Original Article

Immunomodulatory Effects of the Somatic Antigens of *Fasciola Hepatica* and *Teladorgasia Circumcincta* in Mice Immunized with Sheep Red Blood Cells

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Abstract

Background & Objective: Infestation of humans with helminth parasites can reduce the severity of some auto-inflammatory symptoms in humans. This study was done to evaluate the impact of the somatic antigens extracted from Fasciola hepatica (as an indicator Trematode), and Teladorgasia circumcincta (as an indicator nematode) on the immune responses of NMRI mice challenged with sheep red blood cells (SRBCs).

Materials & Methods: The mice in treatment groups were intraperitoneally immunized with 1×10^9 SRBCs twice with 14 days intervals. Concurrent with the immunization, the mice received the extract of each of the parasites (50,100. And 150 µg of protein) or placebo, throughout the study on a daily basis. The specific cellular immune responses and the anti-SRBC antibody titers were detected by footpad thickness and, microhemagglutination test, respectively. Splenocytes were also monitored for cytokine production, proliferation rate, and respiratory burst.

Results: The extracts of F. hepatica and T. circumcincta had an opposite effect on the change of the Anti-sRBC antibody level. The extract of F. hepatica caused a significant decrease in the antibody level whereas, extract T. circumcincta did not show any significant changes in the anti-SRBC antibody. Both extracts caused a significant decrease in the level of delayed-type hypersensitivity. However, the extract of F. hepatica caused a more profound reduction in the severity of delayed-type hypersensitivity. The level of IFN-γ in the splenocytes of immunized mice receiving the F. hepatica showed a more pronounced decrease than the immunized mice receiving the extract of T. circumcincta extract. IL-10 levels were only increased in the immunized mice that received the extract of F. hepatica.

Conclusion: The extract of F. hepatica may have an immunosuppressive property, while the extract of T. circumcincta may have immunomodulatory properties.

Keywords: Fasciola hepatica, Teladorgasia circumcincta, Somatic antigen, Immunoregulation

Introduction

Helminths have evolved to become experts at overthrowing immune responses (1). Despite medical advances, helminths remain one of the most successful families of infectious agents on the earth (1, 2). The immune system has evolved to induce strong responses against pathogens while eluding autoimmunity (1). Prolonged and uncontrolled immune response can result in tissue damage and the development of organ-specific autoimmunity (1, 2). Due to the high potential of parasitic infestations in inducing Th2/regulatory responses, much attention has been paid to the possibility of modulating immune responses by worms (3).

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According to the hygiene theory, one of the reasons for the increase in the rate of allergic, metabolic, and autoimmune diseases is due to increase in public health and reduced the parasitic infections subsequently (4). Parasitic infections are high in less developed countries. The prevalence of immune-pathological diseases such as inflammatory bowel disease (IBD) is significantly high in the health high-level countries (1, 5). Infection with intestinal worms has successfully reduced the severity of IBD, autoimmune diabetes, rheumatoid arthritis, and multiple sclerosis (5). Nevertheless, conflicting results were reported in infections by some of helminths. For example, the infestation with Necator americanus larvae could improve the sign of some patients with IBD (7 out of 9 patients studied), but in two others worsened the condition (6). Artificial infestation with parasitic worms, in addition to the many risks and ethical considerations, will be severe for patients to accept this method (7). It is necessary to find a suitable method instead of using direct infection with the parasite. One logical way is to use parasitic antigens instead of using parasitic infestation directly (8). Ultimately, it is parasitic antigens that modulate immune responses (3). Nematodes and trematodes are two important phyla of worms. However, there is no information about the difference in the immunomodulatory capacity of these two phyla of worms. On the other hand, the commercialization of parasitic worm extracts for immunomodulation requires that these molecules be extracted from worm parasites with a high infection prevalence.

Fasciola hepatica is a famous parasitic trematode. F. hepatica can infect the livers of humans and different species of animals. Previous studies showed that the crude extract of F. hepatica ameliorates the acetic acid-induced ulcerative colitis in Wistar rats (8).

Immunomodulatory benefits due to nematodes infection has been reported previously. For example, the antigens of the rodent nematode Acanthocheilonema viteae have immunomodulatory potential in the rheumatoid arthritis mouse model (9).

Teladorgasia circumcincta, known in the old name of Ostrategia circumcincta, is one of the most famous nematodes that infect goats and sheep that infect abomasum and rarely the small intestine. This parasite has a wide geographical spread but is found mainly in England, Iran, Australia, Africa , and the USA (10).

This study was designed to evaluate the effect of the somatic antigens extracted from Fasciola hepatica (as an indicator Trematode), and Teladorgasia circumcincta (as an indicator nematode) on the immune responses of NMRI mice challenged with sheep red blood cells (SRBCs).

Material & Methods

Chemicals

Hanks' Balanced Salt solution was purchased from GIBCO/Life Technologies Inc. (Gaithersburg, MD, USA). The enzyme-linked immunosorbent assay (ELISA) kits were obtained PeproTech EC, Ltd. (London, UK). Other reagents were procured from Sigma-Aldrich Corporation (St. Louis, MO, USA).

Preparation of parasitic antigens

Live adult flocks of F. hepatica, and T. circumcincta nematodes were isolated from liver of cattle and small intestine of sheep in Urmia abattoir, respectively. The samples were washed with Hank's saline (pH 4.7) and stored at -20 °c. Samples were crushed by a scalpel after thawing and sonicated in the presence of 1 mM PMSF (Phenyl Methyl Sulfonyl Fluoride) in Isopropanol. Then, sonication was performed for the samples (3 times, 10 minutes). Finally, the samples were centrifuged for 15 minutes at 4 °C at 10,000 rpm (Orumtadjhiz, Iran). The supernatant was separated and maintained at -20 °C. The protein content was assumed by the Bradford assay (8).

Animals

Six to seven-week-old male NMRI mice (A Swiss-type mouse given by Clara Lynch to Poiley in 1937) were obtained from the Pasteur Institute of Iran. The mice were kept under the stable conditions of temperature (22-24 °C) and 12 h light/dark cycle.

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Mice accessed food and water ad libitum. Experimental ethics was observed in compliance with the regulations approved and designed by the Ethics Committee of Urmia University, Urmia, Iran.

Experimental design, Immunological challenge and evaluation:

After accommodation time, mice were divided into three groups. Each group had 10 NMRI mice.

Mice were intraperitoneally immunized twice with two-week intervals by 1×10^9 sheep red blood cells (SRBC). Mice in treatment groups received subcutaneously 50,100 and 150 µg of somatic antigens of F. hepatica or T. circumcincta throughout the study, daily. Sheep Red Blood Cells were prepared as described earlier (11). Mice bled from their hearts seven days after the last injection. The sera were collected and the levels of anti-SRBC antibody were monitored by Micro-hemagglutination test as qualified previously (12).

It is necessary to mention that 50 µl of SRBCs (1×10^9 cells) were injected subcutaneously into the right hind footpad of each mouse, and concurrently, the same volume of PBS was injected into the left footpad as a negative control, 72 h before bleeding time. Footpad thickness was evaluated before bleeding time using a digital caliper (Swiss Precision Instruments, USA). The mean percentage enhancement in footpad thickness was reported correspondent to this formula: (Thickness of right footpad) _ (Thickness of left footpad] * 100/ (Thickness of left footpad)(12).

Cytokines analysis

Splenocytes were aseptically removed from mice at the time of bleeding. A single-cell suspension was prepared in RPMI 1640 medium supplemented with 10% fetal calf serum. Red blood cell content was omitted by ACK lysis buffer. Cell suspension (2×10^6 cells/ml) was cultured in 12-well plates and primed with 50µL phytohemagglutinin (1mg/ml). After 72h, the supernatants were collected and centrifuged to delete the cell debris (13).IFN-γ and IL-10 production was monitored by commercial ELISA kits according to the manufacturer's instructions (BenderMed, UK).

Statistical analysis

Normal distribution of data was affirmed by Kolmogorov-Smirnov test analysis. Findings were evaluated by one-way ANOVA, plus Dunnett's post hoc test and presented as means ± SD. P values less than 0.05 were considered statistically significant.

Results

As shown in Chart 1, the administration of F. hepatica parasite extract in a non-dose-dependent manner resulted in a significant reduction in anti-SRBC antibody titer compared to immunized control mice. Although increasing the daily intake of F. hepatica antigen from 50 to 150 micrograms caused a step-by-step decrease in the anti-SRBC titer, these changes were not statistically significant (Chart 1). Unlike F. hepatica extract, the daily administration of somatic antigens of T. circumcincta, could not promote any significant change in the anti-SRBC antibody titer than

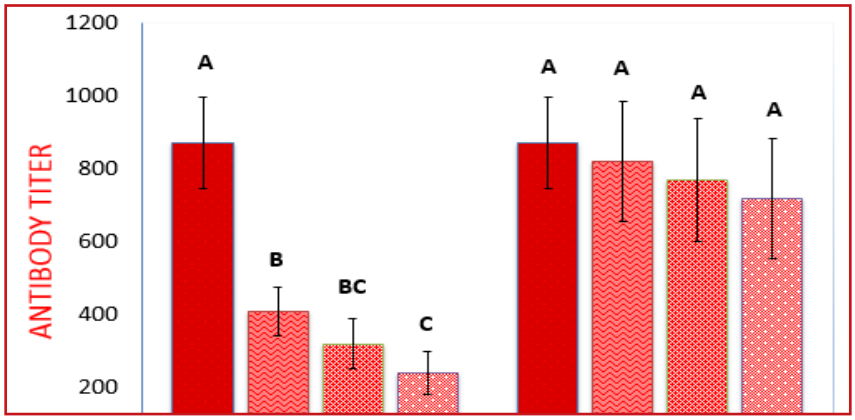

Chart 1. Evaluation of antibody titer responses in SRBC-challenged mice (Different letters indicate significant difference at p> 5% level)

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Daily intake of F. hepatica antigen caused a dose-dependent manner decrease in the level of delayed hypersensitivity responses in immunized mice with sheep red blood cells (Chart 2). This reduction also occurred in mice immunized with SRBC and treated with the somatic antigens of T. circumcincta. However, this reduction was not dose-dependent, so that between 100 µg and 150 µg there was no significant difference. Moreover, compared with the 150 µg doses of F. hepatica and T. circumcincta extracts, F. hepatica reduced the delayed hypersensitivity reaction in mice immunized with SRBC more profound than mice received T. circumcincta extracts (p> 0.01, Chart 2).

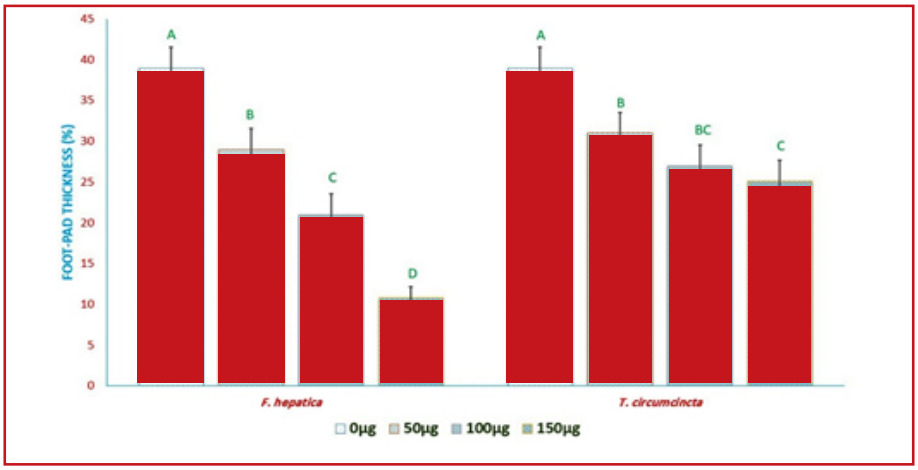

Chart 2. Assessment of delayed-type hypersensitivity responses in SRBC challenged mice (Different letters indicate significant difference at p> 5% level).

A significant reduction in IFN-γ secretion in splenocytes found in immunized mice revived somatic antigens of F. hepatica compared to splenocytes from immunized control mice in a non-dose dependent manner (Chart 3).

Finally, the most significant reduction was related to mice daily receiving 150 µg of somatic antigens of F. hepatica, compared to mice receiving 50 µg of somatic antigens of F. hepatica. (Chart 3).

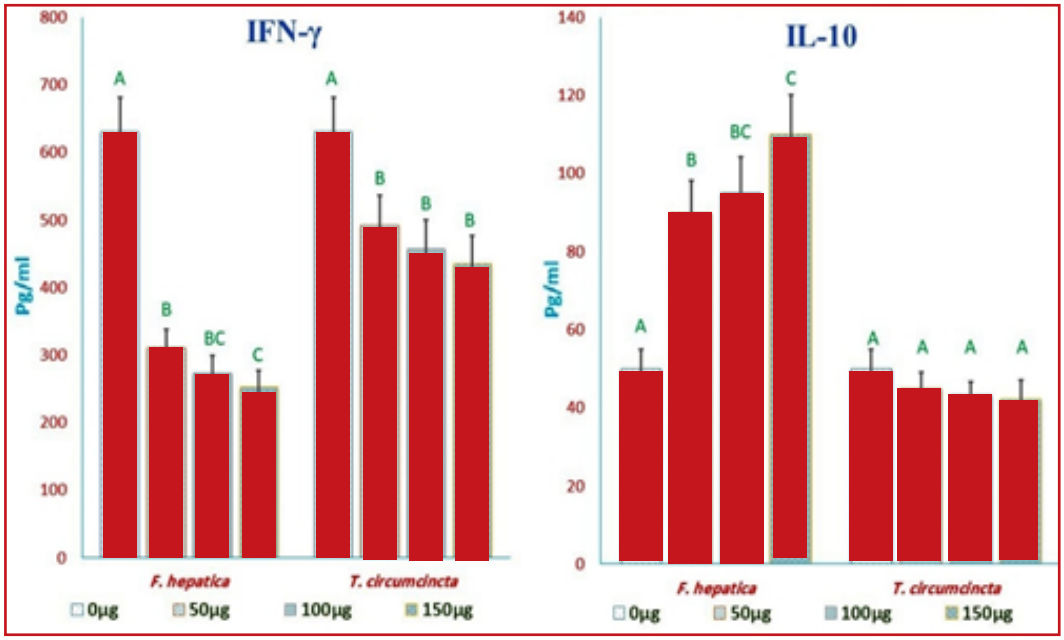

Chart 3. Evaluation of cytokine production in spleen cells of mice challenged with SRBC (Different letters indicate a significant difference at the level of p> 5%).

The levels of IFN-γ secretion in the spleen cell population of immunized mice revived somatic antigens of T. circumcincta extract was decreased compared to splenocytes from immunized control mice. However, there were no significant differences among the antigen-receiving groups (Chart 3). Compared to the corresponding data, this reduction was more significant for F. hepatica extract than T. circumcincta extract ($p> 0.01$).

The levels of IL-10 were significantly increased in immunized mice treated with F. hepatica extract compared to immunized control mice in a non-dose dependent manner (Chart 3). Despite the step by step increase in IL-10 level, there was no significant difference between the two groups receiving doses 50 and 100 µg. The highest increase in IL-10 was significantly observed in the immunized mice that received 150µg of F. hepatica extract (Chart 3).

The levels of IL-10 did not show any significant difference between immunized mice that received different doses of extract and immunized control mice (Chart 3).

Discussion

Modulation of immune responses plays an essential role in improving body function following immunological challenges in which the host's immune system needs to be inhibited or conversely enhanced (14, 15). Today, immunosuppressive, and anti-inflammatory compounds such as glucocorticoids, methotrexate, and non-steroidal anti-inflammatory drugs are used to treat autoinflammatory, autoimmune diseases as well as prophylaxis for graft rejection. Most drugs have severe side effects like osteoporosis, gastrointestinal ulcers, blood disorders, and more importantly immunosuppression (16, 17). nhibiting the immune response can lead to some infections and even cancers (16). Therefore, the use of immunomodulatory compounds instead of the usual immunosuppressive compounds, which changes the polarization of these responses toward non-pathological ones instead of merely inhibiting immune responses, is a logical decision (12, 18, 19) .

An immunomodulatory agent typically has the opposite effect on cell-mediated and humoral immunity responses (12). Therefore, due to the reduction of delayed hypersensitivity reactions and the increase in antibody production by the crude extract of T. circumcincta, this extract acted like a classical immunomodulator. On the other hand, a conventional immunosuppressive compound like glucocorticoids, inhibits cell-mediated immunity with minimal impact on humoral immunity responses (20, 21). Due to the DTH reduction and no change in the antibody level, the crude extract of F. hepatica acts as an immunosuppressant.

Previously, the administration of F. hepatica excretory/secretory antigens has been shown to inhibit diabetes in NOD (Non-Obese Diabetic) mice by inhibiting the production of interferon-gamma by T lymphocytes (22). Recent data also indicated that the extracts Fasciola hepatica and Schistosoma mansoni have vigorous immunomodulatory benefits via helminth defense molecule (23). Molecules of Fasciola hepatica like cathepsin L peptidase, peroxiredoxin, and mucin-like peptides have various immunomodulatory properties that could be used to control immune-related conditions (24, 25). Moreover, F. hepatica contains high levels of phosphorylated glycoproteins and mannose residues that are essential in modifying its host's immune responses (26). Researchers have also shown that the administration of these Fasciola antigens causes the peritoneal macrophages to polarize into M2 anti-inflammatory macrophages and increase the expression of Ym1, Arg-1 , and PD-L1 markers on their surface (22). M2 macrophages secrete large amounts of immunosuppressive agents like TGF-β by which it can dampen DTH reactions. Also, M2 macrophages themselves are involved in forming Treg cells and inhibition of immune responses (27). According to our results, F. hepatica extract could reduce DTH reaction more prominent than T. circumcincta. Secretory extracellular vesicles of F.

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hepatica protect the mice from inflammatory bowel disease by reducing the number of inflammatory cytokines and interfering with both MAPK and NF-kB pathways (28). Also, somatic antigens of Fasciola gigantica attenuate collagen-induced arthritis in rats via reducing serum IL-10, IL-4, IFN-γ, and TNF-α and decrease in the expression level of matrix metalloproteinases and nitric oxide in joints (29).

Most of the nematodes like T. circumcincta nested in the gastrointestinal tract produce immunosuppressive and anti-inflammatory mediators for their survival (30). Pervious documents suggested that the slow development of protective immunity against T. ostertagi may be due to suppressing the host immune response by T. ostertagi infection (31). A recent document indicated that Tck6, an ShKT-domain-containing protein of T. circumcincta larvae can modulate sheep T cell cytokine responses (32). In our finding, T. circumcincta extracts led to a significant decrease in DTH. Similar to our results, Cross and Klesius reported that T. ostertagi larval antigen could suppress the Concanavalin A response of mouse splenic lymphocytes in vitro (33). Some reports showed a lymphocyte proliferation in the lymph nodes of cattle abomasum infected by T. ostertagi (34). It may be due to the formation of type 2 immunity lymphocytes. Type 2 immunity strongly inhibits delayed hypersensitivity responses (12).

In the current study, a significant decrease in IFN-γ level by both parasitic extracts and a remarkable increase in IL-10 level by T. circumcincta extract were observed. Recent data showed that small size excretory/secretory antigens of the Haemonchus contortus (a species closely related to T. circumcincta) significantly reduced the potential of production of IFN-γ (the hallmark cytokine of type 1 immunity) in goat peripheral blood mononuclear cells (35). Moreover, it has been noted that F. hepatica glycans could induce potent production of IL-10 and IL-27p28 by dendritic cells and decrease allogeneic T lymphocyte proliferation, through the induction of anergic/regulatory T lymphocytes (36).

Evaluation of DTH is one of the typical criteria for monitoring cell-mediated immunity. This response implicates the interplay between T-lymphocytes, monocytes, and macrophages without any antibody involvement (37). DTH response is primed and promoted by IFN-γ (38, 39). IL-10 is a famous anti-inflammatory cytokine. It plays an essential role in suppressing and terminating macrophage activity in DTH reactions, regression of antibody production , and preventing respiratory burst (40, 41). Our results showed that treatment with somatic antigens of F. hepatica during challenge with SRBC significantly suppressed IFN-γ and concurrently increased IL-10 production more profound than somatic antigens of T. circumcincta. This justifies a further reduction in the DTH response of the somatic antigens of F. hepatica to the somatic antigens of T. circumcincta. IL-10 was profoundly increased in immunized mice that received the crude extract of F. hepatica. However, the crude extract of T. circumcincta did not lead to any significant reduction in the level of this in mice. The lack of change in antibody production observed in the immunized mice treated with somatic antigens of T. circumcincta could be due to no change in the production of IL-10.

Conclusion

Collectively, the extract of F. hepatica led to a more profound inhibition in the level of cellular and humoral responses and may have an immunosuppressive property. In contrast, the extract of T. circumcincta may have immunomodulatory properties. This extract caused a significant decrease in cellular immunity without any significant change in antibody responses. However, the present study is preliminary. Further studies on autoimmune models will be needed in the future.

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Conflict of Interest

The authors declare no conflicts of interest.

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