

[10] J. Li, C. Sutter, D.S. Parker, T. Blauwkamp, M. Fang, K.M. Cadigan, CBP/p300 are bimodal regulators of Wnt signaling, *EMBO J.* 26 (2007) 2284–2294.

\* Corresponding author.

E-mail address: [hnakano@hirosaki-u.ac.jp](mailto:hnakano@hirosaki-u.ac.jp) (H. Nakano).

Daiki Rokunohe, Hajime Nakano\*, Eijiro Akasaka, Yuka Toyomaki, Daisuke Sawamura  
Department of Dermatology, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki 036-8562, Japan

Received 24 March 2016

<http://dx.doi.org/10.1016/j.jdermsci.2016.06.005>

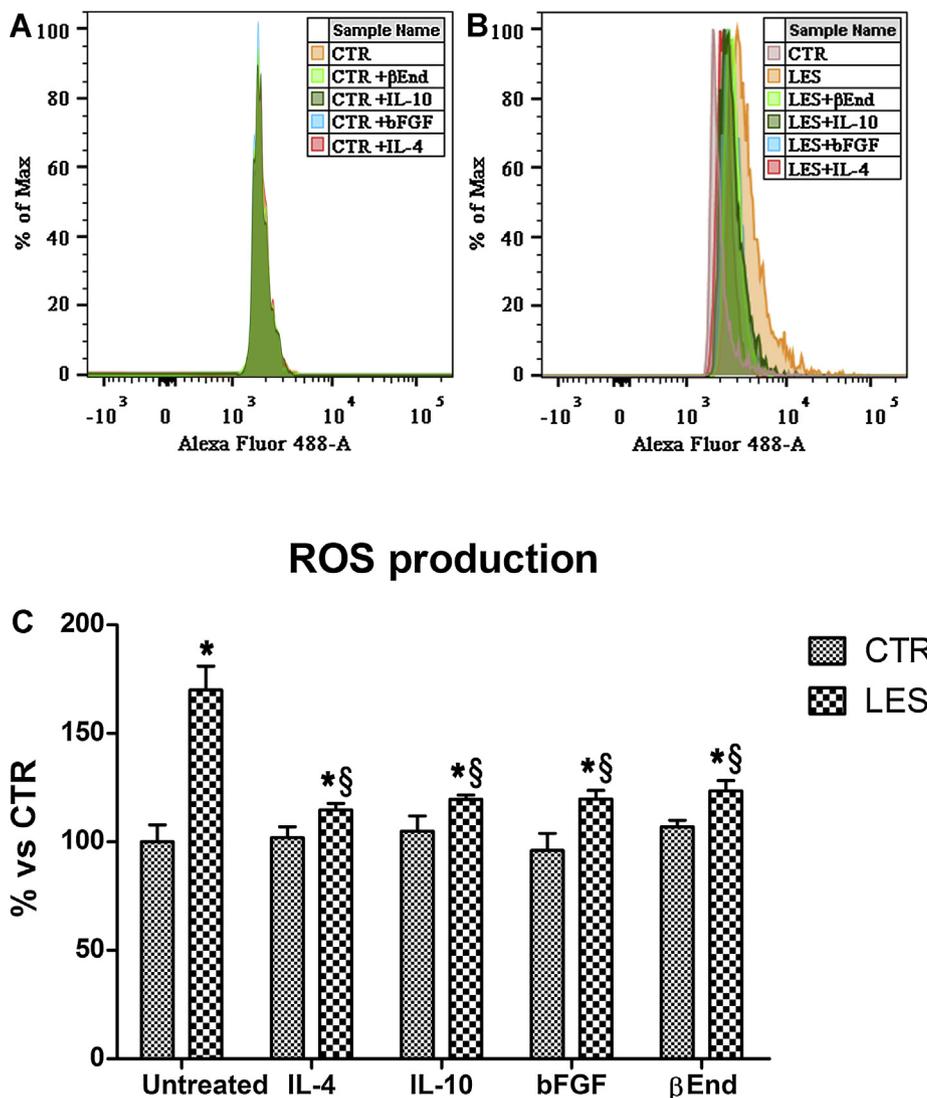
## Letter to the Editor

### Low dose cytokines reduce oxidative stress in primary lesional fibroblasts obtained from psoriatic patients



Psoriasis vulgaris is an inflammatory systemic disease with unclear pathogenesis characterized by the appearance of scaly

erythematous plaques on the skin. These plaques are the sites of keratinocytes hyper-proliferation and misdifferentiation and inflammatory infiltration by T cells skewed toward Th1/Th17 with respect to Th2/TREG. Such disequilibrium between T cells population features psoriasis as an autoimmune disease [1]. A prominent attention is given to biologic drugs aimed to restore the balance between T cells populations in psoriasis: the use of antibodies against the overproduced cytokines or the use of cytokines-antagonists represent two successful strategies that have been shown to ameliorate Psoriasis Area Severity Index



**Fig. 1.** Total ROS production in intact primary fibroblasts obtained from the lesional skin of psoriatic patients (LES) and from healthy donors (CTR). Total ROS production measured by FACS Analysis using  $H_2DCFDA$  fluorescent probe (A, B) following 48 h incubation with 10 fg/ml of sequential-kinetic-activation (SKA) IL-4 (LES + IL-4), IL-10 (LES + IL-10), bFGF (LES + bFGF) or  $\beta$ -endorphin (LES +  $\beta$ End). Treatment of LES with low dose cytokines significantly suppressed the emission of  $H_2DCFDA$  fluorescence in the present experimental condition. (C) Quantitative analysis of ROS production by flow cytometry where ROS production is expressed in percentage versus CTR. Reported values (means  $\pm$  SD) are representative of five independent experiments, each one performed in triplicate. \*Significant difference ( $P \leq 0.05$ ) versus untreated CTR, § Significant difference ( $P \leq 0.05$ ) versus untreated LES.

(PASI). Although targeting specific parts of immune system, such treatments require the use of high dose biologics and are frequently associated with adverse effects [2].

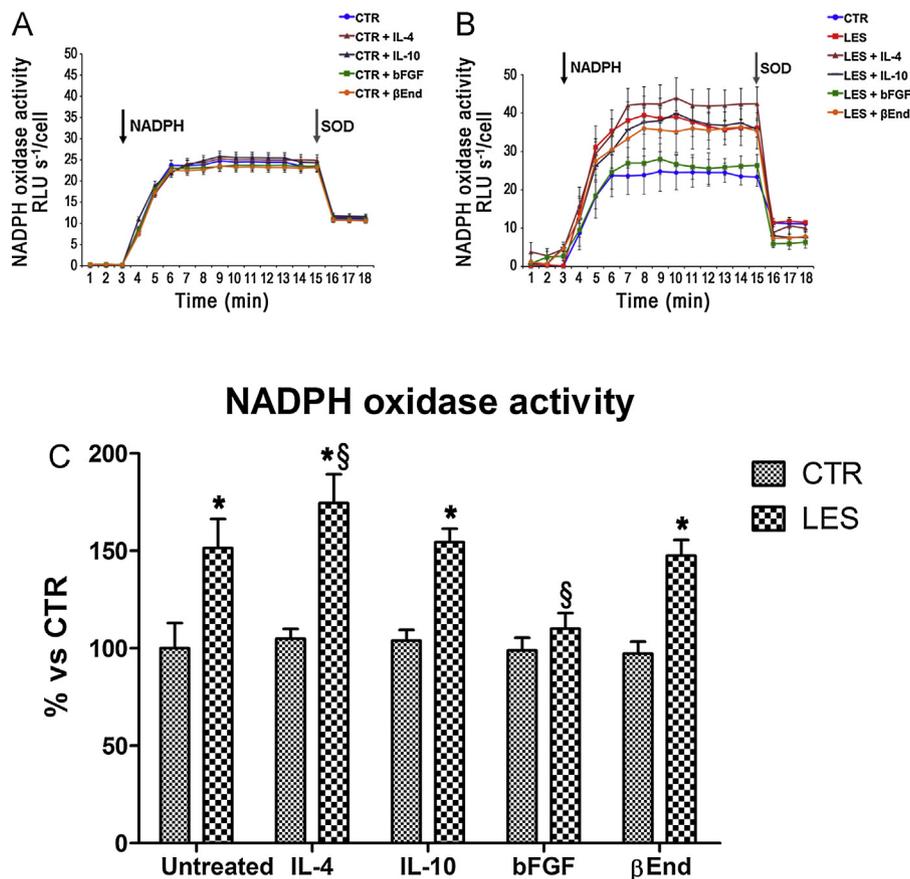
There are evidences that low doses of cytokines prepared by sequential-kinetic-activation (SKA) are effective and reduce adverse effects in psoriasis treatment with respect to standard dose of recombinant cytokines. Thus, in a clinical trial involving 48 patients affected by psoriasis vulgaris, the oral administration of SKA low-dose IL-4, IL-10 and IL-11 led to a significant reduction in PASI index with no adverse events [3]. Previously, signs of oxidative stress were found in the blood and skin of psoriatic patients [4,5] and according to our data, anti-TNF $\alpha$  drug Infliximab reduces oxidative stress in Peripheral Blood Mononuclear cells (PBMC) from psoriatic patients [4]. Moreover, we recently showed that the treatment with low dose SKA (femtograms per ml) IL-4, IL-10, basic fibroblasts growth factor (bFGF) and  $\beta$ -endorphin ( $\beta$ -End) decreases oxidative stress in perilesional keratinocytes obtained from vitiligo skin [6].

In the present study the effect of low dose SKA cytokines in fibroblasts from lesional skin of psoriatic patients was investigated. We focused our attention on dermal fibroblasts because these cells provide a crucial microenvironment for epidermal keratinocyte function and, together with infiltrating polymorphonuclear leukocytes were shown to extensively produce superoxide and H<sub>2</sub>O<sub>2</sub> impairing the redox balance in psoriatic derma and

participating, at least partially, in abnormal keratinocytes growth in psoriasis [7].

The following SKA cytokines (10 fg/ml) were used: (a) IL-10 and IL-4 produced by TREGs and Th2, respectively; (b)  $\beta$ -endorphin, neuropeptide that was shown to be increased in the serum of psoriatic patients and produced directly in psoriatic plaques by inflammatory cells [8]; and (c) bFGF that was shown to increase cellular resistance to oxidative stress *in vitro* [9]. Lesional skin punch biopsies were obtained from four patients affected by plaque psoriasis and primary fibroblasts cell cultures were established. Cells on passages 2–5 were used for experiments. Cellular redox profile was determined by measuring intracellular ROS production by flow cytometry analysis (fluorescent probe H<sub>2</sub>DCFDA) and NADPH oxidase activity by luminometric assay [4]. In Fig. 1 a significantly higher total ROS production by lesional fibroblasts with respect to control fibroblasts is shown 170  $\pm$  11% versus 100  $\pm$  7% respectively. In lesional fibroblasts NADPH oxidase activity significantly raised (by 51.43  $\pm$  15%) compared to control cells (Fig. 2).

In order to investigate the role of low dose SKA IL-4, IL-10, bFGF and  $\beta$ -End on the above parameters, lesional fibroblasts were incubated with each cytokine for 48 h and then total ROS production and NADPH oxidase activity were measured. Interestingly, every cytokine treatment significantly reduced intracellular ROS production (Fig. 1): IL-4 by 29.3  $\pm$  3, IL-10 by 24.3  $\pm$  2, bFGF by 24.3  $\pm$  4 and



**Fig. 2.** NADPH oxidase activity in intact primary fibroblasts obtained from the lesional skin (LES) of psoriatic patients and from healthy donors (CTR). NADPH oxidase activity was measured by a luminometric assay following 48 h incubation with 10 fg/ml of sequential-kinetic-activation (SKA) IL-4 (LES + IL-4), IL-10 (LES + IL-10), bFGF (LES + bFGF) or  $\beta$ -endorphin (LES +  $\beta$ End). Among all cytokines only bFGF led to a significant down-regulation of NADPH oxidase activity in LES fibroblasts. The histogram (C) represents the corresponding values in% normalized to CTR of area under the curve of NADPH oxidase activity. Extracellular ROS production was significantly higher in LES with respect to CTR fibroblasts. Reported values (means  $\pm$  SD) are representative of five independent experiments, each one performed in triplicate. \*Significant difference ( $P \leq 0.05$ ) versus untreated CTR, § Significant difference ( $P \leq 0.05$ ) versus untreated LES.

$\beta$ -End by  $20.5 \pm 5\%$  vs LES fibroblasts. As regards as NADPH oxidase activity, only bFGF among selected cytokines was effective in reducing this parameter in lesional cells to control level (Fig. 2).

Firstly, the results of our study demonstrate an increased ROS production in lesional fibroblasts from psoriatic patients. Moreover, we show that also extracellular ROS production via NADPH oxidase is significantly increased in lesional with respect to control fibroblasts. Importantly, all experiments were performed *in vitro*, in the absence of T cell infiltrates, so our data indicate the existence of an intrinsic oxidative stress condition in psoriatic fibroblasts.

In particular, in psoriatic lesion, extracellular ROS overproduction by fibroblasts can exert a pro-inflammatory role in psoriatic skin independently and in concomitance with infiltrating T cells. Hence, redox balancing agents can represent an adjuvant therapy for psoriasis. In the current work we studied the effect of low dose SKA IL-4, IL-10, bFGF and  $\beta$ -End on the redox balance of lesional fibroblasts. We found that all of selected cytokines decreased significantly intracellular ROS production in lesional fibroblasts, however, only bFGF was effective in reducing NADPH oxidase activity. The inhibitory effect of bFGF on NADPH oxidase was already shown in adipocytes via the mechanism of direct non kinase-dependent coupling of bFGF receptor to G protein of NADPH oxidase [10]. A similar mechanism could occur in psoriatic lesional fibroblasts although further studies are needed. At the same time IL-4, IL-10 and  $\beta$ -End act on intracellular ROS producing systems, such as mitochondrial electron transport chain or xanthine oxidase: other two main sources of ROS in the cell. Taken together, our preliminary data show the effectiveness of low doses SKA IL-4, IL-10, bFGF and  $\beta$ -End in modulation of oxidative stress in lesional fibroblasts obtained from the skin of psoriatic patients. Although further studies should be performed, the possible use of low dose cytokines as a targeting treatment for psoriasis vulgaris can be suggested.

#### Conflict of interest

The authors have no conflict of interest to declare.

#### Funding source

Ente Cassa di Risparmio di Pistoia e Pescia (Grant no. 2011.0365).

#### References

- [1] A. Alikhan, L.M. Felsten, M. Daly, V. Petronic-Rosic, Vitiligo: a comprehensive overview Part I. Introduction, epidemiology, quality of life, diagnosis,

- differential diagnosis, associations, histopathology, etiology, and work-up, *J. Am. Acad. Dermatol.* 65 (2011) 473–491.
- [2] D.E. Brănișteanu, C.M. Voicu, A. Crețu, A. Dimitriu, M.C. Luca, C.M. Sălăvăstru, Adverse reactions of biological therapy for psoriasis, *Rev. Med. Chir. Soc. Med. Nat. Iasi.* 119 (2015) 38–44.
- [3] M.L. Roberti, L. Ricottini, A. Capponi, E. Scლაუzero, P. Vicenti, E. Fiorentini, et al., Immunomodulating treatment with low dose interleukin-4, interleukin-10 and interleukin-11 in psoriasis vulgaris, *J. Biol. Regul. Homeost. Agents* 28 (2014) 133–139.
- [4] V. Barygina, M. Becatti, G. Soldi, F. Prignano, T. Lotti, P. Nassi, D. Wright, N. Taddei, C. Fiorillo, Altered redox status in the blood of psoriatic patients: involvement of NADPH oxidase and role of anti-TNF- $\alpha$  therapy, *Redox Rep.* 18 (2013) 100–106.
- [5] Q. Zhou, U. Mrowietz, M. Rostami-Yazdi, Oxidative stress in the pathogenesis of psoriasis, *Free Radic. Biol. Med.* 47 (2009) 891–905.
- [6] V. Barygina, M. Becatti, T. Lotti, S. Moretti, N. Taddei, C. Fiorillo, Treatment with low-dose cytokines reduces oxidative-mediated injury in perilesional keratinocytes from vitiligo skin, *J. Dermatol. Sci.* 79 (2015) 163–170.
- [7] K.J. Trouba, H.K. Hamadeh, R.P. Amin, D.R. Germolec, Oxidative stress and its role in skin disease, *Antioxid. Redox Signal.* 4 (2002) 665–673.
- [8] W. Glinski, H. Brodecka, M. Glinska-Ferenz, D. Kowalski, Increased concentration of beta-endorphin in sera of patients with psoriasis and other inflammatory dermatoses, *Br. J. Dermatol.* 131 (1994) 260–264.
- [9] A. Burdzińska, U. Bartoszek-Bruzzzone, M.M. Godlewski, A. Orzechowski, Sodium ascorbate and basic fibroblast growth factor protect muscle-derived cells from H<sub>2</sub>O<sub>2</sub>-induced oxidative stress, *Comp. Med.* 56 (2006) 493–501.
- [10] H.I. Krieger-Brauer, P. Medda, H. Kather, Basic fibroblast growth factor utilizes both types of component subunits of Gs for dual signaling in human adipocytes. Stimulation of adenylyl cyclase via Galph(s) and inhibition of NADPH oxidase by Gbeta gamma(s), *J. Biol. Chem.* 275 (2000) 35920–35925.

Victoria Barygina<sup>1,\*</sup>, Matteo Becatti<sup>1</sup>

Department of Experimental and Clinical Biomedical Sciences “Mario Serio”, University of Florence, Viale Morgagni, 50, 50134 Florence, Italy

Torello Lotti

Dermatology and Venereology Division, Guglielmo Marconi University, Via Plinio, 44, 00193 Roma, Italy

Niccolò Taddei, Claudia Fiorillo

Department of Experimental and Clinical Biomedical Sciences “Mario Serio”, University of Florence, Viale Morgagni, 50, 50134 Florence, Italy

<sup>1</sup>These authors contributed equally to this work.

\* Corresponding author.

E-mail address: [v.barygina@gmail.com](mailto:v.barygina@gmail.com) (V. Barygina).

Received 1 April 2016

<http://dx.doi.org/10.1016/j.jdermsci.2016.06.002>

## Letter to the Editor

### Association of interleukin (IL)18 and IL10 gene polymorphisms with oral lichen planus risk; a case-control study



Expression of both pro- and anti-inflammatory cytokines has been reported in oral lichen planus (OLP) [1]. Interleukin-18 (IL18) an interferon gamma (INF $\gamma$ ) inducing agent is involved in OLP as it is a potent pro-inflammatory cytokine besides its inhibitory effect on the synthesis of IL10 [2]. IL-10 is an anti-inflammatory cytokine

having a direct ability to down-regulate IL18 and INF $\gamma$ , which makes it an important immunoregulator as well as a mediator of inflammatory process [3].

Genetic factors producing disproportions in pro- and anti-inflammatory cytokine production have been anticipated to increase the susceptibility for OLP [1].

The above mentioned entangled cytokines network connections drew our attention to investigate a possible association between functional single nucleotide polymorphisms (SNPs) related to these cytokines and OLP. The impact of these polymorphisms on tissue IL18 and IL10 expression was also determined.

This case-control study included 72 consecutive newly diagnosed OLP Egyptian patients and 75 apparently healthy Egyptian volunteers as controls. All subjects were free from any systemic