ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Role of IL-17 and IL-27 in Cutaneous Leishmaniasis

Hussein A A[1] . Al- Marsome H T.[1] and Al-Musawi H N.[2]

1. Department of Microbiology, College of Medicine University of AL-Nahrain, Iraq
2. Department of Medicine, College of Medicine University of AL-Nahrain, Iraq

Manuscript Info

Manuscript History:

Received: 14 April 2015
Final Accepted: 19 May 2015
Published Online: June 2015

Key words:

Breeding season, Buffaloe,Post-partum anoestrus,

Corresponding Author

Hussein A A

Abstract

Patients with CL have a strong type 1 immune response to *Leishmania* antigen, with high production of IFN-γ and TNF-α. Interleukin 17 (IL-17) plays a critical role in inflammation and autoimmunity. IL-27 is a cytokine that can initiate a Th1 response but can also regulate inflammatory response. This study aims to assess the role of IL-17 and IL-27 in cutaneous leishmaniasis to identify its role in disease progression. This study involved 48 (CL) patients and 16 healthy control. Smear stained by Geimsa stain, also swab was taken for culturing. Serum were obtain to measure IL-17 and IL-27 by ELISA method. The results indicate a highly significant increase in the serum level of IL-17 in CL patients (78.36±6.28 pg\ml), no significant increase in the serum level of IL-27 in CL patients (17.81±1.96 pg\ml) when compare with control (21.01±2.77 pg\ml). In other hand, statistically significant important lower in serum level of IL-17A associated with ulcerative lesions 53.42±29.11 pg/ml compared with absence of ulceration, while increase in serum level of IL-27 with ulcerative lesions 21.75±19.34 pg/ml compared with absence of ulceration. The study found that IL-17A and IL-27 associated with progression CL infection.

INTRODUCTION

Leishmaniasis represents a group of tropical diseases caused by infection with protozoan parasites from the genus *Leishmania*. These parasites are widely distributed in 88 tropical and subtropical countries and pose a major public health problem and a risk for people living in or traveling to the endemic areas. The parasite apparent by the many names it has adopted "Baghdad Boil", "Jericho buttons" and "Oriental sore" to name a few. It has an annual estimated worldwide incidence of 600,000 and prevalence of 12 million cases. Leishmaniasis is a vector-transmitted disease, and at least 20 species of *Leishmania* are known to be pathogenic for humans (Banuls, *et al.*, 2007). *Leishmania* parasites have a di-genetic life cycle, multiplying as flagellated promastigotes in the midgut of sand flies and as non-flagellated amastigotes within mammalian phagocytes. Humans and domestic animals are accidental hosts for many *Leishmania* spp., which are maintained in cycles between wild animals and sand flies (WHO 2010).

Disease resolution in mediated by the cell mediated response rather than the humoral immune response. There is strong correlation between activation of different T-cell subsets and outcome of disease (Alexandar and Bryson, 2005). T helper 17 cells are independently regulated CD4+ T-cell, characterized as producing cytokines in the Interleukin 17 family .They are highly proinflammatory and stimulate the production by endothelial and epithelial cells and monocytes. Interleukin-17 (IL-17) enhance T-cell and stimulate fibroblast, endothelial cell,

neutrophil and macrophage (host cell and effector cell that kill the parasite) and epithelial cell (Maira, *et al.,* 2009).

IL-27 is produced by innate cell such as macrophage and dentritic cells (Kastelein, *et al.,* 2007). Initial studies into biological function of IL27 implicated role in promoting Th1 development from naive cells the requirement for IL27 in early Th1 development (Atsunobu, *et al.,* 2003). Many studies and reports point to role of IL-17 and IL-27 the immune response in individuals with *Leishmania braziliensis* infection, focusing on the role of IL-10 and IL-27 in the modulation of immune response and evaluating whether IL-17 production was associated with control of infection(Roberto, *et al.,* 2011), and another study in mucosal leishmaniasis and American cutaneous leishmaniasis point to produce higher levels of IL-17 (Bacellar, *et al.,* 2009).

Material and Methods

Patients and controls:

Sixty four individuals (64) were enrolled in this study, were divided into two groups; forty-eight (48) patients with cutaneous leishmaniasis Those patients were either established or newly diagnosed, the age range from (1.5-50 years), sixteen (16) healthy individuals in different age groups all of them received no treatment with no complaint of other chronic or systemic diseases and. All patients and control from the in two hospitals in Baghdad/ Iraq: AL-Karama Teaching Hospital and Al-Emamain Al-Kadhemain medical city in the period January 2014 till May 2014.

Diagnosis of Cutaneous leishmaniasis

The skin lesion was cleaned with 70% alcohol, and then 0.1-0.2 ml of sterile normal saline was injected in the edge of the ulcer subcutaneously under the pus area. The injected solution was re-aspirated without taking the needle out of the skin. This aspirate was directly used to prepare culture, the aspirates was inoculated onto semi solid media, and then incubated at 27ºC. The culture was microscopically examined for the presence of the promastigote stage after 7-10 days. It was considered negative if absence of this stage during the first four weeks. At the same time slides were prepared to staining by Geimsa stain, the slides were microscopically examined under oil immersion lens for the detection of the parasite (amastigote) within macrophage.

Detection of serum interleukin-17 (IL-17) and IL-27

Using enzyme-linked immunosorbent assay test for the quantitative measurement of human IL-17 in serum (The Ray Bio Human IL-17 kit) and IL-27 in serum (The CUSABIO human IL-27 ELISA kit) . This assay employs an antibody specific for human IL-17 coated on a 96-well plate. 100 µl Standards and samples are pipetted into the wells and IL17 (or IL-27) present in a sample is bound to the wells by the immuobilized antibody. The wells are washed and biotenylated anti-human IL17 Antibody (Ab) (or anti-human IL27 Ab) was added. After washing away unbound biotinylated Ab, HRP-conjugated streptavidin was pipette to the wells. The wells washed again, a TMB substrate solution was added to the well and color develops in proportion to the amount of IL17 bound (or IL-27). The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

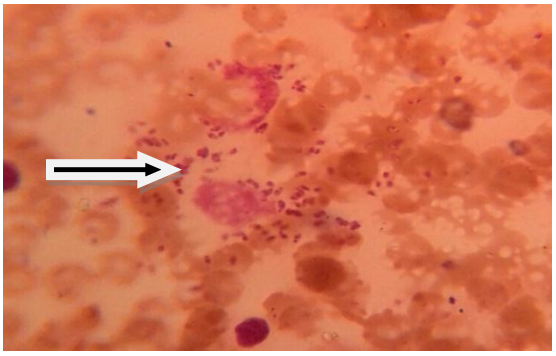
Statistical analysis:

The data were statistically analyzed depending on the nature of the character, according to Snedecor and Cochran (1981). The relationship between the indicators was measured quantitatively by using the correlation coefficient which runs between complete positive and negative correlations, respectively. ANOVA test were used to compare means of numerical variables among more than two groups.

Result and Discussion

All forty eight samples patients were examined microscopically by staining with Giemsa stain the results indicate that 20(42%) of samples were positive, while 28(58%) given negative results (figure 1). These results agree with study by (Ansam, M. 2011). The reason for this may be the incompetence of staining in some slides or the difficulty of the parasite in the emergence of some swabs.

Figure 1: Amastigote in macrophage according to slide stained with Giemsa stain



The results of culturing revealed that one skin aspirate sample give promastigote growth from the 48 isolate in semisolid media. the reason to lower positive results for samples culturing on culture media may be due to the different *leishmania spp*., genetic developments of parasite, the low percent of parasite recovery and difficulties in taking samples especially from children and young patients which may lead to contamination of samples and hence the failure of culturing.

To evaluate expression of IL-17 protein in serum CL patients .the current results by using ELISA test denoted that IL-17 A is highly elevated in serum of CL patients(78.36±43.56 pg/ml) when compared to healthy control group(30.62±6.22pg/ml),which it is significantly important(p =0.001) (table 1). This results agree with many studies (Bacellar, *et al*,. 2009; Anderson, *et al.*, 2009 and Claudia, *et al.*, 2013), IL-17 may contribute to pathogenesis through several mechanisms, including neutrophil activation, tissue injury, and osteoclast activation and the regulated production of IL-17 contributes to infection control, while excessive IL-17 can promote neutrophil influx and tissue damage.

Table 1: Descriptive serum IL-17 level characteristics in the studied groups.

IL-17A (pg/ml)	Mean	Std. Deviation	Std. Error
Healthy control	30.62	24.90	6.22
Patients with CL	78.36	43.56	6.28
P value	0.001		

The results showed that there is no significant elevation in the mean serum level of IL-27 between group CL patients (17.81±1.96 pg\ml) and healthy control group (21.01±2.77pg\ml), P=0.444 (table2), this result agree with study by (Tolouei S, 2012) point to the low level of IL-23 and IL-27 produced by macrophages derived from peripheral blood mononuclear cell culture collected from patients with healing or non-healing form of cutaneous leishmaniasis lesion.

Table 2: Descriptive serum IL-27 level characteristics in the studied groups.

IL-27 (pg/ml)	Mean	Std. Deviation	Std. Error
Healthy control	21.01	11.08	2.77
Patients	17.81	13.64	1.96
p value	0.444		

Many factors other than parasite dose may be important in promoting pathology, such as the genetic background of the patient, the influence of the vector, the site of infection, Co-infection and the microflora in the skin (presence or absence of secondary bacterial infection), and/or the species/strain of the parasite, any of which may contribute to a much more pronounced early immune response after a natural infection (Kaye, *et al*,. 2011 and Naik, *et al.*, 2012), the result of IL-17 level in positive group for Co-infection was mean (53.42±29.11) compare with group without ulceration (83.80±40.82), the different was statistically significant important (p=0.043) between culture positive (co-infection) compare with absence of ulceration. Ulceration with secondary bacterial infection is one of the complications of the disease that can increase the tissue destruction and resulting the scar so the possible presence of co-infection an effect on the lower of IL-17 level, thus leads to ulceration CL and the survival period of the disease to long time, this corresponds with (Hengameh Ziaie 2008). Table 3.

The present study revealed the mean of IL-27 levels (21.75±19.34) higher in CL patients with co-infection than of the mean IL-27 levels (15.89±11.80) in CL patients without co-infection. , this can be through the presence of co-infection stimulate production high percentage of IL-27 this agree with study (Hengameh, 2008). All the different were not statistically significant important. Table 3.

Table 4-11: Serum levels of IL-17 and IL-27 according to Bacterial culture (co-infection) in ulcerative CL patients.

			IL-17 (pg/ml)		IL-27A (pg/ml)	
			Mean	**Standard Deviation**	**Mean**	**Standard Deviation**
Presence of ulceration	**Yes**	**Positive (Co-infection)**	53.42	29.11	21.75	19.34
		Negative (co-infection)	86.20	71.66	23.77	13.42
	No		83.80	40.82	15.89	11.80
culture positive vs culture negative			0.242		0.840	
Culture positive vs absence of ulceration			0.043		0.258	

leishmaniasis depends upon the type of immune response generated and the species of *Leishmania*. IL-17and IL-27 indicate that the cellular immune response is important for disease control especially Th1. IL-17 and IL-27 may play a possible and complementary role with Th1 cytokines in human protection against Leishmania infection. Therefore we need more studies to establish the role of these cytokines as markers in healing process of cutaneous leishmaniasis.. IL-27 are involved in the regulation of Th17 response.

References

Banuls A L, Hide M and Prugnolle F. *Leishmania* and the leishmaniases: a parasite genetic update and advances in taxonomy, epidemiology and pathogenicity in humans. Adv. Parasitol.2007; 64:1–109.

World Health Organization(WHO). Factsheet338. Medicines: rational use of medicines. Geneva.WHO.2010. http://www.who.int/ mediacentre/ factsheets/ fs338/en/.

Alexandar J and Bryson K. T helper 1/Th2 and *Leishmania* :paradox rather than paradigm. Immunology Letters.2005;99:17-23.

Maira GR Pitta, Audrey R and Sandrine C *et al*. IL-17 and IL-22 are associated with protection against human kala azar caused by *Leishmania donovani* .Journal of Clinical Investigation.2009;119(8):2379–2387.

Kastelein, R.A. and C.A. Hunter *et al*. Discovery and biology of IL23 and IL27:related but functionally distinct regulators of inflammation. Annual Review of Immunology.2007;25:221-242.

Atsunobu T, Shinjiro H and Atsushi Y *et al.* Cutting Edge: Role of IL-27/WSX-1 Signaling for Induction of T-Bet Through Activation of STAT1 During Initial Th1 Commitment. Journal Immunology. 2003; 170:4886-4890.

Roberto N, Olívia B and Márcia N and *et al.* IL-17 and Regulatory Cytokines (IL-10 and IL-27) in *L. braziliensis* Infection. Parasite Immunology. 2011; 33(2): 132–136.

Bacellar O, Faria D and Nascimento M, *et al.* IL-17 Production in Patients with American Cutaneous Leishmaniasis. Journal Infection Disease.2009; 1: 200(1):75–78.

Ansam, M. H. Leishmaniasis: immunopathological and diagnostic study in human and mice. MSc Thesis submitted to collage of Veterinary Medicine, University of Baghdad, Iraq. 2011.

Anderson C F, Stumhofer J S and Hunter C A, *et al.* IL-27 regulates IL-10 and IL-17 from CD4+ cells in nonhealing Leishmania major infection. Journal Immunology.2009;183: 4619–4627.

Claudia G L, Ciara G and Olivia B *et al.* IL-17 Mediates Immunopathology in the Absence of IL-10 Following *Leishmania major* Infection. PLoS Pathogens.2013;9(3): e1003243.

Tolouei S, Ghaedi K and Khamesipour A *et al*. IL-23 and IL-27 Levels in Macrophages Collected from Peripheral Blood of patients with Healing Vs Non-Healing Form of Cutaneous Leishmaniasis. Iranian J Parasitol.2012; 7; No.1pp. 18-25.

Kaye P and Scott P. Leishmaniasis: complexity at the host-pathogen interface. Nat Rev Microbiol.2011;9: 604–615.

Naik S, Bouladoux N and Wilhelm C, *et al.* Compartmentalized Control of Skin Immunity by Resident Commensals. Science.2012;31: 337(6098): 1115–1119.

Hengameh Z and Sadeghian G . Isolation of bacteria causing secondary bacterial infection in the lesions of cutaneous leishmaniasis Indian J Dermatol.2008; 53(3):129–131.