Isolation and Structure-functional Characterization of Human Colostral Lactoferrin

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ABSTRACT

Human lactoferrin has been the focus of extensive research because of its antimicrobial, ferrochelating, immunomodulatory, and diagnostic potentialities. To meet the need for homogenous lactoferrin preparations, here we report simple procedures for colostrum processing, chromatographic isolation, and quality control techniques for the evaluation of the most relevant structure-functional properties of lactoferrin. Purity and identity were determined by combination of electrophoretic, densitometric, and immunochromatographic techniques. Structural integrity was assayed by high-performance liquid chromatography (HPLC), and iron-chelating properties were evaluated by spectroscopic analysis of lactoferrin derivatives. Antimicrobial activity was tested in vitro against Vibrio cholerae C7258, a regional epidemic strain. These procedures allowed the isolation and characterization of substantially pure (98–99%), structurally intact (92–93%), non-toxic, lipopolysaccharide (LPS)-free (less than 0.40 EU/mg of protein), low-iron saturated (below 7% saturation), biologically active (2.50 µM lactoferrin inhibited V. cholerae growth in 50%), and consistently homogenous preparations. The quality of this lactoferrin preparation allows its use in basic studies of the mechanisms of microbial virulence, and in the development of new tools for the diagnosis of gastrointestinal inflammation which are currently being performed by our group.

Keywords: antibacterial activity, human colostrum, iron, lactoferrin, LPS, protein purification, Vibrio cholerae

Introduction

Recognition of the immunoprotective potential of breast-feeding at epidemiological level [1], and the absence of similarly safe and effective pharmaceutical tools for prophylaxis of microbial infections in newborns [2] have promoted research on immunologically-active factors in human milk.

Lactoferrin, a monomeric high-affinity non-haem iron-binding glycoprotein (calculated isoelectric point [pI] ≈ 9.7, 77–80 kDa), is present in the milk of all mammals except rabbits, rats and dogs [3]. In human milk, its concentration ranges from 37.5 to 87.5 µM in colostrum to about 12.5 µM in mature milk [4], from where it was firstly purified in 1960 [5–7]. It occurs in all body fluids at different concentrations, from higher values in secretions of exocrine glands [8] to lowest ones in human blood (~20 nM) [9, 10]. Its main biological role is to keep concentrations of free iron to amounts insufficient to support microbial growth in vivo, together with other high-affinity iron-binding glycoproteins. The significance of these proteins in host-parasite interactions and the mechanisms developed by microbial pathogens for iron uptake to overcome their effects, were recently reviewed [11, 12].
Lactoferrin has been involved in multiple effects in vitro, but no definitive corroboration of their physiological significance has yet been provided. To our knowledge, there are three structural properties directly related to its biological activity, which have also been used in purification strategies [9]. Firstly, lactoferrin comprises two homologous domains, each with one iron-binding site. Its antimicrobial effects are based on the capacity to withhold iron with high affinity even at low pH values (pH = 2.0), which can occur in certain body locations [12]. Its capacity to bind multivalent cations has been used for purification by metal-ion affinity chromatography [13]. Secondly, each lactoferrin domain comprises an N-linked complex biantennate glycosylation site whose main function seems to be proteolytic resistance [14]. Glycosylation differences allowed separation by concanavalin A affinity chromatography of bovine and human lactoferrins from the milk of transgenic cows [9]. Thirdly, non-specific binding of lactoferrin’s highly basic N-terminal to several eukaryotic and prokaryotic structures [15] has been implicated in antiviral effects against human immunodeficiency virus type 1 (HIV-1) [16], human cytomegalovirus (HCMV) [17], herpes simplex virus (HSV) types 1/2 [18], and hepatitis C virus (HCV) [19]. Immunomodulatory and anti-endotoxic effects mediated by direct binding to bacterial lipopolysaccharides (LPS) have been reported [20], as well as bactericidal effects due to interactions with anionic cell wall components of Gram-negative bacteria [21]. Cytokine-like activities ascribed to this protein have also been discussed [22]. The cationic nature of lactoferrin and the unique distribution of its superficial charges are widely used for purification by cation-exchange chromatography [9, 15].

Human milk lactoferrin has been used as a tool for research into the mechanisms of iron uptake by pathogens [12] and the potential of bacterial lactoferrin receptors for vaccine development has been mentioned [23]. The therapeutic potential of lactoferrin has been tested in animal models of diseases such as rheumatoid arthritis [24], staphylococcal kidney infection [25] and endotoxin shock [26], as well as in humans with chronic HCV infection [27]. Lactoferrin concentrations are a reliable biochemical marker for the diagnosis of bacterial infection-related diarrhoea [28], as well as gastrointestinal inflammation of autoimmune origin [29]. The realization by scientific, medical, and pharmaceutical communities of the therapeutic and prophylactic implications of iron metabolism in host-parasite relationships [30], along with the forthcoming availability of commercial recombinant lactoferrin [31, 32], suggest that there could be widespread clinical and research uses of this protein in the near future. In this context, the availability of a high-quality and homogenous lactoferrin preparation is crucial for the study of its multiple biological effects. Although several effective procedures for purification of lactoferrin from human milk have been developed [5–7, 9, 31], differences in biological activity—mainly due to the partial degradation of the N-terminus—and different degrees of iron saturation have been detected between commercial preparations. This indicates that there is a need for an accurate control of the structural properties directly related to the biological effects of lactoferrin [9, 31, 32]. On the other hand, the costs of commercially available lactoferrin preparations may preclude its use for research in some locations. Here, we describe practical procedures for the purification and characterization of non-toxic, highly-pure and functionally-active colostral lactoferrin, as well as techniques for the quality control of its most important structure-functional relationships.

**Materials and Methods**

**Human colostrum**

Colostrum was kindly provided by healthy nursing women during the first five postpartum days at the Neonatological Units of Havana Gynecobothetic Hosp. All donors were seronegative to hepatitis B virus (HBV), HCV, HIV, Treponema pallidium and Toxoplasma gondii by the Diagnostic Services of the Mother-Infant Care Program of Cuba. Standard milk bank regulations were carefully followed to control microbial contamination [33]. Samples were stored at −20°C for no longer than three weeks before processing.

**Colostrum fractionation**

This was performed as previously reported [34] with major modifications. Frozen colostrum was thawed with running tap water at room temperature, diluted 9:1 (v:v) with 4.0 M NaCl, 0.1 M phosphate buffer (65.5 mM NaH_2PO_4, 31.5 mM Na_2HPO_4), adjusted to pH 4.6 with 0.1 M glacial acetic acid (Merck, Germany), and gently agitated for 1 h at 4°C. After centrifugation at 56,000 × g for 30 min at 4°C, three clearly separated layers were obtained: fats were in the top layer and casein plus cellular debris were at the bottom. The middle whey-containing layer was carefully collected and immediately adjusted to pH 6.5 with 0.1 M NaOH (Merck, Germany). Whey was further clarified by sequential filtration using a glass fiber prefiltre on top and three nitrocellulose membrane filters of 0.8, 0.45 and 0.2 µm pore-sizes placed on each other with polyester nitrate supply (Amicon, USA). Lactoferrin samples were carefully followed to control microbial contamination [33]. Samples were stored at −20°C for no longer than three weeks before processing.

**Chromatographic processing**

A XK-50 column (Pharmacia-LKB, Sweden) packed with 100 mL of a strong cationic exchanger (SP Sepharose Fast Flow, Pharmacia-LKB, Sweden) and equilibrated with 0.4 M NaCl, 0.01 M phosphate buffer, pH 6.50, was used for cation-exchange chromatography. Lactoferrin purification was performed essentially as reported [9, 29] with some variations. Clarified whey was passed through the column at a linear flow rate of 0.67 cm/min. Bound lactoferrin was eluted in a single peak with 0.8 M NaCl, 0.01 M phosphate buffer, pH 6.5. This fraction was concentrated against 0.01 M phosphate buffer, pH 6.5, and desalted to 0.15 M NaCl in an Amicon ultrafiltration stirred cell with controlled nitrogen supply (Amicon, USA). Lactoferrin samples were aliquoted and stored either frozen at −20°C or at 4°C after conventional freeze-drying (Cryodios Telstar, Spain). Eluates were saved and used as source for purification of secretory IgA (Center for Immunoassays, Cuba, data not shown).


Final ultrafiltration, filling and sealing steps were carried out under aseptic conditions. The Laboratory for Analytical Technical Control (Finlay Institute, Cuba) tested colostral whey and lactoferrin samples for microbial sterility.

Analytical methods

Protein concentrations were determined by the method of Lowry [35] using bovine serum albumin (BSA) as standard (BDH, UK). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was routinely performed in a discontinuous gel system [36]. All samples were boiled for 5 min under reducing conditions and 60 µg of total proteins were applied onto 12.5% polyacrylamide gels. Human lactoferrin and molecular weight markers (Sigma, USA) were used for antigenic identity and molecular weight estimation, respectively. Quantification of relative concentrations and molecular weight estimation of the bands antigenically identified as lactoferrin were performed by densitometry of silver nitrate (Sigma, USA)-stained gels [37] using an in-built software (Laser UltraScan XL Densitometer, Pharmacia-LKB, Sweden). To ensure that all bands were taken into account, the low density rejection function was set to zero.

Western blotting was performed as reported elsewhere [38]. After electrophoresis, gels were soaked in TBS (Tris-buffered saline: 50 mM Tris-HCl, 150 mM NaCl, pH 8.0) to reduce SDS concentration and incubated in transfer buffer (20 mM Tris, 150 mM glycine, 20% methanol, 0.1% SDS; pH 8.3) for 10 min. After transfer for 2 h at 4 °C, 0.2-µm nitrocellulose membranes (Pharmacia-LKB, Sweden) were incubated in TBS-T blocking solution (TBS plus 0.05% Tween 20, pH 8.0) containing 1% BSA Fraction V (Sigma, USA). After three washes, membranes were incubated in primary binding solution containing 1:1000 TBS-T-diluted anti-human lactoferrin rabbit polyclonal antibodies. After three washings, the membranes were incubated in a secondary binding solution containing horseradish peroxidase (HRPO)-conjugated goat anti-rabbit IgG (SAPU, Scotland) diluted 1:2000 in TBS-T. The gels were washed five times, and then exposed to HRPO substrate solution (0.02% [w/v] 4-chloro-1-naphtol [Sigma, USA] and 0.06% [v/v] hydrogen peroxide [BDH, UK] in TBS) until color developed. The reaction was stopped by acetate, 40 mM EDTA, and adjusted to pH 4.0 by dropwise addition of concentrated glacial acetic acid. The solution was firstly equilibrated and dialyzed against the same buffer overnight at 4 °C. Hololactoferrin, the 100% iron-saturated form, was obtained by incubation of 1% lactoferrin (w/v) in a ferric nitrotriacetic acid (FeNTA) solution (Fe:NTA molar ratio 1:4) overnight at 20 °C. To ensure full iron saturation, a twofold molar excess of FeNTA per iron binding site was added to lactoferrin solution. In both procedures, final samples were extensively dialyzed against 0.15 M NaCl, 0.01 M phosphate buffer, pH 6.5. Iron saturation was judged by comparing absorbance values at 280 and 465 nm [14, 40].

Preparation of lactoferrin derivatives

Apolactoferrin, the iron-free form, was produced as reported [40]. Briefly, the protein was diluted to 1% (w/v) in 0.1 M phosphate, 0.1 M sodium acetate, 40 mM EDTA, and adjusted to pH 4.0 by dropwise addition of concentrated glacial acetic acid. The solution was firstly equilibrated and dialyzed against the same buffer overnight at 4 °C. Hololactoferrin, the 100% iron-saturated form, was obtained by incubation of 1% lactoferrin (w/v) in a ferric nitrotriacetic acid (FeNTA) solution (Fe:NTA molar ratio 1:4) overnight at 20 °C. To ensure full iron saturation, a twofold molar excess of FeNTA per iron binding site was added to lactoferrin solution. In both procedures, final samples were extensively dialyzed against 0.15 M NaCl, 0.01 M phosphate buffer, pH 6.5. Iron saturation was judged by comparing absorbance values at 280 and 465 nm [14, 40].

Preparation of lactoferrin antiserum

Male New Zealand adult white rabbits were subcutaneously immunized with 0.05 mg of human lactoferrin (Sigma, USA) using Freund’s complete adjuvant (Difco Laboratories, USA) in the first injection. The same dose and route were used at 2, 4, and 6 weeks but in Freund’s incomplete adjuvant (Difco Laboratories, USA). Terminal bleeds were performed 2 weeks after the final injection and the antiserum was found to be adequate for the specific identification of lactoferrin by Western blotting at a 1:2000 dilution. The antiserum was aliquoted and stored at −20 °C.

Bacterial culture medium

Chemicals of the highest purity were purchased from Sigma, USA. Chemically-defined medium (CDM) was prepared as previously reported [41] with some modifications [42]: 59 mM glucose, 12 mM Na4HPO4, 69 mM NaCl, 9.3 mM NH4Cl, 7.3 mM KH2PO4, and 2.8 mM MgSO4. Amino acids were added to the following final concentrations (mg/L): glutamic acid, 1200; aspartic acid, 1200; tyrosine, 100; tryptophan, 30; cysteine, 120; isoleucine, 300; valine, 240; methionine, 90; phenylalanine, 100; leucine, 300; glycine, 1200; alanine, 1200; proline, 1200; lysine, 300; histidine, 240; arginine, 360; threonine, 2400; and serine, 2400. Vitamins were added to the following final concentrations (µM): biotin, 0.003; thiamine, 0.5; calcium pantothenate, 0.5; niacin, 0.5; riboflavin, 0.003. A mineral and trace element solution was added to the following final concentrations (µM): CaCl2·6H2O, 0.5; CoCl2·6H2O, 0.05; CuSO4·5H2O, 0.05; ZnSO4·7H2O, 0.5; MgSO4·7H2O, 0.005; K2HPO4·7H2O, 0.002; Na2HPO4·7H2O, 0.002; MnSO4·H2O, 0.001; FeSO4·7H2O, 0.0005; Na2EDTA·6H2O, 0.0005. Amino acids were added to the fol-
Inhibiting the growth of V. cholerae, the minimal concentration of lactoferrin capable of inhibition was determined by the Student's t test. Values of \( P < 0.05 \) were considered statistically significant.

Results

Human colostrum

All colostrum samples retained their typical organoleptic properties. A pH value of 7.0 ± 0.2 was consistently measured in these samples, which positively correlated with the absence of microbial contamination as observed in the microbial sterility assays (data not shown).

Colostrum fractionation

In a previous work, we observed that colostral lactoferrin bound to an SP Sepharose FF matrix consistently eluted as a single peak at 0.6–0.7 M NaCl in a 0.0 M–1.0 M NaCl linear gradient. No more peaks were detected thereafter, even at ionic strengths of up to 2.0 M NaCl [33]. As a result, ionic strength and phosphate concentration in whey samples were increased to 0.4 M NaCl and 0.01 M phosphate, respectively, before acid precipitation, and the cationic matrix was equilibrated with the same buffer composition. Addition of 0.4 M NaCl and 0.01 M phosphate to colostrum samples practically eliminated precipitation of lactoferrin in the casein fraction. Lactoferrin concentration in whey fractions rose by 20–30%, compared to non-treated samples from the same batch.

Chromatographic processing

Previous treatment with 0.4 M NaCl increased the efficiency of the chromatographic process by allowing nearly the triplication of the volume of whey to be applied per volume of bed—1.0 L vs. 0.35 L of whey per 100 mL of resin—without breakthrough occurrence [33] and reducing the time for chromatographic processing in about 50%. Lactoferrin consistently eluted at 0.8 M NaCl in a single symmetric peak at a 1.5 ± 0.1 bed volume (Figure 1). The protein yield represented 83–85% of the initial concentration, nearly the double of the values reached when lactoferrin was directly isolated from the whey samples (3.6 g vs. 6.0 g per liter of colostrum) [33]. Endotoxin levels were significantly lower in the samples isolated from treated vs. non-treated colostral whey, and were within the limit of acceptability—less than 0.4 EU/mg of protein. Taken together, these results indicate a complete disruption of the non-specific interactions between lactoferrin and caseins during acid precipitation, as well as a separation of these proteins from other whey components.

Figure 1. Pattern of elution of lactoferrin purified from human colostral whey from SP Sepharose FF Matrix. For specific conditions see the text.

Lactoferrin antibacterial activity

As we previously reported for Staphylococcus aureus [42], the minimal concentration of lactoferrin capable of inhibiting the growth of V. cholerae to 50% during exponential phase (MIC) was determined by the addition to CDM of lactoferrin concentrations between 0.3 μM and 20 μM at the time of inoculation. Lactoferrin-free CDM was used as negative control. To confirm the bacteriostatic effect of iron-deprivation upon the growth of V. cholerae C7258 strain, an identical culture was supplemented with 800 μM EDDHA (Sigma, USA), a specific ferrochelating reagent. Cultures were observed for 10 h.

Iron reversibility of lactoferrin antibacterial effect

The iron reversibility of the growth inhibitory activity of lactoferrin against V. cholerae was tested by adding a 20-fold molar excess of FeNTA at 0, 3 and 6 h, respectively, to three different CDM cultures which were supplemented with MIC lactoferrin at the time of inoculation. As control, non-iron supplemented but lactoferrin-containing CDM cultures were kept under similar conditions.

Statistical analysis

All experiments described were performed at least three times, and the standard error, the mean and the coefficient of variation were calculated for all data. The mean values for OD measurements in all cultures were compared with the control culture and among them by the Student’s t test. Values of \( P < 0.05 \) were considered statistically significant.

Densitometric analysis of electrophoretically separated protein bands consistently showed a major band at 78–80 kD, which represented 98–99% of the total protein applied per lane. Few lower molecular weight bands were detected in both eluted and Sigma lactoferrin samples (Figure 2). These bands were antigenically identified as degraded forms of lactoferrin [4, 9].

Contrary to a previous report [39], no IgA contamination could be detected, even in very concentrated lactoferrin samples purified from treated colostral whey, while minor non-quantified IgA contamination was detected when non-treated colostral whey was used [33, 34] (data not shown).

Mono S HR HPLC separation of lactoferrin samples indicated that 92.8 ± 1.12% of the protein eluted between 0.6 M and 0.69 M NaCl. Three minor peaks eluted at 0.17 M, 0.5 M and 0.56 M NaCl, which altogether represented less than 7% of the total protein eluted. No statistically significant differences in the levels of purity and the pattern of elution from Mono S HR HPLC were detected between the commercial reference and the preparation assayed under similar conditions. Lactoferrin from Sigma and the commercial reference and the preparation assayed under similar conditions. Lactoferrin from Sigma and the samples eluted at 0.615 M and 0.624 M NaCl, and at 64.04 and 64.88-min retention times, respectively (Figure 3). Apo and iron-saturated forms of purified lactoferrin were obtained as described above, and the ratio of absorbance values at 465 and 280 nm of a 1% lactoferrin were obtained as described above, and the ratio of absorbance values at 465 and 280 nm of a 1% lactoferrin solution was in agreement with previously reported values; i.e. 0.60 for 100% iron-saturated lactoferrin and nearly zero for apolactoferrin [40]. No differences in absorbance were found for the lactoferrin from Sigma, either in the apo or in the holo forms (data not shown).

**Antibacterial activity**

The growth of *V. cholerae* strain C7258 in CDM was inhibited by the addition of lactoferrin in a dose-dependent manner. The inhibitory effect was either undetectable or only transiently observed at concentrations below 1.25 µM. The minimal concentration of lactoferrin which consistently reduced *V. cholerae* growth in 50% during the logarithmic phase was 2.5 µM. This effect was saturable as no significantly higher growth inhibition could be achieved by increasing lactoferrin concentration up to 8 times above this value (Figure 4). A greater and more stable inhibition was observed in cultures treated with 800 µM EDDHA, which corroborated the requirement of iron for the normal growth of this strain under the conditions of this study [12, 43].

![Figure 2. Patterns of processed human colostral whey (lane 1), eluate (lane 2), eluted lactoferrin (lane 3), and Sigma lactoferrin (lane 4) after electrophoretic separation and silver staining in a 12.5% SDS-PAGE. Observe the similarities in the electrophoretic profiles of the two lactoferrin samples. Estimated molecular weights (kD) are shown at the left side.](image)

![Figure 3. Chromatograms of commercial (upper) and colostrum-purified (lower) lactoferrin samples analysed by Mono S HPLC under identical conditions. No differences in retention time (64.04 min vs. 64.88 min) and molarity of elution (0.615 M vs. 0.624 M) between samples could be detected. The solid diagonal line represents the linear saline elution gradient from 0.0 M to 1.0 M NaCl.](image)

![Figure 4. Concentration dependence of lactoferrin microbicidal effect against *Vibrio cholerae* C7258. A 2.5-µM lactoferrin consistently halved bacterial growth during logarithmic phase. The solid horizontal line represents 50% of growth inhibition. This effect was saturable, as no further growth inhibitory effect was observed for lactoferrin concentrations up to eight times higher (20 µM).](image)
Iron reversibility of lactoferrin antibacterial effect

A 20-fold molar excess of FeNTA, i.e. 50 µM, completely reversed the growth inhibitory effect of MIC$_{50}$ lactoferrin (2.5 µM). Interestingly, it was noted that the kinetics of iron reversion seemed to be affected by the time of exposure of the bacterial cultures to lactoferrin. A longer delay before resumption of normal growth after addition of FeNTA was consistently observed in cultures treated with 2.5 µM lactoferrin for more than 4–5 h. This effect does not seem to be dose-dependent, as a similar delay was observed at higher lactoferrin concentrations (data not shown). We did not observe this effect in previous experiments using S. aureus strains [42]. It suggests possible differences in the microbistatic activity of lactoferrin against V. cholerae.

Discussion

Since lactoferrin is the most cationic protein present in human milk, its non-specific electrostatic interactions with anionic moieties of molecules such as caseins, lysozyme, IgA secretory component, α-lactalbumin, and β-lactoglobulin [9, 14, 33], as well as bacterial LPS [20], are disrupted only at ionic strengths above 0.3 M NaCl. Consequently, addition of up to 0.4 M NaCl and 0.01 M phosphate prior to the delipidation and casein precipitation steps prevents coprecipitation with caseins and increases the yield and purity of lactoferrin before chromatographic processing.

Because lactoferrin has the highest affinity for cationic exchange resins and a clustered distribution of its positive charges at the N-terminus, it can be selectively separated in a substantially pure, intact and complex-free form in a single chromatographic step at a high ionic strength [14, 33]. Therefore, lactoferrin preparations were consistently 98–99% pure and essentially free of LPS contamination (below 0.4 EU/mg of protein).

The structural integrity of the purified protein is not affected (Figure 3), as shown by the pattern of elution in Mono S HPLC, a reliable and more affordable technique for monitoring the degree of structural integrity of lactoferrin preparations as previously reported [9].

The lack of toxicity of this preparation was confirmed in two different experimental settings. Doses as high as 30 mg were injected intravenously into adult Balb/c mice without causing any detectable clinical or histopathological damage (data not shown). Concentrations as high as 4–5 mg/mL of lactoferrin were required before any cytotoxicity against cultured Vero cells was observed (data not shown).

Lactoferrin concentrations 30-fold lower than those present in human colostrum showed a significant bacteriostatic activity against a regional epidemic strain of V. cholerae. This demonstrates the biological activity of these preparations, corroborates the potential of lactoferrin as a broad-spectrum natural antimicrobial agent, as shown both in vitro [16–21, 31] and in vivo [24–27], and reinforces the importance of breast-feeding for the prevention of gastrointestinal microbial infections in newborns [1].

As with other El Tor strains, V. cholerae C7258 strain secretes the hemagglutinin/metalloenzyme Hap with proteolytic activity against lactoferrin and other protective components of the immune system [44]. Clinical trials using genetically-attenuated V. cholerae CTXφ vaccine strains seem to implicate Hap in cholera virulence, as detection of intestinal inflammatory effects and increased levels of lactoferrin and proinflammatory cytokines were limited to stools of human volunteers immunized with a hap+ strain [45], but not with the parental hap- strain (146], García L, Finlay Institute, Havana, personal communication). In contrast, Toma et al. reported that the proteolytic degradation of lactoferrin by Hap does not affect its antimicrobial activity in vitro against a non-O1 strain of V. cholerae isolated from humans [47]. We observed that V. cholerae, but not the S. aureus strains assayed under similar conditions, showed a direct correlation between the time of exposure to lactoferrin and the delay in resumption of normal growth after addition of iron [42]. Experiments are being carried out to elucidate the actual effect of Hap-mediated proteolysis on the mechanisms of antimicrobial activity of lactoferrin against V. cholerae, and on the mechanisms of iron uptake by V. cholerae strains in vivo.

We conclude that the procedures described in this article for the purification and quality control of lactoferrin provide fast, simple and state-of-the-art methods for obtaining a high quality, substantially pure, structurally intact, and biologically functional lactoferrin from human colostrum. This lactoferrin preparation shows quality control parameters equivalent to commercially available preparations. Therefore, this lactoferrin preparation may be used in basic studies on the mechanisms of microbial virulence related to iron metabolism and in the development of new tools for the diagnosis of gastrointestinal inflammation of infectious or autoimmune origin.

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