553–9.
7 Beltrani VS. The clinical spectrum of atopic dermatitis. *J Allergy Clin Immunol* 1999; **104**: S87–92.
8 Kayagaki N, Yamaguchi N, Nakayama M *et al.* Involvement of TNF-related apoptosis-inducing ligand in human CD4+ T cell-mediated cytotoxicity. *J Immunol* 1999; **162**: 2639–47.

Carcinoma with eccrine differentiation arising in a seborrhoeic keratosis

SIR, Rare malignancies have been reported in association with seborrhoeic keratosis (SK). Most of them were basal cell epitheliomas (BCEs) and *in situ* squamous cell carcinomas (SCCs).[1–3] Infiltrating SCCs, and two cases of eccrine poroma with areas of malignant changes, have also been described.[4–7] To our knowledge, an overtly malignant carcinoma showing sweat gland differentiation has not yet been described in association with SK. We report an invasive carcinoma (IC) that showed eccrine differentiation and metastasized to regional lymph nodes, on a background of an SK.

A 89-year-old woman presented with a 2·5-cm ulcerated plaque on her left axilla, that had been present for 20 years, and rapidly grew over several months. Left axillary lymph nodes were enlarged. The skin lesion and axillary lymph nodes were removed. One year later the patient was well without evidence of recurrences or metastases. Histological examination documented an IC lying under an SK (Figs 1,2). The IC consisted of anastomosing islands and irregular cords of atypical oval-to-spindle cells, showing eosinophilic cytoplasm; foci of eccrine duct (ED) formation were recognizable, suggesting a sweat gland differentiation. The IC was in direct continuity with the SK, and transitional zones (TZs) sharing features of both lesions were evident in between. There was no evidence of actinic damage. Search for human papillomavirus (HPV) by HPV screening *in situ* hybridization with biotin-labelled HPV cocktail probes[8] was negative.

On immunohistochemistry (Figs 1,2), cytokeratins 8, 13, 18, 19 (Biogenex, Menarini, Florence, Italy; 1 : 400; 1 : 100; 1 : 1000; 1 : 5000, respectively) and 17 (Dako, Milan, Italy; 1 : 20) were positive in the IC and in EDs, and almost negative in the SK; cytokeratin 14 (BioOptica, NeoMarkers, Milan, Italy; 1 : 50) was strongly positive all over the lesion. Cytokeratins 5 (Sigma, Milan, Italy; 1 : 5000) and 7 (Biogenex; 1 : 400) were negative in the SK, strongly positive in the EDs, and showed variable degrees of positivity in the IC. Cytokeratin 10 (Dako; 1 : 10) was almost completely negative in the IC, and positive in some cells of the EDs and TZs and in keratinized areas of the SK. Carcinoembryonic antigen (BioOptica; 1 : 400) stained the inner layer of the EDs. MIB-1 (Immunotech, Delta Biologicals, Rome, Italy; 1 : 100) labelled 20% of nuclei in the IC and TZs, and 2% of basal and suprabasal nuclei of the SK. p53 (Immunotech; 1 : 100) was moderately to strongly positive in 20% of nuclei in the IC, in 10–15% of nuclei in the TZs, and in 5–10% of nuclei in the SK. Metastatic deposits were documented in two lymph nodes.

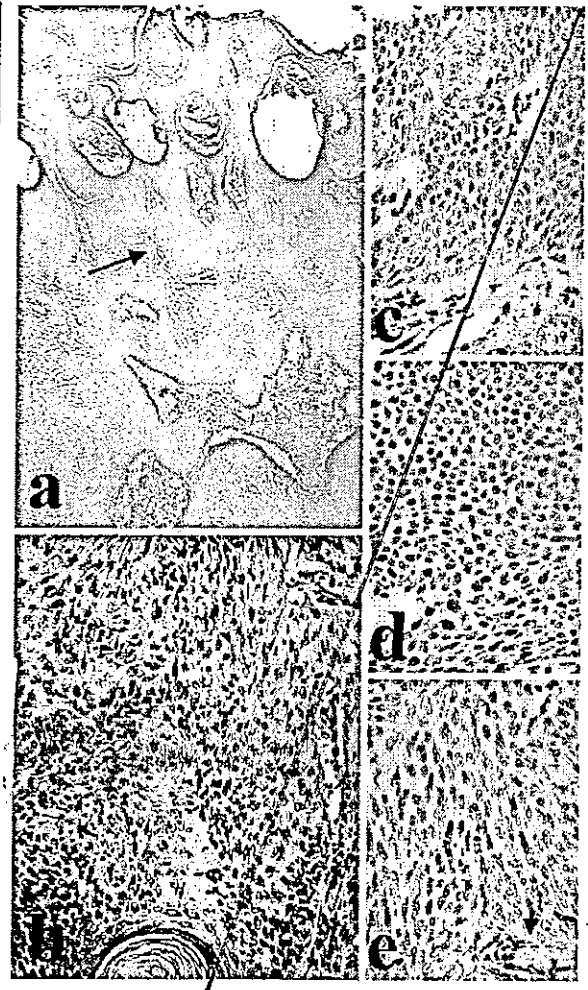

Figure 1. (a) Hyperkeratotic seborrhoeic keratosis showing a transition (arrow) into an invasive carcinoma; (b) detail of a transitional zone; p53 expression (c) in seborrhoeic keratosis, (d) in a transitional zone and (e) in invasive carcinoma. A ductal structure is shown (arrow). (a,b) Haematoxylin and eosin; original magnification: (a) × 50; (b) × 200; (c,d,e) alkaline phosphatase-antialkaline phosphatase; original magnification × 400.

A diagnosis was made of infiltrating carcinoma showing eccrine differentiation, arisen on an SK, and metastasized to axillary lymph nodes. An eccrine poroma, which may share some histological features with SK, was excluded as the background benign lesion in our case lacked ductal differentiation, and showed evident pseudohorn cysts; furthermore, it

Journal of Dermatological Science (2003) 31, 53–58

ELSEVIER

Journal of Dermatological SCIENCE

www.elsevier.com/locate/jdermsci

TGF-β/Smad signaling inhibits IFN-γ and TNF-α-induced TARC (CCL17) production in HaCaT cells

Koji Sumiyoshi[a,b], Atsuhito Nakao[a,*], Yasuhiro Setoguchi[c], Ryoji Tsuboi[b], Ko Okumura[a], Hideoki Ogawa[a,b]

[a] *Atopy (Allergy) Research Center, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*
[b] *Department of Dermatology, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*
[c] *Department of Respiratory Medicine, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*

Received 10 July 2002; received in revised form 25 September 2002; accepted 25 September 2002

KEYWORDS
TGF-β;
TARC;
HaCaT cells;
Smad;
Atopic dermatitis

Summary ***Background:*** A Th2 chemokine, thymus and activation regulated chemokine (TARC/CCL17), produced by keratinocytes, is implicated in the development of atopic dermatitis by recruiting CLA^+CCR4^+ lymphocytes into lesional skin and its expression was induced by proinflammatory cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). However, it remains unknown how TARC expression is negatively regulated in keratinocytes. ***Objective:*** We sought to determine whether transforming growth factor-β1 (TGF-β1) regulated TARC expression in keratinocytes. ***Methods:*** The effect of TGF-β1 on mRNA and protein expression of IFN-γ and TNF-α-induced TARC in a human keratinocyte cell line, HaCaT cells, was evaluated by using RT-PCR and ELISA. Adenovector-mediated gene transfer was used to determine the effect of Smad proteins on TARC expression in HaCaT cells. ***Results:*** TGF-β1 inhibited mRNA and protein expression of IFN-γ and TNF-α-induced TARC in HaCaT cells. The inhibitory effect of TGF-β1 on the TARC expression was suppressed by overexpression of Smad7, a major inhibitory regulator of Smad pathway for transforming growth factor-β (TGF-β) signaling, but not by PD98059, an inhibitor for ERK/mitogen-activated protein kinase (MAPK) pathway. In addition, overexpression of Smad2 or Smad3, major signal transducing Smads, was sufficient to inhibite the IFN-γ and TNF-α-induced TARC production in HaCaT cells. ***Conclusion:*** TGF-β1 inhibited IFN-γ and TNF-α-induced TARC production in HaCaT cells via Smad2/3, suggesting that modulation of TGF-β/Smad signaling pathway may be beneficial for the treatment of atopic dermatitis.

Abbreviations: CLA, cutaneous lymphocyte-associated antigen; MAPK, mitogen-activated protein kinase; TARC, Thymus and activation regulated chemokine; TGF-β, Transforming growth factor-β.

*Corresponding author. Tel.: +81-3-5802-1591; fax: +81-3-3813-5512.
E-mail address: anakao@med.juntendo.ac.jp (A. Nakao).

0923-1811/02/$20.00 PII: S0923-1811(02)00141-X

1. Introduction

TARC (CCL17) is an 8 kDa CC chemokine that attracts Th2 lymphocytes exclusively expressing CC chemokine receptor 4-positive ($CCR4^+$) [1–4] and thought to be involved in the development of Th2-mediated inflammation such as atopic dermatitis [5]. In atopic dermatitis, TARC was expressed in keratinocytes and may selectively recruit cutaneous lymphocyte-associated antigen $(CLA)^+CCR4^+$ lymphocytes into the lesional skin [6]. Thus, it would be important to explore the regulatory mechanisms of TARC expression in keratinocytes for better understanding of the pathophysiology of atopic dermatitis.

Vestergaard et al. showed that interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) work synergistically to induce TARC production in primary human keratinocytes and a human keratinocyte cell line, HaCaT cells [6]. They also showed that TARC expression in keratinocytes, which was colocalized with IFN-γ and TNF-α, was implicated in the development of atopic dermatis-like skin lesions in NC/Nga mice [7]. Since we found that transforming growth factor-β (TGF-β) significantly suppressed atopic dermatitis-like skin lesions in NC/Nga mice [8], we hypothesized that TGF-β could regulate IFN-γ and TNF-α-induced TARC production in keratinocytes.

In this study, we thus sought to determine whether TGF-β regulated IFN-γ and TNF-α-induced TARC production in keratinocytes. We showed that TGF-β significantly inhibited IFN-γ and TNF-α-induced TARC production in HaCaT cells. In addition, we showed that Smad signaling intermediates for TGF-β mediated the inhibitory effect. These findings suggest that modulation of TGF-β/Smad signaling pathway may have therapeutic potential for atopic dermatitis.

2. Materials and methods

2.1. Cell culture and stimulation

HaCaT cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 100 μg/ml streptomycin. HaCaT cells were stimulated with 10 ng/ml of human recombinant IFN-γ (R&D, MN) and/or 10 ng/ml of human recombinant TNF-α (R&D, MN) in the presence or absence of several doses of recombinant human transforming growth factor-β1 (TGF-β1) (R&D, MN) or a control vehicle (4 mM HCl containing 1 mg/ml of BSA) for 24 h. PD98059 (Biomol Research Labo, Inc, PA) was added for some experiments. Culture supernatant were collected and stored at −80 °C for ELISA assay. Concentration of IFN-γ and TNF-α used for stimulation and time course for ELISA assay were determined according to the study by Vestergarrd et al. [6].

2.2. ELISA

The amount of TARC in the culture supernatant of HaCaT cells was determined using human ELISA kits (Endogen) according to the manufacture's recommendation.

2.3. RT-PCR

Total RNA was prepared from cultures of HaCaT cells after stimulation with indicated cytokines by using Isogen solution (Nippon Gene, Japan). Complementary DNA (cDNA) was synthesized from 3 μg of total RNA using 1st strand cDNA synthesis kit (Ready To Go) (Amersham Pharmacia Biotech AB, Sweden). PCR amplification (95 °C for 45 s, 55 °C for 60 s, and 72 °C for 60 s; 35 cycles) was performed in a DNA thermal cycler (Perkin–Elmer). The PCR products were size-fractionated by agarose gel electrophoresis using 2.0% agarose, and stained with 1.0 μg/ml ethidium bromide. Primers used in this study were as follows; TARC (5′-CTTCTCTGCAGCACATCC-3′ and 5′-AAGACCTCT-CAAGGCTTTG-3′), and hypoxanthine phosphoribosyltransferase (HPRT) (5′-TTCTTTGCTGAC-CTGCTG-3′ and 3′-TTTCTACCAGTTCCAGCG-5′).

2.4. Adenovirus vector construction, virus purification, and transfection

The recombinant E1-deleted adenoviral vectors carrying mouse Smad2, Smad3, Smad7, or lacZ cDNA under cytomegarovirus promoters, AdCMV-Smad2, AdCMV-Smad3, AdCMV-Smad7 or AdCMV-lacZ, were generated, purified, and transfected as previously described [9,10]. Infection of recombinant adenoviruses was performed at a multiplicity of infection (m.o.i.) of 1×10^2 plaque forming units (pfu)/cell in 1 ml of serum-free DMEM for 60 min. HaCaT cells infected with the adenovirus solutions were cultured in DMEM supplemented with 10% FCS for 72 h and thereafter used for experiments.

2.5. In situ β-galactosidase staining

In situ β-galactosidase staining was performed using In situ β-galactosidase staining kit (STRATA-

GENE) according to the manufacture's recommendation.

2.6. Western blots

Immunoblotting with anti-Flag M2 antibody (Sigma, MO) was performed as previously described [11].

2.7. Data analysis

Data are summarized as mean ± S.D. The statistical analysis of the results was performed by the amount of variance using Fisher's least significant difference test for multiple comparisons. $P < 0.05$ was considered to be significant.

3. Results

3.1. TGF-β1 significantly suppresses IFN-γ and TNF-α-induced TARC production in HaCaT cells

When HaCaT cell cultures were grown to confluence and stimulated with 10 ng/ml IFN-γ and 10 ng/ml TNF-α for 24 h, they produced substantial amount of TARC whereas stimulation with either IFN-γ or TNF-α only induced a small production of TARC as previously described [6] (Fig. 1A). When the cell cultures were incubated with 10 ng/ml IFN-γ and 10 ng/ml TNF-α for 24 h in the presence of 10 ng/ml TGF-β1, the TARC production was suppressed by 65% (Fig. 1A). TGF-β1 dose-dependently (0.01–10 ng/ml) suppressed the IFN-γ and TNF-α-induced TARC production (data not shown). In addition, RT-PCR analysis showed that increased expression level of TARC mRNA at 3 h after stimulation INF-γ and TNF-α was inhibited by TGF-β1 (Fig. 1B). There was no significant difference in total cell numbers between TGF-β1-treated and non-treated HaCaT cells at 24 h after incubation of these cytokines (Fig. 2). These findings indicated that TGF-β1 significantly suppressed the IFN-γ and TNF-α-induced TARC expression both at mRNA and protein levels.

3.2. TGF-β1 suppresses IFN-γ and TNF-α-induced TARC production via Smad signaling pathway

TGF-β acts through the TGF-β type I and type II receptors to activate intracellular mediators, such as Smad proteins and mitogen-activated protein kinase (MAPK) pathways including ERK and p38

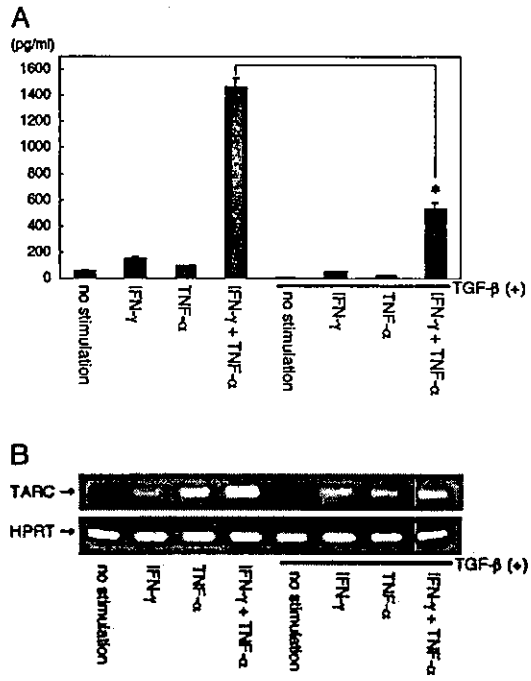

Fig. 1 Effect of TGF-β1 on IFN-γ and TNF-α-induced TARC production in HaCaT cells. (A) HaCaT cells were stimulated with IFN-γ and/or TNF-α in the presence or absence of TGF-β1 for 24 h. Supernatant were collected and TARC production was assessed by ELISA. Values represent the mean ± S.D. ($n = 3$). *, $P < 0.05$, significantly different from the mean value of the corresponding control response. (B) RT-PCR analysis of TARC from RNA prepared from HaCaT cells that were stimulated with IFN-γ and TNF-α in the presence or absence of TGF-β1 for 3 h.

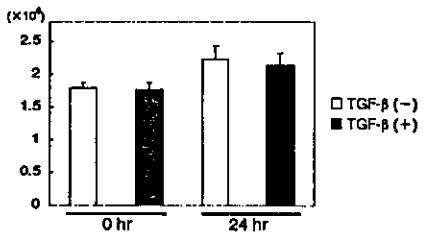

Fig. 2 Comparison of cell number between TGF-β1-treated and non-treated HaCaT cells. HaCaT cells stimulated with IFN-γ and TNF-α in the presence or absence of TGF-β1 for 24 h and viable cells were counted using trypan blue staining. Values represent the mean ± S.D. ($n = 3$).

[12–14]. Therefore, we determined which signaling pathways mediated the inhibitory effect of TGF-β1 on IFN-γ and TNF-α-induced TARC production in HaCaT cells.

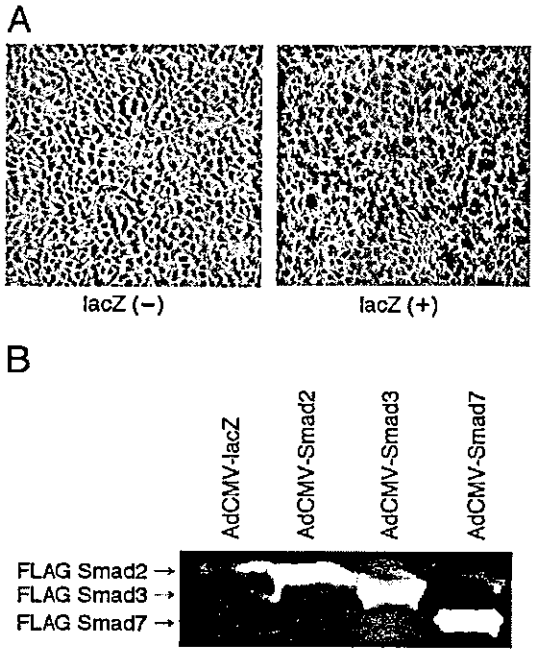

Fig. 3 Expression of exogenous Flag-tagged Smad2, Smad3 or Smad7 in HaCaT cells after infection with adenoviral constructs carrying the Smad cDNAs. (A) In situ β-galactosidase staining. HaCaT cells infected with lacZ were fixed and stained with X-gal solution at 48 h after the infection (right panel). Left panel is negative control. (B) Expression of exogenous Smad2, Smad3 or Smad7 in HaCaT cells by adenoviral-mediated transfer of the Smads. Cell lysates from HaCaT cells infected with adenoviruses carrying lacZ (AdCMV-lacZ), Flag-tagged Smad2 (AdCMV-Smad2), Flag-tagged Smad3 (AdCMV-Smad3), or Flag-tagged Smad7 (AdCMV-Smad7) in adenoviral vectors were immunoblotted with anti-Flag antibody.

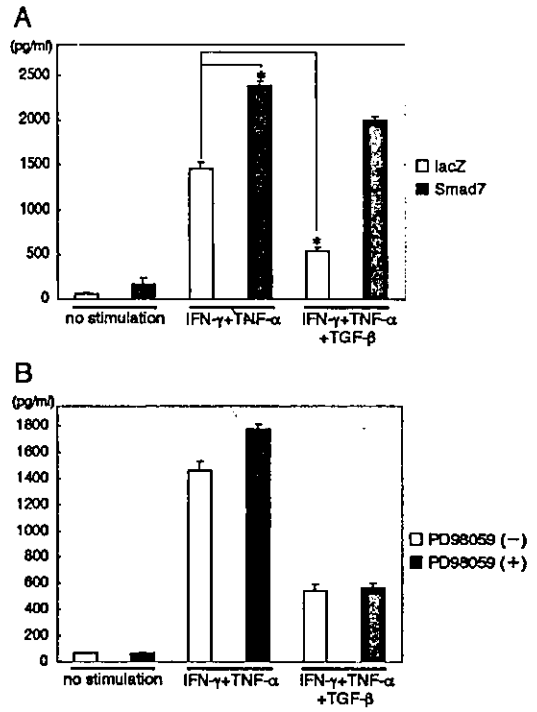

Fig. 4 Abrogation of TGF-β1-mediated inhibition of IFN-γ and TNF-α-induced TARC production in HaCaT cells by overexpression of Smad7. (A) HaCaT cells infected with adenoviral constructs carrying Flag-Smad7 cDNA or lacZ cDNA were stimulated with IFN-γ and TNF-α in the presence or absence of TGF-β1 for 24 h. Supernatant were collected and TARC production was assessed by ELISA. (B) HaCaT cells were stimulated with the indicated cytokines in the presence or absence of 5 μM PD98059 for 24 h. Supernatant were collected and TARC production was assessed by ELISA. Values represent the mean ± S.D. ($n = 3$). *, $P < 0.05$, significantly different from the mean value of the corresponding control response.

To investigate whether Smad signaling pathway was involved in the IFN-γ and TNF-α-induced TARC production in HaCaT cells, we examined the effect of Smad7, a major inhibitor of Smad pathway [12], on the TARC production in HaCaT cells. Adenoviruses carrying Flag-tagged Smad7, or control lacZ cDNA were used for introduction of the cDNAs into HaCaT cells. Transfection efficiency was almost 100% as judged by X-gal staining of HaCaT cells at 48 h after the infection with adenovirus carrying a control lacZ cDNA (Fig. 3A). Expression of exogenous Smad7 (and also Smad2 and Smad3; see below) was confirmed by Western blotting with anti-Flag antibody at 72 h after the infection (Fig. 3B).

As shown in Fig. 4A, overexpression of Smad7 abrogated the inhibitory effect of TGF-β1 on the IFN-γ and TNF-α-induced TARC production in HaCaT cells when compared with overexpression of a control lacZ. Interestingly, overexpression of Smad7 alone (without addition of TGF-β) in HaCaT cells significantly enhanced IFN-γ and TNF-α-induced TARC production when compared with a control lacZ overexpression. In contrast, addition of PD98059, an inhibitor of ERK activity, did not affect the inhibitory effect of TGF-β1 on IFN-γ and TNF-α-induced TARC production in HaCaT cells (Fig. 4B). Thus, the inhibitory effect of TGF-β1 on IFN-γ and TNF-α-induced TARC production in HaCaT cell was mediated by Smad signaling pathway, but not ERK pathway.

3.3. Overexpression of Smad2 or Smad3 is sufficient for suppression of IFN-γ and TNF-α-induced TARC production in HaCaT cells

The activated TGFβ receptors phosphorylate the cytosolic receptor-activated Smads (R-Smads) (Smad2 and Smad3), which form heteromeric complex with Smad4, and enter the nucleus, bind to DNA, and regulate gene transcription in cooperation with various transcriptional factors [12–14]. We thus asked whether Smad2 or Smad3 mediated the inhibitory effect of TGF-β1 on IFN-γ and TNF-α-induced TARC production in HaCaT cells. As shown in Fig. 5, overexpresson of either Smad2 or Smad3 using adenoviral vector system suppressed IFN-γ and TNF-α-induced TARC production in HaCaT cells. The effect of Smad3 appeared to be more potent than Smad2. Thus, overexpression of Smad2 or Smad3 was sufficient for inhibition of IFN-γ and TNF-α-induced TARC production in HaCaT cells.

4. Discussion

We have previously shown that TGF-β induces phosphorylation of endogenous Smad2 or Smad3 in HaCaT cells [15]. Taken together with the current results that (1) Smad7, a major inhibitor of Smad pathway, abrogated the inhibitory effect of TGF-β on IFN-γ and TNF-α-induced TARC production in HaCaT cells. (2) Overexpression of Smad2 or Smad3, major signal transducing Smads, was sufficient to inhibit IFN-γ and TNF-α-induced TARC production in HaCaT cells, we concluded that

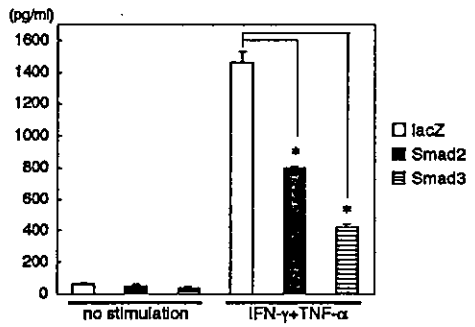

Fig. 5 Effect of Smad2 or Smad3 overexpression on IFN-γ and TNF-α-induced TARC production in HaCaT cells. HaCaT cells infected with adenoviruses carrying lacZ, Smad2, or Smad3 cDNA were stimulated with IFN-γ and TNF-α for 24 h. Supernatant were collected and TARC production was assessed by ELISA. Values represent the mean ± S.D. ($n = 3$). *, $P < 0.05$, significantly different from the mean value of the corresponding control response.

TGF-β significantly inhibited IFN-γ and TNF-α-induced TARC production in HaCaT cells via Smad pathway.

Overexpression of Smad7 alone (without addition of TGF-β) in HaCaT cells significantly enhanced IFN-γ and TNF-α-induced TARC production when compared with a control lacZ overexpression (Fig. 4A). This might be explained by the Smad7 blockade of signaling by endogenous TGF-β that might be slightly produced from HaCaT cells or Smad7 overexpression itself might have some capacity to affect TARC production by unknown mechanisms. This issue should be investigated in future study.

Smads function by carrying signals from the TGF-β receptors directly to the nucleus and they then regulate gene transcription in cooperation with various transcriptional factors and coactivators/corepressors [12–14]. Several inhibitory mechanisms of transcriptional activation of certain genes by Smads have been reported; e.g. competition of Smads with other transcriptional factors for limiting amounts of transcriptional coactivators such as p300 [16]. The detailed mechanisms how Smad2/3 functions as inhibitory for TARC expression in HaCaT cells are currently under investigation.

Accumulating data suggests that Smad-independent pathways also exist for TGF-β action [12,14]. MAPKs, including ERKs, p38, and JNKs through their upstream kinase activators such as TAK1 and protein kinase B (PKB, also called Akt) have been suggested to function downstream of TGF-β receptors. However, our findings suggested that, at least, ERKs were not involved in the TGF-β inhibition of TARC production in HaCaT cells.

Overexpression of Smad2 or Smad3 alone significantly inhibited IFN-γ and TNF-α-induced TARC production in HaCaT cells (Fig. 4). It has been reported that overexpression of R-Smads alone transduced TGF-β signals without stimulation of TGF-β1 [11,17,18] and Fujii et al. showed that some fractions of R-Smads spontaneously translocated into the nucleus and were activated when they were overexpressed [10]. Although the precise mechanisms are not yet clear, this could explain the current results.

In summary, we showed that TGF-β significantly inhibited IFN-γ and TNF-α-induced TARC production in HaCaT cells via Smad pathway. Given the implication of IFN-γ and TNF-α-induced TARC production by keratinocytes in atopic dermatitis, the results suggested that modulation of TGF-β/Smad signaling might have therapeutic potential for atopic dermatitis.

Acknowledgements

We thank Hiroko Ushio, Chiharu Nishiyama, Keiko Maeda, Toshiro Takai, Tomoko Tokura, Masayuki Mizoguchi, Nobuhiro Nakano for discussion and technical assistance, Emiko Kawasaki, Michiyo Matsumoto for secretarial assistance. This work was supported in part by the grant from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

References

[1] Murphy PM, Baggioline M, Charo IF, Hebert CA, Horuk R, Matsushima K, Miller K, Miller LH, Oppenheim JJ, Power CA. International union of pharmacology. XXII. Nomenclature for chemokine receptors. Pharmacol Rev 2000;52:145–76.

[2] Imai T, Baba M, Nishimura M, Kakizaki M, Takagi S, Yoshie O. The T cell-directed CC chemokine TARC is a highly specific biological ligand for CC chemokine receptor 4. J Biol Chem 1997;272:15036–42.

[3] Imai T, Baba M, Nishimura M, Kakizaki M, Nishimura M, Wang J, Gray P, Matsushima K, Yoshie O. Selective recruitment of $CCR4^+$ bearing Th2 cells toward antigen-presenting cells by the CC chemokine thymus and activation-regulated chemokine and macrophage-derived chemokine. Int Immunol 1999;11:81–8.

[4] Campbell JJ, Haraldsen G, Pan J, Rottman J, Qin S, Ponath P, Andrew DP, Warneke R, Ruffing N, Kassam N, Wu L, Butcher EC. The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. Nature 1999;400:776–80.

[5] Sandoval-Lopez G, Teran LM. TARC: novel mediator of allergic inflammation. Clin Exp Allergy 2001;31:1809–12.

[6] Vestergaard C, Bang K, Gesser B, Yoneyama H, Matsushima K, Larsen CG. A Th2 Chemokine, TARC, produced by keratinocytes may recruit CLA^+CCR4^+ lymphocytes into lesional atopic dermatitis skin. J Invest Dermatol 2000;115:640–6.

[7] Vestergaard C, Yoneyama H, Murai M, Nakamura K, Tamaki K, Terashima Y, Imai Y, Yoshie O, Irimura T, Mizutani H, Matsushima K. Overproduction of Th2-specific chemokines in NC/Nga mice exhibiting atopic dermatitis-like lesions. J Clin Invest 1999;104:1097–105.

[8] Sumiyoshi K, Nakao A, Ushio H, Mitsuishi K, Okumura K, Tsuboi R, Ra C, Ogawa H. TGF-β1 suppresses atopic dermatitis-like skin lesions in NC/Nga mice. Clin Exp Allergy 2002;31:309–14.

[9] Setoguchi Y, Danel C, Crystal RG. Stimulation of erythropoiesis by in vivo gene therapy: physiologic consequences of transfer of the human eythropoietin gene to experimental animals using an adenovirus vector. Blood 1994;84:2953–64.

[10] Fujii M, Takeda K, Imamura T, Aoki H, Sampath TK, Enomoto S, Kawabata M, Kato M, Ichijo H, Miyazono K. Roles of bone morphogenetic protein type I receptors and Smad proteins in osteoblast and chondroblast differentiation. Mol Biol Cell 1999;10:3801–13.

[11] Nakao A, Imamura T, Souchelnytskyi S, Kawabata M, Ishisaki A, Oeda E, Tamaki K, Hanai J, Heldin CH, Miyazono K, ten Dijke P. TGF-β receptor-mediated signaling through Smad2, Smad3, and Smad4. EMBO J 1997;16:5353–62.

[12] Massague J. How cells read TGF-β signals. Nat Rev Mol Cell Biol 2000;1:169–78.

[13] Attisano L, Wrana JL. Signal transduction by the TGF-β superfamily. Science 2002;296:1646–7.

[14] Piek E, Heldin CH, ten Dijke P. Specificity, diversity, and regulation in TGF-β superfamily signaling. FASEB J 1999;13:2105–24.

[15] Shimizu A, Kato M, Nakao A, Imamura T, ten Dijke P, Heldin CH, Kawabata M, Shimada S, Miyazono K. Identification of receptors and Smad proteins involved in activin signaling in a human epidermal keratinocyte cell line. Genes Cells 1998;3:125–34.

[16] Werner F, Jain MK, Feinberg MW, Sibing NE, Pellacani A, Wiesel P, Chin MT, Topper JN, Perrella MA, Lee ME. Transforming growth factor-β1 inhibition of macrophage activation is mediated via Smad3. J Biol Chem 2000;275:36653–8.

[17] Furuhashi M, Yagi K, Yamamoto H, Furukawa Y, Shimada S, Nakamura Y, Kikuchi A, Miyazono K, Kato M. Axin facilitates Smad3 activation in the transforming growth factor-β signaling pathway. Mol Cell Biol 2001;21:5132–41.

[18] Yuan W, Varga J. Transforming growth factor-β repression of matrix metalloproteinase-1 in dermal fibroblasts involves Smad3. J Biol Chem 2001;276:38502–10.

Journal of Dermatological Science (2003) 33, 31–40

ELSEVIER

Journal of Dermatological SCIENCE

www.elsevier.com/locate/jdermsci

Up-regulation of interleukin-13 receptor α1 on human keratinocytes in the skin of psoriasis and atopic dermatitis

Jongkonnee Wongpiyabovorn[a,b], Hajime Suto[a,b], Hiroko Ushio[b,*], Kenji Izuhara[c], Koichi Mitsuishi[a], Shigaku Ikeda[a], Atsuhito Nakao[b], Ko Okumura[b,d], Hideoki Ogawa[a,b]

[a] *Department of Dermatology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*
[b] *Atopy (Allergy) Research Center, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*
[c] *Department of Biomolecular Sciences, Saga Medical School, 5-1-1, Nabeshima, Saga 849-8501, Japan*
[d] *Department of Immunology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*

Received 18 February 2003; received in revised form 2 June 2003; accepted 3 June 2003

KEYWORDS
Interleukin-13;
Interleukin-4;
IFN-γ;
Psoriasis;
Atopic dermatitis

Summary *Background:* Interleukin (IL)-13 is a pleiotropic cytokine, which shares many biological functions with IL-4. The receptor subunits of IL-13 consist of IL-4Rα, IL-13Rα1 and IL-13Rα2. The regulatory mechanisms of the IL-13Rα expression in the keratinocytes of certain skin disease have not been known. *Objective:* To clear the roles of IL-13 and the regulatory mechanisms of its receptor in atopic dermatitis (AD) and psoriasis. *Method:* The expression of IL-13Rα1 in the skin of AD and psoriasis was investigated by immunohistochemistry. The regulation of IL-13Rα mRNA in the skin and human primary keratinocyte (HPK) was investigated by quantitative PCR. The secretion of IL-6 and RANTES from HPK was measured by ELISA. *Results:* The expression of IL-13Rα1 was more prominent on the suprabasal keratinocytes in the skin of AD and striking increase of staining was observed on all layers of keratinocyte in the skin of psoriasis. The mRNA of IL-13Rα1, but not of IL-13Rα2 was overexpressed in both skin of AD and psoriasis. In vitro experiment using HPK demonstrated that IFN-γ, IL-13 but not IL-4 could up-regulate the mRNA expression of IL-13Rα1. In contrast, IL-13Rα2 mRNA expression was up-regulated by IFN-γ plus IL-4. Furthermore, the stimulation of HPK with IFN-γ plus IL-13 and/or IL-4 resulted in significant enhancement of IL-6 and RANTES secretion. *Conclusion:* These findings indicate that IL-4 and IL-13 have different regulatory effects on the expression of IL-13Rα1 and α2, and the overexpression of IL-13Rα1 may play some roles in the pathogenesis of chronic stage of AD or psoriasis.

*Corresponding author. Tel.: +81-3-5802-1591; fax: +81-3-3813-5512.
E-mail address: hushio@med.juntendo.ac.jp (H. Ushio).

0923-1811/03/$30.00
doi:10.1016/S0923-1811(03)00148-8

1. Introduction

Interleukin (IL)-13 is an immunoregulatory cytokine secreted predominantly by activated Th2 cells and mast cells [1,2]. IL-13 shares many functional properties with IL-4, including the induction of IgE synthesis, CD23 expression by B cells [3], the up-regulation of major histocompatibility complex class II on monocytes [4,5], and the induction of vascular cell adhesion molecule-1 on endothelial cells [6]. IL-13 exerts its actions via binding to the complex receptor consisting of IL-4Rα and at least two other cell surface proteins, IL-13Rα1 and IL-13Rα2 [7–10]. IL-13Rα1 itself, binds IL-13 with low affinity, but when paired with IL-4Rα, it binds IL-13 with high affinity and form functional receptor which signals [7,11]. Whereas, IL-13Rα2 that can bind IL-13 with high affinity, has been postulated to be a decoy receptor because of its short cytoplasmic tail without having any signaling motifs [12]. We have previously demonstrated that IL-13Rα1 is expressed on the keratinocyte in healthy skin [13]. But the physiological and pathological roles of this receptor in the function of keratinocyte are largely unknown.

Also the regulation of IL-13Rα1 and IL-13Rα2 expression has not been understood completely. It is reported that these receptor components are expressed on both haematopoietic (T, B lymphocyte, monocyte, mast cell, basophil) and non-haematopoietic cell (endothelial cell, keratinocyte, fibroblast, respiratory epithelium, heart muscle and hepatocyte) [13,14]. IL-4 increases IL-4Rα mRNA transcription in T cells [14], whereas IL-13 and IL-4 down-regulate IL-4Rα and IL-13Rα1 expressions on monocytes [14–16] but up-regulate the mRNA and the surface expression of IL-13Rα1 on B lymphocyte [17]. In addition, it is reported that IFN-γ up-regulates IL-13Rα2 surface expression on U937 cell line, A549 cell line and primary nasal epithelial cells [18]. Thus, it is possible that each cell has specific regulatory mechanisms of the expression of IL-13Rα1 and IL-13Rα2.

It is generally believed that IL-4Rα/IL-13Rα1 serves as a functional receptor complex for both IL-4 and IL-13 in non-haematopoietic cells, such as endothelial cell, fibroblast, respiratory epithelium, smooth muscle cell, all of which lack the common γ chain of IL-2 receptor (IL-2Rγ) [17,19]. Thus, the most of the biological effects and the signaling events elicited by IL-4 and IL-13 are overlapping. IL-13 utilizes Janus tyrosine kinase (JAK)-signal transducer and activation of transcription (STAT) pathways like IL-4. It has been demonstrated that Tyk2, Jak1, member of JAK family, and STAT6, STAT3, a member of the STAT family, are activated by IL-13 [20,21]. But recent accumulating evidence in the murine asthma model suggests that IL-13 can exert its biological function by acting directly on the bronchial epithelial cell or on the smooth muscle cell, independent of IL-4 and IgE synthesis [22,23]. However, there is not enough information how IL-13 exerts its specific biological function that is different from IL-4 via same subunits IL-4Rα/IL-13Rα1 in different cell types.

In this study, we first demonstrate that the expression of IL-13Rα1 is increased in the chronic inflammatory skin disease, such as atopic dermatitis (AD) or psoriasis in which the roles of IL-13 have been implicated [24–26]. Furthermore, we demonstrate the expression of each receptor, IL-13Rα1 and IL-13Rα2, on keratinocyte is differently regulated by IFN-γ, IL-13 and IL-4.

2. Materials and methods

2.1. Cell cultures

Human primary keratinocytes (HPKs) were prepared from human foreskin as previously described [27] and cultured in the serum-free Keratinocyte Growth Medium (Kurabo, Osaka, Japan) supplemented with 0.1 ng/ml human epidermal growth factor, 2 ml of bovine pituitary extract, 10 μg/ml Insulin, 0.5 μg/ml hydrocortisone and antibiotics. Cells were grown at 37 °C in 5% CO_2, two to three passages of the cells were used for all experiments. Human premyeloid erythroleukemia (TF-1) and human basophilic cell line (KU812) were cultured in the RPMI 1640 medium, supplemented with 10% heat inactivated fetal bovine serum (Sigma-Aldrich, Tokyo, Japan), 10 mM HEPES and antibiotics (50 U/ml penicillin, 50 μg/ml streptomycin). Human endothelial cell was grown as previously described [28].

2.2. Immunohistochemistry

Skin punch biopsies from the forehand of chronic AD ($n = 6$), psoriasis ($n = 6$) or the breast of normal ($n = 6$) skin were obtained with written informed consent under the approval of the ethical committee of the University. Chronicity of AD was diagnosed based on the standard criteria [29]. Frozen sections were made and fixed with cold acetone for 5 min. The specimens were probed with anti-human IL-13Rα1 (UU15) antibody [13] or isotype control, mouse IgG2aκ (DAKO JAPAN Co., Ltd, Kyoto, Japan) followed by incubation with biotin-conjugated rabbit anti-mouse IgG (DAKO JAPAN Co., Ltd) and then alkaline phosphatase-conju-

gated streptavidin (DAKO JAPAN Co., Ltd). The signal was detected by the alkaline phosphatase method according to the manufacturer's instruction (DAKO JAPAN Co., Ltd).

2.3. RT reaction and quantitative PCR

Total RNA was extracted using RNA STAT-60 (Teltest inc., Fiends wood, TX) according to the manufacturer's instruction from the stimulated primary keratinocytes or the 5-mm skin biopsies of chronic atopic, psoriatic skin lesions and normal skin of plastic surgery. Three μg of total RNA was reverse-transcribed using superscript II RT (Invitrogen Japan, Tokyo) and oligo dT primer (Invitrogen Japan, Tokyo). The specific pairs of primers used for detecting human IL-13Rα1, IL-13Rα2, IL-4Rα, common γ chain and GAPDH were 5′ CAG TGT AGC ACC AAT GAG AGT GAG 3′ and 5′ TCA GGT TTC ACA CGG GAA GTT A 3′ for IL-13Rα1; 5′ ATA CCT TTG GGA CCT ATT CC 3′ and 5′ TGA ACA TTT GGC CAT GAC TG 3′ for IL-13Rα2; 5′ GTC TGC AGA TGA GGACTA GGG G3′ and 5′ TAC TCT CAT GGG ATG TGGG CG 3′ for IL-4Rα; 5′ GAC AGG CCA CAC AGA TGC TA 3′ and 5′ GTT CAC TGT AGT CTG GCT GC 3′ for common γ chain; and 5′ ACC ACA GTC CAT GCC AT 3′ and 5′ ACC ACC CTG TTG CTG TA 3′ for GAPDH, respectively.

The competitive cDNA fragment for IL-13Rα1, IL-13Rα2 and GAPDH were constructed by deletion of a 51-bp fragment of the IL-13Rα1 target cDNA (386 bp) by cleaving between position 395 and 446 by Nsi; by deletion of a 141-bp fragment of the IL-13Rα2 target cDNA (428 bp) by cleaving between position 1101 and 1242 by BsI, and by deletion of a 106-bp fragment from GAPDH target cDNA (451 bp) by cleaving between position 711 and 816 by XcmI, respectively. The PCR reactions were carried out with 1 μl of cDNA as a template with specified primers and various amounts of competitors (0.1 fg/μl–100 pg/μl) using Ampli Taq Gold polymerase (Applied Biosystems, Branchburg, NJ). The PCR cycles were composed of the denature at 94 °C for 5 min and 45 cycles of 94 °C for 45 s, 60 °C for 1 min, 72 °C for 1 min for IL-13Rα1; 35 cycles of 94 °C for 1 min, 60 °C for 2 min, 72 °C for 2 min for IL-13Rα2: 35 cycles of 94 °C for 30 s, 60 °C for 45 s, 72 °C for 1 min for IL-4Rα and common γ chain; and 27 cycles of 94 °C for 1 min, 60 °C for 2 min, 72 °C for 2 min for GAPDH. The 8 μl of each PCR product was run on a 2% agarose gel, stained with 2 μg/ml of Ethidium Bromide solution for 30 min and quantified with a Storm Fluor Imager (Molecular Dynamics, Inc, Uppsala Sweden). Data was normalized according to the GAPDH expression and expressed as the ratio of each α chain of mRNA to GAPDH mRNA. Semi-quantitative PCR was performed using normalized amount of cDNA according to the GAPDH expression and specific primers without competitors. PCR product was analyzed as described above using a Storm Fluor Imager after staining with EtBr and expressed as the value of EtBr volume.

2.4. Measurement of cytokine concentrations

Human keratinocytes (1×10^5 cell/ml) in serum-free Keratinocyte Growth Medium were stimulated with 100 ng/ml of recombinant human IFN-γ (Genzyme Techne, Minneapolis, MN) alone or combination with 100 ng/ml of recombinant human IL-13 and IL-4 (PeproTech EC Ltd, London, England) for 48 h. The levels of IL-6 and RANTES in the supernatant were measured by ELISA kit (Genzyme Tech Corp). The viability of the cells under each condition was more than 98% and there were no significant differences in the number of viable cells under each condition after 48 h incubation. The experiments were performed four times using different keratinocyte preparations. Results were shown as pg/10^6 cell ±S.D.

2.5. Statistical analysis

Statistical analysis was performed using the Student *t*-test. Values of $P \leq 0.05$ were considered as significant.

3. Results

3.1. Immunohistochemical detection of IL-13Rα1 in the skin of AD and psoriasis

Since we have previously demonstrated that the keratinocyte was a main cell expressing IL-13Rα1 in the skin [13], we examined the expression of this receptor in the chronic inflammatory skin of AD and psoriasis patients. The distribution of IL-13Rα1 in normal skin was consistent with the previous results, in that, mostly lower layer of keratinocytes expressed IL-13Rα1 (Fig. 1a and b). There were some positive staining cells in the dermis, which had mast cell like futures (Fig. 1a and b). When we performed immunohistochemical staining on the skin of chronic AD, positive results were observed in the upper, more differentiated keratinocytes (Fig. 1d and e). Also positive staining was observed among the infiltrating cells in the dermis. Even though we have not specified phenotype of these

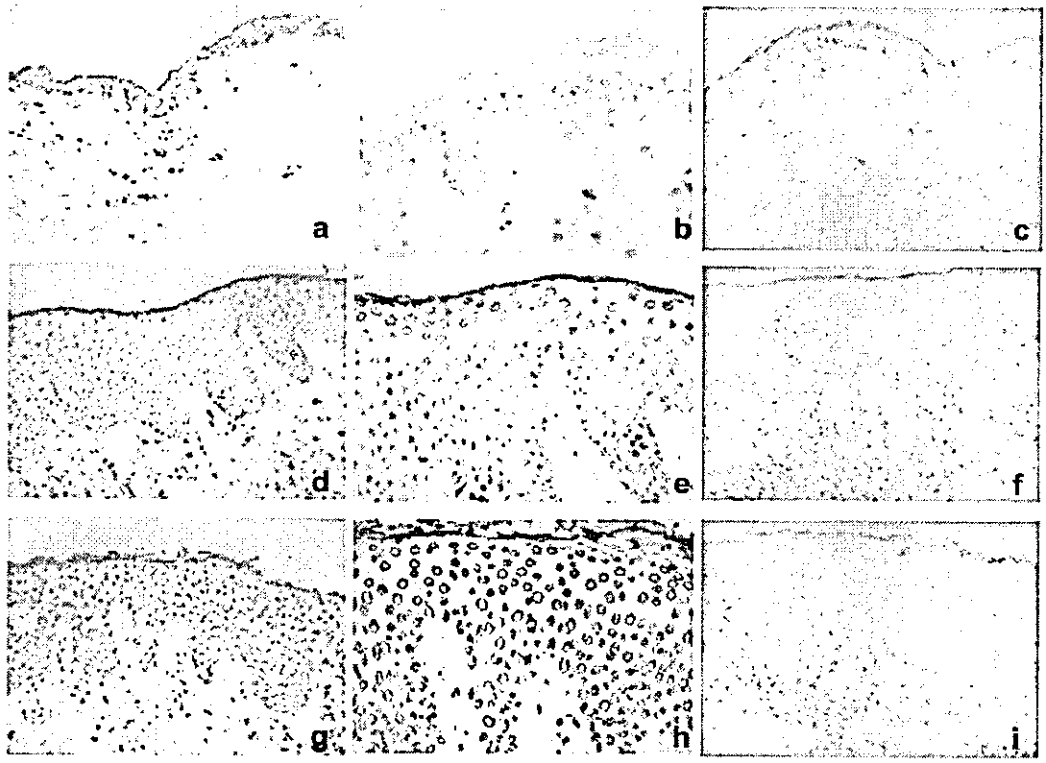

Fig. 1 Immunohistochemical detection of IL-13Rα1 in the skin of AD and psoriasis. Immunohistochemical staining was performed on the skin specimens from normal (a–c), AD (d–f) or psoriasis patients (g–i), using anti-IL-13Rα1 (UU15) or isotype control, mouse IgG2a antibody (c, f and i), as described in Method. The signal development was done using alkaline phosphatase method according to the manufacture's instruction. Nuclear staining was done by methyl green. Data shown is one representative result from several experiments that had similar results. (b, e, h: 120 × , a, c, d, f, g, i: 60 ×).

cells, the size and shape showed mast cell- and monocyte-like features (Fig. 1a, b, d and e). No clear differences were observed in the staining of IL-13Rα1 in the dermis of healthy and AD patients except that there were increased numbers of mast cell-like, positive-stained cells in the AD lesion. Striking increase of positive staining of IL-13Rα1 was observed in all stages of keratinocytes from the psoriatic skin (Fig. 1g and h) and the staining intensity was higher in the skin of psoriasis than that of normal or AD. No positive staining was observed in all specimens stained with isotype control, mouse IgG2a (Fig. 1c, f and i).

3.2. The expression of IL-13Rα1 and IL-13Rα2 mRNA in the skin of chronic AD and psoriasis

The mRNA expressions of IL-13Rα1 and IL-13Rα2 in the skin biopsies from chronic AD and psoriasis were analyzed by quantitative PCR. IL-13Rα1, but not IL-13Rα2 mRNA expression, was significantly increased in the chronic skin lesion of AD and psoriasis compared to the normal skin (Fig. 2a and b). The result, the increment of IL-13Rα1 mRNA was much higher in the skin of psoriasis than AD, was consisted with the immunohistochemical data. We aware of that we can not completely exclude the possibility that the increment of IL-13Rα1 mRNA expression we observed was due to the increase of infiltrating cells in the dermis but not due to the expression of this receptor on keratinocyte, since we used whole skin for the isolation of RNA. But immunohistochemical data and the differences in the expression of IL-13Rα1 mRNA between AD and psoriasis (both have almost same amount of infiltrating cells) strongly support that the keratinocyte is a major cell contributing to the increment of IL-13Rα1 mRNA expression in AD and psoriasis. Since the high affinity IL-13 receptor consists of heterodimeric unit of IL-13Rα and IL-4Rα, we also measured the levels of IL-4Rα mRNA

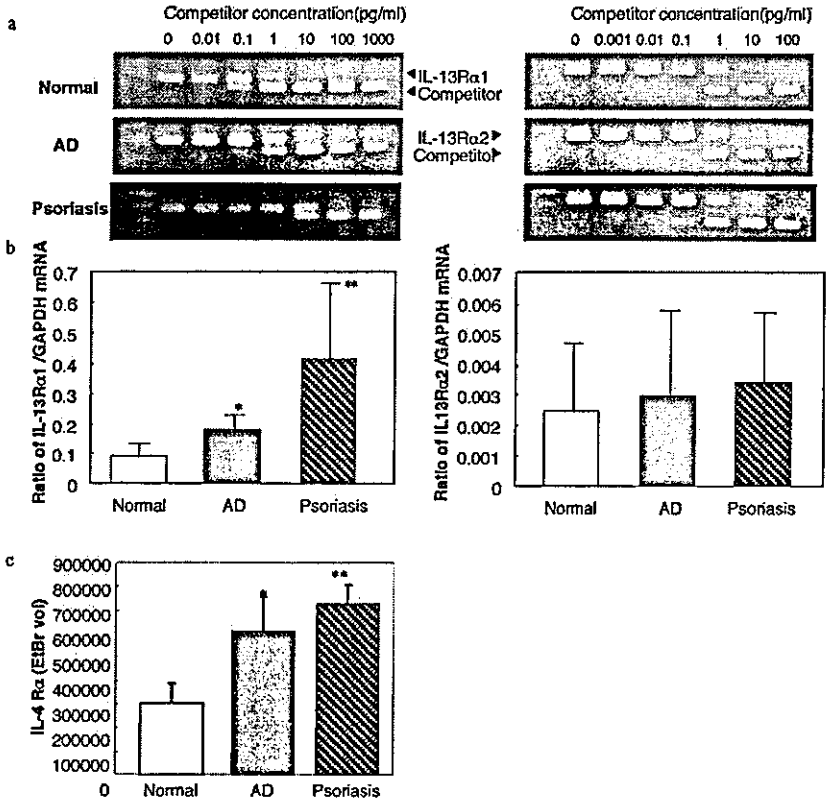

Fig. 2 The expression of IL-13Rα1 and IL-13Rα2 mRNA in the skin of chronic AD and psoriasis. Total RNA was isolated from the skin specimens of normal, AD and psoriasis, and quantitative PCR was performed as described in Section 2. The amount of cDNA was normalized according to the expression of GAPDH. Representative result from each skin sample were shown in (a) upper; normal, middle; AD and lower; psoriasis. The quantitation of each band was done using image analyzing soft and showed as the ratio of mRNA of IL-13Rα1 to GAPDH (left) or IL-13Rα2 to GAPDH (right) (b). The expression of IL-4Rα mRNA was measured semi-quantitatively as described in Section 2 (C). Data shown are mean ± S.D. of six experiments conducted with different skin preparations from different patients. $^{**}P < 0.01$, $^{*}P < 0.05$, significantly different from the mean value of the normal skin.

by semi-quantitative PCR. Consisted with previous report [25,35,38], IL-4Rα was significantly increased in the chronic skin lesion of AD and psoriasis compared to the normal skin (Fig. 2c).

3.3. Modulation of IL-13Rα1 and IL-13Rα2 mRNA expression in primary human keratinocyte by IFN-γ, IL-4 and IL-13

Having results that IL-13Rα1 was overexpressed on human keratinocyte with the chronic skin disease (Fig. 2a and b), next we investigated how the expression of IL-13Rα1 and α2 were regulated in the keratinocytes. Since IL-13, IL-4 or IFN-γ have been reported to have regulatory role in IL-13 receptor expression on both haematopoietic and non-haematopoietic cells, we first examined the effects of these cytokines on the expression of IL-13Rα1 and IL-13Rα2 mRNA using HPKs in vitro. As shown in Fig. 3a, IL-13 or IFN-γ alone significantly enhanced the IL-13Rα1 mRNA expression. In contrast, the same concentration of IL-4 had no effect on the IL-13Rα1 mRNA expression. Furthermore, simultaneous addition of IL-4 and IL-13 abolished this effect of IL-13. Combined stimulation of IL-4 with IFN-γ suppressed, but not significantly, the IL-13Rα1 mRNA expression which was up-regulated by IFN-γ alone. In contrast, as shown in Fig. 3b, IL-4 combined with IFN-γ significantly increased the IL-13Rα2 mRNA expression which was not observed by the combined stimulation with IFN-γ and IL-13. These results demonstrated that not only IL-13 or IL-4 could modulate their own receptor expressions, but also IFN-γ could regulate the IL-13Rα mRNA expression in primary human keratinocyte,

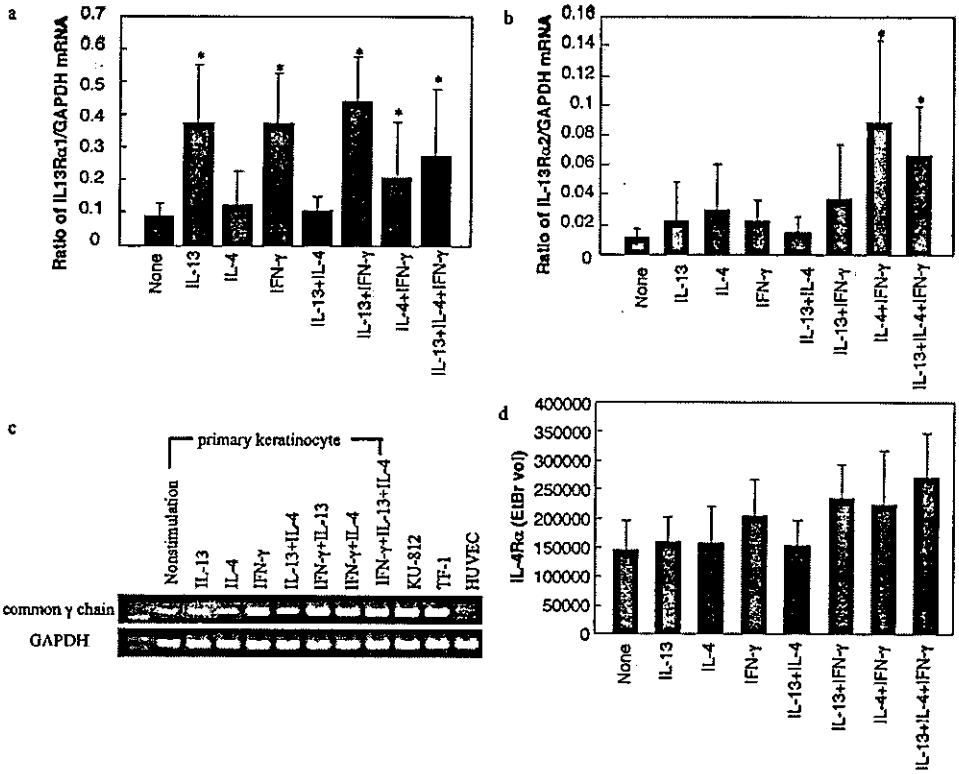

Fig. 3 Modulation of IL-13Rα1 and IL-13Rα2 mRNA expression in primary human keratinocyte by IFN-γ, IL-4 and IL-13. Primary human keratinocytes were stimulated with IFN-γ (100 ng/ml) alone or combination of either IL-13 (100 ng/ml) or IL-4 (100 ng/ml) for 48 h and total RNA was isolated and proceed for quantitative PCR of IL-13Rα1 and α2 or for semi-quantitative PCR for IL-4Rα. The amount of cDNA was adjusted according to the expression of GAPDH and the data was showed as the ratio of mRNA of IL-13Rα1 to GAPDH (a) or IL-13Rα2 to GAPDH (b). Data shown were mean ± S.D. of four experiments conducted with different preparation of keratinocytes. (c) The mRNA expression of common γ-chain and (d) IL-4Rα was detected as described in Section 2. Human premyeloid erythroleukemia (TF-1), human basophilic cell line (KU812) and human umbilical endothelial cell (HUVEC) were used as positive or negative control, respectively. *$P < 0.05$, significantly different from the mean value of unstimulated keratinocytes.

and the mRNA expressions of IL-13Rα1 and IL-13Rα2 were differently regulated by IL-4 and IL-13.

To address the possibility that the different effects of IL-13 and IL-4 on the expression of each IL-13R component may come from that IL-4 signals through type I IL-4R consisting of IL-4Rα1 and common γ chain, we investigated the expression of common γ chain in HPK. Semi-quantitative PCR revealed that, the mRNA for the common γ chain was slightly expressed in the resting HPK and the expression was enhanced by IFN-γ, comparable to the levels of other haematopoietic cells, such as KU812 or TF-1 (Fig. 3c). Same condition of PCR did not reveal the detectable expression of the mRNA of common γ chain in the human endothelial cells as has been reported (Fig. 3c) [6]. But, no detectable protein for the common γ chain was confirmed by FACS analysis even in the IFN-γ-stimulated keratinocytes (Data not shown). Also we tried to confirm the increased surface expression of the IL-13Rα1 and IL-13Rα2 on keratinocytes, there were no differences in the expression of these receptors upon stimulation with IFN-γ, IL-4, IL-13 analyzed by FACS (Data not shown). Semi-quantitative PCR for IL-4Rα mRNA revealed that its expression was seemed to increase upon stimulation with IFN-γ, and simultaneous stimulation with IL-4 and IL-13, but not significant. (Fig. 3d).

3.4. Inflammatory cytokine secretion from the primary keratinocytes stimulated with IL-4, IL-13 and IFN-γ

To examine the biological consequence of the enhanced expression of IL-13Rα1, we measured inflammatory cytokine production from keratino-

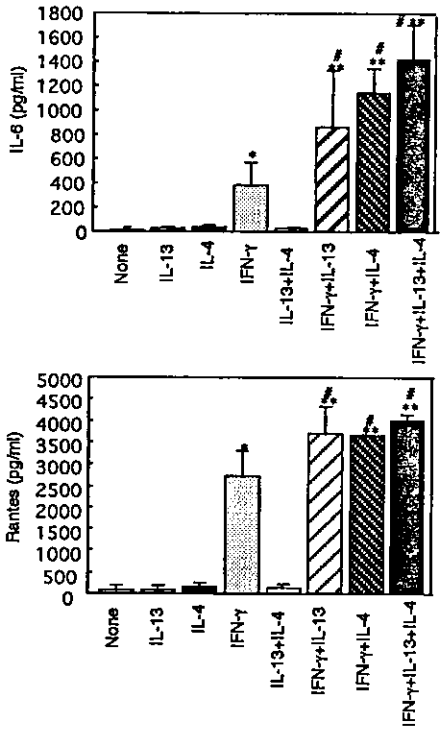

Fig. 4 Inflammatory cytokine secretion from the primary keratinocytes stimulated with IL-4, IL-13 and IFN-γ. Primary human keratinocytes were stimulated with IFN-γ (100 ng/ml) alone or combination of either IL-13 (100 ng/ml) or IL-4 (100 ng/ml) for 48 h and the levels of IL-6 and RANTES were measured by ELISA kit. Data shown are mean ±S.D. of four experiments conducted using different sources of keratinocytes. $^{**}P < 0.01$, $^{*}P < 0.05$, significantly different from the mean value of the unstimulated control. $\#P < 0.05$, significantly different from the mean value of the IFN-γ-stimulated cells.

cytes upon stimulation with these cytokines. As shown in Fig. 4, even though IL-4 or IL-13 alone could not induce secretion of IL-6 and RANTES from the HPKs 48 h after stimulation, the presence of IL-13 or IL-4 significantly enhanced the IFN-γ-stimulated secretion of these cytokines. IL-8 and IP-10, two cytokines involves in the psoriasis, were not produced by the primary keratinocyte stimulated by IL-4 or IL-13 alone. Also the IFN-γ-induced secretion of these cytokines was tend to increase but not significantly modified by the presence of IL-4 or IL-13 (Data not shown).

4. Discussion

The biological effects of IL-4 and IL-13 are thought to be mediated by a shared receptor composed of IL-4Rα and IL-13Rα1 chain, expressed on a variety of cell types. In this study, we examined the expression of IL-13Rα1 in the skin of chronic inflammatory disease and the regulation of IL-13Rα1, IL-13Rα2 mRNA expression in HPK.

We first demonstrated that there were differences in the expression patterns of IL-13Rα1 in the skin, especially epidermal layer of keratinocytes of normal, AD and psoriasis. Although the epidermal hyperplasia is a characteristics observed in both AD and psoriasis, the biochemical mechanisms or mediators responsible for this remain unclear. Recent reports have suggested that the signaling through the IL-20 receptor, which binds both IL-19 and IL-20, causes proliferation and aberrant differentiation of keratinocytes in the psoriasis [31,32]. Thus, it is tempting to hypothesis that different staining patterns of IL-13Rα1 observed in AD vs. psoriasis reflect the different mechanism of hyperplasia between them. Another possibility to explain this phenomenon may come from the different cytokine environment in AD and psoriasis. AD is the chronic inflammatory disease that has biphasic cytokine response, predominant Th2 cytokine such as IL-4, IL-5, IL-10 and IL-13 in acute phase, while Th1 cytokine, especially IFN-γ accompanied by Th2 cytokines in chronic phase [33]. Whereas, the predominant cytokine produced in psoriasis is reported to be IFN-γ, IL-15 and less prominent, IL-13 [34]. Consisted with our in vitro data in which IFN-γ appears to be a major cytokine for the induction of IL-13Rα1, the expression of IL-13Rα1 was much higher in the skin of psoriasis than AD. These phenomena may reflect the predominance of IFN-γ in the psoriasis compared to the AD that may have other inhibitory cytokine, such as IL-4. Cancino-Diaz et al. [35] have recently demonstrated that overexpression of the mRNA but underexpression of the protein of IL-13Rα1 in psoriatic keratinocyte. The clear discrepancy in the detection of IL-13Rα1 protein observed between them and us may come from the different antibody used for the detection of IL-13Rα1, since we have not get similar staining using commercially available antibody and same skin sample (Data not shown). Or because of we used frozen section instead of parafolmalized-paraffin section. We have observed different regulation of IL-13Rα1 or IL-13Rα2 mRNA in primary keratinocyte by IL-4 and IL-13 (Fig. 3). Since the signaling unit for the IL-4 and IL-13 on non-haematopoietic cell is though to be a heterodimer consisting of IL-4Rα and IL-13Rα1 subunit, we do expect that IL-4 and IL-13 have similar effect. To explain this phenomenon, we look for the IL-4 specific signaling unit, common γ chain. Even though we could detect the slight

mRNA expression for common γ chain in resting keratinocyte and the enhancement of the expression in keratinocyte with IFN-γ stimulation, no detectable surface protein was observed by FACS analysis in any conditions of keratinocytes. Thus, it is reasonable to think that IL-4 and IL-13 signal through the same receptor unit, IL-4Rα/IL-13Rα1, or keratinocytes express new signaling unit as has been reported in the myofibroblast (IL-13Rα1, IL-4Rα plus IL-13Rα2 or IL-13Rα1, IL-4Rα plus common γ chain) [36,37].

Consisted with previous reports [25,35,38], the expression of IL-4Rα mRNA was simultaneously overexpressed in the skin of patients with psoriasis and AD. Since the affinity difference of IL-4 and IL-13 to each subunit has been reported [39], the proper ratio of IL-13Rα1 to IL-4Rα may determine the responsibility of the cell to IL-4 or IL-13.

So far, we do not know how IL-4 and IL-13 has different biological effects on keratinocytes through the same receptor unit consists of IL-13Rα1/IL-4Rα. Recent experiment using HaCaT cell line has reported that IL-4 and IL-13 have different effect on the TNF-α-and IFN-γ-induced secretion of Thymus and activation-regulated chemokine and interferon-induced protein (IP)-10 [40]. Thus further experiment is required to elucidate the signaling unit shared or unshared by IL-4 and IL-13 in human keratinocytes.

The regulation of decoy receptor, IL-13Rα2 has been recently reported in different cells. IFN-γ stimulates the rapid mobilization of IL-13Rα2 from intracellular storage to the cell surface without protein synthesis and resulted in diminished IL-13 signaling in nasal epithelial cell, monocytic cell (U937) and lung carcinoma (A549) [18]. Other report using HaCaT showed that IL-4 and IL-13 itself induce the IL-13Rα2 mRNA by STAT6, ERK and p38 MAPK dependent manner [41]. In contrast to their data, our experiment revealed that IL-4 or IL-13 alone could not, but IFN-γ plus IL-4 could induce the IL-13Rα2 mRNA in primary keratinocyte. The difference between our data and their data may come from primary vs. immortalized cell line. Since several differences in the response have been reported between HaCaT and primary human keratinocytes [42].

Much has not been known about the functional significance of IL-13 on the HPK. There were several reports about the secretion of cytokines or chemokines using immortalized keratinocytes, A432, SVK14 and HaCaT but not in the primary keratinocytes [30,40]. Although the induction of CD60w has been demonstrated in primary keratinocytes by IL-4 and IL-13 and its suppression by IFN-γ [43], the biological significance of CD60w in psoriasis remains unclear. Recent report has suggested the subcutaneous administration of IL-4 improved the psoriasis by antagonizing the IFN-γ-producing T cells and reducing the concentration of IL-8 and IL-19 in the skin, two cytokines directly involved in psoriasis [31]. The target cell to this treatment is not clear. In addition to T cells, the keratinocyte, which express IL-4Rα/IL-13Rα1, may be a direct target to IL-4. It is interesting to know whether IL-13 has same effect or not.

Collectively we found that up-regulation of IL-13Rα1 in the skin of chronic inflammatory disease, such as AD or psoriasis and suggest the possibility that the expression of IL-13Rα1 and IL-13Rα2 is modulated by the IFN-γ, IL-13 or IL-4 or combination of these cytokines. We also found using primary human keratinocytes that regulatory mechanism of IL-13Rα by IL-13 and IL-4 was not the same. These results may help to explain complicated pathology observed in the chronic phase of AD or psoriasis and to understand the role of IL-13 in the function of keratinocyte, such as proliferation or differentiation or any other unknown function in physiological and pathological conditions.

Acknowledgements

This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology. Dr Wongpiyabovorn Jongkonnee was supported by grants from Japan International Cooperation Agency (JICA). The authors thank to Drs Kamolwat, P., Suga, Y., Hira, K., Hasegawa, T for providing us valuable clinical samples and Drs Nakano, N, Nakayama, K. and Ms Tokura, T. for technical assistances.

References

[1] Minty A, Chalon P, Derocq JM, Dumont X, Guillemot JC, Kaghad M, et al. Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. Nature 1993;362:248–50.

[2] Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. Science 1998;282:2258–60.

[3] Defrance T, Carayon P, Billian G, Guillemot JC, Minty A, Caput D, et al. Interleukin 13 is a B cell stimulating factor. J Exp Med 1994;179:135–43.

[4] Doherty TM, Kastelein R, Menon S, Andrade S, Coffman RL. Modulation of murine macrophage function by IL-13. J Immunol 1993;151:7151–60.

[5] de Waal Malefyt R, Figdor CG, Vries JE. Effects of interleukin 4 on monocyte functions: comparison to interleukin 13. Res Immunol 1993;144:629–33.

[6] Kotowicz K, Callard RE, Friedrich K, Matthews DJ, Klein N. Biological activity of IL-4 and IL-13 on human endothelial