Archives of Dermatological Research (2019) 311:563–571
https://doi.org/10.1007/s00403-019-01932-x

CONCISE COMMUNICATION

Alterations in IL-4, IL-10 and IFN-γ levels synergistically decrease lipid content and protein expression of FAS and mature SREBP-1 in human sebocytes

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Received: 21 December 2018 / Revised: 2 May 2019 / Accepted: 14 May 2019 / Published online: 24 May 2019

Abstract
When anti-acne alternatives from dietary and plant sources are ingested, systemic alterations of interleukin (IL)-4, IL-10, IL-12 and interferon (IFN)-γ, individually or simultaneously, are induced at a 0.1–10.0-fold (×) range of normal physiological concentrations (1×). However, little is known about the effects of these cytokines on excess sebum, a pathophysiological factor of acne development. In this study, human sebocytes were treated with 0.1–10.0× of IL-4, IL-10, IL-12 and IFN-γ for 3 or 5 days to elucidate the effects on lipid content. Treatment with individual cytokines decreased the lipid content at specific concentrations rather than in a concentration-dependent manner. Specifically, 5.0× of IL-4, 5.0× of IFN-γ (5.0IFN), and 0.5×, 5.0× and 10.0× of IL-10 for 3 days, and 0.5× of IL-4 (0.5IL4) for 5 days decreased lipid content to 87.6–93.0% of the control. Treatment with other concentrations of IL-4, IL-10 and IFN-γ, and 0.1–10.0× of IL-12 did not alter lipid content. Combined treatment with 0.5IL4, 5.0IFN and 0.5× of IL-10 for 3 or 5 days decreased the lipid content more than each individual treatment. However, this effect was more evident after 3 days, in parallel with decreased levels of triglycerides, cholesterol esters and free fatty acids, the major lipid compositions of sebocytes, and decreased protein expression of fatty acid synthase (FAS) and mature sterol response element-binding protein-1 (SREBP-1), the lipogenesis-related factors, without altered cell proliferation. We demonstrated that suppressed IL-4 and IL-10 with enhanced IFN-γ synergistically decreased lipid content and protein expression of FAS and mature SREBP-1 in human sebocytes.

Keywords Sebocytes · Cytokines · Synergism · Lipid content · FAS · Mature SREBP-1

Introduction

Sebum is mainly composed of triglycerides (TG), cholesterol esters (CE), free fatty acids (FFA), and cholesterols (Chol), which are synthesized de novo in sebocytes [25, 43]. Sebum is initially stored as lipid droplets in the cytoplasm of premature sebocytes. During the final maturation process, the lipid droplets disintegrate, and sebum is excreted into follicles [28]. The lipid barrier formed by the excreted sebum protects skin from ultraviolet irradiation and environmental pathogens [34]. However, excess sebum along with follicular colonization of *Propionibacterium acnes*, which induces secretion of chemotactic and pro-inflammatory cytokines in sebocytes, is a major factor in the pathophysiology of acne vulgaris [8, 22].

The recognition that excess sebum is a pathophysiological factor in acne development, coupled with the undesirable side effects of pharmaceutical agents, has led to the search for anti-acne alternatives from dietary and plant sources [12, 27, 42]. Daily ingestion of n-3 polyunsaturated fatty acids (PUFAs) or γ-linolenic acid (18:3n-6) decreases the clinical severity of acne and reduces the expression of interleukin (IL)-8 in acne lesions [12]. Ingestion of n-3 or n-6 PUFAs also has been reported to alter the levels of IL-1, IL-4, IL-10 and interferon (IFN)-γ in plasma [24]. Cannabidiol, the major nonpsychotropic phytocannabinoid of *Cannabis sativa*, decreases lipogenesis and mRNA expression of IL-1, IL-6, and tumor necrosis factor-α (TNF-α) in sebocytes [27]. In addition, cannabidiol alters the IL-10 and

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IL-12 production in macrophages in vivo and in vitro [33]. Together, these observations suggest that ingested dietary or plant alternatives with anti-acne efficacy can reduce levels of principal pro-inflammatory cytokines of innate immunity, such as IL-1, IL-6, IL-8 and TNF-α [2, 10], and induce systemic alterations of adaptive immunity cytokines, such as IL-4, IL-10, IL-12 and IFN-γ. However, little is known about the effect of adaptive immunity cytokines, individually or in combination, on excess sebum, a major pathophysiological factor of acne development [8, 22]. In this study, to elucidate possible mechanisms for the anti-sebum effect of dietary or plant alternatives with anti-acne efficacy, we examined the effects of individual or combined treatments of IL-4, IL-10, IL-12, and IFN-γ on lipid content, lipid composition, and protein expression of lipogenesis-related factors in sebocytes.

Materials and methods

Cell culture and treatment

Primary human sebocytes (Cat. No. 36079-01, Celprogen, Torrance, CA, USA) were maintained in human sebocyte complete media containing 10% fetal bovine serum (Cat. No. M36079-01S, Celprogen), as described previously [26, 42]. Based on normal serum concentrations of cytokines in healthy adults (IL-4: 5 pg/ml [9, 14], IL-10: 10 pg/ml [16, 19], IL-12: 30 pg/ml [16, 19], IFN-γ: 10 pg/ml [9, 15]), which we referenced as a 1.0-fold (×) treatment, sebocytes were treated with various concentrations of IL-4, IL-10, IL-12 and IFN-γ (0.1, 0.5, 1.0, 5.0 or 10.0×) for 3 or 5 days. For the combined cytokine treatments, sebocytes were treated with 0.5 × IL-4 (0.5IL4) + 5.0 × IFN-γ (5.0IFN) (0.5IL4 + 5.0IFN); 0.5IL4 + 0.5 × IL-10 (0.5IL10) (0.5IL4 + 0.5IL10); 5.0IFN + 0.5IL10; 0.5IL4 + 5.0IFN + 0.5IL10; 0.5IL4 + 5.0IFN + 5.0 × IL-10 (5.0IL10) (0.5IL4 + 5.0IFN + 5.0IL10); 5.0 × IL-4 (5.0IL4) + 5.0IFN + 0.5IL10 (5.0IL4 + 5.0IFN + 0.5IL10); or 5.0IL4 + 5.0IFN + 5.0IL10 for 3 or 5 days. As a control, sebocytes were treated with phosphate buffered saline (PBS) (control), 10.0 ng/ml TNF-α (positive control) [5], 0.1 μM 13-*cis*-retinoic acid (RA) or 20.0 μM epigallocatechin-3-gallate (EGCG) (negative controls) [42, 44]. TNF-α and EGCG were dissolved in distilled water and diluted with PBS. RA dissolved in 0.2% dimethyl sulfoxide was kept in dark and diluted with PBS.

Lipid content analysis by Oil Red O staining

Cells were fixed with 10% formaldehyde, stained with Oil Red O working solution (isopropanol: H_2O, 6:4, v/v), and washed with distilled water, as described previously [5, 18]. The Oil Red O staining of lipid droplets was quantified by elution into 100% isopropanol. The staining intensity of each sample was determined at 500 nm using a plate reader and evaluated as described previously [5, 18].

Lipid composition analysis by high-performance thin-layer chromatography (HPTLC)

Total lipids were extracted from cell homogenates, and TG, CE, FFA and Chol, as well as squalene and wax esters that co-migrated with the respective standards, were fractionated by hexane:diethyl ether:acetic acid (70:30:1, *v*/*v*/*v*) in HPTLC, as described previously [7]. The evaluation method for the intensity of each fraction is described elsewhere [7].

Sebocyte proliferation

Cell proliferation was determined using a Cell Counting Kit-8 colorimetric assay (CCK-8; Enzo Life Science, Farmingdale, NY, USA) according to the manufacturer's instructions [41]. After cells were treated with cytokines for 3 days, 10 μl of CCK-8 solution, which contained the highly water-soluble tetrazolium salt (WST-8), was added to each well. After 2 h at 37 °C, the level of WST-8 formazan in the culture medium, which is proportional to the number of living cells, was determined at 450 nm by a plate reader.

Western blotting

Protein extracts (20 μg/lane) of cell lysates were separated on 8% SDS-PAGE gels and blotted to nitrocellulose membranes, as described previously [5]. Membranes were incubated with primary antibodies against fatty acid synthase (FAS) (sc-55580), peroxisome proliferator-activated receptor-γ (PPARγ) (sc-7273), sterol response element-binding protein-1 (SREBP-1) (sc-17755), and anti-human β-actin (sc-1616) (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA), followed by incubation with immunoglobulin G-horseradish peroxidase secondary antibodies. Immunoreactive proteins were visualized and quantified as described previously [5].

Statistical analysis

Data are expressed as mean ± SEM. Differences between treatment and control in Tables 1 and 2 were determined by unpaired Student's *t* test. Differences among all treatments in Fig. 1, and treatments (control; TNF-α; RA; EGCG; 0.5IL4; 5.0IFN; 0.5IL10; 0.5IL4 + 5.0IFN; and 0.5IL4 + 5.0IFN + 0.5IL10) in Table 1 were determined by one-way ANOVA coupled with Duncan's multiple comparison test using SPSS software for windows (ver. 23.0, IBM,

Table 1 Effects of individual or combined cytokine treatments on lipid content in sebocytes

Treatment[1]	Concentration	Relative lipid content (% control)	
		3 days	5 days
Control	–	100.0 ± 1.8[2ab]	100.0 ± 1.7[b]
Tumor necrosis factor-α	10.0 ng/ml	103.9 ± 2.5[a]	116.2 ± 4.5**[a]
13-*cis*-Retinoic acid	0.1 μM	95.2 ± 2.2[bc]	89.4 ± 2.9**[c]
Epigallocatechin-3-gallate	20.0 μM	85.7 ± 2.3***[d]	97.6 ± 2.1[bc]
IL-4			
0.1×	0.5 pg/ml	93.1 ± 4.4	102.8 ± 3.4
0.5×	2.5 pg/ml	95.7 ± 4.4[bc]	92.8 ± 2.9*[bc]
1.0×	5.0 pg/ml	99.8 ± 4.8	102.7 ± 4.2
5.0×	25.0 pg/ml	87.6 ± 3.0**	101.5 ± 4.0
10.0×	50.0 pg/ml	96.2 ± 3.5	108.2 ± 3.8
IL-12			
0.1×	3.0 pg/ml	96.5 ± 4.0	101.9 ± 3.0
0.5×	15.0 pg/ml	99.3 ± 3.2	99.6 ± 3.1
1.0×	30.0 pg/ml	105.4 ± 3.3	106.5 ± 3.1
5.0×	150.0 pg/ml	98.1 ± 2.9	98.2 ± 3.0
10.0×	300.0 pg/ml	97.6 ± 2.6	100.9 ± 2.5
IFN-γ			
0.1×	1.0 pg/ml	92.8 ± 3.9	100.3 ± 3.2
0.5×	5.0 pg/ml	97.0 ± 4.0	103.4 ± 3.5
1.0×	10.0 pg/ml	99.0 ± 3.7	102.2 ± 4.7
5.0×	50.0 pg/ml	88.7 ± 2.2***[cd]	97.5 ± 3.7[bc]
10.0×	100.0 pg/ml	97.6 ± 2.1	103.9 ± 3.8
IL-10			
0.1×	1.0 pg/ml	95.4 ± 2.8	103.7 ± 3.4
0.5×	5.0 pg/ml	93.0 ± 2.3*[bcd]	96.1 ± 3.3[bc]
1.0×	10.0 pg/ml	101.5 ± 2.4	107.7 ± 5.0
5.0×	50.0 pg/ml	88.7 ± 3.5**	95.7 ± 2.9
10.0×	100.0 pg/ml	91.4 ± 2.0**	94.5 ± 2.8
0.5IL4 + 5.0IFN	2.5 + 50.0 pg/ml	88.7 ± 2.1***[cd]	92.0 ± 1.7**[bc]
0.5IL4 + 0.5IL10	2.5 + 5.0 pg/ml	94.2 ± 1.3	94.2 ± 1.7
5.0IFN + 0.5IL10	50.0 + 5.0 pg/ml	95.8 ± 1.3	99.2 ± 1.8
0.5IL4 + 5.0IFN + 0.5IL10	2.5 + 50.0 + 5.0 pg/ml	86.2 ± 2.4***[d]	88.3 ± 2.1***[c]
0.5IL4 + 5.0IFN + 5.0IL10	2.5 + 50.0 + 50.0 pg/ml	99.6 ± 3.5	105.4 ± 1.6
5.0IL4 + 5.0IFN + 0.5IL10	25.0 + 50.0 + 5.0 pg/ml	95.0 ± 1.5	106.5 ± 2.7
5.0IL4 + 5.0IFN + 5.0IL10	25.0 + 50.0 + 50.0 pg/ml	95.5 ± 1.4	107.0 ± 1.9

[a,b,c,d]Means with different letters in the same column indicate significant differences among treatments (control; TNF-α; RA; EGCG; 0.5IL4; 5.0IFN; 0.5IL10; 0.5IL4 + 5.0IFN; and 0.5IL4 + 5.0IFN + 0.5IL10) at $P < 0.05$ by one-way ANOVA and Duncan's multiple comparison test

[1]Sebocytes were treated with either individual cytokines at the indicated concentrations, which are shown as fold (×) change based on the normal serum concentration (1.0×) or combined cytokines of 0.5× interleukin (IL)-4 (0.5IL4: 2.5 pg/ml) + 5.0× interferon (IFN)-γ (5.0IFN: 50.0 pg/ml) (0.5IL4 + 5.0IFN); 0.5IL4 + 0.5× IL-10 (0.5IL10: 5.0 pg/ml) (0.5IL4 + 0.5IL10); 5.0IFN + 0.5IL10; 0.5IL4 + 5.0IFN + 0.5IL10; 0.5IL4 + 5.0IFN + 5.0× IL-10 (5.0IL10: 50.0 pg/ml) (0.5IL4 + 5.0IFN + 5.0IL10); 5.0× IL-4 (5.0IL4: 25 pg/ml) + 5.0IFN + 0.5IL10 (5.0IL4 + 5.0IFN + 0.5IL10); or 5.0IL4 + 5.0IFN + 5.0IL10. As a control, sebocytes were treated with phosphate buffered saline (control), 10.0 ng/ml tumor necrosis factor-α (TNF-α) (positive control), 0.1 μM 13-*cis*-retinoic acid (RA) or 20.0 μM epigallocatechin-3-gallate (EGCG) (negative controls). After 3 or 5 days, lipid content was analyzed by Oil Red O staining. The staining intensity of each sample was first normalized to the corresponding protein concentration and then to the intensity of the control (100%)

[2]Values are mean ± SEM ($n = 15$) from three independent experiments. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ (***) compared with control by unpaired Student's *t* test

Table 2 Effects of individual or combined cytokine treatments on sebocyte proliferation

Treatment[a]	Concentration	Proliferation (% control)
Control	–	100.0 ± 2.5[b]
Tumor necrosis factor-α	10.0 ng/ml	91.4 ± 3.6
13-*cis*-Retinoic acid	0.1 μM	97.8 ± 2.8
Epigallocatechin-3-gallate	20.0 μM	40.9 ± 2.1***
0.5 × IL-4 (0.5IL4)	2.5 pg/ml	119.7 ± 1.7***
5.0 × IFN-γ (5.0IFN)	50.0 pg/ml	109.0 ± 1.6*
0.5 × IL-10 (0.5IL10)	5.0 pg/ml	102.5 ± 2.0
0.5IL4 + 5.0IFN	2.5 + 50.0 pg/ml	101.1 ± 0.8
0.5IL4 + 5.0IFN + 0.5IL10	2.5 + 50.0 + 5.0 pg/ml	94.2 ± 1.4

[a]Sebocytes were treated with cytokines at the indicated concentrations, which are shown as fold (×) change based on the normal serum concentration (1.0×): 0.5 × interleukin (IL)-4 (0.5IL4: 2.5 pg/ml); 5.0 × interferon (IFN)-γ (5.0IFN: 50.0 pg/ml); 0.5 × IL-10 (0.5IL10: 5.0 pg/ml); 0.5IL4 + 5.0IFN; or 0.5IL4 + 5.0IFN + 0.5IL10. As a control, sebocytes were treated with phosphate buffered saline (control), 10.0 ng/ml tumor necrosis factor-α (positive control), 0.1 μM 13-*cis*-retinoic acid or 20.0 μM epigallocatechin-3-gallate (negative controls). After 3 days, cell proliferation was measured using a Cell Counting Kit-8 colorimetric assay

[b]Values are mean ± SEM ($n = 5$). *$P < 0.05$ and ***$P < 0.001$ compared with control by unpaired Student's *t* test

Armonk, NY, USA). Differences with $P < 0.05$ were considered statistically significant.

Results

Effects of individual or combined cytokine treatments on lipid content in sebocytes

Ingestion of anti-acne alternatives frequently induces systemic alterations of IL-4, IL-10, IL-12 and IFN-γ at a 0.1–10.0× range of their normal physiological concentrations (1×) [23, 24, 33]. To elucidate a possible mechanism for the anti-sebum effect of ingested anti-acne alternatives, we first compared the effects of individual or combined treatments with 0.1–10.0× of IL-4, IL-10, IL-12 and IFN-γ on lipid content with those of positive or negative controls (Table 1). In Oil Red O staining, TNF-α (positive control) and RA (negative control) [5, 44] for 3 days had no effect on lipid content. However, after 5 days, TNF-α increased lipid content to 116.2%, and RA decreased it to 89.4%. EGCG, another negative control that is reported as an anti-sebum and anti-acne alternative [42], for 3 days significantly decreased lipid content to 85.7%, but had no effect after 5 days. TNF-α, RA and EGCG altered the lipid content to 85.7–116.2% of the control, but their effects varied depending on treatment duration.

Individual treatments with 0.1–10.0× of IL-4, IL-10 and IFN-γ for 3 or 5 days decreased the lipid content at specific concentrations rather than in a concentration-dependent manner. 5.0IL4, 5.0IFN, or 0.5×, 5.0× and 10.0× of IL-10 for 3 days decreased the lipid content to 87.6–93.0% of the control. 0.5IL4 for 5 days decreased the lipid content to 92.8% of the control. Neither IFN-γ and IL-10 treatments for 5 days nor IL-12 treatment for 3 and 5 days at any concentration altered lipid content. The combined treatment at the minimum effective concentrations of IL-4 (0.5 × IL4), IFN-γ (5.0 × IFN) and IL-10 (0.5 × IL10) (0.5IL4 + 5.0IFN + 0.5IL10) for 3 days decreased lipid content to a greater extent (86.2% of the control) than each individual treatment, which was comparable to the effect with EGCG, the anti-sebum and anti-acne alternative [42], for 3 days. The combined treatment with 0.5IL4 and 5.0IFN (0.5IL4 + 5.0IFN), but not with 0.5IL4 + 0.5IL10 or 5.0IFN + 0.5IL10, for 3 days decreased lipid content to 88.7% of the control, which was comparable to the effect with 5.0IFN, but modestly less than that with 0.5IL4 + 5.0IFN + 0.5IL10. Although 5.0IL4 or 5.0IL10 for 3 days decreased lipid content, combined treatments with 0.5IL4 + 5.0IFN + 5.0IL10, 5.0IL4 + 5.0IFN + 0.5IL10, or 5.0IL4 + 5.0IFN + 5.0IL10 for 3 days did not alter the lipid content. Notably, 0.5IL4 + 5.0IFN + 0.5IL10 or 0.5IL4 + 5.0IFN for 5 days decreased lipid content to 88.3–92.0% of the control. Other combined treatments for 5 days did not alter lipid content. Individual treatments with IL-4, IL-10 and IFN-γ at specific concentrations or combined treatments with 0.5IL4 + 5.0IFN + 0.5IL10 and 0.5IL4 + 5.0IFN decreased lipid content after 3 or 5 days, but these effects were more evident after 3 days. Therefore, we examined the effects of individual treatments with 0.5IL4, 5.0IFN, or 0.5IL10, and of combined treatments with 0.5IL4 + 5.0IFN + 0.5IL10 or 0.5IL4 + 5.0IFN for 3 days in further studies.

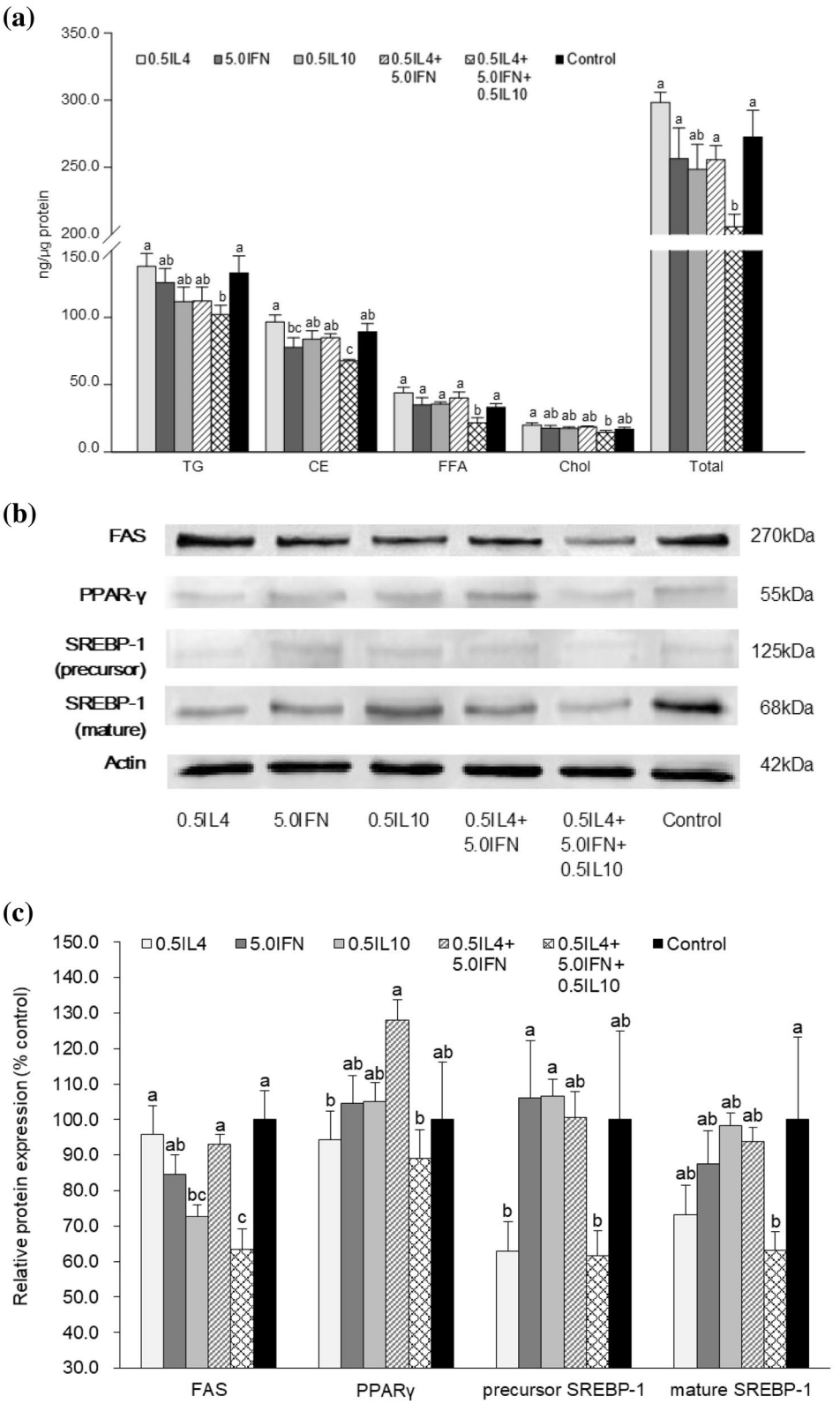

Fig. 1 Effects of individual or combined cytokine treatments on lipid composition and protein expression of lipogenesis-related factors in sebocytes. Sebocytes were treated with 0.5 × interleukin (IL)-4 (0.5IL4: 2.5 pg/ml), 5.0 × interferon (IFN)-γ (5.0IFN: 50.0 pg/ml), 0.5 × IL-10 (0.5IL10: 5.0 pg/ml), 0.5IL4 + 5.0IFN, or 0.5IL4 + 5.0IFN + 0.5IL10 for 3 days. Fold (×) changes are based on the normal serum concentration (1.0×) of each cytokine. As a control, sebocytes were treated with phosphate buffered saline for 3 days. **a** Lipid composition was fractionated by high-performance thin-layer chromatography for triglycerides (TG), cholesterol esters (CE), free fatty acids (FFA) and cholesterols (Chol), and expressed as ng/μg protein of cell homogenates. **b** Representative protein expression of fatty acid synthase (FAS), peroxisome proliferator response receptor-γ (PPARγ), precursor and mature sterol response element-binding protein-1 (SREBP-1), and actin. Protein extracts (20 μg/lane) of cell lysates were separated on 8% SDS-PAGE gels and immunoblotted with primary antibodies against FAS, PPARγ, SREBP-1 or actin (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA). **c** The signal intensities from multiple experiments of **b** were quantified, and integrated areas were first normalized to the corresponding value of actin (loading control) and then to the signal of the control (100%). Values are mean ± SEM ($n = 5$). [a,b,c]Means with different letters were significant different ($P < 0.05$) by one-way ANOVA and Duncan's multiple comparison test

Effects of individual or combined cytokine treatments on lipid composition and protein expression of lipogenesis-related factors in sebocytes

In HPTLC analysis, 0.5IL4 + 5.0IFN + 0.5IL10, but neither 0.5IL4 + 5.0IFN nor the individual treatments with 0.5IL4, 5.0IFN and 0.5IL10, decreased the level of total lipids, reflecting the decreased lipid content determined by Oil Red O staining (Fig. 1a). Moreover, 0.5IL4 + 5.0IFN + 0.5IL10, but neither 0.5IL4 + 5.0IFN nor the individual treatments with 0.5IL4, 5.0IFN and 0.5IL10, decreased the levels of TG, CE, and FFA, the fractionated major lipid compositions in sebocytes [25, 43]. The level of Chol was not altered by any of the treatments. The levels of squalene and wax esters could be altered by 0.5IL4 + 5.0IFN or the individual treatments with 0.5IL4, 5.0IFN and 0.5IL10, but they were detected at low or trace levels in the HPTLC analysis (data not shown).

FAS is a lipogenic enzyme for the de novo biosynthesis of FFA, and its expression is upregulated by nuclear transcription factors such as SREBP-1 and PPARs [28]. The precursor SREBP-1 is localized in the cytoplasm and is cleaved to yield mature SREBP-1 [17]. After translocated to the nucleus, mature SREBP-1 is involved in the synthesis of FFA [21, 30] that is then esterified into TG and CE [32]. Of the various PPAR isoforms, PPARγ plays a major role in sebocyte lipogenesis and inflammation [20]. 0.5IL4 + 5.0IFN + 0.5IL10 decreased protein expression of FAS and mature SREBP-1 (Fig. 1b, c). 0.5IL10 also decreased protein expression of FAS, but that decrease was modestly less than that with 0.5IL4 + 5.0IFN + 0.5IL10. Protein expression of FAS and mature SREBP-1 was not altered by other treatments. Protein expression of PPARγ and precursor SREBP-1 was not altered by any of the treatments.

Effects of individual or combined cytokine treatments on sebocyte proliferation

0.5IL4 + 5.0IFN + 0.5IL10, 0.5IL4 + 5.0IFN or 0.5IL10 did not alter sebocyte proliferation, similar to TNF-α and 13-RA (Table 2). However, 0.5IL4 or 5.0IFN increased sebocyte proliferation, whereas EGCG decreased it. Together, we demonstrated that 0.5IL4, 5.0IFN and 0.5IL10 synergistically decreased the levels of total lipids including TG, CE and FFA, and the protein expression of FAS and mature SREBP-1, the lipogenesis-related factors, with no altered sebocyte proliferation.

Discussion

When sebocytes were treated with IL-4, IL-10, IL-12 and IFN-γ individually, IL-4, IL-10, and IFN-γ, but not IL-12, decreased the lipid content at specific concentrations. Among adaptive immunity cytokines, IL-12 induces the differentiation of Th lymphocytes (Th-0) toward Th-1 cells that produce IFN-γ [1]. In contrast, IL-4 induces differentiation of Th-0 lymphocytes toward Th-2 cells, and promotes the production of IgE [40]. IL-10, whose production varies depending on stage of inflamed acne [3, 13], suppresses the Th-1-mediated immune response [6]. Ingestion of anti-acne alternatives, such as n-3 PUFA, decreases the clinical severity of acne and levels of IL-1, IL-6, IL-8 and TNF-α [12], the principle pro-inflammatory cytokines of innate immunity that are increased in inflamed acne [2, 10]. Moreover, increased IFN-γ or IL-12 level (by up to ~ 3-fold) and decreased IL-4 or IL-10 level (< 0.5-fold) are observed following ingestion of anti-acne alternatives, such as cannabidiol, n-3 or n-6 PUFAs [24, 33]. 0.5IL4, 5.0IFN and 0.5IL10, with fold alterations similar to those after ingestion of anti-acne alternatives in prior studies [24, 33], as well as 5.0IL4, 5.0IL10 and 10.0IL10, decreased the lipid content significantly in sebocytes after 3 or 5 days. Other prior studies demonstrate that 0.01 ng/ml IL-4 stimulates the migration of endothelial cells, whereas 1 ng/ml IL-4 inhibits it [38]. Moreover, 0.005–0.5 μM cannabidiol, the major nonpsychotropic phytocannabinoid of *Cannabis sativa*, increases IL-12 and decreases IL-10 production, but 1–5 μM cannabidiol has no effect on their production in murine macrophages [33]. The biphasic effect of cannabinoid on anxiety response of in vivo animal model is explained by its binding to glutamatergic CB1 receptor at 1 μg/kg vs. GABAergic CB1 receptor at 50 μg/kg, inducing different signaling pathways for either anxiolytic or anxiogenic effects [31]. As such, when IL-4, IL-10 and IFN-γ bind to their various receptors, such as type I vs. type II IL-4 receptors consisting of IL-4Rα with either γC or IL-13Rα1, IFN- γR1 vs. IFN-γR2 receptors, and IL-10R1 vs. IL-10R2 receptors, different signaling pathways are likely to be induced in sebocytes, which vary depending on the treated concentrations [4, 35, 38]. Although mechanisms for the concentration-independent effect of cytokines on lipid content remain to be elucidated in further studies, these results, together with prior studies [33, 38], suggest that specific concentrations (or fold alterations) of certain Th-1 or Th-2 cytokines, namely IL-4, IL-10 or IFN-γ, could be systemic mediators of ingested anti-acne alternatives, ultimately decreasing lipid content in sebocytes.

Combined treatment with 0.5IL4 + 5.0IFN + 0.5IL10 decreased the lipid content more than their individual treatment or 0.5IL4 + 5.0IFN. Moreover, 0.5IL4 + 5.0IFN + 0.5IL10 decreased the levels of total lipids

including TG, CE, and FFA, the fractionated major sebum lipids in HPTLC [25, 43], and protein expression of FAS and mature SREBP-1. Because the effect of 0.5IL4 + 5.0IFN on decreased lipid content was less than that of 0.5IL4 + 5.0IFN + 0.5IL10, 0.5IL10 may play an important role in the synergistic effect of 0.5IL4 + 5.0IFN + 0.5IL10. However, our results indicated no effect on lipid content of either 0.5IL4 + 0.5IL10 or 5.0IFN + 0.5IL10 as well as of 0.5IL4 + 5.0IFN + 5.0IL10, 5.0IL4 + 5.0IFN + 0.5IL10 or 5.0IL4 + 5.0IFN + 5.0IL10. Therefore, suppressed IL-4 (0.5IL4) and IL-10 (0.5IL10) with enhanced IFN-γ (5.0IFN) all seem to be required to decrease lipid content, lipid composition, and protein expression of FAS and mature SREBP-1. When anti-acne alternatives are ingested, the altered level of IFN-γ (by up to ~ 3-fold) is far higher than that of IL-4 or IL-10 (< 0.5-fold) [24, 33]. In terms of the balance between Th-1- and Th-2-type responses, which limit each other in adaptive immunity [1, 6, 40], 5.0IFN could be comparable with the decreased two, not one, Th-2 cytokines (0.5IL4 and 0.5IL10).

In sebocyte lipogenesis, activation of SREBP-1 is regulated by the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin complex 1 (mTORC1) pathway, which is induced by specific receptors of various stimuli including cytokines [39]. When bound to their receptors, IL-4 and IL-10 activate the PI3K/Akt/mTORC1 pathway [29], whereas chronic treatment of IFN-γ over 24 h inhibits the mTORC1 pathway [36]. Therefore, suppressed IL-4 and IL-10, and enhanced IFN-γ in the combined treatment of 0.5IL4 + 5.0IFN + 0.5IL10 could function synergistically to inhibit the mTORC1 pathway, which then inactivates SREBP-1, a major transcription factor for most lipid synthesizing enzymes including FAS [21, 28, 30]. However, the protein expression, but not mRNA expression, of FAS and mature SREBP-1 was decreased in 0.5IL4 + 5.0IFN + 0.5IL10-treated sebocytes, which did not seem to be related to inactivation of SREBP-1. Alternatively, the PI3K/Akt/mTORC1 pathway induces mRNA translation and cell proliferation by phosphorylation of S6 kinase and eukaryotic translational initiation factor (eIF) 4E-binding protein [21, 29, 39]. FAS and mature SREBP-1, but not precursor SREBP-1, are likely to be mTORC1 target mRNAs, whose translation is inhibited by 0.5IL4 + 5.0IFN + 0.5IL10. In contrast, the protein and mRNA expressions of PPARγ, another transcriptional factor that upregulates the expression of FAS [28], are induced by IL-4, but not by IL-10 and IFN-γ [11]. However, the protein expression of PPARγ was not altered by any of the treatments in this study. Moreover, unlike EGCG, a negative control used in this study, which inhibits the PI3K/Akt/mTORC1 pathway [21, 37], and thereby decreases proliferation and lipid content of sebocytes, 0.5IL4 + 5.0IFN + 0.5IL10 did not alter sebocyte proliferation. Together, these results suggest that the inhibited lipogenesis in sebocytes treated with 0.5IL4 + 5.0IFN + 0.5IL10 is not due to decreased cell proliferation, but is mediated by decreased protein expression of FAS and mature SREBP-1. The individual treatments of 0.5IL4, 5.0IFN and 0.5IL10, or 0.5IL4 + 5.0IFN increased cell proliferation (0.5IL4; 5.0IFN), inhibited lipid content in Oil Red O staining (5.0IFN; 0.5IL4 + 5.0IFN), or inhibited the protein expression of FAS (0.5IL10). Thus, these individual and combined treatments might alter the PI3K/Akt/mTORC1 pathway in different ways to regulate cell proliferation and protein/mRNA expressions of other lipid-synthesizing enzymes for squalene and wax esters with altered SREBP-1 activation. However, these mechanisms remain to be elucidated in future studies.

In summary, we demonstrated that the combined treatment with 0.5IL4, 5.0IFN and 0.5IL10 synergistically decreased the lipid content. In addition, levels of TG, CE and FFA, the major lipid composition of sebocytes, and protein expression of FAS and mature SREBP-1, the lipogenesis-related factors, were decreased with this combined treatment. Therefore, based on the results of this in vitro study, we conclude that alterations in IL-4, IFN-γ and IL-10 levels (i.e., suppressed IL-4 and IL-10 with enhanced IFN-γ) synergistically decrease lipid content, and protein expression of FAS and mature SREBP-1 in human sebocytes. Clinical studies in acne patients may allow confirmation of systemic alterations of Th-1- and Th-2-type cytokines, simultaneously suppressing IL-4 and IL-10, and enhancing IFN-γ, after ingestion of anti-sebum alternatives.

Acknowledgements This study was supported by CJ Foods R & D Center, CJ CheilJedang Corporation in Suwon-si, Gyeongggi-do, Republic of Korea (KHU Grant No. 20150943).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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