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

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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 3×10^{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 specie 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

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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 leuckocytes 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*.

Table 1. Leukogram (mean ± SE), one week post treatment with lactoferrin in immunosuppressed rats with *E. coli*.

Groups	TLC 10^3 /μL	Neutrophil 10^3 /μL	Eosinophil 10^3 /μL	Basophil 10^3 /μL	Lymphocyt 10^3 /μL	Monocyte 10^3 /μL
Control (I)	$7.75^a \pm 0.45$	$2.74^a \pm 0.25$	$0.154^b \pm 0.041$	$0.016^a \pm 0.01$	$4.39^a \pm 0.31$	$0.47^a \pm 0.072$
E. coli (II)	$14.27^c \pm 1.24$	$9.06^c \pm 0.89$	$0.021^a \pm 0.019$	0.00	$4.65^a \pm 0.41$	$0.54^a \pm 0.075$
Lactoferrin & *E. coli* (III)	$9.51^b \pm 0.41$	$4.35^b \pm 37$	$0.125^b \pm 0.035$	0.0	$4.51^a \pm 0.39$	$0.52^a \pm 0.063$
Lactoferrin (IV)	$8.35^a \pm 0.59$	$2.52^a \pm 0.29$	$0.138^b \pm 0.0.39$	$0.017^a \pm 0.01$	$5.09^a \pm 0.36$	$0.56^a \pm 0.071$

Means in the same column not followed by the same letter differ significantly ($P < 0.05$).

Table 2. Leukogram (mean ± SE), one week post treatment with lactoferrin in immunosuppressed rats with *E. coli*.

Groups	TLC 10^3 /μL	Neutrophil 10^3 /μL	Eosinophil 10^3 /μL	Basophil 10^3 /μL	Lymphocyt 10^3 /μL	Monocyte 10^3 /μL
Control (I)	$8.35^a \pm 0.51$	$2.53^a \pm 0.28$	$0.165^b \pm 0.044$	$0.016^a \pm 0.016$	$5.18^b \pm 0.36$	$0.46^a \pm 0.051$
E. coli (II)	$8.30^a \pm 0.48$	$3.46^a \pm 0.35$	$0.034^a \pm 0.016$	0.00	$4.02^a \pm 0.34$	$0.78^b \pm 0.055$
Lactoferrin & *E. coli* (III)	$8.68^a \pm 0.42$	$2.89^a \pm 0.29$	$0.174^b \pm 0.030$	0.00	$4.91^a \pm 0.31$	$0.71^b \pm 0.048$
Lactoferrin (IV)	$8.91^a \pm 0.57$	$2.98^a \pm 0.31$	$0.178^b \pm 0.034$	$0.089^a \pm 0.09$	$5.29^b \pm 0.34$	$0.37^a \pm 0.065$

Means in the same column not followed by the same letter differ significantly ($P < 0.05$).

Table 3. Some selective immunological parameters one week post treatment with lactoferrin mean ± SE) in immunosuppressed rats with *E. coli*.

Groups	T. Protein gm/dl	Albumin gm/dl	*α*-globulin gm/dl	*β*-globulin gm/dl	*γ*-globulin gm/dl	TNF-P g/ml
Control (I)	$7.45^a \pm 0.41$	$3.40^a \pm 0.25$	$1.41^a \pm 0.18$	$1.35^a \pm 0.17$	$1.29^b \pm 012$	$21.2^a \pm 2.45$
E. coli (II)	$7.67^a \pm 0.54$	$3.21^{ab} \pm 0.34$	$1.74^a \pm 0.12$	$1.81^b \pm 0.14$	$0.91^a \pm 0.08$	$54.6^b \pm 6.25$
Lactoferrin & *E. coli* (III)	$7.64^a \pm 0.61$	$3.25^{ab} \pm 0.31$	$1.69^a \pm 0.11$	$1.72^b \pm 0.10$	$0.98^a \pm 0.06$	$44.6^b \pm 5.41$
Lactoferrin (IV)	$7.52^a \pm 0.62$	$3.38^a \pm 0.46$	$1.46^a \pm 0.19$	$1.32^a \pm 0.16$	$1.36^b \pm 0.13$	$20.4^a \pm 2.01$

Means in the same column not followed by the same letter differ significantly ($P < 0.05$).

Table 4. Some selective immunological parameters three week post treatment with lactoferrin (mean ± SE) in immunosuppressed rats with *E. coli*.

Groups	T. Protein gm/dl	Albumin gm/dl	*α*-globulin gm/dl	*β*-globulin gm/dl	*γ*-globulin gm/dl	TNF-Pg/ml
Control (I)	$7.48^a \pm 0.48$	$3.46^a \pm 0.23$	$1.39^a \pm 0.16$	$1.31^a \pm 0.17$	$1.32^b \pm 014$	$18.9^a \pm 2.08$
E. coli (II)	$6.39^a \pm 0.42$	$2.86^a \pm 0.21$	$1.35^a \pm 0.13$	$1.25^a \pm 0.14$	$0.94^a \pm 0.08$	$46.1^b \pm 5.15$
Lactoferrin & *E. coli* (III)	$7.21^a \pm 0.56$	$3.26^{ab} \pm 0.20$	$1.34^a \pm 0.14$	$1.35^a \pm 0.13$	$1.28^b \pm 0.10$	$23.7^a \pm 2.82$
Lactoferrin (IV)	$7.52^a \pm 0.49$	$3.38^a \pm 0.32$	$1.33^a \pm 0.16$	$1.32^a \pm 0.18$	$1.48^b \pm 0.15$	$19.4^a \pm 1.95$

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, calve 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 Shaoo [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 responses in blood plasma. This result also agreed with Zimecki *et al.* [30] who reported that I.V. administration of lethal dose of *E. coli* leading to an increase of TNF-α level. In the presented study, TNF-α was significantly decreased in *E. coli* and LF treated group comparing with infected one at 2nd week and return to the normal at 3rd week. In the same line, the Zimecki *et al*., [30] concluded the protective action of LF in *E. coli* induced bacteremia by revealing the phenomenon of accelerated neutrophil recruitment and down regulation of *E. coli*-induced TNF alpha serum level. Also, Adamik & Wlaszczyk [31] contribute the protection action of LF against pathogens and their metabolites to enhance phagocytosis, cell adherence and controlling release of proinflammatory cytokines such as TNF-alpha. Spagnuolo *et al*. [32] reported that short-term dietary LF decreased TNF alpha expression in intestinal lymphocyte in healthy mice. Crouch *et al*. [33] concluded that LF may modulate immune responses by inhibiting cytokine activity (TNF-α). This result is in contradiction with Teraguchi, *et al.* [34] who observed increase cytokine (IFN-γ, TNF-α, IL-12, and IL-18) production in response to LF administration in several animal models.

During the experiment period, there are none significant changes in the LF treated group comparing with control group in all investigated items which indicate high safety profile of LF. This result agreed with Yamauchi *et al.* [35], who reported no significant toxilogical results in male and female rats treatment with 2000 mg/kg. BW/of LF is once daily for 13 weeks.

In conclusion, LF has been thought to be powerful antibacterial 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

[1] D. Mark, C. Annette and B. Jean, "Estimated Annual Costs Due to Selected Food-Borne Pathogens," Data from the Centers for Disease Control and Prevention, Food-Related Illness and Death in the United States Economic Research Service, US Department of Agriculture, 2001.

[2] K. Shin, K. Yamauchi, S. Teraguchi, H. Hayasawa, M. Tomita, Y. Otsuka and S. Yamazaki, "Antibacterial Activity of Bovine Lactoferrin and Its Peptides against Enterohaemorrhagic *Escherichia coli* O157:H7," *Letters in Applied Microbiology*, Vol. 26, 1998, pp. 407-411. http://dx.doi.org/10.1046/j.1472-765X.1998.00358.x

[3] W. J. van der Velden, N. M. Blijlevens and J. P. Donnelly, "The Potential Role of Lactoferrin and Derivatives in the Management of Infectious and Inflammatory Complications of Hematology Patients Receiving a Hematopoietic Stem Cell Transplantation," *Transplant Infectious Disease*, Vol. 10, No. 2, 2007, pp. 68-71.

[4] C. C. Rosario, A. C. Lopaz, I. G. Tellez, O. A. Navarro, R. C. Anderson and C.C. Eslava, "Serotyping and Virulence Genes Detection in *Escherichia coli* Isolated from Fertile and Infertile Eggs, Dead-in-Shell Embryos, and Chickens with Yolk Sac Infection," *Avian Diseases*, Vol. 48, No. 4, 2004, pp. 791-802. http://dx.doi.org/10.1637/7195-041304R

[5] R. R. Arnold, M. F. Cole and J. R. McGhee, "A Bactericidal Effect for Human Lactoferrin," *Science*, Vol. 197, No. 4300, 1977, pp. 263-265. http://dx.doi.org/10.1126/science.327545

[6] S. Teraguchi, K. Ozawa, S. Yasuda, K. Shin, Y. Fukuwatari and S. Shimamura, "The Bacteriostatic Effects of Orally Administered Bovine Lactoferrin on Intestinal Enterobacteriaceae of SPF Mice Fed Bovine Milk," *Bioscience, Biotechnology, and Biochemistry*, Vol. 58, No. 3, 1994, pp. 482-487. http://dx.doi.org/10.1271/bbb.58.482

[7] T. Susumu, S. Kouichirou, O. Tomohiro, K. Michiko, K. Akira, M. Hirofumi, F. Yasuo and S. Seiichi, "Orally Administered Bovine Lactoferrin Inhibits Bacterial Translocation in Mice Fed Bovine Milk," *Applied and Environmental Microbiology*, Vol. 61, No. 11, 1995, pp. 4131-4134.

[8] E. Lynn, B. H. Ronaldo, F. Y. Freda, R. H. Denis, A. S. Robert and P. S. Michael, "Lactoferrin Protects Neonatal Rats from Gut-Related Systemic Infection," *The American Journal of Physiology—Gastrointestinal and Liver Physiology*, Vol. 281, No. 5, 2001, pp. 1140-1150.

[9] M. Zimecki, J. Mazurier, G. Spik and J. A. Kapp, "Human Lactoferrin Induces Phenotypic and Functional Changes in Murine Splenic B Cells," *Immunology*, Vol. 86, No. 1, 1995, pp. 122-127.

[10] E. H. Coles, "Textbook of Veterinary Clinical Pathology," 4th Edition, W. B. Saunders Co., Philadelphia, 1986.

[11] K. S. Latimer, E. A. Malaffey and K. W. Prasse, "Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology," 4th Edition, Iowa State Press, Iowa City, 2003.

[12] R. J. Henry, D. C. Cannon and J. W. Winkelman, "Clinical Chemistry: Principles and Techniques," Harper and Row, Hagerstown, 1974.

[13] B. Beutler and A. Cerami, "Cachectin: More than Tumor Necrosis Factor," *The New England Journal of Medicine*, Vol. 316, No. 7, 1987, pp. 379-385. http://dx.doi.org/10.1056/NEJM198702123160705

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

Inflammatory Processes," *Biochemistry and Cell Biology*, Vol. 90, No. 3, 2012, pp. 252-268. http://dx.doi.org/10.1139/o11-056

[15] H. Tanaka, N. Tanaba, M. Shoji, T. Katono, S. Sato, M. Motohashi and M. Maeno, "Nicotine and Lipopolysaccharide Stimulate the Formation of Osteoclast-Like Cells by Increasing Macrophage Colony-Stimulating Factor and Prostaglandin E2 Production by Osteoblasts," *Life Sciences*, Vol. 78, No. 15, 2006, pp. 1733-1740. http://dx.doi.org/10.1016/j.lfs.2005.08.017

[16] P. Visca, C. Dalmastri, D. Verzili, G. Antonini, E. Chiancone and P. Valenti, "Interaction of Lactoferrin with *Escherichia coli* Cells and Correlation with Antibacterial Activity," *Medical Microbiology and Immunology*, Vol. 179, No. 6, 1990, pp. 323-333. http://dx.doi.org/10.1007/BF00189610

[17] A. H. Liliana, E. Inga, B. Lars, D. Gunnar, A. H. Lars and M. Inger, "Human Lactoferrin and Peptides Derived from a Surface-Exposed Helical Region Reduce Experimental *Escherichia coli* Urinary Tract Infection in Mice," *Infection and Immunity*, Vol. 68, No. 10, 2000, pp. 5816-5823.

[18] T. Zagulski, Z. Jarzabek, A. Zagulska, M. Jaszczak, I. E. Kochanowska and M. Zimecki, "Lactoferrin Stimulates Killing and Clearance of Bacteria but Does Not Prevent Mortality of Diabetic Mice," *Archivum Immunologiae et Therapia Experimentalis*, Vol. 49, No. 6, 2001, pp. 431-438.

[19] A. D. Von, P. O. Hasselgren and J. E. Fischer, "Hepatic Protein Synthesis in a Modified Septic Rat Model," *Journal of Surgical Research*, Vol. 48, No. 5, 1990, pp. 476-480. http://dx.doi.org/10.1016/0022-4804(90)90016-U

[20] M. Kinsbergen, R. M. Bruckmaier and J. W. Blum, "Metabolic, Endocrine and Haematological Responses to Intravenous *E. coli* Endotoxin Administration in 1-Week-Old Calves," *Zentralblatt fur Veterinarmedizin Reihe A*, Vol. 41, No. 7, 1994, pp. 530-547.

[21] N. A. Andréa and G. G. Loreny, "Lactoferrin and Free Secretory Component of Human Milk Inhibit the Adhesion of Enteropathogenic *Escherichia coli* to HeLa Cells," *BMC Microbiology*, Vol. 1, 2001, p. 25. http://dx.doi.org/10.1186/1471-2180-1-25

[22] J. Tanaka, T. Sato, R. T. Jones, B. F. Trump and R. A. Cowley, "The Pathophysiology of Septic Shock: Responses to Different Doses of Live *Escherichia coli* Injection in Rats," *Advance in Shock Reserch*, Vol. 9, 1983, pp. 101-114.

[23] N. C. Jain, J. L. Vegad, A. B. Shrivastava, N. K. Jain, U. K. Garg and G. N. Kolte, "Haematological Changes in Buffalo Calves Inoculated with *Escherichia coli* Endotoxin and Corticosteroids," *Research in Veterinary Science*, Vol. 47, No. 3, 1989, pp. 305-308.

[24] R. P. Richardson, C. D. Rhyne, Y. Fong, D. G. Hesse, K. J. Tracey, M. A. Marano, S. F. Lowry, A. C. Antonacci and S. E. Calvano, "Peripheral Blood Leukocyte Kinetics Following *in Vivo* Lipopolysaccharide (LPS) Administration to Normal Human Subjects. Influence of Elicited Hormones and Cytokines," *Annals of Surgery*, Vol. 210, No. 2, 1989, pp. 239-245. http://dx.doi.org/10.1097/00000658-198908000-00018

[25] J. Tong, H. Kishi, T. Matsuda and A. Muraguchi, "A Bone Marrow-Derived Stroma Cell Line, ST2, Can Support the Differentiation of Fetal Thymocytes from the $CD4^-CD8^-$ Double Negative to the $CD4^+$ $CD8^+$ Double Positive Differentiation Stage *in Vitro*," *Immunology*, Vol. 97, No. 4, 1999, pp. 672-678. http://dx.doi.org/10.1046/j.1365-2567.1999.00822.x

[26] B. Adamik, A. Wlaszczyk, M. Zimecki and A. Kübler, "Lactoferrin Effects on Immune Response in Critically Ill," 17*th International Symposium on Intensive Care and Emergency Medicine*, Brussels, 18-21 March 1997.

[27] K. T. Jaya and P. K. Sahoo, "Dietary Bovine Lactoferrin Induces Changes in Immunity Level and Disease Resistance in Asian Catfish Clarias Batrachus," *Veterinary Immunology and Immune Pathology*, Vol. 94, No. 1-2, 2003, pp. 1-9. http://dx.doi.org/10.1016/S0165-2427(03)00065-5

[28] P. M. Joseph, R. S. Matt and M. D. Claire, "Early Response Cytokines and Innate Immunity: Essential Roles for TNF Receptor 1 and Type I IL-1 Receptor during *Escherichia coli* Pneumonia in Mice," *The Journal of Immunology*, Vol. 166, No. 6, 2001, pp. 4042-4048.

[29] J. S. Theodore, "Anti-Inflammatory Cytokines and Cytokine Antagonists," *Current Pharmaceutical Design*, Vol. 6, No. 6, 2000, pp. 633-649. http://dx.doi.org/10.2174/1381612003400533

[30] M. Zimecki, J. Artym, G. Chodaczek, M. Kocieba and M. L. Kruzel, "Protective Effects of Lactoferrin in *Escherichia coli*-Induced Bacteremia in Mice: Relationship to Reduced Serum TNF Alpha Level and Increased Turnover of Neutrophils," *Inflammation Research*, Vol. 53, No. 7, 2004, pp. 292-296. http://dx.doi.org/10.1007/s00011-004-1257-1

[31] B. Adamik and A. Wlaszczyk, "Lactoferrin—Its Role in Defense against Infection and Immune Tropic Properties," *Postępy Higieny i Medycyny Doświadczalnej*, Vol. 50, No. 1, 1996, pp. 33-41.

[32] P. A. Spagnuolo, R. P. Bird and L. Hoffman-Goetz, "Effect of Short-Term Dietary Intake of Bovine Lactoferrin on Intestinal Lymphocyte Apoptosis in Healthy Mice," *Nutrition*, Vol. 23, No. 11-12, 2007, pp. 812-817. http://dx.doi.org/10.1016/j.nut.2007.07.006

[33] S. P. Crouch, K. J. Slater and J. Fletcher, "Cytokine Release from Mononuclear Cells by the Iron-Binding Protein Lactoferrin," *Blood*, Vol. 80, No. 1, 1992, pp. 235-240.

[34] W. H. Teraguchi, H. Kuwata, K. Yamauchi and Y. Tamura, "Protection against Infections by Oral Lactoferrin: Evaluation in Animal Models," *BioMetals*, Vol. 17, No. 3, 2004, pp. 231-234. http://dx.doi.org/10.1023/B:BIOM.0000027697.83706.32

[35] K. Yamauchi, T. Toida, S. Nishimura, E. Nagano, O. Kusuoka, S. Teraguchi, H. Hayasawa, S. Shimamura and M. Tomita, "13-Week Oral Repeated Administration Toxicity Study of Bovine Lactoferrin in Rats," *Food and Chemical Toxicology*, Vol. 38, No. 6, 2000, pp. 503-512. http://dx.doi.org/10.1016/S0278-6915(00)00036-3

