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INVESTIGATIVE REPORT

Indomethacin-induced Reduction in CRTH2 in Eosinophilic Pustular Folliculitis (Ofuji's Disease): A Proposed Mechanism of Action

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Eosinophilic pustular folliculitis is an inflammatory skin disease characterized by pruritic follicular papulopustules. It is usually resistant to topical and/or systemic corticosteroids, but it responds well to systemic indomethacin. We report here two patients with classical-type disease who were treated with systemic indomethacin. As indomethacin is an inhibitor of cyclo-oxygenases and a potent agonist of the prostaglandin D2 (PGD2) receptor, CRTH2 (chemoattractant receptor homologous molecule expressed on Th2 cells), we investigated the effects of indomethacin on CRTH2 expression by leukocytes. CRTH2 was expressed on blood eosinophils and lymphocytes. *In vitro* treatment with indomethacin suppressed the expression of CRTH2 on these cells. In addition, systemic treatment with indomethacin reduced eosinophil CRTH2 expression in another patient in association with improvement of skin lesions and blood eosinophilia. A number of inflammatory cells expressed haematopoietic PGD synthase, an essential enzyme for generating PGD2 in skin lesions of eosinophilic pustular folliculitis. A PGD2-CRTH2 interaction may be involved in the pathogenesis. Moreover, indomethacin may exert its therapeutic effect via reducing CRTH2 expression, as well as by inhibiting PGD2 synthesis. *Key words: CRTH2; eosinophils; indomethacin; prostaglandin D2; PGD synthase.*

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Eosinophilic pustular folliculitis (EPF) is an inflammatory skin disease characterized by pruritic follicular papulopustules that tend to form an annular configuration, accompanied by blood and tissue eosinophilia. Prior reports have suggested that EPF is mediated by Th2 cells producing interleukin-5 (1). EPF is usually resistant to topical or systemic corticosteroids; however, it responds well to systemic administration of indomethacin (2, 3). In some cases, indomethacin treatment can even be used as a diagnostic tool for EPF, although the mechanisms of its therapeutic effect are unknown.

Prostaglandin D2 (PGD2) is a metabolite of arachidonic acid released from activated mast cells and Th2 cells (4, 5). It is synthesized by multiple reaction steps, the last of which is the isomerization of prostaglandin H2 (PGH2) to PGD2 by haematopoietic PGD synthase (H-PGDS) (6). PGD2 exerts a wide range of biological activities through the classical PGD2 receptor (DP) and the recently identified receptor CRTH2 (chemoattractant receptor homologous molecule expressed on Th2 cells) (7). CRTH2 was initially thought to be preferentially expressed on Th2 cells (8), but it was later found to be expressed on eosinophils and basophils (7). Stimulation of CRTH2 in these cells leads to chemotaxis or Ca^{2+} mobilization or both (7, 9). An increase in the proportion of $CRTH2^+$ cells among $CD4^+$ cells in the blood is observed in patients with atopic dermatitis (10). In human allergic rhinitis, CRTH2 seems to be involved in the inflammation of mucosal tissues (11). In a number of animal models of allergic airway or skin disease, CRTH2-mediated signals have been shown to contribute to the inflammatory reactions where eosinophil infiltration is prominent (12–16).

A recent study revealed that indomethacin is a potent agonist of CRTH2 (17). Indomethacin is also an inhibitor for cyclo-oxygenases, which are involved in the generation of prostaglandins (18). We report here two cases of patients with EPF who were treated successfully with indomethacin. We sought to identify the effects of indomethacin on CRTH2 expression on blood cells, based on the hypothesis that indomethacin may exert its therapeutic effect via regulating PGD2-CRTH2 interaction.

CASE REPORTS AND RESULTS

A 21-year-old woman presented with a 1-month history of itchy facial eruption. Physical examination revealed multiple annular plaques consisting of follicular papules and pustules (Fig. 1A). Laboratory investigations indicated increased blood eosinophil counts (882/μl) and serum levels of IgE (391 IU/ml). Histologically, there was a marked cellular infiltrate consisting of lymphocytes and eosinophils in the dermis and around hair follicles (Fig. 1B). Eosinophils infiltrated into the hair follicle epithelium and sebaceous glands. Antibody against human immunodeficiency virus (HIV) was

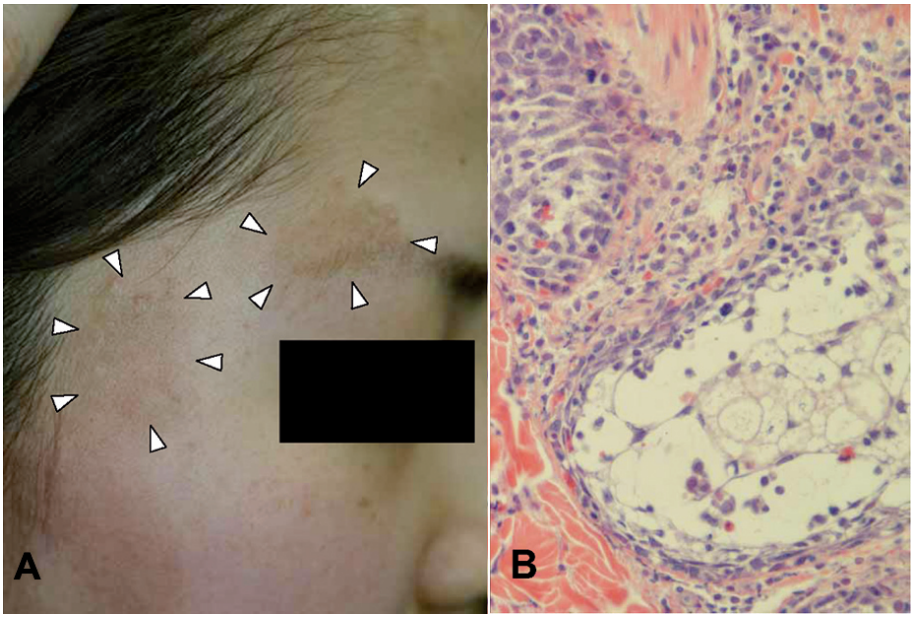

Fig. 1. Clinical and histological features of the case. (A) Facial lesions; annular configuration of follicular papulopustules (*arrowheads*). (B) Histological features. Eosinophils and mononuclear cells are located within and around hair follicles (haematoxylin and eosin staining, original magnification: ×400).

negative. The patient was treated orally with indomethacin (50 mg/day). Skin lesions rapidly responded to the treatment and blood eosinophil levels normalized. However, papulopustules recurred several weeks after the cessation of indomethacin. The condition is being controlled with occasional administration of 25 mg indomethacin when skin lesions recur.

To verify the mechanisms by which indomethacin exerted its therapeutic effects, whole blood cells obtained from the patient before treatment were incubated with indomethacin for 5 h. Expression of CRTH2 (R-phycoerythrin (RPE)-conjugated anti-CRTH2 antibody, clone BM16, Miltenyi Biotechnology Inc., Auburn, MI, USA) by eosinophils was analysed by direct immunofluorescence using whole-blood flow cytometry as previously described (19). For determining CRTH2 expression on CD4- and/or CD8-positive lymphocytes, whole blood cells were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-CD4 (L3T4, eBioscience, San Diego, CA, USA) or anti-CD8 (RPA-T8, eBioscience) antibodies together with RPE-conjugated anti-CRTH2 antibody. Nearly all eosinophils and approximately 3% of the lymphocytic population expressed CRTH2. *In vitro* treatment with indomethacin at 10^{-5} to 10^{-7} M (concentrations within the therapeutic range) remarkably reduced eosinophil CRTH2 expression (Fig. 2A). Relative fluorescence intensities (RFIs) were 0.627 and 0.287 at 0 M and 10^{-5} M indomethacin, respectively. Similar results were obtained with cells of the lymphocytic population. Among these cells, CRTH2 expression was detected not only on $CD4^+$ cells, but also on $CD8^+$ cells, at a much lower level. Treatment with indomethacin reduced CRTH2 levels in both of these lymphocyte types (Fig. 2B) (RFIs were 4.59 and 2.15 at 0 M and 10^{-5} M, respectively, for $CD4^+$ cells, and 2.09 and 0.83 at 0 M and 10^{-5} M, respectively for $CD8^+$ cells). The inhibitory effect on CRTH2 was most marked at an incubation time of 5 h and was dose dependent (data not shown).

We next attempted to determine the *in vivo* effects of indomethacin on CRTH2 expression. A 60-year-old male patient with EPF presented with 3-year history of recurrent annular facial plaques. He had high blood eosinophil counts (583/μl) and serum IgE (327 IU/ml). The patient was treated with indomethacin (50 mg/day) for 2 weeks. Interestingly, eosinophil CRTH2 expression was reduced after treatment compared with that before treatment (Fig. 2C).

With samples from a healthy donor, we also confirmed the down-regulation of eosinophil CRTH2 by two of its agonists, DK-PGD2 (Cayman Chemicals, Ann Arbor, MI, USA), a synthetic compound specific for CRTH2, and PGD2 (Cayman Chemicals). Eosinophil RFIs were 1.79, 1.09, 0.93, and 0.70 for no treatment, 10^{-5} M indomethacin, 10^{-5} M DK-PGD2, and 10^{-5} M PGD2, respectively. Suppressive effects of DK-PGD2 and PGD2 thus seemed relatively more potent than those of indomethacin. Similar data were obtained in lymphocytes (data not shown).

To determine whether PGD2, the endogenous ligand for CRTH2, can be produced in the lesional skin, immunohistochemical staining for haematopoietic PGD synthase (H-PGDS) with a rabbit polyclonal anti-human H-PGDS antibody (kindly provided by Dr Y. Urade, Osaka Bioscience Institute, Osaka, Japan) was performed. We used pretherapy skin biopsy specimens obtained from the first two patients and one additional EPF patient (female, 39 years old). A number of H-PGDS-positive cells were observed within the cellular infiltrate in the dermis and around hair follicles (Fig. 3).

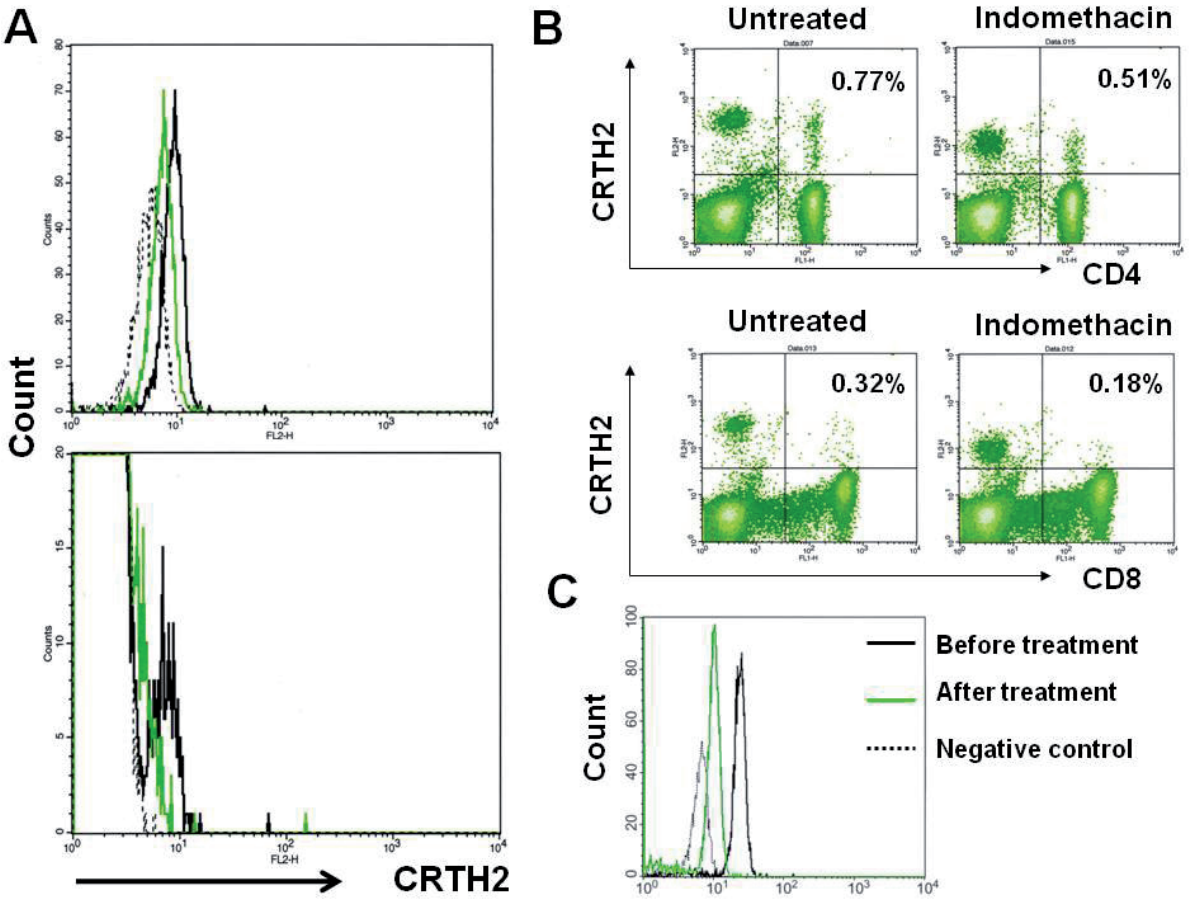

Fig. 2. Indomethacin-induced inhibition of eosinophil and lymphocyte CRTH2 (chemoattractant receptor homologous molecule expressed on Th2 cells) expression investigated by whole blood flow cytometry. (A) Eosinophils (*upper*) and a small percentage of cells of lymphocytic region (*lower*) expressed CRTH2 (*bold lines*). CRTH2 expression by these cells was reduced by 5-h incubation with 10^{-5} M indomethacin (*green line*). Dotted lines indicate negative control (isotype control antibody). (B) Indomethacin inhibited CRTH2 expression on both CD4- and CD8-positive cells. (C) In another patient with eosinophilic pustular folliculitis, expression of eosinophil CRTH2 was reduced after systemic treatment with indomethacin (*green line*). *Black bold line*: before treatment.

DISCUSSION

We report here two patients with classical-type EPF treated with oral indomethacin. Indomethacin exhibited therapeutic effects on EPF, as demonstrated in previous reports (2, 3). It is also known to be a potent agonist of the receptor CRTH2 (17); this receptor mediates chemotaxis of Th2 cells, eosinophils, and basophils (7). These two somewhat inconsistent findings prompted us to investigate the effect of indomethacin on CRTH2 expressed by $CD4^+$ and $CD8^+$ lymphocytes and by eosinophils. The expression of CRTH2 on eosinophils, $CD4^+$ cells, and $CD8^+$ cells taken from an EPF patient before treatment was reduced after *in vitro* incubation with indomethacin at concentrations within the therapeutic range (Fig. 2A and B). We also confirmed the suppressive *in vitro* effects of indomethacin on CRTH2 expression in eosinophils and lymphocytes obtained from three healthy volunteers (data not shown). In ad-

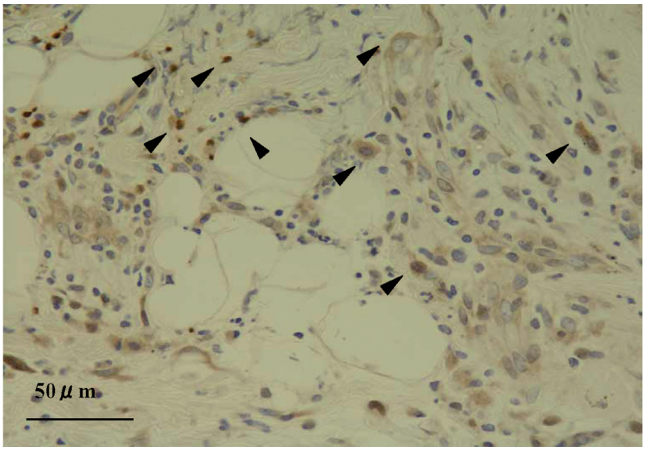

Fig. 3. Immunohistochemical staining for H-PGDS. A representative image of immunohistochemical findings from one of the three patients with eosinophilic pustular folliculitis. H-PGDS-positive cells (*arrowheads*) were observed throughout the dermis. Reaction products were visualized using diaminobenzidine.

dition, CRTH2 expression on eosinophils in another EPF patient was down-regulated during the treatment with systemic indomethacin (Fig. 2C), although we were unable to completely rule out the possibility that changes in CRTH2 might represent day-to-day variation. These data agree with the previous observation that treatment of CRTH2-expressing T-cell lines with indomethacin reduced CRTH2 expression, while inducing Ca^{2+} mobilization and chemotaxis in the cells (17). It was likely that indomethacin made eosinophils and Th2 lymphocytes "desensitized or low responder cells" to PGD2 via its agonistic effect on CRTH2 and down-regulation of CRTH2 expression. We observed a number of infiltrative cells expressing H-PGDS, an essential enzyme for generating PGD2, in the lesional skin of EPF patients. PGD2, if it is produced by H-PGDS$^+$ infiltrative cells, probably contributes to the induction of Th2-predominant skin inflammation, such as that seen in EPF (1). This is evidenced by the previous observation that transgenic mice overexpressing PGDS demonstrated strong allergic lung responses and eosinophilia associated with the production of cytokines biased towards the Th2 type (20). In addition, stimulation with PGD2 enhanced IL-4, IL-5, and IL-13 production by Th2 cells *in vitro* (21).

Recently, we have demonstrated that in atopic dermatitis, blood CCR4$^+$ Th2 cells expressing H-PGDS increased, and lesional skin contained more H-PGDS$^+$ T cells than in psoriasis (22). CRTH2 expression on eosinophils was higher, not only in atopic dermatitis, but also in chronic urticaria and prurigo nodularis, than in healthy donors (23). Although we have not been able to detect a statistical significance in the difference of eosinophil CRTH2 expression between EPF patients and healthy donors because of the limited number of patients (data not shown), the involvement of a PGD2-CRTH2 interaction does not appear to be specific for EPF. However, there is evidence that indomethacin inhibits cyclo-oxygenases, leading to the suppression of prostaglandin generation (18), and down-regulates CRTH2 expression, thus probably explaining why EPF can be treated successfully with indomethacin. This also suggests that the PGD2-CRTH2 interaction contributes to the pathological mechanisms of EPF, perhaps even to a greater degree than in atopic dermatitis, in which indomethacin does not exert therapeutic effects. The concomitant inhibition of PGD2 production and CRTH2 expression may become a specific and useful new approach to the treatment of EPF.

The authors declare no conflicts of interest.

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