Increased Expression of Haematopoietic Prostaglandin D Synthase in CCR4-positive T Cells From Patients with Atopic Dermatitis

Chieko Shimura, Takahiro Satoh* and Hiroo Yokozeki

*Department of Dermatology, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan. *E-mail: tasa-1688.derm@tmd.ac.jp*

Accepted April 29, 2008.

Sir,

Prostaglandin D2 (PGD2) is an arachidonic acid metabolite that has a wide range of biological activities, including vasodilatation, bronchoconstriction and inhibition of platelet aggregation. PGD2 has also been implicated in allergic diseases. For example, PGD2 production is observed in bronchoalveolar lavage (BAL) fluid during the asthmatic response (1), and mice overproducing PGD2 show enhanced allergic lung responses, eosinophilia and increased Th2-type cytokine production (2). We have also demonstrated that PGD2 produced in the skin plays an essential role in IgE-mediated skin responses in mice (3). Thus, PGD2 may contribute to Th2-predominant inflammation. However, it has been reported that PGD2 also provides a braking signal for delayed hypersensitivity type (4).

PGD2 synthesis is mediated by the isomerization of PGH2 into PGD2 through the enzymatic activity of haematopoietic PGD synthase (H-PGDS) (5). Mast cells express H-PGDS and have been thought to rapidly secrete PGD2 in response to antigen stimulation, thereby contributing to inflammation in the early stages of allergic responses (6, 7). Some Th2 cells, but not Th1 cells, also possess H-PGDS and produce PGD2 when stimulated with CD3/CD28 (8). These data suggest that PGD2 is produced not only in immediate-type reactions, but also in the later stages of inflammation. Indeed, in IgE-mediated skin responses in mice, levels of PGD2 transiently increased in the immediate-type reaction, followed by a second increase in the very late-phase response (chronic allergic inflammation) (3); the latter response may share morphological similarities with inflammation in atopic dermatitis (9). However, little is known about the involvement of PGD2-producing T cells in human skin diseases.

Atopic dermatitis (AD) and psoriasis vulgaris (PV) are chronic skin diseases of unknown aetiology. While AD is mediated by a biphasic T helper cells response (Th2 in acute and Th1 in chronic) (10, 11), Th1 as well as Th17 cells appear to contribute to the pathogenesis of PV (12, 13). In this study, we analysed the expression of H-PGDS by CCR4(+)/CD3(+) T cells, which represent a subpopulation of Th2 cells, in AD compared with PV.

MATERIALS AND METHODS

Study participants included patients with extrinsic AD ($n=12$; 8 males, 4 females; mean age 30.8 years) (serum IgE = 5393 ± 1786 IU/ml), patients with PV ($n=11$; 9 males, 2 females; mean age 62 years) and healthy volunteers (H; $n=9$; 2 males, 7 females; mean age 25.1 years). Informed consent was obtained from all patients. This study was approved by the ethics committee of Tokyo Medical and Dental University.

Intracellular staining for H-PGDS in Th2 cells was performed using the Intrastain Kit (Dako Cytomation, Kyoto, Japan). Platelet-rich plasma was removed from whole blood anticoagulated with EDTA, and washed with phosphate-buffered saline (PBS). Cells were suspended in PBS containing 0.1% NaN_3, 3% foetal calf serum (FCS), and were then incubated with carboxyfluorescein (CFS)-conjugated mouse anti-human CCR4 mAb (clone: 205410) (R&D Systems, Minneapolis, USA), and PE-Cy5-conjugated mouse anti-human CD3 mAb (Clone: UCHT1) (Beckman Coulter, Fullerton, USA) for 30 min on ice. After washing and cell permeabilization, cells were incubated with rabbit anti-human H-PGDS polyclonal Ab (a gift from Drs K. Aritake and Y. Urade from Osaka Bioscience Institute, Osaka, Japan), or with control rabbit IgG (Beckman Coulter, Fullerton, USA) for 30 min at room temperature, followed by staining with R-PE-conjugated goat F(ab')2 anti-rabbit IgG (H+L) Ab (Southern Biotechnology, Birmingham, USA).

Frozen tissue sections were fixed with methanol and incubated in PBS containing 10% normal goat serum, 0.01% Triton-X and 0.1% NaN_3. Sections were then incubated with anti-H-PGDS Ab or control rabbit IgG, followed by incubation with CFS-conjugated anti-human CCR4 mAb, PE-Cy5-conjugated anti-human CD3 mAb, and R-PE-conjugated goat F(ab')2 anti-rabbit IgG (H+L) Ab.

Multiple comparison analysis by Scheffe's F test was used to assess the statistical significance of differences between mean values.

RESULTS

A subpopulation of CCR4(+)/CD3 (+) cells possess H-PGDS in their cytoplasm (Fig. 1A). The percentage of H-PGDS (+) cells among CCR4 (+)/CD3 (+) cells was significantly higher in patients with AD than in healthy donors (Fig. 1B). There were no differences in H-PGDS (+) cells/CCR4 (+) T cells from patients with AD with or without bronchial asthma and/or allergic rhinitis (data not shown). Patients with psoriasis had comparable levels of H-PGDS (+)/CCR4 (+) T cells as healthy donors. In the lesional skin of AD, infiltration of H-PGDS (+)/CCR4(+)/CD3 (+) cells was detected by immunohistochemical analysis (Fig. 2). Nevertheless, there was no correlation between H-PGDS (+) cells in the peripheral blood and clinical severity as assessed by the Eczema Area and Severity Index (EASI score (14)), serum IgE levels or blood eosinophil counts (data not shown).

DISCUSSION

Increased numbers of H-PGDS (+)/CCR4 (+) T cells in blood and infiltration into the skin suggest that these

 ISSN 0001-5555
doi: 10.2340/00015555-0515

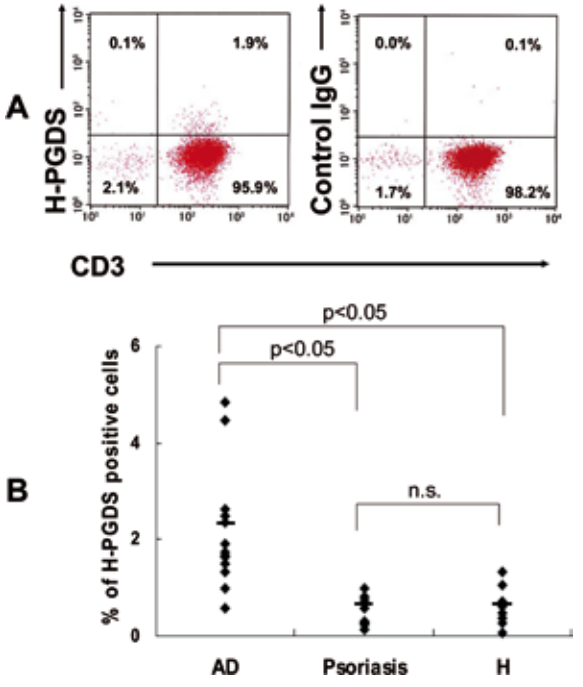

Fig. 1. H-PGDS expression in blood T cells. (A) A representative figure for intracellular H-PGDS staining (*left panel*). CCR4 (+) cells in the lymphocyte/lymphoblast region were gated. 97.8 % of CCR4 (+) cells were CD3 (+) T cells and 1.9 % of CCR4 (+) cells were H-PGDS expressing T cells. Negative control staining (*right panel*). (B) Percentage H-PGDS (+) T cells. AD patients had higher numbers of H-PGDS-expressing cells among CD3 (+)/CCR4 (+) cells than psoriasis patients and healthy donors. AD: atopic dermatitis; H: healthy donors.

cells are an important source of PGD2. It is conceivable that persistent production of PGD2 by infiltrative T cells in lesional skin stimulates eosinophil and basophil chemoattraction to chronically inflamed skin via CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells); a PGD2 receptor (15). Moreover, PGD2 stimulates Th2 cells to produce interleukin (IL)-2, IL-4, IL-5 and IL-13 via the CRTH2 receptor (16), resulting in further modulation of Th2-mediated inflammation.

We were unable to detect statistically significant increases in the percentage of CCR4(+)/CD3 (+) cells in the blood of patients with AD when compared with psoriasis patients and healthy donors (data not shown). This is in contrast to previous observations that Th2 cells were elevated in the blood of patients with AD (17). Although the reasons for this discrepancy are uncertain, it might be due to the fact that disease activity and stages of inflammation of the adult patients with AD in our study varied. In addition, CCR4 (+)/CD3(+) cells might include not only Th2 cells, but also Th0 and Th1 cells (18).

Although the molecular mechanisms underlying the regulation of H-PGDS expression are unknown, the present data suggest that H-PGDS (+)/CCR4 (+) T cells contribute to inflammation in AD.

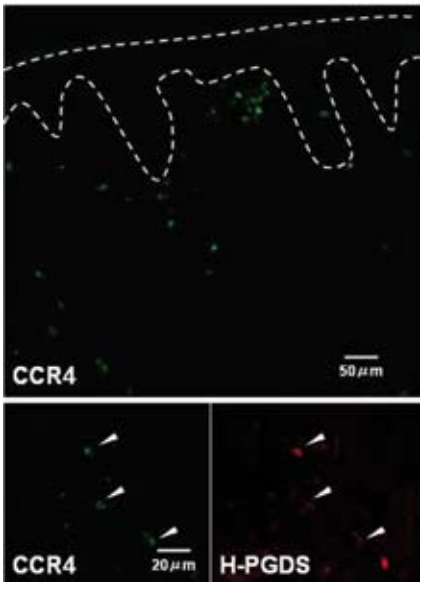

Fig. 2. H-PGDS expression in tissue T cells. In lesional skin of atopic dermatitis (AD), a number of CCR4 (+) cells were observed in the dermis (n=6). H-PGDS was co-localized to CCR4 (+) cells (*arrowheads*). These cells were also positive for CD3 (data not shown).

REFERENCES

1. Miadonna A, Tedeschi A, Brasca C, Folco G, Sala A, Murphy RC. Mediator release after endobronchial antigen challenge in patients with respiratory allergy. J Allergy Clin Immunol 1990; 85: 906–913.
2. Fujitani Y, Kanaoka Y, Aritake K, Uodome N, Okazaki-Hatake K, Urade Y. Pronounced eosinophilic lung inflammation and Th2 cytokine release in human lipocalin-type prostaglandin D synthase transgenic mice. J Immunol 2002; 168: 443–449.
3. Satoh T, Moroi R, Aritake K, Urade Y, Kanai Y, Sumi K, et al. Prostaglandin D2 plays an essential role in chronic allergic inflammation of the skin via CRTH2 receptor. J Immunol 2006; 177: 2621–2629.
4. Trivedi SG, Newson J, Rajakariar R, Jacques TS, Hannon R, Kanaoka Y, et al. Essential role for hematopoietic prostaglandin D2 synthase in the control of delayed type hypersensitivity. Proc Natl Acad Sci USA. 2006; 103: 5179–5184.
5. Kanaoka Y, Urade Y. Hematopoietic prostaglandin D synthase. Prostaglandins Leukot Essent Fatty Acids 2003; 69: 163–167.
6. Peters SP, MacGlashan DW Jr., Schulman ES, Schleimer RP, Hayes EC, Rokach J, et al. Arachidonic acid metabolism in purified human lung mast cells. J Immunol 1984; 132: 1972–1979.
7. Naclerio RM, Proud D, Togias AG, Adkinson NF Jr., Meyers DA, Kagey-Sobotka A, et al. Inflammatory mediators in late antigen-induced rhinitis. N Engl J Med 1985; 313: 65–70.
8. Tanaka K, Ogawa K, Sugamura K, Nakamura M, Takano S, Nagata K. Cutting edge: differential production of prostaglandin D2 by human helper T cell subsets. J Immunol 2000; 164: 2277–2280.
9. Sato E, Hirahara K, Wada Y, Yoshitomi T, Azuma T, Matsuoka K, et al. Chronic inflammation of the skin can be induced in IgE transgenic mice by means of a single challenge of multivalent antigen. J Allergy Clin Immunol 2003; 111: 143–148.

10. Grewe M, Gyufko K, Schopf E, Krutmann J. Lesional expression of interferon-gamma in atopic eczema. Lancet. 1994; 343: 25–26.
11. Tsicopoulos A, Hamid Q, Haczku A, Jacobson MR, Durham SR, North J, et al. Kinetics of cell infiltration and cytokine messenger RNA expression after intradermal challenge with allergen and tuberculin in the same atopic individuals. J Allergy Clin Immunol 1994; 94: 764–772.
12. Schlaak JF, Buslau M, Jochum W, Hermann E, Girndt M, Gallati H, et al. T cells involved in psoriasis vulgaris belong to the Th1 subset. J Invest Dermatol 1994; 102: 145–149.
13. Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, et al. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature 2007; 445: 648–651.
14. Hanifin JM, Thurston M, Omoto M, Cherill R, Tofte SJ, Graeber M. The eczema area and severity index (EASI): assessment of reliability in atopic dermatitis. EASI Evaluator Group. Exp Dermatol 2001; 10: 11–18.
15. Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, et al. Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. J Exp Med 2001; 193: 255–261.
16. Tanaka K, Hirai H, Takano S, Nakamura M, Nagata K. Effects of prostaglandin D2 on helper T cell functions. Biochem Biophys Res Commun 2004; 316: 1009–1014.
17. Okazaki H, Kakurai M, Hirata D, Sato H, Kamimura T, Onai N, et al. Characterization of chemokine receptor expression and cytokine production in circulating CD4+ T cells from patients with atopic dermatitis: up-regulation of C-C chemokine receptor 4 in atopic dermatitis. Clin Exp Allergy 2002; 32: 1236–1242.
18. Cosmi L, Annunziato F, Galli MIG, Maggi RME, Nagata K, Romagnani S. CRTH2 is the most reliable marker for the detection of circulating human type 2 Th and type 2 T cytotoxic cells in health and disease. Eur J Immunol 2000; 30: 2972–2979.