THE INFLUENCE OF ADJUVANT AND IMMUNOGENIC SUBSTITUTION FOLLOWING IMMUNIZATION WITH DIFFERENT ANTIGEN[+]

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Abstract

The nature of the antigen and the presence of the adjuvant are the major factors, which influence the level of then immune reactivity following immunization. This study examines the quantity and isotype of antibody produced in rabbit immunized with different antigens in combination with different adjuvant. The primary and secondary antibody levels to various antigens in Freund's complete adjuvant (FCA) and alum adjuvant were compared between the groups. Results using ELISA assay show that animals immunized with antigens in adjuvant produced lower level ($p < 0.05$) of the antibody compared with animals that were immunized with direct antigens in the marginal vein. The antibody titer was higher significantly in animals given oil-emulsion adjuvant than the alum.

المستخلص

أن طبيعة المستضد ووجود العامل المساعد هما من العوامل الرئيسية التي تؤثر في مستوى الاستجابة المناعية بعد التلقيح. هذه الدراسة فحصت كمية ونوع الضد المنتج في أرانب لقحت بمستضدات مختلفة ومستويات مختلفة من العامل المساعد. أظهرت النتائج ان معيار الضد عند الحيوانات التي أعطت المستضد مع العامل الدهني في الماء كان أعلى وبمستوى إحصائي ذو دلالة معنوية عن التي أعطت مواد الشب كعامل مساعد.

Introduction

Vaccines are used widely in preventative veterinary medicine [5]. However, there are little critical data, which examine the myriad of immune pathways activated following vaccination or experimental immunization. An evaluation of the degree of immunological primary that occurs following antigen injection is therefe relevant for the optimization of immunization regimes and procedures . Allegro number of factors influence the development of humoral immunity following immunization . These include the dose and nature of antrigens [3] , presence of adjuvant , [13 , 6] , route of inoculation [2] , maturity of animal [9] and the immunization regime [5] .Two of the these factors , immunologically of antigen and presence of adjuvant are the least understood immunologically , though the most imprtant factor for the vaccine development [11]. Adjuvant is widely known to have potential immune reactivity to otherwise ineffectual antigens[1 , 13]. As demonstrated in the response to the attenuated vaccines , chemical or physical of an antigens can render it more or less immunogenice [7]. The aims of this study is to examine the quantity of antibody reflecting T

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and B cell priming produced of adjuvant and mmunogenicity All other factors , does and route of injection , immunization regime and animal maturity atrue primary , stimulus as well as avoiding the complication of virulence antigens. Animals were immunized via the subcutaneous (S.C) route with different antigens in different adjuvants . Another groups of animals was immunized via the marginal vein of ear route with different antigens.

Materials and Methods

Buffers:

Buffers included the carbonate –coating buffer at PH 9 . 6(1.5 gNa2 Co 3 ; 2.93 g NaN3 : 0.5 ml Tween 20; 1LH2O) , phosphate –buffered saline Tween (PBS-Tween) at PH 7.4(8g NaCl ; 0-2g KH2PO4 ; 0.2G NaN3 ; 0.5 ML Tween 20; 1L H2O) .

Antigen

Entrotoxigenic Escherichia Coli (ETEC) strain 0128: K78

Adjuvants:

The two adjuvants used in this study were aluminum hydroxide (alum), and Freund's complete adjuvants (FCA). Alum adjuvanta were prepared by mixing protein solution containing up to mg/ml of protein with half its volume of 1m NaHCo3 followed by one volume of 0.2m potassium aluminum sulfate solution . The protein is adsorbed on to the resulting aluminum hydroxide precipitate was the mixture which is slowly stirred and left for 30 min. The precipitate is pelleted by centrifugation for 15 min. at 300 xg and washed three times with phosphate buffered saline. The precipitate was suspended in 2 ml of PBS. Freund's complete Adjuvants was homogenized with antigen.

Preparation of O Antigen:

1-ETEC 0128: K 78 was cultured in Liquid Minimal Medium (L.M.M.) according to the method described by [8]at 37°C for 18-24 hr without agitation .
2-The bacterial suspension was seeded and collected into asterile centrifuge tube by repeated washing with PBS.
3-The bacterial suspension was placed into a boiling water bath for 21/2 hours .
4-The sterility of the bacterial suspension was checked by inoculating a plate of nutrient agar , incubated for 24 hours at 37°C
5-For injection into animals, the bacterial suspension was diluted with saline to a final concentration of 8 x10 bacterial per ml on the McForland scale (3 or 4 tube).

Preparation of H Antigen:

1-ETEC 0128:K78 was cultured in LMM at 37° C for 18-24 hr .
2- The bacteria was seeded and collected into astrile centrifuge tube by repeated washing with PBS , followed by addition of an equal volume of 0.6% formation saline at room temp. for 24 hours .
3-The sterility of the bacterial suspension was checked by inoculating a plate of nutrient agar . Incubate for 24 hours inoculating a plate of nutrient agra . Incubate for 24 hours at 37° C .
4-The bacteria were colleted by centrifugation in a refrigerated centrifuge for 10 min . Discard the supernatant and re-suspend the bacteria in a 0.3% formalized saline and stored in the refrigerator .
5-For injection into animals , the bacteria suspension was washed and diluted with saline to a

final concentration of 8 x 10 bacteria per ml on the McFarland scale (3 or 4 tubes) .

Preparation of OH Antigen:

1-ETEC 0128 K 78 was cultured in LMM at 37°C for 18-24 hr .
2-The bacteria were collected into a sterile centrifuge tube by repeated washing with PBS.

3- The bacteria were sonicated at force of 10 implitade for 5 min . in ice bath and stored in the refrigerator .
4-For injection into animals, the bacteria suspension was washed and diluted with saline to a final concentration of 8x 10 bacteria per ml on the McFarland scale (3 or 4 tube)

Inoculation:

The bacteria suspension was injected into the marginal vein of the ear of group No .1 of rabbit weighing at least 3kg.As approximately 15 days 1).0.25 ml of killed suspension.3) 1ml of killed suspension. 5) 3 ml of killed suspension+1 ml heparin . 9 , 12 , 15) 0.5 ml of freshly prepared living suspension+1 ml heparin Group No.2 of rabbit received bacterial suspension via the S.C route combined with a different adjuvant.The animals were boosted with 0.125 mg/ml and their respective alum after 21 days from the primary inoculation, and after 14 days.

Bleeding

Seven to eight days after the last injection, 20 ml blood was taken from the marginal vein of the ear and collected in a sterile universal container .
After a further five to seven days another 20 ml was taken . Separation of serum and preparation for use the blood was left on the bench for 24 hr to allow the clot to retract . The supernatant was removed by pipette and centrifuged lightly to remove red blood corpuscles and any small blood clots . Serum from the samples was pooled and stored at 4°C with sodium azid (0.1 %) as preservative .

ELISA (Enzyme – linked immunosorbant assay)

ELISA was done essentially according the method described by [12] . ELISA was performed using the bacterial suspension as the antigen.
Flat – bottomed microtiter elisa plates (96 wells) were used as the solid support. Wells were coated with 100 ml of E. coli (9 x 10 cell /well) in carbonate buffer, and incubated for 1 h at 37° C over night at 4° C . Plates were washed three times with PBS-Tween. Plates were incubated with the appropriate dilution of the serum for 1hr at 37°C .The plates were then washed again three times with PBS-Tween and 100 ml of anti-rabbit 1 g coupled to horseradish peroxide (Sigma Chemical Co. 1:100 in PBS –Tween) were added to each well . After 1 hr incubation at 37° C, the plates were washed again followed by addition of 100 ml freshly prepared substrate solution (25 mg / ml orthopheny diamine in citrate buffer (pH 9.8) the reaction was stopped by addition of 100 ml 12.5% from 3M H_2SO_4. Adsorbance was read at 492 nm.

Results and Discussion

A peak anti-H Ag antibody response (Fig.1) was significantly different at 7 days after the last injection compared with the last controls ($P < 0.05$). The aim of the present study is to use ELISA method for the determination of extremely low concentration of antibody. In principle, non-purified antigens or antigen mixtures can also be used for the production of the antibody.

Results indicate that adjuvancy and immunogenicity as well as quantity of antibody are produced by the use of adjuvants Injection of H antigen without adjuvant induced strong primary and secondary response.

A peak of anti – H antibody response (Fig.1) was significantly compared with controls ($P < 0.05$) Similar results were obtained by Reynolds and Griffin (1990) who studied the elisa titer of antibody from serum ewe that was injectied with clostridial vaccine using different inoculation schedules .

The anti – OH Ag response of animal immunized with sonicated antigen gave similar primary and secondry responses. A peak of anti OH Antigen response (Fig.1) was singnificantly different at day 7 compared with anti- H antigen.

The rabbit immunized with H – antigen in FCA in the primary injection produced higher antibody levels than the secondary injection of animal immunized with H- antigen in alum while the response of animals immunized with O and OH antigen in FCA produced lower antibody levels than the antigen (Fig .2).

These forms of antigens seem to be able to intiate antibody production much more effectively than the same antigen non – particulate form. . The advantages of FCA of immunization are that both the primary and secondary and that the beak level of antibody are maintained over a long period by the small quantities of antigen released from the depot [10]. Depot adjuvant (FCA) produced strong cellular activation and plateau levels of 1gG antibody which was typically unaffected by the booster inoculum [1].
Qualitative changes may have occurred with increasingly high affinity reccptors being selected progressively by persisting antigen [10]. In all groups that responded to the H antigen, the antibody peak is stable at day 7 to day 21.

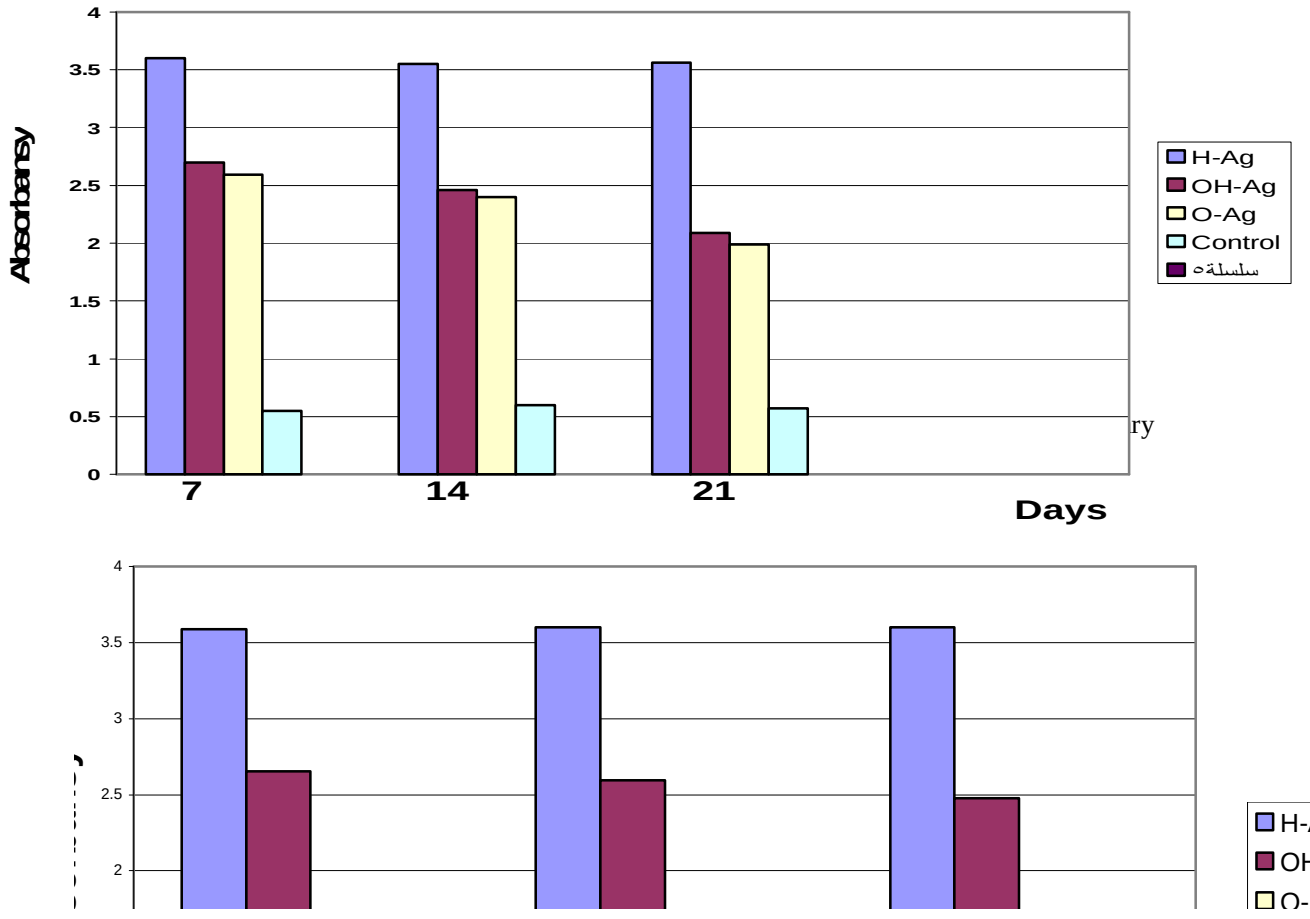

Fig.2 The antibody response immunized with different Antigen FCA . All groups were then injected with antigens in alum for their secondary injection

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