Treatment with low-dose cytokines reduces oxidative-mediated injury in perilesional keratinocytes from vitiligo skin

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 red itchy 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 (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 the signs of oxidative stress were found in the blood and skin of psoriatic patients (4, 5) and according to our data anti-TNFα 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 β-endorphin (β-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. The following SKA cytokines (10 fg/ml) were used: a) IL-10 and IL-4 produced by TREGs and Th2, respectively; b) β-endorphin, neuropeptide that was shown to be increased in the serum of psoriatic patients and produced directly in psoriatic plaques by inflammatory cells (7); and c) bFGF that was shown to increase cellular resistance to oxidative stress *in vitro* (8). 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_2DCFDA) and NADPH oxidase activity by luminometric assay (4). In Fig.1 (A, B) a significantly higher total ROS production by lesional fibroblasts with respect to control fibroblasts is shown (170 ± 11 % versus 100 ± 7 % respectively (Fig. 1, B)). In

lesional fibroblasts NADPH oxidase activity significantly raised (by 51.43 ± 15%) compared to control cells (Fig.1, C, D).

In order to investigate the role of low dose SKA IL-4, IL-10, bFGF and β-End on the above parameters, lesional fibroblasts were incubated with each cytokine for 48 hours and then total ROS production and NADPH oxidase activity were measured. Interestingly, every cytokine treatment significantly reduced intracellular ROS production (Fig.1, A, B): IL-4 by 29.3 ± 3, IL-10 by 24.3 ± 2, bFGF by 24.3 ± 4 and β-End by 20.5 ± 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.1, C,D).

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-infiammatorial 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 β-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 (9). A similar mechanism could occur in psoriatic lesional fibroblasts although further studies are needed. At the same time IL-4, IL-10 and β-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 (10). Taken together, our preliminary data show the effectiveness of low doses SKA IL-4, IL-10, bFGF and β-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.

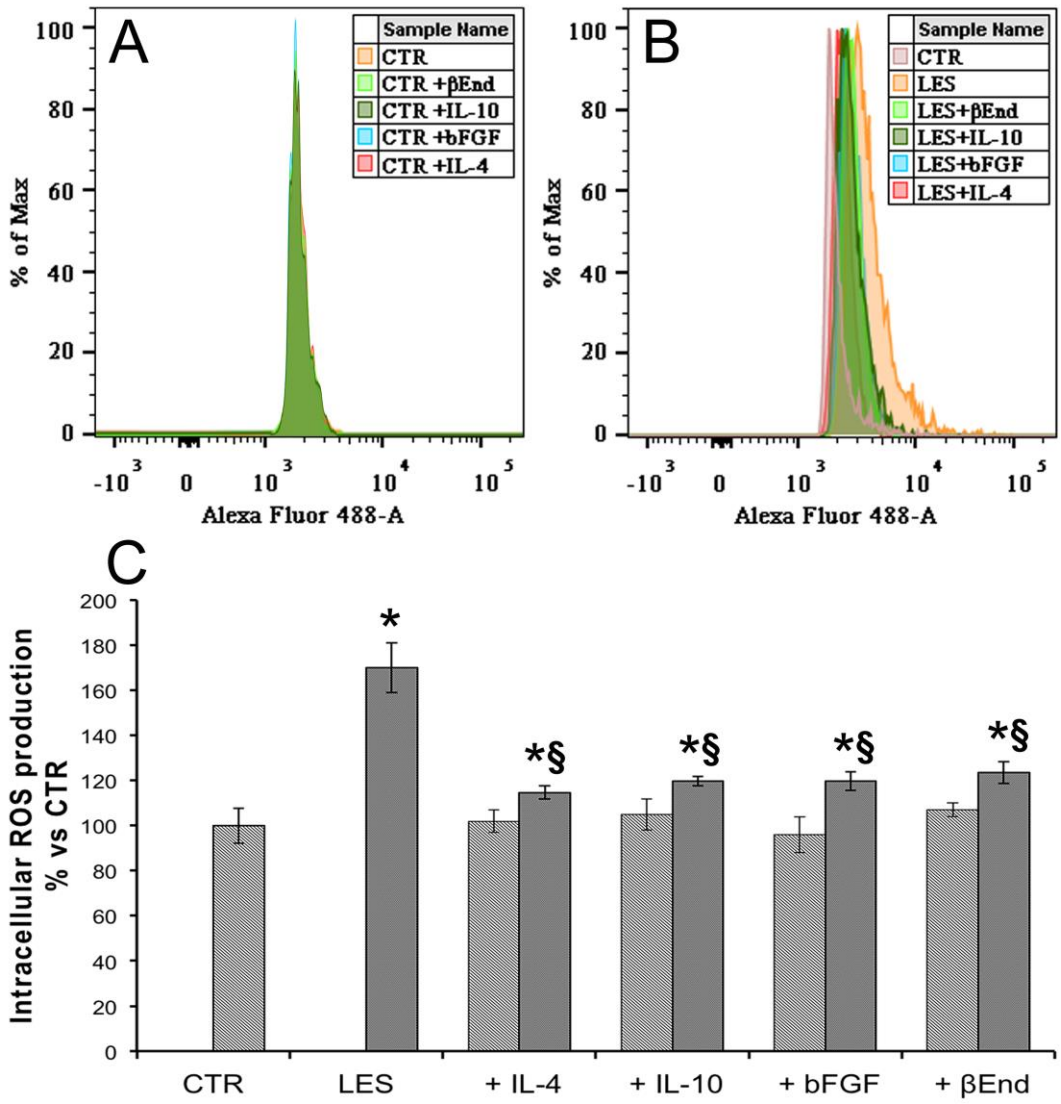

Fig. 1. Total ROS production in intact primary fibroblasts obtained from healthy donors (CTR) and from the lesional skin (LES) of psoriatic patients. Following 48 h incubation with 10 fg/ml of sequential-kinetic-activation (SKA) IL-4 (LES + IL-4), IL-10 (LES + IL-10), bFGF (LES + bFGF) or β-endorphin (LES + βEnd) the total ROS production and NADPH oxidase activity were measured by FACS analysis using H_2DCFDA fluorescent probe in CTR (A) and LES (B). Treatment of CTR with low dose cytokines didn't change significantly the emission of H2DCFDA fluorescence in the present experimental condition (A), well seen by quantitative analysis of ROS production by flow cytometry (C) where the ROS production is expressed in percentage versus untreated CTR. Instead, low dose cytokines suppressed significantly the emission of H2DCFDA fluorescence in LES (B, C). Reported values (means ±SD) are representative of three independent experiments. * Significant difference ($P \leq 0.05$) versus untreated CTR, § Significant difference ($P \leq 0.05$) versus untreated LES.

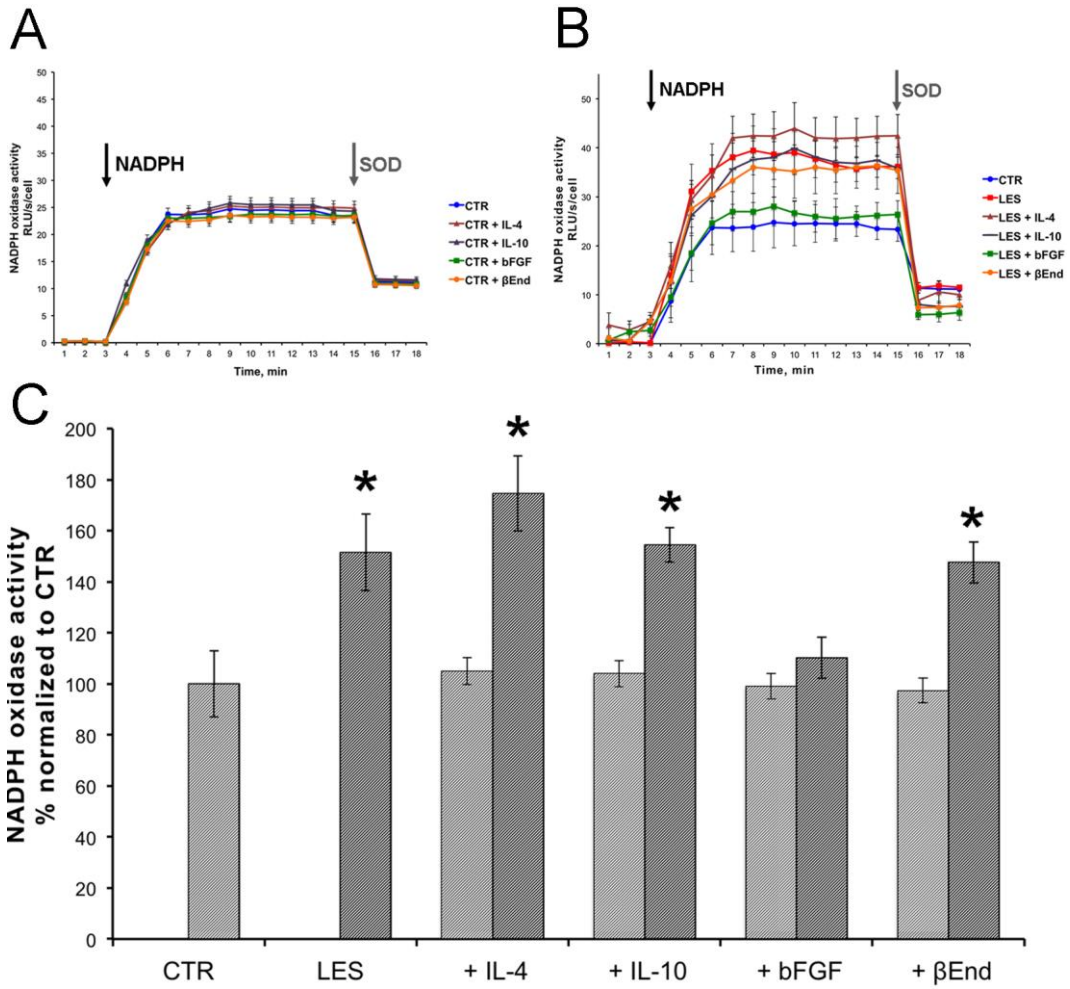

Fig. 2. NADPH oxidase activity in intact primary fibroblasts obtained from healthy donors (CTR) and from the lesional skin (LES) of psoriatic patients. NADPH oxidase activity was measured in CTR and LES following 48 h incubation with 10 fg/ml of sequential-kinetic-activation (SKA) IL-4 (LES + IL-4), IL-10 (LES + IL-10), bFGF (LES + bFGF) or β-endorphin (LES + βEnd) by luminometric assay. Treatment of CTR with low dose cytokines didn't change significantly the NADPH oxidase activity in the present experimental condition (A), well seen in the histogram (C) which represents the values of area under the curve of NADPH oxidase activity expressed in % versus untreated CTR. Extracellular ROS production was significantly higher in LES with respect to CTR fibroblasts (B, C). Among all cytochines only bFGF leaded to a significant down-regulation of NADPH oxidase in LES fibroblasts. Reported values (means ±SD) are representative of five independent experiments, each one performed in triplicate. * Significant difference ($P \leq 0.05$) versus untreated CTR.

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