

Antibody response in Hamsters Immunized against experimental Leishmaniasis

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Abstract:

Direct agglutination test was used to evaluate the immunogenicity of three different antigens inoculated in hamsters as one , two and three doses which were: Group (1) inoculated with autoclaved killed *Leishmania tropica* , Group (2) inoculated with BCG vaccine alone while Group (3) inoculated with mixed antigens (autoclaved killed *Leishmania* "AKL"+ BCG).(4) Control animals inoculated with phosphate buffer saline.

The maximum level of antibody titers were evaluated in animal inoculated with one , two or three dose of mixed antigens (320 , 640 and 1280) respectively when it compared with animals inoculated with corresponding doses of AKL antigen (80 , 160 and 320) respectively. While the minimum level of antibody titers were observed in animal inoculated with two and three dose of BCG (20 and 40) respectively.

Our findings suggest that administration of BCG with AKL could lead to a potentially associated antibody response in animals, as well as, such response may evaluate the immunogenicity of some antigens.

Key word: *Leishmania tropica* , Immunogenicity , Antibody titers

Introduction:

Leishmaniasis refers to a spectrum of a clinical disease produced by *Leishmania spp.*[1],Which belong to the order Kinetoplastida , family Trypanosomatidae [2]. *Leishmanain spp.* reside solely within mononuclear phagocytes as intracellular amastigotes in human and other mammals and as flagellated extracellular promastigotes in the gut of their sand fly vectors[3]. The clinical manifestations of disease depend on complex interactions between virulence factors of the infecting *Leishmania spp.*and genetically determined , cell-mediated immune response of its mammalian host[2]. The spectrum of disease has traditionally been divided into three major syndromes ; cutaneous, mucosal and visceral leishmaniasis [4].

The importance of *Leishmania* as a human pathogen has stimulated a large number of researches to deal with immunization against Leishmaniasis especially in experimental animals [5,6,7]. Evaluating the potency and immunogenicity of antigens used in vaccination against Leishmaniasis is not an easy target , investigators examined several parameters to measure immune response provoked by antigens used in immunization , such as the delayed type of hypersensitivity test (skin test) ,lymphocyte transformation , Interferon gamma production[6, 7 ,8] and evaluation of humoral immune response by determined specific antibody titer[9].This paper aimed to compare the immunogenicity of

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different antigens by antibody response profiling.

Materials and Methods:

Isolation of the *Leishmania* parasite

Leishmania tropica was isolated from a lesion in the left arm of a 21 year-old male at Baghdad teaching hospital / Baghdad according to method of Al-Aloussi ,1979 [10].it was isolated in 26/12/1999.

Media

1-Semi –Solid media: The medium was prepared according the method of Alder and Theodor (1926) [11] and was used for parasite isolation .

2- Biphasic Medium: The medium was prepared according the method of Kagan and Norman (1970) [12] and was used for parasite cultivation in order to prepare antigens used in inoculation and for direct agglutination test.

Autoclaved Killed *Leishmania* (AKL) antigen preparation

Moheballi *et al* . (1998) method [13] was used in preparing *Leishmania tropica* vaccine with little modification [14] and as the following:

1. Promastigote of *Leishmania tropica* was cultivated in Biphasic Medium at 26°C .
2. Promastigotes were harvested and concentrated using centrifuge at 3200 rpm.
3. The promastigotes were washed five times with phosphate buffer saline (PBS), and was counted using Haemocytometer to get the final concentration of immunization dose which was 10^7 parasites / 0.2 ml.

The promastigotes were separated and transferred into several autoclavable containers and autoclaved at 121°C for 15 minutes, and then the containers were kept at 4°C.

BCG Vaccine

BCG vaccine was obtained from "The National Centre for Drug Control and Researches / Baghdad / Iraq" .The vaccine was made by " Japan BCG Libratory" and each vial was contained 0.5 mg of lyophilized vaccine and each 1 ml of vaccine contained 8.26×10^6 cells of *Mycobaterium bovis*.

Animals

Sixty male Golden hamsters (*Mesocricetus auratus*) aged 8-10 weeks were obtained from "The National Centre for Drug Control and Researches / Baghdad / Iraq". The animals were separated into four groups each group contained 15 animal which inoculated as following:

- 1- Group (1) was inoculated with 1×10^7 Autoclaved killed *Leishmania* (AKL) per 0.2 ml.
- 2- Group (2) was inoculated with 1.4×10^6 unit of BCG per 0.2 ml [15].
- 3- Group (3) was inoculated with mixed inoculums of both (1×10^7 AKL /0.2 ml) and (1.4×10^6 unit of BCG / 0.2 ml).
- 4- Group (4) was considered as control group, the animal in this group were inoculated with 0.2 ml phosphate buffer saline.

One, two or three doses of each the previous antigen were used with an interval of 15 days between each dose and the other.

All previous animals were inoculated intradermally in the left hind footpad using 1 ml sterile syringe for each animal. After 15 days of inoculation, blood was taken from all animal then sera were separated by blood centrifugation at 3000 rpm for 5 minutes, and sera were stored at -20° C until tested.

Direct Agglutination Test

This test was done according Allian & Kagan (1975) method , [16] which was as following:

The promastigotes were grown in biphasic medium, and the flask was incubated at 25°C until a peak growth was reached. The organisms were harvested by pooling the overlays from the flasks and filtering them through a funnel loosely packed with glass wool to remove any particles of agar. The antigen suspensions were consisted of formalin-fixed

Leishmania promastigotes that were treated with trypsin. Twenty-five hundredths of an ml (0.025 ml) of PBS, pH 7.2, containing 1 :25,000 Evans blue dye was dispensed in each well of a clear microtitration "V" plate. Serial dilutions of serum (0.025 ml), starting at a dilution of 1:2 and continuing through a dilution of 1 :4,096, were made by using microtitration loops. Twenty-five hundredths of an ml (0.025 ml) of the antigen suspension was added to each well, while the plate is shaking on a vibrating apparatus. Each plate was sealed with 3-inch transparent plastic or cellophane tape. After the organisms settle for 4 hours at room temperature or overnight at 4°C, the agglutination results were read and recorded.

Results:

Different levels of antibody titers were observed among different inoculated animal group with different antigens and various boosting doses. The maximum level of antibody titers were observed in animal inoculated with one, two or three dose of mixed antigens (320, 640 and 1280 respectively) when it was compared with animals inoculated with corresponding doses of AKL antigen (80, 160 and 320 respectively). While negative results were seen in all control groups and animal inoculated with single dose of BCG.

The minimum level of antibody titers were observed in animal inoculated with two and three dose of

BCG (20 and 40) respectively. Important significant ($P \leq 0.01$) differences were noticed among the different antigens with one, two or three dose, but the most efficient antigen in provoking antibody response was the mixed antigen preparations (AKL+BCG), (Figures 1, 2 and 3).

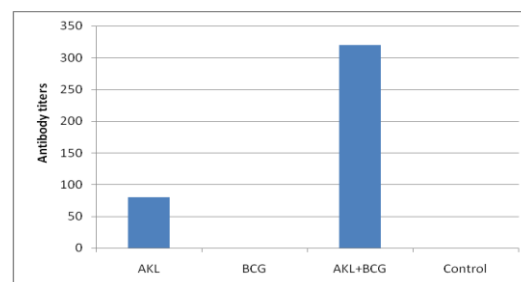


Fig. 1: Antibody titers for animals inoculated with "one" dose of different antigen preparations

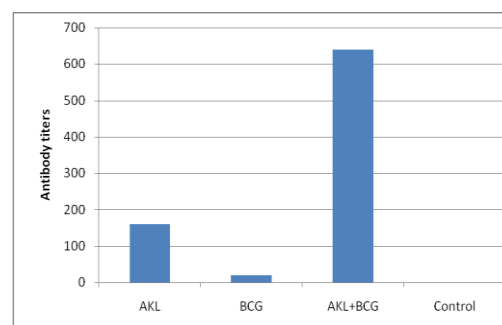


Fig. 2: Antibody titers for animals inoculated with "two" dose of different antigen preparations

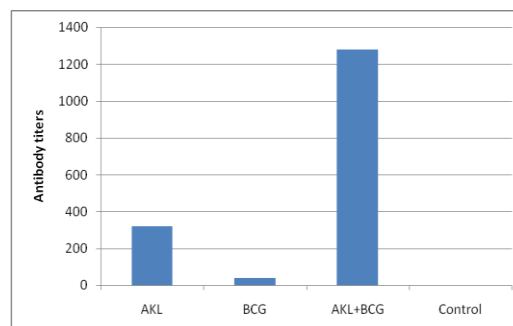


Fig. 3: Antibody titers for animals inoculated with "three" dose of different antigen preparations

Discussion:

Golden hamsters were used in this study because some investigators proved that these animals were the suitable host for *Leishmania tropica* experimental design [17]. In other hands gender and age of the hosts are also play an important role in the immune response provoked by *Leishmania* [18, 19], so we used only male aged 8-10 weeks.

Promastigotes (infected stage of *Leishmania spp.*) were used in antigen preparations, which were harvested in stationary phase, because the promastigote in stationary phase can be considered to be more virulent and immunogenic than promastigotes in log phase [20], as well as the second and third boosting dose of these antigens were administrated due to the recommendation of Goldsby *et al.*, 2000 who recommended to use more than one dose for killed vaccine [21].

In comparison with other standard serologic procedures used in the diagnosis of leishmaniasis or in the evaluating of specific antibodies, the direct agglutination test is simple to perform, and the antigen suspensions are stable and sensitive after prolonged storage at 4°C. [16] so the direct agglutination test was used in this study.

Results showed that the most efficient antigen preparation in provoking antibody response were the mixed antigens AKL+BCG, when it compare with AKL antigen and BCG vaccine alone, these results agreed with the results of Sharples *et al.* (1994) [9], who demonstrated that the AKL+BCG can stimulate high antibody response, these results may be explained according to the presence of cross-reacting antigenic determinants between *Leishmania* and *Mycobacterium bovis*, which may stimulate cellular and humoral immune response [22], as well as BCG was

considered as good adjuvant in stimulating T-helper 2 immune response which may correlate with the high antibody titer against *Leishmania* antigens [7,9], depending on this results it is possible to suggest that the administration of BCG could lead to a potentially exacerbatory T-helper 2 associated antibody response in hamsters. In other hand most articles revealed that the antibodies may play simple role in healing of *Leishmania tropica* infection [17], but we can evaluate the immunogenicity of some antigens depending on their antibody response.

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استجابة الأجسام المضادة في حيوانات الهامستر الممنعة ضد الخمج بداء اللشمانيات التجريبي

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الخلاصة:

اختبرت في هذه الدراسة القابلية التمنيعية لثلاث مستضدات مختلفة باستخدام فحص التلازن المباشر في اربعة مجاميع من حيوانات الهامستر الذهبي التي حقنت بجرعة وجرعتين وثلاث جرع من هذه المستضدات وكما يلي : المجموعة الأولى حقنت بمستضد طفيلي اللشمانيا المقتولة بحرارة المؤصده , المجموعة الثانية حقنت بمستضد لقاح الـ BCG , المجموعة الثالثة حقنت بخليط المستضدين السابقين اما المجموعة الرابعة فقد حقنت بمحلول دارى الفوسفات الملحي واعتبرت مجموعة سيطرة .
اظهرت النتائج ان اعلى معيار للأضداد كان في مجموعة الحيوانات المحقونه بجرعة , جرعتين وثلاث جرع من خليط المستضدين (460,320 و 1280 على التوالي) , اذا ماقورنت بالحيوانات المحقونه بجرعه , جرعتين وثلاث جرع من مستضد طفيلي اللشمانيا المقتولة بحرارة المؤصده(80, 160 و 320 على التوالي) , بينما كان اقل معيار للأجسام المضاده في الحيوانات المحقونه بجرعتين وثلاث جرع من مستضد لقاح الـ BCG (40,20) على التوالي , ومن خلال هذه الدراسة نستطيع القول بان مستضد لقاح الـ BCG يمكن ان يحفز استجابة الأضداد النوعية , كما يمكن اعتبار استجابة الأضداد مؤشرا جيدا لأختبار القابلية التمنيعية لمستضدات معينة.