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Research Article

Bovine Lactoferrin: A Nutritional Supplement for Down-Regulation of Inflammatory Response in Cutaneous Disorder

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Abstract

Background: Lactoferrin (LF)is an innate-defence non-heme iron binding glycoprotein of 80 kDa able to support the immune system and influence immune cell activity by antioxidant, antibacterial and antiviral properties. This work focusses on the study of the in vitro anti-inflammatory activity of Bovine LF(bLF)on Lipopolysaccharide (LPS)-induced cytokines expression.

Methods: We investigated the immunomodulatory effect of bLF on tumor necrosis factor alpha (TNF-α), interleukin-10 (IL-10) and interleukin-12 (IL-12) cytokines on human keratinocytes NCTC2544 and human myelomonocytic leukaemia cells, THP-1.

Results: Bovine LF exerted an anti-inflammatory activity since early phase to 8h of treatment, by modulating cytokines expression and secretion.

Conclusions: These data encourage the use of bLF as immunomodulatory agent in the treatment of different dermatological conditions linked to inflammatory processes.

Keywords: Acne Vulgaris; Cutaneous Disorder; Cytokines; Immunomodulation; Interleukin-10 (IL-10); Interleukin-12 (IL-12); Lactoferrin; Lipopolysaccharide (LPS); Nutritional Supplement; Tumor Necrosis Factor Alpha (TNF-α)

Abbreviations:

LF	:	Lactoferrin
TNF-α	:	Tumor Necrosis Factor Alpha
LPS	:	Lipopolysaccharide
IL-10	:	Interleukin-10
IL-12	:	Interleukin-12
hLF	:	Human Lactoferrin
bLF	:	Bovine Lactoferrin
LF10	:	Lactoferrin 10 µg/mL
LF40	:	Lactoferrin 40 µg/mL
PC	:	Positive Control
FBS	:	Fetal Bovine Serum
MTT	:	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

GAPDH	:	Human glyceraldehyde-3-phosphate dehydrogenase
SD	:	Standard Deviation
SEM	:	Standard Error from Mean
ROS	:	Reactive Oxygen Species

Introduction

Lactoferrin (LF), is an 80 kDa glycoprotein belonging to the transferrin family of non-heme iron binding proteins together with transferrin [1-3], ovotransferrin [4], melanotransferrin [5] and a recently identified carbonic anhydrase inhibitor [6]. The main role of these proteins is to control the levels of free iron in biological fluids. LF was discovered for first time from bovine milk by Sorensen and Sorensens in 1939 [7] and then purified by three independent laboratories in 1960 [8-10].

It consists of a single polypeptide chain of 703 amino acids, folded in two globular lobes, C (carboxy) and N(amino) terminal respectively, connected by a α-helix, with one iron binding site on each lobe. LF binds primarily Fe^{+2} or Fe^{+3} but is also capable of binding other metals ions traces like Al^{3+}, Ga^{3+}, Mn^{3+}, Co^{3+}, Cu^{2+}, Zn^{2+} but with much lower affinity [11].

LF is found primarily in exocrine secretions such as milk [12,13] tears, nasal exudate or bronchial mucus [11,14-18], and is also known as a major component of the secondary granules of neutrophils [19]. Conversely, blood, plasma and serum levels of LF are very low [16]. An increase of LF in blood may occur during infection, inflammation or tumor growth [20]. Due to its higher affinity for iron [21], the ability to retain iron over a broad pH range [22,23], and differential tissue relative distribution than other transferrins, LF owns unique functional properties.

Following infection or inflammation, pH levels on sites of inflammation become very low (<4.5) due to metabolic activity of bacteria. In such a condition LF prevents bacterial proliferation by its bound to iron, also the one released from transferrin [24]. Being a multifunctional molecule, LF owns several physiological functions: i) iron absorption and metabolism [25,26]; ii) as a part of the innate immune system, LF protects against microbial infections, both Gram-positive and negative bacteria, viruses, protozoa, or fungi [27]; iii) antibacterial [27] and antiviral activity [28]; stimulation of bone growth [29]; prevention of inflammation by reducing production of pro-inflammatory cytokines [30,31] and diminishing oxidative stress [32]; antiparasitic activity [33]; antitumor activity [34-36].

Recently, LF has been named "Nutraceutical Protein", due to its multiple properties and the potential for use as a therapeutic protein [37]. Until recently, the main source of LF was from human breast milk. Nowadays, however, LF from bovine source, Bovine Lactoferrin (bLF) is ready available. bLF has about 69% amino acids identity with Human LF(hLF) [38] but, despite slight changes in domains orientation and closure any functional differences are found [39]. Therefore, the carbohydrate structure of bLF is much better defined than that of hLF [40] and it has been shown that bLF bind to human neutrophils with higher affinity than hLF [41]. bLF is generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) and permitted as food and dietary supplement ingredient in many Countries. Supplements of bLF are reported to have the ability to support the immune system by anti-infective, anti-cancer, and anti-inflammatory effects [42] highlighting the bLF potential as therapeutic agent.

Nowadays several studies have re-evaluated the role of dietary interventions in the development and therapy of skin disease. This led to an increasing interest in nutraceutical and dietary supplement as novel therapeutic agents. Due to its various and interesting functions, LF has attracted a growing interest for potential clinical applications and there are increasing evidences for its use in treatments of different dermatological conditions [43]. The aim of the present work was to study the effects of bLF on Lipopolysaccharide (LPS)-induced cytokines expression and secretion in two cell lines (human keratinocytes NCTC2544 and THP-1 myelomonocytic leukaemia cells) to deeper understand its potential in the treatment of skin related disease.

Materials and Methods

Chemicals

Lactoferrin Moringa Low endotoxin bovine milk (LF) (<1 EU/mg, <20% iron saturated, >95% purity) was provided by C.F.M. CO. Farmaceutica Milanese S.p.A. (Milan, Italy). Cell culture media and all supplements were from Lonza Inc. (Barcelona, Spain), for NCTC2544 cell and from Sigma (St Louis, MO, USA) for THP-1 cells. Bacterial Lipopolysaccharide (LPS) (Escherichia coli, Serotype 0111: B4, 3×10^6 EU/mg) and ELISA reagents were purchased from Sigma. Antibodies and protein for ELISA assay were from R&D System (Minneapolis, MN, USA).

Cell culture and Viability

Normal human keratinocyte NCTC 2544 (Istituto Nazionale di Ricerca sul Cancro–Italy) were cultured under humidified atmosphere (5% CO_2, 37°C) on Eagle's Minimum Essential Medium Balanced with salt solution (EMEM-EBSS) containing 2mM l-glutamine, 1% of Non-Essential Amino Acids (NEAA) and penicillin (100 U/ml)/streptomycin (100 U/ml) supplemented with 10% Fetal Bovine Serum (FBS) (basal medium). THP-1 cells (Istituto Zooprofilattico di Brescia, Bres-cia, Italy) were cultured in RPMI 1640 containing 2 mM l-glutamine, 0.1 mg/ml streptomycin, 100 IU/ml penicillin, 50μM-2-mercaptoethanol and supplemented with 10% heated-inactivated fetal calf serum (basal medium).

Both cell types were incubated in 25 cm^2 surface culture flasks at 37°C with 5% CO_2 until ca. 80% of confluence was reached. Fol-

lowing harvesting with trypsin/EDTA cells were seeded at $5x10^4$ cells per well into 96 well plates or $1x10^6$ cells per well into 12 well plates for 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide proliferation (MTT) assay and cytokines quantification, respectively.

Effect of bLF Preparations on Viability of Cells

Cytotoxicity of LF on NCTC2544 and THP-1 cell lines was analyzed by MTT assay according to the method of Hansen [44] with minor modifications. After reaching 80% confluence, cells were exposed to bLF at the following concentrations: 2.5, 5, 10, 20, 40, 80 and 160µg/mL, for 24h at 37˚C, under 5% of CO_2. Cells in basal medium alone were used as control. At the end of treatment, the medium was replaced with 100 µl per well of the MTT solution and cells were incubate for 3h in darkness, at 37˚C, under CO_2 5%. MTT was previously dissolved (5 mg/ml) in PBS and diluted 1:10 in the cell culture medium without phenol red. Following incubation, 100 µl per well of Dimethylsulphoxide (DMSO) were added to dissolve purple formazan product and the solution shaked for 15 min at room temperature. Finally, the absorbance of the solutions was read at 550 nm in a microplate reader (BioTek Instruments Inc., Bad Friedrichshall, Germany). Each experiment was carried out in triplicate. Data were expressed as the mean percentage of viable cells compared with control.

TNF-α, IL-10 and IL-12 Immunomodulation by bLF

Immunomodulatory properties of bLF were investigated first by mean of qRT-PCR. Total RNA was isolated at different times of treatment using a commercial available kit (TriReagent from Sigma) as described by Chomczynski and Mackey [45]. 2µg of total RNA were retro-transcribed in cDNA using a high-capacity cDNA kit from Applied Biosystems (Foster City, CA, USA) in a thermal cycler (Stratagene Mx3000P Real Time PCR System, Agilent Technologies Italia S.p.A., Milan, Italy) according to these conditions: 25˚C for 10 min, 37˚C for 120 min and 85˚C for 60 s. mRNA levels where then quantified by using TaqManTM-PCR technology. Following 20X TaqMan® assay (Applied Biosystems) were used: Hs00174128-m1 (Tumor Necrosis Factor Alpha, TNF-α), Hs00961622_m1 (Interleukin-10, IL-10), Hs01011518_m1 (Interleukin-12, IL-12) and Hs999999-m1 (Human glyceraldehyde-3-phosphate dehydrogenase, GAPDH). PCR amplifications were carried out using 40 ng of cDNA in a 20 µl of mixture reaction containing 10 µl of 2XPremix Ex Taq (Takara, Clontech Laboratories, Inc, Mountain View, USA), 1 µl of 20× TaqMan gene expression assay, 0.4 µl of 50X Rox TM reference dye II (Takara, Clontech Laboratories, Inc,), 4.6 µl of water and 4 µl of cDNA. PCR conditions were 95˚C for 30 secs (for Amplitaq activation) followed by 40 amplification cycles (95˚C for 5 s; 60˚C for 20sec). Analyses were carried out in triplicate. Average value of Human GAPDH gene was used as endogenous reference for target gene and the quantification of transcripts levels was performed by the $2^{-\Delta\Delta CT}$ method [46].

The expression of cytokines at protein levels was also assessed in cell free supernatants, stored at -80˚C until measurement, after centrifugation at 1200 rpm for 5 mins. Before ELISA assay samples were concentrated with Vivaspin Sartorius centrifugal concentrators (with cut off 10000 and 30000 MW, respectively for IL-10 and IL-12/TNF-α). Protein expression was assessed by custom sandwich Elisa assays, according to manufacturer recommendations as regards antibodies and standards dilutions and following a custom home-made protocol for coating and detection of antibodies. Results were expressed as pg/mg protein.

Statistical Analysis

All experiments were repeated at least three times, with representative results shown. Data are expressed as Mean ± SEM for qRT-PCR analysis and Standard Deviation (SD) for other experiments. Results were checked for normal distribution using Shapiro-Wilk test before further analyses. Analysis of Variance (ANOVA) was carried out following by Sidak's multiple comparison test, using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com). P-values equal to or less than 0.05 were considered significant.

Results

In vitro Effects of bLF on TNF-α, IL-10 and IL-12 Anti-Inflammatory Cytokines

Primarily, lactoferrin 10 µg/mL (LF10) and 40 µg/mL (LF40) effect on LPS-induced cytokines gene expression was determined by mean of qRT-PCR. Down-regulation in cytokines expression reflects into an anti-inflammatory action. Both LFs exerted a significant ($P<0.0001$) immunomodulatory action on TNF-α since 1 to 8h of treatment (Figure 1a) respect to Positive Control (PC), when tested on human keratinocytes NCTC2544. On THP-1 cells (Figure 1b), even if treatment with both LFs produced a significant ($P<0.0001$) down-regulation of TNF-α after 1h of treatment, the strongest LF dose-independent immunomodulation was found from 5h to 7h of incubation. At 8h LF40 was most effective than LF10. In addition, IL-10 expression was strongly significant ($P<0.0001$) influenced by LFs (vs PC), independently from concentrations and in general, independently from cell line (Figure 2a and 2b). Most interesting, on THP-1 cells this effect was evident since 1h of incubation (Figure 2b).

On the contrary, immunomodulation of IL-12 cytokine was strongly dependent from cell line used. Both LFs showed to exert a similar significant ($P<0.0001$) anti-inflammatory action on NCTC2544 cells from 2h of incubation (Figure 3a). This effect wears off after 5h and 6h of incubation for LF40 and LF10, respectively. On THP–1 cells, immunomodulation by LFs respect to PC was less time and dose dependent (Figure 3b). The strongest

significant (P<0.0001) effect was found for LF10 from 3 to 5h of incubation and LF40 after 7h. Statistical significance (p value) is reported in Table S1.

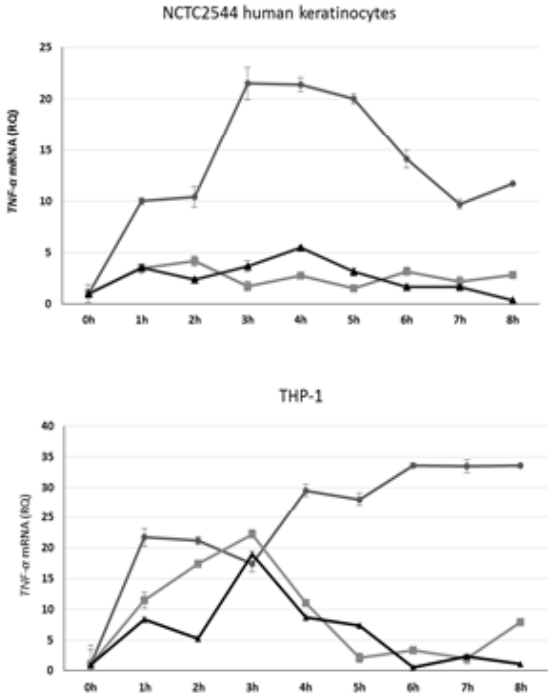
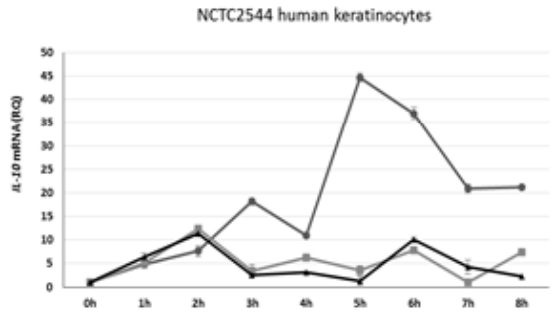


Figure 1: Time course of Tumor Necrosis Factor-α (*TNF-α*) gene expression on NCTC2544 (a) and THP-1 cells (b), respectively. Cells were seeded at 10^6 cells/well and, after reaching 80% confluency, treated for different times (1-8h) with LF10 (Lactoferrin 10µg/mL) (■), LF40 (Lactoferrin 40µg/mL) (▲) or basal medium 2.5% FBS (positive control-PC) (•). Simultaneously inflammation was induced by adding basal medium at 2.5% FBS with LPS (10µg/mL). Data are the means±SEM of three separate experiments, n = 3. Statistical analysis was performed with Sidak's.



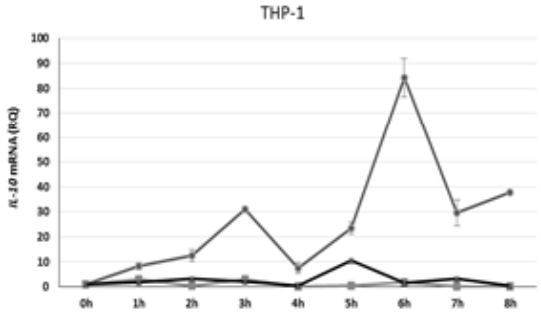


Figure 2: Interleukin 10 (*IL-10*) gene expression on NCTC2544 (a) and THP-1 cells (b), respectively. Cells were seeded at 10^6 cells/well and, after reaching 80% confluency, treated for different times (1-8h) with LF10 (Lactoferrin 10µg/mL) (■), LF40 (Lactoferrin 40µg/mL) (▲) or basal medium 2.5% FBS (Positive Control-PC) (•). Simultaneously inflammation was induced by adding basal medium at 2.5% FBS with LPS (10µg/mL). Data are the means±SEM of three separate experiments, n = 3. Statistical analysis was performed with Sidak's.

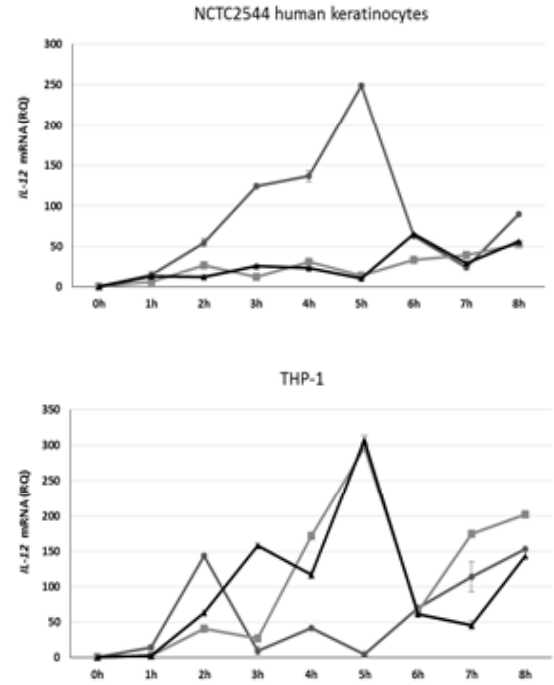


Figure 3: Interleukin 12 (*IL-12*) gene expression on NCTC2544 (a) and THP-1 cells (b), respectively. Cells were seeded at 10^6 cells/well and, after reaching 80% confluency, treated for different times (1-8h) with LF10 (Lactoferrin 10µg/mL) (■), LF40 (Lactoferrin 40µg/mL) (▲) or basal medium 2.5% FBS (Positive Control-PC)(•). Simultaneously inflammation was induced by adding basal medium at 2.5% FBS with LPS (10µg/mL). Data are the means±SEM of three separate experiments, n = 3. Statistical analysis was performed with Sidak's.

qRT-PCR results were mostly confirmed by ELISA assay and this was according to the delayed expression of a protein respect to the related gene. In particular, according to *TNF-α* gene expression, ELISA assay (Table 1) confirmed LFs anti-inflammatory activity, independently from cell line used. On NCTC2544 cells IL-10 protein expression was delayed at 6h of treatment and continued up to 8h. LF10 and LF40 produced the same anti-inflamamtory effect, respect to PC, with the exception of 7h treatment, following which LF40 was most effective than LF10 (Table 2). On THP-1, IL-10 expression was delayed above 8h of treatment (Table 2). Same results were found for IL-12 expression on NCTC2544 (Table 3).

On the contrary, according to gene expression evaluation, on THP-1 cell line, LFs immunomodulation was most evident from 6 to 7h of incubation (Table 3), independently from doses.

Cell line	Time	TNF-alpha		
		PC	LF10	LF40
NCTC 2544	1h	110.04±41.29^{a}	0.00±7.75^{b}	0.00±11.30^{b}
	2h	588.75±14.85^{c}	250.16±7.73^{d}	274.71±16.95^{d}
	3h	588.72±27.82^{e}	83.84±13.08^{f}	340.70±103.56^{g}
	4h	614.64±56.46^{h}	395.81±50.52^{i}	345.98±5.21^{i}
	5h	746.99±33.59^{j}	570.56±28.10^{k}	534.79±18.63^{k}
	6h	1,303.43±41.26^{l}	575.38±75.47^{m}	776.19±69.08^{m}
	7h	916.62±15.98^{n}	709.62±148.83^{n}	876.99±54.07^{n}
	8h	964.28±27.54^{o}	601.01±14.01^{p}	601.44±81.34^{p}
THP–1	1h	606.25±29.16^{q}	347.69±24.54^{r}	347.69±24.54^{r}
	2h	542.64±2.29^{s}	445.50±82.38^{s}	531.47±118.42^{s}
	3h	586.46±60.31^{t}	633.83±47.18^{t}	663.50±22.65^{t}
	4h	654.80±42.97^{u}	740.03±29.86^{u}	741.39±41.98^{u}
	5h	729.36±87.82^{v}	680.03±12.26^{v}	713.96±30.70^{v}
	6h	896.85±7.22^{w}	796.16±18.35^{x}	922.55±43.55^{y}
	7h	889.30±15.70^{z}	963.07±24.35^{z}	832.72±53.91^{z}
	8h	1,296.04±29.07^{aa}	916.66±14.88^{ab}	777.08±71.85^{ab}

Table 1: Tumor Necrosis Factor-α (TNF-α) protein expression as determined by ELISA. Cells were seeded at 10^6 cells/well and, after reaching 80% confluency, treated with LF10 (Lactoferrin 10µg/mL), LF40 (Lactoferrin 40µg/mL) or basal medium 2.5% FBS (Positive Control-PC). Simultaneously inflammation was induced by adding basal medium at 2.5% FBS with LPS (10µg/mL). Analyses were carried on collected cell free supernatant after incubation at 37°C for 1-8h, under 5% CO_2. Data are the means±SD of three separate experiments, n = 3. Statistical analysis was performed with Sidak's multiple comparison test. N.d. = Not Detectable. $^{a-ab}$Values with different superscript letters, differ significantly (P < 0.001).

Cell line	Time	IL–10		
		PC	LF10	LF40
Cell line	1h	n.d.	n.d.	n.d.
	2h	n.d.	n.d.	n.d.
	3h	n.d.	n.d.	n.d.
	4h	n.d.	n.d.	n.d.
	5h	n.d.	n.d.	n.d.
	6h	53.17±1.87^{a}	21.94±0.83^{b}	12.92±1.31^{b}
	7h	49.42±3.81^{c}	8.15±9.99^{d}	2.79±2.07^{e}
	8h	60.17±2.96^{f}	13.49±8.74^{g}	5.24±0.60^{g}
THP–1	1h	n.d.	n.d.	n.d.
	2h	n.d.	n.d.	n.d.
	3h	n.d.	n.d.	n.d.
	4h	n.d.	n.d.	n.d.
	5h	n.d.	n.d.	n.d.
	6h	n.d.	n.d.	n.d.
	7h	n.d.	n.d.	n.d.
	8h	n.d.	n.d.	n.d.

Table 2: Interleukin 10 (IL-10) protein expression as determined by ELISA. Cells were seeded at 10^6 cells/well and, after reaching 80% confluency, treated with LF10 (Lactoferrin 10µg/mL), LF40 (Lactoferrin 40µg/mL) or basal medium 2.5% FBS (Positive Control-PC). Simultaneously inflammation was induced by adding basal medium at 2.5% FBS with LPS (10µg/mL). Analyses were carried on collected cell free supernatant after incubation at 37°C for 1-8h, under 5% CO_2. Data are the means±SD of three separate experiments, n = 3. Statistical analysis was performed with Sidak's multiple comparison test. N.d. = Not Detectable. $^{a-g}$Values with different superscript letters, differ significantly (P < 0.001).

Cell line	Time	IL-12		
		PC	LF10	LF40
NCTC2544	1h	n.d.	n.d.	n.d.
	2h	n.d.	n.d.	n.d.
	3h	n.d.	n.d.	n.d.
	4h	n.d.	n.d.	n.d.
	5h	n.d.	n.d.	n.d.
	6h	n.d.	n.d.	n.d.
	7h	n.d.	n.d.	n.d.
	8h	n.d.	n.d.	n.d.
THP-1	1h	n.d	n.d	n.d
	2h	n.d	n.d	n.d
	3h	n.d	n.d	n.d
	4h	n.d	n.d	n.d
	5h	n.d	n.d	n.d
	6h	12.53±0.13^{a}	0.33±0.56^{b}	1.57±0.23^{b}
	7h	15.28±0.14^{c}	11.70±0.19^{d}	10.51±0.22^{d}
	8h	25.71±0.18	n.d	n.d

Table 3: Interleukin 12 (IL-12) protein expression as determined by ELISA. Cells were seeded at 106 cells/well and, after reaching 80% confluency, treated with LF10 (Lactoferrin 10µg/mL), LF40 (Lactoferrin 40µg/mL) or basal medium 2.5% FBS (Positive Control-PC). Simultaneously inflammation was induced by adding basal medium at 2.5% FBS with LPS (10µg/mL). Analyses were carried on collected cell free supernatant after incubation at 37°C for 1-8h, under 5% CO2. Data are the means±SD of three separate experiments, n = 3. Statistical analysis was performed with Sidak's multiple comparison test. N.d. = Not Detectable. [a-d]Values with different superscript letters, differ significantly ($P < 0.001$).

The inhibitory effects exerted by LFs on cytokine expression, following the LPS-mediated inflammation were not due to cytotoxicity as assessed by MTT assay (Table 4).

Treatment	µg/mL	MTT reduction (% of the control)±SD
Control		100.000±1.407[a]
LF	2.5	101.012±0.727[a]
	5	93.051±3.729 [a]
	10	94.859±2.345 [a]
	20	95.721±0.516 [a]
	40	94.232±0.962 [a]
	80	90.299±3.166 [a]
	160	91.177±6.895 [a]

Table 4: Effect of Lactoferrin on the cell viability. Cells were seeded at 10^5 cells/well and, after reaching 80% confluency, treated with Lactoferrin (LF) (2.5-160µg/mL) or basal medium (control), for 24h at 37°C, under 5% of CO_2. The percentage of viable cells was measured through the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Data are the means of three independent experiments±SD, n = 3. [a:] Values with different superscript letters, differ significantly ($P < 0.05$).

Discussion

bLFis an iron-binding glycoprotein that consists of a single polypeptide chain of 689 amino acids; the sequence homology with human LF is 69% [39]. As an integral part of the innate immune system LF is considered a well-known immunomodulator of leukocyte populations [47-52] and many recent evidences support the role of Lf in regulation of host acting by inhibition of several cytokines [30,53] and acts primarily as key modulators and regulators of immune processes. Produced also by cells other than immune cells, after the binding to specific receptors, cytokines produce multiple signals and induce target cell to new mRNA and protein synthesis [54]. This results in a specific biological response.

Among cytokines, TNF-α [55-58], IL-10 [59-63], IL-12 [64–66], are key effectors both in immune and infectious response [67]. Studies on various human monocytic cell lines, included THP-1 cells, showed that LF, both bovine and human, can modulate basal and LPS-mediated pro-inflammatory cytokines release [31,68,69]. LPS is the major constituent of the outer membrane of bacterial pathogens and is a well-known initiator of inflammation [70]. Among others, Appelmelk and coworkers [71] suggested that, in systemic infections, the inhibition of primary inflammatory response TNF-α-linked could be due to the binding between LF and the lipid A moiety of LPS released from bacteria, inhibiting subsequent LPS-mediated pro-inflammatory response.

LF, both human and bovine, present similar binding to THP-1 cells [72]. In a more recent study on THP-1 monocytes, Haversen [31] demonstrated that LF regulates the LPS-induced cytokine expression on a transcriptional level, by interference with the intracellular events leading to NF-kB activation. Therefore, in the same study, bLF seemed somewhat more efficient compared to human LF and that TNF-α, the most inhibited cytokine, down-regulates IL-10 expression. Moreover, many receptors have been identified both on the surfaces of immunocompetent cells [73-75] and epithelial cells [19] suggesting also a direct involvement of LF in the signaling pathways of pro-inflammatory cytokines.

Inflammation plays an important role in pathogenesis of many cutaneous disorders such as for example psoriasis [76], atopic dermatitis [77], contact dermatitis [78], acne vulgaris [79,80], UV-induced inflammation [81-83]. Although there are still few studies evaluating LF usage for dermatological conditions, the reported studies encourage the use of LF for these purposes [43]. In a first explorative study, Muller and coworkers [84] demonstrated the efficacy and tolerability of oral bLF supplementation in subjects with mild to moderate facial acne vulgaris. Previously, Kim and coworkers [85] showed the ameliorating effect of Lactoferrin-enriched fermented milk on acne vulgaris. LF exerted these effects probably due to its anti-bacterial and anti-inflammatory effects [86].

LF has also the potential to prevent UV-induced skin damage by the inhibition of UV-stimulated cytokines [87] and acting as a sacrificial scavenger for Reactive Oxygen Species (ROS) [88]. Most interesting, also topical exposure to LF is able to influence inflammatory responses acting on local production pro-inflammatory cytokines [89]. The *in vitro* experiment presented in this work confirmed the immunomodulatory activity of bLF, which exerted its action at a transcriptional level since to the early phase to 8h of treatment. The anti-inflammatory activity occurs by immune-modulation of cytokines expression in an LPS-mediated inflammatory system on normal human keratinocytes and THP-1 cells. Most likely bLF exerted its effect both by inhibition of binding of lipopolysaccharide endotoxin to cells, as well as acting directly on cells cytokines production.

Conclusions

Our results add an important element to the knowledge on the anti-inflammatory potential of bovine LF in an LPS-mediated system. bLF is capable of immunomodulate pro-inflammatory cy-

tokines at a transcriptional level. These findings encourage the use of bLF as immunomodulator agent in the treatment of different cutaneous disorder (eg. *acne vulgaris*) both as dietary supplement and topically administered. However larger randomized clinical trials are necessary to better define its role and pharmacokinetic behavior for dermatological purposes.

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