

The skin fungus-induced Th1- and Th2-related cytokine, chemokine and prostaglandin E₂ production in peripheral blood mononuclear cells from patients with atopic dermatitis and psoriasis vulgaris

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Summary

Background It is suggested that skin fungi may be involved in the development of atopic dermatitis (AD) and psoriasis vulgaris (PV).

Objective We studied skin fungus-induced Th1- or Th2-related cytokine, chemokine and prostaglandin E₂ (PGE₂) secretion in peripheral blood mononuclear cells (PBMC) from patients with AD and PV and normal subjects.

Methods PBMC were cultured with the extracts of *Malassezia furfur* (MF), *Candida albicans* (CA) and *Trichophyton rubrum* (TR). The cytokine, chemokine and PGE₂ amounts in the supernatants were measured by enzyme-linked immunosorbent assays.

Results MF induced IL-4 and macrophage-derived chemokine (MDC) secretion in AD patients, while induced IFN- γ and interferon-inducible protein of 10 kDa (IP-10) secretion in PV patients, however, did not induce either secretion in normal subjects. CA induced IL-4, MDC, IFN- γ and IP-10 secretion in AD and PV patients and normal subjects. In AD patients, the magnitude of IL-4 and MDC responses to CA was higher than that to MF. Compared with PV patients and normal subjects, the magnitude of IL-4 and MDC responses to CA was higher while that of IFN- γ and IP-10 responses to CA was lower in AD patients. TR induced moderate IL-4 and MDC secretion only in AD patients. The three fungi induced higher levels of PGE₂ secretion in AD patients than in PV patients and normal subjects. Cyclooxygenase-2 inhibitor NS-398 suppressed PGE₂ responses to MF, CA and TR, and partially suppressed IL-4 and MDC responses to MF, CA and TR, while enhanced IFN- γ and IP-10 responses to CA in AD patients, and these effects of NS-398 were reversed by cyclic AMP analogue.

Conclusion AD patients manifest Th2-skewed responses to MF, CA and TR, which may be partially attributable to the enhanced PGE₂ responses to these fungi. PV patients manifest Th1-skewed responses to MF.

Keywords atopic dermatitis, *Candida albicans*, chemokine, cytokine, *Malassezia furfur*, prostaglandin E₂, psoriasis vulgaris, Th1, Th2, *Trichophyton rubrum*

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Introduction

It is reported that fungi causing cutaneous infection, *Malassezia furfur* (MF), *Candida albicans* (CA) and *Trichophyton rubrum* (TR), may be related to the development of atopic dermatitis (AD) [1]. MF is a part of normal microflora of human adult skin and is preferentially colonized in the seborrheic area. PO-specific IgE antibodies are detected in the sera of patients with AD [2]. AD lesions located on the seborrheic area responded favourably to anti-mycotic treatment [3], suggesting the linkage between MF and AD on the seborrheic area. MF may also be related to the pathogenesis of psoriatic eruption on the

seborrheic area [4]. The relationship between intestinal CA and AD or psoriasis is also suggested [2, 5]. On the other hand, there are fewer reports on the relations between TR and AD or psoriasis [6].

It is known that T cells from AD patients preferentially produce Th2-type cytokines, such as IL-4 inducing B cell IgE production, in response to environmental antigens like house dust mite [7]. On the other hand, the mite-induced production of Th1-type cytokines, such as IFN- γ inhibiting B cell IgE production, is down-regulated in AD patients [7]. In contrast, psoriasis is characterized by Th1-shifted immunity [8]. It is thus anticipated that IL-4 or IFN- γ responses to fungal antigens may be enhanced in AD or psoriatic patients, respectively.

Fungus cells or components are internalized by antigen-presenting cells (APC) like monocyte/macrophages, B cells or dendritic cells [9], and induce their prostaglandin E₂ (PGE₂) secretion [10, 11]. PGE₂ inhibits IFN- γ production, although

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it does not alter or up-regulate IL-4 production in T cells [12]. These effects of PGE₂ are mainly mediated by increasing intracellular cyclic AMP (cAMP), as PGE₂ receptor is coupled to cAMP-synthesizing adenylate cyclase [13]. It is reported that spontaneous or lipopolysaccharide-induced PGE₂ secretion by monocytes is enhanced in AD patients compared with normal subjects [14, 15], indicating the contribution of PGE₂ to Th2-shifted immunity in AD patients.

A CXC chemokine, IFN-inducible protein of 10 kDa (IP-10) preferentially attracts Th1 cells, while a CC chemokine, macrophage-derived chemokine (MDC) preferentially attracts Th2 cells [16]. The effective interaction of APC and T cells induces IP-10 or MDC production by both APC and T cells [16, 17]. IFN- γ promotes IP-10 but suppresses MDC production, while IL-4 has opposite effects [16, 17]. PGE₂ inhibits IP-10, although it promotes MDC production by APC [16]. Thus IP-10 or MDC may be produced in Th1- or Th2-favouring conditions, respectively.

In this study, we examined IL-4, IFN- γ , IP-10, MDC and PGE₂ secretion in response to MF, CA or TR extracts by peripheral blood mononuclear cells (PBMC) from patients with AD and psoriasis vulgaris (PV). We also investigated if the inhibition of PGE₂ responses to fungi may alter the Th1/Th2 responses in these patients, in order to know the involvement of PGE₂ in the Th1/Th2 response to fungi.

Materials and methods

Patients and controls

We examined patients with AD ($n = 20$; 10 men and 10 women; age 34.2 ± 4.8 years, mean $\pm$ SEM), PV ($n = 15$; eight men and seven women; age 37.6 ± 5.3 years) and normal healthy subjects ($n = 15$; eight men and seven women; age 35.6 ± 4.9 years). The diagnosis of AD was based on the criteria suggested by Hanifin and Rajka [18]. Among 20 AD patients, 11 patients had acute skin lesions with erythema, papulation and excoriation, while nine had chronic skin lesions with scaling, dryness and lichenification. The diagnosis of PV was based on clinical and histological criteria. Among 15 PV patients, six patients had chronic stationary plaque lesions without peripheral spread, six with active plaque lesions spreading peripherally, and three with active guttate lesions. None of the normal subjects had symptoms indicative of allergic diseases or seborrheic dermatitis and none was receiving medication. Serum total IgE value of AD, PV patients and normal subjects was 4729 ± 573 , 78.5 ± 9.6 and 74.2 ± 10.8 IU/mL (mean $\pm$ SEM), respectively. All the AD patients were positive for serum IgE RAST against MF, CA and TR (≥ 0.7 U_A/mL) and the value for MF, CA or TR was 8.0 ± 0.9 , 13.2 ± 1.2 or 4.1 ± 0.4 U_A/mL (mean $\pm$ SEM), respectively. Sera from normal subjects and PV patients were negative for any fungus-specific IgE (< 0.34 U_A/mL).

At the time of the study, 16 AD patients and 12 PV patients were treated with topical corticosteroids of mild to very strong rank. Twelve AD patients and eight PV patients were taking anti-allergic or antihistaminic drugs, and there was at least 18 h between the last drug intake and the blood examination. None of the AD and PV patients were taking systemic corticosteroids

or other immunosuppressants or receiving desensitization immunotherapy. None of the AD and PV patients and normal subjects were treated with topical or systemic antimycotics and had active infection by fungi, viruses or bacteria at the time of the investigation. All the AD and PV patients and normal subjects were informed of the objectives and methods of this study and consented to participate.

Reagents

The extracts from CA and TR cells and spores were purchased from Greer (Lenoir, NC, USA), and were prepared as described [19]. MF extract was purchased in lyophilized form from Allergon AB (Angelholm, Sweden), and was reconstituted in phosphate buffered saline. Tetanus toxoid (TT) of *Clostridium tetani*, 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethylpyrazole (SC-560), N-(2-cyclohexyloxy-4-nitrophenyl)methanesulphonamide (NS-398) and dibutyrylcAMP (Bt₂cAMP) were from Calbiochem (San Diego, CA, USA). Indomethacin was purchased from Sigma (St Louis, MO, USA).

Cell culture

Heparinized blood from AD and PV patients and normal subjects was diluted 1/1 with Hanks' balanced salt solution, and was layered over discontinuous Percoll (Pharmacia, Upssala, Sweden) gradients with densities of 1.070 and 1.079 g/mL followed by centrifugation as described [20]. PBMC were harvested from the upper cell layer. As examined by Toluidine blue and Diff-Quick staining, basophils, neutrophils and eosinophils were not detectable in the PBMC fraction. The PBMC fraction routinely consisted of 64 to 75% CD3⁺ T cells, 5 to 10% CD20⁺ B cells, 15 to 20% CD14⁺ monocytes and 5% CD16⁺ NK cells. PBMC (2×10^6 cells/well) were cultured in triplicate with or without 10 μ g/mL MF extract, 0.1% CA extract, or 0.1% TR extract or 5 μ g/mL TT in 1 mL of culture medium in 24-well plates at 37°C for 18 h to examine PGE₂ secretion, for 5 days to examine IFN- γ and IL-4 secretion, or for 3 days for MDC and IP-10. The medium used was RPMI 1640 (Gibco/BRL, Grand Island, NY, USA), supplemented with 10% fetal bovine serum, 100 U/mL penicillin G and 100 μ g/mL streptomycin (Gibco/BRL).

To examine the effects of cyclooxygenase (COX) inhibitors and cAMP analogue, PBMC were pre-incubated with NS-398, SC-560 or indomethacin (each 1 μ M) in the presence or absence of Bt₂cAMP 50 μ M for 1 h, then fungal extract was added and the cells were incubated during the indicated periods. The amount of PGE₂ in the supernatants was measured by enzyme-linked immunosorbent assay (ELISA) (Cayman Chemicals, Ann Arbor, MN, USA). The MDC amount was measured by ELISA (R & D Systems, Minneapolis, MN, USA), and that of IP-10 was measured by ELISA (HyCult Biotechnology, b.v. Uden, Netherlands). The IFN- γ and IL-4 amounts were measured by ELISA (Biosource, Camarillo, CA, USA). The detection limit for PGE₂, MDC and IP-10 was 15, 62.5 and 20 pg/mL, respectively, and that for IFN- γ and IL-4 was 4 and 0.27 pg/mL, respectively. The fungus-induced PGE₂, cytokine or chemokine secretion was calculated as each concentration with fungus minus that without (background secretion). We

considered positive response to fungus if the fungus-induced secretion was more than background.

Statistical analyses

One-way analysis of variance with Scheffe's multiple comparison test was used for the comparison of means in Figs 1 to 4. Spearman's correlation coefficient was determined as shown in Table 1. A value of $P < 0.05$ was considered significant.

Results

IL-4 and IFN- γ responses to fungi

All the AD patients showed IL-4 response to MF, while this was seen in very few PV patients and normal subjects (Fig. 1a). IL-4 response to CA was commonly seen in AD, PV and normal groups (Fig. 1c); however, the magnitude of the response was higher in AD patients than in the other two groups. AD patients showed moderate IL-4 response to TR, while this was seen in very few PV patients and normal subjects (Fig. 1e). In AD patients, the magnitude of IL-4 response to CA was highest, followed by MF, while that to TR was lowest. Very few AD and PV patients and normal subjects showed IL-4 response to Th1-type recall antigen TT (Fig. 1g).

Most of the PV patients showed IFN- γ response to MF, while this was seen in very few AD patients and normal subjects

(Fig. 1b). Very few AD and PV patients and normal subjects showed IFN- γ response to TR (Fig. 1f). IFN- γ response to CA was common to AD, PV and normal groups; however, the magnitude of the response was lower in AD patients than in the other two groups (Fig. 1d). IFN- γ responses to TT was common to AD, PV and normal groups and the magnitude of the response was similar in the three groups (Fig. 2h), indicating that the low IFN- γ response to CA in AD patients was not caused by the general defect in IFN- γ production. When IL-4 or IFN- γ responses to fungi were compared among different subgroups in AD or PV patients, such as acute vs. chronic AD, there were no significant subgroup-to-subgroup differences (data not shown).

Table 1. The correlation of MF-, CA- or TR-specific IgE values with cytokine, chemokine or PGE₂ responses to the respective fungi in AD patients ($n = 20$)

	MF		CA		TR	
	r_s^*	P	r_s	P	r_s	P
Δ IL-4	0.671	0.0032	0.717	0.0018	0.612	0.0046
Δ IFN- γ	-0.120	0.6019	-0.639	0.0054	0.284	0.2153
Δ MDC	0.864	0.0002	0.869	0.0002	0.607	0.0081
Δ IP-10	0.342	0.1710	-0.529	0.0212	-0.097	0.6532
Δ PGE ₂	0.603	0.0102	0.644	0.0048	0.612	0.0093

*Spearman's correlation coefficient.

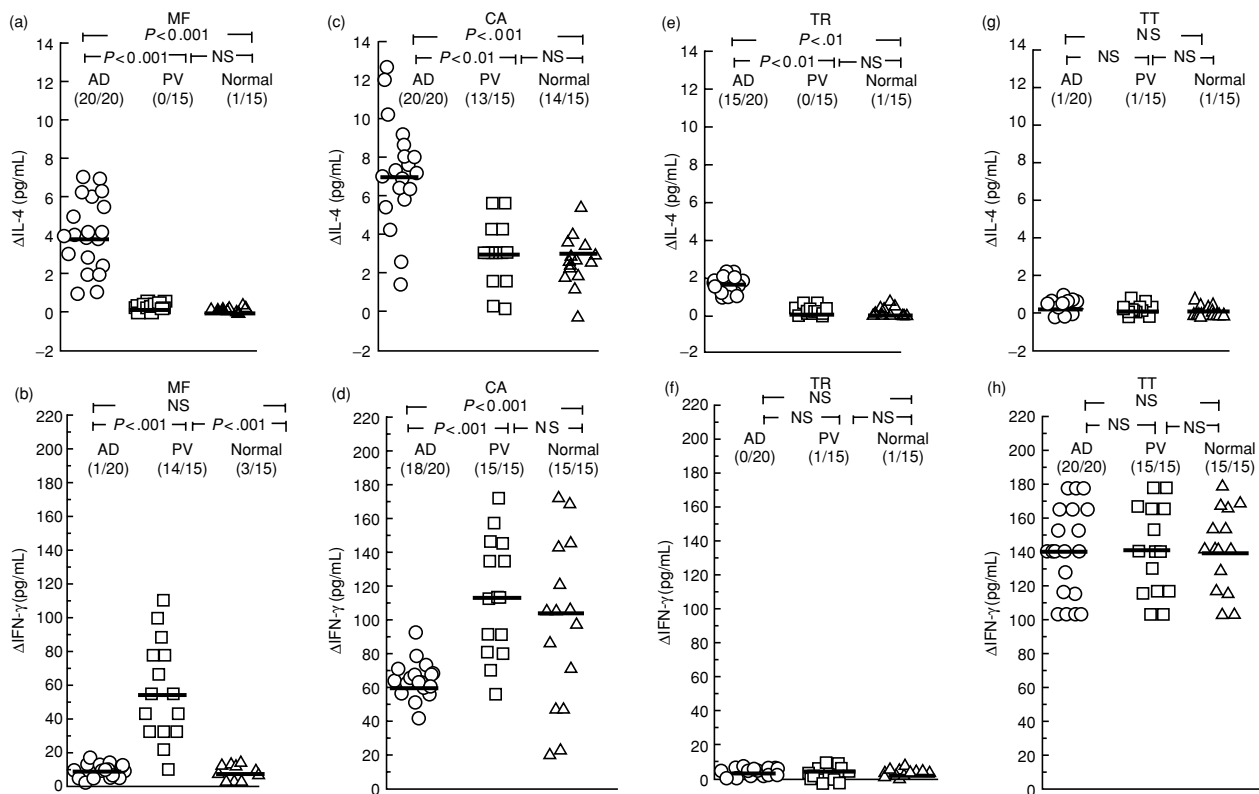


Fig. 1. IL-4 (a, c, e, g) and IFN- γ (b, d, f, h) responses to MF, CA, TR and TT. Each point represents the mean of triplicate cultures from each donor. Bars show the group means. Values in parentheses are the number of positive responders/total donor number. The following are background cytokine secretions in AD, PV patients or normal subjects, respectively: IL-4 0.50 ± 0.06 (mean $\pm$ SEM), 0.45 ± 0.07 , or 0.45 ± 0.04 pg/mL; IFN- γ 14.8 ± 2.0 (mean $\pm$ SEM), 15.1 ± 2.2 , or 14.0 ± 1.2 pg/mL. There were no significant differences in the background cytokine secretion among the three groups. NS = not significant.

MDC and IP-10 responses to fungi

MDC response to each fungus was associated with IL-4 response to the respective fungus; MDC responses to MF (Fig. 2a) and TR (Fig. 2e) were only seen in AD patients. MDC response to CA was common to AD, PV and normal groups, and the magnitude of the response was higher in AD patients than in the other two groups (Fig. 2c). In AD patients,

the magnitude of MDC response to CA was highest, followed by MF, while that to TR was lowest. MDC response to TT was not seen in any of the three groups (Fig. 2g). IP-10 response to each fungus was associated with IFN- γ response to the respective fungus; IP-10 response to MF was only seen in PV patients (Fig. 2b). IP-10 response to CA was common to AD, PV and normal groups (Fig. 2d), and the magnitude of the response was lower in AD patients than in the other two groups. IP-10

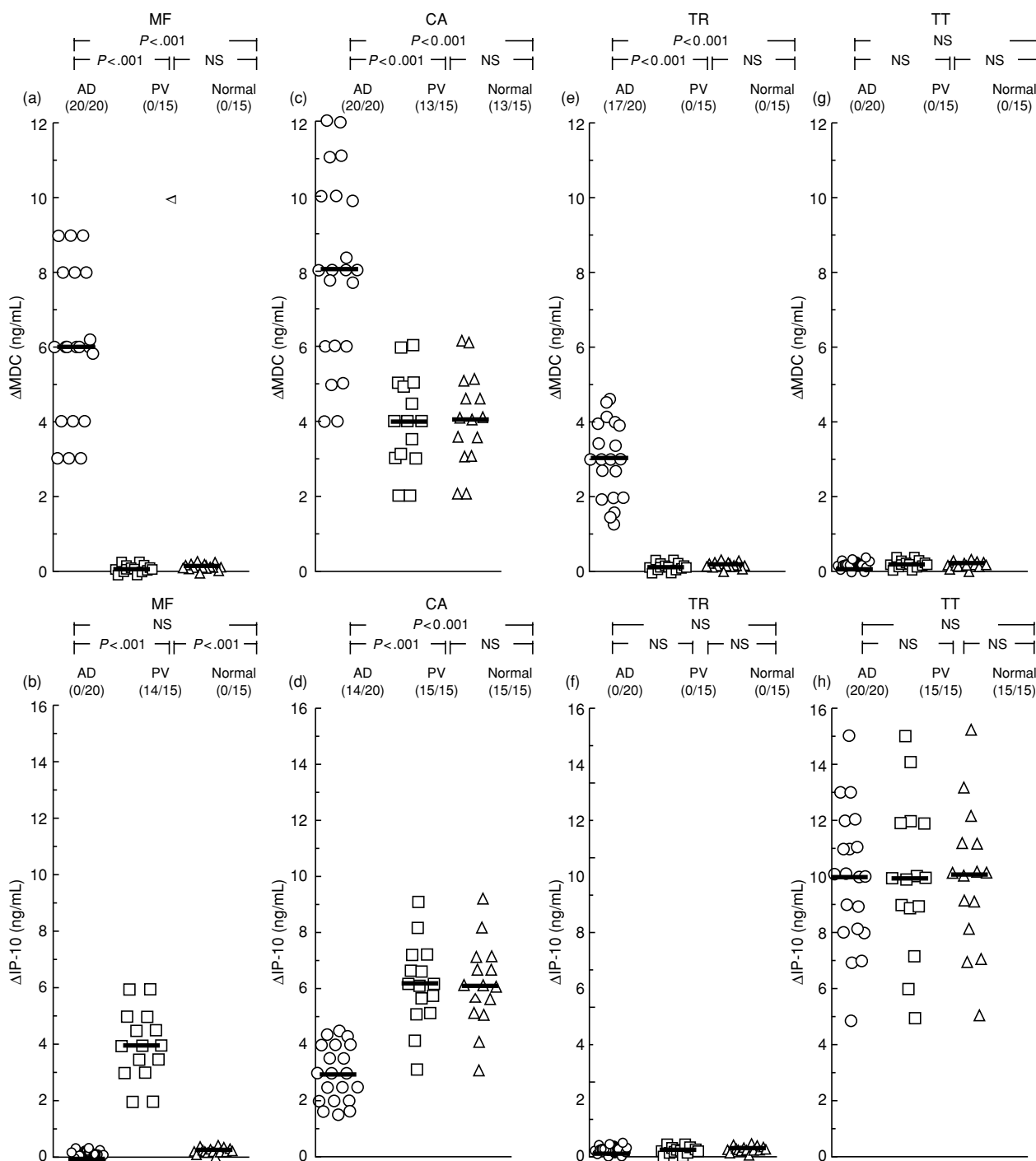


Fig. 2. MDC (a, c, e, g) and IP-10 (b, d, f, h) responses to MF, CA, TR and TT. The following are background chemokine secretions in AD, PV patients or normal subjects, respectively: MDC 2.1 ± 0.3 (mean $\pm$ SEM), 2.2 ± 0.4 , or 1.9 ± 0.3 ng/mL; IP-10 2.3 ± 0.3 , 2.2 ± 0.4 , or 2.1 ± 0.4 ng/mL. There were no significant differences in the background chemokine secretion among the three groups.

response to TR was not seen in any of the three groups (Fig. 2f). All the AD and PV patients and normal subjects showed IP-10 responses to TT (Fig. 2h). When MDC or IP-10 responses to fungi were compared among different subgroups in AD or PV patients, there were no significant subgroup-to-subgroup differences (data not shown).

PGE₂ responses to fungi

PGE₂ responses to MF and CA were common to AD, PV and normal groups (Fig. 3a, b); however, the magnitude of the responses was higher in AD patients than in the other two groups. The incidence and the magnitude of PGE₂ response to TR were higher in AD patients than in the other two groups (Fig. 3c). TT did not induce PGE₂ response in any of the three groups (Fig. 3d). In AD, PV and normal groups, the magnitude of PGE₂ response to CA was highest, followed by MF, while that to TR was lowest. When PGE₂ responses to fungi were compared among different subgroups in AD or PV

patients, there were no significant subgroup-to-subgroup differences (data not shown).

PGE₂ synthesis requires the activity of cyclooxygenase (COX), which converts arachidonic acid to PGH₂, a precursor of PGE₂ [21]. There are two kinds of COX; COX-1 is constitutively expressed, while COX-2 is induced by certain stimuli [21]. We thus examined the contribution of COX-1 or COX-2 to fungus-induced PGE₂ release. COX-1-specific inhibitor SC-560 and COX-1 and COX-2 inhibitor indomethacin almost completely suppressed basal (control) PGE₂ secretion without fungi in AD (Fig. 3e), PV (Fig. 3f) and normal groups (Fig. 3g), while COX-2-specific inhibitor NS-398 did not alter the basal PGE₂ secretion. This indicates that basal PGE₂ secretion is mainly mediated by COX-1. On the other hand, NS-398 reduced PGE₂ secretion in the presence of MF, CA or TR to the basal level in AD, PV and normal groups, while indomethacin almost completely suppressed the PGE₂ secretion in the presence of each fungus. This indicates that each fungus-induced PGE₂ secretion

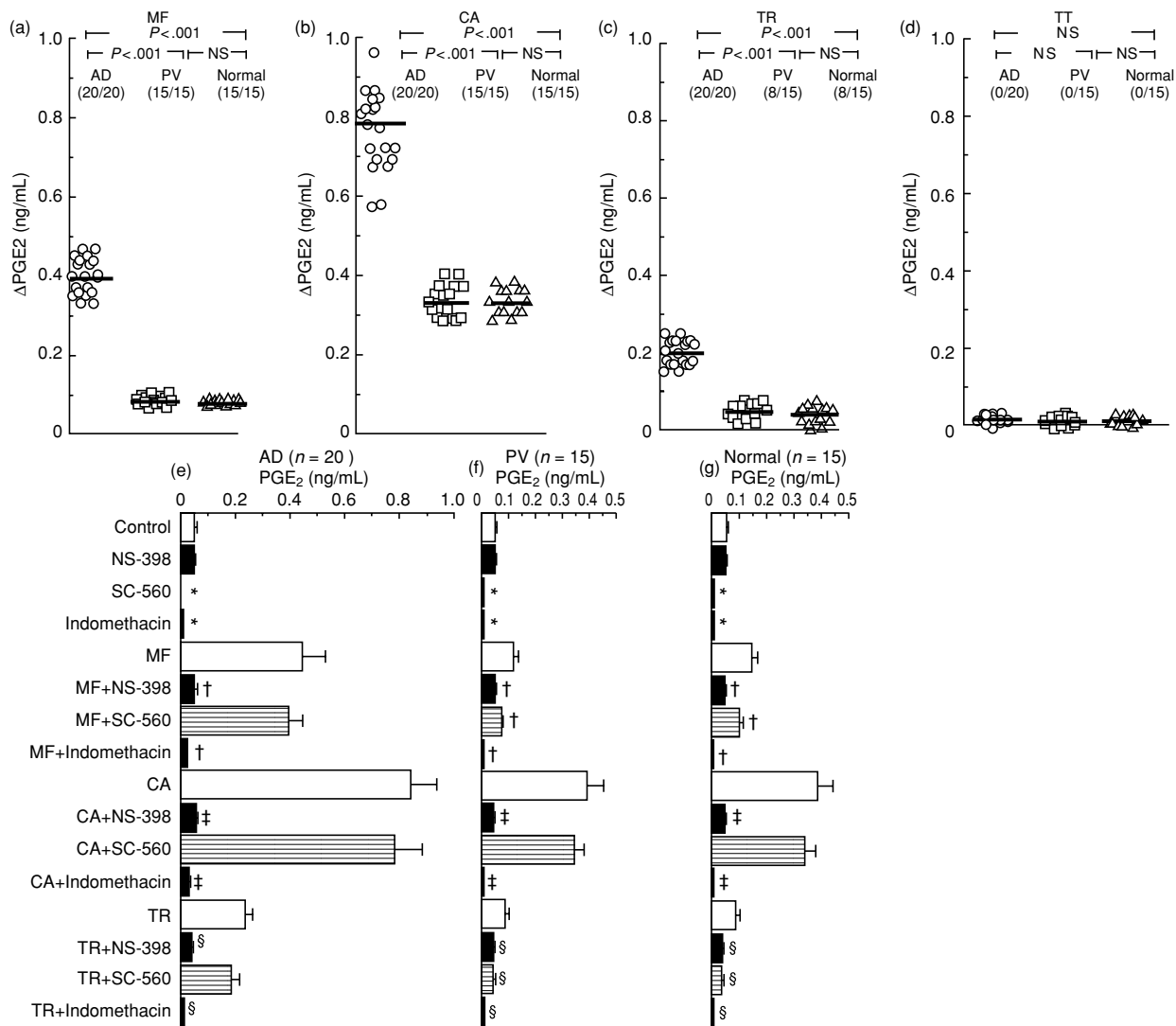


Fig. 3. MF (a), CA (b), TR (c) or TT (d) induced PGE₂ secretion and the effects of COX inhibitors on PGE₂ secretion in AD (e), PV (f) patients and normal subjects (g). There were no significant differences among the three groups in the basal (control) PGE₂ secretion showing in (e)(f)(g). In (e)(f)(g), the data are expressed as mean $\pm$ SEM. **P* < 0.05 vs. control values with medium alone, †*P* < 0.05 vs. values with MF alone, ‡*P* < 0.05 vs. values with CA alone, §*P* < 0.05 vs. values with TR alone.

may be mainly mediated by COX-2. Compared with NS-398 or indomethacin, the inhibition by SC-560 on PGE₂ secretion in the presence of each fungus was moderate. This indicates that SC-560 may mainly inhibit basal PGE₂ secretion and may not affect that induced by fungi.

The effects of COX-2 inhibitor on Th1 and Th2 responses to fungi

It is reported that PGE₂ promotes IL-4 and MDC production while inhibiting IFN- γ and IP-10 production by elevating the cAMP level in the target cells [12, 13, 16]. We thus examined if the inhibition of each fungus-induced PGE₂ release by NS-398 may alter IL-4, IFN- γ , IP-10 and MDC responses to the respective fungi and if cAMP analogue Bt₂cAMP may reverse the effects of NS-398. In AD (Figs 4a and b), PV (Figs 4e and f) and normal groups (Figs 4i and j), NS-398 reduced IL-4 and MDC responses to CA by 35 to 50% of controls, and Bt₂cAMP reversed the reduction by NS-398. In AD patients, NS-398 reduced IL-4 and MDC responses to MF and TR by 40 to 50% of controls, and Bt₂cAMP reversed the reduction by

NS-398. In PV and normal groups, IL-4 and MDC responses to MF and TR were not altered by NS-398 or NS-398 plus Bt₂cAMP. These results suggest that PGE₂ induced by CA may enhance IL-4 and MDC responses to CA in AD, PV and normal groups, and that cAMP may mediate the effects of PGE₂. PGE₂ induced by MF and TR may similarly enhance IL-4 and MDC responses to the respective fungi in AD patients, but may not in PV and normal groups. IL-4 and MDC responses to TR were not altered by NS-398 or NS-398 plus Bt₂cAMP in AD, PV and normal groups (data not shown).

NS-398 increased IFN- γ and IP-10 responses to CA by 30 to 53% of controls in AD (Figs 4c and d), PV (Figs 4g and h) and normal groups (Figs 4k and l), and the increase by NS-398 was reversed by Bt₂cAMP. IFN- γ and IP-10 responses to MF and TR in AD and normal groups and those to TR in PV patients were not significantly altered by NS-398 or NS-398 plus Bt₂cAMP. NS-398 slightly (by 15% and 13% of controls, respectively) increased IFN- γ and IP-10 responses to MF in PV patients; however, the increase was not statistically significant. These results indicate that PGE₂ induced by CA may suppress

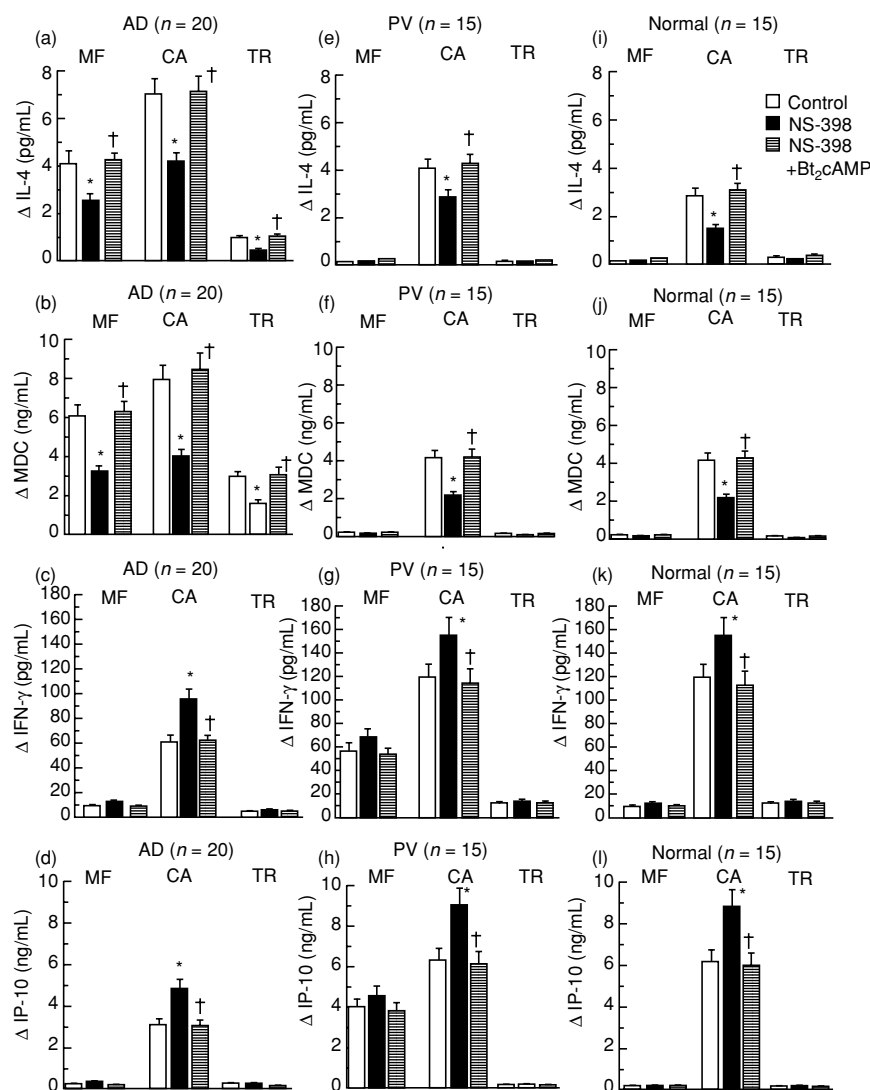


Fig. 4. The effects of NS-398 on IL-4 (a, e, i), MDC (b, f, j), IFN- γ (c, g, k) or IP-10 (d, h, l) responses to fungi and the reversal by Bt₂cAMP. PBMC from AD or PV patients or normal donors were incubated with MF, CA or TR extracts in the presence or absence of NS-398 (1 μ M) or NS-398 plus Bt₂cAMP (50 μ M), and cytokine or chemokine production was analysed. The left three columns show the responses to MF, the middle three columns show the responses to CA, and the right three columns show the responses to TR in each figure. The data are expressed as mean $\pm$ SEM. * P < 0.05 vs. control values with each fungus alone, and † P < 0.05 vs. values with each fungus plus NS-398. The background cytokine or chemokine secretion was shown in legends to Figs 1 and 2.

IFN- γ and IP-10 responses to CA via cAMP in AD, PV and normal groups, while that induced by MF or TR may not. IFN- γ and IP-10 responses to TT were not altered by NS-398 or NS-398 plus Bt₂cAMP in AD, PV and normal groups (data not shown).

The correlation of MF, CA or TR-specific IgE values with cytokine, chemokine and PGE₂ responses to the respective fungi in AD patients

We finally examined the correlation of serum fungus-specific IgE values with cytokine, chemokine or PGE₂ responses to the respective fungi in AD patients. IL-4, MDC and PGE₂ responses to MF, CA or TR positively correlated with the respective fungus-specific IgE values (Table 1). On the other hand, IFN- γ and IP-10 responses to CA inversely correlated with CA-specific IgE value.

Discussion

MF induced IL-4 response in PBMC from AD patients and induced IFN- γ response in PV patients but did not induce either response in normal subjects. In this study, the main source of IL-4 and IFN- γ in PBMC may be T cells, as basophils or eosinophils were not contaminated. Thus T cells of AD and PV patients may be *in vivo* more potently sensitized to MF than those of normal subjects. We should further examine if T cells derived from PV or AD skin lesions may also show the Th1- or Th2-shifted responses to MF, respectively.

Though IL-4 and IFN- γ responses to CA were common to AD and PV patients and normal subjects, the response in AD patients was shifted to Th2. However, conflicting results have been reported on Th1 responses to CA in AD patients. Some studies reported that contact hypersensitivity to CA was suppressed in AD patients [22], indicating the suppressed Th1 response to CA. However, the other studies reported that IFN- γ response to CA was rather enhanced in AD patients [1, 23]. Such discrepancy may be due to the difference in experimental conditions, such as antigen preparation or patient population.

IL-4 response to TR was specific to AD patients. However, the magnitude of the response was much lower than that to the other two fungi, indicating the lower antigenicity of TR. Though mannans or mannoproteins are major components of fungus cell walls, and may be the cross-reactive fungal antigens [1], their structure and physico-chemical properties may differ among the fungus species, which may lead to the difference in antigenicity. Recently, several immunogenic proteins unique to each fungus have been sequenced; Malf 7, Malf 8 and Malf 9 from MF [24], or Tri r 2 from TR [25]. The immune responses to these proteins should be further examined.

The MDC or IP-10 response to each fungus was closely associated with that of IL-4 or IFN- γ , respectively. It is thus anticipated that MDC may attract Th2 cells into the atopic skin lesions where fungal antigen-presenting APC interact with the antigen-activated Th2 cells, and thus amplify the Th2-mediated immune responses. In contrast, IP-10 may similarly amplify the Th1-mediated inflammation in psoriatic skin lesions.

PGE₂ responses to fungi were mediated by COX-2. The inhibition of CA-induced PGE₂ release by COX-2 inhibitor resulted in the reduction of IL-4 and MDC responses and in

the enhancement of IFN- γ and IP-10 responses to CA in AD and PV patients and normal subjects. These effects of COX-2 inhibitor were reversed by cAMP analogue. Taken together, it is suggested that PGE₂ induced by CA may promote Th2 responses and suppress Th1 responses to CA by elevating cAMP level in the target cells either from AD or PV patients or normal donors. In PV patients, the amount of PGE₂ induced by MF was much lower than that induced by CA and may be insufficient to suppress Th1 responses to MF. In AD patients, PGE₂ responses to MF and TR appear to promote IL-4 and MDC responses to the respective fungi as well as CA. PGE₂ responses to MF, CA and TR were all enhanced in AD patients, and this may contribute to the Th2-shifted responses to these fungi. Besides PGE₂ is known to enhance IgE production of B cells [19]. PGE₂ responses to MF, CA or TR did correlate with the respective fungus-specific IgE values in AD patients as well as IL-4 and MDC responses. Collectively, PGE₂ induced by each fungus may potentiate the production of the respective fungus-specific IgE *in vivo* in AD patients directly or through the induction of IL-4 and MDC or the suppression of IFN- γ production.

It should be elucidated how fungus extract induces COX-2 in APC. It is anticipated that the binding of fungus extracts to APC may trigger the activation of signalling molecules like protein kinase C or mitogen-activated protein kinases, which activate transcription factors required for COX-2 gene expression [21, 26]. Fungus cells or components bind to mannose receptor, β -glucan receptor or complement receptor on APC [9]. As fungus extracts used in this study were not opsonized, their major receptors may not be a complement receptor but mannose or β -glucan receptor. It should also be clarified why PGE₂ responses to fungi were enhanced in AD patients. One possible reason is the increase of mannose receptors on APC in AD patients. As IL-4 or PGE₂ enhances mannose receptor expression [27, 28], the *in vivo* increased IL-4 or PGE₂ secretion in AD patients may have promoted the mannose receptor expression [9]. The second possibility is the presence of additional receptor(s) for fungal extracts in AD patients; fungus-specific IgE, which is bound to Fc ϵ RI on APC, may be one candidate. The cell surface expression of Fc ϵ RI and IgE on monocytes is enhanced in AD patients [29]. It is reported that the house dust mite allergen interacted with surface IgE on monocytes from AD patients, which led to the ligation of Fc ϵ RI and resulted in PGE₂ secretion [30].

Our results suggest that Th2-shifted responses to fungi in AD patients may be at least partially attributable to the enhanced PGE₂ responses to fungi. It is also indicated that COX-2 inhibitor can be therapeutically used to alleviate the Th2-shifted responses to fungi in AD patients. However, PGE₂ may not be the single inducer for the Th2 shift, which may involve the other chemical mediators or cytokines such as adenosine or IL-10. We should further examine the fungus-induced production of these chemical mediators or cytokines in AD patients.

References

- Viander M, Pene J, Kalimo K, Terho EO, Bousquet J. *Pityrosporum* and *Candida* specific and non-specific humoral, cellular and cytokine responses in atopic dermatitis patients. *Clin Exp Allergy* 2001; 31:125–34.

- 2 Savolainen J, Broberg A. Crossreacting IgE antibodies to *Pityrosporum ovale* and *Candida albicans* in atopic children. *Clin Exp Allergy* 1992; 22:469–74.
- 3 Back O, Scheynius A, Johansson SGO. Ketoconazole in atopic dermatitis: therapeutic response is correlated with decrease in serum IgE. *Arch Dermatol Res* 1995; 287:448–51.
- 4 Rosenberg EW, Noah PW, Skinner RB Jr, Zwaag RV, West SK, Browder JF. Microbial associations of 167 patients with psoriasis. *Acta Derm Venereol (Stockh)* 1989; 146 (Suppl.):72–5.
- 5 Buslau M, Menzel I, Holzman H. Fungal flora of human faeces in psoriasis and atopic dermatitis. *Mycoses* 1990; 33:90–4.
- 6 Dahl MV, Grando SA. Chronic dermatophytosis: what is special about *Trichophyton rubrum*? *Adv Dermatol* 1994; 9:97–111.
- 7 Wierenga EA, Snoek M, de Groot C et al. Evidence for compartmentalization of functional subsets of CD4+ T lymphocytes in atopic patients. *J Immunol* 1990; 144:4651–6.
- 8 Austin LM, Ozawa M, Kikuchi T, Walters IB, Krueger JG. The majority of epidermal T cells in psoriasis vulgaris lesions can produce type 1 cytokines, interferon- γ , interleukin-2, and tumor necrosis factor- α , defining TC1 (cytotoxic T lymphocyte) and TH1 effector populations: a type 1 differentiation bias is also measured in circulating blood T cells in psoriatic patients. *J Invest Dermatol* 1999; 113:752–9.
- 9 Buentke E, Zarugari A, Heffler LC, Avila-Carino J, Savolainen J, Scheynius A. Uptake of the yeast *Malassezia furfur* and its allergenic components by human immature CD1a+ dendritic cells. *Clin Exp Allergy* 2000; 30:1759–70.
- 10 Shibata Y, Metzger WJ, Myrvik QN. Chitin particle-induced cell-mediated immunity is inhibited by soluble mannan. Mannose receptor-mediated phagocytosis initiates IL-12 production. *J Immunol* 1997; 159:2462–7.
- 11 Castro M, Ralston NVC, Morgenthaler TI, Rohrbach MS, Limper AH. *Candida albicans* stimulates arachidonic acid liberation from alveolar macrophages through α -mannan and β -glucan cell wall components. *Infect Immun* 1994; 62:3138–45.
- 12 Snijderwint FGM, Kalinski P, Wierenga EA, Bos JD, Kapsenberg ML. Prostaglandin E₂ differentially modulates cytokine secretion profiles of human T helper lymphocytes. *J Immunol* 1993; 150:5321–9.
- 13 Phipps RP, Stein SH, Roper RL. A new view of prostaglandin E regulation of the immune response. *Immunol Today* 1991; 12:349–52.
- 14 Chan SC, Kim J-W, Henderson WR Jr, Hanifin JM. Altered prostaglandin E₂ regulation of cytokine production in atopic dermatitis. *J Immunol* 1993; 151:3345–52.
- 15 Snijders A, van der Pouw Kraan TCTM, Engel M et al. Enhanced prostaglandin E₂ production by monocytes in atopic dermatitis (AD) is not accompanied by enhanced production of IL-6, IL-10 or IL-12. *Clin Exp Immunol* 1998; 111:472–6.
- 16 Kuroda E, Sugiura T, Okada K, Zeki K, Yamashita U. Prostaglandin E₂ up-regulates macrophage-derived chemokine production but suppresses IFN-inducible protein-10 production by APC. *J Immunol* 2001; 166:1650–8.
- 17 Galli G, Chantry D, Annunziato F et al. Macrophage-derived chemokine production by activated human T cells *in vitro* and *in vivo*: preferential association with the production of type 2 cytokines. *Eur J Immunol* 2000; 30:204–10.
- 18 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* Supplement 1980; 92:44–7.
- 19 Zargari A, Harfast B, Johansson S, Johansson SGO, Scheynius A. Identification of allergen components of the opportunistic yeast *Pityrosporum orbiculare* by monoclonal antibodies. *Allergy* 1994; 49:50–6.
- 20 Kopley H, Craig S, Schwartz L. Purification of human basophils by density and size alone. *J Immunol Meth* 1994; 175:1–9.
- 21 Herschman HR. Prostaglandin synthase 2. *Biochim Biophys Acta* 1996; 1299:125–10.
- 22 Tanaka M, Aiba S, Matsumura N et al. IgE-mediated hypersensitivity and contact sensitivity to multiple environmental allergens in atopic dermatitis. *Arch Dermatol* 1994; 130:1393–401.
- 23 Savolainen J, Kosenen J, Lintu P et al. *Candida albicans* mannan- and protein-induced humoral, cellular and cytokine responses in atopic dermatitis patients. *Clin Exp Allergy* 1999; 29:824–31.
- 24 Rasool O, Zargari A, Almqvist J, Eshashi H, Whitley P, Schenynius A. Cloning, characterization and expression of complete coding sequences of three IgE binding *Malassezia furfur* allergens, Mal f 7, Mal f 8 and Mal f 9. *Eur J Biochem* 2000; 267:4355–61.
- 25 Woodfolk JA, Sung S-SJ, Benjamin DC, Lee JK, Platts-Mills TAE. Distinct human T cell repertoires mediate immediate and delayed-type hypersensitivity to the *Trichophyton* antigen, Tri r 2. *J Immunol* 2000; 165:4379–87.
- 26 Ogasawara A, Arakawa T, Kaneda T et al. Fluid shear stress-induced cyclooxygenase-2 expression is mediated by C/EBP β , cAMP-response element-binding protein, and AP-1 in osteoblastic MC3T3–E1 cells. *J Biol Chem* 2001; 276:7048–54.
- 27 Stein M, Keshav S, Harris N, Gordon S. Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med* 1992; 176:287–92.
- 28 Scriber S, Perkins SL, Teitelbaum SL, Chappel J, Stahl PD, Blum JS. Regulation of mouse bone marrow macrophage mannose receptor expression and activation by prostaglandin E and IFN- γ . *J Immunol* 1993; 151:4973–81.
- 29 Maurer D, Fiebiger E, Reininger B et al. Expression of functional high affinity immunoglobulin E receptors (Fc ϵ RI) on monocytes of atopic individuals. *J Exp Med* 1994; 179:745–50.
- 30 Takenaka M, Tanaka Y, Anan S, Yoshida H, Ra C. High affinity IgE receptor-mediated prostaglandin E₂ production by monocytes in atopic dermatitis. *Int Arch Allergy Immunol* 1995; 108:247–53.