Plant Archives Volume 20 No. 2, 2020 pp. 6492-6494 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

THE ROLE OF ANTI-INTERLEUKIN-4 IN BALB/C MICE INFECTED WITH VISCERAL LEISHMANIASIS

Manahel H. Mjefy* and Altamemy A.K. Aakool

Department of Biology, College of Science, University of Wasit, Iraq.

Abstract

Leishmaniasis is endemic in Iraq with the visceral form of the disease, caused by the *L. donovani* complex, is principally endemic in the central part of the country. 45 mice (male) were divided into three groups, the first was injected with (3×10^6) promastigote as control positive, the second was injected with (3×10^6) promastigote and treated with anti-interleukin 4 and the third was injected with PBS as control negative. After 30, 45 and 60 days the serum was taken and examined by the ELISA test to find out the level of interferon gamma (IFN-γ).

The present study revealed that the level of (IFN-γ) at day 30, was higher in group 2 (539.45±2.352), than in group 1 (173.26±1.21) and group 3 (109.69 ± 0.25). At day 45, the level recorded in group 2 (393.89±2.012), than group 1 (286.6±1.521) and group 3 (109.93±0.22). At day 60, the level was in group 2 (259.48±1.841), than group 1 (310.22±1.632) and group 3 (109.69±0.23). The aim of present study was to determine the efficient role of anti IL-4 on IFN-γ activity in mice infected with *L. donovani*. We concluded our results that the level of interferon gamma (IFN-γ) was higher in group 2 at all periods.

Key words: Visceral leishmaniasis, interferon gamma, anti-interleukin 4, ELISA, mice.

Introduction

Visceral leishmaniasis consider as a vital community health problem in Iraq and the foremost lethal form, classically which is, known as Kala-azar or black fever (Jarallah, 2016), caused by *Leishmani donovani* complex that include three specie; L. *infantum,* L. *chagasi* and L. *donovani*. This form of the infection is the majority severe shape, it is progressive and can be deadly if not treated will typically result in death and the fatality rate can be as high as 100% (Chappuis *et al*., 2007).

The life cycle of *Leishmania* parasites is distinguished by its alternating generation have and exist in two particular shapes: the amastigote in human host and the promastigote in sand fly vector (Dostálová and Volf, 2012). During a blood feeding, motile *Leishmania* promastigotes are exchanged from the sand fly into a human or animal host where they are phagocytized by neutrophils, macrophages, or dendritic cells; *Leishmania* parasites distinguish into amastigotes and reproduce inside phagolysosomes, finally rupturing the host cell and releasing the parasites (Halsey, 2019).

The classical symptom of visceral leishmaniasis includes; delayed fever, serious weight loss, hepatosplenomegaly, anemia, leucopenia and hypergammaglobulinemia (Andargie and Ejara, 2015). Immunity against *leishmaniasis* requires Th1 induced IFN-γ for activation of infected macrophages to produce nitric oxide (NO), antileishmanial immunity is mediated through both innate (macrophages, dendritic cells (DCs) and neutrophils) and adaptive (T cells) immunity, but the CD4+ T cell subset is crucial for resistance (Muli, 2013). IL-2 and IFN-γ were related to Th1 activation and disease healing, whereas IL-4 and IL-5 were related to Th2 activation and disease progression (Castellano *et al*., 2009).

Most of the drugs available to treat *leishmaniasis* are not able to reliably remove the infection, but they are able to reduce the load of parasites (Miro *et al*., 2008). For this time, no effective vaccine has been found against at all form of *leishmaniasis* for general human use (Kevric *et al*., 2005).

Materials and Methods

In total, group of (45) BALB/c mice, males, (6-8) weeks old and weight range about 25-30g, divided to three subgroup (15 mice of each group).

***Author for correspondence :** E-mail: manahelhadi58@gmail.com

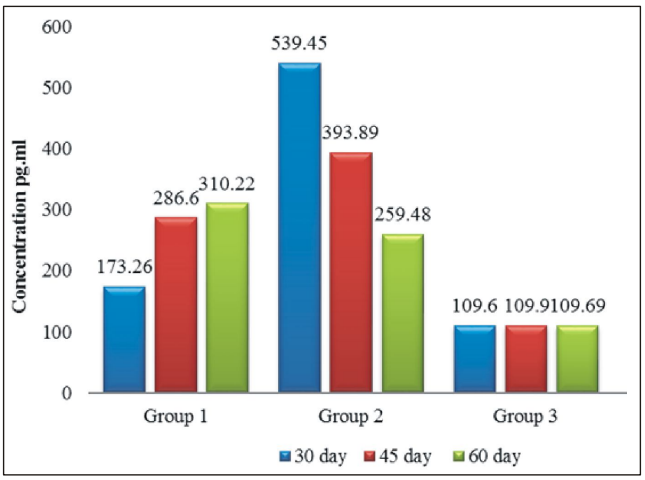

Fig. 1: Level of interferon gamma in 30, 45 and 60 days.

Group 1 injected (3×10^6) promastigotes as control +, group 2 Injected (3×10^6) promastigotes and treated (50) µg of anti IL-4 intraperitoneally, twice weekly after the first week of infection for four connection weeks post-infection and group 3 Injected (0.25) ml phosphate buffer saline only as control. Blood samples were collected from five mice of each group for (30-45-60) days post-infection to determine the level of interferon gamma (IFN-γ), by ELISA test.

Results

A total of 45 sample of serum taken from mice was examined by ELISA and detected the level of INF-γ. In our study the result indicated that, the level of interferon gamma INF-γ during time periods (30, 45 and 60) days in three groups,in group (3) negative control there is no significant difference in level of INF-γ during time periods, in group (2) infected mice and treated with anti IL-4 enhanced to INF-γ production and peak at 30th day that help the mice to control infection then began to decrease until 60th day, which lead to progressive the disease, while in group (1) infected mice observed the level of INF-γ was low, this associated with parasites multiply rapidly during the first weeks, after four weeks recover their ability to produce IFN-γ, reach to the peak at 60th day when parasite growth was controlled.

Discussion

Leishmaniasis is endemic in 98 countries and 350 million people are at risk of contracting this disease, according to the World Health Organization. Each year,

Table 1: Distribution the level of INF-γ in 30 days.

Groups	Mean±SEM	LSD
Group 1 (control +) **	173.26±1.21	0.564
Group 2 (Anti-IL4) ***	539.45±2.352	2.251
Group 3 (control -) *	109.69±0.25	0.025

Table 2: Distribution the level of INF-γ in 45 days.

Groups	Mean±SEM	LSD
Group 1 (control +) **	286.6±1.521	1.521
Group 2 (Anti-IL4) ***	393.89±2.012	1.652
Group 3 (control -) *	109.93±0.22	0.024

approximately 58,000 cases of visceral leishmaniasis are diagnosed. It is usually, accepted that IFN-γ is required for the management of and therefore protection from *Leishmania* infections, IFN-γ is one of the Th1 prototype cytokines responsible for activating macrophages, resulting to an efficient killing of the parasites (Holzmuller *et al*., 2005). The mechanisms of control of *Leishmania* parasites in mice are very clear and require the activation of Th1 responses, which leads to iNOS up regulation by phagocytic cells (Kaye and Scott, 2011). The low level of IFN-γ in group 1 (Table 1), agreement with Loeuillet *et al*., (2016) through first 4 weeks after starting infection, parasite replication is associated with, the immune cell inability to produce IFN-γ and IL2 (macrophage-activating cytokines), whereas production of IL4 or IL5 is preserved. Treatment with anti IL-4 induces a resistance phenotype with matching induction of INF-γ (Fromm, 2010), this agreed with our results that appeared the level of interferon gamma significant increased in group (2) in comparative with group (1) control + and group (3) control – in table 1.

After the first 4 weeks of infection, CD4+ T, CD8+ T and natural killer (NK) cells recover their capacity to produce IFN-γ, thus promoting the macrophage microbicidal activity with (NO) synthesis (Kopf *et al*., 1996), this observed in group (1) (Table 2).

Mice treated with anti-IL-4 antibody, demonstrating that they heal infection. This explains the significant increase in the level of interferon gamma in group (2) in comparative with other groups (Faleiro *et al*., 2014).

During experimental VL in genetically susceptible mice, parasite growth was controlled after 6-8 weeks post infection (Engwerda *et al*., 2004), this suggested agree with our result, that observed in (Table 2) group (1) the level of INF-γ increased in compare with first 4 weeks.

In visceral *leishmaniasis*, the infection will be resolved within 6 to 8 weeks due to development of Th1, then granuloma response will occur, which is caused by the production of IFN-γ, this suggested agree with our

Table 3: Distribution the level of INF-γ in 60 days.

Groups	Mean±SEM	LSD
Group 1 (control +) **	310.22±1.632	2.312
Group 2 (Anti-IL4) ***	259.48±1.841	1.325
Group 3 (control -) *	109.69±0.23	0.025

result, that observed in (Table 3) group (1).

The anti-IL-4 treated mice eventually developed progressive infection, as evidenced by the increase in tissue parasites at 8 wk (Heinzel *et al*., 1989), this agreement with our result, that observed in (Table 3) group (2) the level of INF-γ decreased in day 60.

Ethical Clearance

The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Andargie, T.E. and E.D. Ejara (2015). Pro-and anti-inflammatory cytokines in visceral leishmaniasis. *Journal of Cell Science & Therapy*, **6(3):** 1.

Castellano, L.R., D. Correia Filho, L. Argiro, H. Dessein, A. Prata, A. Dessein and V. Rodrigues (2009). Th1/Th2 immune responses are associated with active cutaneous leishmaniasis and clinical cure is associated with strong interferon-γ production. *Human immunology*, **70(6):** 383-390.

Chappuis, F., S. Sundar, A. Hailu, H. Ghalib, S. Rijal, R.W. Peeling, J. Alvar and M. Boelaert (2007). Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? *Nature reviews microbiology*, **5(11):** 873-882.

Dostálová, A. and P. Volf (2012). Leishmania development in sand flies: parasite-vector interactions overview. *Parasites & vectors*, **5(1):** 276.

Faleiro, R.J., R. Kumar, L.M. Hafner and C.R. Engwerda (2014). Immune regulation during chronic visceral leishmaniasis. *PLoS neglected tropical diseases*, **8(7)**.

Fromm, P.D. (2010). Dissociation of interferon-gamma production and resistance to leishmaniasis in the absence of tumor necrosis factor (Doctoral dissertation, James Cook University).

Engwerda, C.R., M. Ato, S. Stäger, C.E. Alexander, A.C. Stanley and P.M. Kaye (2004). Distinct roles for lymphotoxin-α and tumor necrosis factor in the control of *Leishmania donovani* infection. The American journal of pathology, **165(6):** 2123-2133.

Halsey, G. (2019). Use of the ITK/BTK Inhibitor Ibrutinib for the Treatment of Experimental Visceral Leishmaniasis Caused by *Leishmania donovani* (Doctoral dissertation).

Heinzel, F.P., M.D. Sadick, B.J. Holaday, R.L. Coffman and R.M. Locksley (1989). Reciprocal expression of interferon gamma or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. *The Journal of experimental medicine*, **169(1):** 59-72.

Holzmuller, P., M. Cavaleyra, J. Moreaux, R. Kovacic, P. Vincendeau, G. Papierok and J.L. Lemesre (2005). Lymphocytes of dogs immunised with purified excreted-secreted antigens of *Leishmania infantum* co-incubated with Leishmania infected macrophages produce IFN gamma resulting in nitric oxide- mediated amastigote apoptosis. *Veterinary immunology and immunopathology*, **106(3-4):** 247-257.

Jarallah, H.M. (2016). Pathological Effects of Leishmania donovani promastigotes on liver and spleen of experimentally infected Balb/c mice. *Medical Journal of Babylon*, **13(1):** 134-140.

Kevric, I., M.A. Cappel and J.H. Keeling (2015). New world and old world Leishmania infections: a practical review. *Dermatologic clinics*, **33(3):** 579-593.

Kaye, P. and P. Scott (2011). Leishmaniasis: complexity at the host–pathogen interface. Nature Reviews Microbiology, **9(8):** 604-615.

Kopf, M., F. Brombacher, G. Kohler, G. Kienzle, K.H. Widmann, K. Lefrang, C. Humborg, B. Ledermann and W. Solbach (1996). IL-4-deficient BALB/c mice resist infection with *Leishmania major*. The Journal of experimental medicine, **184(3):** 1127-1136.

Loeuillet, C., A.L. Bañuls and M. Hide (2016). Study of *Leishmania pathogenesis* in mice: experimental considerations. *Parasites & vectors*, **9(1):** 144.

Miro, G., L. Cardoso, M.G. Pennisi, G. Oliva and G. Baneth (2008). Canine leishmaniosis-new concepts and insights on an expanding zoonosis: part two. *Trends in parasitology*, **24(8):** 371-377.

Muli, M.J. (2013). Evaluation of crude *Leishmania donovani* antigen co-administered with th1 adjuvants as a potential vaccine for leishmaniasis in vervet monkey model (doctoral dissertation, kenyatta university).