Immunity of Outer Membrane of Salmonella Typhi in Rabbits

Duaa Hassan[1], Frial G. Abd[2], Lubna Abdulazeem[3]
[1]Al-Furat Al-Awsat Technical University, Babylon Technical Institute, Medicine Laboratory/Iraq
[2]Department of Biology College of Science, University of Babylon, Iraq
[3]DNA research center, University of Babylon, Iraq
Email: albayatilubna@yahoo.com

Abstract
Objectives: The release of various types of membrane vesicles is widespread, from prokaryotes to more complex eukaryotic cells. It has been known for decades that Gram-negative bacteria shed outer membrane vesicles (OMVs), but they are also released by other groups such as Gram-positive bacteria. There is increasing interest in vesicles and recruitment of the immune system for medical applications which could be linked to the finding that 60% of the available pharmaceutical drugs exert their effect through interaction with membrane proteins A few years after their discovery, research started to explore OMVs as vaccine product, in particular for the application against many diseases. They present a range of surface antigens in a native conformation and have natural properties like immunogenicity, self-adjuvation and uptake by immune cells which make them attractive for application as vaccines against pathogenic bacteria. The aim of this research as determination effect of o outer membrane protein of salmonella on some immunological parameters on rabbits' animals. Methodology: in this research This study prepare Outer membrane protein (OMPs) and Molecular Weight of Outer membrane protein (OMPs) was determinated by SDS- page, The animals was injected with 0.1ml outer membrane of intradermal of skin, As for the control animals, they were injected with normal saline and the same amount, and recording the observed skin change after (4,24,48,72) hr. in comparison with control animals. Use this test to find out the effect of outer membrane on stimulating the cellular response of rabbits, This study also determinated Interleukin (1β, 2 and17) concentration Results: the results of present study exhibited The SDS-PAGE OMP analysis of Salmonella typhi revealed the molecular weight (MW) of the gels was estimated at 44KD by comparison with standard MW markers, the present study also investigated how outer membranes influenced on the skin sensitivity and cell immunity of the rabbit. The results of the production of cytokines were estimated using the equation from the standard curve carried out with the same test. Statistic analysis on IL-2 showed a mean value (38.548±4.771,49.781±5.264 and 72.410±4.221) of the control group when compared to the immunized group with OMPs antigen and mean value (23.434±2.501) respectively. Conclusion: the outer membranes of Salmonella typhi influenced on the cell immunity of the rabbit

Keywords: Salmonella typhi, OMV, 1β, 2 and17

1. Introduction

Gram-negative microorganism are surrounded by means of each an inner membrane (IM) and outer membrane (OM). The OM is unique in its composition and asymmetrical distribution of lipids, with the inner leaflet containing phospholipids, whereas the outer leaflet is composed of lipopolysaccharide (LPS), a fantastically negatively charged molecule that protrudes into the bacterial environment [1]. This uncommon membrane environment is domestic to lipoproteins and critical membrane proteins known as outer membrane proteins (OMPs). As an terrible lot as 3% of the Gram- bad bacterial genome may additionally code for OMPs [2].

OMVs have been implicated in many different carrier functions, However OMVs also have a great potential as endogenous vaccine. The presence of several antigens on OMVs limits the possibilities for pathogens to mutate all the target antigens present in the vaccine and thereby limits the possibility to generate vaccine escape variants. Furthermore, OMV isolation is relatively low-cost, compared to manufacturing of synthetic molecules for instance. This makes OMVs of great interest for vaccine development [3].

In early research, bacterial ghosts have been derived and in addition designed as advanced focused on shipping carriers for the centered shipping of drugs and RNA [4]. The diverse houses of bacterial membranes constitute a promising subject matter to discover and develop them as coating materials for artificial nanoparticles. Hence, nanoparticle penetration into the immune cells, which reasons cytokine induction, stimulation of T cells, activation of the immune response genes, improved antigen processing, and antibody secretion by using B cells, offers an excellent possibility of the usage of nanoparticles as carriers and adjuvants within the guidance of antibodies and vaccines towards infections [5] .

OMVs have a wide variety of interactions with immune cells showing their potential to be used for immunization [6]. The first studies into immune responses evoked by OMVs already showed promising inductions of cytokines and chemokines in macrophages and other cell types. sOMVs isolated

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from Brucella melitensis were used to stimulate bone marrow-derived macrophages and showed induction of interleukin (IL)-6, IL-10, IL-12, or tumor necrosis factor (TNF) α, depending on the LPS structure of the strain used [7]. sOMVs from E. coli were shown to induce CXCL1 expression in mouse endothelia, leading to an increased influx of neutrophils [8].

The aim of this research as determination effect of outer membrane protein of Salmonella on some immunological parameters on rabbits animals

2. The Methods

Outer membrane protein (OMPs) preparation

The Method was used to prepare Outer membrane protein (OMPs) according to [9] .

Determination of Molecular Weight by SDS- page

Molecular Weight of Outer membrane protein (OMPs) was determinated according to (Green and Sambrook [10] .

Lab Animals

Used 6 adult males Newzland rabbits (Oryctylagus conniculus) at 3-5 months and weight 1-1.5 kg. were used to detect immune response for antigen.It was kept in cages specialized for animals in laboratory animal house and left there for two weeks for adaptation with consideration of use clean food and water for animals during the period of experiments [11].

Experiment Design and Injection Method

Table(1): Program experiment design and injection of method in rabbits

Type of antigen		Group of animal's outer membrane	Group of animals control
NO. of animals		3	3
Dose	First Week	0.5ml Ag with 0.5ml of oil	0.5ml Ag with 0.5ml of oil
	Second Week	1ml for each kg	1ml for each kg
	Third week	1ml for each Kg	1ml for each Kg
concentration		1.5mg/ml	0
Administrations method		0.25 ml right intramuscular	0.25 ml right intramuscular
		0.25 ml left intramuscular	0.25 ml left intramuscular
		0.5 ml Subcutaneous	0.5ml subcutaneous

Skin Test

Use This test conducted in the fourth week, when each group of injected animals. The animals was injected with 0.1ml outer membrane of intradermal, As for the control animals, they were injected with normal saline and the same amount, and recording the observed skin change after (4,24,48,72) hr. in comparison with control animals. Use this test to find the effect of outer membrane on stimulating the cellular response of rabbits [12].

Interleukin (1β, 2 and17) Assay Procedure

The following procedures were performed at room temperature according to manufacturer's instructions (Elabscience –china).

3. Results and Discussion

Salmonella typhi was utilized in OMP isolation and antigen prepared in immunological studies because it results in phenotypic and genotypic detections for the majority of the virulence factor. The In SDS-PAGE OMP analysis of Salmonella typhi revealed the molecular weight (MW) of the gels was estimated at 44KD by comparison with standard MW markers (Fig1). The protein concentration was (30 g/L) (4-10).

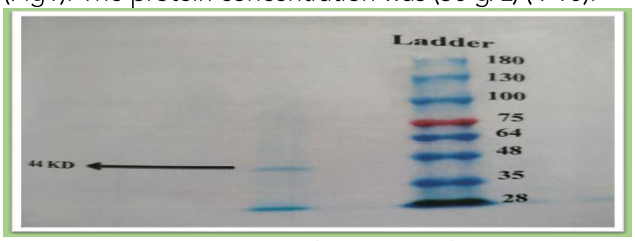

Figure (1) protein profile. protein from SDS-PAGE(10%W/V) of whole cell lysate preparation from Salmonella typhi at 150V for 6 hours visualized after staining with coomasise Lane (L): Protein ladder 28-180 KD Arrow :44-KD band.

The gel had an intensity of 44KD fig(1),[13]said that the SDS-primary PAGE's and important OMPs were a 40KD band. Salmonella typhi include other main protein, including traces of additional proteins, with molecular weights of 33,36, and 44 KD, including 22,28, and 30KD and 50KD. The entire SDS OMPs were insoluble with 44 KD protein and no detectable protein content above 40KD. This might be attributed to a tiny molecular protein release from the degraded lysozyme of peptidogly can [14].Although the protein profile is similar under different growth conditions, the osmolality and temperature affected the expression of the total OMPs the expression of all OMPs decreased by two fold when the temperature was raised from 30C to 37C.OMPs from Gram negative bacteria are anchored to the outer membrane by N-terminal domain ,which consists of integral trans membrane domain .OMPs are well-know core players in maintaining of bacterial membranes ,selectively permeability and bacterial pathogenesis in host cell[15,16].

[17] demonstrate the insulation and characterization from the outside membranes of Salmonella typhi of an immunogenic protein . The OMPs of gram-negative bacteria are immunologically important because of their accessibility to the host defense system. Major OMPs identified from SDS-PAGE gels on the basis of their molecular weights were used as eliciting antigens in studies.

This study investigated how outer membranes influenced on the skin sensitivity and cell immunity of the rabbit.

Table (2): Skin sensitivity of the immunized rabbits				
rabbet groups	Mean±S.D Measure in mm			
Time of result	After 4 hr.	After 24 hr.	After 48 hr.	After 72 hr.
control	00.00	00.00	00.00	00.00
Outer membrane	1.83"±0.28	9.00"±2.00	10.66"±2.15	7.66"±0.57
" Means a significant difference				

The results of our test is consistent ([18] The delayed hypersensitivity test for the skin used for the indicator of cellular immunity in rabbits immunized by the different kinds of Salmonella antigens revealed a substantial increase in inoculated animals. Testing of the final result. [19], who found that after 24 hours the skin response was higher. The favorable results of this study are consistent with the findings of previous studies [20,21].

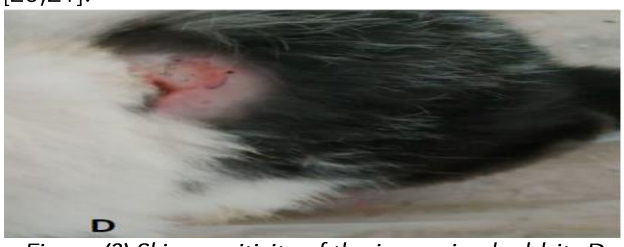

Figure (3) Skin sensitivity of the immunized rabbits D immunized rabbits with outer membrane)

[22] that the induration diameter of all injected rabbit groups with antigens after 4,24,48,72 hr had significant increase in the induration diameter of control group at P<0.05. These results referred that these three types of antigens induce the delayed type hypersensitivity (DTH) which belong to the type IV hypersensitivity that reaction result from the T cell inflammation cell-initiated inflammation and do not involve antibody. Inflammatory responses result from the manner in which T cells encounter and respond to antigen.

Immune system drives the specialized immune cells T cells to the skin where they release chemical messengers called lymphokines, which have been sensitized by prior infection. These lymphokines produce induration at and around the injection site (a hard, elevated region with distinct borders) by promoting local vasodilation (increases in blood vessel diameter), producing edema, fibrin deposition and other types of inflammatory cells [23].

The results of the production of cytokines were estimated using the equation from the standard curve carried out with the same test. Statistical analysis on IL-2 showed a mean value (38.548±4.771,49.781±5.264 and 72.410±4.221) of the control group when compared to the immunized group with OMPs antigen and mean value (23.434±2.501) respectively.

Table (3) Concentration of IL-1β, IL-17 and IL-2 (pg/ml) in immunized and control rabbits					
LDS	IL-2 Mean±S.D	IL-17 Mean±S.D	IL-1β Mean±S.D	No. of animals	Parameter
24.155	49.781±5.264	101.444±16.943	36.860±9.534	3	Outer membrane
	23.434±2.501	50.310±5.498	17.700±2.545	3	Control
P=Value0.000*" Means a significant difference					

Soluble molecules play an important role in clinical immunology as a group. Macrophages separate them and can function as stimulating or inhibitory signals between cells. Chemokines are considered cytokines that induce leukocyte chemotaxis. Among cytokines, several of their stimulating activities are of particular interest. The secondary function of interleucines (IL-1β, IL-2 and IL 17) in the enhancement of immunological responses is of particular importance. A wide variety of cells, including T and B, are susceptible to IL-1β. IL-2 is largely involved with lymphocytes, although the IL-receptor B cells and the natural cell killed have identical tropic.

[24] explained generated antigen-presenting virus-like nanoparticles (VPN) that co-express IL-2 bound to different membrane anchors. They found that the fusion of the C-terminus of IL-2 with a minimal glycosylphosphtidylinositol anchor acceptor sequence with two intervening immunoglobulin-like domains of CD16b led to an optimal stimulation of T cells and induction of CD8+ T cell effectors function in vivo . Other studies reported the generation of IL-2 surface conjugates on PEGylated liposomes using covalently bonded succinimidyl-4-p-maleimidophenyl butyrate-modified IL-2 or an Fc scaffold fused to the C-terminus of the murine IL-2 .Using an adoptive cell therapy model, [25] found that the vast majority of antigen-specific T cells reacted to IL-2-decorated liposomes in vivo after a single injection and that repeated administrations increased cytotoxic T cell activation and proliferation in melanoma-bearing mice. We functionalized nanocapsules with different amounts of IL-2 and studied how they interact with different T-cell populations [26].

For regulatory T cell maintenance and T cell memory responses, interleukin-2 (IL-2) cell growth factor is crucial. In allograft rejection and autoimmune and inflammatory diseases, the low dose of the IL-2 injection considered promising, whereas the first FDA-approved treatment was the first IL-2 cancer immunotherapy. However, its therapeutic potential is restricted because of its pleiotropic nature and the fact that many IL-2 receptors with varying affinity are present. Consequently, for increasing clinical application of cytokine a specific receptor assignment is needed.

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