Study of Some Changes in Spleen among Golden Hamsters Vaccinated with Killed *Leishmania tropica*

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**Abstract**

Different factors related with spleen (histopathological changes, spleen weight ratio, length, number of parasite, impression and culture of spleen) were screened in four groups of golden hamsters, inoculated with one, two and three doses of different antigens as following: Group 1 inoculated with autoclaved killed *Leishmania tropica*, Group 2 inoculated...
with BCG vaccine alone while Group 3 inoculated with mixed vaccines (autoclaved killed *Leishmania* + BCG) and Group 4 inoculated with phosphate buffer saline which considered as control group, in order to evaluate the efficacy of the previous antigen against challenge infection with virulent *Leishmania tropica* isolate along five times follow up (30, 45, 60, 75, and 90) days after infection. Group 3 which inoculated with one, two and three doses of mixed vaccine, was considered as the best vaccine in this study because animal inoculated with this vaccine showed the following results compared with other antigens:

- Low or moderate histopathological changes.
- Less significant spleen weight ratio (1.36±0.1) mg/gm, (1.24 ±0.08) and (1.21±0.05) mg/gm respectively.
- Less significant spleen length (24.4±0.07) mm, (23.7±0.6) mm and (23.4±0.1) mm respectively.
- Less but no significant amastigote count (1.4 ±0.01), (1.32±0.05) and (1.42±0.03) million parasites respectively.
- Negative spleen culture along 90 days of following up.

**INTRODUCTION**

Leishmaniasis is a group of diseases caused by over 20 known species of pathogenic protozoan parasites of the genus *Leishmania* with diverse clinical features ranging from self-limiting cutaneous leishmaniasis to visceral disease(1,2). The various species of *Leishmania* are transmitted by sand flies, amastigotes, liberated from host cells in the insect’s gut, transform into promastigotes, which multiply there and finally introduced into a new host when sandfly again feed(3). The importance of *Leishmania* as a human pathogen has stimulated a large number of researches deal with immunization against Leishmaniasis especially in experimental animals (4, 5, 6).

The spleen is one of the organs that determine the severity of the *Leishmania* infection, because it contains a large proportion of phagocytic cells (7).

Evaluation the success of vaccines against Leishmaniasis is not so easy, 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 and following up of experimentally infected animal after immunization (8,9,10). In this paper we addressed the use of some changes in spleen to evaluate the success of vaccine against experimental infection.
of *Leishmania tropica* using three different antigens with one, two and three doses for each antigens.

**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 [11].

**Media**

1- **Semi –Solid medium:** This medium was prepared according to the method of Alder & Theodor, 1926 [12] and was used for parasite isolation from man and reclaim from the infected animal tissue.

2- **Biphasic Medium:** This media was prepared according to method of Kagan & Normab, 1970 [13] and used for parasite cultivation in order to prepare vaccine antigens and for preparing injecting dose.

**Autoclaved Killed Leishmania (AKL) Vaccine preparation**

Mohebali , *et al*., (1998) method (14) was used in preparing *Leishmania tropica* vaccine with little modification according to AL-Warid, (15) as following:

1. Promastigote of *Leishmania tropica* was cultivated in Biphasic Medium at 26°C instead of RPMI-1640.
2. Promastigotes were harvested and concentrated using centrifuge at 3200 rpm.
3. The promastigotes were washed five times with phosphate buffer saline (PBS), and counted using Haemocytometer to get the final concentration of immunization dose which was 1×10^7 parasites / 0.2 ml.

The promastigotes were separated and transferred into several autoclavable containers and was put in autoclave 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 Laboratory" each vial contained (0.5) mg of lyophilized vaccine and each 1 ml of vaccine contained 8.26 × 10^6 cells of *Mycobacterium bovis*.

**Animals**

One hundred eighty male of Golden hamsters (*Mesocricetus auratus*) aged (8-10) weeks were obtained from "The National Centre for Drug Control and Researches / Baghdad / Iraq". Animals were separated into four groups each group contained (45) animal which inoculated as following:

1- Group (1): inoculated with Autoclaved killed *Leishmania* (AKL) per 0.2 ml.
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2- Group (2): inoculated with \(1.4 \times 10^6\) cell per 0.2 ml.

3- Group (3): inoculated with mixed inoculums of both \((1 \times 10^7\) AKL/0.2 ml) and \((1.4 \times 10^6\) unit of BCG/0.2 ml).

4- Group (4): was considered as control group. Animals in this group were inoculated with 0.2 ml phosphate buffer saline.

One, two and three doses for each of 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 immunization, all animals were inoculated with (challenge dose) \(5 \times 10^7\) promastigotes of virulent *Leishmania tropica* isolates / 0.2 ml.

**Dissections**

All animals of the four groups were dissected along five times of follow up (30, 45, 60, 75, and 90) days after challenge infection, the follow up of *L. tropica* infection was done using different parameters:

1- Histopathological of the spleen (5), for histopathological examinations a biopsy was taken at the site of infection fixed in 10% formalin in PBS, washed in water for 4 hours, dehydrated and embedded in paraffin, cut (3-4μm thick) and stained with hematoxylin and eosin for optical microscopic examination (16).

2- Spleen weight ratio (spleen weight (mg) / body weight (gm)) (17)

3- Spleen length (7)

4- Estimated number of amastigote in spleen (18).

5- Impression and cultivation of spleen (17)

**RESULTS AND DISCUSSION**

Golden hamsters were used in this study because some investigators showed that these animals were the suitable host for *Leishmania tropica* experimental design (19). As well as gender and age of the hosts are also play an important role in the immune response stimulated by *Leishmania* (20, 21), so male aged 8-10 week was used in this study.

Promastigotes (infected stage of *Leishmania spp.*) were used as killed antigen, 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 (22), 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 (23).

Spleen showed sever histopathological changes in both control animals and animal administrated with BCG from the fifteen days post
infection till the end of the following up period, these changes represented by white pulp dilatation, infiltration of macrophage and lymphocytes, follicular hyperplasia, blood congestion and fibrosis, while both AKL and mixed vaccine administrated animal in different boosting dose showed low or moderate histopathological changes in their spleens (figure 1, 2 and 3), this result agreed with other studies done in Iraq (24, 25) who showed moderate histopathological changes in immunized animal with live attenuated vaccine when it compare with non immunized group. The moderate histopathological changes which showed in AKL and mixed vaccine administrated animal may due to high level of T-lymphocyte which produced interleukins that in turn activate macrophage which reduce the number of amastigote in spleen (9, 15).

**Figure 1:** Section in spleen of mixed vaccine administrated group, showing white pulp dilation after 60 days post infection, Hematoxylin - Eosine stain (400 X)

**Figure 2:** Section in spleen of BCG vaccine administrated group, showing hyperplasia and hyper trophy of spleen cells after 60 days post infection, Hematoxylin - Eosine stain (400 X)
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![Image](209x594 to 408x737)

**Figure 3: Section in spleen of Control group, showing white pulp dilatation, infiltration of macrophage and lymphocytes after 60 days post infection, Hematoxylin-Eosine stain (400 X)**

Spleen weight ratio, spleen length and number of parasites in spleen were scored also during five times follow up (30, 45, 60, 75, and 90) days after challenge infection with virulent *Leishmania tropica* isolate. Statistical analysis showed that there was a significant differences (p≤0.05) among these parameters in experimented animal group following up intervals.

Animal inoculated with one, two and three doses of mixed vaccine showed less spleen ratio as their rat were (1.36±0.1) mg/gm, (1.24 ±0.08) and mg/gm (1.21±0.05) mg/gm respectively, Animal inoculated with one, two and three doses of AKL showed the following spleen ratio respectively (2.31 ±0.02) mg/gm, (1.73±0.14) mg/gm and (1.69±0.18) mg/gm, while the three control group showed high spleen weight ratio (4.04 ±0.7) mg/gm, (4.3 ±0.12) mg/gm and (4.73) mg/gm respectively. Animals inoculated with one, two and three dose of BCG vaccine showed the following spleen ratio (1.95 ±0.07) mg/gm, (2.04 ±0.12) mg/gm and (2.62±0.31) mg/gm respectively after 75 day post infection (figure 4). Statistical analysis showed that was a significant differences (p≤0.05) among the experimented animal groups and this results agreed with Tonui & Titus (26) who showed that spleen weights in control mice increased over time, while spleen weights in mice immunized with *L. major* promastigote exogenous antigens(LmSEAgs) remained relatively constant.
Animal inoculated with one, two and three doses of mixed vaccine showed minimum spleen length as their values were (24.4±0.07) mm, (23.7±0.6) mm and (23.4±0.1) mm respectively which were approximately equal to the normal value of spleen length (24.2±0.33). Animal inoculated with one, two and three doses of AKL showed the following spleen length (28.6 ±0.1) mm, (28.5±0.6) mg/gm and (28.3±0.59) mm respectively, while the three control group showed maximum spleen length (34.2 ±0.14) mm, (34.3 ±0.22) mm and (35.9±2.7) mm respectively, followed by animals inoculated with one, two and three dose of BCG vaccine which showed the following spleen length (33.6 ±0.46) mm, (31.11 ±0.22) mm and (35.9±2.7) mm respectively, after 75 day post infection (figure 5). Statistical analysis showed that was significant differences (p≤0.05) among the experimented animal groups. This result agreed with Al-Najjar who showed that both ultraviolet Leishmania vaccine and gamma Leishmania vaccine animal group had the lowest spleen length values compared with highest spleen length values in control groups.

Reductions in spleen weights and lengths in mixed antigen vaccinated hamsters with one, two and three doses were also accompanied by very low
parasite burdens, where it reached (1.4 ±0.01), (1.32±0.05) and (1.42±0.03) million parasites respectively. Animals inoculated with one, two and three doses of AKL showed the following parasites number in spleen (2.31±0.01) mm, (2.18±0.01), (2.4±0.0) respectively, while the three control group showed maximum amastigote count (4.62 ±0.18) mm, (5.2±0.04) and (4.9±0.9) million parasites respectively. Animals inoculated with one, two and three dose of BCG vaccine which showed the following amastigote count (3.5 ±0.09), (4.49 ±0.22) and (3.2±0.01) million amastigotes respectively, after 75 days post infection (figure 6). Although there were big differences in number of parasites, statistical analysis showed that there was no significant differences (p≤0.05) among the experimented animal groups.

These results agreed with the result of Afrin & Ali (27) who noticed that increase in parasite burden in the control hamsters paralleled an increase in the weight of the spleen, similarly, the Leishmania Antigen-liposome-immunized group with significantly reduced parasite load had spleens comparable to those of normal, uninfected hamsters, and also agreed with Tonui & Titus (26) who noticed that there was very low parasite burdens, as much as a 4,913-fold reduction in parasite burden in L. major promastigote exogenous antigens (LmSEAgs)-immunized mice compared with controls (P<0.001). Furthermore other study showed significant lower parasite burdens in both spleens and livers compared with non-immunized mice or mice injected with adjuvant alone (28). The moderate changes (spleen weight ratio, spleen length and count of parasites) in spleens of immunized animals especially the mixed group and AKL vaccinated group can be discussed as both mixed and AKL antigens have the ability to provoke high level of T- lymphocyte which activate both macrophage and natural killer cells before reducing the number of parasite in spleen.

Figure 6: Graph showing the number of amastigote in four study groups at five successive follow up periods after challenge.
Finally spleen impression and cultivation of spleen were scored during six times follow up (15, 30, 45, 60, 75, and 90) days after challenge infection with virulent *Leishmania tropica* isolate, results of spleen impressions were negative up to 15 days of infection in animals immunized with different dose of each (AKL) and (AKL+BCG). While spleen cultivation results were negative in the above mentioned animals along 90 days of follow up (table 1). These results may correlate with the high levels of lymphocytes and macrophages which induce different immunological factors that killed the parasites especially in spleen, which have large amount of macrophages (29). These factors can reduce or prevent the transforming of amastigotes to promastigotes in culture. This may be the reason of the negative cultivation results in the spleen belonging to the animals immunized with different doses of each (AKL) and (AKL+BCG).

Table 1: Impressions and parasite culture of spleen after challenge infection

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Study of Some Changes in Spleen among Golden Hamsters Vaccinated with Killed 

*Leishmania tropica*

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AKL : Autoclaved killed *Leishmania*

PBS: phosphate buffer saline

REFERENCES

11. Al-Alousi, R.S. "Investigation into possible existence of more than one strain of Leishmania tropica in Iraq" M.Sc. thesis University of Baghdad(1979).
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