Immunological and pathological effect of lactoferrin against murine leishmaniasis

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Abstract
The total numbers of animals in the experimental 40 mice of both sexes divided into 2 groups: The first group (30 mice) was immunized with lactoferrin orally with a dose of 0.25mg/10g (B.w.) in a volume of 0.1 ml daily for 10 days and then challenged with L. major. The 2nd group (10 mice) were non-immunized serve as a positive control. Then each group sacrificed by chloroform overdosing and blood was collected to measure serum IgG through by using ELISA Kit (mouse IgG Elisa Kite). The IgG levels were measured both in mice immunized with lactoferrin and in healthy controls and the results were then compared with each other. In our study, IgG levels were significantly higher in sera than in sera of the control group (P<0.001) in the mean values of the group that immunized by lactoferrin which was (3.378±0.171) as compared with control group (PBS) (0.282±0.035).

The pathological lesion varied between moderate acanthosis with slight basal cells degeneration together with dermal blood vessels dilation accompanied by neutrophils in their lumen, as well as proliferation of dermal collagen fiber in another section MNCs infiltration with few PMNs leukocytes mainly around hair follicle. Another section, the upper dermis showed collagen fibers proliferation.

Keywords: Leishmania major, lactoferrin, mice, igg, histopathology

1. Introduction
Leishmaniasis is a major parasitic disease that affect on human health and society caused by genus of leishmania. Their prevalence has been widespread closed to the poorest countries of the world, and they receive less care than other infectious diseases such as malaria, tuberculosis and AIDSF [1]. or the aggressive of individual species, their organ preference and the immune status host determine the course of the disease. There are a variety of clinical features that are determined by site of sand fly bites which is cutaneous leishmaniasis, visceral Leishmaniasis, destructive mucositis and mucosal leishmaniasis [2].

There are two species present in Iraq: L.tropica which is the agent of anthroponotic cutaneous leishmaniasis (ACL) and L.major which is the agent of zoonotic cutaneous leishmaniasis (ZCL). Both ACL and ZCL were reported as causative agents of leishmaniasis in Iraq, but the ACL is found mainly in the suburban areas [3]. In general the cutaneous leishmaniasis causes skin lesions and the lesions typically evolve from papules to nodal plaques of ulcerative lesions, with elevated boundaries and central depression, which can be covered with a scab or crust. Some lesions continue as nodules. The lesions usually are painless but can be painful, especially if ulcerative lesions become infected with bacteria [4].

Immune responses are designed to interact with the environment to protect the host against pathogenic invaders and conferring a state of health through effective elimination infectious agents (bacteria, viruses, fungi and parasites) and modulation of systemic responses that include host immunologica surveillance. Recent research has identified lactoferrins [5]. Milk and Colostrum are a vital nutritional source for the offspring of humans and all mammals. In addition to its nutritional value, it is a rich source of proteins, including lactoferrin (Lf) [6].

The aims of the study: Due to a little researches about the anti-parasitic effect of lactoferrine, the current research is an attempt to Investigate the immunmodulatory effect of lactoferrine against murine leishmaniasis

2. Materials and Methods
2.1 Experimental design
The total numbers of animals in the experimental 40 mice of both sexes divided into 3 groups: The first group (30 mice) was immunized with lactoferrin orally with a dose of 0.25mg/10g
(B.w.) in a volume of 0.1 ml daily for 10 days and then challenged with L. major. The 2nd group (10 mice) was non-immunized serve as a positive control. In 30 days of the first immunization 10 mice from each 1st and 3rd group were sacrificed by chloroform overdosing and blood was collected to measure serum IgG through by using ELISA Kit (mouse IgG Elisa Kite). The remaining animals were challenged with L.major 0.5ml/mouse subcutaneous (S/c) containing (1x10^7)

to investigate the histopathological changes.

2.2 Statistical analysis: Data were analyzed using the program SPSS.22 (statistic package for social science) and use Excel.10 program. A P values of 0.05 or less were considered to be significant.

3. Results
3.1 ELISA test for detection levels of (IgG) titer
IgG levels were measured both in mice immunized with lactoferrin and in healthy controls. and the results were then compared with each other (Table 1) In our study, IgG levels were significantly higher in sera than in sera of the control group (P<0.001) in the mean values of the group that immunized by lactoferrin which was (3.378±0.171) as compared with control group (PBS) (0.282±0.035).

Table 1: Evaluation of IgG antibody from mice immunized with Bovine lactoferrin (bLf)

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Mice immunized group</th>
<th>Mice Control group</th>
</tr>
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<tbody>
<tr>
<td>IgG titer</td>
<td>3.378±0.171</td>
<td>0.282±0.035</td>
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<tr>
<td>p-values</td>
<td>P&lt;0.001</td>
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3.2 Histopathological study
3.2.1 Non immunized infected mice
Histopathological changes in animal mainly with L. major epidermal lesion includes multifocal point of necrosis with focal epidermal ulceration with necrotic debris (Fig.1), intra epidermal abscess with severe epidermal infiltration by amastigote (Fig.2). The upper dermis diffuses histocytes contain numerous amastigote (Fig.3), Numerous intracellular amastigotes were seen in the hypodermis with plasma cell and eosinophil with hyperplasia of follicular cells (Fig.4).

3.2.2 Immunized with lactoferrin
Histopathological changes in the group immunized with lactoferrin post challenge with L.major the cutaneous lesion varied between moderate acanthosis with slight basal cells degeneration together with dermal blood vessels dilation accompanied by neutrophils in their lumen, as well as the proliferation of dermal collagen fiber (Fig.5) in another section MNCs infiltration with few PMNs leukocytes mainly around hair follicle (Fig.6). Another section, the upper dermis showed collagen fibers proliferation (Fig.7).

Fig 1: Histopathological section in skin infected with L.major shows multifocal points of epidermal necrosis and focal epidermal ulceration with necrotic debris (H&E x400)

Fig 2: Histopathological section in skin infected with L.major shows intra epidermal abscess with severe epidermal infiltration by amastigote (H&E x400)

Fig 3: Histopathological section in Skin post challenge with L.major shows Diffuse infiltration of histocytes filled with numerous amastigote with keratin degeneration of hair follicle (H&E x400)

Fig 4: Histopathological section in Skin infected with L.major showed Numerous intracellular amastigotes in the hypodermis with plasma cell and eosinophil with hyperplasia of follicular cells. (H&E stain x400)

Fig 5: Histopathological section in skin in group immunized with lactoferrin and infected with L.major shows moderate acanthosis with slight basal cells degeneration together with dermal blood vessels dilation accompanied with neutrophils in their lumen (H & Estain x400)
Progressive cutaneous leishmanial lesions have been reported in the current experimental study mainly associated with epidermal abscess containing a number of neutrophils with intracellular amastigotes together with follicle rupture this observation may indicate that Neutrophils are rapidly recruited to the site of Leishmania infection is consistent with [17] and parasites promastigotes are killed by neutrophil extracellular traps (NETs) consistent with [18]. However, several studies showed that Lactoferrin modulates the phagocytic capacity of neutrophils and macrophages in the resolution of infections [19] and this evidence is in agreement with present study expressed moderate to severe MNCs infiltration in the examined tissue there are several parasites in which Lf and its peptides have been used to control infection such as Toxoplasma gondii, amoebiasis.

5. Conclusions: Lactoferrin is effective for treating and presents suitable oral immune prevention therapy against Leishmaniasis. Lactoferrin oral immunization of Leishmania with (0.25mg/10g B.w.) for 10 days induced inhibition and reduction in Leishmania growth and load.

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7. References
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