

Vaccine Trail against *Trypanosomia evansi*

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Abstract

Back ground: Trypanosomiasis is one of the transmissible, zoonotic diseases that infect man & animals.

Objectives: The present study was designed to investigate the immunization of adult Balb/mice with the immunomodulator LPG alone or mixed with NPG or BCG, by studying the hematological, immunopathological changes and immune response against *Trypanosoma evansi* infection.

Methodes: 50 male Balb/c mice at (3-4) weeks of age divided in to 5 equal groups, animals of groups 1&2 were considered as control groups, animals of groups 3, 4&5 were injected 4 times i/p with equal dosage of different biological adjuvant as antigens: LPG, LPG+BCG, LPG+NPG; 20/0.08, 20/0.08+50/0.1, 20/0.08+50/0.25; µg/ml/mice, respectively. Immunized & +ve control animals were infected with trypanosome evansi by injection of parasite, experiment conducted for 12 days. Hematological parameters were determined using the MS9. Immunopathological changes were refereed as liver and spleen weight /body

weight Immune response detected by using IHA test.

Results: Results revealed a significant variation in hematological, and liver & spleen weight between immunized infected, and non immunized infected animals. The biological adjuvant (LPG alone or mixed with BCG or NPG) had high immunogenic and less toxicity against the experimental infection of *Trypanosoma evansi*.

Conclusion: The most successful immune responses (increase Ab) was in combination between LPG +BCG. While the more effective in decrease of severity of the disease (hematological & pathological changes) was in combination of LPG+NPG.

Keywords: Immunomodulators; *Trypanosoma evansi*; Hematological and pathological changes; IHA.

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Introduction

Trypanosomiasis is one of the major parasitic disease for which control is still far from reality. The classical vaccination approaches by using dominant surface proteins, has not been successful, mainly due to antigenic variation of the parasite surface coat. Current methods of treatment of African sleeping sickness are unsatisfactory because the number of available drugs is limited, the period of treatment is long, and the associated with severe side effects⁽¹⁾

For Chagas disease and the leishmaniasis, the existing drugs are also inadequate because of their variable efficacy, toxicity, and required long courses of treatment^(2, 3). *Trypanosoma evansi* (*T. evansi*), which is normally the causative agent of animal trypanosomiasis known as Surra⁽⁴⁾, was reported as human infection in India⁽⁵⁾. The chronic form of the disease is most common and is likely to be associated with secondary infections due to immunosuppression⁽⁶⁾. Protozoa are a diverse group of unicellular, eukaryotic organisms. Only a few of the many tens of thousands of protozoan species are pathogenic for humans and animals. These pathogens are of two general kinds; those that parasitize the intestinal

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and urogenital tracts, and those that parasitize blood cell and tissues ie: Trypanosoma and Leishmania species⁽⁷⁾. According to the zoological classification of protozoa ⁽⁸⁾Trypanosma and Leishmani genera are below the suborder; Trypanosomatina, under the order Kinetoplastida. The major constraint to developing a trypanosome vaccine is the ability of the parasite to undergo antigenic variation. (Murray M, & Urquhart GM) ⁽⁹⁾, reviewed the various attempts made to vaccinate both domestic livestock and laboratory animals and it was obvious from the reported studies that complete protection was readily achieved only if the same variable antigen type (vat) was used for immunization and challenge. Antigenic variation, the major obstacle to developing a trypanosome vaccine, is the process whereby trypanosomes sequentially express a series of surface antigens; it is these antigens that are capable of inducing protective immunity. The immune response against each variant, although rapid and highly effective in destroying any trypanosomes that possess that particular antigen, is invariably too late to affect that proportion of the population that has altered its antigenic identity. Thus, parasitaemia rises and falls in waves with each parasite population carrying different surface antigens reviewed by ^(10, 11, 12). Hematological ;pathological ; and immunological responses to trypanosomiasis were studied in naturally and experimentally infection with and without vaccination^(13,14,15,16,17,18). The present study aimed to evaluate the potential of some biological immunomodulators on the ways in which animals can build up resistance to T. evansi infection . Research activities focused on some

hematological and pathological changes and immunresponse.

Materials and methods

Immunization: Immunomodulators which were used in this study including: Lipophosphoglycan (LPG) were prepared from *Leishmania donovani* promastigotes, Nocardia peptidoglycan (NPG) , or Mycobacterium tuberculosis (BCG) .LPG adjuvant was extracted from harvested promastigotes during the log phase of growth according to the procedure described by ⁽¹⁹⁾ briefly as the following: The harvested promastigotes were delipidated using the mixture of chloroform methanol ; water(4:8;3V:V) then LPG was extracted by water saturated Butanol, then lyophilized and stored at -20C°. NPG was extracted from harvested Nocardia microorganism cultured in brain heart infusion as described by ⁽²⁰⁾. BCG was obtained from the Institute of serum and vaccines /Ministry of Health. Experimental animal groups were immunized with two doses of different types of adjuvant at intervals with 4 days by intraperitoneal injection.

Parasite and experimental host: Iraqi local strain of *Trypanosoma evansi* was isolated from blood samples of naturally infected camels were brought out to the laboratory of parasites, college of Vet, Med .at Abu-ghareb, the parasite was preserved in laboratory mice by i/p injection of 0.1ml of +ve camels blood sample, then it was reused after preparing according to the method described by ⁽²¹⁾.

Challenge infection 50 male Balb/c mice, 3-4 weeks of age, were obtained from Alrazi center and kept in air conditioned place with food and water, divided into equal five groups, as following: group -1 treated as _ve control injected with normal saline,

group -2 as +ve control, infected only with *T.evansi*, group -3 immunized with (20µg/0.08ml/animal)LPG,group -4 immunized with a mixture of (20µg/0.08ml/ +50µg/0.25ml/animal) LPG +NPG, group-5 immunized with (20µg/0.08ml/ +50µg/0.1ml/animal) LPG +BCG. Experimental animals of second, third, fourth, and fifth groups were challenged by intraperitoneal injection with 0.1ml diluted infected blood (, the infected dosage used 0.2×10^3 parasites), after 7 days of the last immunization dose. Experimental animals kept under observation for 10 days from infection. During the third day of infection fresh blood sample obtained from the tail of lived mice were examined directly to confirm the continues of infection.At the end of the experiment body, spleen, and liver weights were recorded; blood samples were collected for Hb, total & differential WBCs, and platelet count. Serological test for detection of antibody titer was determined using commercial kit from al-razi center /Baghdad, using indirect hem agglutination test (IHA).Post mortem examination of animal carcass was done

Statistic analysis All data are expressed as mean±SE. F test was used to test the differences between groups, using a significant level of $P < 0.05$

Results

Result showed in table(1) refer to the hematological changes of immunized and *Trypanosome evansi* infected mice in compare with control negative,(non infected) positive,(infected non-immunized),.Hb showed significant decreased($P < 0.05$) in infected non-immunized mice(control positive)(6.1 ± 0.07 gm/dl)in compare with non-infected,non immunized(12.8 ± 0.06 mg/dl),while other

experimental groups immunized with different immunomodulators revealed slightly decrease in Hb in comparison with non-infected control and increased in comparison with infected group control as follow: 9.3 ± 0.08 , 8.9 ± 0.13 , 7.1 ± 0.39 in ,LPG, LPG+N, L+BCG respectively.The above Hb level represent mean of pool sample of 10 blood sample from groups of 10 mice.Total W.B.C. elevated significantly ($P < 0.05$)in infected immunized mice groups in compare with group-1(3.9 ± 0.11 cells/c.mm) but decreased significantly ($P < 0.05$) in compare with infected non-immunized mice group-2which show Leucocytosis(65.4 ± 2.0 cells/c.mm),while infected immunized groups- 3,4,5(15.2 ± 0.44 13 ± 0.44 , 19.3 ± 0.40 cell/.mm) respictevly. Differential WBCs count showed: Lymphocytes (%) increased significantly ($P < 0.05$)in infected control positive group-2(94 ± 0.44)but they remained in the same level in other groups -1, 3, 4, 5(89.4 ± 0.55 , 84.7 ± 2.6 , 88 ± 0.89 , 85.8 ± 0.17 %) respectively.Granulocytes decreased (granulocytopenia)significantly ($P < 0.05$) in infected control positive ($1.8 \pm 0.08\%$)and increased in group- 3(11.5 ± 0.21) , other groups stayed within normal level..Monocytes(%) showed no changes in all groups.

Platellate count increased (thrombocytosis) significantly ($P < 0.05$) in infected control group (1141.5 ± 0.01 ce./c.mm),there was no significant difference with immunized infected mice groups-3, 4, 5 and control negative (355 ± 0.10 , 378 ± 0.22 , 171 ± 0.9 and 241 ± 0.46 ce./c.mm) respectively.

(Table2) showed results for liver and spleen weight/body weight (B.W.) ratio. There was significant increase in liver and spleen/B.W, 10.7 ± 0.24 , $8.86 \pm 0.42\%$

respectively, in control positive, in compare with control negative (5.2 ± 0.22 and $(0.63 \pm 0.17\%)$, groups- 3,4,5 reveled significant decrease in liver and spleen/ body weight ratio in compare with group-1 ,but increase in compare with group-2 (6.37 ± 0.28 & 1.04 ± 0.06 , 6.88 ± 0.04 & 1.07 ± 0.02 , 8.97 ± 0.05 and 1.8 ± 0.07 %) respectively

(Figure1) showing the values of antibody (Ab) titer by using Indirect hemagglutination test(IHA).

The highest titer was in mice immunized with LPG+BCG and infected (1/640) in compare with LPG (1/320) and LPG+N, (1/160). While the control positive showed (1/320) and control negative (0).

Table-1: Hematological changes in immunized with different immunomodulators and *Trypanosome evansi* infected mice(mean $\pm$ SE)

Animal groups	Hb. gm/dl	WBCs cell $\times 10^3$ /mm	Lymph. %	Granul. %	Mono. %	Plat. cell $\times 10^3$ /mm
Group -1	a 12.8 ± 0.06	c 03.9 ± 0.11	b 89.4 ± 0.55	c 6.6 ± 1.55	a 5.0 ± 0.43	c 241 ± 0.46
Group -2	d 6.1 ± 0.07	a 65.0 ± 2.00	a 94.0 ± 0.44	d 1.8 ± 0.08	b 4.1 ± 0.40	a 1141 ± 0.01
Group -3	c 8.9 ± 0.13	b 15.2 ± 0.44	b 84.7 ± 2.60	a 11.5 ± 0.21	c 3.8 ± 0.01	b 355 ± 0.10
Group -4	b 9.3 ± 0.8	b 13.0 ± 0.44	b 88.0 ± 0.89	b 8.6 ± 0.27	c 3.4 ± 0.26	b 378 ± 0.22
Group -5	d 7.1 ± 0.4	b 19.3 ± 0.40	b 85.8 ± 0.17	b 8.5 ± 0.24	a 5.6 ± 0.26	d 171 ± 0.09

n=10, Group -1: negative control, Group -2: positive control, Group -3: immunized with LPG and infected with *T. evansi*, Group -4: immunized with LPG+N and infected with *T. evansi*, Group -5: immunized with LPG+BCG and infected with *T. evansi*

Different letters denote significant differences between groups ($P < 0.05$)

Table 2: Liver and spleen weight changes in immunized with different immunomodulators and *Trypanosoma evansi* infected mice (mean $\pm$)

Animal groups	Body weight(gm)	Liver Weight(gm)	Liver/body %	Spleen weight(gm)	Spleen/body %
Group -1	a 7.95 $\pm$ 0.31	d 0.41 $\pm$ 0.22	d 5.2 $\pm$ 0.22	c 0.05 $\pm$ 0.17	c 0.63 $\pm$ 0.17
Group -2	c 7.25 $\pm$ 0.26	a 0.78 $\pm$ 0.24	a 10.7 $\pm$ 0.24	a 0.64 $\pm$ 0.42	a 8.86 $\pm$ 0.42
Group -3	c 7.37 $\pm$ 0.84	d 0.47 $\pm$ 0.20	c 6.37 $\pm$ 0.20	c 0.077 $\pm$ 0.06	b 1.04 $\pm$ 0.06
Group -4	a 7.88 $\pm$ 0.05	c 0.54 $\pm$ 0.04	c 6.88 $\pm$ 0.04	c 0.085 $\pm$ 0.02	b 1.07 $\pm$ 0.02
Group -5	b 7.53 $\pm$ 0.11	b 0.67 $\pm$ 0.05	b 8.97 $\pm$ 0.05	b 0.137 $\pm$ 0.07	b 1.8 $\pm$ 0.07

n=10, Group -1: negative control, Group -2: positive control, Group -3: immunized with LPG and infected with *T. evansi*, Group -4: immunized with LPG+N and infected with *T. evansi*, Group -5: immunized with LPG+BCG and infected with *T. evansi*
Different letters denote significant differences between groups (P< 0.05)

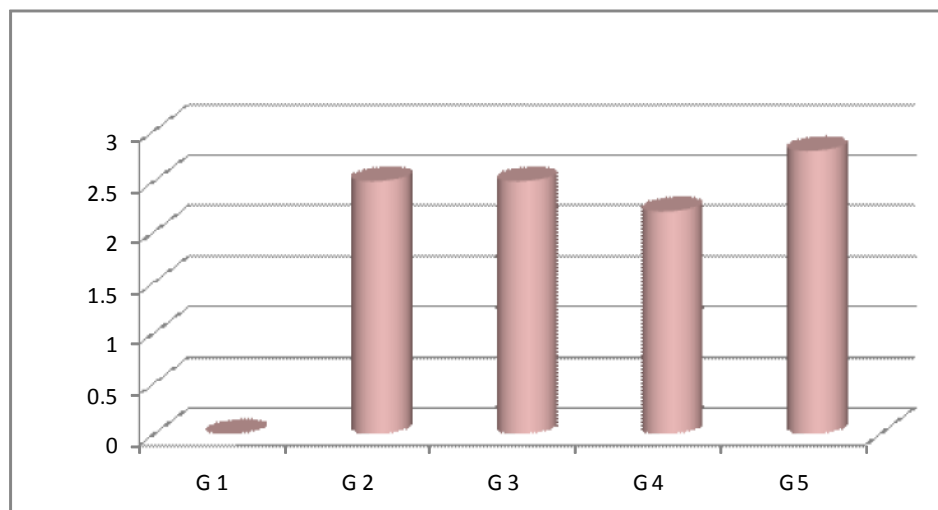


Figure.1: Antibody titers values in immunized with different immunomodulators and *Trypanosoma evansi* infected mice. n=10, Group -1: negative control, Group -2: positive control, Group -3: immunized with LPG and infected with *T. evansi*, Group -4: immunized with LPG+N and infected with *T. evansi*, Group -5: immunized with LPG+BCG and infected with *T. evansi* horizontal numbers represent the log of antibody titer values in experimental groups: Group 1:0 , Group 2:1\320, Group 3:1\320 , Group 4:1\160 , Group 5:1\640.

Discussion

Anemia caused by *T. evansi* infection represented by sharp decrease in Hb specially in group -2 in compare with group -1 (Table1) .Trypanosomiasis usually multiplied rapidly in the blood and is evenly dispersed through out the cardiovascular system and tend to be aggregated in some blood vessels and capillaries of the heart, brain, skeletal muscle, and rarely heavy parasitemias and are excreta their effect mainly by causing sever anemia and mild to moderat organ damage. The anemia has a complex pathogenesis involving mainly increasing erythrophagocytosis, some hemolytic and dyshemopoiesis ⁽²²⁾. Severity of anemia was reduced in mice immunized with different immunomodulators (table-1), but in different levels, since the preinfection immunization increased the capability of mice to control parasitemias better and have less sever anemia and organ damage ⁽²³⁾.

Value of total WBCs count was highly elevated (leukocytosis) in infected non immunized mice, and slightly elevated in preinfection immunized mice (Table-1). During the acute phase of experimentally trypanosomiasis the total WBCs revealed leukocytosis, with relative lymphocytosis ^(17,18).Preinfection vaccination of mice group -3,4,5 with different immunomodulators was effective in modulation of the immune response to decrease the severity of the infection with *T. evansi* represented by the decrease in total and differential WBCs in comparison with group-2. Several antigens showed increase in immune response (cellular and humoral) attributed to either increase in macrophages stimulation, and lymphocytes number ⁽²⁴⁾, or direct effects on progenitor cells in bone

marrow to produce more lymphocytes and monocytes ⁽²⁵⁾. The platelets count showed extreme elevation in mice of group-2, and mild elevation in preinfection vaccinated mice of groups -3, 4, 5, almost near to group-1. Production of platelets from the bone marrow progenitor's cells is highly affected by reduction of RBCs as a compensatory mechanism ⁽²⁶⁾.

Liver and spleen are the main constituents of the reticuloendothelial system, which affected by the infection and enlarged specially in cases of blood parasites ⁽⁸⁾, these changes attributed to increase in accumulation of macrophages ⁽²⁷⁾, or follicular hyperplasia ⁽²⁸⁾.The hepatosplenomegalia in mice group-2 referred to the severity of the infection which was modulated in preinfected vaccinated mice of groups -3, 4, 5, specially the spleen /body ratio (Table 1). Similar findings cited by ⁽²⁹⁾ against *L.donovani* infection in mice used different antigens, suggesting that the vaccine induced more than 90%elimination of parasite from both liver and spleen, due to an immunomodulation towards Th1 is effective for successful vaccination.

Anti body titer is regarded as indicators to immune response against the antigens either from infection or vaccination. The non immunized non infected mice of group-1 showed nil antibody titer, while non immunized infected mice of group -2 and immunized infected mice of group -3 showed the same responses, since they are belong to the suborder;Trypanosomatina, and contain the same antigenic characters ⁽⁸⁾. Mice of group -4 vaccinated with LPG+N revealed an a low antibody titer, this could be explained by the antigenic variation of LPG+N with trypanosome,

The cross reaction between LPG and BCG due to the obligatory intracellular of both of these microorganisms caused increase in the antibody titer in mice of group -5, which vaccinated with LPG+BCG and infected with *T. evansi*. These finding are agree with previous research to evaluate potentially of the same vaccine against *L. donovani* in mice^(30, 19) and against hydatid cyst in mice⁽³¹⁾.

Conclusion

A crucial issue for assessment of the potential of any new compound against human and animal trypanosomosis is the ability of such a compound to decrease the severity of the disease and increase the resistance of the host against the infection. A combination between two or more cellular fragment of heterologus microorganism, with multiple intraperitonail administration gives the most successful immune responses as in combination between LPG and BCG.

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