Tikrit Journal of Veterinary Sciences (2023) 1(1): 1-7

IRAQI Academic Scientific Journals

TJVS Tikrit Journal of Veterinary Sciences

Tikrit Journal of Veterinary Science

Immunomodulator effects of lactoferrin in Rats immunized with the Rev1 Vaccine

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ARTICLE INFO.

Article history:
-Received: 25/ 4/2023
-Accepted: 13/6/2023
-Available online:

Keywords:
Immunomodulator, cytokines, Rev1, Bovine Lactoferrin, vaccination, synergistic effect

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ABSTRACT

Lactoferrin has many medical effects such as anti-microbial, anti-inflammatory and antioxidant. The current study was designed to investigate the immunomodulatory effect lactoferrin For this purpose lactoferrin and Rev1vaccine had been given to three animal experimental (rats) groups, while the fourth group served as the control negative group.

The results of the current study showed that total leukocytic count and immune markers (IL6, IL12 and TNFα) significant increases in 14 and 21 days after immunization in the 1st, the 2nd and 3rd groups. The study concluded lactoferrin and Rev1 vaccine have a synergistic effect when they are given in the same group.

1. Introduction

A state of health is conferred by the effective elimination of infectious agents (bacteria, viruses, fungi, and parasites) and the modulation of systemic responses, both of which are accomplished by immune responses that are designed to interact with the environment to protect the host against pathogenic invaders[1]. The immune system defends the body from potentially hazardous environmental stimuli by identifying them and mounting a variety of immunological responses[2]. Approximately 690 amino acid residues make up the monomeric, 80-kDa single polypeptide chain glycoprotein known as lactoferrin(LF's) [3]. Neutrophil granules also include lactoferrin, which is primarily found in

mucosal secretions and is made by epithelial cells[4]. First-line defence proteins such as lactoferrin are involved in the prevention of systemic inflammation and defence against a wide range of microbial infections [5]. The cellular effects of lactoferrin are mediated via receptors[6]. The intelectin 1 receptor and the 105 kDa lactoferrin receptor (LFR) are 100% identical[7]. The LFR is found in pigs' and humans' intestinal brush borders of cell membranes[8]. The nutritional immunity in the vertebrate host includes numerous proteins such as calprotectin, calgranulin C, hemoglobin, ferritin, transferrin, and lactoferrin. Lactoferrin is highly abundant in host tissues infected with bacterial pathogens such as streptococcal species. Interestingly, several of these nutritional immunity proteins also have immunoregulatory properties. This review will focus on the intersection of lactoferrin's involvement in antimicrobial activity and immune regulation and pathogenesis[9]. Vaccinating animals has been shown to be the most efficient method of brucellosis control in recent years. Human vaccines have not yet been created, despite the necessity of immunizing those who live in brucellosis endemic areas, as well as cattle, laboratory workers, veterinarians, and those who work with humans [10]. The best vaccines for preventing animal brucellosis are live-attenuated vaccines [11]. Inactivated, live-attenuated, and rough-attenuated vaccines have all been used in the development of brucellosis vaccines. Live-attenuated vaccinations, which are more successful in terms of immunogenicity, have replaced inactivated vaccines as the primary method of brucellosis control [12]. (Rev.1 vaccine) is the most effective vaccine against caprine and ovine brucellosis. Although these two vaccines provide good immunity for animals against brucellosis, the expense of persistent serological responses is one of the main problems of both vaccines[13]. The purpose of the present in vitro study was to evaluate the single and synergistic effect of lactoferrin and Rev1 vaccine on immunity as well as an immune modulator.

2. Materials and Methods

2.1Study design

Animal groups: twenty-four animals(rats) divided into four groups each group containing 6 rats in age 3months as follows:

First group: Each rate given 100 µg/kg of lactoferrin (Ingredia Nutritional-France) orally by stomach tube for three weeks.

Second group: Each rat was given as 1st group and then given *Brucella melitensis* Rev1 strain (Brucevac -jovac- Jordan) which contain 0.1 x 10^9 CFU sub-continuous in single dosage at 2nd week.

Third group: Given *Brucella melitensis* Rev1 strain (Brucevac -jovac- Jordan) which contains 0.1 x 10^9 CFU sub-continuous in single dosage at 2nd week, was inoculated S/C.

Fourth group: This would be served as the control negative group, administer 0.2 ml s/c normal saline.

Blood sample collected after 1day, 7days, 14days, 21days for :

- Complete blood count: by use of hematology analyzer (CBC Analyzer Misba Count Germany
- Immune marker :
- TNF: determined by used of (Rat TNF-alpha ELISA Kit - Thermofisher)
- IL6: determined by use of (Rate IL-6 ELISA KIT- CUSABIO-USA) and according to the manufacturer's instructions
- IL12: determination by use of (Rat IL12) (Sandwich ELISA) ELISA Kit - LS-F23156- LSBIO-USA and according to the manufacturer's instructions

3.Results and Discussion:

Total Leukocytic Count (WBCS×103 /µl): The results showed in table (1) that the means and SD of the total leukocytic count at 1 day were 7.23±0.52, 8.61±0.42, 7.9±0.36 and 7.51±0.81in 1st, 2nd, 3rd and 4th groups respectively, while at 7 days were 9.7±0.61, 9.8±0.72, 7.91±0.79 and 7.81±0.81 in 1st, 2nd, 3rd and 4th groups respectively. the mean serum levels of total leukocytic count At day 14 were 11.2±0.37, 14.8±0.58, 8.11±0.81 and 7.38±0.95 respectively in the study groups. At day 21 the results were 11.4±0.51, 14.1±0.92, 13.2±0.81 and 7.6±0.91 respectively. Table (1) results showed a significant difference between the 3rd group in (14, 21) days with the study groups.

Table (1): Total Leukocytic Count (WBCS×103 /µl) in the experimental group

Animal groups	The period from the experimental beginning			
	1days	7days	14days	21days
1st group	7.23±0.52 (b)	9.7±0.61 (b)	11.2±0.37(a)	11.9 ±0.51(a)
2nd group	7.61±0.42 (b)	9.8±0.72 (b)	14.6±0.58 (a)	14.8±0.92 (a)
3rd group	7.9±0.36 (b)	7.91±0.79 (b)	12.11±0.81 (a)	12.2±0.81 (a)
4th group	7.51±0.81 (b)	7.81±0.81 (b)	7.38±0.95 (b)	7.6±0.91 (b)

The different small letters horizontally refer to the presence of significantly different at (P<0.05).

Determination of serum level of Rat TNF α by ELISA assay: At the and 21 days post-immunization with lactoferrin and Rev1, the means and SD serum levels of TNFα titers according to ELISA assay in the 3rd and 4th groups showed significant differences as compared with other study groups table (2).

Table (2): The mean and standard error of serum level of (TNFα) in immu immunized and non-immunized animals at (1-21) days post-immunization levels (pg/ml).

Animal groups	The period from the experimental beginning			
	1days	7days	14days	21days
1st group	57.1±2.1 (b)	63.3 ±1.3 (b)	64.1±1.9 (a)	68.2 ±3.1 (a)
2nd group	58.2±3.1(b)	63.5 ±2.3 (b)	69.3±3.1 (a)	74.34 ±1.8 (a)
3rd group	57.3±2.7(b)	58.4±3.1 (b)	63.7± 4.1 (a)	69.6 ±41 (a)
4th group	58.1±2.7(b)	57.3 ±1.3 (b)	58.1±1.9 (b)	58.2 ±3.1(b)

The different small letters horizontally refer to the presence of significantly different at (P<0.05).

Determination of the IL6 levels by ELISA assay (pg/ml): As shown in table (3), results indicated an increase in IL6 levels in the lactoferrin group (1st), (2nd) and (3rd) group at 14 days and 21 days, after mating when compared to the control group and other study groups in 1 and 7 days. The results showed a significant difference between study groups.

Table (3): The mean and standard error of serum level of (IL6) in immunized and non-immunized animals at(1-21) days post-immunization levels (pg/ml).

Animal groups	The period from the experimental beginning			
	1days	7days	14days	21days
1st group	44.1±3.1(b)	54.3±1.8 (b)	73.1±3.1 (a)	74.2±3.1 (a)
2nd group	44.2±3.1 (b)	54.5±1.3 (b)	99.3±3.1 (a)	115.4±2.1 (a)
3rd group	44.3±2.7 (b)	44.4±3.1 (b)	49.7± 4.1 (a)	89.6±5.1 (a)
4th group	43.1±4.1 (b)	44.3±1.9 (b)	42.1±3.1 (b)	44.2±3.1 (b)

The different small letters horizontally refer to the presence of significantly different at ($P<0.05$).

Determination of the IL12 levels by ELISA assay (pg/ml): As shown in table (4), results indicated an increase in IL12 levels in the lactoferrin group (1st), (2nd) and (3rd) group at 14 days and 21 days, after mating when compared to the control group and other study group in 1and 7 days. The results showed a significant difference between study groups.

Table (4): The mean and standard error of serum level of (IL12) in immunized and non-immunized animals at(1-21) days post-immunization levels (pg/ml).

Animal groups	The period from the experimental beginning			
	1days	7days	14days	21days
1st group	86.22 ± 4.1 (b)	97.34 ± 4.9 (b)	110.23 ± 4.77 (a)	132.51 ± 5.32 (a)
2nd group	88.3 ± 7.3 (b)	98.34± 5.25 (b)	114.2 ± 7.1 (a)	134.4 ± 6.1 (a)
3rd group	86.55 ± 3.8 (b)	89.34 ± 4.29 (b)	87.7 ± 6.1 (a)	125.45 ± 6.98 (a)
4th group	84.52 ± 2.8 (b)	88.7 ± 4.3 (b)	86.51 ± 6.1 (b)	89.53 ± 32 (b)

4. Discussion

When interpreting the findings, it is important to keep in mind that we are primarily concerned with the concentrations of various immune markers, such as total leukocytic count in plasma or immunity markers serum to identify LF's and Rev1 impact on immunity. According to recent results, the data showed to increase in the tittering of leukocytic count in 3rd and 4th groups due to the ability of LF's and Rev1 to activation of many inflammatory cells and, the particulate nature of LF's and Rev1 enhance and/or facilitate the uptake of adsorbed antigen by antigen-presenting cells (APCs), such as dendritic cells or macrophages, this probably being the most important function attributed to the adjuvanticity [14], also previous study indicated white blood cells number increased at 5hrs and 3 days after insemination and decreased at 7 days after insemination

in the lactoferrin [15], initial phase of vaccination is associated with recruitment neutrophils and macrophages to the site of inoculation and these cells act as antigen-presenting cell APCs that expose peptides of Ags to CD4T lymphocytes which proliferate and differentiate into T helper 1 cell and T helper 17 that produced cytokines and chemokines that attracted other immune cells, moreover, T helper cells produced IFN y that attracted and activated macrophages [16]. While the immunity cytokines in current results increase from 14 to 21 days post-immunization with LF's and Rev1 in 1st, 3rd groups and remarkably in 2nd group due to the ability of LF's and Rev1 to Enhance both IFN-y, IL- 10 and TNFα production by stimulation of many immune cells responsible for the production of these cytokines this idea is consistent with many previous studies that showed the functions rely not only on the capacity of–LF'sto bind iron but also on its immunomodulatory effect by its cellular and molecular mechanisms with both host and pathogenlactoferin can interact with antigen-presenting cells, reduce excessive inflammation and stimulate host immune responses, as well as identify cell targets and receptors and this was important in the maintenance of immune system homeostasis [17], the adjuvants can be used in vaccine formulation to improve the protective immune response [18]**.** As well as adjuvant expressed a good stimulation ability for both cell-mediated and humoral immune responses, and they revealed that these substances can activate CD4 and CD8 T cells to produce immune cytokines such as TNF-α and IFNy which are associated with class switch immunoglobulin to IgG2a [19]. both IFN-y and IL- 10 production by stimulated and unstimulated MLN cells. The production level of IFN-y by MLN cells was correlated with that of IL- 10.

Conclusion

The study concluded that there was a significant increase in immunogenic values in the immunized groups hence the Lactoferrin promotes immune response by activation two arm of immune system.

Acknowledgment

The authors are grateful to the University of Tikrit /College of Veterinary Medicine for all the facilities to achieve this study.

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التأثيرات المناعية للاكتوفيرين في الجرذان المحصنة بـ Rev1

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الملخص

يمتلك اللاكتوفيرن العديد من التأثير الطبي كمضاد بكتيري ومضاد للالتهاب ومضاد للاكسدة. صممت الدراسة الحالية لتحري عن تأثير المعدل المناعي للاكتوفيرن , ولهذا الغرض اعطي كل من اللاكتوفيرن ولقاح 1Rev لثلاث مجاميع من الحيونات المختبرية(الجرذان) بينما اعتبرت المجموعة الرابعة كمجموعة سيطرة سالبة. اظهرت نتائج الدراسة الحالية زيادة في معدل خلايا الدم البيضاء والمؤشرات المناعية (IL6, IL12 and TNFα) مع فرق معنوي في اليوم 14 واليوم 21 من التجربة بعد التمنيع في المجاميع الاولى والثانية والثالثة. خلصت هذه الدراسة الى ان اللاكتوفيرن و اللقاح Rev1 vaccine يمتلكون تاثير تأزري عندما يتم اعطائهم سوية في نفس المجموعة.