Genetic Predisposition to Colitis and Immune Response in Mice Induced with Disease and Treated with Helminth Antigen

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Abstract

The study aims to know the role of parasitic worm's antigens in immunomodulation as its great importance in understanding the interactions between the host and the parasites, due to the immune modulation allows the survival of both the host and the parasite through the control of immune disorders and the sustainability of parasites. The worms have the ability to persist in the host and are also mainly responsible for the chronic infection, despite the strong immune response developed by the host-parasite. Here, the whole worm antigens was used treatment of one of autoimmune disease (colitis).The experiment included the use of 50 adult male albino mice , Mus musculus, with ages ranging between 8-10 weeks, average weights ranged between 18-32 g. The experiment includes inducing three groups (induced colitis mice, treated mice with worm's antigens and control). The blood sample was collected from all individuals in all groups and divided into two samples one them used for measured of cytokines (IL-4,IL-10,IL-17) and the other was used for study of gene CXcl1 expression .The current study, in which (IL-4,IL-10) levels were measured, showed a clear and significant decrease in (IL-4,IL-10) levels in the ulcerative colitis induced group, While the results of the current study showed that the mean concentration of IL- 17 in the serum of the experimental group induced by ulcerative colitis was high compared to the control group and the other groups. The result of gene expression was discussion in this study and explain its relation with other criteria.

Keywords: helminth antigen, ulcerative colitis, immunomodulation.

1. Introduction

Hymenolepis diminuta, commonly known as "rat tapeworm," is a common intestinal parasite in small rodents, like mice and rats, they are widely spread all over the world, especially in temperate and tropical climatic regions. Although the parasite is a rodent parasite, accidental infections have been recorded in people [1-3] . Previous studies reported that H. diminuta and its ESP products have an inhibitory effect on pro-inflammatory cytokines and chemokines. As well as reducing the expression of transcription factors and the scavenger receptor CD36, which is involved in many innate immune processes including, removing dead cells and inhibiting tumor angiogenesis, in this way, the parasite-derived molecules interact with the host's immune system as antigens linked to three sources: Secretory secretion, surface proteins, and somatic proteins [4, 5]. Many of these molecules are proteins involved in parasite metabolism and survival strategies. Secretion-excretory proteins (ESPs), which have been found as antigens with a potential effect on parasite-host interaction in H. diminuta [6]. Some of proteins with antigenic potential are actins, annexin, lamin, myosin, paramyosin, tubulin that are a skeleton proteins and major egg antigen [6].

In an attempt to understand the mechanism of the contribution of worms to treatment, it has been observed that in areas that recorded high epidemics with parasitic intestinal worms, there is a decrease in the number of those infected with autoimmune diseases. It was found in patients infected with parasitic worms that these worms lead to a modification of immunity either to enhance its stability or because the host has found a process to reduce the damage of infection. The worms stimulate the immune response in acute infection, but they suppress the immune response in chronic infections, as they activate the regulator cytokines and suppress those responsible for initiating inflammatory [7]. The worms had a clear protective effect against immunological disorders familiar to the population of countries with high rates of worm infestation, as in the study of Greenwood [8], who showed that the incidence of rheumatic and autoimmune diseases decreases in African countries.

It was clarified that in susceptible individuals, environmental factors and genetic predisposition cause damage to the epithelial barrier of the intestine and lead to an immune response in the mucosa, and increased production of macrophages in high amounts that produce pro-inflammatory cytokines (TNF-α and others) and production of tissue-destroying molecules (NO, ROS)., this lead to the loss of healthy epithelial cell and insufficient mucous layer, and therefore it facilitates the transmission of bacterial infection to the sub-epithelial areas. The dendritic DCs that are stimulated after recognition of the bacteria are then activated and migrate to the gut, where naive cells are induced and they produce IL-12/23, This leads to the differentiation of CD4 + T cells into Th1 and Th17 cells. These, in turn, migrate to the intestine and perpetuate the inflammatory response through, for example, the production of IFN which supports the activation of macrophages. Conversely, dendritic cells stimulated by molecules released from tapeworms prime Th2 cells to produce IL-4/-13.Tissue-produced cytokines such as IL-25 support the development of a Th2-cell response and inhibit

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pro-inflammatory by macrophages and dendritic cells. The Th2 response supports the growth and development of T cells, IL-4/13 activates macrophages and activates the mobilization of factors such as arginase and RELM-α that are essential for wound repair. IL-4/13 also drives increased epithelial cell production, goblet cell hyperplasia, and increased mucus production.Secreted products of tapeworms modulate the response of dendritic cells and inhibit their expression of MHC II and the production of Th1- and Th17. Prefer Certain species of nematodes directly prefer T-cell regulator Foxp3+ stimulation by secretion of TGF-β, Dendritic cells assemble in the gut and support IL-10 production by regulatory T cells, which limits the functional downregulation of Th1 and Th17 as well as Th2 cells [9]. The present study aimed to assessment of the levels of cytokines such as IL-4, IL-10, and IL-17- in the serum of experimental groups to estimate their contribution in either inducing, inhibiting, or regulating IBD and investigate the gene expression of Cxcl1 gene in experimental animals to estimate its role in inducing IBD.

2. Materials and Methods

The experiment included 50 adult male of albino mice, Mus musculus, with ages ranging between 8-10 weeks, average weights ranged between 18-32 g. They were obtained from the University of Tikrit and were housed in the animal house of the College of Education for Pure Sciences / University of Diyala,

The mice were placed in plastic cages furnished with sawdust, it was equipped with water and food for the duration of the study, and cleaned every two days. And they kept at room. The rodents (mice and rats) were collected from the local markets of Baquba they dissected and the worms (Hymenoleps diminuta) were collected after cleaning and preserving them with PBS until use.the antigen of the worm was prepared

Preparation of Helminth extract

The method by Wang et al. [7] was used for the purpose of preparing the worm extract H.diminuta Adults from the intestine of rats were cleaned (four times) in normal saline at room temperature (±25°C). 15 g (wet weight) of H. diminuta was weighed and 15 ml of sterile PBS buffer was added and homogenized. Using a speed of 4000 rpm for 30 minutes at C°4, the impurities were removed. The soluble supernatant was collected in PBS and subjected to two additional rounds of centrifugation. The supernatant was then collected, the protein concentration determined by Bradford assay, and this "crude extract" kept at C°-80

Animals groups

The animals were divided into five groups:

First: The first group, the negative control group: includes 10 healthy (untreated) mice, which were given distilled water.

Second: The second group includes the positive control group

Colitis induced mice only (not vaccinated with helminth antigen) 10 male mice were injected with acetic acid at a concentration of 40% of body weight via the anus

Mice injected with intestinal helminth antigen only (not induced with colitis) at a concentration of 100 µl of 10 male mice intraperitoneum

Third: Treatment groups

Injected with antigen after induction of inflammation in 10 male mice (treatment)

10 male mice injected with antigen before induction of inflammation (prevention)

ELISA

After inducing inflammation and treating worms the blood sample, which was previously drawn from the heart, was placed in gel-containing tubes and then left at room temperature for about an hour until the blood clot formed. Then the samples were separated by centrifugation at a speed of 4000 rpm for 4 minutes, then the serum was withdrawn from the gel tube by the micropipette and placed in the appnedrof, and then kept at -40 ° C until the tests were performed on it to measure for IL-4, IL-10, IL-17.

Real-time quantitative RT-PCR (qRT-PCR)

The Colon tissue samples were collected at 4 ± 0.5 cm from the anus and placed immediately into TRIzol reagent for total RNA isolation. Treated colon were lysed with 0.5 ml of TRIzol reagent total RNA purification system (both from Invitrogen), and the RNA was extracted and purified according to the manufacturer's instructions. Portion of RNA was used for reverse transcription (RT) using an iSCRIPT cDNA synthesis kit (Bio Molecular System, Australia).

To confirm the gene expression, RT-PCR were performed by using TRIzol ™ qPCR Master Supermix Taq®MAN ,with the following specific primers: Gapdh: forward 5-TTGTCTCCTGCGACTTCA, reverse 5-CCTGTTGCTGTAGCCGTATT; Cxcl1: forward 5-AC TGCACCCAAACCGAAGTC, reverse 5-CAAGGGAGCTTCAGG-GTCAA, The cycles passing threshold (CT) were recorded and relative expression level of a target gene = 2Δct, where

ΔCT = CT of Gapdh CT of the target gene.

3. Results and Discussion

Immunomodulatory function of helminths and their products could be used as antiinflammatory drugs. Hymenolepis diminuta has been tested recently to treat patients with inflammatory bowel disease and success. The current study, in which IL-4 levels were measured in the serum of 50 experimental mice , A clear and significant decrease in the levels of IL-4 in the group induced ulcerative colitis by acetic acid(4.40549 ± 11.2635) pg/ml, Compared with the control group on average(23.6383 ± 2.14323)pg/ml ,And other groups dosed with worm extract(75.0500 ± 39.03307) .and groups before and after inductions, respectively(33.3507 ± 4.60014),(65.3732 ± 21.92055) ,at P<0.05. Since IL-4 is produced by T cells, mast cells, and basophils, it has many biological roles in innate and adaptive immunity. The differentiation and activation of B cells is of particular interest to the current study, IL-4 contributes to an increase in antibody responses after infection and this is consistent with Wang et al. [7]. The results of the significant decrease of IL-4 in the group induced by colitis, as shown in Figure (1) agree with the Versini et al. [9],Which indicated that IL-4 is a highly regulated cytokine through the improvement of the mucosal layer, and IL-4 is the most efficient enhancer of goblet cell numbers .Goblet cell

surface vascular congestion In addition to the production and transport of myosin vesicles within the cell, IL-4 has a known set of effects on B-cell proliferation, immunoglobulin production, and homotype switching.

IL-4-deficient mice had fewer IgM-producing cells and similar numbers of IgA-producing cells suggesting that there is no obligatory role for IL-4 in switching the homologous pattern from IgM to IgA.However, it is unclear whether this effect is a cause or a consequence of the disease improvement process in the colon. One of the main activities of IL-4 is to act as a switching factor for Wagner et al. [10]. Thus, IL-4-deficient mice have been described as having low levels of IgG1 and this is consistent with [11].

While the results of the current study showed that the average concentration of IL-10) in the serum of the experimental group induced with ulcerative colitis, was also low compared to the control group and other groups, and the differences between groups were statistically significant. The average concentration in the serum of the infected group compared to the control group(8.5346 ± 7.78061) pg/ml,(± 26.7037 10.57566) respectively, This is consistent with the study [12-14] They report that IL-10, one of the cytokines of Th2 cells, has a biological function to determine and terminate the inflammatory response through its ability to inhibit the production of some proinflammatory cytokines And it works to stop the inflammatory response by targeting some cells such as phagocytes, Neutrophils, Eosinophils, Mast cells and lymphocytes while reducing nitric oxide production As in Figure(2) .

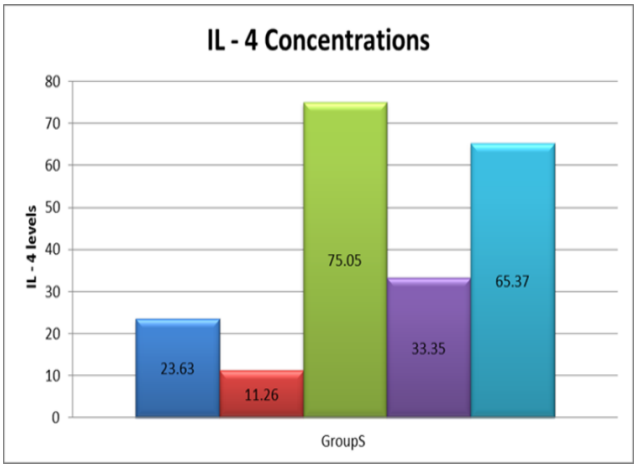

Figure (1): IL-4 concentration rates among the study groups

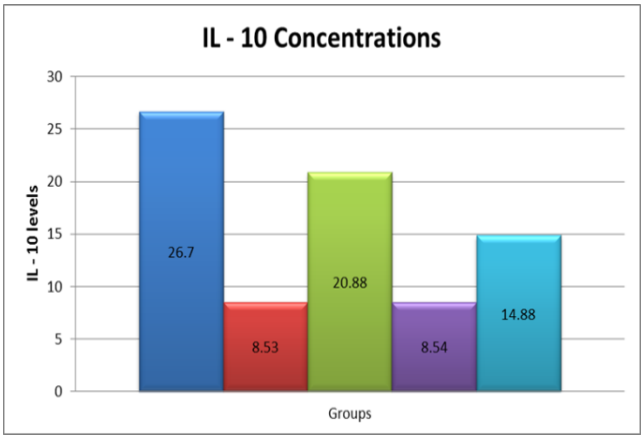

Figure (2): The IL-10concentration rates among the study groups

Also the current study showed that the results of the mean concentration of IL- 17 in the serum of the experimental group induced by ulcerative colitis was high compared to the control group and the other groups, and the differences between groups were statistically significant (P<0.05) .The average concentration in the serum of the induced group was (8.5179 ± 4.29753)While in the control sample (4.6103 ± 2.70188),As for the worms-dosed groups, it reached (2.7810 ± 1.19085), while in the challenge group and the treated group, it reached (3.3569 ± 2.77479), (4.3028 ± 3.05024) .

This is consistent with studies [15, 16] showing that the low concentration of IL-17 in the control group described by previous studies in IBD. Some clinical studies found high levels of Th17 and IL-17 in the mucosa of IBD patients. Compared to healthy controls,Th17 cells are distributed mainly in the lamina propria of the intestinal mucosa in ulcerative colitis and in the submucosal and intramuscular mucosa of patients with Crohn's disease (CD) [17].

Reported that IL-17 directly correlates with the secretion of proinflammatory factors and is also responsible for stimulating the transport of immune cells to peripheral tissues. After this process, IL-17 binds to surface receptors and finally activates NF-kB, releasing proinflammatory factors [18].

It has been observed in studies that showed elevated serum IL-17 expression in IBD patients, which is consistent with our results. As in Figure (3).

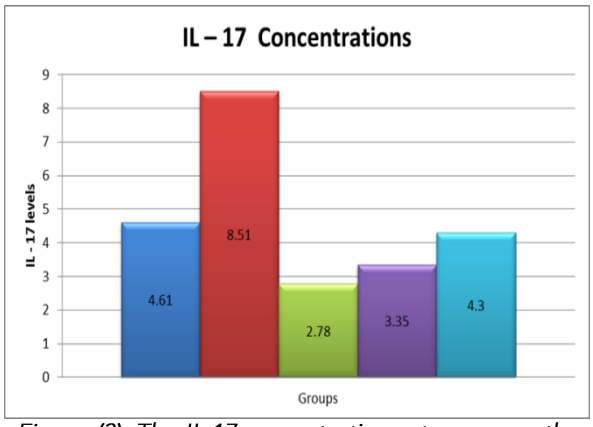

Figure (3): The IL-17concentration rates among the study groups

The results of (Cxcl1) gene expression showed high levels of folding with an average of (52.71 ± 23.50) in the case of Ulcerative Colitis induced groups with 4% acetic acid. Perhaps the reason for the appearance of a high expression in infected groups is because it plays an important role in the formation of Reactive Oxygen Species (ROS), which leads to an increase in oxidative stress and thus will disrupt the mucosal barrier. Where (ROS) works to push the enzyme (myeloperoxidase) MPO from the mucous layer of the colon, which works on the shedding of the mucous membrane and the emergence of an increased infiltration of neutrophils to the mucous membrane of the colon.It plays an essential role in the development of colitis, mucosal damage and triggering an acute inflammatory response that begins in the original lamina. MPO stimulates the oxidation of chloride ions and hydrogen peroxide to the cytotoxic form of hypochlorous

acid This corresponds to [19] They show that persistent and uncontrolled oxidative stress with increased ROS production and/or insufficient removal of ROS by antioxidant systems will cause apoptosis and tissue injury As in Figure (4)

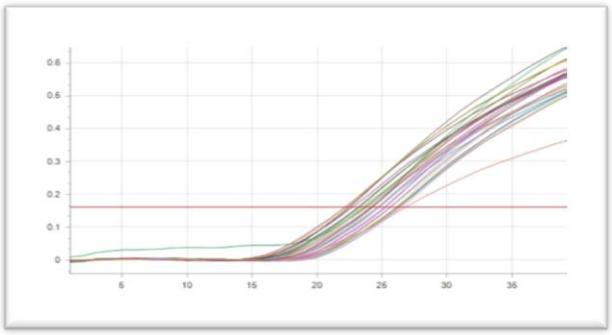

Figure (4): A graph of the relationship between the gene expressions of the CXCL1 gene for the study groups by PCR technique

Helminthes regulate immune system by modifying of both the innate and adaptive immune system. Helminthes may stimulate the response of Th2- and down-regulate the differentiation of Th1/Th17, leading to increased Th2-type cytokine (IL-4, IL-5, IL-9, IL-10, and IL-13) and decreased the secretion of Th1/Th17-type cytokine (TNF-α, IFN-γ, IL-6, IL-12, and IL-17). Furthermore, the products of worm enhance the reproduction of Treg cell, that impeding Th1/Th2/Th17 polarization mainly by the secretion of IL-10 and TGF-β. Helminthes may stimulate a regulatory of B-cells, Denteric Cells, and macrophages, which may contribute to switching from a Th1/Th17 to a Th2/Treg profile. Finally, these parasites may impeding the proliferation of ILC2, that responsible for allergic responses. Thus, helminthes prepare environment ensuring their survival and protecting the host by limiting inflammatory factors and autoimmune phenomena [9].

4. Conclusions

The cytokines of IL-17 showed a high concentration in patients with ulcerative colitis in comparison with other samples. While the cytokines of IL-4 and IL-10 showed low concentrations in the infected samples, also the treatment with H.diminuta worm protein extract for the groups induced with ulcerative colitis caused a significant difference represented by a decrease in the gene expression of Cxcl1 gene in the treated and challenge groups and the treatment group.

Ethics approval

In this study, all procedures followed and the animals that used were in accordance with the ethical standards of the university in which the study was carried out(2019/09/29)

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Conflict of interest

There was no conflict of interest among the authors in presenting this article for publication.

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