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# Hypoxia, hypoxia-inducible factor-1α and vascular endothelial growth factor in a murine model of *Schistosoma mansoni* infection

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ABSTRACT

*Schistosomiasis mansoni* is a chronic parasitic disease where much of the symptomatology is attributed to granuloma formation, an immunopathological reaction against *Schistosoma* eggs. To more clearly understand the immunopathology of schistosomiasis, the tissue microenvironment generated by *S. mansoni* infected mice was investigated. Using the hypoxia marker pimonidazole, we provide immunohistochemical evidence that hypoxia occurred in inflammatory cells infiltrated around the eggs and cells surrounding granulomas in the liver, intestine, spleen and lungs of infected mice. Hypoxia-inducible factor-1α (HIF-1α) was mainly expressed in inflammatory cells surrounding the eggs and in hepatocytes surrounding cellular and fibrocellular granulomas in infected mouse liver. HIF-1α expression was also verified in granulomas in the other tissues tested (intestine, spleen and lungs). Vascular endothelial growth factor (VEGF) expression was observed in the extracellular space surrounding inflammatory cells in liver granuloma. The VEGF expression pattern verified in infected mouse liver was very similar to that observed in the other tissues tested. A strong positive correlation occurred between pimonidazole binding and HIF-1α and VEGF expression in the tissues tested, except for lung. This work is the first evidence that infection by a helminth parasite, *S. mansoni*, produces a hypoxic tissue microenvironment and induces HIF-1α and VEGF expression.



## Introduction

*Schistosomiasis mansoni* is a chronic parasitic disease and a serious public health problem in many parts of the tropical and subtropical areas of the world (Pearce and MacDonald, 2002). The disease is mainly caused by eggs released by adult worm pairs residing in mesenteric veins (Burke et al., 2009). Some eggs are trapped in the microvasculature of the liver, where they induce a vigorous granulomatous response (Stavitsky, 2004). The granulomas destroy the eggs and sequester or neutralize otherwise pathogenic egg antigens, but this process also leads to fibrinogenesis in host tissues (Stavitsky, 2004). Egg granulomas can occur in several organs, including the spleen, lungs and intestine. Consequently, much of the symptomatology of schistosomiasis is attributed to this egg-induced granulomatous response (Wilson et al., 2007). Studies in mice, rhesus monkeys and pigs have demonstrated that the granulomatous response around the *Schistosoma* egg is characterized by transitory stages (Cheever et al., 2002). It is a gradual accumulation of mononuclear cells, neutrophils and eosinophils that surrounds the freshly deposited egg. Mature granulomas show histiocytes and epithelioid cells and, in the later stages, fibrocytes and collagen fibers become prominent, while the eggs degenerate and disintegrate (Stavitsky, 2004; Cheever et al., 2002; Lenzi et al., 2006). Thus, granulomas are the main cause of the lesion responsible for morbidity and mortality in schistosomiasis (Pearce and MacDonald, 2002; Burke et al., 2009). Interestingly, a role in the development and growth of the granulomas has been attributed to the degree of angiogenesis (Baptista and Andrade, 2005). Newly formed blood vessels within and throughout the periphery of granulomas has been demonstrated, although in one third of granulomas, angiogenesis is minimal in both outbred and inbred mice (Baptista and Andrade, 2005). Angiogenesis is also minimal or absent in older granulomas (Baptista and Andrade, 2005). Since a deficient oxygen supply is one consequence of poor angiogenesis and inadequate perfusion, we hypothesized that schistosomal granulomas are hypoxic. To test the presence of hypoxia in infected tissues, a chemical hypoxia marker, pimonidazole, was used. Pimonidazole is metabolically reduced under hypoxia to produce adducts that can be detected using antibodies, it is also used as an investigational oncology probe (Raleigh et al., 1999). Recently, it has been used to demonstrate hypoxia in pulmonary granulomas induced by *Mycobacterium tuberculosis* (Via et al., 2008).

In the present study, the expression of hypoxia-inducible factor-1α (HIF-1α), a prominent regulator of genetic response to hypoxia, was also examined in murine *S. mansoni* infected tissues. HIF-1α is composed of α and β subunits and while the β subunit is constitutively expressed, the α subunit is degraded under normoxic conditions (Semenza et al., 2000), such that the intracellular content of HIF-1α increases immediately following a decrease in oxygen tension. HIF-1α is up regulated in several tumors and, for a number of persistent infections,

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HIF-1α has been shown to be activated in *in vitro* and *in vivo* systems (Zinkernagel et al., 2007; Nizet and Johnson, 2009). Its activation is also apparent during viral (e.g. respiratory tract respiratory syncytium virus) and bacterial infection (Haeberle et al., 2008; Kempf et al., 2005). HIF-1α in association with parasites has been much less studied. *Toxoplasma gondii* induces HIF-1α in infected fibroblasts and cutaneous lesions in mice infected with *Leishmania amazonensis* express HIF-1α (Arrais-Silva et al., 2005; Spear et al., 2006).

Since hypoxia and HIF-1α transactivate vascular endothelial growth factor (VEGF), a cytokine which can induce angiogenesis in schistosomal granulomas (Loeffler et al., 2002), VEGF expression in the tissues of mice infected with *S. mansoni* was also investigated.

## Materials and methods

### *Mice and parasite*

Four-week-old female Swiss mice were purchased from the Animal Center of the Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil. All experiments and procedures were approved by the University's Animal Research Ethics Committee (protocol number 1892-1). The mice were tail-infected for 2 h with 70 cercariae each of the BH *S. mansoni* strain (Belo Horizonte, MG, Brazil), in water, shed from *Biomphalaria glabrata* snails.

### *Tissue hypoxia analyses*

The mice were intraperitoneally administered pimonidazole hydrochloride (Hypoxyprobe-1, Chemicon International Inc., Billerica, MA) dissolved in sterile, filtered physiological saline at 60 mg/kg of body weight. After 90 min, the liver, intestine, spleen and lungs were perfused fixed with 4% paraformaldehyde and embedded in paraffin, followed by immunohistological detection of pimonidazole protein adducts, in accordance with the manufacturer's recommendations and as detailed. Serial 5 μm thick paraffin sections of liver, intestine, spleen and lungs were treated as follows: deparaffination, rehydration, endogenous quenching and citrate buffer microwave antigen retrieval using citrate buffer. To detect pimonidazole, the sections were incubated with mouse anti-pimonidazole antibody (Chemicon), developed with secondary polyclonal anti-mouse antibody conjugated with peroxidase (Sigma Chemical Co., Saint Louis, MO) and visualized with a peroxidase substrate solution containing 3,3′-diaminobenzidine and hydrogen peroxide (Arrais-Silva et al., 2005; Shi et al., 1991). Tissues sections were counterstained with hematoxylin, dehydrated in serial alcohol solutions and mounted in cytoseal-60 mounting medium (Sigma). The images were recovered with a digital imaging system, a light microscope (Eclipse E800, Nikon, Tokyo, Japan), a Cool Snap-Pro Color camera (Media Cybernetics, Silver Spring, MD), and capture software Image-Pro plus (Media Cybernetics).

### *Evaluation of infection*

The mice were sacrificed by cervical dislocation, the adult worms were collected, counted and sorted by sex after perfusion of the portal system. The number of granulomas in each organ was determined by analyzing six random optical fields within each tissue section of at least three slides, as described previously in Yoshioka et al., 2002.

### *Immunohistochemical analysis*

Immunochemical analyses of HIF-1α and VEGF were performed using rabbit polyclonal anti-HIF-1α antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and rabbit polyclonal anti-VEGF antibody (Chemicon), respectively, and developed with goat polyclonal anti-rabbit antibody conjugated with peroxidase (Sigma). Serial 5 μm thick paraffin sections of liver, intestine, spleen and lungs were treated as follows: deparaffination, rehydration, endogenous quenching and citrate buffer microwave retrieval and visualized with a peroxidase substrate solution containing 3,3′-diaminobenzidine and hydrogen peroxide, as previously described (Arrais-Silva et al., 2005; Shi et al., 1991). Control sections were incubated with normal rabbit serum, rabbit polyclonal antibody and mouse normal serum as primary antibodies. Tissues sections were counterstained with hematoxylin, dehydrated in serial alcohol solutions and mounted in cytoseal-60 mounting medium (Sigma). The images were recovered with a digital imaging system, a light microscope (Eclipse E800, Nikon), a Cool Snap-Pro Color camera (Media Cybernetics) and capture software Image-Pro plus (Media Cybernetics).

### *Data presentation*

The results presented were verified in four mice for each time point after *S. mansoni* infection. Control mice (four per time point) remained uninfected. For each mouse, at least three sections per slide of four slides from each organ were quantitatively analyzed for immunohistochemical detection of pimonidazole protein adducts, HIF-1α and VEGF. The data are presented as the mean ± SD or percentage and analyzed by Student t test to determine which groups were divergent. The level of significance was set at $p<0.05$.

## Results

All the mice exposed to 70 cercariae became infected, which was easily verified after perfusion by the recovery of worms in the portal system and mesenteric veins. Worm burden was similar at 8 weeks (a median of 49 worms per mouse) and 12 weeks postinfection (a median of 30 worms per mouse). Parasite eggs were detected in the liver, spleen, lungs and intestine in mice at 8 and 12 weeks postinfection (data not shown). Periovular granulomas occurred in all infected mice; the number of hepatic granulomas was similar at 8 and 12 weeks postinfection (6 ± 0.6 and 5 ± 0.7 per 0.84 $mm^2$ of liver, respectively). Hepatic granulomas with an intense inflammatory reaction composed of mononuclear and polymorphonuclear cells (i.e. cellular granulomas) were observed at 8 weeks postinfection; while granulomas with similar features and more fibrotic granulomas (i.e., fibrocellular granulomas) due to diminished cellularity, were observed in mouse liver at 12 weeks postinfection.

The question whether infection with *S. mansoni* leads to tissular hypoxia was evaluated using pimonidazole as a surrogate of hypoxia (Via et al., 2008). Pimonidazole was administered to the mice and 90 min later they were sacrificed and adducts produced by pimonidazole with thiol containing protein were detected by peroxidase-antibody (Via et al., 2008). Mouse liver at 8 week postinfection showed evidence of pimonidazole immunostaining located in inflammatory cells infiltrated around the eggs (Fig. 1B). Most of the granulomas (97%) were pimonidazole positive. Many hepatocytes surrounding the granuloma and Kupffer cells also presented positive immunostaining for pimonidazole (Fig. 1). Most of the granulomas (84%) observed in the mouse liver at 12 weeks postinfection showed fibrocytes and disintegrated eggs, while hepatocytes within these areas showed clear evidence of pimonidazole immunostaining (Fig. 1C). It should be noted that extensive areas of infected liver without granulomas were uniformly pimonidazole negative (Fig. 1C). As shown in Fig. 1A, no significant pimonidazole immunostaining of liver tissue was observed in uninfected mice.

Since HIF-1α accumulation is triggered primarily by hypoxia, the expression of HIF-1α immunoreactivity was analyzed in the tissues studied. As shown in Fig. 1D, liver from uninfected mice showed no immunostaining for HIF-1α. Immunostaining revealed the presence of infiltrated inflammatory cells around the schistosomal eggs expressing HIF-1α at 8 weeks postinfection. Hepatocytes presenting positive nuclear and cytoplasmic staining for HIF-1α around the granulomas

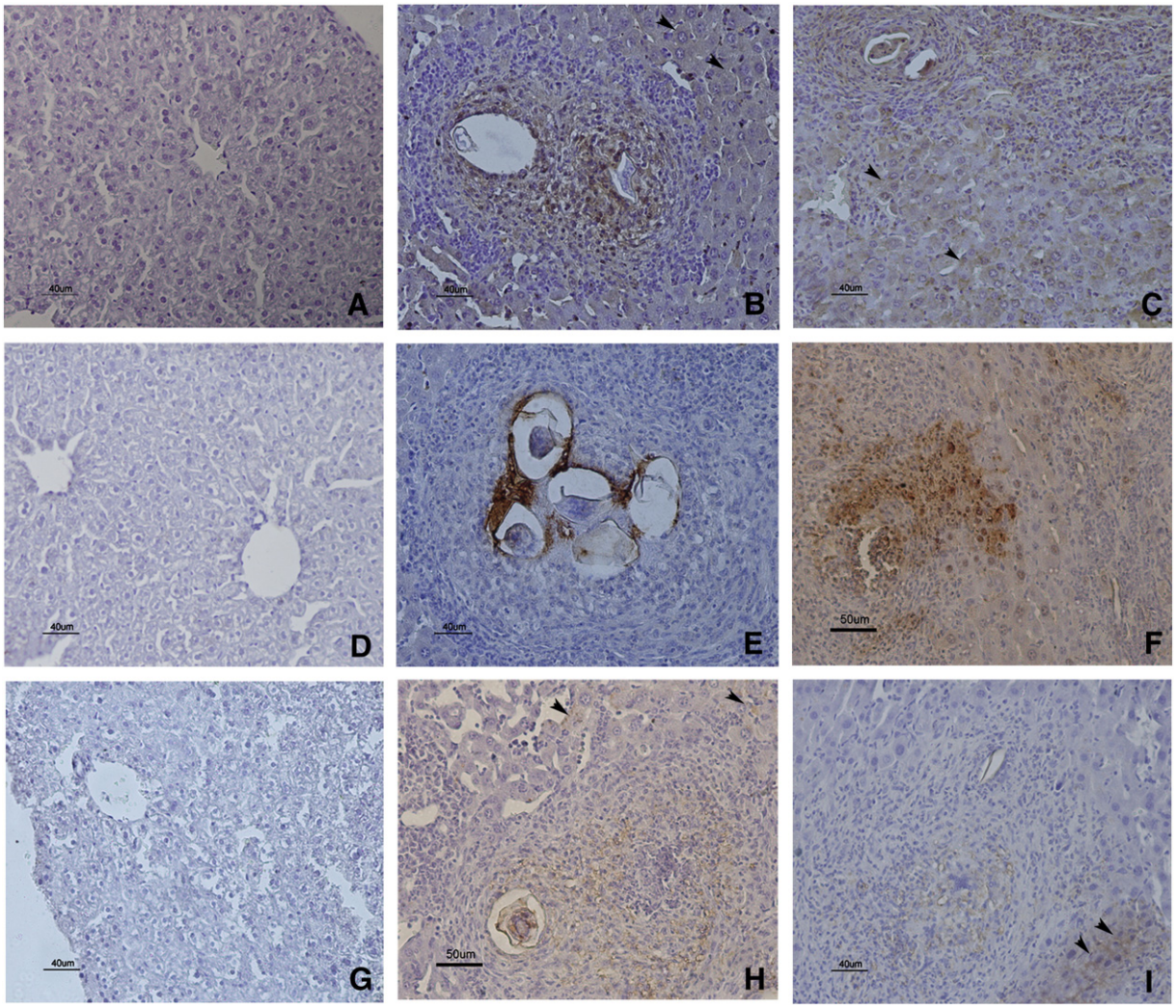


**Fig. 1.** Evaluation of hypoxia, HIF-1α and VEGF expression in mouse liver tissue. Liver tissue of uninfected mouse stained with anti-pimonidazole complex antibody, note that no immunoreactivity was observed (A); *S. mansoni* infected mouse at 8 weeks postinfection stained with anti-pimonidazole complex antibody, the arrow indicates positively stained hepatocytes (B); *S. mansoni* infected mouse at 12 weeks postinfection stained with anti-pimonidazole complex antibody, the arrow indicates positively stained hepatocytes (C); uninfected mouse stained with anti-HIF-1α polyclonal antibody, note that no immunoreactivity was observed (D); *S. mansoni* infected mouse at 8 weeks postinfection stained with anti-HIF-1α polyclonal antibody (E); *S. mansoni* infected mouse at 12 weeks postinfection stained with anti-HIF-1α polyclonal antibody (F); uninfected mouse stained with anti-VEGF polyclonal antibody, note that no immunoreactivity was observed (G); *S. mansoni* infected mouse at 8 weeks postinfection stained with anti-VEGF polyclonal antibody, the arrow indicates positively stained hepatocytes (H); *S. mansoni* infected mouse at 12 weeks postinfection stained with anti-VEGF polyclonal antibody, the arrow indicates positively stained hepatocytes (I). All sections were counterstained with hematoxylin. Data are representative of three independent experiments. 200× magnification for all photomicrographs.

(Figs. 1G and 2) and no necrosis were observed in infected hepatic tissue. Examination of the liver at 12 weeks postinfection verified inflammatory cells (Fig. 1F) and many hepatocytes staining positive for HIF-1α surrounding cellular and fibrocellular granulomas (data not shown). On average, 83% and 64% of the liver granulomas expressed HIF-1α at 8 and 12 weeks postinfection, respectively.

VEGF expression in diseased tissues can be governed by hypoxia and HIF-1α (Pugh and Ratcliffe, 2003) and angiogenesis appears to be a factor in the development of the granuloma (Baptista and Andrade, 2005; Loeffler et al., 2002). VEGF expression was investigated in *S. mansoni* infected murine liver, as shown in Fig. 1. VEGF staining was located in the extracellular space surrounding inflammatory cells in granulomas (Fig. 1); 92% and 61% of the granulomas were positive for VEGF at 8 and 12 weeks postinfection, respectively. In addition, VEGF staining was also verified in a small percentage of hepatocytes in mouse livers at 8 and 12 weeks postinfection (data not shown).

Others organs may be affected in schistosomiasis (Burke et al., 2009). Intestine, spleen and lungs contained periovular granulomas. Granulomas were observed in the serosa and mucosa of the large intestine of mice at 12 weeks postinfection (data not shown and

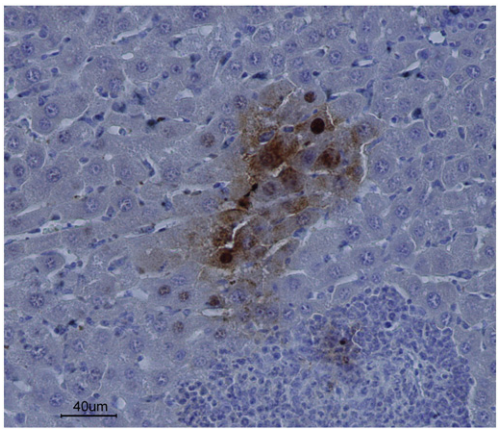


**Fig. 2.** Determination of HIF-1α in hepatocytes. Liver tissue of *S. mansoni* infected mouse at 8 weeks postinfection stained with anti-HIF-1α. Note HIF-1α immunostaining in the nuclei and cytoplasm of hepatocytes. Liver tissue was counterstained with hematoxylin. 200× magnification for all photomicrographs.

Edungbola and Shciller, 1979; Lopes et al., 2006). The staining of intestine with antibody against pimonidazole demonstrated hypoxia in smooth muscle cells and in granulomas at different stages of development; 92% of the granulomas presented pimonidazole immunostaining. Granulomas were observed in the submucosal layer (Fig. 3D). A similar pattern of immunoreactivity was observed for HIF-1α, i.e., staining of smooth muscle cells and granuloma inflammatory cells; 89% of the granulomas showed evidence of HIF-1α (Fig. 3E). VEGF reactivity was observed in the extracellular space surrounding inflammatory cells in granulomas and in smooth muscle cells; 70% of the intestinal granulomas were positive for VEGF (Fig. 3F). Pimonidazole, HIF-1α and VEGF were consistently nonreactive in normal intestinal tissue (Figs. 3A and C). Granulomas were also present in the white and red pulps of mouse spleen at 12 weeks postinfection (data not shown and (Stavitsky, 2004; Lopes et al., 2006). Pimonidazole and HIF-1α immunostaining were located in inflammatory cells surrounding schistosomal eggs and splenic cells, as shown on Figs. 4D and E; 86% and 63% of these granulomas were positive for pimonidazole and HIF-1α, respectively. VEGF expression was restricted to the extracellular space surrounding inflammatory cells in 50% of splenic granulomas (Fig. 4F). Granulomas frequently occurred in all the lobes of both the right and left lungs (data not shown and Lopes et al., 2006). In lung tissue, pimonidazole-positive areas were more extensive than that of HIF-1α and VEGF positive immunostaining regions (Figs. 5D, E, F). On average, 100% of the lung granulomas presented pimonidazole immunostaining, but only 21% were positive for HIF-1α and 18% were positive for VEGF. It should be noted that pimonidazole, HIF-1α and VEGF were consistently nonreactive in normal spleen and lung tissues (Figs. 4B, C, 5B and C).

## Discussion

The importance of tissue and cellular response to hypoxia in the pathogenesis of tumors is well appreciated (Höckel and Vaupel, 2001; Vaupel and Harrison, 2004); however, these processes are much less frequently examined in infectious diseases (Zinkernagel et al., 2007; Nizet and Johnson, 2009). For example, hypoxic and HIF-1α induction were demonstrated in experimental infection with *T. gondii*, *L. amazonensis* and *Bartonella* (Arrais-Silva et al., 2005; Kempf et al., 2005; Spear et al., 2006). To our knowledge, there are no studies involving hypoxia and hypoxia-induced factors for helminth infection. The summarized findings of this study are: 1) hypoxia, determined by pimonidazole staining, occurred in inflammatory cells infiltrated around the eggs and cells surrounding the granulomas in the liver, intestine, spleen and lungs of mice infected with *S. mansoni*; 2) HIF-1α was mainly expressed in periovular inflammatory cells and hepatocytes surrounding cellular and fibrocellular granulomas in the liver of infected mice. HIF-1α expression was observed for granulomas in the other three tissues tested, intestine, spleen and lungs; 3) VEGF expression was observed in the extracellular space surrounding inflammatory cells in the granulomas, such that the pattern of VEGF expression verified in infected mouse liver was very similar to that in the other tissues tested; and 4) a strong positive correlation occurred between pimonidazole binding and HIF-1α and VEGF expression in the tissues tested, except for lung tissue.

Between 84 and 100% of the granulomas in the liver, intestine, spleen and lungs of mice infected with *S. mansion* were pimonidazole positive. Indeed, the presence of hypoxic regions within tissues presenting *Schistosoma* egg granulomas is to be expected, since proliferation and increased migration of immunologically active cells into granulomas (Stadecker, 1999) and the deposition of eggs containing live embryos should result in increased demand for oxygen (Feldman et al., 1990). We also hypothesized that the vascular obstructive process occurring during infection (Andrade and Cheever, 1971) is involved in the development of hypoxia. Consistent with the data obtained here, the presence of hypoxia in granuloma lesions induced by *M. tuberculosis* was recently demonstrated by analyzing pimonidazole adducts (Via et al., 2008). Lung tissue from guinea pig, rabbit and nonhuman primate showed more hypoxic necrotic lesions than hypoxic diffuse inflammatory infiltrate (Via et al.,

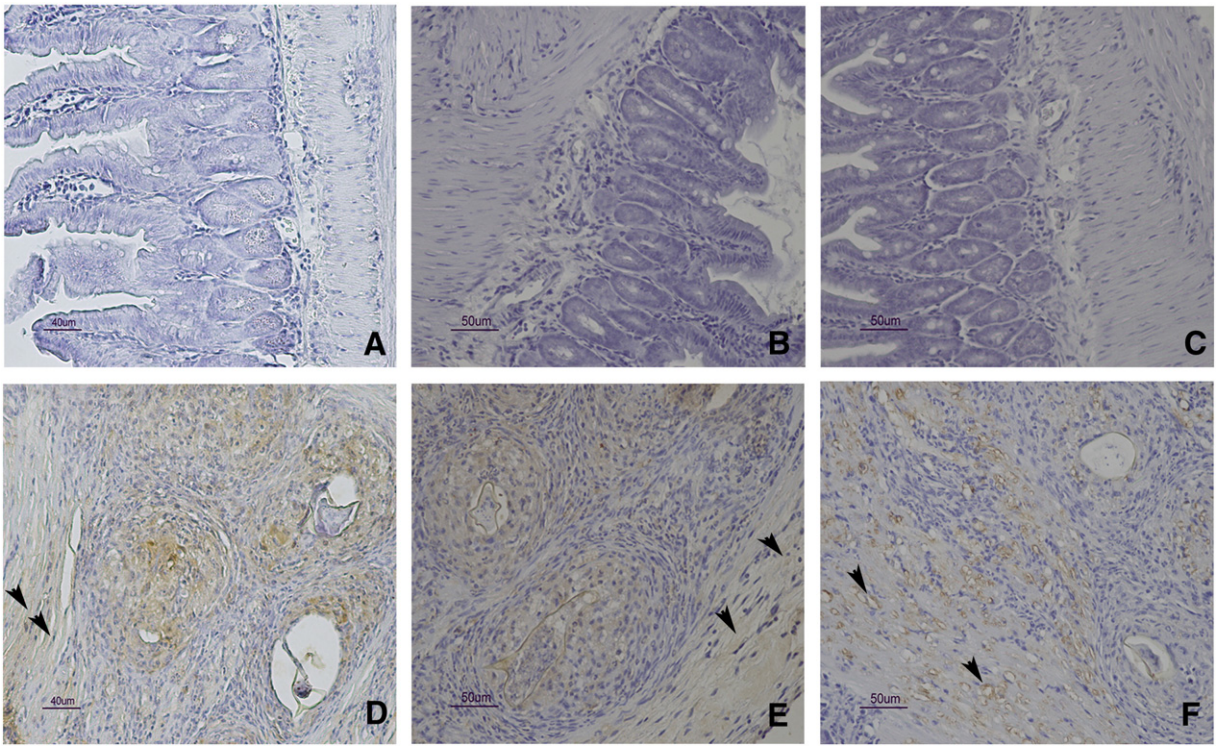


**Fig. 3.** Evaluation of hypoxia, HIF-1α and VEGF expression in mouse intestine tissue. Intestine tissue of uninfected mouse stained with anti-pimonidazole complex antibody (A) or anti-HIF-1α polyclonal antibody (B) or anti-VEGF polyclonal antibody (C); note that no immunoreactivity was observed. Intestine tissue of *S. mansoni* infected mouse at 12 weeks postinfection stained with anti-pimonidazole complex antibody (D) or anti-HIF-1α polyclonal antibody (E) or anti-VEGF polyclonal antibody (F). The arrows indicate positively stained smooth muscle cells. All sections were counterstained with hematoxylin. Data are representative of three independent experiments. 200× magnification for all photomicrographs.

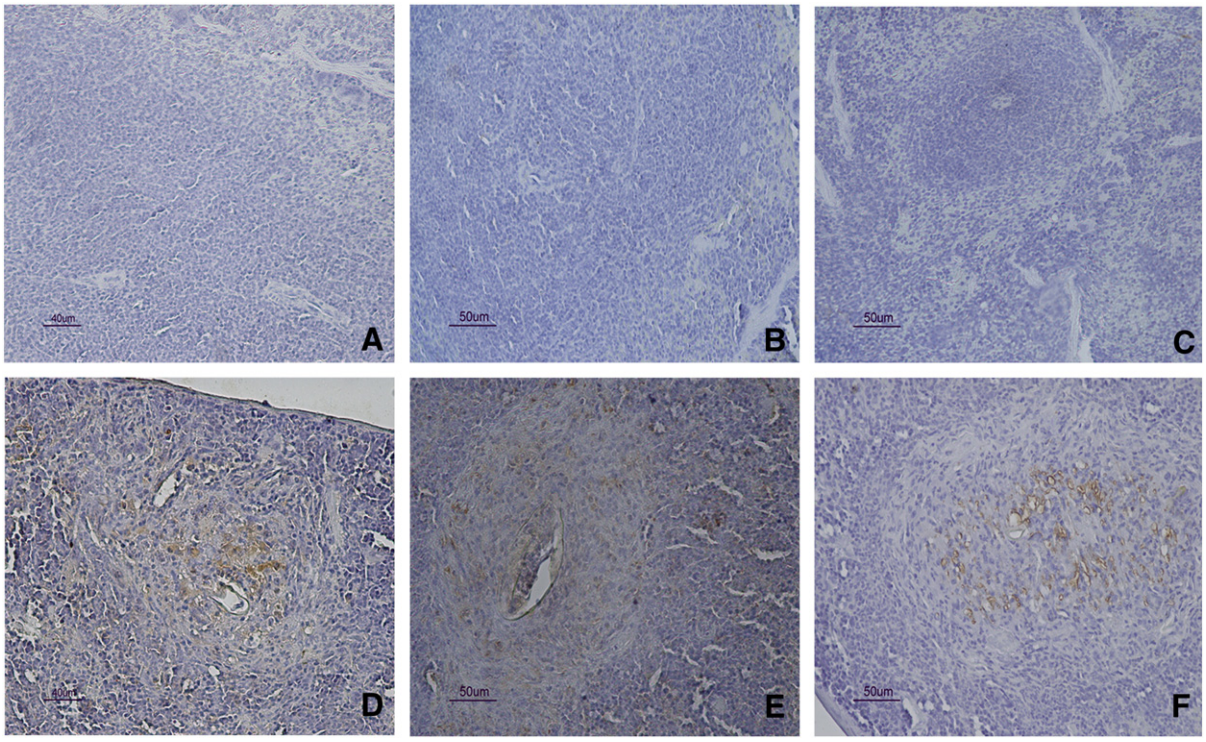


**Fig. 4.** Evaluation of hypoxia, HIF-1α and VEGF expression in mouse spleen tissue. Spleen tissue of uninfected mouse stained with anti-pimonidazole complex antibody (A) or anti-HIF-1α polyclonal antibody (B) or anti-VEGF polyclonal antibody (C); note that no immunoreactivity was observed. Spleen tissue of *S. mansoni* infected mouse at 12 weeks postinfection stained with anti-pimonidazole complex antibody (D) or anti-HIF-1α polyclonal antibody (E) or anti-VEGF polyclonal antibody (F). All sections were counterstained with hematoxylin. Data are representative of three independent experiments. 200× magnification for all photomicrographs.

2008). However, in a granuloma model of latent tuberculosis (mouse hollow-fiber model), hypoxic perifiber containing inflammatory cells and encapsulated live bacilli stained positive for pimonidazole (Klinkenberg et al., 2008). The fact that pimonidazole adducts are considered to form only when $O_2$ tension is below 1–2% (Rasey et al., 1999; Bassnett and McNulty, 2003; Sobhanifar et al., 2005) and that HIF-1α presents half

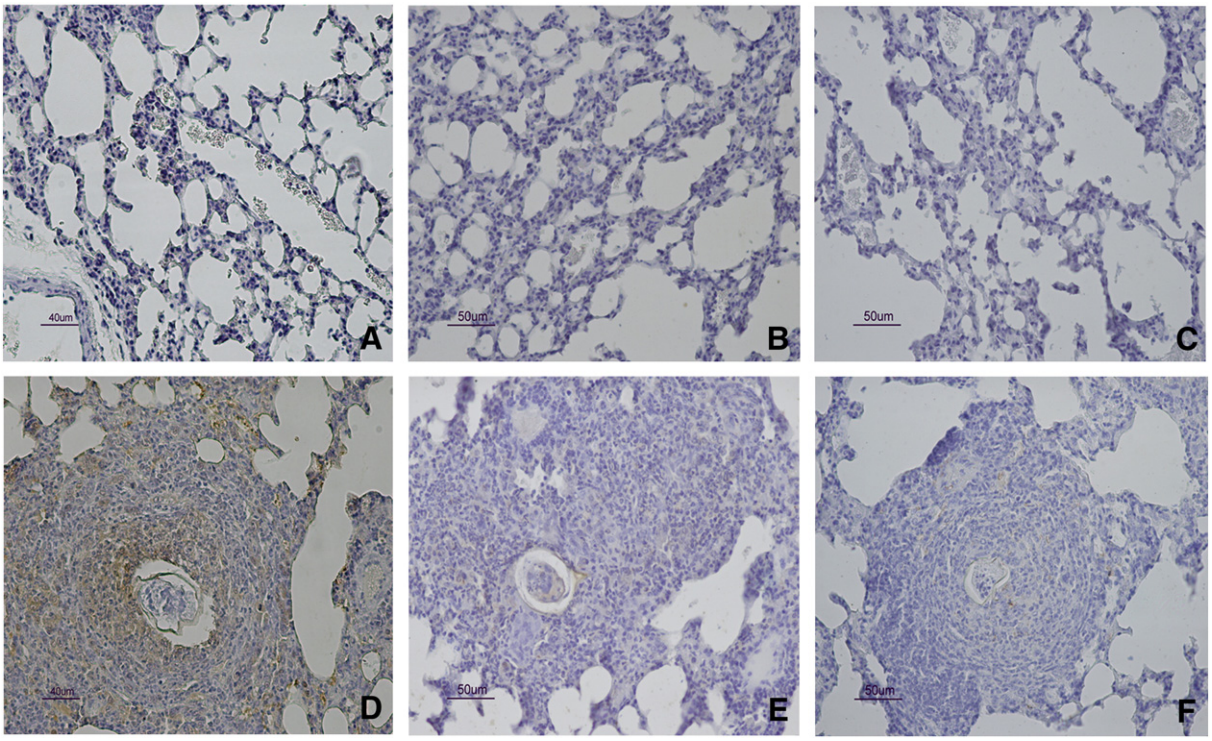


**Fig. 5.** Evaluation of hypoxia, HIF-1α and VEGF expression in mouse lung tissue. Lung tissue of uninfected mouse stained with anti-pimonidazole complex antibody (A) or anti-HIF-1α polyclonal antibody (B) or anti-VEGF polyclonal antibody (C); note that no immunoreactivity was observed. Lung tissue of *S. mansoni* infected mouse at 12 weeks postinfection stained with anti-pimonidazole complex antibody (D) or anti-HIF-1α polyclonal antibody (E) or anti-VEGF polyclonal antibody (F). All sections were counterstained with hematoxylin. Data are representative of three independent experiments. 200× magnification for all photomicrographs.

maximal activation at $O_2$ tension between 1.5 and 2% (Jiang et al., 1996), led us to suppose that hypoxic schistosomal lesions induces HIF-1α. Indeed, a strong positive correlation between HIF-1α expression and pimonidazole binding occurred in the tissues studied. Liver, intestine, spleen and lungs exhibited HIF-1α staining that was primarily located in infiltrated inflammatory cells surrounding the schistosomal eggs, both in cellular and fibrocellular granulomas. Although expression independent of hypoxia has been demonstrated by our group and other researchers using an *in vitro* model of *Leishmania* infection, lipopolysaccharides, proinflammatory cytokines and reactive oxygen species (Albina et al., 2001; Blouin et al., 2004; Kietzmann and Görlach, 2005; Degrossoli et al., 2007; Pagé et al., 2008), in this report, the results strongly suggest hypoxia-dependent HIF-1α activation in *S. mansoni* infected mice. In agreement with these findings, several studies have shown that HIF-1α is present in many common diseases, such as cancer, pulmonary hypertension and myocardial ischemia (Semenza et al., 2000).

In schistosomiasis, angiogenesis plays a role in the neovascularization of granulomas and the remodeling of blood vessels (Lenzi et al., 2006; Baptista and Andrade, 2005). VEGF released from accumulating leukocytes and shown to be upregulated in endothelial cells *in vitro* due to egg-derived factors may contribute to these vascular changes (Freedman and Ottesen, 1988; Carmeliet, 2005; Kanse et al., 2005). This study demonstrated for the first time that VEGF expression is increased in schistosomal infection. Since hypoxia and HIF-1α are major inducers of VEGF, we expected that VEGF would be expressed in tissues of *S. mansoni* infected mice. Observation revealed that VEGF is located in the extracellular space surrounding inflammatory cells in granulomas present in the liver, intestine, spleen and lungs. These findings are in agreement with previous reports that VEGF is located mainly on cell surfaces and within extracellular matrixes (Ramos et al., 1998; Park et al., 1993); for example VEGF is located in the extracellular space surrounding smooth muscle cells and macrophage-derived foam cells in atherosclerotic lesions (Ramos et al., 1998). The present data for *S. mansoni* infected murine liver, intestine and spleen showed that a positive correlation occurred between pimonidazole binding and HIF-1α and VEGF expression, suggesting that hypoxia plays a role in the maintenance of the angiogenic response in granulomas.

Interestingly, the results obtained revealed a lack of correlation between pimonidazole binding and HIF-1α and VEGF expression in lung tissue, such that 100%, 21%, and 18% of lung granulomas were positive for pimonidazole, HIF-1α and VEGF, respectively. The reasons why pulmonary granulomas do not respond to hypoxia by expressing HIF-1α and VEGF, as demonstrated in granulomas of the other organs, are not clear. One possible explanation is that when immature worms pass through the lung, rupturing the blood vessels and enter the alveoli, during the pre-granuloma stage, this attracts an inflammatory response that causes nonspecific damage (von Lichtenberg et al., 1977; Gobert et al., 2007) that may produce molecular sequelae in the tissue. The present results appear to support previous reports showing unexpected lack of hypoxic-induced HIF-1α in lung epithelial cells, xenografts of human pharyngeal carcinoma, colon carcinoma, astrocytoma, biopsies of human head-and-neck tumor, and diabetic ulcers (Sobhanifar et al., 2005; Vordermark et al., 2005; Uchida et al., 2004; Janssen et al., 2002; Botusan et al., 2008). *In vitro* studies have identified HIF-1α inhibition by hyperglycemia in hypoxic primary dermal fibroblasts and endothelial cells (Catrina et al., 2004), and by hypoglycemia and serum depletion in several tumor cell lines (Sobhanifar et al., 2005; Vordermark et al., 2005). In the latter studies, the authors demonstrated that normal glucose and serum concentrations restore the HIF-1α response to hypoxia (Sobhanifar et al., 2005; Vordermark et al., 2005), suggesting that inadequate delivery of nutrients leads to lack of HIF-1α expression in tissues. Although hypoglycemia, i.e., low serum glucose concentration, has been verified in *S. mansoni* infected mice (Couto et al., 2008), the extent of nutrient and glucose depletion in different mouse organs remains unknown and requires further investigation. Since VEGF, a HIF-1α-regulated gene, was repressed in the lungs of *S. mansoni* infected mice, it seems probable that HIF-1α is the causative link for VEGF suppression in this tissue.

In conclusion, the findings presented a first hint that murine *S. mansoni* infection generates a local hypoxic microenvironment and that a strong positive correlation exists between hypoxia and HIF-1α and VEGF expression in the liver, intestine and spleen, but not in the lungs. Further clinical and experimental studies are required to substantiate these findings.

## Conflict of interest statement

The authors declare that there are no conflicts of interest.

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