Studies on the Immunomodulatory Effects of Lactoferrin in Rats Infected with *E. coli*

Mohamed El-Sayed El-Boshy1,2*, Osama Ali Abdalla3, Adal Hassan3

1Department of Clinical Pathology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt
2Laboratory Medicine Department, Faculty of Applied Medical Science, Umm Al-Qura University, Makkah, Saudi Arabia
3Department of Clinical Pathology, Faculty of Veterinary Medicine, Ismaïlia, Egypt

Email: *ahmedmed3@yahoo.com

Received May 17, 2013; revised June 24, 2013; accepted July 10, 2013

Copyright © 2013 Mohamed El-Sayed El-Boshy et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**ABSTRACT**

Eighty male albino rats of Westar strain (350 ± 10 g), 10 to 12 weeks old were divided into four groups. The groups treated are as following. The first control group (Gp. I) was given intraperitoneally in normal saline (1 mL). The second group (Gp. II) was orally infected with $3 \times 10^{12}$ CFU of *E. coli* /Kg. BW. The third group (Gp. III) was infected with *E. coli* and treated with (0.5%) lactoferrin (LF) 72 hours before *E. coli* infection in filtered tap water for the duration of the experiment (21 days). The fourth group (Gp. IV) was administrated with LF only (0.5%) in drinking water. Two separate blood samples were collected from heart puncture at the end of 1st, and 3rd week post-treatment for immunological studies. The Leukogram in *E. coli* treated group was insignificant compared with the control group while lymphocytosis was clear compared with the infected group. Total protein, albumin, α-globulin and β-globulin were insignificantly changed in LF & *E. coli* treatment group comparing with infected and control groups. TNF-α and γ-globulin are significantly increased in infected group comparing with other treated groups. In conclusion, lactoferrin has powerful antibacterial activity in a variety of ways as well as a safe immunostimulant protein when it is orally administrated.

**Keywords:** Lactoferrin; Immunomodulatory; *E. coli*; Rats

1. Introduction

*Escherichia coli* is probably the best-known bacterial species and one of the most frequently isolated organisms from clinical specimens [1].

Lactoferrin is (LF) a very important part of the human body’s natural defense system, sequestering free iron result in inhibition, adhesion and growth of (pathogens) like *H. pylori* and *E. coli* to the intestinal wall. The cellular structure of pathogens loses its integrity due to the iron deprivation and necrosis [2]. LF and its derivatives have pleiotropic functions including broad-spectrum antimicrobial activity, regulation of cell growth and differentiation, and modulation of inflammatory as well as humoral and cellular immune responses [3].

*Escherichia coli* is one of the main species of bacteria that normal inhabitants lower intestines of worm blooded animals, including birds and mammals [4].

LF is a natural defense protein belonging to the innate immune system present in several body fluids and secretions, as well as in the secondary granules of polymorphonuclear neutrophils [3]. LF has been thought to protect against gram-negative bacteria in a variety of ways. It sequesters iron that is essential for bacterial growth [5]. It has reported that orally administered bovine lactoferrin (bLF) inhibits the proliferation of endogenous members of the family *Enterobacte-riaceae* in the guts of mice fed bovine milk [6]. (It was shown also that orally administered bLF into mice inhibits bacterial translocation [7]. Therefore, the goals of this study are safety evaluation of antimicrobial activity of LF in rat experimentally infected with *E. coli* through measuring some selective immunological parameters.

2. Material & Methods

2.1. Materials

**Experimental Animals (Rats)**

Eighty male albino rats of Westar strain (350 ± 10 g,) 10
to 12 weeks old procured from College of Veterinary Medicine Zagazig University, Egypt, were used for the study. Animals were fed with commercially available standard and balanced rat ration and water was provided ad libitum. The rats were housed under controlled conditions of humidity, temperature (25°C ± 2°C) and light (12 h light/12 h dark) and had free access to water and food. All animals were acclimatized for 1 week before experimentation and experiment extended for 21 days.

Bovine lactoferrin (bLF) was purchased from (Symbiotics Co lustrum U.S.A. (lot no. MLF 160996;) was obtained from bovine colostrums. It was a light red pink powder, almost odorless, with a purity of 100% bLF. E. coli O78, strain was provided by (Animal Health Research Center, Ismailia, Egypt).

2.2. Methods

Rats were randomly divided into four equal groups, each consisting of twenty rats. Each group separated in Plastic cages. The groups treated as following. 1st control group (Gp. I) was given intraperitoneally normal saline (1 mL). 2nd group (Gp. II) was orally infected with 3 × 10^12 CFU of E. coli /Kg. BW according to Lyn et al. [8]. 3rd group (Gp. III) infected with E. coli and treated with bLF (0.5%) in filtered tap water for the duration of the experiment (21 days). 4th group (Gp. IV) administrated lactoferrin only (0.5%) in drinking water according to Zimecki et al. [9]. The experiment extends for 21 days post treatment.

2.2.1. Blood Sampling

Two separate blood samples were collected from heart puncture at end of 1st and 3rd week post treatment. One sample was taken in epindorf tubes at which mixed with EDTA for total and differential leukocytes counts which were measured according to [10,11] respectively. The second blood samples were taken in test tube without anticoagulant. The samples were centrifuged at 3000 rpm for 10 minutes and the clear serum was separated carefully and determination of some biochemical parameters (total protein and albumin) by using commercial diagnostic kits which were obtained from Human-Germany and Spinreact-Spanish).

Immunoelectrophoresis of serum protein has been done using cellulose acetate gel according to [12].

Tumor necrosis factor—α (TNF) was measured by Enzyme Amplified Sensitivity Immunoassay (EASIA) performed on microplate. The assay used monoclonal antibodies (MAbs) directed against distinct epitopes of TNF-according to [13].

2.2.2. Statistical Analysis

The results were analyzed by analysis of variance (ANOVA) followed by LSD using SPSS.18 for window. Two groups were significantly different if P was statistically lower than 0.05.

3. Results & Discussion

Lactoferrin is a protein found in cow milk and human milk. Colostrum, the first milk produced after a baby is born, contains high levels of lactoferrin, which is about seven times the amount found in milk produced later on. Lactoferrin is also found in fluids in the eye, nose, respiratory tract, intestine, and elsewhere [14].

Infected group in the present work showed marked leukocytosis and neutrophilia in agreement with Shin et al. [2] who found that Shiga toxin of E. coli caused marked (seven fold) granulocytosis in the peripheral blood. Tanka et al. [15] demonstrated that i/p injection of E.coli lipopolysaccharid (LPS) stimulates inflammatory response. The response in turn, caused release of chemical mediators such as macrophage colony stimulating factor which in turn activates various cell systems as macrophage and neutrophils.

The current results of our study revealed that the LF and E. coli treated group show significant decrease in leucocytes comparing with infected group and return to normal level during 3rd weeks (Tables 1 & 2). The antibacterial activity of LF is not only due to its iron binding capacity Visca et al. [16] but also due to neutralizing the endotoxins, binding to the bacterial cell and protective activity against lethal E. coli infection by lactoferrin. This result agreed with Lynn et al. [8] who reported that orally administered LF protected neonatal rats from systemic bacterial infection, illness, and death following massive intestinal infection with E. coli. In the same line, Liliana et al. [17] recorded that the numbers of bacteria in the kidneys and bladder of E. coli treated mice were significantly reduced 24 h later by the LF treatments compared to the findings for the control group. Zagulski et al. [18] reported that LF I/V injected to mice, with dose of E. coli, lead to strong clearance of E. coli from blood as well as liver, lungs, spleen and kidney.

Current result revealed significant lymphocytosis in LF and E. coli treated group comparing with infected group. Also increased lymphocyte counts in LF group comparing with control one. This could be attributed to LF which activated natural killer cells as well as promoted maturation of T and B cells from neonatal mice [9].

Concerning to plasma protein profile of this result, insignificant alteration of total plasma protein, alpha and beta globulins in infected group comparing with control one was shown (Tables 3 & 4). This may be as a result of increased synthesis of acute phase protein by E. coli infection von et al. [19] as well as dehydration. Current result partially in hand with Kinsbergen et al. [20] who reported insignificant change in total plasma protein in calves I/V injected with E. coli.
The present result showed hyoalbuminemia in the 3rd week in E. coli infected group comparing with control one. E. coli infection caused hypoalbuminemia in rats due to fall in the levels of albumin mRNA in response to infection parallel to a decrease in intrahepatic albumin synthesis. Also, infection can lead to increased catabolic rate and/or redistribution of albumin from plasma to interstitial compartment. Also, infection could be attributed to malabsorption as a result of the diseases affecting the GIT especially the intestine. Total plasma protein and albumin were insignificantly different in E. coli and LF in comparison with the control one. In addition, Andréa and Loreny [21] recorded that LF inhibit the adhesion of enteropathogenic E. coli to the intestinal epithelial cells. γ globulin in the present work was decreased in E. coli infected group comparing with the control one. This could be due to the immunosuppression of E. coli. Immunosuppression of E. coli, has been reported with different authors; [22-24] in rats, calves and healthy volunteers respectively.

Regarding to γ-globulins in LF and E. coli, treated group showed significant increase of γ-globulins comparing with infected group and returned to normal at 3rd week. LF and its derivatives have pleiotropic functions regulation of cell growth and differentiation, and modulation of inflammatory as well as humoral and cellular immune responses [3]. LF could induce cytokine production in stromal cells, who are able to support differentia-
tion of T and B cells [25]. LF exerts a beneficial action on the immune response, inducing lymphocyte proliferation and good inducer of IL-6 [26]. γ-globulins in LF group showed insignificant differences comparing with control group. This result was the same with Jaya and Shao [27] who reported that oral feeding of LF potentially stimulate non-specific immune response while specific immunity was not influenced by LF feeding.

Tumor necrosis factor α is the principal cytokines produced by activated T cells, mononuclear phagocytes and NK cells to induce cell mediated immune responses to foreign agents [28]. TNF-α was significantly increased in E. coli infected group comparing with the control one. This result goes with Theodore [29] who recorded the most marked acute phase reactions in responses to E. coli are the greatest TNF-α produced by activated T cells, mononuclear phagocytes and partially stimulate non-specific immune response while specific immunity was not influenced by LF feeding.

In conclusion, LF has been thought to be powerful anti-bacterial agent in a variety of ways as well as is a safe immunostimulant protein when orally administrated, for a long time without any observed adverse effect.

REFERENCES


[14] D. Legrand “Lactoferrin, a Key Molecule in Immune and


