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# Therapeutic effect of soluble worm protein acting as immune regulatory on colitis

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ABSTRACT

**Objective:** To investigate the anti-inflammatory effect of the protein derived from the soluble factor of *Heligmosomoides polygyrus* (*H. polygyrus*) excretory-secretory in a colitis model.

**Methods:** Colitis was induced by providing drinking water containing 3% dextran sodium sulfate (DSS) for a week. DSS was administrated in a cycle protocol, each cycle consisted of 7 days of 3% DSS in the drinking water and followed by 7 days of regular water. This study consisted of five treatment groups, including Groups A (control) received untreated water, B (DSS only, without excretory-secretory), and C–E injected (*i.p.*) with excretory-secretory protein (*H. polygyrus* excretory-secretory total, excretory-secretory 28 kDa and excretory-secretory 55 kDa, respectively). Mice received injection every week. The injection of excretory-secretory was started from the 6th weeks and continued until 11 weeks. At the end of 11 weeks of the experiment, mice were sacrificed, colon tissue was removed and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, flow cytometry, real-time PCR and histology examination.

**Results:** Mice received *H. polygyrus* excretory-secretory 55 kDa reduced mono-nuclear cell infiltrations. *H. polygyrus* excretory-secretory 55 kDa induced the down-regulation of mRNA interferon-γ expression. There were significant differences in the expression of mRNA interferon in the colon of mice after the administration of the excretory-secretory 55 kDa protein fraction compared with other groups ($P < 0.001$), whereas mRNA transforming growth factor-β expression up regulated in the colon of mice after the administration of the excretory-secretory 55 kDa protein fraction compared with total excretory-secretory group ($P < 0.05$). The treatment of colitis in mice with excretory-secretory 55 kDa protein fractions modulated interleukin-10 (IL-10) expression, whereas excretory-secretory total and excretory-secretory 28 kDa protein fractions insufficient promoted IL-10 expression. Excretory-secretory 55 kDa proteins fraction promoted IL-10 expression via Foxp3-independent pathways.

**Conclusions:** Excretory-secretory 55 kDa protein could reduce inflammation and have potential therapy. *H. polygyrus* excretory-secretory 55 kDa was the soluble factor that may help in the development of novel treatments to cure colitis.

## 1. Introduction

Ulcerative colitis is a form of inflammatory bowel disease, or chronic inflammation of the colon. Colitis is characterized by spontaneous recurrences and the social conditions of the patient. Ulcerative colitis patients have a high risk of complications, including colon carcinoma and rectal carcinoma [1].

The prevalence and incidence of ulcerative colitis have increased in developing countries in recent years [1,2]. The highest prevalence of worm infections in developing countries

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is likely the cause of the low incidence of certain diseases, such as inflammatory bowel disease.

The prevalence and incidence of colitis in Asia have been increasing. An experimental model of ulcerative colitis in rodent was induced by administration of drinking water containing dextran sodium sulfate (DSS) [3]. Colitis is induced by DSS causing rodents to show many symptoms of human ulcerative colitis, such as bloody feces, diarrhea, loss of body weight, shortening of the large intestine and mucosal ulceration as well as lesions of human ulcerative colitis [3,4].

The number of $GR1^+$ and $CD11b^+$ cells of DSS-induced mice increased compared to controls [5]. In patients with colitis, macrophages are activated and show increased production of cytokines, such as interferon-γ (IFN-γ), interleukin-12 (IL-12) and IL-23 [6,7]. Th1 cells activate $CD4^+$ T cells, resulting in them secreting IL-2 and IFN-γ. The secretion of IFN-γ plays a role in macrophage activation. Activated macrophages secrete IFN-γ, which is a pro-inflammatory cytokine [8].

Worm therapy has recently been developed. This therapy is widely used in China, Russia and Japan. *Schistosoma mansoni* infection has a protective response to trinitro-benzene-sulfonic acid-induced colitis in mice [9,10]. *Hymenolepis diminuta* infection is able to cure colitis in mice [11], whereas *Trichuris suis* is a therapy for Crohn disease [12] and *Trichinella spiralis* protects against colitis induced by dinitrobenzene sulfonic acid [13]. The mechanisms of human disease modification by these various worm species can be studied using a mouse model of *Heligmosomoides polygyrus* (*H. polygyrus*) infection [14,15].

Many studies have shown that living nematodes have therapeutic uses. The use of worms has become an alternative therapy, but this method has disadvantages. Patients must be infected by the parasite into the body, thus, a large number of living nematodes can implement these therapies. It has been studied about the inflammatory response caused by a parasite and the consequences resulting from the nematode that lives in the body during therapy.

The inflammatory responses to a parasite and the consequences resulting from the presence of living nematodes in the body during therapy have been studied. During worm infection, macrophages are activated to produce Th2 cells, which inhibit Th1 response. However, allowing nematodes to infect and remain alive inside the human body is still difficult for patients to accept [9,16]. Many studies have reported that treatment with living worm infection or worm extracts could reduce inflammation associated with autoimmune diseases, such as rheumatoid arthritis. However, living parasites have the disadvantage of potential side effects due to invasion of the parasite to other tissues in the human host [17–20].

Based on the above reason, the worm infection causes pathological disorders. Therefore, the therapy using living parasites is necessary to be replaced with a soluble protein produced by parasites [12,21–23]. Treatment with excretory-secretory proteins could overcome the disadvantages of treatment using living parasites. We examined potential mechanisms that contribute to DSS infection by nematode *H. polygyrus*. Therefore, therapies using excretory-secretory proteins are more favorable than therapies that allow living nematodes to be maintained in the host. We also examined the effect of *H. polygyrus* excretory-secretory on mice with DSS-induced colitis.

## 2. Materials and methods

### 2.1. Animals

Female BALB/c mice, aged 8–10 weeks and weighing 20–25 g were maintained in a relative humidity of 50%–55% with a preset light–dark cycle (12:12 h). Mice were given normal drinking water *ad libitum* during the experimental periods. The mice were housed under specific pathogen-free conditions. All experimental procedures involving animals were conducted in accordance to institutional guidelines and the current regulations and approved by the Ethical Committee Brawijaya University, Malang, Indonesia (No. 288/EC/KEPK-JK/11/2012).

### 2.2. Worm establishment

BALB/c mice were infected with 300 third-stage larvae (L3) *H. polygyrus*. L3 *H. polygyrus* were provided by Jikei University of Tokyo and maintained in Laboratory of Parasitology, Faculty of Medicine, Brawijaya University, Indonesia. Adult worms were collected 14–20 days post-infection and were used for *in vitro* culture to measure secreted proteins.

### 2.3. Excretory-secretory antigens of H. polygyrus

Excretory-secretory was collected from adult worms. Briefly, the worm was washed several times with sterile culture medium (RPMI-1640, 100 IU/mL penicillin, 100 IU/mL streptomycin) (Sigma–Aldrich, St. Louis, MO, USA) and incubated in 10 mL of culture medium at 37 °C in a 5% $CO_2$ atmosphere. Culture supernatants were removed every 24 h for 3 days and were stored at −20 °C, and the conditioned medium containing the excretory-secretory products was collected, centrifuged at 4000 r/min for 30 min, fractionated into low and high-molecular-weight fractions using 50 kDa MWCO Amicon Ultra Centrifugal (Amicon, Danvers, MA) and stored at −20 °C until use.

### 2.4. Induced colitis and injected excretory-secretory in mice

BALB/c mice were given drinking water containing 3% DSS (ICN Biomedical Inc, CA, USA) for 7 days. DSS was administered in a cycle protocol, with each cycle consisting of 7 days of 3% DSS and followed by 7 days of regular water. Colitis was induced by cyclical DSS treatments, which consisted of 7 days of 3% DSS followed by 7 days of untreated water. The injection of excretory-secretory (40 μg/mL) was started from 6th weeks and then, continued until 11 weeks. The body weights of the mice were measured every week. Induction of colitis was determined by the observations of weight loss, fecal blood. Blood in the feces was detected using an occult blood detection kit (Hemoccult). Female BALB/c mice were adaptively fed for 1 week and then randomly divided into five groups. Group A was regarded as normal control group with free access to drinking water. Group B was DSS model group, freely drinking 3% DSS solution for 7 days followed water for 7 days. Group C was *H. polygyrus* excretory-secretory total treatment group, with the same drinking solution as in Group B, with intraperitoneal