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An *in vitro* study on suppressive effects of *Leishmania major* on IL-2Rα expression on peripheral human T lymphocyte

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Abstract. *Leishmania* sp. is an intracellular protozoan parasite that causes significant morbidity and mortality in many parts of the world. The parasite can escape from host immune system by several mechanisms. Understanding biological behavior of the parasite can help us to control and treatment leishmaniasis. Therefore current study was conducted to determine suppresive effect of *Leishmania major* on IL-2Rα expression in the human peripheral T Lymphocytes. Human peripheral T Lymphocyte were co-cultured with standard strain of *Leishmania major* (MRHO/IR/75/EK) in RPMI1640 medium. Infected cells were stained with FITC-labelled anti-CD25 (IL-2Rα chain MAb) and Picoerithrin-labelled anti-CD_4 (CD_4 MAb) and analyzed by flow cytometry. The results showed that *L. major* suppressed IL-2Rα expression in activated T cells as well as inhibited lymphocyte proliferation 6h after infection and was increased up to 36 hour later. This finding also indicated that suppressed IL-2R expression was increased when the number of promastigote was added up to 7.5×10^6 cells/ml. Inhibition of IL-2R expression by the parasite might play a critical role for escaping from host immune system. Understanding biological characterization of the Leishmania can be useful for vaccine development and also cytokine therapy.

INTRODUCTION

Leishmaniasis is one of the most important zoonotic diseases which is caused by an obligate intracellular protozoan parasite. The parasite replicates in parasitophorous vacuoles within mononuclear phagocyte cells such as macrophage, langerhans cells and monocytes (Abdullah *et al.*, 1998; Antoine *et al.*, 1998). The disease has worldwide distribution specially existing in tropical and subtropical regions. The clinical signs of leishmaniasis were included cutaneous, mococutaneous and visceral disorder (Giorgio *et al.*, 2000). The parasite approximately infects 12 million people with 1.5-2 million new cases in a year. About 350 million people live in risk zone. The disease is endemic in 88 countries of 5 continents. More than 90% of cutaneous leishmaniasis patients live in Iran, Afghanestan, Syria, Saudi Arabia, Brazil and Peru (Umakant & Sarman, 1998). Twenty one *Leishmania* species have been identified as pathogens for human. Cutaneous leishmaniasis is a self-limiting disease but visceral leishmaniasis is lethal if left untreated (Umakant & Sarman, 1998).

Leishmania parasites live in hostile environment such as mononuclear phagocyte cells and they have great potential for escaping from the host immune system. Glycoprotein 63 kd (metalloproteinase), LPG

(lipophosphoglycan) and cystein protease are major tools of *Leishmania* sp. to survive in mononuclear cells (Descoteaux *et al.*, 1999; Poot *et al.*, 2005; Laurence & Buxbaum, 2006).

The interaction between immune responses of host and parasite defines resistance or sensitivity outcome. The activity of Th1 responses cause control of infection but Th2 responses are associated with progressive disease (Awasthi *et al.*, 2004). One effective evade mechanism of *Leishmania* sp. is suppression of IL2 production and reduction of IL2 receptors expression on stimulated T cells.

The role of IL2 and its receptors are essential for proliferation of T cells by promoting G1 to S cell growth cycle. Stimulation of NK (Natural killer cells), IFN γ production, and T and B lymphocytes for killing intracellular parasites are the other immune role of IL2 (Murray *et al.*,1987). The biological role of IL2 is performed via its receptors IL2-R (Cillari *et al.*,1986; Murray *et al.*, 1993). IL2-R is constituted of three hetrodimer protein domains called α (TAC Antigen or CD 25), β (CD122) and γ (CD132) chain. Each chain alone has low affinity for attachment to IL2 cytokine and βγ dimer have intermediate affinity for it but a complex of IL2R αβγ attaches with high affinity to cytokine and is essential for induction of initiation signal to stimulate proliferation of T cells (Cillari *et al.*,1986; Murray *et al.*, 1993). Many investigations indicated that human immune system was suppressed by *Leishmania* sp. in the first week after infection because of suppressed IL2 production (Carvalho *et al.*,1985; Cillari *et al.*,1988, 1991). Patients with visceral leishmaniasis have shown a decrease on IL2 production (Smith 1980; Harel-Bellan *et al.*, 1983) but controversy in some study the production of IL2 was not reduced (Leichuk *et al.*, 1984). The production of IL2 is reduced by other infectious diseases such as trypanosomiasis (Watson *et al.*, 1985), malaria (Mohagheghpour *et al.*, 1985), histoplasmosis (Reiner & Finke, 1983) and lepromatosus leprosy (Smith 1988). Experimental infection in mice with *Leishmania donovani* indicated that spleen cells produce lower levels of IL2 than normal group (Abbas *et al.*, 2007).

Treatment of the disease is taken place by classes of anti Leishmania drugs such as Glucantim, Amphotricne B, Stibogluconate with major side effects and drug resistant problems. In the other hand, the prevention of disease by control of vectors and reservoir host is very difficult because of variety in geographical distribution of vector and reservoir host. There have been attempts to produce an effective conventional vaccine for human immunization but unfortunately it is not achievable yet. Thus understanding parasite escaping ways from host immune system is needed for designing a perfect treatment against leishmaniasis. Therefore, we decided to investigate the suppressive effect of *Leishmania major* promastigotes on IL2-R α (CD25) expression in T lymphocytes and their inhibitory action on T cells proliferation. The findings of this study shall increase our knowledge about parasite biological behavior and thus help in controlling the disease.

MATERIAL AND METHODs

Leishmania parasites

The standard strain of *L. major* (MRHO/IR/75/EK) was kindly provided by Parasitology Department of Shiraz University of Medical Sciences, Shiraz, Iran. Promastigotes were cultured in conventional medium (NNN) at 25ºC and PH 7.2 for five days. After this time, the promastigotes were collected and cultured in RPMI 1640 medium containing penicillin 100 IU/ml , streptomycine 100 IU/ml, FCS (fetal calf serum) 10% inactivated by heating (56ºC for 20 min). The number of promastigotes were counted microscopically with hematocytometer and viability of organisms were tested by trypan blue staining. Samples viability more than 99% were accepted for examination.

Isolation of peripheral blood mononuclear cells (PBMC):

PBMC was separated from heparinated blood of healthy volunteers by density gradient – centrifuging using Ficoll-Hypaque with a

density of 1.077. The PBMC were washed three times with RPMI 1640 and viability of cells was determined by trypan blue staining and cells with more than 90% viability were used for examination. The cells were suspended at a concentration of 1×10^6 cells/ml and transferred to a 75 ml flask. After culturing , the cells (1.25×10^6 cells) were seeded in 96 well plates and incubated at 37ºC, 5% CO2 for various times with PHA (Phytohaemagglutinin 30-50µg/ml) for lymphocyte stimulation with or without the various dilutions of *L. major* promastigotes.

Reagents

Purified monoclonal antibody (mAb) and mouse anti-human antibody conjugated with FITC for CD25 (Anti-TAC (FITC conjugated) were obtained from Dako/Denmark. Purified monoclonal antibody (mAB) and mouse anti-human antibody conjugated with Phycoeritrin for CD4 (Anti-TAC (FITC conjugated) were purchased from Dako Denmark for flow cytometry test.

Flow cytometry

After incubation of PBMC at 37ºC and 5% CO2 with parasites and PHA, non adherent cells were recovered and washed three times with phosphate buffered saline solution containing 1% bovine serum albumin and spun at 1500 rpm for 3 min. The cells were stained with appropriated MAb (Anti-TAC) and MAb (Anti-CD4) conjugated with FITC (Fluorescein isothiocyanate) and phycoerythrine respectively for 30 min at 4ºC in dark place and followed by one washing with phosphate buffered saline solution. The cells were fixed by 1% formaldehyde and were prepared for flow cytometry (Partec Germany class 1 laser producer) technique. The obtained results from flow cytometry were analyzed statistically by Flomax® and WIN MDI soft ware version 2.8. A minimum of 10,000 cells were gated. Percentages of decrease in the proportion of cells expression were calculated by using the following equation:

Percentage of suppression = (lymphocyte + PHA) % – (lymphocyte+PHA+Parasite)*100/ (lymphocyte + PHA) %

RESULTS

Suppressive effect of *Leishmania major* on IL-2Rα expression of stimulated T lymphocyte by PHA (phytohemaglotinin) in various incubation times.

The finding of flow cytometry showed a reduction of IL2-Rα expression in infected T lymphocyte. The most suppressive effects were seen at 24-36 hours after incubation. The results of suppressive effects of *Leishmania* promastigotes on expression of IL2 receptor are shown in Table 1. The most suppressive effects of *Leishmania* promastigote were obtained in logarithmic phase of promastigote culture. The suppression percentages at 12, 24, 36 and 48 hours after infection were 27.22%, 47.95%, 44.22% and 16.65% respectively (Table 2). ANOVA test indicated significant difference between test and control groups in reducing IL2-Rα expression on T lymphocytes. The tests were repeated three times.

The suppression potential of *L. major* on expression of IL2-Rα on T lymphocyte according to various dilutions of promastigotes

The flow cytometry finding indicated that the increase in number of promastigotes has a

Table 1. The percentage of IL2-α receptor on T cells in various incubation time infected by *L. major* (Tcells= $2/5\times10^6$/ml, *L. major*=5×10^6/ml)

	Time (hour)	Lymph+PHA	Lymph+PHA+*L. major*	Percentage of suppression
A	12	76.23	55.48	27.22
B	24	49.26	25.64	47.95
C	36	67.62	37.72	44.22
D	48	51.81	35.16	16.65

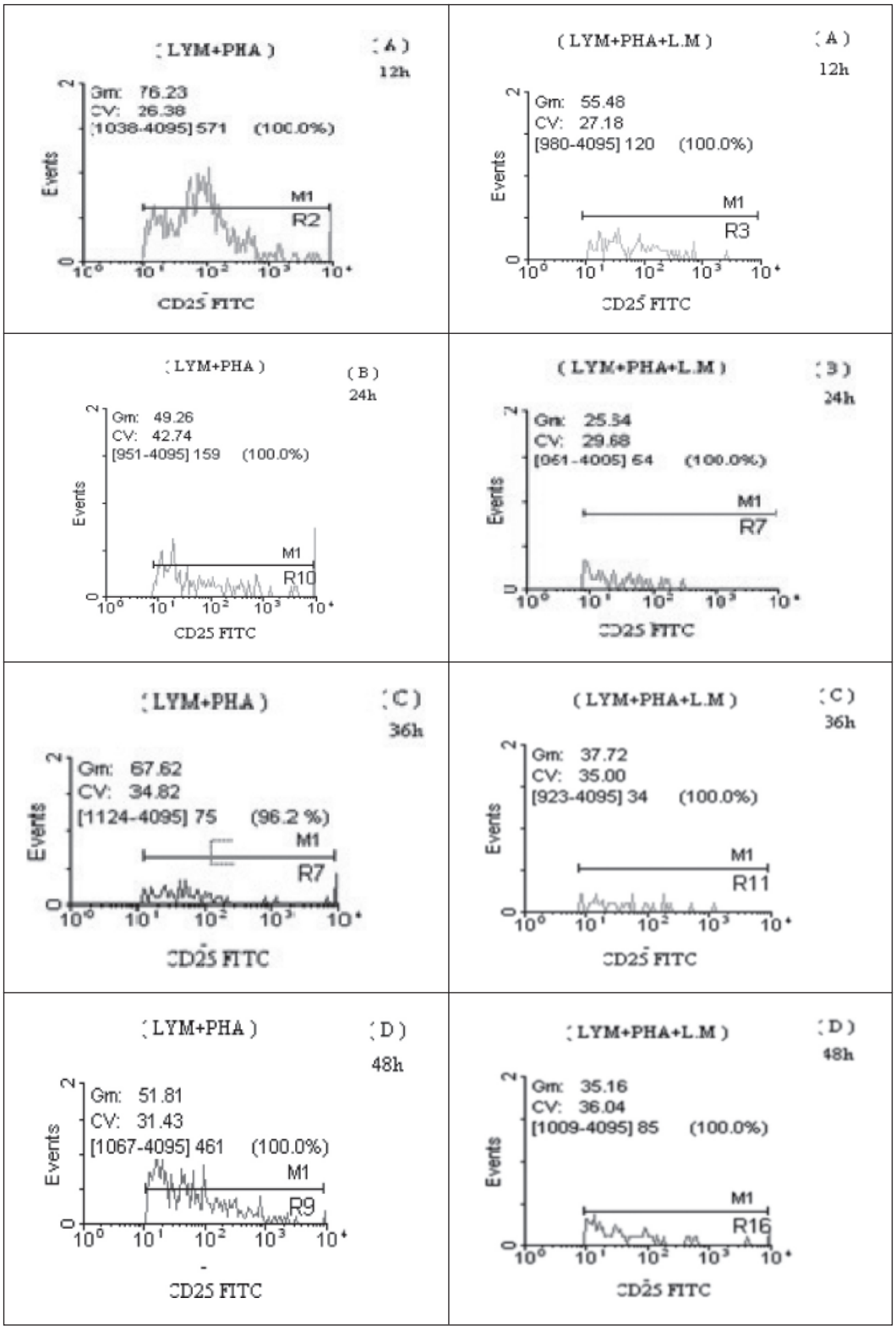

Figure 1. The suppression effect of Leishmania promastigotes on expression of IL2 receptor on T lymphocytes in various time incubation in flow cytometry test

direct effect on suppression potential of *L. major* and the maximal effect was obtained in 7.5×10^6 cells/ml concentration. The suppression rate was 26.47%, 42.08% and 50.65% for 2.5×10^6 cells/ml, 5×10^6 cells/ml and 7.5×10^6 cells/ml dilution respectively in triple examination ($p<0.05$). The detail data was shown in Table 2 and Figure 2.

The reduction in proliferation of infected T lymphocyte by *L.major* promastigotes

The proliferation assay was performed in various dilutions of promastigotes numbers. The suppression of lymphocyte proliferation increased according to concentration of promastigotes when compared to control group ($P<0.05$). The maximal effect was

Table 2. The suppression effect of various concentration of *L. major* promastigotes on expression of IL2-Rα on T lymphocyte

	Promastigotes concentration	(Lymph+PHA)	(Lymph+PHA+*L. major*)	Percentage of suppression
A	2.5×10^6	55.40	40.00	26.47
B	5×10^6	55.40	32.02	42.08
C	7.5×10^6	55.40	27.34	50.65

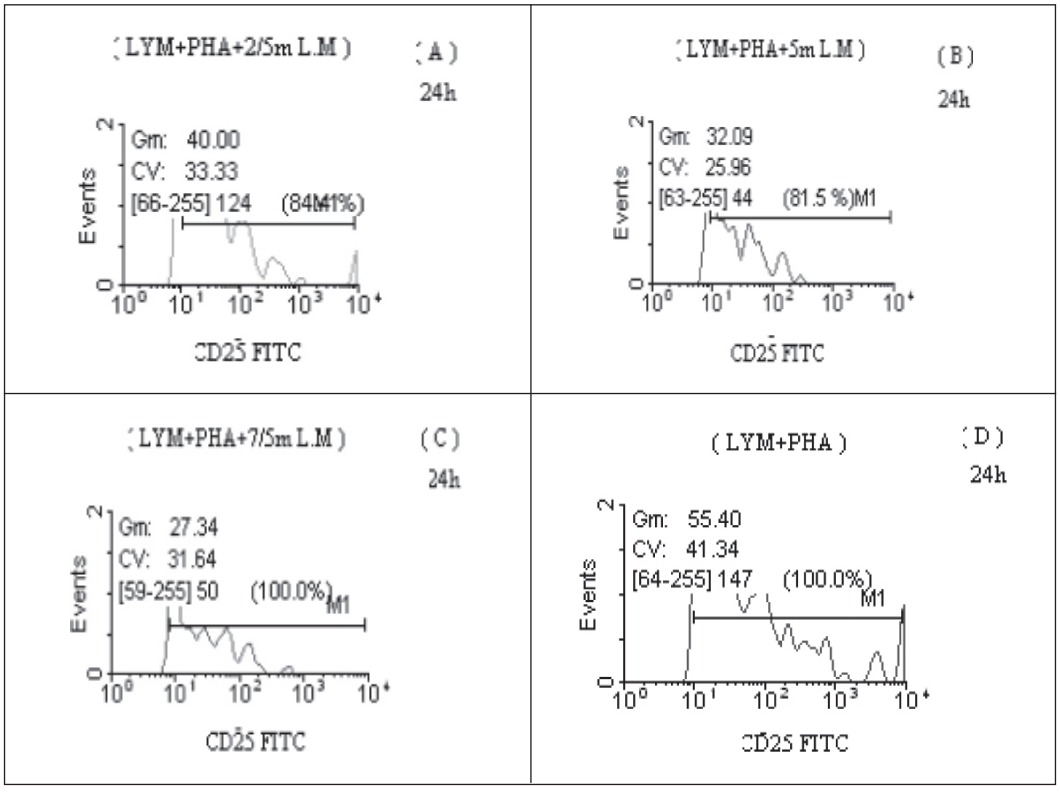

Figure 2. The suppression effect of various concentration of *L. major* promastigotes on expression IL2-Rα T lymphocytes in contrast to control group in flow cytometry test

Table 3. The suppression potential effect of various concentration of *L. major* promastigotes on T lymphocytes (1.25×10^6 /ml) proliferation

Incubation Time (hour)	Promastigotes concentration	(lymph+PHA)	(lymph+PHA+*L. major*)	Percentage of suppression
24	$2/5\times10^6$	39/59	26/53	32/99
24	5×10^6	39/59	20/94	48/88
24	$7/5\times10^6$	39/59	15/41	61/07

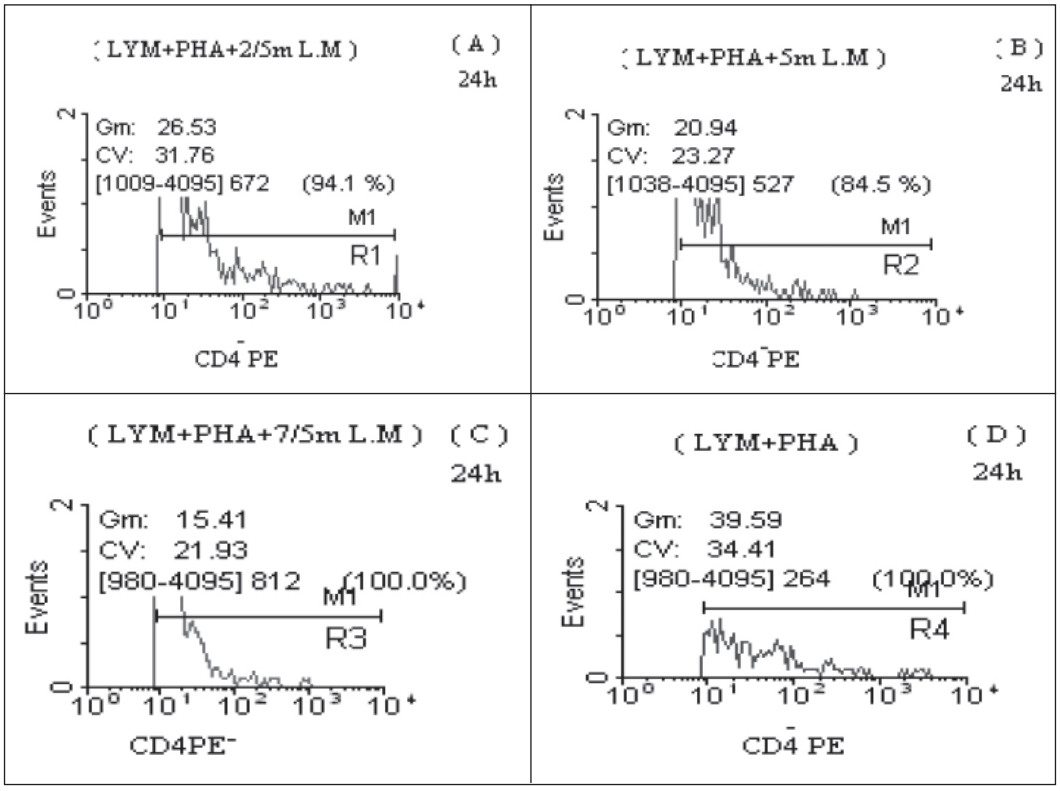

Figure 3. The suppression potential effect of various concentration of *L. major* promastigotes on T lymphocytes proliferation in flow cytometry test

obtained in 7.5×10^6concentration of *L.major* promastigote (Table 3 and Figure 3) .

DISCUSSION

Like other intracellular parasites, *Leishmania* sp. has several mechanisms for escaping from immune system of vertebrate host. One of these effective mechanisms is suppression of IL2 production and reduction in expression of IL2-Rα (CD25) on stimulated T lymphocyte. The parasite also blocks the physiologic role of IL2. Many studies indicated that BALB/c mice are more sensitive to *Leishmania* infection than C57/BALB mice due to suppressed IL2 production and IL2-R reduction (Frederick *et al.*, 1998). IL2 cytokine stimulates proliferation and differentiation of T lymphocytes and triggers cell growth cycle of T cells. It also increases IL4 and INF γ

production for promoting intracellular killing action (Majumder & Kierszenbaum, 1995).

This study investigated the ability of promastigotes to suppress *in vitro* IL2-Rα (CD25) expression in infected human T cells. The results of current study indicates that the suppressive effect of *L. major* started at six hour after infection. At this time the number of IL2-R^+ T cells and density of IL2-R were markedly reduced by the parasite. The maximum suppression was seen at 24 hours after infection and decreased in 48 hours because of consumption of antigen and nutrition components by T cells and parasite as well as accumulation of waste products in cell culture ($P < 0.05$).

The results of current study indicates that *L. major* promastigotes specially in logarithmic phase, can suppresses IL2-Rα expression in T cells in early stage of infection. There is a direct correlation between the number of *L. major* promstigotes and their potential suppressive effect ($P < 0.05$). It is an effective mechanism for promastigotes to evade from cell mediated immune system of their host.

These finding are in agreement with previous investigations. Cillari *et al.*, 1991 showed that infected spleen cells with promastigotes of *L. major* in BALB/c mice produced significantly lower level of IL2 in response to concanavalin A stimulation in comparison with control group. They postulated that the increased level of IL2 production closely associated with healing of lesion (Cillari *et al.*, 1991). The same results were obtained in infected spleen cells of mice by *L. donovoni* due to impairment of IL2 production when the cells were stimulated by phytohemagglutinin (Abbas *et al.*, 2007).

Other infective agents including *Plasmodium berghei* (Mohagheghpour *et al.*, 1985), and *Histoplasma capsulatum* in mice (Reiner & Finke, 1983) as well as *Mycobacterium leprae* in humans (Smith 1980) can also suppress IL2 production. There are many studies to investigate effect of *Trypanosoma cruzi* on immune system specially on IL2 production by T cells. Majumder and Kierszenbaum showed a marked inhibition of IL2 production by adding a *T. cruzi* filtered suspension to human PBMC. They also showed a decline of mRNA expression level in stimulated T cells (Majumder and Kierszenbaum, 1995; Lisa *et al.*, 1990). In other study co-culture of *T. cruzi* with human PBMC inhibited the production of IL2 and its receptor on T cells (Lisa *et al.*, 1989; Kierszenbaum *et al.*, 2002). Some studies indicated that the suppressive effect of *T. cruzi* depends on a glycoprotein membrane called AGC10 which can inhibit the production of IL2 and CD25, CD122 and CD132 (the components of IL2 receptor). This component of *T. cruzi* membrane can also diminish the production IFNγ and TNFα IL2-dependent in immunosuppressive role (Kierszenbaum *et al.*, 1990).

Our study confirms that there are significant differences between infected cells and non infected cell (control group) in lymphocytes proliferation test and *L. major* promastigotes cause reduction of T cells proliferation in infected group ($P < 0.05$). This finding is in agreement with other studies. The inhibition of T. cells (human PBMC) proliferation have been reported in *T. cruzi* infection (Lisa *et al.*, 1989; Sileghem *et al.*, 1989; Kierszenbaum *et al.*, 1990, 2002), *Trypanosoma brucei* (Kierszenbaum *et al.*, 1990) and *L. major* (Isakov & Tamir, 1994).

Why the parasite can suppress IL2-Rα expression on T cells while it lives in macrophages and other antigen presenting cells is unknown but it seems that the parasite can act so by soluble excretory factors which are secreted to lymphocyte cell culture. It is confirmed that excretory factor (EF) of *L. major* also causes a decrease in proliferation of T cells and reduces production of IL2 at both early and late stages of infection in a dose dependent manner (Isakov & Tamir, 1994). Khodadadi *et al.* (2010) showed that *L. major* promastigotes cause down regulation of MHC I expression on infected dendritic cells (as antigen presenting cell). Dendritic cells have an important role in initiating immune responses for killing interacellular parasite, and interaction between denderic cells and T cells is essential for stimulation of cell mediated immunity (Khodadadi *et al.*, 2010).

In conclusion, the major finding of the present study is that *L. major* can escape from host immune system via suppression of IL2 and its receptors production on T lymphocytes in early stage of infection. Understanding the interactions between parasite and immune system help us to find effective tools for treatment and control of leishmaniasis via producing drugs and vaccines.

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REFERENCES

Abbas, K.A., Lichtman, A.H. & Pillai, S. (2007). *Cellular and Molecular Immunology*. Saunders.

Abdullah, S.M., Flath, B. & Presber, W. (1998). Mixed infection of human U-937 cells by two different species of Leishmania. *American Journal of Tropical Medicine and Hygiene* **59**: 182-188.

Antoine, J.C., Prina, E., Lang, T. & Courret, N. (1998). The biogenesis and properties of the parasitophorus vacuoles that harbour *Leishmania* in murine macrophages. *Trends in Microbiology* **7**: 392-401.

Awasthi, A., Mathur, R.M. & Saha, B. (2004). Immune response to *Leishmania* infection. *Indian Journal of Medical Research* **119**: 238-258.

Carvalho, E.M., Badaró, R., Reed, S.G., Jones, T.C. & Johnson, W.D. (1985). Absence of gamma interferon and interleukin 2 production during active visceral leishmaniasis. *Journal of Clinical Investigation* **76**: 2066-2069.

Cillari, E., Liew, F.Y., Lo Campo, P., Milano, S., Mansueto, S., Salerno, A. (1986). Suppression of IL-2 production by cryopreserved peripheral blood mononuclear cells from patients with active visceral leishmaniasis in Sicily. *Journal of Immunology* **140**: 2721-2726.

Cillari, E., Liew, F.Y. & Lelchuk, R. (1986). Suppression of interleukin-2 production by macrophages in genetically susceptible mice infected with *Leishmania major*. *Infection and Immunity* **54**: 386-394.

Cillari, E., Milano, S., Dieli, M., Maltese, E., Di Rosa, S., Mansueto, S., Salerno, A., Liew, F.Y. (1991). Reduction in the number of UCHL-1_ cells and IL-2 production in the peripheral blood of patients with visceral leishmaniasis. *Journal of Immunology* **146**: 1026-1030.

Descoteaux, A., Salvatore, J. & Turco, B. (1999). Glycoconjugates in Leishmania infectivity. *Biochimica et Biophysica Acta* **1455**: 341-352.

Frederick, P.H., Ronald, M.R., Andrea, M.H. & Richard, A.M. (1998). Increased capacity for interleukin-2 synthesis parallels disease progression in mice infected with *Leishmania major*. *Infection and Immunity* **66**: 4537-4540.

Giorgio, C.D., Ridoux, O., Delmas, F., Azas, N., Gasquet, M., Timon, D.P. (2000). Flow cytometric detection of *Leishmania* parasites in human monocyte-derived macrophages: Application to anti leishmanial-drug testing. *Antimicrobial Agents and Chemotherapy* **44**: 3074-3078.

Harel-Bellan, A., Joskowicz, M., Fradelizi, D. and Eisen, H. (1983). Modification of T cell proliferation and interleukin-2 production in mice infected with *Trypanosoma cruzi*. *Proceedings of the National Academy of Sciences of the United States of America* **80**: 3466-3469.

Isakov, N., Tamir, A. & el-On, J. (1994). Suppression of antigen-specific T cell responses by *Leishmania major* excreted factor: inhibition of activation signals linked to the T cell antigen receptor and interleukin 2 receptor. *Israel Journal Of Medical Sciences* **30**: 673.

Khodadadi, A., Rezvan, H., Asteal, F.A. & Ali, A. (2010). Inhibitory effect of Leishmania on MHC I expression on mouse dendric cells. *International Immunology Meeting Abstracts* **22**: 23-35.

Kierszenbaum, F., Fresno, M. & Sztein, M.B. (2002). The *Trypanosoma cruzi* membrane glycoprotein AGC10 inhibits human lymphocyte activation by a

mechanism preceding translation of both, interleukin-2 and its high-affinity receptor subunits. *Molecular and Biochemical Parasitology* **125**: 91-101.

Kierszenbaum, F., Cuna, W.R., Beltz, L.A. & Sztein, M.B. (1990). Trypanosomal immunosuppressive factor: a secretion product(s) of *Trypanosoma cruzi* that inhibits proliferation and IL-2 receptor expression by activated human peripheral blood mononuclear cells. *Journal of Immunology* **144**: 4000-4004.

Laurence, U. & Buxbaum. (2003). Cysteine Protease B of *Leishmania mexicana* Inhibits Host Th1 responses and protective immunity. *The Journal of Immunology* **171**: 3711-3717.

Leichuk, R., Rose, G. & Playfair, J.H.L. (1984). Changes in the capacity of macrophages and T cells to produce interleukins during murine malaria infection. *Cellular Immunology* **84**: 253-263.

Lisa, A., Beltz, L.T., Kierszenbaum, F. & Marcelo, B.S. (1989). Selective suppressive effects of *Trypanosoma cruzi* on activated human lymphocytes. *Infection and Immunity* **57**: 2301-2305.

Lisa, A., Beltz, L.T., Kierszenbaum, F. & Marcelo, B.S. (1990). *Trypanosoma cruzi*-induced suppression of human peripheral blood lymphocytes activated via the alternative (CD2) pathway. *Infection and Immunity* **58**: 1114-1116.

Majumder, S. & Kierszenbaum, F. (1995). *Trypanosoma cruzi* immunosuppressive factor decreases the interleukin-2 mRNA level in cultured normal activated human lymphocytes. *Infection and Immunity* **63**: 4546-4549.

Mohagheghpour, N., Gelber, R.H., Larrick, J.W., Sasaki, D.T., Brennan, P.J. & Engleman, E.G. (1985). Defective cell mediated immunity in leprosy: failure of T cells from lepromatous patients to respond to *Mycobacterium leprae* is associated with defective expresion of interleukin-2 receptors and is not reconstituted by interleukin-2. *Journal of Immunology* **135**: 1443-1449.

Murray, H.W., Stern, J.J., Welte, K., Rubin, B.Y., Carriero, S.M. & Nathan, C.F. (1987). Experimental visceral Leishmaniasis: production of interleukin 2 and interferon tissue immune reaction, and response to treatment with interleukin 2 and interferon *Journal of Immunology* **138**: 2290-2297.

Murray, H.W., Miralles, G.D., Stoeckle, M.Y. & McDermott, D.F. (1993). Role and effect of IL-2 in experimental visceral Leishmaniasis. *Journal of Immunology* **151**: 929-938.

Poot, J.H., Denise, D. & Herrmann, C. (2005). Virulence and protective potential of several Cysteine peptidase knockout strains of *Leishmania infantum* in hamsters. Chapter 5. Experimental challenge models for canine Leishmaniasis in hamsters and dogs, optimization and application in vaccine research. Printed by Febodruk b.v., Enschede, the Netherlands.

Reiner, N.E. & Finke, J.H. (1983). Interleukin-2 deficiency in murine *Leishmania donovani* and its relationship to depressed spleen cell responses to phytohemagglutinin. *Journal of Immunology* **131**: 1487-1491.

Sileghem, M., Darji, A., Hamers Marnix, R., Patrick, V.D.W. & Baetselier, D. (1989). Dual role of macrophages in the suppression of interleukin 2 production and interleukin 2 receptor expression in trypanosome-infected mice. *European Journal of Immunology* **19**: 829-835.

Smith, K. (1980). T-cells growth factor. *Immunological Reviews* **51**: 337-357.

Smith, K.A. (1988). Interleukin-2: a 10-year perspective. In: *Interleukin-2*. (Editor, K.A. Smith) pp. 1-35. Academic Press, New York.

Umakant Sharma, Sarman Singh. Immunobiology of Leishmaniasis. *Indian Journal of Exprimental Biology* **47**: 412-423.

Watson, S.R., Schmitt, S.K., Hendricks, D.E. & Bullock, W.E. (1985). Immunoregulation in disseminated murine histoplasmosis: disturbances in the production of interleukins 1 and 2. *Journal of Immunology* **135**: 3487-3493.