Protection of Chicken against Bacterial Infection by Immunization with Bacterial Subcellular Fractions

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الخلاصة :

أجريت هذه الدراسة لتعين قابلية الأجزاء المختلفة دون الخلوية من البكتريا القولونية كالغشاء السايتوبلازمي الجدار الخلوي والمستضد الجسمي لمنح الدجاج وقاية ضد الإصابة بالبكتريا القولونية المميتة .

إن تمنيع الدجاج بحدود تركيز 12.5 مايكرو جرام من الغشاء السايتوبلازمي منح نسبة وقاية بحدود 65-70% ضد الإصابة بجرع مميتة من البكتريا القولونية مقارنة بدجاج السيطرة الذي حقن بمحلول فسيولوجي (بدلاً من الأجزاء دون الخلوية) وبجرع مميتة مماثلة من البكتريا القولونية أما تمنيع الدجاج بتراكيز بحدود 50 مايكرو جرام من الجدار الخلوي أو 100 مايكرو جرام من المستضد الجسمي فقد منح الدجاج وقاية بحدود 55% و 25% على التوالي ضد الإصابة بجرع مميتة من البكتريا القولونية .

لقد بينت هذه النتائج إمكانية استعمال الأجزاء دون الخلوية من البكتريا القولونية كالغشاء السايتوبلازمي والجدار الخلوي كلقاح في الدجاج ضد الإصابة بالبكتريا القولونية المرضية .

Abstract:

The current studies were undertaken to assess the ability of various subcellular fraction of E.coli to induce protection against challenge with E.coli in chicken. Immunization with as little as 12.5 µg of cytoplasmic membrane conferred 65% protection. In addition cell wall and somatic antigen conferred 55% and 25% protection respectively. These results indicate the usefulness of cytoplasmic membrane and cell wall in the induction of active immunity against pathogenic E.coli in chicken.

Introduction

Enterotoxigenic E.coli continue to be problematic for both human and live stock disease, primarily affects adults travelors to areas where it is endemic (1,2) and particularly children under 3 years of age living in these regions (3) . live stock-specific strains afflict newborn calves and piglets, which may lead to mortality (4,5) .

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On the other hand colibacillosis is a common and economically important disease of chicken. It was found that 10-15% of E.coli present in the intestine of chicken are pathogenic strains. There are different clinical syndromes recognized in chicken, among them colisepticemia, polyserositis, omphalitis, arithritis, peritonitis, and usually associated with mycoplasma as a secondary pathogen in chronic respiratory disease (CRD).

The mode of entry of E.coli are either direct or indirect through the intake of healthy chicken a large number of E.coli via contaminated water or food or through the respiratory system, this will cause the microbes to get access to the important organs such as Kidneys, liver, spleen, heart , and air sacs(6). When the microbe enter the body, the immune system responds through a diverse sets of mechanisms in an attempts to eliminate the infectious agent (7).

As yet, little is known about the microbial factors that determine pathogenicity and those that elicit a protective immune response in human and various animals infected with various pathogens.

There are many studies showed that lipopolysaccharide (LPS) from g(-) bacteria enhance non-specific resistance to bacterial infection (8,9,10). Sato K and his associates reported that cell fractions of Lactobacillus casei enhance host resistance in mice against infection due to listeria monocytogenes (11).

Recently, attentions have been addressed to the role of the subcellular fraction of (g-) bacteria in the induction of a specific immunity. Studies in mice have shown that immunization with outer membrane proteins from Neisseria gonorrhaeae and Neisseria meningitides (12) results in protection against the infection caused by these bacteria. Armando and his associates (13) studied the role of the outer membrane proteins (OMPs) of Salmonella typhi in the inductions of protection against an intraperitioneal challenge with S. typhi in mice. They found that immunization as little as 30µg of omp conferred 100% protection to mice challenged with up to 1000 times the 50% lethal dose of S. typhi and about 30% protection against challenge with up to 500 times the LD50 of S. typhimurium .

The aim of the study was to examine the role of some subcellular fraction of E.coli 088 in the induction of protection in chicken against an intraperitoneal challenge with lethal dose of E.coli 088 .

Materials and methods

Culture and growth conditions :

The bacterial strains used in this study was Escherichia coli 088 provided by Department of Poultry and Fish disease, college of Veterinary Medicine, University of Baghdad.

Twenty liters of fresh nutrient broth was inoculated with cell suspension in broth. The cells were then cultivated in water bath shaker at 37°c for 24 hours, organisms were harvested in a late logarithmic phase by centrifugation at 4000 rpm for 1 hour. The bacterial sediment was washed with sterile saline and was used for subcellular fraction isolation and challenge experiments.

Breakage of cells and isolation of envelope fractions

the method of schnaitman C.A. (14) was used for separation of envelope fraction cultures were harvested by centrifugation at 0ºc and suspended to a volume of 100 ml. in 0.05 M tris (hydroxymethyl) amino-methane (tris) buffer (pH7.8) containing 1mM ethylene diaminotetraacetic acid (EDTA). This suspension was placed in a Sorval Omnimixer blender and treated for 1 min at speed control setting of 7.5. This procedure removes flagella, pilli, and capsular material. The cells were again centrifuged at 0ºc and suspended to 25 ml. in the same buffer solution . Approxmately 1mg each of pancreatic ribonuclease and deoxyribonuclease were stirred into the suspension, and the cells were broken by sonnication at 7-7.5 kc for 15min, the broken cell suspension was then made 2mM in Mg+2 by the addition of Mgcl2 and centrifuged at 5000 rpm in a sorval SS-34 roter for 5 min to remove intact cells and debris. The supernatant fluid was then centrifuged in a Spinco 50Ti roter for 45 min at 50000 rpm.

The pellet from this centrifugation, which is designated as the crude envelope fraction, was suspended to a final protein concentration of 10-30 mg/ml. in a 0.01 M N-2-hydroxyethyl piperazine-N-2-ethan sulfonic acid (HEPES) buffer (pH7.5). Portions (1ml) of the crude envelope suspension were then layered on 28 ml. Sucrose gradient prepared in the same buffer. The exact sucrose gradients are 10-ml. 0.077M sucrose, 10ml. 1.44M sucrose and 8 ml. 2.02M sucrose, the gradient were then centrifuged for 16 hrs in a Spinco SW 25.1 roter at 25000 rpm. The gradient were fractionated by pumping the solution out through a metal capillary tube inserted into the bottom of the gradient, the upper band (cytoplasmic membrane enriched fraction) and the lower band (cell wall enriched fraction) was separated into different test tubes and diluted with buffer to reduce the sucrose concentration and centrifuged as above. This procedure removes any remaining intact cells. Finally the fractions were recovered by centrifugation in the Spinco 50 Ti rotor for 1.5 hr at 50000-rpm . the samples were suspended in a small volume of distilled water.

Somatic Antigen Separation

Lipopolysaccharide (LPS) was extracted using hot phenol-water procedure of Westphal and Jann (15). The somatic antigen (0A) was obtained by hydrolysis of LPS in 1% acetic acid at 100ºc for 2 hrs (16), the water-soluble 0A antigen was dialyzed against distilled water and lyophilized, while water insoluble free lipid A was washed several times with distilled water and dried in vacuum.

Immunization of Chicken & Challenge with E.coli 088

One-day-old broiler type chicken were obtained from a commercial hatchery and reared at 28 days of age. One hundred and sixty chicken were divided randomly into 5 groups, each group between 30-36 chicken. The first group was immunized intraperitoneally for 3 days with OA antigen (100µg/chick) before bacterial challenge. The second group was immunized in the same manner with cell wall antigen (50µg/chick) and the third and fourth groups were immunized intraperitoneally with 12.5µg/chick and 50µg/chick cytoplasmic membrane respectively. While the fifth group (control chicks) was received only PBS buffer in the same way. On the fourth day, all the five groups of chicks were injected intraperitoneally with lethal dose of E.coli 088 (109 cell/chick) and the mortality in the following 30 days was recovered.

Results & Discussion

The E.coli 088 cytoplasmic membrane, cell wall and somatic antigens were suspended after partial purification in a sterile PBS buffer, pH 7.2 and kept frozen at -20ºc until use .

All the groups of chicken were immunized with three injections of 0.2 ml. volume containing either 50µg cytoplasmic membrane antigen, or 12.5µg cytoplasmic membrane antigen or 50µg cell wall antigen or 100µg somatic antigen (OA) without adjuvant, all immunized chicken were challenged with lethal dose of E.coli 088 .

The results of active protection induced by immunization with doses of cytoplasmic membrane from E.coli 088 ranging from 12.5 to 50µg are depicted in fig.1a. Immunization with as little as 12.5µg cytoplasmic membrane give almost 65-70% protection in comparison with control chicks, where as increasing immunization dose to 50µg induce the same protective immunity capacity during the 30 days observation periods.

Fig. 1b and Fig. 1c showed the protection immunity of 55-60% and 25-30% obtained by immunization with 50µg cell wall and 100µg somatic antigen respectively in comparison with control chicks during the same observation period. The same experiments were repeated three times and similar results were obtained, these results indicate that these subcellular fractions contain an immunogens capable of inducing active protection against a challenge with live bacteria.

No toxic effects such as a loss of body weight observed during the four days of various doses of subcellular fractions were injected intraperitoneally.

In a previous studies (17) it was shown that Lipopolysaccharide (LPS) isolated from E.coli 088 confer 50% protection against challenge with E.coli 088, in contrast, the 55-60% protection determined by this study might due to the cell wall associated outer membrane proteins and to the LPS contaminated cell wall preparations as it was always the case with isolated cell wall in gram negative bacteria (13). It is noteworthy that higher percentage of protection among the subcellular fraction studied was induced by cytoplasmic membrane.

To the best of our knowledge, this is the first study dealing with the protective efficiency of various subcellular fractions on chicken. Therefore these results indicate that cytoplasmic membrane and cell wall fractions could probably be used as vaccines against pathogenic E.coli in chicken.

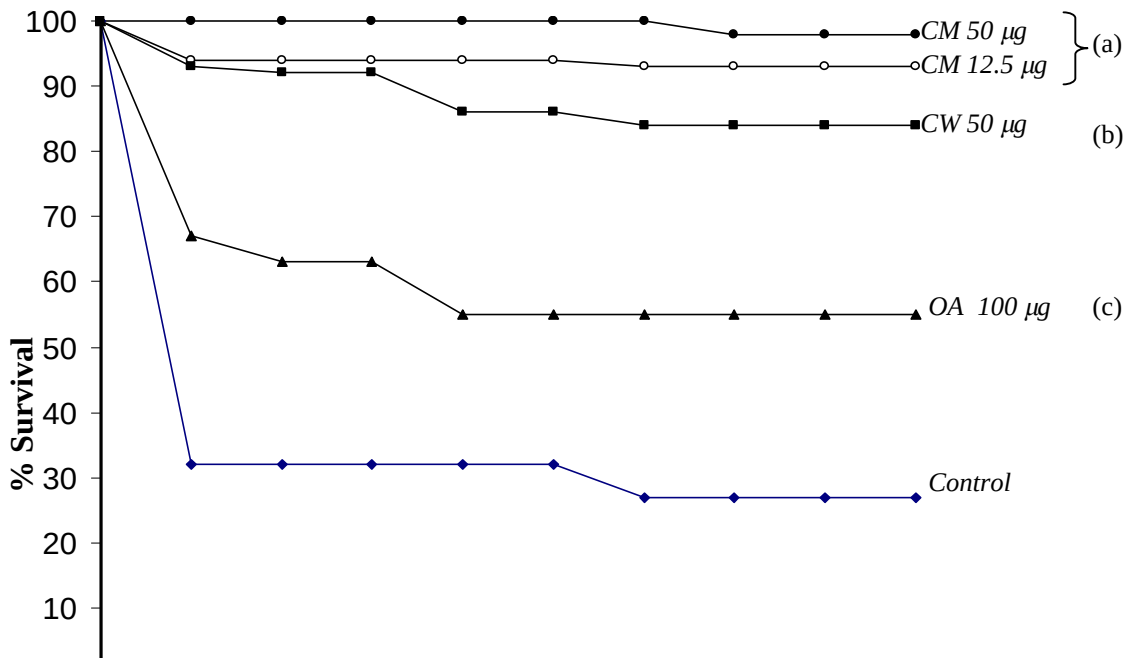

Fig.1 Active protection of chicken immunized with

a- 50 µg and 12.5 µg cytoplamic membrane (CM) of E-coli 088 challenged with lethal dose of E-coli.088 in comparison with control chicken.

b- 50 µg cell wall (CW) of E-coli.088 challenged with lethal dose of E-coli 088 in comparison with control chicken.

c- 100 µg somatic antigen (OA) of E-coli.088 challenged with lethal dose of E-coli in comparison with control chicken.

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