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Oral Liposomal Lactoferrin in Healthy Volunteers Activates Leukocyte Gene Expression for Lactotransferrin, Angiotensinogen, and Granulocyte Colony Stimulating Factor Increasing Innate Immunity Against SARS-CoV-2 Infection

Gabriel Serrano[1]*, Iulia Kochergina[1], Arturo Albors[2], Juan M Serrano[3], Guillen Hueso[3], Mar Oroval[3], Silvia Mir[3], Irene Mor[3], and Eva Martí[3]

[1]Dermatology Clinic, Valencia, Spain

[2]Medical Department of Sesderma Laboratories, Valencia, Spain

[3]Research Department of Sesderma Laboratories, Valencia, Spain

***Corresponding author:** Serrano G, Department of Dermatology Clinic, Valencia, Spain; Tel: +34 615 810 159; E-mail: gabriels[At]sesderma.com

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Abstract

Severe acute respiratory syndrome coronavirus 2, SARS-CoV-2, emerged in later December 2019 in Wuhan, China, as a new epidemic form of respiratory life treating infection caused by a novel coronavirus. Lactoferrin (LF), a milk protein used as a food supplement has been reported to be effective in vitro and in vivo against SARS-CoV-2 both as a potential preventative or therapy treatment. Lactoferrin or lactotransferrin, is a protein found in cow and human milk with great affinity for iron ions and with a significant role as immunomodulator. It belongs to the components of the innate immune system and is present in several mucosal secretions and in neutrophil leukocytes. Increases in lactoferrin are seen with inflammatory bowel disease but also with other inflammatory conditions, with intestinal bacterial infections, some parasitic infections, and with colon cancer. LF gen and other genes such us S100A9, and Lipocalin 2 are over expressed in patients with SARS which supports that LF has immunomodulatory properties derived from its ability to bridge innate and adaptative immunity. In the present study we analyze the effects of the oral intake of a single dose of 24 mg of liposomal lactoferrin (LLF) in 4 human volunteers on leucocyte gene expression at five times points, before (0h) and 2-4-6 and -24 hours after its oral intake. LLF absorption was measured in blood serum by ELISA and the expression of genes for Lactotransferrin (LTF), Angiotensinogen (AGT), Granulocyte-colony stimulating factor (G-CSF) and Granulocyte-macrophage colony-stimulating factor (GM-CSF) were measured using Reverse Transcription Polymerase Chain Reaction (RT-qPCR) on blood cells. Oral administration of LLF increased lactoferrin levels in blood serum and enhanced the expression of LTF, AGT, and GC-SF genes while GM-CSF levels remained unchanged. These findings support that LLF activates the innate-immune response and increase our defense system against bacterial and viral infections.

Keywords: ***Lactoferrin; Innate immunity; SARS-CoV-2; COVID-19.***

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1. Introduction

Lactoferrin is a multifunctional protein found in bovine and human milk with a significant role as immunomodulator, binding and transport of iron ions, antibacterial, antiviral, antiparasitic, catalytic, anti-cancer, and anti-allergic properties. It is present in the tears, nose, mouse, respiratory tract, bowels, as well as in the neutrophils in the form of granules which are secreted in the blood and sites of infection [1]. LF also exhibits a natural antimicrobial synergistic interaction with other components of the innate immune system, such as IgA, lysozyme and beta defensin. LF can contribute to antimicrobial defense by limiting the availability of iron to bacteria. Lactoferrin have shown antiviral activity against viral pathogens that cause common infections such as the common cold, influenza, gastroenteritis, summer cold, hepatitis C, herpes and SARS-2 [2,3]. A recent *in vitro* study from Brazil showed the inhibitory effect of bovine lactoferrin on SARS-CoV-2 infection in African green monkey kidney epithelial cells (Vero E6) and in adenocarcinoma human alveolar basal epithelial cells (A549). [2]. Inhibitory effects of LF on SARS- 2 were also observed in vitro in Vero E6, Caco2, and Huh7 cells in another study from the United States that also showed that LF potentiate the effects of Remdesivir and Hydroxychloroquine [3]. Lactoferrin stimulates also an antiviral host cell response and retains its inhibitory activity in induced pluripotent stem cell (iPSC)-derived alveolar epithelial cells [3]. Regarding *in vivo* clinical studies, a very recent prospective observational study with COVID-19 patients revealed that the intake of liposomal lactoferrin allows a complete and fast recovery from the disease within the first 4-5 days [4]. The virus infects host cells using its spike glycoprotein (S-protein) has significantly higher infectivity and mortality rates among the aged population. Liposomal LF can block viral entry into host cells by interacting with viral and/or cell surface receptors [5-8]. ACE2 is the functional receptor for the SARS-CoV-2 that uses its spike proteins to invade cells via this receptor [7,8]. LF is frequently used as a food supplement to support a well-functioning immune system and improve the ability of our body to fight infections (Fig. 1). LF may also have a role in sequestering iron and inflammatory molecules that are severely increased during the cytokine burst [1,4]. In the present paper we study on its role as an oral supplement to enhance innate immunity and its antibacterial and antiviral properties and study the functional effects of the oral treatment with Lactyferrin after its intake in 4 human volunteers, through analysis of lactoferrin levels by using ELISA, and *Lactotransferrin (LTF), Angiotensinogen (AGT) and Granulocyte-colony stimulating factor (G-CSF)* gene expression using RT-qPCR [9,10]. *LTF, lactotransferrin,* is the gene codifying lactoferrin. It is a member of the transferrin family of genes. Angiotensinogen gen is involved with the Renin Angiotensin System (RAS) and plays an important role in the control of blood pressure [11]. G-CSF (Granulocyte Colony- Stimulating Factor) activate the bone marrow to produce granulocytes and stem cells and release them into the bloodstream. In addition, it stimulates several functions of neutrophils [12]. GM-CSF (Granulocyte-Macrophage Colony-Stimulating Factor) is secreted by macrophages, T-cells, mast cells, natural killer cells, endothelial cells and fibroblasts.[13]. LF may have an inhibitory role on its production via interleukin-1 (IL-1). LF may be involved in the transcriptional regulation of GM-CSF gene expression [14].

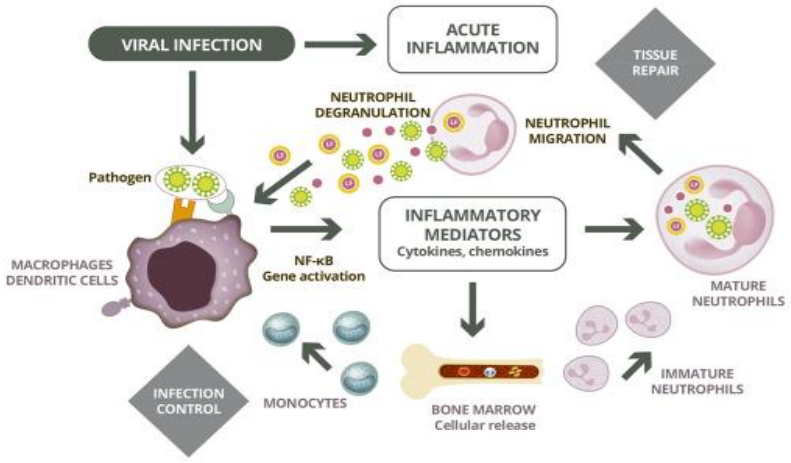

Fig. 1. *Lactoferrin* (LF) mediates cellular responses to environmental insults.

Injury defined by infection, or trauma leads to activation of the NF-κB signal transduction pathway within monocyte/macrophages and/or dendritic cells. This in turn stimulates the production of inflammatory mediators, which subsequently stimulates the production of fresh neutrophils and monocytes from bone marrow and activates circulating neutrophils. Activated neutrophils degranulate to release secondary mediators, including LF. By interacting with specific receptors on monocytes/macrophages and other immune and non-immune cells, LF attenuates inflammation and contributes to tissue repair and limits spread of infectious agents.

2. Material and Methods

2.1 Products tested

The commercial product Lactyferrin drinkable was used in the present study. A single dose of 24 mg was given 1-2 hours after breakfast. Lactoferrin was encapsulated in positively charged PC-liposomes. The liposomes were unilamellar, with a single phospholipid bilayer sphere enclosing the aqueous solution, flexible and have a size of 80 and 150 nm (Delsa Nanosizer C, Beckman Coulter Inc., Brea, California, USA). The polydispersity index was below 0.20, and a zeta potential of (30-150) mV.

2.2 Analytical equipment

Blood extraction fingerstick system, EDTA tubes for blood samples, quantifier Nano-Drop spectrophotometer, laminar flow hood, Quant Studio 5 (Applied Biosystem) Quantitative real-time PCR, vortex, Glomax Discovery Promega Multi-mode detection system.

2.3 Reagents

Distilled water (Braun), Phosphate buffered saline (Sigma), Trizol LS (Fisher), chloroform (Sigma-Aldrich), isopropanol (Sigma-Aldrich), Prime Script RT reagent kit (Perfect Real Time- Takara Clontech), oligonucleotides for RT-PCR amplification of LTF, AGT, G-CSF, GM-CSF and ACT, SYBR ® master mix, liquid nitrogen, ethanol (Sigma-Aldrich), ELISA kit for Lactoferrin quantification.

2.4 Procedure

2.4.1 Blood serum extraction and processing

Two tubes of 200 µl of blood (one for plasma isolation and the other for mRNA extraction) were taken through fingerstick from each of the 4 human volunteers submitted to the treatment, previously (0h) to oral intake and after 2 hours (2h), 4 hours (4h), 6 hours (6h) and 24 hours (24h). 30 ml of Lactyferrin (95 % content in lactoferrin) were ingested for each volunteer. Immediately after extraction, for lactoferrin protein quantification, plasma was extracted from blood samples through centrifugation and kept frozen at -80ºC until analysis, whereas samples for gene expression analysis were directly processed with Trizol for mRNA extraction from blood cells.

2.4.2 Gene expression using RT-qPCR

mRNA expression levels were determined in the blood serum samples obtained using fingerstick at different time points. *LTF, AGT,* and *G-CSF* and *ACT* (internal control) were amplified using four technical replicates of cDNAs.

Total RNA was extracted from blood samples using Trizol reagent following manufacturer´s instructions. Quantitative PCR was performed in a real time PCR machine (Quant Studio 5 Applied Biosystem). One biological replicate with four technical replicates per condition were performed. To perform raw data analysis, we used the Pfaffl method [15] to calculate the gene relative expression ratio to ACT (internal control-housekeeping gene).

2.4.3 Panel

The following were criteria for the inclusion: healthy volunteers, male or female, between 25-50 years, who has no participation in any clinical trial or who participated in a clinical study of this type, at least 15 days before the start of the present study. Volunteers were on fasting conditions for 10 hours before the experiment (except for water), in the day of measurements. They were advised not to consume any supplement or specific food containing lactoferrin during the week before the start of the experiment and finally all signed an informed consent. Obligations for the volunteers include to respect the product's (food) conditions of use (food), to keep the personal eating habits during the week before the start of the study and not to take any drug or dietary supplement, aimed at reducing weight or regulating gastrointestinal metabolic parameters, 15 days before the start of the study. On the other hand, the criteria for exclusion include people with allergy to the components of the product, diabetes, gastrointestinal and cardiorespiratory diseases, pregnant or lactating women or who plan to become pregnant during the study and people under medical treatment in the weeks prior to the study, that could interfere with the evaluations of the present study.

2.4.4 Ethics

The study protocol was in accordance with the Scientific Committee on Consumer Safety (SCCS) guidance. It meets all international standards for research studies involving human subjects, the Good Clinical Practices (ICH-GCP) and World Medical Association. It has been conducted pursuant to the Declaration of Helsinki (1864), with the amendments of Tokyo (1975), Venice (1983), Hong Kong (1989) and Seoul (2008).

3. Results

In this assay, we determine the functional effects of the treatment with Lactyferrin after oral intake in 4 human volunteers, through analysis of lactoferrin levels using ELISA, and Lactotransferrin (LTF), Angiotensinogen (AGT) and Granulocyte-colony stimulating factor (G-CSF) gene expression using RT-qPCR; at different timepoints before and after the single treatment (0h, 2h, 4h, 6h and 24h). Results showed that oral treatment with Lactyferrin increased Lactotransferrin (LTF) gene expression by 1.3 ± 0.5 -fold ($p > 0.05$), 8.1 ± 3.8 -fold ($p > 0.05$) and 4.3 ± 0.6 -fold (**), respectively, 4 hours, 6 hours and 24 hours after treatment, compared to basal values at 0h. In the same way, the treatment stimulated Angiotensinogen (AGT) gene expression by 10.2 ± 8.9 -fold ($p > 0.05$), 20.1 ± 7.9 -fold ($p > 0.05$) and 9.8 ± 4.9 -fold (*), respectively, 2 hours, 4 hours and 6 hours after treatment. Regarding Granulocyte-colony stimulating factor (G-CSF), gene expression was increased by 3.2 ± 2.6 -fold ($p > 0.05$), 12.2 ± 8.6 -fold ($p > 0.05$) and 17.4 ± 13.9 -fold ($p > 0.05$), respectively, 2 hours, 4 hours and 6 hours after treatment; as shown in Figs 3-4 and Table 1. It is important to remark that no quantifiable mRNA levels were detected at any condition for Granulocyte-macrophage colony-stimulating factor (GM-CSF). For this reason, it is not represented in graphs. Lactoferrin levels curves are represented in 6. Oral intake of Lactyferrin [R] increased lactoferrin levels in blood serum by 6.0 ± 10. Fig. 8 %, 33.4 ± 27.2 %, 15.6 ± 17.3 % and 27.0 ± 10.8 %, respectively, 2 hours, 4 hours, 6 hours and 24 hours after treatment, even though results were not statistically significant ($p > 0.05$) compared to basal values at 0h; as shown in in Fig. 2 and Table 1 and 2. *Represents statistical significance with p-value < 0.05. **Represents statistical significance with p value < 0.01.

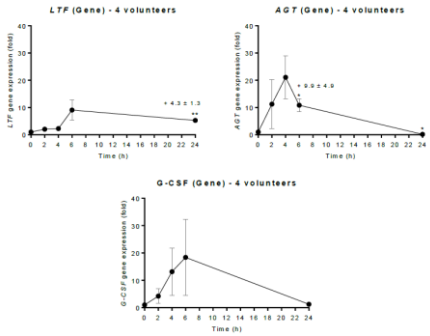

Fig. 2. Mean results (N=4).

*Pharmacokinetics graphical representation of Lactotransferrin (LTF), Angiotensinogen (AGT) and Granulocyte-colony stimulating factor (G-CSF) gene expression, after single treatment with Lactyferrin (30 ml) in 4 human volunteers. Mean values for the 4 volunteers are shown. Blood samples were obtained using fingerstick at 0h (before), 2h, 4h, 6h and 24h after intake. * Represents statistical significance with p-value < 0.05. ** Represents statistical significance with p value < 0.01.*

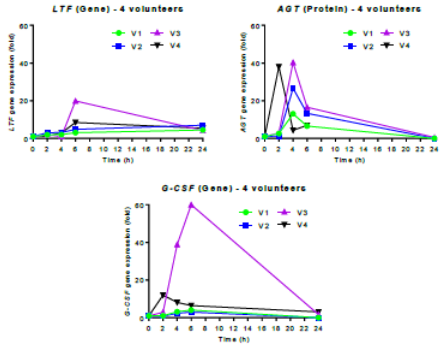

Fig. 3. Individual results (4 vol.).

Pharmacokinetics graphical representation of Lactotransferrin (LTF), Angiotensinogen (AGT) and Granulocyte-colony stimulating factor (G-CSF) gene expression, after single treatment with Lactyferrin (30 ml) in 4 human volunteers. Individual values are shown. Blood samples were obtained using fingerstick at 0h (before), 2h, 4h, 6h and 24h after intake.

The lactoferrin ELISA Kit is an enzyme immunoassay developed for detection and quantification of lactoferrin in different type of samples (cellular, blood samples, serum, etc.). The quantity of lactoferrin is determined by comparing its absorbance with that of a known lactoferrin standard curve provided by the kit's manufacturer. Results indicated Lactyferrin oral intake increased lactoferrin levels in blood serum by 6.0 ± 10.8 %, 33.4 ± 27.2 %, 15.6 ± 17.3 % and 27.0 ± 10.8 %, respectively, 2 hours, 4 hours, 6 hours and 24 hours after treatment, even though results were not statistically significant ($p > 0.05$) compared to basal values at 0h; as shown in in Fig. 4 and Table 1.

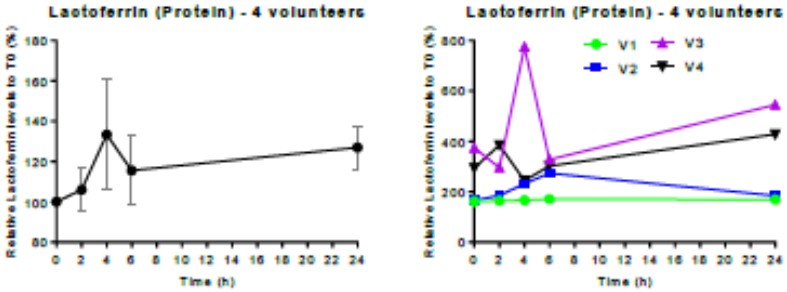

Fig. 4. Pharmacokinetics graphical representation of lactoferrin protein levels in serum samples of 4 human volunteers, after single treatment with *Lactyferrin* (30 ml). Blood samples were obtained using fingerstick at 0h (before), 2h, 4h, 6h and 24h after intake. Mean results (N=4) are shown at left, whereas individual results (4 vol.) are shown at right.

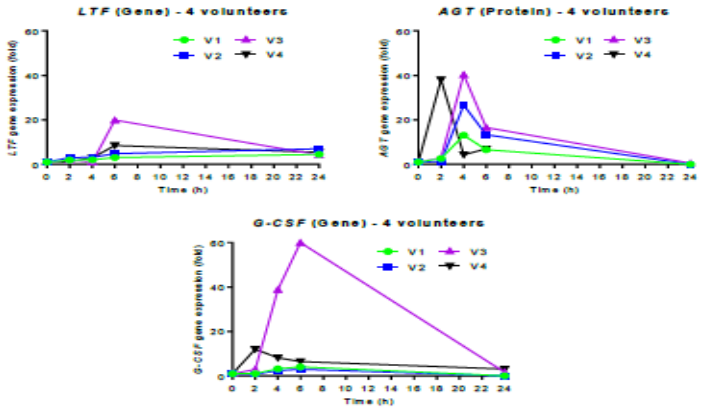

Fig. 5. Individual results (4 vol.).

Pharmacokinetics graphical representation of Lactotransferrin (LTF), Angiotensinogen (AGT) and Granulocyte-colony stimulating factor (G-CSF) gene expression, after single treatment with Lactyferrin (30 ml) in 4 human volunteers. Individual values are shown. Blood samples were obtained using fingerstick at 0h (before), 2h, 4h, 6h and 24h after intake.

Table 1: Lactoferrin Increased Levels in Blood Serum.

Absorbance (OD 450 nm)				
	V1	V2	V3	V4
0h	5164	5301	13736	10513
2h	5275	6119	10565	14165
4h	5373	7986	29826	8497
6h	5550	9678	11853	10840
24h	5478	6093	20593	15902

LF protein levels (pg/ml)				
	V1	V2	V3	V4
0h	161,4	164,8	374,7	294,5
2h	164,1	185,1	295,8	385,3
4h	166,6	231,6	775,0	244,3
6h	171,0	273,7	327,8	302,6
24h	169,2	184,5	545,3	428,6

Table 2: Statistical Analysis of Results Shown in Figure 5.

Table Analyzed	**LTF – Gene Expression**			
Column B	2h	4h	6h	24
vs.	vs,	vs,	vs,	vs,
Column A	0h	0h	0h	0h
Paired t test				
P value	0,0747	0,0787	0,1224	0,0065
P value summary	ns	ns	ns	**
Significantly different? (P<0.05)	No	No	No	**Yes**
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed
t, df	t=2,686 df=3	t=2,625 df=3	t=2,135 df=3	t=6,820 df=3
How big is the difference?				
Mean of differences	1,036	1,300	8,086	**4,298**
SD of differences	0,7712	0,9902	7,574	1,261
SEM of differences	0,3856	0,4951	3,787	0,6303
95% confidence interval	-0,1916 to 2,263	-0,2762 to 2,875	-3,966 to 20,14	2,292 to 6,304
R square	0,7062	0,6966	0,6031	0,9394
Table Analyzed	**AGT – Gene Expression**			
Column B	2h	4h	6h	24
vs.	vs,	vs,	vs,	vs,
Column A	0h	0h	0h	0h
Paired t test				
P value	0,3350	0,0840	0,0277	0,0453
P value summary	ns	ns	*	*
Significantly different? (P<0.05)	No	No	**Yes**	**Yes**
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed
t, df	t=1,146 df=3	t=2,550 df=3	t=4,019 df=3	t=4,536 df=2
How big is the difference?				
Mean of differences	10,24	20,08	**9,856**	**-0,8083**
SD of differences	17,87	15,75	4,904	0,3086
SEM of differences	8,937	7,876	2,452	0,1782
95% confidence interval	-18,20 to 38,68	-4,985 to 45,14	2,052 to 17,66	-1,575 to -0,04167
R square	0,3044	0,6842	0,8434	0,9114

Table Analyzed	G-CSF - Gene Expression			
Column B	2h	4h	6h	24
vs.	vs,	vs,	vs,	vs,
Column A	0h	0h	0h	0h
Paired t test				
P value	0,3108	0,2547	0,2988	0,7657
P value summary	ns	ns	ns	ns
Significantly different? (P<0.05)	No	No	No	No
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed
t, df	t=1,217 df=3	t=1,405 df=3	t=1,253 df=3	t=0,3263 df=3
How big is the difference?				
Mean of differences	3,210	12,15	17,39	0,2398
SD of differences	5,278	17,30	27,75	1,470
SEM of differences	2,639	8,649	13,87	0,7348
95% confidence interval	-5,188 to 11,61	-15,37 to 39,68	-26,76 to 61,54	-2,099 to 2,578
R square	0,3303	0,3969	0,3437	0,03427

Table 3: Statistical Analysis of Results Shown in Figure 7.

Table Analyzed	Lactoferrin (ELISA)			
Column B	2h	4h	6h	24
vs.	vs,	vs,	vs,	vs,
Column A	0h	0h	0h	0h
Paired t test				
P value	0,6203	0,3076	0,4346	0,0883
P value summary	ns	ns	ns	ns
Significantly different? (P < 0.05)	No	No	No	No
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed
t, df	t=0,5506 df=3	t=1,226 df=3	t=0,8996 df=3	t=2,493 df=3
How big is the difference?				
Mean of differences	5,966	33,40	15,58	26,97
SD of differences	21,67	54,47	34,63	21,64
SEM of differences	10,84	27,24	17,31	10,82
95% confidence interval	-28,52 to 40,45	-53,28 to 120,1	-39,52 to 70,67	-7,461 to 61,40
R square	0,09177	0,3339	0,2124	0,6744

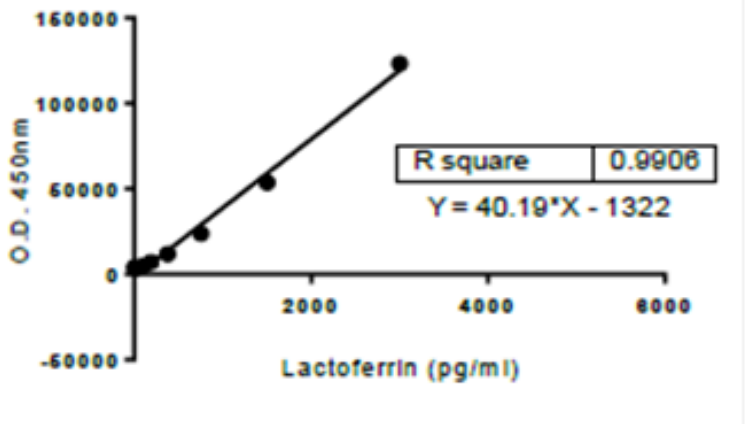

Fig. 6. LF standar curve used for Elissa assay.

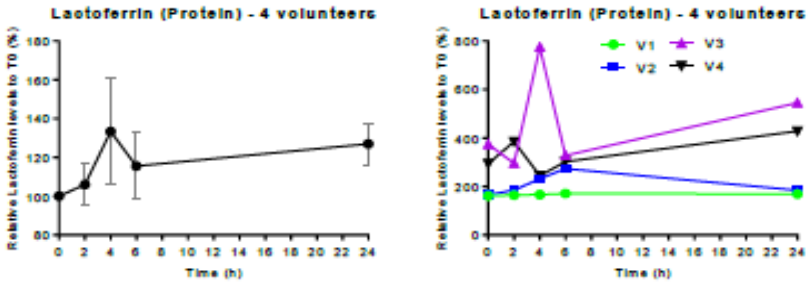

Fig. 7. Pharmacokinetics graphical representation of lactoferrin protein levels in serum samples of 4 human volunteers, after single treatment with *Lactyferrin* (30 ml). Blood samples were obtained using fingerstick at 0h (before), 2h, 4h, 6h, and 24h after intake. Mean results (N=4) are shown at left, whereas individual results (4 vol.) are shown at right.

4. Discussion

Oral administration of a single dose of 24 mg of liposomal lactoferrin to four volunteers increased blood lactoferrin levels (ELISA) and enhanced the expression of leukocyte genes (RT-qPCR) including Lactotransferrin (LTF), Angiotensinogen (AGT) and Granulocyte-colony stimulating factor (G-CSF). Volunteer 3 has the higher LF levels and volunteer 1, was the one with lowest levels of LF and who also showed less gene response. Translating these values to percentages we found and increase in LF levels of 5,61 % (V1), 39,79% (V2), 50,37% (V3) and 31,29 (V4) in the four volunteers. Lactoferrin is involved with regulation of bone marrow function (myelopoiesis), and it can boost the body's defense immune mechanisms [16,17]. Gene expression profiles of peripheral blood mononuclear cells (PBMCs) from SARS patients shows that several genes are highly upregulated such as, the genes coding for Lactoferrin, S100A9 and Lipocalin 2 which suggests that the response of affected patients is related with an innate inflammatory response, rather than a specific immune response against the viral infection [18]. S100A9 plays an important role in the regulation of inflammatory processes and immune response by activating neutrophils (chemotaxis, adhesion, bactericidal activity, phagocytosis and degranulation). Lipocalin-2, is a protein that sequester iron and prevents its use by bacteria, thus limiting their growth. It is also expressed in neutrophils and in low levels in the kidney, prostate, and epithelia of the respiratory and gastro-intestinal tracts [18]. These mechanisms which are enhanced by lactoferrin, can be also operative in SARS-CoV-2 infections by inducing the upregulation of several genes involved in the innate defense. LTF belongs to the transferrin class of genes and its protein, lactoferrin, can be found in several epithelial secretions (tears, saliva, bronchial fluids, etc.) and in the form of granules in neutrophil leukocytes [1,19,20]. Increased levels of LF may be have a significant antimicrobial action in part by depriving iron from bacteria and have an important anti-inflammatory action in association with the downregulation of pro-inflammatory cytokines, as IL-6 (Fig. 1). Elevated IL-6 is implied in iron homeostasis disorders. LF has an immunomodulatory effect that may be related with the increase in G-CSF gene expression and subsequent changes in the number of cells in the leukocyte subsets in the peripheral blood after its oral administration. G-CSF also stimulates the survival, proliferation, differentiation, and function of neutrophil precursors and mature neutrophils. G-CSF is an important regulator of granulopoiesis and stimulates the bone marrow to produce granulocytes and stem cells which are released into the bloodstream [16,17]. It is produced by several different tissues (endothelium, macrophages, and several other immune cells. Neutrophils are among the first line

defense against infection and closely involved in the initiation of inflammatory responses. Neutrophils participate in fighting the infection through phagocytosis, intracellular degradation, releasing of several types of granules (primary, secondary, tertiary), building of extracellular nets and production of both pro-inflammatory (IL-6) and anti-inflammatory cytokines (IL-10) upon stimulation. Furthermore, neutrophils may interact with other cells such as dendritic cells, macrophages, natural killer cells, T and B cells to balance or modulate innate and adaptive immunity. Regarding the Granulocyte-macrophage colony-stimulating factor (GM-CSF) no quantifiable mRNA levels were detected at any condition. GM-CSF was originally defined by its ability to generate in vitro granulocyte and macrophage colonies from bone marrow precursor cells [18]. This factor acts mainly on mature myeloid cells of granulocyte and macrophage origin, mainly during host defense and inflammatory reactions. Microorganisms which enter the body are normally met, recognized and destroyed by cells of the nonspecific immune system: polymorphonuclear granulocytes (PMN), macrophages, natural killer cells and cytotoxic lymphocytes. During this battle against infection, an inflammatory response with the characteristic symptoms of local hyperthermia, swelling, redness and pain may occur. In all cases, the oral intake of LLF increased the levels of angiotensinogen. Angiotensinogen encodes the only known precursor of angiotensin II, a critical regulator of the cardiovascular system [20]. Transcriptional control of angiotensinogen in hepatocytes is an important regulator of circulating angiotensinogen concentrations. Angiotensinogen is a heterogeneous glycoprotein composed of 452 amino acids and different degrees of glycosylation, which is mainly produced in the liver. It is a member of the serpin family and is the main substrate of the renin-angiotensin-aldosterone system [21,22]. This molecule is catabolized by renin, giving rise to the angiotensin I molecule. Plasma angiotensinogen levels are increased by corticosteroids, estrogens, thyroid hormone, and angiotensin II levels. Angiotensinogen transcription is increased by the inflammatory cytokine tumor necrosis factor (TNF)-α by a nuclear factor- κB-like protein binding to an inducible enhancer called the acute-phase response element. Angiotensinogen encodes the only known precursor of angiotensin II, a critical regulator of the cardiovascular system [20]. Transcriptional control of angiotensinogen in hepatocytes is an important regulator of circulating angiotensinogen levels. Angiotensinogen is a heterogeneous glycoprotein composed of 452 amino acids and different degrees of glycosylation, which is mainly produced in the liver. It is a member of the serpin family and is the main substrate of the renin-angiotensin-aldosterone system [21,22]. This molecule is catabolized by renin, giving rise to the angiotensin I molecule. Plasma angiotensinogen levels are increased by corticosteroids, estrogens, thyroid hormone, and angiotensin II levels. Angiotensinogen transcription is increased by the inflammatory cytokine tumor necrosis factor alfa (TNF-a) by a nuclear factor- NB-like protein binding to an inducible enhancer called the acute-phase response element. Angiotensinogen gen levels were found increased in the blood serum of all volunteers which in turn may theoretically increase the levels of angiotensin in order to regulate blood pressure [23,24]. LF or its derivative peptides are a good source of oral antihypertensive agents [11]. LF may act as Angiotensin Converting Enzyme inhibitor and play significant anti- inflammatory effects on some aspects of vascular inflammation, resulting in reduced expression of pro inflammatory cytokines (IL-6) and modulation of cell activation (macrophages). SARS-CoV-2, which is the virus that causes COVID-19 infection, uses the angiotensin converting enzyme 2 receptor (ACE 2 receptor) to gain entry into the human host cell and lactoferrin competes with the S protein of the virus and blocks the ACE2 receptors and possibly also interfere with the cell transmembrane serine 2 protease that primes viral S protein [5]. Lactoferrin and its hydrolytic peptides are a good source of orally active antihypertensive agents that might act on several molecular targets and interacts with different components of the renin-angiotensin (RAS) and endothelin (ET) systems [23-25]. These peptides may have the ability

to modify the expression of genes encoding proteins involved in the nitric oxide (NO) pathway and prostaglandin synthesis [11].

5. Conclusion

Oral intake of LLF displays functional effects by increasing lactoferrin levels in blood serum and activation of lactoferrin related gene expression (LTF), angiotensinogen, and granulocyte colony stimulating factor. The significance of these facts may be related with immune activation, stimulation or immune modulation mechanisms. GM-CSF is not activated in healthy volunteers but possible may be activated once the infection or inflammatory process begins to develop.

6. Limitations

We postulate that the encapsulation of LF in a phosphatidylcholine liposome may provide to the protein a longer release beyond the 24 hours of the present study. However, we did not include a wash out period for clearing of the protein from the blood after the first 24 hours. The present sample is very small and more volunteers would be needed to statistically confirm the functional effects provided by the oral treatment with liposomal lactoferrin.

7. Conflict of Interest

All authors are at present working for Sesderma laboratories and there involves no conflict of interest.

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