Journal of Dermatological Science 76 (2014) 90–95

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Expression of thymus and activation-regulated chemokine (TARC) by human dermal cells, but not epidermal keratinocytes

Tetsuo Shoda [a,b,*], Kyoko Futamura [a], Fumio Kobayashi [b], Hirohisa Saito [a], Kenji Matsumoto [a], Akio Matsuda [a,*]

[a] Department of Allergy and Immunology, National Research Institute for Child Health and Development, 2-10-1, Okura, Setagaya-ku, Tokyo 157-8535, Japan
[b] Department of Health and Psychosocial Medicine, Aichi Medical University School of Medicine, 1-1 Yazakokarimata, Nagakute, Aichi 480-1195, Japan

ARTICLE INFO

Article history:
Received 25 June 2014
Received in revised form 6 August 2014
Accepted 19 August 2014

Keywords:
Atopic dermatitis
TARC
TNF-α
IL-4
Dermal cells
Corticosteroid

ABSTRACT

Background: Serum levels of thymus and activation-regulated chemokine (TARC/CCL17) have served as a reliable biomarker of disease progression of atopic dermatitis (AD). However, it remains to be scientifically explained why serum TARC levels correlate well with the degree of AD progression.
Objective: We hypothesized that dermal cells, but not epidermal keratinocytes, are major cellular sources of TARC and thus responsible for subclinical skin inflammation. This study aimed to identify the skin cells that can produce TARC protein.
Methods: Primary normal human epidermal keratinocytes (NHEK), dermal microvascular endothelial cells (HMVEC-dBl) and dermal fibroblasts (NHDF) were stimulated with TNF-α and IL-4, alone and in combination. TARC mRNA and protein levels were quantified by qPCR and ELISA, respectively. We also investigated the effects of such immunosuppressants as a corticosteroid (dexamethasone) and tacrolimus (FK506) on TARC production, and used various signaling inhibitors to evaluate the signaling pathways involved in TARC expression.
Results: Although neither TNF-α nor IL-4 alone induced TARC production by any of the tested cell types, together they induced expression of TARC mRNA and appreciable amounts of TARC protein by HMVEC-dBl and NHDF, but not by NHEK. TARC production by those dermal cells was not inhibited by dexamethasone or FK506. TARC production by HMVEC-dBl was completely inhibited by NF-κB and p38 MAPK inhibitors, but not by an ERK inhibitor.
Conclusion: Dermal cells, but not epidermal keratinocytes, may be important cellular sources of TARC in AD skin. Therefore, even if epidermal eczematous lesions seem to be improved, complete inhibition of inflammation in the dermis is thought to be particularly important for suppressing both the TARC blood level and progression of AD. However, immunosuppressants did not directly inhibit TARC production by the dermal cells. Anti-inflammatory therapy may decrease TARC blood levels in AD patients indirectly, via its inhibitory effects on TNF-α- and/or IL-4-producing cells in the dermis.

1. Introduction

Atopic dermatitis (AD) is characterized by chronic or relapsing eczematous lesions, as well as by pruritus [1]. Traditionally, objective disappearance of the eczema and subjective relief from pruritus have been considered to be clinical signs of remission of AD. However, those signs are insufficient for assuming full recovery of the skin inflammation because of concern that subclinical inflammation may persist despite apparent remission. Furthermore, subclinical inflammation is frequently associated with severe and refractory disease, and it can be detected only by additional techniques such as skin biopsy, or indirectly by assaying biomarkers [2]. However, skin biopsy is invasive and impractical in most clinical settings.

Abbreviations: AD, atopic dermatitis; CCR4, CC chemokine receptor 4; CTACK, cutaneous T-cell-attracting chemokine; NHDF, normal human dermal fibroblasts; NHEK, normal human epidermal keratinocytes; HMVEC-dBl, human microvascular endothelial cells from dermal blood vessels; MDC, macrophage-derived chemokine; SCCA2, squamous cell carcinoma antigen 2; TARC, thymus and activation-regulated chemokine.

* Corresponding authors at: Department of Allergy and Immunology, National Research Institute for Child Health and Development, 2-10-1, Okura, Setagaya-ku, Tokyo 157-8535, Japan. Tel.: +81 3 5494 7120.
E-mail addresses: shoda-t@ncchd.go.jp (T. Shoda), matsuda-a@ncchd.go.jp (A. Matsuda).

http://dx.doi.org/10.1016/j.jdermsci.2014.08.009
0923-1811/

Thymus and activation-regulated chemokine (TARC/CCL17) is a member of the CC chemokine family and is a potent and selective chemoattractant for Th2 cells via CC chemokine receptor 4 (CCR4) [3]. There is increasing evidence that TARC is involved in the development of allergic diseases, such as AD and bronchial asthma, and elevated blood levels of TARC have been observed in AD patients [4,5]. Importantly, serum TARC levels have served as a clinically reliable biomarker of AD disease progression [6]. In particular, it is well-known that AD patients with elevated serum TARC levels tend to relapse easily and fail to achieve good control of AD symptoms, even when their skin lesions seem to disappear after initial anti-inflammatory therapy [7]. This clinical evidence strongly suggests that serum TARC levels in AD patients correlate well with the degree of subclinical inflammation in the skin. Based on the evidence, measurement of serum TARC levels in AD patients is covered by medical insurance in Japan [8].

However, it remains to be scientifically explained why serum TARC levels correlate well with the degree of AD progression and subclinical inflammation in the skin. Since visual assessment of eczema recovery is totally unreliable, we hypothesized that dermal cells, but not epidermal keratinocytes, may be major cellular sources of TARC and play an important role in subclinical inflammation that persists in AD patients in spite of anti-inflammatory therapy. In this context, we examined which types of human skin tissue cells, including epidermal keratinocytes and dermal cells, can produce TARC protein in a Th2 cytokine milieu. Besides TARC, recent insights into the complex network of cytokines and chemokines in AD might lead to promising candidates for an AD biomarker [9]. Various molecules, such as cutaneous T-cell attracting chemokine (CTACK/CCL27) [10], macrophage-derived chemokine (MDC/CCL22) [5], squamous cell carcinoma antigen (SCCA2/SERPINB4) [11] and periostin [12], have shown a correlation with AD disease activity. To elucidate the differences in biological implications between TARC and other biomarker candidates, we examined the expression of these AD-related genes in skin tissue cells. We also investigated the effects of such immunosuppressants as a corticosteroid (dexamethasone) and tacrolimus (FK506) on TARC production by skin tissue cells, and used various signaling inhibitors to evaluate the signaling pathways involved in TARC expression.

2. Materials and methods

2.1. Reagents

Recombinant human TNF-α and IL-4 were purchased from PeproTech (Rocky Hill, NJ). PD98059, SB202190, PDTC and Bay 11-7082 were purchased from Calbiochem (La Jolla, CA). Dexamethasone and tacrolimus (FK506) were purchased from SIGMA (St. Louis, MO). RU486 was purchased from Cayman Chemical (Ann Arbor, MI).

2.2. Cell culture and stimulation

Normal human epidermal keratinocytes (NHEK), normal human dermal fibroblasts (NHDF) and human microvascular endothelial cells from dermal blood vessels (HMVEC-dBl) were purchased from Lonza (Walkersville, MD) and maintained exactly as recommended by the manufacturer. All the experiments described in this study were performed using second- or third-passage cells in 70–80% confluent monolayers. Furthermore, in order to eliminate effects of the different genetic and environmental backgrounds of the primary cell donors, we performed the same experiments using each type of cell originating from at least two different donors; we obtained reproducible results.

All cells were treated with 10 ng/ml TNF-α and/or 10 ng/ml IL-4 for up to 48 h. In some experiments, HMVEC-dBl were treated with PD98059, SB202190, PDTC or Bay 11-7082 for 60 min before stimulating with TNF-α and IL-4, and those inhibitors were allowed to remain in the culture medium throughout the culture period. Both KGM-Gold™ BulletKit and EGM-2MV BulletKit (Lonza), which are optimized for use with NHEK and HMVEC-dBl, respectively, contain hydrocortisone. Therefore, all experiments were performed after hydrocortisone deprivation for 24 h, as described previously [13,14].

2.3. Quantitative real-time PCR

Total RNA samples were extracted using RNeasy (QIAGEN, Valencia, CA) and digested with RNase-free DNase I (QIAGEN) in accordance with the manufacturer's instructions. First-strand cDNA was synthesized using an iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Quantitative real-time PCR (qPCR) was performed as described previously [13,14]. Primer sets for six genes were synthesized at Fasmac (Kanagawa, Japan): TARC (sense, 5′-ACCTGCAAAGCCTTGAGAGGT-3′; antisense, 5′-ACGGTGGAGGTCCCAGGTA-3′), periostin (sense, 5′-CACACCCGTGAGGAAGTTGC-3′; antisense, 5′-TTTCACTGAGAACGACCTTCCC-3′), macrophage-derived chemokine (MDC) (sense, 5′-TACTCTGATGACCGTGGCCTTG-3′; antisense, 5′-AGAGAGTTGGCACAGGCTTCTG-3′), cutaneous T-cell attracting chemokine (CTACK) (sense, 5′-CCGAAAGCCACTCTCAGACAA-3′; antisense, 5′-GCCTCCTGCAGTTCCACCT-3′), squamous cell carcinoma antigen 2 (SCCA2) (sense, 5′-GGAGCCACGGTCTCTCAGTATCTAA-3′; antisense, 5′-CAGCTTCCACTCCCTCCTCAGT-3′) and β-actin (sense, 5′-CCCAGCCATGTACGTTGCTAT-3′; antisense, 5′-TCACCGGAGTCCATCACGAT-3′). To determine the exact copy number of the genes, quantified concentrations of the purified PCR products of TARC, periostin, MDC, CTACK, SCCA2 and β-actin were serially diluted and used as standards in each experiment. An aliquot of cDNA equivalent to 2 ng of the total RNA sample was used for each qPCR. The mRNA expression levels were normalized to the β-actin level in each sample.

2.4. ELISA

The TARC protein concentration in each cell-free supernatant was measured with a specific ELISA kit (R&D Systems, Minneapolis, MN) in accordance with the manufacturer's instructions.

2.5. Western blotting

HMVEC-dBl were seeded into 6-well plates at 1×10^5 cells/well and cultured until subconfluent (2 or 3 days). The cells were then treated for 15 min with 10 ng/ml TNF-α and 10 ng/ml IL-4. Whole-cell preparations were extracted with 200 μl of NuPAGE® sample buffer (Invitrogen) containing 5% 2-mercaptoethanol and lysed by sonication. Equal volumes of whole-cell lysates were separated by SDS-PAGE (5–15% Ready Gels J; Bio-Rad) gel electrophoresis and transferred to nitrocellulose membranes (iBlot™ Gel Transfer Stacks, mini; Invitrogen). Immunoblotting was performed using the following antibodies: clone D13.14.4E, rabbit mAb for phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204), rabbit polyclonal Ab for phospho-p38 MAPK (Thr180/Tyr182), clone 93H1, rabbit mAb for phospho-NF-κB p65(ser536), clone L35A5, mouse mAb for IκBα (Cell Signaling Technology, Danvers, MA) and clone AC-15, mouse mAb for β-actin (SIGMA) in accordance with the manufacturers' instructions.

2.6. Statistical analysis

All data are presented as the mean ± SD. Differences between groups were analyzed using analysis of variance (ANOVA) with