Integrative Biosciences 10: 15-20, 2006

Integrative
Biosciences
http://www.ibiosci.or.kr

Cytokines Regulate the Expression of the Thymus and Activation-Regulated Chemokine (TARC; CCL17) in Human Skin Fibroblast Cells

Ji-Sook Lee, In Sik Kim[1], Dong-Hee Kim[2] and Chi-Young Yun*

Department of Biology, Daejeon University, Daejeon 300-716, Korea; [1]Department of Clinical Laboratory Science, School of Medicine, Eulji University, Daejeon 301-832, Korea; [2]Department of Pathology, College of Oriental Medicine, Daejeon University, Daejeon 300-716, Korea

Abstracts: Allergic inflammation is thought to be a Th2 cell-dominant immune response during which tissue-resident fibroblasts produce chemokines which contribute to the recruitment of migratory leukocytes to sites of tissue injury. Thymus and activation-regulated chemokine (TARC; CCL17) is a potent member of the CC chemokine family and a selective chemoattractant for Th2 cells. In order to study the regulatory profiles of TARC production by TNF-α, IFN-γ, and IL-4 in human normal skin fibroblast, CCD-986sk cell line was used. The expression of TARC protein was measured using ELISA, and mRNA level was detected by RT-PCR. The combination of TNF-α and IL-4 induced a time- and dose-dependent synergistic increase in the expression of TARC at both protein and mRNA levels in the cultured human skin fibroblasts. Exposure of the cells to single cytokine had no effect on TARC expression. The high concentration (100 ng/ml) and long incubation time (72 h) of IFN-γ further enhanced the TARC production induced by TNF-α/IL-4 in the skin fibroblast. This synergistic effect of Th1 and Th2 type cytokines on TARC production by skin fibroblasts may contribute to the inflammatory cell infiltration and tissue damage with allergic inflammation.

Keywords: Allergic inflammation, skin fibroblast cell, thymus and activation-regulated chemokine (TARC;CCL17), CCD-986sk cell

The term "allergy" refers to some clinical event that is caused by immune mechanisms and is harmful to the host (Terr, 2001). Allergic inflammation is thought to be a Th2 cell-dominant immune response, given the pronounced accumulation of Th2 cells apparent at sites of such inflammation in individuals with vernal keratoconjunctivitis, atopic dermatitis, or asthma (Robinson et al., 1992; van Reijsen et al., 1992). Atopic dermatitis is an inflammatory skin disease in which the inflammation is characterized by the influx of lymphocytes into the dermis (Vestergaard et al., 2000). The clinical manifestations of allergic diseases differ among various organs, including the eye (ocular surface), skin and lungs, possibly at least in part, as a result of differences in the resident fibroblasts.

Fibroblasts are abundant in connective tissue, where they provide mechanical strength by providing a supporting framework of extracellular matrix. Tissue-resident fibroblasts thus function as sentinel or effector cells during allergic inflammation, modulating the activities of immune cells by producing cytokines and chemokines (Smith et al., 1997). Knowledge of the regulation of chemokine synthesis by fibroblasts is thus important to understand the pathogenesis of inflammation.

Thymus and activation-regulated chemokine (TARC;CCL17) is a potent member of the CC chemokine family and a selective chemoattractant for Th2 cells, which express the corresponding chemokine receptor (CCR4) on their surfaces (Imai et al., 1997). Moreover, serum levels of TARC were shown to correlate well with severity of disease in Th2-prone skin disorders, such as atopic dermatitis (Kakinuma et al., 2001). Keratinocytes also express TARC *in vivo* and *in vitro* under an appropriate condition (Vestergaard et al., 2000; Kakinuma et al., 2001).

Therefore, TARC- and CCR4-mediated chemotaxis may play a crucial role in the initiation and perpetuation of

Abbreviations: TARC, thymus and activation-regulated chemokine; IFN-γ, interferon-γ; IL-4, interleukin-4

*To whom correspondence should be addressed.
Tel: +82-42-280-2432; Fax: +82-42-285-2432
E-mail address: chyun@dju.ac.kr

allergic inflammation. To shed light on the mechanism by which local infiltration of Th2 cells is regulated, we have investigated TARC production by cultured human skin fibroblasts, as well as, the possible effects of IL-4, TNF-α and IFN-γ on such production.

MATERIALS AND METHODS

Materials

Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum, L-glutamine, non essential amino acids, penicillin, streptomycin, and fetal bovine serum (FBS) were obtained from Invitrogen Life Tecnologies. Human recombinant TNF-α, IFN-γ, and IL-4 were obtained from PeproTech. Human TARC protein for ELISA was from R&D Systems. The ImProm-II Reverse transcription system kit for isolation of total RNA and the PCR master Mix were from Promega. DNA molecular size standards were from Takara Shuzo and agarose was from FMC Bioproducts.

Cell culture

Normal human skin fibroblast CCD-986sk cells were from the Korean Cell Line Bank (KCLB). The cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1% non essential amino acids, 25 U/ml penicillin and 25 μg/ml streptomycin, and maintained in a humidified incubator aerated with 95% air and 5% CO_2 at 37°C. Culture medium was exchanged twice a week.

Cells (4×10^5 cells per well) were plated into 24-well plates and cultured for 24 h. Before stimulation, all of the culture medium in each well was replaced by 0.1% FBS-DMEM. The cells were treated with the medium only or with various concentrations (0.1-100 ng/ml) of TNF-α, IFN-γ, and IL-4. After incubation for the indicated times, the medium was collected from each culture dish and centrifuged at 120 g for 5 min. The resulting supernatant was stored at –80°C for subsequent assay of TARC. The fibroblasts remaining in the dish were exposed to trypsin-EDTA, and cell number was determined with a hemocytometer.

Determination of TARC concentration by means of ELISA

Concentration of TARC in the supernatant was measured with an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems) according to the manufacturer's instructions using an ELISA reader, BIO-TEK ELx808. This ELISA method enabled detection of TARC concentrations of greater than 15.6 pg/ml.

RT-PCR

TARC mRNA was detected by reverse transcriptase polymerase chain reaction (RT-PCR) in human skin fibroblasts. Extracted RNA was subjected to reverse transcription using the ImProm-II Reverse transcription system kit. Transcripts of the constitutively expressed gene for GAPDH served as control in each sample. The sequences of the PCR primers were as follows: TARC sense, 5'-ACTGCTCCAGGGATGCCATCGTTTTT-3'; TARC antisense, 5'-ACAAGGGGATGGGATCTCCCTCACTG-3'; and GAPDH sense, 5'-ACCACAGTCCATGCCATCAC-3'; GAPDH antisense, 5'-TCCACCACCCTGTTGCTGTA-3'. These primers yielded PCR products of the expected sizes of 270 bp for TARC mRNA and 452 bp for GAPDH mRNA. The PCR protocol comprised an initial denaturation step at 95°C for 30 s followed by 35 cycles of amplification. For the amplification of TARC cDNA, the cycles consisted of denaturation at 95°C for 1 min, annealing at 58°C for 1 min, and elongation at 72°C for 1 min. For the amplification of GAPDH cDNA, the cycles included denaturation at 95°C for 1 min, annealing at 56°C for 1 min, and elongation at 72°C for 1 min. To verify the specificity of the amplification, we also subjected PCR products to electrophoresis on a 3% agarose gel, which was then stained with ethidium bromide (1 μg/ml) and examined with a Nighthawk system. The latter comprised a charge-coupled device camera, an ultraviolet transilluminator, and an analysis program (QuantityOne).

Statistical analysis

Data are expressed as means ± SD and differences were analyzed by Student's *t* test or by ANOVA and Fisher's PLSD test. A *P* value of <0.01 was considered statistically significant.

RESULTS

Synergistic effect of TNF-α and IL-4 or TNF-α, IL-4, and IFN-γ on TARC secretion by human skin fibroblast cells

The cells were cultured for 72 h with each cytokine at a concentration of 10 ng/ml either separately or in combination. TARC was not detectable in the culture medium of cells incubated in the absence of cytokine or in the presence of TNF-α, IFN-γ, or IL-4 or alone (Fig. 1A).

Synergistic effect of TNF-α and IL-4 on the TARC mRNA in skin fibroblast cells

Then, we investigated the effects of TNF-α and IL-4 or TNF-α, IFN-γ, and IL-4 on the abundance of TARC mRNA in human skin fibroblasts. Cells were cultured for 72 h in the absence or presence of cytokines, each at a concentration of 10 ng/ml, after which the amount of TARC mRNA in cell lysates was assayed by RT-PCR. Exposure of cells to TNF-α or IL-4 alone had no significant effect on the amount of TARC mRNA. In contrast, stimulation with both TNF-α and IL-4 or TNF-α, IFN-γ, and IL-4 induced an increase in the abundance of TARC

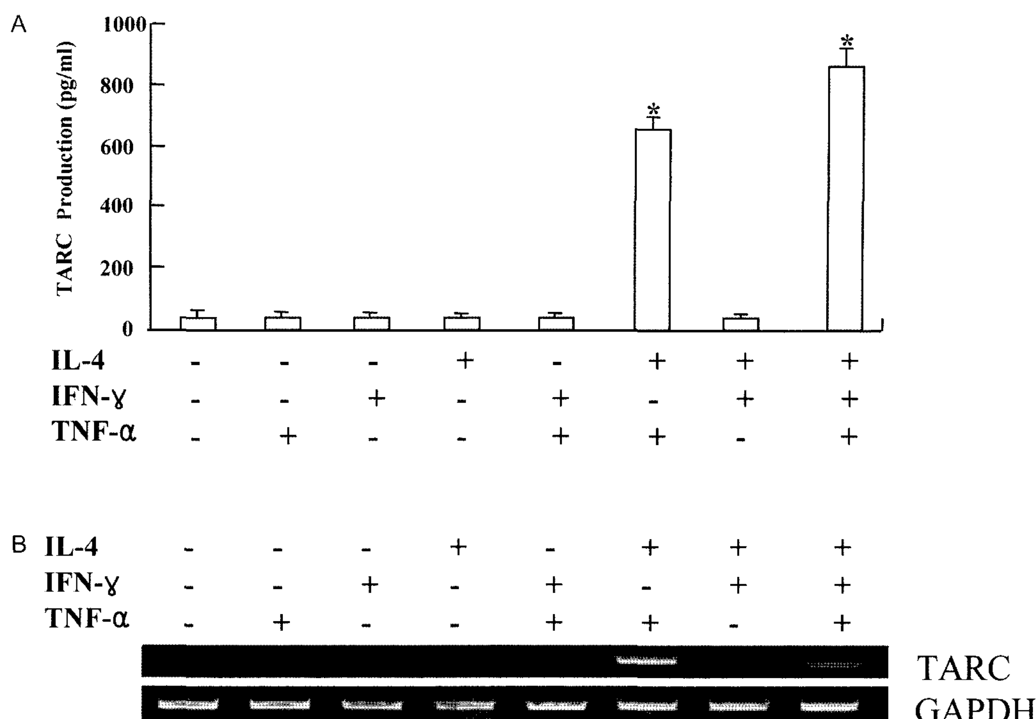

Fig. 1. Effects of cytokines on TARC releases and mRNA by human skin fibroblast cells. Skin fibroblasts were incubated for 72 hours in the absence or presence of the indicated cytokines (10 ng/ml), after which the concentration of TARC in the culture supernatants was measured by means of ELISA (A). Data are means ± SD ($n = 3$) from a representative experiment that was repeated three times with similar results. *$p < 0.01$ (Student's t test) vs the media only point. At the same time, the cell lysates were assayed for TARC mRNA by semi-quantitative RT-PCR. The amount of TARC mRNA was normalized by that of GAPDH mRNA (B).

mRNA compared with that apparent in unstimulated cells (Fig. 1B).

Time course of TARC secretion from skin fibroblasts stimulated with the combination of cytokines

The time course of TARC was released by skin fibroblasts incubated for up to 72 h with TNF-α (10 ng/ml), IL-4 (10 ng/ml), IFN-γ (10 ng/ml), or that combination. Whereas incubation of cells with TNF-α or IL-4 alone did not induce detectable release of TARC during the incubation period, exposure of the skin fibroblasts to the combination of TNF-α and IL-4 or TNF-α, IFN-γ, and IL-4 resulted in a time-dependent increase in TARC release that was statistically significant at 48 and 72 h (Fig. 2A, B).

Dose dependence of TARC secretion from skin fibroblasts stimulated with the combination of cytokines

To further elucidate the regulatory effects, we then examined the dose dependence of the effect of the combination of TNF-α and IL-4 or TNF-α, IFN-γ, and IL-4 on the production of TARC by skin fibroblasts. Cells were cultured for 72 h with various concentrations of TNF-α in the absence or presence of IL-4 (10 ng/ml) (Fig. 3) or with various concentrations of IL-4 in the absence or presence of TNF-α (10 ng/ml) (Fig. 4). In the absence of IL-4, skin fibroblasts did not release detectable amount of TARC into the culture medium at any concentration of TNF-α; however, in the presence of IL-4, TNF-α induced a dose-dependent increase in TARC secretion that was statistically significant at concentrations of 0.1, 1, 10, and 100 ng/ml (Fig. 3). Similarly, in the absence of TNF-α, IL-4 did not induce detectable release of TARC. But, in the presence of TNF-α, IL-4 induced a dose-dependent increase in TARC release that was statistically significant at concentrations of 0.1, 1, 10 and 100 ng/ml (Fig. 4). Finally, in the presence of TNF-α (10 ng/ml) and IL-4 (10 ng/ml), IFN-γ induced a dose-dependent increase in TARC release that was statistically significant at concentrations of 0.1, 1, 10 and 100 ng/ml (Fig. 5). Low concentration of IFN-γ (0.1 ng/ml) suppressed TARC release.

DISCUSSION

We have shown that the combination of TNF-α and L-4 induces a synergistic increase in the expression of TARC at both the protein and mRNA levels in cultured human skin fibroblasts, CCD-986sk cell line. Exposure of the cells to either cytokine alone had no effect on TARC expression. Given that TARC is a potent chemoattractant for Th2 cells,

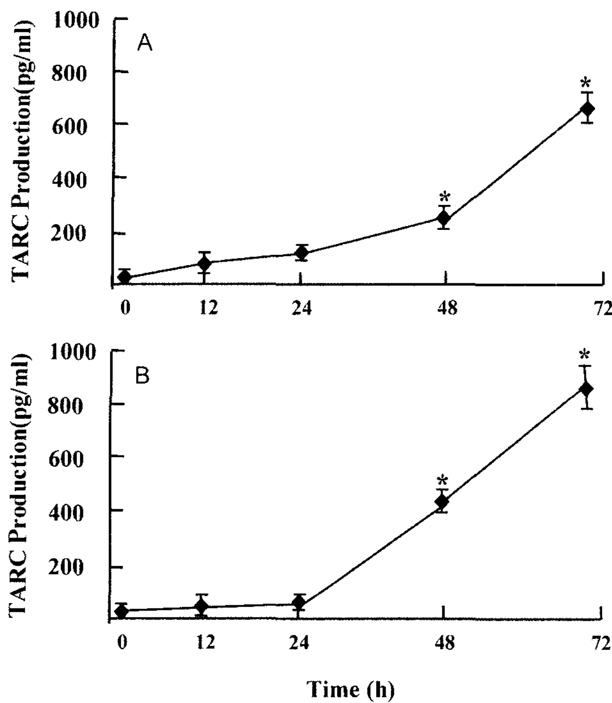

Fig. 2. Time course of TARC releases from human skin fibroblast cells. Skin fibroblast cells were cultured for the indicated times in the presence of both TNF-α (10 ng/ml) and IL-4 (10 ng/ml) (A) or TNF-α (10 ng/ml), IFN-γ (10 ng/ml), and IL-4 (10 ng/ml) (B), after which the culture supernatants were assayed by ELISA. Data are means ± SD (*n* = 3) from a representative experiment that was repeated three times with similar results. *$p < 0.01$ (Student's *t* test) vs the zero time point.

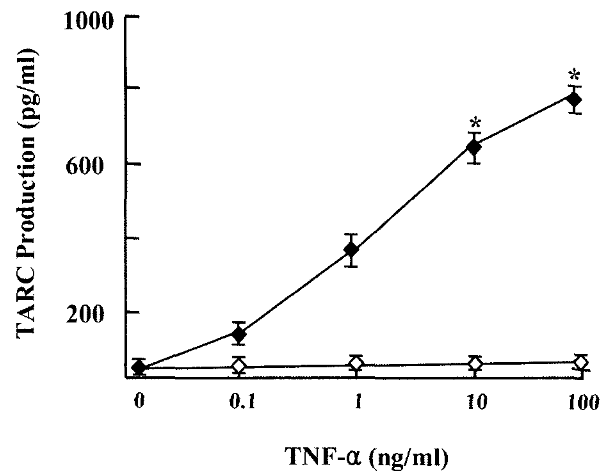

Fig. 3. Dose dependence of the effect of TNF-α in the absence or presence of IL-4 on TARC release by human skin fibroblast cells. Cells were cultured for 72 h with the indicated concentrations of TNF-α in the absence (◇) or presence (◆) of IL-4 (10 ng/ml). Culture supernatants were then assayed for TARC by ELISA. Data are means ± SD (*n* = 3) from a representative experiment that was repeated three times with similar results. *$p < 0.01$ (Fisher's PLSD test) vs the corresponding value for cells incubated in the absence of IL-4.

our results suggest that cytokine-induced activation of fibroblasts may contribute to the pathogenesis Th2 cell-mediated allergic reactions in skin tissue by promoting the infiltration of Th2 cells. Also, this result suggests that Th2 cytokine microenvironments in skin increase TARC production by skin fibroblast cells, providing attraction of Th2 cells in skin. This may be an amplification circuit in a Th2-dominant inflammatory skin disease like atopic dermatitis (AD).

It is generally believed that AD is a Th2 type disease, as the Th2 type lymphocytes produce IL-4, IL-5, IL-10, and IL-13. However, it has become evident in recent years that the cytokine profile in the skin changes during the course of the disease towards a Th1-Th2 mixed cytokine profile (IFN-γ, TNF-α, and IL-2) (Cheng et al., 1997; Nakazawa et al., 1997; Yamamoto et al., 1997; Spergel et al.,1999).

Th2 type cytokines (IL-4 and IL-13) are important in cell recruitment, inflammation and tissue damage; moreover TARC has been proven to paly an important role in the Th2 type immune response by selectivity recruiting the Th2-polarized memory/effector T cell into inflamed tissue. Also, TARC was expressed in epidermis of lesional skin from AD patients and NC/Nga mice exhibiting AD-like lesions (Vestergaard et al., 1999, 2000; Imai et al., 1999). In accordance with this, inhalant allergen-specific Th2 type cells have been found at increased frequency both in lesional atopic skin and in skin lesions that have been provoked in the skin of AD patients by epicutaneous application of inhalant allergens to which patients were sensitized (atopy patch test) (Neumann et al., 1996; Werfel et al., 1996; Reinhold et al., 1991; Van der Heijden et al., 1991).

It is notable that IFN-γ (Th1 type cytokine), when in the presence of both IL-4 (Th2 type cytokine) and TNF-α, induces skin fibroblast cell to express TARC which acts a specific ligand for Th2 type CC chemokine receptor (CCR4) (Fig. 5). IFN-γ is also the potent inducer of the trio of Th1 type CXC chemokines Mig/CXCL9, IP-10/CXCL10 and I-TAC/CXCL11 in various types of cells including human keratinocytes (Tensen et al., 1999). Mig, IP-10 and I-TAC commonly act on CXCR3 and selectively attract Th1 cells (Yoshie et al., 2001; Muller et al., 1993). Previous studies on the cytokine pattern of AD skin lesions have demonstrated that a Th2 cytokine profile (IL-4, IL-5 and IL-13) is predominant during the initial phase of skin inflammation, but both Th2 and Th1 cytokines (IL-5 and IFN-γ) are up-regulated in chronic lesions (Grewe et al., 1998; Leung et al., 2000; Ong et al., 2002; Kim et al., 2005).

Even though most AD patients had undetectable levels of IFN-γ in their plasma samples as control subjects (<8 pg/ml), IFN-γ may be the key factor in the chronic phase of AD because of its unique ability to simultaneously induce both Th1 and Th2 attracting chemokines from epidermal

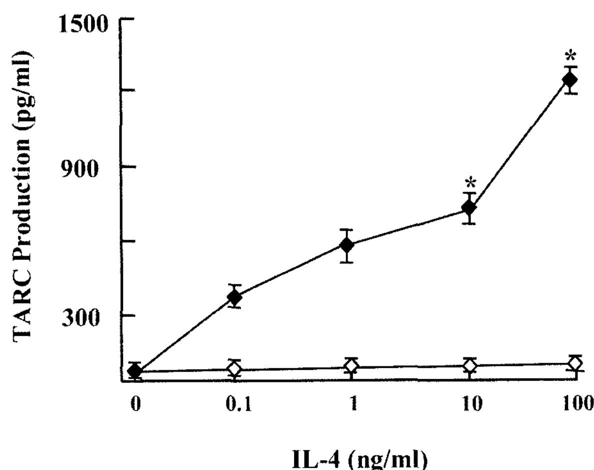

Fig. 4. Dose dependence of the effect of IL-4 in the absence or presence of TNF-α on TARC release by human skin fibroblast cells. Cells were cultured for 72 h with the indicated concentrations of IL-4 in the absence (◇) or presence (◆) of TNF-α (10 ng/ml). Culture supernatants were then assayed for TARC by ELISA. Data are means ± SEM (n = 3) from a representative experiment that was repeated three times with similar results. *p < 0.01 (Fisher's PLSD test) vs the corresponding value for cells incubated in the absence of TNF-α.

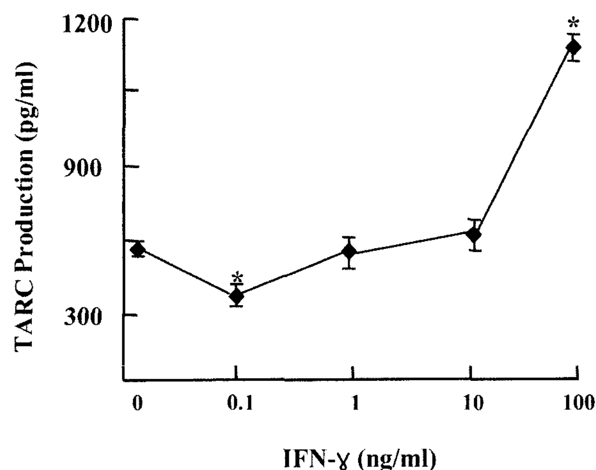

Fig. 5. Dose dependence of the effect of IFN-γ in the presence of TNF-α and IL-4 on TARC release by human skin fibroblast cells. Cells were cultured for 72 h with the indicated concentrations of IFN-γ in presence (◆) of TNF-α (10 ng/ml) and IL-4 (10 ng/ml). Culture supernatants were then assayed for TARC by ELISA. Data are means ± SD (n = 3) from a representative experiment that was repeated three times with similar results. *p < 0.01 (Fisher's PLSD test) vs the corresponding value for cells incubated in the absence of IFN-γ with the stimulation from both TNF-α and IL-4.

keratinocytes in AD skin. Indeed, the critical role of IFN-γ in AD pathogenesis has been amply demonstrated (Grewe et al., 1998). Although low concentrations of IFN-γ (0.1 ng/ml) suppressed TARC release, the time or dose dependence of IFN-γ was induced TARC release (Fig 2, 5). At the molecular level, the percentage of T cells producing IFN-γ in 48-72 h atopy patch-test sites significantly increased, matching the range found in chronic lesional atopic skin. The kinetics of this two-phase response is consistent with recent data generated in a mouse model, in which Th1 or Th2 cells were injected into skin to induce a local inflammatory reaction (Xiao et al., 2003). Whereas Th2 cells induced a rapid but short-lasting inflammation, the Th1 cell induced the reaction showed a later onset with prolonged kinetics. Also, TARC production is differently regulated by Langerhans cell, fibroblast and keratinocytes in various cytokines (Kumagai et al., 2000; Kakinuma et al., 2002; Fukuda et al., 2003). Sumiyoshi et al. (2003) suggested that IFN-γ and TNF-α-induced TARC production was inhibited by TGF-β in HaCaT cells via Smad pathway. The CCR4-mediated chemotaxis was mediated by p38-MAPK and MEK/ERK pathways (Moroi et al., 2004). It would be important to explore the regulatory mechanisms of TARC expression in fibroblasts for better understanding of the pathophysiology of atopic dermatitis. This mechanism will require some further studies.

In summary, the combination of TNF-α and IL-4 induces a time- and dose-dependently synergistic increase in the expression of TARC at both the protein and mRNA levels in cultured human skin fibroblasts. The high concentration (100 ng/ml) and long time incubation (72 h) of IFN-γ further enhanced the TNF-α/IL-4-induced TARC production in the skin fibroblast. This synergistic effect of Th2 and Th1 type cytokines on TARC production of skin fibroblast may contributes to an *in vitro* model of pathogenesis of atopic dermatitis. These observations may best be explained by a model in which the initiation of AD is driven by activation of Th2 type cells, whereas the chronic inflammatory reaction is dominated by a Th1 type response.

A distinct advantage of cell culture assays, as opposed to *in vivo* studies, is that it allows much broader range of experiments, observations, and therapies. The normal skin fibroblast CCD-986sk cell culture can be used to study for simple assay atopic dermatitis.

ACKNOWLEDGMENTS

This work was supported by $RIC_{(R)}$ grants from the Traditional and Bio-Medical Research Center, Daejeon University (RRC04730, 2005) by Korea Institutes of Industrial Technology Evaluation and Panning (ITEP).

REFERENCES

Cheng JF, Ott NL, Peterson EA, George TJ, Hukee MJ, Gleich GJ, and Leiferman KM (1997) Dermal eosinophils in atopic dermatitis undergo cytolytic degeneration. *J Allergy Clin Immunol* 99: 683-692.

Fukuda K, Fujitsu Y, Seki K, Kumagai N, and Nishida (2003) T Differential expression of thymus- and activation-regulated chemokine (CCL17) and macrophage-derived chemokine (CCL22) by human fibroblasts from cornea, skin, and lung. *J*

Allergy Clin Immuno 111: 520-526.

Grewe M, Bruijnzeel-Koomen CA, Schopf E, Thepen T, Langeveld-Wildschut AG, Ruzicka T, and Krutmann J (1998). A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. *Immunol Today* 19: 359-361.

Imai T, Baba M, Nishimura M, Kakizaki M, Takagi S, and Yoshie O (1997). The T cell-directed CC chemokine TARC is a highly specific biological ligand for CC chemokine receptor 4. *J Biol Chem* 272: 15036-15042.

Imai T, Yoshie O, Irimura T, Mizutani H, and Matsushima K (1999). Overproduction of Th2-specific chemokines in NC/Nga mice exhibiting atopic dermatitis-like lesions. *J Clin Invest* 104: 1097-1105.

Kakinuma T, Nakamura K, Wakugawa M, Mitsui H, Tada Y, Saeki H, Torii H, Asahina A, Onai N, Matsushima K, and Tamaki K (2001). Thymus and activation-regulated chemokine in atopic dermatitis: Serum thymus and activation-regulated chemokine level is closely related with disease activity. *J Allergy Clin Immunol* 107: 535-541.

Kakinuma T, Nakamura K, Wakugawa M, Yano S, Saeki H, Torii H, Komine M, Asahina A, and Tamaki K (2002). IL-4, but not IL-13, modulates TARC (thymus and activation-regulated chemokine)/CCL17 and IP-10 (interferon-induced protein of 10kDA)/CXCL10 release by TNF-alpha and IFN-gamma in HaCaT cell line. *Cytokine* 20: 1-6.

Kim JE, Kim BJ, Jeong MS, Seo SJ, Kim MN, Hong CK, and Ro BI (2005). Expression and modulation of LL-37 in normal human keratinocytes, HaCaT cells, and inflammatory skin diseases. *Korean Med Sci* 20: 649-654.

Kumagai N, Fukuda K, Ishimura Y, and Nishida T (2000). Synergistic induction of eotaxin expression in human keratocytes by TNF-α and IL-4 or IL-13. *Invest Ophthalmol Vis Sci* 41: 1448-1453.

Kumagai N, Fukuda K, and Nishida T (2000). Synergistic effect of TNF-α and IL-4 on the expression of thymus- and activation-regulated chemokine in human corneal fibroblasts. *Biochem Biophys Res Commun* 279: 1-5.

Leung DY (2000). Atopic dermatitis: new insights and opportunities for therapeutic intervention. *J Allergy Clin Immunol* 105: 860-876.

Moroi Y, Yu B, Urabe K, Koga T, Nakahara T, Dainichi T, Uchi H, Furue M (2004) Effects of MAPK inhibitors on CCR4-mediated chemotaxis against thymus and activation-regulated chemokine (TARC/CCL17). *J Dermatol Sci* 36: 186-188.

Muller KM, Jaunin F, Masouye I, Saurat JH, and Hauser C (1993) .Th2 cells mediate IL-4-dependent local tissue inflammation. *J Immunol* 150: 5576-5584.

Nakazawa M, Sugi N, Kawaguchi H, Ishii N, Nakajima H, and Minami M (1997). Predominance of type cytokines-producing CD4+ and CD8+ cells in patients with atopic dermatitis. *J Allergy Clin Immunol* 99: 673-682.

Neumann C, Gutgesell C, Fliegert F, Bonifer R. and Herrmann F (1996). Comparative analysis of the frequency of house dust mite specific and nonspecific Th1 and Th2 cells in skin lesions and peripheral blood of patients with atopic dermatitis. *J Mol Med* 74: 401-406.

Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, Gallo RL, and Leung DY (2002). Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Eng J Med* 347: 1151-1160.

Reinhold U, Kukel S, Goeden B, Neumann U, and Kreysel HW (1991). Functional characterization of skin-infiltrating lymphocytes in atopic dermatitis. *Clin Exp Immunol* 86: 444-448.

Robinson DS, Hamid Q,Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, and Kay AB (1992). Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 326: 298-304.

Smith RS, Smith TJ, Blieden TM, and Phipps RP (1997). Fibroblasts as sentinel cells. Synthesis of chemokines and regulation of inflammation. *Am J Pathol* 151: 317-322.

Spergel JM, Mizoguchi E, Oettgen H, Bhan AK, and Geha RS (1999). Roles of TH1 and TH2 cytokines in a murine model of allergic dermatitis. *J Clin Invest* 103: 1103-1111.

Sumiyoshi K, Nakao A, Setoguchi Y, Tsuboi R, Okumura K, Ogawa H (2003). TGF-beta/Smad signaling inhibits IFN-gamma and TNF-alpha-induced TARC (CCL17) production in HaCaT cells. *J Dermatol Sci* 31: 53-58.

Tensen CP, Flier J, Van Der Raaij-Helmer EM, Sampat-Sardjoepersad S, Van Der Schors RC, Leurs R, Scheper RJ, Boorsma DM, and Willemze R (1999). Human IP-9: A keratinocyte-derived high affinity CXC-chemokine ligand for the IP-10/Mig receptor (CXCR3). *J Invest Dermatol* 112: 716-722.

Terr AI (2001). The atopic diseases. In: Parslow TG, Stites DP, Terr AI, and Imboden JB (eds), Medical Immunology. 10th Ed. Lange Medical Books, New York, p 349.

Van der Heijden FL, Wierenga EA, Bos JD, and Kapsenberg ML (1991). High frequency of IL-4-producing CD4+ allergen-specific T lymphocytes in atopic dermatitis lesional skin. *J Invest Dermatol* 97: 389-394.

van Reijsen FC, Bruijnzeel-Koomen CA, Kalthoff FS, Maggi E, Romagnani S, Westland JK, and Mudde GC (1992). Skin-derived aeroallergen-specific T-cell clones of Th2 phenotype in patients with atopic dermatitis. *J Allergy Clin Immunol* 90: 184-193.

Vestergaard C, Yoneyama H, Murai M, Nakamura K, Tamaki K, Terashima Y, Imai T, Yoshie O, Irimura T, Mizutani H, and Matsushima K (1999). Overproduction of Th2-specific chemokines in NC/Nga mice exhibiting atopic dermatitis-like lesions. *J Clin Invest* 104: 1097-1105.

Vestergaard C, Bang K, Gesser B, Yoneyama H, Matsushima K, and Larsen CG (2000). A Th2 chemokine, TARC, produced by keratinocytes may recruit CLA+CCR4+ lymphocytes into lesional atopic dermatitis skin. *J Invest Dermatol* 115: 640-646.

Werfel T, Morita A, Grewe M, Renz H, Krutmann J, and Kapp A (1996). Allergen specificity of skin-infiltrating T cells is not restricted to a type-2 cytokine pattern in chronic skin lesions of atopic dermatitisJ. *Invest Dermatol* 107: 871-876.

Xiao T, Fujita H, Saeki H, Mitsui H, Sugaya M, Tada Y, Kakinuma T, Torii H, Nakamura K, Asahina A, and Tamaki K (2003). Thymus and activation-regulated chemokine (TARC/CCL17) produced by mouse epidermal Langerhans cells is upregulated by TNF-alpha and IL-4 and downregulated by IFN-gamma. *Cytokine* 23: 126-132.

Yamamoto S, Hamasaki Y, Ishii E, Ichimaru T, and Miyazaki S (1997). Unbalanced production of interleukin-5 and interleukin-2 in children with atopic dermatitis. *Ann Allergy Asthma Immunol* 78: 517-523.

Yoshie O, Imai T, and Nomiyama H (2001). Chemokines in immunity. *Adv Immunol* 78: 57-110.

[Received January 23, 2006; accepted March 14, 2006]