# Differential effects of t helper-17 cytokines on the functions of granulocytes isolated from *schistosoma mansoni*-infected patients and healthy individuals

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**Received** 7 April 2016
**Accepted** 28 April 2016

**Journal of The Arab Society for Medical Research** 2016, 11:29–36

**Background and aim**
The role of T helper-17 lymphocytes in the regulation of the immune response against *Schistosoma* soluble egg antigens is still controversial. In this study, the in-vitro effects of T helper-17 cytokines [interleukin (IL)-17 and IL-22] on granulocyte functions isolated from *Schistosoma*-infected patients or healthy individuals were examined.

**Patients and methods**
Twenty-seven *Schistosoma mansoni*-infected patients and 13 healthy individuals from Kasr Al-Aini Viral Hepatitis Center were enrolled in the present study. Granulocytes were isolated from whole blood of patients and controls by Ficoll–Paque density gradient for removal of the mononuclear layer and then lysis of red blood cells. Granulocytes were stimulated *in vitro* with soluble egg antigen in the presence of IL-17, IL-22, or both. After 24 h, the supernatants were collected for the measurement of tumor necrosis factor (TNF)-α , hydrogen peroxide ($H_2O_2$), myeloperoxidase (MPO), and nitric oxide (NO) using enzyme-linked immunosorbent assay as surrogate markers for granulocyte functions.

**Results**
The results indicated that the presence of IL-17 significantly decreased ($P<0.05$) TNF-α , $H_2O_2$, MPO, and NO production by granulocytes isolated from *Schistosoma*-infected patients. In contrast, in the presence of IL-22 or both IL-17 and IL-22, there were significant increases in the production of $H_2O_2$ and TNF-α by granulocytes isolated from *Schistosoma*-infected patients. Moreover, in the presence of both IL-17 and IL-22, nonsignificant changes were observed in MPO or NO levels compared with those in the control participants.

**Conclusion**
IL-17, in contrast to IL-22, inhibited the functional activity of granulocytes isolated from *S. mansoni*-infected patients. Therefore, neutralization of IL-17 may work as a therapeutic strategy for these patients.

**Keywords:**
cytokines, granulocytes, Schistosoma spp, T helper-17 cells

J Arab Soc Med Res 11:29–36

1687-4293

## Introduction

Schistosomiasis, an infectious disease caused by parasitic Trematodes (schistosomes), is a major public health problem in tropical and subtropical regions [1]. The disease causes health problems and labor loss, with a strong impact on socioeconomic development [2]. Approximately 207 million individuals are infected and 779 million are at risk of being infected in 76 endemic countries (mostly in Africa), leading to the loss of about 4.5 million disability-adjusted life years [1–4]. In Egypt, two species, *Schistosoma haematobium* and *Schistosoma mansoni*, cause urinary and intestinal schistosomiasis, respectively [5].

*Schistosoma* eggs and their secreted products provide a continuous antigenic stimulus for the immune response. If these antigens are not sequestered or neutralized effectively, they can damage the affected tissues. Hepatocytes are particularly sensitive to toxins secreted by the *Schistosoma* eggs [6]. The lesions are mediated and orchestrated by $CD4^+$ T cells as reported by several studies [7–10].

Several studies suggested that T helper-17 (Th17) cells, a new $CD4^+$ T-cell lineage, regulate the immune responses by secreting interleukin (IL)-17/IL-22 and thereby stimulating the production of additional proinflammatory and chemotactic molecules [11–17]. The role of Th17 cells and their secreted cytokines (IL-17 and IL-22) in the

recruitment of granulocytes in the presence of *Schistosoma* soluble egg antigens (SEA) has been partially studied [18,19]. IL-17 aids the recruitment of granulocytes during the inflammatory response against *Schistosoma* SEA and the development of granuloma. However, the effect of IL-22 is still unclear [16,20]. In the present study, the effects of Th17 cytokines (IL-17 and IL-22) on granulocyte functional activities *in vitro* were studied using human granulocytes isolated from the whole blood of *Schistosoma*-infected and control participants. Granulocytes were stimulated with SEA in the presence of Th17 cytokines and cultured overnight. Their mediators such as tumor necrosis factor (TNF)-α , hydrogen peroxide ($H_2O_2$), myeloperoxidase (MPO), and nitric oxide (NO) were measured in the culture supernatant as surrogate markers for granulocyte functions.

## Patients and methods

### Patients

Twenty-seven *S. mansoni*-infected patients (20 men with a mean age of 47.4±14.6 years and seven women with a mean age of 48±12 years) and 13 healthy individuals with no history of *Schistosoma* infection (four men with a mean age of 40.88±16 years and nine women with a mean age of 40.6±14.6 years) were enrolled in the present study. This study is a part of our project no. 1814 funded by Science and Technology Development Fund, Egypt, and enrolled 150 patients, most of whom were coinfected with hepatitis C virus. The present study focuses on *S. mansoni* monoinfected patients.

Participants were enrolled from Kasr Al-Aini Viral Hepatitis Center, Faculty of Medicine, Cairo University, from October 2013 to June 2015. All patients were subjected to a physical examination and a clinical history was obtained. All patients had a history of schistosomiasis, detection of *S. mansoni* ova in stool or rectal biopsy samples, and seropositivity for schistosomal antibodies (indirect hemagglutination; Femouz Laboratories, Asniéres, France). The patients enrolled in the study had no serological markers for the presence of all hepatitis viruses, cytomegalovirus infection, Epstein–Barr virus infection, or other hepatic or intestinal parasites. None of the patients had a history of habitual alcohol consumption or hepatocellular carcinoma.

Approximately 15 ml of blood was withdrawn from all the enrolled participants using ethylenediaminetetraacetic acid (EDTA) anticoagulated vacutainer tubes. The study was approved by the Research Ethics Committee of Cairo University, Egypt. All participants signed an informed consent.

### Isolation of granulocytes from whole blood

Isolation of human granulocytes from whole blood was performed by the Ficoll–Paque density gradient as described by Mollinedo *et al.* [21]. Whole blood in EDTA anticoagulant (15 ml) was diluted 1 : 1 in phosphate buffer saline (Sigma, St Louis, Missouri, USA) and slowly layered on the Ficoll–Paque solution (Axis-Shield PoC AS, Oslo, Norway) in a sterile tube. The tubes were centrifuged at 1500 rpm for 25 min at 4°C in the cooling centrifuge (). The upper layers were discarded and the pellet-containing granulocytes and erythrocytes (around 5 ml) were collected. Erythrocytes were lysed by adding ACK lysis buffer (8.024 mg $NH_4Cl$, 1.001 mg $KHCO_3$, and 3.722 mg EDTA-$Na_2 \cdot 2H_2O$ were added to 1 : 1 of $H_2O$) and mixing slowly by inversion for 5 min and left for 20 min to lyse erythrocytes completely. The granulocyte suspension was centrifuged at 2000 rpm for 10 min, the supernatant was discarded, and cells were washed with Dulbecco's modified eagle medium media (). Finally, granulocytes were resuspended in Dulbecco's modified eagle medium and 10% fetal bovine serum (HyClone, USA) and cells were counted. The viability of isolated granulocytes was greater than 98% as measured by trypan blue dye (ADWIC, Cairo, Egypt) exclusion.

### Overnight cultures of granulocytes

Isolated granulocytes were stimulated by different antigens such as lipopolysaccharide (LPS) (100 ng/ml) as a positive control, SEA (1 ng/ml), or SEA in the presence of IL-17 (125 pg/ml), or IL-22 (300 pg/ml) or both IL-17 and IL-22 in a 96-well cell culture plate. The concentrations of antigens and cytokines were used according to the study of Nady and Shata [22]. Each experiment was conducted in triplicate. The cultures were maintained at 37°C in a 5% carbon dioxide incubator for 18 h. The supernatant was collected and stored in −70°C for further analysis.

### Measured granulocytes mediators

NO was measured according to the method of Green *et al.* [23]. NO in the supernatant was assayed by the Griess reaction, which has the ability to produce a chromophore with the Griess reagent. Reading of the color changes was measured using a microtiter plate reader (Bio Tec, Winooski, USA) at dual wavelength (450 and 640 nm). A standard curve was used to measure the concentration of nitrite.

TNF-α was measured according to the method of Fossati *et al.* [24] using the enzyme-linked

immunosorbent assay kit (BosterImmunoleader, Pleasanton, CA, USA).

$H_2O_2$ was measured using $H_2O_2$ colorimetric methods ('Bio-diagnostic Com. Giza, Egypt) according to the method of Segal [25].

Human MPO was measured using the human MPO/MPO enzyme-linked immunosorbent assay kit (Booster Immunoleader) according to the method of Segal [25].

### Statistical analysis

Statistical analysis was carried out using a *t*-test to compare granulocyte functions of *Schistosoma*-infected individuals with those of noninfected individuals using Graph Pad Prism 6 Software (GraphPad, San Diego, California, USA). The data are presented as mean±SD. Percent change from nonactivated granulocytes was calculated. Results with a *P* value of less than 0.05 were considered significant.

## Results

### Tumor necrosis factor-α production by overnight activated granulocytes

The present results as shown in Fig. 1 indicated that LPS-stimulated granulocytes isolated from control participants produced significantly higher levels of TNF-α ($P$=0.0193) than that produced by granulocytes isolated from *Schistosoma*-infected patients. However, there was no significant difference in the levels of TNF-α produced in response to SEA by granulocytes isolated from either controls or *Schistosoma*-infected patients. In contrast, the presence of IL-17 significantly ($P$=0.0127) decreased the TNF-α level in *Schistosoma*-infected granulocytes compared with that in the control granulocytes. However, in the presence of IL-22 or both IL-17 and IL-22, highly significant ($P$<0.0001) levels of TNF-α were produced by *Schistosoma*-infected granulocytes compared with those produced by control granulocytes (Table 1).

### Hydrogen peroxide production by overnight activated granulocytes

The differences in the levels of $H_2O_2$ produced by LPS-stimulated granulocytes isolated from either control participants or *Schistosoma*-infected patients were not statistically significant. SEA induced the release of significant ($P$=0.0021) levels of $H_2O_2$ from granulocytes isolated from control participants compared with that produced from *Schistosoma*-infected patients. In addition, the presence of IL-17 significantly ($P$=0.0302) inhibited the release of $H_2O_2$ by *Schistosoma*-infected granulocytes compared with that produced by control granulocytes. The presence of IL-22 alone had no significant effect on the levels of $H_2O_2$ produced by granulocytes isolated from either controls or *Schistosoma*-infected patients. A marked increase ($P$<0.001) was observed in the levels of $H_2O_2$ produced by granulocytes isolated from *Schistosoma*-infected patients compared with the controls in the presence of both IL-17 and IL-22 (Fig. 2 and Table 2).

### Nitric oxide production by overnight activated granulocytes

LPS-stimulated granulocytes isolated from control participants produced significantly ($P$=0.021) higher levels of NO than those produced by granulocytes isolated from *Schistosoma*-infected patients. In contrast, SEA induced a significant ($P$=0.0053) increase in NO production by *Schistosoma*-infected granulocytes compared with that produced by control granulocytes. However, in the presence of IL-17, a significant ($P$=0.0018) inhibition in NO production was observed in *Schistosoma*-infected granulocytes compared with that produced by control granulocytes. In the presence of IL-22 or both IL-17 with IL-22, no significant changes in the levels of NO were produced by granulocytes isolated from either

**Table 1 Tumor necrosis factor-α level (pg/ml) produced by granulocytes stimulated overnight with soluble egg antigen in the presence of T helper-17 cytokines**

| | Stimulus used to activate granulocytes | | | | | |
|---|---|---|---|---|---|---|
| Groups (granulocyte source) | No stimulus | LPS (100 ng/ml) | SEA (1 ng/ml) | SEA+IL-17 (125 pg/ml) | SEA+IL-22 (300 pg/ml) | SEA+IL-17+IL-22 |
| Controls participants | 1.7±0.5 | 20.36±7.8 (1126.4) | 8.6±1.7 (405.7) | 15±0.7 (782) | −11±2.8 (−747) | −5.7±2.6 (−435.3) |
| *Schistosoma*-infected patients | 3.2±3.1 | 6.8±4.5 (112.5)* | 5.9±3.4 (84.4) | 6.2±9.4 (93.75)* | 2.5±3.1 (−22)** | 4.3±3.1 (34.38)** |

All data are represented as mean±SD and percentage change from nonactivated granulocytes are given in parentheses. IL, interleukin; LPS, lipopolysaccharide; SEA, soluble egg antigen. *Significantly different from control participants at P<0.05. **Significantly different from control participants at P<0.0001.

**Table 2 Hydrogen peroxide level (μmol/l) produced by granulocytes stimulated overnight with soluble egg antigen in the presence of T helper-17 cytokines**

| | Stimulus used to activate granulocytes | | | | | |
|---|---|---|---|---|---|---|
| Groups (granulocyte source) | No stimulus | LPS (100 ng/ml) | SEA (1 ng/ml) | SEA+IL-17 (125 pg/ml) | SEA+IL-22 (300 pg/ml) | SEA+IL-17+IL-22 |
| Controls participants | 499.8±5.7 | 511.7±20.9 (2.5) | 524.8±4.9 (5.2) | 512.4± 9.3 (2.7) | 514.1±16.3 (3.0) | 515.6±10.4 (3.3) |
| *Schistosoma*-infected patients | 295.8±2.1 | 292.8±0.0 (–1.0) | 294.3±2.1 (–0.5)* | 270.3±0.0 (–8.6)* | 294.3±2.1 (–0.5) | 381.4±22.1 (28.9) ** |

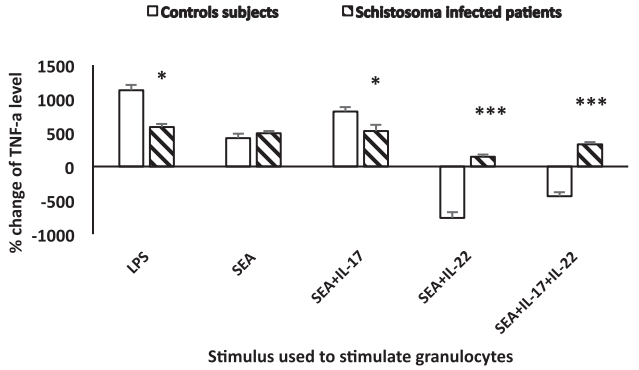
All data are represented as mean±SD and percentage change from nonactivated granulocytes are given in parentheses. IL, interleukin; LPS, lipopolysaccharide; SEA, soluble egg antigen. *Significantly different from control participants at P<0.05. **Significantly different from control participants at P<0.0001.

**Table 3 Nitric oxide level (μM/g) produced by granulocytes stimulated overnight with soluble egg antigens in the presence of T helper-17 cytokines**

| | Stimulus used to activate granulocytes | | | | | |
|---|---|---|---|---|---|---|
| Groups (granulocyte source) | No stimulus | LPS (100 ng/ml) | SEA (1 ng/ml) | SEA+IL-17 (125 pg/ml) | SEA+IL-22 (300 pg/ml) | SEA+IL-17+IL-22 |
| Controls participants | 78.4±3.6 | 115.6±10.7 (47.6)* | 74.3±1.6 (–5.2) | 99.8±9.1 (27.4) | 73.9±3.2 (–5.5) | 80.5±4.6 (2.8) |
| *Schistosoma*-infected patients | 75.8±5.3 | 76.1±4.4 (0.4) | 78.4± 2.6 (3.3)* | 65.9±2.3 (–13)* | 74.7±5.5 (–1.5) | 73.5±1.4 (–3.14) |

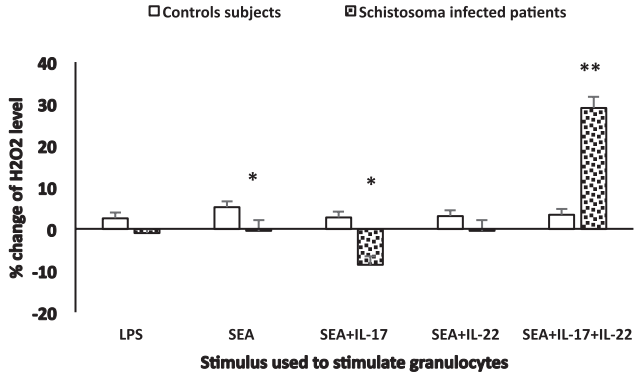
All data are represented as mean±SD and percentage change from nonactivated granulocytes are given in parentheses. IL, interleukin; LPS, lipopolysaccharide; SEA, soluble egg antigen. *Significantly different from control participants at P<0.05.

**Figure 1**




TNF-α produced by granulocytes stimulated overnight with SEA in the presence of Th17 cytokines. *, significant different as compared to control subjects at $P < 0.05$. ***, significant different as compared to control subjects at $P < 0.0001$.

**Figure 2**




Hydrogen peroxideproduced by granulocytes stimulated overnight with SEA in the presence of Th17 cytokines. *, significant different as compared to control subjects at $P < 0.05$. **, significant different as compared to control subjects at $P < 0.001$.

controls or *Schistosoma*-infected patients (Fig. 3 and Table 3).

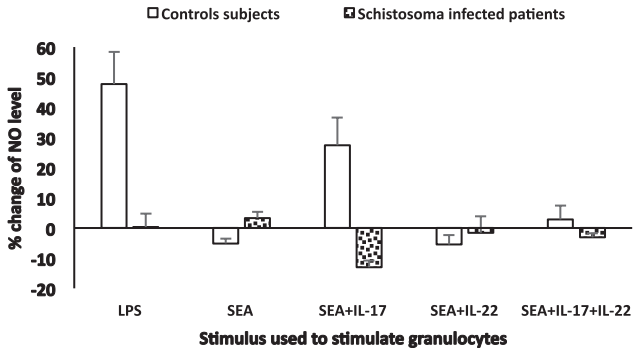
### Myeloperoxidase production by overnight activated granulocytes

No significant changes were observed in the levels of MPO produced by granulocytes isolated from either controls or *Schistosoma*-infected patients in response to LPS or SEA. In the presence of IL-17 alone were significant changes ($P$=0.0054) in the MPO levels observed in *Schistosoma*-infected granulocytes compared with that produced by control granulocytes (Fig. 4 and Table 4).

## Discussion

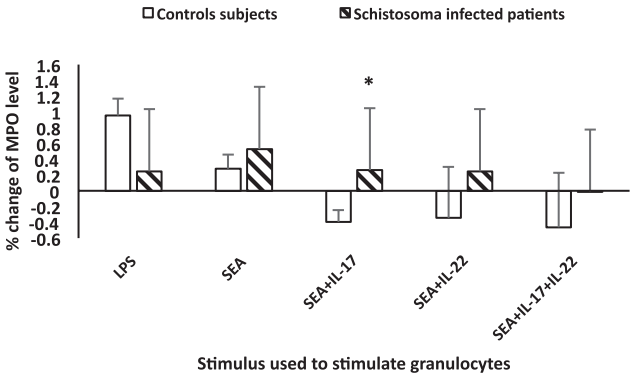
The role of Th17 cells in schistosomiasis has been partially explored in mice models [26–33]; however, limited data are available in terms of human schistosomiasis. Previous studies have indicated that enhanced neutrophil recruitment and activation is an important factor in Th17 cell-mediated inflammation [16,20]. A recent study suggested that one apparent mechanism used by Th17 cells to regulate the immunopathology is through the recruitment of granulocytes [34]. Both granulocytes

**Figure 3**




NO produced by granulocytes stimulated overnight with SEA in the presence of Th17 cytokines. *, significant different as compared to control subjects at $P < 0.05$.

**Figure 4**




Myeloperoxidase produced by granulocytes stimulated overnight with SEA in the presence of Th17 cytokines. *, significant different as compared to control subjects at $P < 0.05$.

**Table 4 Myeloperoxidase level (ng/ml) produced by granulocytes stimulated overnight with soluble egg antigen in the presence of T helper-17 cytokines**

| | Stimulus used to activate granulocytes | | | | | |
|---|---|---|---|---|---|---|
| Groups (granulocyte source) | No stimulus | LPS (100 ng/ml) | SEA (1 ng/ml) | SEA+IL-17 (125 pg/ml) | SEA+IL-22 (300 pg/ml) | SEA+IL-17+IL-22 |
| Controls participants | 156.9±1.8 | 157.5±2.1 (0.38) | 156.4±1.7 (–0.32) | 155.4±1.5 (–0.95) | 155.5±0.6 (–0.89) | 155.3±0.7 (–1) |
| *Schistosoma*-infected patients | 156.4±0.5 | 156.8±0.6 (0.26) | 156.8±0.7 (0.25) | 160.4±1.4 (2.5)* | 156.5±0.8 (0.1) | 155.9±0.8 (–0.32) |

All data are represented as mean±SD and percentage change from nonactivated granulocytes are given in parentheses. IL, interleukin; LPS, lipopolysaccharide; SEA, soluble egg antigen. *Significantly different from control participants at P<0.05.

and eosinophils contribute significantly toward immunopathology induced by *Schistosoma* SEA [35]. The aim of the present study was to examine the role of Th17 cells or their secreted cytokines (IL-17 and IL-22) in the functions of granulocytes in response to *Schistosoma* SEA *in vitro* in healthy individuals and *Schistosoma*-infected patients.

In the present study, LPS-stimulated granulocytes isolated from control participants produced significant levels of TNF-α and NO that were higher than those produced by granulocytes isolated from *Schistosoma*-infected patients, suggesting a potential defect in those granulocytes isolated from *Schistosoma*-infected patients. Previous studies reported that TNF-α is produced through the activation of Toll-like receptors, which are the appropriate receptors of LPS [36]. No significant changes in the levels of $H_2O_2$ or MPO were produced by LPS-stimulated granulocytes isolated from either control participants or *Schistosoma*-infected patients, suggesting that the activations pathways for TNF-α and NO may be different from that of $H_2O_2$ or MPO.

The current results showed that overnight stimulation of granulocytes with SEA did not induce significant levels of TNF-α in either controls or *Schistosoma*-infected patients. However, in the presence of IL-22 alone or both IL-17 and IL-22, significant levels of TNF-α were produced by *Schistosoma*-infected granulocytes compared with those produced by control granulocytes, suggesting higher sensitivity to IL-22 or IL-22+IL-17 of the granulocytes isolated from *Schistosoma*-infected patients compared with those from controls for TNF-α secretion. However, this sensitivity is blocked, in the presence of IL-17 alone, which significantly decreased TNF-α level in *Schistosoma*-infected granulocytes compared with that in control granulocytes.

The release of TNF-α is triggered by binding to one of two distinct receptors designated tumor necrosis factor receptor 1 and tumor necrosis factor receptor 2, which are differentially expressed on various cell types in normal and diseased tissues [37,38]. Therefore, the unresponsiveness to SEA may be because SEA has no binding capacity to these receptors, whereas the presence of IL-22 enhanced this capacity and induced the production of TNF-α . In our previous work, it was observed that SEA exerted inhibitory effects on TNF-α production by granulocytes and this antigen might work on the same granulocyte receptors and may have similar activation pathways [39].

TNF-α primes the neutrophil respiratory burst; upregulates the expression of adhesion molecules, cytokines, and chemokines; and at high local concentrations, can stimulate reactive oxygen species production in adherent granulocytes [37,40]. The oxidative pathway involves the release of NO, which is generated either by the constitutively expressed enzymes nitric oxide synthase (NOS)-1 and NOS-3 or the induced enzyme NOS-2. NOS-2 is not expressed in naive cells, but is induced by immunological stimuli such as bacterial LPS or cytokines such as TNF-α [41].

In the current study, SEA induced the release of significant levels of $H_2O_2$ from granulocytes isolated from control participants more than that produced by granulocytes isolated from *Schistosoma*-infected patients, which suggested a potential defect of granulocytes isolated from *Schistosoma*-infected patients compared with controls. However, this defect is unique to $H_2O_2$ because a significant increase in NO production by *Schistosoma*-infected granulocytes was observed compared with that produced by control granulocytes.

Limited studies have investigated the role of different cytokines in NO production from granulocytes. In the present study, both cytokines IL-17 with IL-22 did not induce NO production, which may possibly indicate that IL-17/IL-22 cytokines play no role in NO production in contrast to the findings reported in previous studies [42].

It is well known that neutrophil azurophilic granules contain a rich supply of the green heme enzyme MPO, which, in combination with $H_2O_2$ and chloride, constitutes a potent antimicrobial system [43]. The current results showed that nonsignificant levels of MPO were produced by granulocytes isolated from either controls or *Schistosoma*-infected patients in response to SEA. In contrast to our results, a recent study reported the production of high levels of MPO in *Schistosoma japonicum*-infected mice [44].

However, in the presence of IL-17 alone, significant levels of MPO were observed in *Schistosoma*-infected granulocytes compared with those produced by control granulocytes. Many studies have reported the antibacterial role of MPO, but few studies have investigated the effect of Th17 cytokines on MPO secretion by granulocytes [45]. Previous studies have reported that IL-17 plays a central role in pulmonary host defense by recruiting and inducing the activity of granulocytes in the bronchoalveolar space. Other studies showed that IL-17 increases potentially in association with neutrophilic inflammation and mucus excess, as well as dysregulation of acquired immunity [46–48].

Similar to its effect on TNF-α production in the present study, the presence of IL-17 significantly inhibited the release of $H_2O_2$ or NO by *Schistosoma*-infected granulocytes compared with those produced by control granulocytes. However, a marked increase in the $H_2O_2$ level and no significant changes in the levels of NO were observed in the presence of both IL-17 and IL-22. However, in the presence of IL-22 alone, no significant changes in the levels of $H_2O_2$ or NO were produced by granulocytes isolated from either controls or *Schistosoma*-infected patients. Previous studies reported that IL-17 exerts no effect on peroxide production by granulocytes activated with formyl-methionyl-leucyl phenylalanine for up to 2 h of activation [49]. In terms of IL-22, some studies [50,51] indicated its involvement in the response against bacterial infections by inducing the release of innate immune mediators.

Therefore, the current results clearly showed a potential defect of granulocytes isolated from *Schistosoma*-infected patients. It also showed that Th17 cytokines, IL-17 and IL-22, might modulate the response of granulocytes to *Schistosoma* SEA and not just the recruitment of granulocytes as reported by several studies [52,53]. In agreement with our results, a previous study reported that Th17 cytokines modulate the inflammatory response of keratinocyte pathways [54].

Previous studies indicated that both cytokines, although secreted from the same cell, might exert differential effects on other cells [52]. *S. japonicum* products promote Th17 proliferation and differentiation through their effect on granulocyte functions [55].

In conclusion, as observed from the current results, the presence of IL-17 in contrast to IL-22 inhibited the functional activity of granulocytes isolated from either control participants or *S. mansoni*-infected patients. Therefore, blocking of the IL-17 effect will leave the microenvironment to IL-22 to stimulate the release of granulocytes mediators that will work on the destruction of *Schistosoma* eggs and the accompanying granuloma. Therefore, anti-IL-17 antibodies may be used as a therapeutic agent for *Schistosoma*-infected patients.

**Acknowledgements**

The authors thank the Egyptian Science and Technology Development Fund (STDF) for supporting this study through grant no. 1814 awarded to Dr Soad Nady under the framework of the Egypt/US cooperation program.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

## References

1 Chitsulo L, Engels D, Montresor A, Savioli L. The global status of schistosomiasis and its control. Acta Trop 2000;77:41–51.

2 King CH, Dickman K, Tisch DJ. Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. Lancet 2005;365:1561–1569.

3 Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. Lancet 2006;368:1106–1118.

4 Utzinger J, Raso G, Brooker S, de Savigny D, Tanner M, Ornbjerg N *et al.* Schistosomiasis and neglected tropical diseases: towards integrated and sustainable control and a word of caution. Parasitology 2009;136:1859–1874.

5 Al-Sherbiny MM, Osman AM, Hancock K, Deelder AM, Tsang VC. Application of immunodiagnostic assays: detection of antibodies and circulating antigens in human schistosomiasis and correlation with clinical findings. Am J Trop Med Hyg 1999;60:960–966.

6 Damian RT, Roberts ML, Powell MR, Clark JD, Lewis FA, Stirewalt MA. Schistosoma mansoni egg granuloma size reduction in challenged baboons after vaccination with irradiated cryopreserved schistosomula. Proc Natl Acad Sci USA 1984;81:3552–3556.

7 Cheever AW, Williams ME, Wynn TA, Finkelman FD, Seder RA, Cox TM *et al.* Anti-IL-4 treatment of *Schistosoma mansoni*-infected mice inhibits development of T cells and non-B, non-T cells expressing Th2 cytokines while decreasing egg-induced hepatic fibrosis. J Immunol 1994;153:753–759.

8 Cheever AW, Finkelman FD, Cox TM. Anti-interleukin-4 treatment diminishes secretion of Th2 cytokines and inhibits hepatic fibrosis in murine *Schistosomiasis japonica*. Parasite Immunol 1995;17:103–109.

9 Cheever AW, Jankovic D, Yap GS, Kullberg MC, Sher A, Wynn TA. Role of cytokines in the formation and downregulation of hepatic circumoval granulomas and hepatic fibrosis in *Schistosoma mansoni*-infected mice. Mem Inst Oswaldo Cruz 1998;93(Suppl 1):25–32.

10 Rutitzky LI, Lopes da Rosa JR, Stadecker MJ. Severe CD4 T cell-mediated immunopathology in murine schistosomiasis is dependent on IL-12p40 and correlates with high levels of IL-17. J Immunol 2005;175:3920–3926.

11 Paradowska A, Maśliński W, Grzybowska-Kowalczyk A, Łacki J. The function of interleukin 17 in the pathogenesis of rheumatoid arthritis. Arch Immunol Ther Exp (Warsz) 2007;55:329–334.

12 Cooke A. Th17 cells in inflammatory conditions. Rev Diabet Stud 2006;3:72–75.

13 Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. Lancet 2007;369:1627–1640.

14 Kullberg MC, Jankovic D, Feng CG, Hue S, Gorelick PL, McKenzie BS *et al.* IL-23 plays a key role in *Helicobacter hepaticus*-induced T cell-dependent colitis. J Exp Med 2006;203:2485–2494.

15 Gaddi PJ, Yap GS. Cytokine regulation of immunopathology in toxoplasmosis. Immunol Cell Biol 2007;85:155–159.

16 Matsuzaki G, Umemura M. Interleukin-17 as an effector molecule of innate and acquired immunity against infections. Microbiol Immunol 2007;51:1139–1147.

17 Schmidt-Weber CB, Akdis M, Akdis CA. TH17 cells in the big picture of immunology. J Allergy Clin Immunol 2007;120:247–254.

18 Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH *et al.* A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol 2005;6:1133–1141.

19 Wilson MS, Mentink-Kane MM, Pesce JT, Ramalingam TR, Thompson R, Wynn TA. Immunopathology of schistosomiasis. Immunol Cell Biol 2007;85:148–154.

20 Nakae S, Suto H, Berry GJ, Galli SJ. Mast cell-derived TNF can promote Th17 cell-dependent neutrophil recruitment in ovalbumin-challenged OTII mice. Blood 2007;109:3640–3648.

21 Mollinedo F, Nieto JM, Andreu JM. Cytoplasmic microtubules in human neutrophil degranulation: reversible inhibition by the colchicine analogue 2-methoxy-5-(2', 3', 4'-trimethoxyphenyl)-2, 4, 6-cycloheptatrien-1-one. Mol Pharmacol 1989;36:547–555.

22 Nady S, Shata M. Th17 cytokines modulates neutrophil response to co-infection with Schistosoma SEA and HCV core protein. International Conference on Biological; 3–4 February 2015; Bali, Indonesia: Civil and Environmental Engineering (BCEE); pp. 93-99.

23 Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem 1982;126:131–138.

24 Fossati P, Prencipe L, Berti G. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem 1980;26:227–231.

25 Segal AW. How neutrophils kill microbes. Annu Rev Immunol 2005;23:197–223.

26 Vennervald BJ, Dunne DW. Morbidity in schistosomiasis: an update. Curr Opin Infect Dis 2004;17:439–447.

27 Hsu CK, Hsu SH, Whitney RA Jr, Hansen CT. Immunopathology of schistosomiasis in athymic mice. Nature 1976;262:397–399.

28 Eltoum IA, Wynn TA, Poindexter RW, Finkelman FD, Lewis FA, Sher A, Cheever AW. Suppressive effect of interleukin-4 neutralization differs for granulomas around *Schistosoma mansoni* eggs injected into mice compared with those around eggs laid in infected mice. Infect Immun 1995;63:2532–2536.

29 Mentink-Kane MM, Cheever AW, Thompson RW, Hari DM, Kabatereine NB, Vennervald BJ *et al.* IL-13 receptor alpha 2 down-modulates granulomatous inflammation and prolongs host survival in schistosomiasis. Proc Natl Acad Sci USA 2004;101:586–590.

30 Domingo EO, Warren KS. Endogenous desensitization: changing host granulomatous response to schistosome eggs at different stages of infection with Schistosoma mansoni. Am J Pathol 1968;52:369–379.

31 Alves Oliveira LF, Moreno EC, Gazzinelli G, Martins-Filho OA, Silveira AM, Gazzinelli A *et al.* Cytokine production associated with periportal fibrosis during chronic Schistosomiasis mansoni in humans. Infect Immun 2006;74:1215–1221.

32 Hesse M, Modolell M, La Flamme AC, Schito M, Fuentes JM, Cheever AW *et al.* Differential regulation of nitric oxide synthase-2 and arginase-1 by type 1/type 2 cytokines in vivo: granulomatous pathology is shaped by the pattern of L-arginine metabolism. J Immunol 2001;167:6533–6544.

33 Grünig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM *et al.* Requirement for IL-13 independently of IL-4 in experimental asthma. Science 1998;282:2261–2263.

34 Laura I, Patrick MS, Miguel JS. T-bet protects against exacerbation of schistosome egg-induced immunopathology by regulating Th17 cell-mediated inflammation. Eur J Immunol 2009;39:2470–81.

35 Bass DA, Szejda P. Eosinophils versus neutrophils in host defense. Killing of newborn larvae of Trichinella spiralis by human granulocytes in vitro. J Clin Invest 1979;64:1415–1422.

36 Hayashi F, Means TK, Luster AD. Toll-like receptors stimulate human neutrophil function. Blood 2003;102:2660–2669.

37 Bradley JR. TNF-mediated inflammatory disease. J Pathol 2008;214:149–160.

38 Petit-Bertron AF, Tabary O, Corvol H, Jacquot J, Clement A, Cavaillon JM, Adib-Conquy M. Circulating and airway neutrophils in cystic fibrosis display different TLR expression and responsiveness to interleukin-10. Cytokine 2008;41:54–60.

39 Afdhal NH. The natural history of hepatitis C. Semin Liver Dis 2004;24 (Suppl 2):3–8.

40 Dewas C, Dang PM, Gougerot-Pocidalo MA, El-Benna J. TNF-alpha induces phosphorylation of p47(phox) in human neutrophils: partial phosphorylation of p47phox is a common event of priming of human neutrophils by TNF-alpha and granulocyte-macrophage colony-stimulating factor. J Immunol 2003;171:4392–4398.

41 Coleman JW. Nitric oxide in immunity and inflammation. Int Immunopharmacol 2001;1:1397–1406.

42 Klebanoff SJ, Kettle AJ, Rosen H, Winterbourn CC, Nauseef WM. Myeloperoxidase: a front-line defender against phagocytosed microorganisms. J Leukoc Biol 2013;93:185–198.

**43** Klebanoff SJ. Myeloperoxidase: friend and foe. J Leukoc Biol 2005;77:598–625.

**44** Wang S, Xie Y, Yang X, Wang X, Yan K, Zhong Z *et al.* Therapeutic potential of recombinant cystatin from *Schistosoma japonicum* in TNBS-induced experimental colitis of mice. Parasit Vectors 2016;9:6.

**45** Lindén A, Laan M, Anderson GP. Neutrophils, interleukin-17A and lung disease. Eur Respir J 2005;25:159–172.

**46** Aujla SJ, Dubin PJ, Kolls JK. Interleukin-17 in pulmonary host defense. Exp Lung Res 2007;33:507–518.

**47** Bettelli E, Oukka M, Kuchroo VK. T(H)-17 cells in the circle of immunity and autoimmunity. Nat Immunol 2007;8:345–350.

**48** Cua DJ, Tato CM. Innate IL-17-producing cells: the sentinels of the immune system. Nat Rev Immunol 2010;10:479–489.

**49** Pelletier M, Maggi L, Micheletti A, Lazzeri E, Tamassia N, Costantini C *et al.* Evidence for a cross-talk between human neutrophils and Th17 cells. Blood 2010;115:335–343.

**50** Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA *et al.* IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nat Med 2008;14:275–281.

**51** Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q *et al.* Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat Med 2008;14:282–289.

**52** Eyerich S, Eyerich K, Cavani A, Schmidt-Weber C. IL-17 and IL-22: siblings, not twins. Trends Immunol 2010;31:354–361.

**53** Kolls JK, Lindén A. Interleukin-17 family members and inflammation. Immunity 2004;21:467–476.

**54** Nograles KE, Zaba LC, Guttman-Yassky E, Fuentes-Duculan J, Suarez-Farinas M, Cardinale I *et al.* Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. Br J Dermatol 2008;159:1092–1102.

**55** Wen X, He L, Chi Y, Zhou S, Hoellwarth J, Zhang C *et al.* Dynamics of Th17 cells and their role in Schistosoma japonicum infection in C57BL/6 mice. PLoS Negl Trop Dis 2011;5:e1399.