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IL-17 in Protective Immunity to Vaginal Candidiasis

Meethaq S. Abood* ***Khalid A. Habib**** ***Eman N. Najee*****

*Biology Department, College of Education, Thi-Qar University .
**Biology Department, College of Science for Women, University of Baghdad.
***Biology Department, College of Science, Al-Mustansiriya University.

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Abstract:

Vulvovaginal candidiasis (VVC) is caused by *Candida albicans* affects a significant number of women during their reproductive ages. Th17 cells play a major role in coordinating the host defense in oropharyngeal candidiasis. In this study we investigated the involvement of the Th17 response in an animal model of vulvovaginal candidiasis (VVC). The present study aimed to shed light on detect concentration of the IL-17 of infected animal and control . A direct Enzyme Linked Immunosorbent Assay (ELISA) was used to quantify IL-17 concentrations in 30 infected animal with VVC and 10 control group. Rats were intravaginally inoculated with *C.albicans*, and vaginal lavage fluids, serum were evaluated for proinflammatory cytokine IL-17 The data suggest that IL-17, produced by vaginal cells, particularly CD4 T cells, detected in the vaginal wash and serum during the infection, reaching a maximum 14 days after the challenge.

Key words: IL-17, ELISA, Vaginal Candidiasis

Introduction:

Candida albicans, an opportunistic polymorphic fungus and resident of the normal vaginal microbiota, is the leading causative agent of vulvovaginal candidiasis (VVC) and presents major quality of life issues for women worldwide [1] about 5–8% (approximately 150 million worldwide) suffer from recurrent VVC (RVVC), resulting in idiopathic chronic episodes of vaginal irritation that require antifungal maintenance therapy (e.g., azoles) to partially control symptoms[1]. It has been demonstrated that the vaginal mucosa, its tissue structure and cervicovaginal fluids, contains both humoral and cellular components of innate and acquired immune responses [2]. Animal models are frequently used to evaluate host defense mechanisms against Candida vaginitis [3] Th17 cells belong to a lineage different from that of Th1 and Th2 cells, and they are characterized by the production of IL-17A, IL-17F and IL-22 [4]. The protective action of IL-17 against extracellular pathogens also involves neutrophil recruitment to the infection sites .[5] IL-17 has a central role in protective immunity against *C. albicans* systemic and oral infections[6,7,8] In response to a systemic challenge with *C. albicans*, IL-17ARdeficient mice showed a reduced survival rate and a significant increase in kidney fungal burden. Mobilization and influx of neutrophils to infected

organs were also impaired and delayed. [6] In another study, the Th17 response also conferred protection against oropharyngeal candidiasis through neutrophil recruitment and antimicrobial factor production . [8] several studies using a mouse model of *Candida* vaginitis and many cross-sectional studies evaluating women with RVVC have shown that protection was not mediated by *Candida*-specific adaptive immunity. [9,10] In contrast, results from a human live challenge study revealed that protection occurs in the absence of any inflammatory response, whereas symptomatic infection is associated with a vaginal cellular infiltrate consisting exclusively of polymorphonuclear neutrophils (PMNs) [11] .In the present work, we focused on the role of IL-17 in protecting rats against vaginal candidiasis.

Materials and Methods:

1. Labortary animals

Adult female Albino rats (*Rattus norvegicus* , animal house /College of Science / Thi-Qar University) weighing between(120-200) gm and in averege age (8) weeks were used in this study .Number of rats were 40 animals which divided into 3 groups , each group was contained 10 animal , as well as control group that included 10 animals , all animals were grew under intensive healthy conditions .

2. Yeast suspension

In this study *C. albicans* isolated from infected women with VVC was used. Stationary phase organisms were obtained from a culture at Sabraud's Distrose Broth (SDB) medium and incubated for 24 hours in 37 ˚C, cells were precipitated by centrifuge and the sediment was suspended in normal saline to reach 5x 10^5cell/ml [10].

3- Vaginal *Candida* inoculation

Rats were intravaginally inoculated by introducing 20µl of phosphate buffered saline(PBS) containing 5×10^5 *C.albicans* into vaginal lumen . Uninoculated control rats were intravaginally challenged with sterile PBS .

After 24 hr post injection of rats by *Candida* suspension, 40 rats were divided into four equal groups each group contain 10 rats , group 1,2 and 3 were prepared to detect level of IL-17 in serum and vaginal washing fluid as flow post-inoculation as well as control group. Rats in the control group received normal saline Vaginal washes and serum were obtained at different times (1 , 2 and 3 weeks), vaginal washes were centrifuged at 600x g and the supernatants were recovered and stored at -20˚C to determent level of IL-17 by using (Enzyme Linked Immunosorbent Assay) ELISA kit . IL-17 concentrations were quantitatively determined in serum and vaginal washing fluid of infected animal and healthy control subjects by means of ELISA using ready kits manufactured by USBiological company (USA).

Statistical analysis

All analysis were performed using the statistical package (SPSS) version 15, the data were expressed as mean, standard deviation SD, percentage. ANOVA was used to analyze repeated measurement. Results were determined as very high significant at ($P< 0.05$) and non significant at ($P> 0.05$)

Results and Discussion:

Results reported in table and figure (1,2) demonstrated that there is an production of IL-17 , starting in 7 days after the challenge , reaching a maximum 14 days post infection , and subsequently decreasing to return to basal levels after 3 weeks of infection. These results agreed with Pietrella *et al* [12] who found that IL-17 produced by

vaginal cells, particularly CD4 T cells, was detected in the vaginal wash during the infection, reaching a maximum 14 days after the challenge.The production of IL-17 in the vaginal wash could presumably be attributed to PMN and epithelial cells, which are known to be innate system cells capable of producing IL-17 [13]. Th 17 responses showed to be involved in the protective response against fungal and bacterial mucosal infections.[14]. In a mouse model of systemic candidiasis a protective role was attributed to IL-17 because of its ability to induce neutrophil recruitment [8]

The study of Yano *et al* [15]showed that the increase of IL-17 in the vaginal lumen and its secretion by vaginal cells seems to be independent of the neutrophil influx. As a matter of fact the robust early neutrophil migration observed soon after infection seems mainly attributable to chemotactic molecules, produced by epithelial cells following interaction with *C. albicans* .Indeed the level of neutrophils also remained high during the resolution of infection, while the IL-17 production paralleled the course of infection. Given that a correlation between infiltration of polymorphonuclear neutrophils and symptomatic vulvovaginal candidiasis has been observed [16].The lack of correlation between the presence of IL-17 and neutrophil infiltration suggests the role of IL-17 may be to protect from, rather than to participate in the inflammatory response. No proinflammatory cytokines and chemokines showed a remarkable increase in response to *Candida* in vivo.

The results of statistical analysis showed significant (P≤0. 05) for group 1 with control group and highly significant (P≤0.005) for group 2,3 with control group

Table (1) Levels of interleukin IL-17 in serum of rats infected with VVC

Study groups	Number	Mean of IL-17 pg/ml
Group1	10	22.0913
Group 2	10	160.5633
Group 3	10	65.9356
Control	10	12.1135

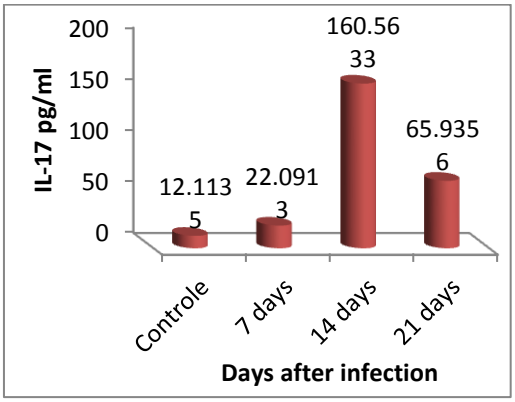

Fig. (1) IL-17 concentration in serum of rats infected with *Candida albicans*

Table (2) Levels of interleukin IL-17 in vaginal washes in rats infected with Candida albicans

Study groups	Number	Mean of IL-17 pg/ml
Group1	10	22.3007
Group 2	10	136.8824
Group 3	10	56.0571
Control	10	11.0676

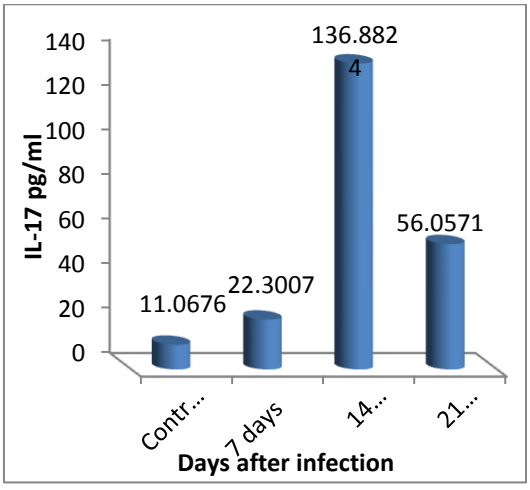

Fig.(2)IL-17 concentration in murine vaginal washes of rats infected with *Candida albicans*

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السايتوكين17-IL في الحصانة الواقية لداء المبيضات المهبلي

ميثاق ستار عبود* **خالد عبد الرزاق حبيب**** **ايمان ناطق ناجي*****

* قسم علوم الحياة / كلية التربية / جامعة ذي قار
** قسم علوم الحياة / كلية العلوم للبنات / جامعة بغداد
*** قسم علوم الحياة / كلية العلوم/ الجامعة المستنصرية

الخلاصة:

داء المبيضات المهبلي يتسبب عن طريق الخميرة *C.albicans* ويؤثر على عدد كبير من النساء في عمر الاخصاب . تلعب الخلايا Th17 دور رئيسي في تنسيق اليات الدفاع عند المضيف ضد داء المبيضات . في هذه الدراسة تم التقصي عن شمولية استجابة خلايا Th17 في نماذج حيوانية مصابة بداء المبيضات المهبلي كما سلطت الدراسة الحالية الضوء على تحديد تركيز الانترلوكين IL-17 في الجرذان المصابة ومجموعة السيطرة. اذ تم استخدام اختبار الاليزا المباشر لتقدير تركيز الانترلوكين IL-17 في 30 جرذ مصاب بالتهاب المهبل الكانديدي و10 جرذان كمجموعة سيطرة. تم حقن الجرذان مهبليا بالخميرة *C.albicans* وتم جمع المصل وسائل الغسل المهبلي لتحديد مستوى الانترلوكين IL-17 في الحيوانات المصابة ومجموعة السيطرة . بينت النتائج ان تركيز IL-17 المنتج من قبل الخلايا المهبلية وخاصة CD4 T cells تم تحديده قي المصل وسائل الغسل اثناء الاصابة ووصل الى اقصى ارتفاع عند اليوم 14 بعد الحقن بالخميرة .

الكلمات المفتاحية: داء المبيضات المهبلي، الاليزا، الانترلوكين-17.