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ORIGINAL ARTICLE

Endothelial P2X7 receptors' expression is reduced by schistosomiasis

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Abstract Endothelial cells control vascular tone, permeability and leukocyte transmigration and are modulated by pro-inflammatory mediators. Schistosomiasis is an intravascular disease associated with inflammation, therefore altering endothelial cells' phenotype. Purinergic P2X7 receptors (P2X7R) play an important role in inflammation; however, the impact of the disease upon endothelial P2X7R function or expression has not been explored. Using ethidium bromide uptake to investigate P2X7R function, we observed that the effects of ATP (3 mM) and the P2X7R agonist 3′-*O*-(4-benzoyl)-ATP (BzATP) were smaller in mesenteric endothelial cells from the *Schistosoma mansoni*-infected group than in the control group. In the control group, BzATP induced endothelial nitric oxide production, which was blocked by the P2X7R antagonists KN-62 and A740003. However, in the infected group, we observed a reduced effect of BzATP and no effect of both P2X7R antagonists, suggesting a downregulation of endothelial P2X7R in schistosomiasis. We observed similar results in both infected and P2X7R$^{-/-}$ groups, which were also comparable to data obtained with KN-62- or A740004-treated control cells. Data from Western blot and immunocytochemistry assays confirmed the reduced expression of P2X7R in the infected group. In conclusion, our data show a downregulation of P2X7R in schistosomiasis infection, which likely limits the infection-related endothelial damage.

Keywords P2X7 receptors · Endothelial cells · *Schistosoma mansoni* · Nitric oxide · Murine

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Introduction

Schistosomiasis is a neglected disease caused by the intravascular helminth *Schistosoma mansoni*. According to the World Health Organization, the disease affects at least 240 million of people in the world [1] and represents a substantial public health burden for low- and middle-income countries [2].

After infection, *S. mansoni* migrates to the host vascular mesenteric bed where each female can lay approximately 300 eggs per day [3]. Worms and eggs, as well as their soluble antigens, interact with host endothelium [4–6], causing its activation [7, 8].

The endothelium produces vasoactive mediators playing an important role in the control of vascular homeostasis [9]. In this context, activation of endothelial cells changes their phenotype causing the synthesis of cytokines, increasing vascular permeability and the expression of adhesion molecules that favours leukocyte adhesion and migration [10, 11]. Nitric oxide (NO) is a potent endothelium-derived vasodilator that at physiological concentrations inhibits endothelium-leukocyte adhesion and cytokine-induced cellular migration [12–14]. Recently, we showed that

schistosomiasis reduces the expression of the constitutive endothelial NO synthase (eNOS) and the related ATP-induced NO production [15].

ATP can be released by endothelial cells under both physiological [16] and pathological [17] conditions. ATP acts as an autocrine and paracrine molecule [17–19] and sometimes as a danger signal if released by harmed or infected cells [20]. Extracellular ATP activates purinergic P2 receptors [21, 22] and is hydrolysed by ectonucleotidases [23]. P2 receptors are subdivided into eight G protein-coupled P2Y receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$), leading to a second messenger system cascade, and seven ionotropic P2X receptors (P2X1–7) that mediate permeability to Na^+, K^+ and Ca^{2+} [24].

Endothelial cells from different vascular beds express both P2Y and P2X receptors, among which $P2Y_1$, $P2Y_2$, $P2Y_{11}$, [25, 26], P2X1, P2X4 and P2X7 [25, 27–29] seem to be the main endothelial subtypes. Amongst the well-described physiological functions, both P2Y and P2X receptors stimulate endothelial NO production [29–33].

S. mansoni interacts with the mesenteric endothelium, and the associated inflammation alters endothelial cell phenotype [15, 34, 35]. Additionally, the worms are able to modulate the host purinergic system through the expression of ectonucleotidases [36]. However, little information is available on the receptors and signalling pathways triggered by this interaction. Of relevance to this study, the P2X7 receptor has been implicated in inflammation [37, 38]. Therefore, the objective of the present study was to investigate the influence of schistosomiasis on murine mesenteric endothelial purinergic P2X7 receptor function and expression.

Materials and methods

Animals and infection with *S. mansoni*

Male Swiss and C57BL/6 (wild-type and P2X7 receptor knockout [$P2X7R^{-/-}$] mice; 55 to 80 days old) were used in all procedures. C57BL/6 mice were obtained from the animal facilities of the Microbiology Institute Paulo de Goes, Federal University of Rio de Janeiro, Brazil. The $P2X7R^{-/-}$ mice were supplied by ATCC (USA) and kept in the Transgenic Mice Laboratory at the Biophysics Institute Carlos Chagas Filho, Federal University of Rio de Janeiro. All experiments were conducted in compliance with ethical standards of our institution (Ethics Committee of the Federal University of Rio de Janeiro, approved under the licenses DFBC-ICB-011 and IBCCF 039) and with the recommendations of both the National Council on Experimental Animal Control (Brazil) and the Committee of Care and Use of Laboratory Animals of the National Research Council of the United States. The animals were kept under a light/dark cycle of 12/12 h and had access to water and food *ad libitum*. All efforts were made to minimise both animal suffering and the number of animals used in association with valid statistical evaluation.

Swiss mice (7 days old) were infected percutaneously with 80 cercariae of both genders (BH strain; obtained from infected *Biomphalaria glabrata* snails) for 8 min as previously described [39]. The animals were used after at least 45 days of infection to allow full establishment of the infection. Age-matched control Swiss, C57BL/6 and $P2X7R^{-/-}$ mice were used for each set of comparisons with infected mice.

Endothelial cell culture

Mesenteric endothelial cells were obtained from Swiss mice (control and *S. mansoni*-infected mice) and C57BL/6 (wild type and $P2X7R^{-/-}$ mice). The animals were anesthetised, sacrificed by cervical dislocation and bathed in 70 % ethanol. The vessels were carefully removed, cut into small pieces and covered with DMEM medium supplemented with 20 % heat-inactivated foetal bovine serum, $NaHCO_3$ (44 mM), glucose (5.5 mM) and gentamicin (30 μg/ml; pH 7.4). The explants were removed after 48 h, and the medium was replaced every 48 h [40] until cells reached confluence.

Characterisation of endothelial cell culture

The identity of mesenteric endothelial cells was confirmed by the expression of the platelet-endothelial cell adhesion molecule-1 (PECAM-1, CD31) [41] and by their typical cobblestone monolayer appearance, which can be observed by optical microscopy (Olympus IX71 inverted microscope).

Primary cultures of endothelial cells (10^5 cells) were incubated with Fc blocker (Clone 2.4G2, BD Pharmingen) for 10 min before incubation with rat anti-mouse PECAM-1 primary antibodies (Santa Cruz Biotechnology; 1:100) or with fluorochrome-conjugated isotype control antibodies (isotype control, BD Pharmingen; 1:100) for 30 min at 4 °C. The cells were then washed twice in cold FACS buffer (1 % bovine serum albumin and 0.1 % sodium azide; 600×*g*, 20 min) and incubated with the secondary antibody FITC-conjugated (Alexa Fluor 488 Goat Anti-Rat; Invitrogen; 1:100) by 30 min at 4 °C. After washing, the cells were analysed using fluorescence-activated cell sorting (FACScan System, BD). Fluorescence was detected in the fluorescence 1 channel (FL1; 488 nm for excitation and 520 nm for emission, argon-ion laser), and 10,000 events per sample were collected and analysed using CellQuest Software (BD Pharmingen). Cell gating, forward (FSC) and side (SSC) scatter and

fluorescence histograms (FL1) were used for analysis and revealed that in both experimental groups, a single population of cells was positive for PECAM-1.

Fluorescent dye uptake assay

Endothelial cells from control, *S. mansoni*-infected and P2X7R$^{-/-}$ mice (10^5 cells; first passage; viability higher than 95 % in all groups) were used to investigate the response to ATP (3 mM) or 3′-*O*-(4-benzoyl)-ATP (BzATP, 10 μM) using 2.5 μM ethidium bromide as tracer (15 min; 37 °C). PECAM-1-positive cells were analysed by flow cytometry (FACScan System, BD Pharmingen). Briefly, cell populations were gated to create a histogram that showed auto-fluorescence at left in the *x*-axis (baseline) and the number of events in *y*-axis and then placed a marker at the end of the histogram. The fluorescence intensity due to ethidium bromide uptake in the absence or presence of agonists was obtained from the shift to the right of the histograms in relation to the baseline and expressed as percentage of permeabilised cells. Five thousand events were obtained with CellQuest program, and the fluorescent dye uptake observed in FL2 channel was analysed using WINMDI 2.9 software.

Assay of the NO measurement

Confluent endothelial cells (first passage) were used in fluorimetric NO measurements using the probe 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate (DAF-FM DA; 2.5 μM) in the presence of physiological solution (NaCl 140 mM, KCl 5 mM, $MgCl_2$ 1 mM, $CaCl_2$ 2 mM, glucose 5 mM and HEPES 5 mM, pH 7.4) enriched with L-arginine (1 mM) (37 °C, 5 % CO_2) [38]. NO production was measured in the absence (basal), in the presence of 100 μM BzATP (15 min) or in the presence of 1 μM KN-62 or 50 nM A740003 (P2X7 receptor antagonists), which were pre-incubated for 45 min (37 °C) followed by 100 μM BzATP for 15 min. After incubation, the fluorophore trapped in live cells was excited at 488 nm, and emission was measured at 515 nm using a microplate fluorometer (Fmax; Molecular Devices, USA). The basal production of NO was considered as 100 %, and the responses to drugs were expressed as percentage over basal.

Immunocytochemistry

Endothelial cells (10^4 cells/well, first passage) were fixed with 4 % paraformaldehyde plus 4 % sucrose (10 min), washed and then incubated for 30 min with ammonium chloride (50 mM, pH 8.0). After three washings with phosphate buffered saline (PBS), nonspecific binding sites were blocked with 10 % foetal bovine serum plus 0.1 % bovine serum albumin (30 min). Next, the cells were washed twice with PBS–Triton X-100 0.2 %, followed by an overnight incubation with a rabbit anti-P2X7 receptor polyclonal primary antibody (Alomone Labs; 1:100; peptide (C)KIRKEFPKTQGQYSGFKYPY, intracellular epitope). Then, the cells were incubated with an anti-rabbit secondary antibody (1:300, 1 h at 4 °C) and fluorophore-conjugated streptavidin (Texas Red, 1:100; Vector, USA). After the incubation, cell nuclei were stained with DAPI (Vectashield). P2X7 receptors expression was observed by fluorescence microscopy (Olympus IX71 inverted microscope; magnification, ×400), and the images were acquired using CellSens 1.5 software (Olympus America Inc., USA).

Western blot assay

Confluent endothelial cells (first passage) were washed with sterile PBS (NaCl 137 mM, Na_2HPO_4 8.1 mM, NaH_2PO_4 1.5 mM and KCl 2.7 mM, pH 7.4) and 200 μl RIPA buffer was added (1 % Nonidet P40, 0.25 % sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 1 mM NaF, 10 μg/ml aprotinin, 10 μg/ml leupeptin and 50 mM Tris–HCl, pH 7.4). The cell lysates were incubated for 30 min at 4 °C, centrifuged (8,100×*g*, 10 min, 4 °C), and the protein content was determined by the Lowry assay [42]. Protein (30 μg) was loaded on SDS–PAGE (10 %). After electrophoresis, the proteins were transferred to a PVDF membrane, incubated for 1 h with non-fat milk (2 %) followed by primary antibody (polyclonal rabbit anti-P2X7 receptors; 1:200) or monoclonal anti-β-actin treatment (1:500; Santa Cruz Biotechnology, USA) for 3 h, and treatment with a peroxidase-conjugated secondary antibody (mouse TrueBlot™ ULTRA HRP anti-mouse IgG or rabbit TrueBlot™ ULTRA HRP anti-rabbit IgG (eBioscience 1:1,000) for 1 h. The protein blot images were scanned by Storm 860 (Molecular Dynamics). Ponceau dye and the monoclonal anti-β-actin antibody were used as internal controls of protein loading and did not vary between the samples. Protein analysis was measured using the ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, MD, USA, http://imagej.nih.gov/ij/, 1997–2011), and the relative expression was normalised as percentage in relation to controls.

Data analysis

Data were expressed as the mean and S.E.M. The differences between two or more groups were analysed by unpaired Student's *t* test or one-way analysis of variance (ANOVA) and followed by the post hoc Newman–Keuls test, with $P<0.05$ considered significant.

Drugs

ATP, 3′-*O*-(4-benzoyl)-ATP (BzATP), KN-62, phenylmethylsulphonyl fluoride, sodium orthovanadate, aprotinin, leupeptin and bovine serum albumin were purchased from SIGMA Chemical Co. (St. Louis, MO, USA); DAF-FM DA was obtained from Molecular Probes (Eugene, OR, USA), A740003 was obtained from Tocris (USA). DMEM, foetal bovine serum and gentamicin reagent solution were acquired from GIBCO BRL Products (Grand Island, NY, USA). Vectashield mounting medium with DAPI was obtained from Vector (Burlingame, CA, USA). Stock solutions were prepared in 100 % dimethylsulphoxide (DAF-FM DA, 2.5 mM; KN-62, 5 mg/ml; A740003, 10 mM), buffered physiological solution (ATP, 10 mM) and water (BzATP, 10 mM). The highest final concentration of the solvent was 0.1 % (*v*/*v*) and had no effect on the experiments. All other reagents used were of analytical grade.

Results

Endothelial cells' characterisation

Mesenteric endothelial cells from all groups showed a typical and similar morphology as observed by optical microscopy (*data not shown*). Flow cytometry analysis of the endothelial cells from control and infected mice showed similar profiles regarding the FSC and SSC scatter (which provide information about cell size and granularity, respectively; Fig. 1a). Moreover, cells from all groups (control, infected and P2X7R$^{-/-}$ mice) presented similar levels of PECAM-1 expression (Fig. 1b), which were similar to data reported for murine endothelial cells [41].

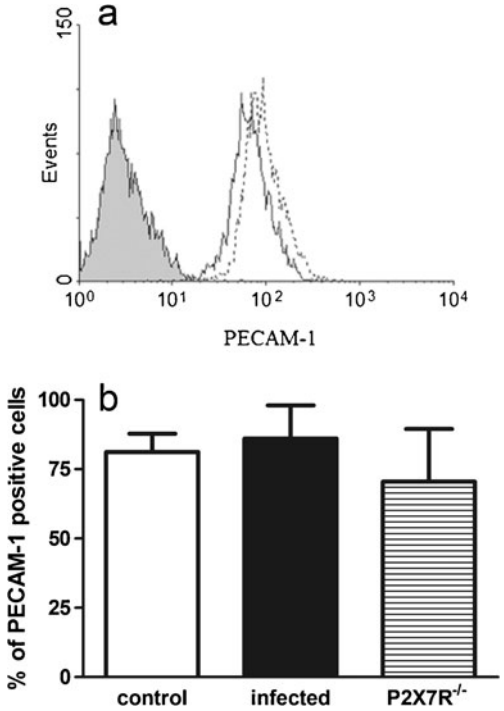

Fig. 1 Characterisation of mesenteric endothelial cells from control, *S. mansoni*-infected and P2X7R$^{-/-}$ mice. **a** A typical flow cytometry histogram shows that cells from control (*solid line*) and from infected mice (*traced line*) express PECAM-1 (*gray histogram*: isotype control). Isotype control staining was similar among all cell types (data not shown). **b** Percentage of endothelial cells expressing PECAM-1, which is classically used as a marker of endothelial cells. *N*=3 (control) or two different cultures (infected and P2X7R$^{-/-}$)

ATP- and BzATP-induced permeabilisation of endothelial cells

Because P2X receptor activation by high ATP concentrations opens a large pore in cell plasma membrane that allows the access of ethidium bromide, we evaluated if ATP (3 mM) would induce permeabilisation of mesenteric endothelial cell from control, infected and P2X7R$^{-/-}$ mice. The basal ethidium bromide uptake was similar in all groups. In the control group, 3 mM ATP induced ethidium bromide uptake, which was the first evidence of the presence of functional P2X7 receptors in mesenteric murine endothelial cells (Fig. 2). The P2X7 receptor agonist BzATP (10 μM) also induced endothelial cell permeabilisation corroborating the ATP data (Fig. 2). Both agonists did not elicit permeabilisation in endothelial cells from P2X7R$^{-/-}$ mice confirming that their effects were due to the activation of P2X7 receptors. However, endothelial cells from infected mice showed a smaller permeabilisation in response to 3 mM ATP and 10 μM BzATP than the control, suggesting a reduced function of the receptor (Fig. 2).

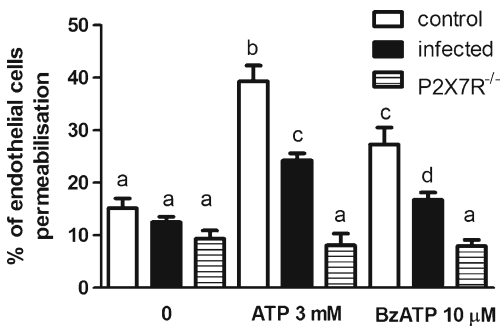

Fig. 2 Fluorescent dye uptake by mesenteric endothelial cells. Assays used endothelial cells from control (*white bars*), *S. mansoni*-infected mice (*black bars*) and P2X7R$^{-/-}$ mice (*hatched bars*) in the absence (*0*) and presence of 3 mM ATP or 10 μM BzATP. Experiments used ethidium bromide (2.5 μM) as a tracer and were analysed by flow cytometry. Data are expressed as the mean and S.E.M. *N*=10–18 replicates (control and infected groups) or six replicates (P2X7R$^{-/-}$ group) using four to six or three different cultures for each group, respectively. *a*, *b*, *c* and d differ from each other ($P<0.05$, one-way ANOVA followed by post hoc Newman–Keuls test)

P2X7 receptor-induced NO production in endothelial cells is impaired in schistosomiasis

Next, we used the potent agonist of P2X7 receptors (BzATP) to assess whether their activation would induce endothelial NO production. In the control group, we observed that 100 μM BzATP increased by 22 % NO synthesis. This effect was blocked by the P2X7 receptor antagonist KN-62 (1 μM; Fig. 3). Conversely, in the infected group, the same BzATP concentration increased NO production only 4.7 % (21.9±2.4 %, n=17 and 4.7±1.8 %, n=14, control and infected groups, respectively), and KN-62 (1 μM) did not alter its effect. A similar profile was observed in endothelial cells from P2X7R$^{-/-}$ mice (Fig. 3) reinforcing the relevance of P2X7 receptors for the effect of BzATP.

In addition to being a known P2X7 receptor antagonist, KN-62 has another pharmacological action inhibiting the calcium/calmodulin-dependent protein kinase II [43, 44]. Therefore, we used a high-affinity and selective P2X7 receptor antagonist. Pre-incubation of A740003 (50 nM) blocked the effect of BzATP in the control group and did not alter the small effect observed in the infected group (Fig. 4), reinforcing the reduced P2X7 receptor function due to schistosomiasis.

P2X7 receptor expression is reduced in mesenteric endothelial cells from *S. mansoni*-infected mice

Immunocytochemistry was used to characterise P2X7 receptors in murine mesenteric endothelial cells. We observed their presence in two mice backgrounds: Swiss and C57BL/6 wild type (Fig. 5a, c) as well as their absence in endothelial cells from P2X7R$^{-/-}$ mice used as an internal control (Fig. 5b). Furthermore, there was a reduction of their expression in the cells from the infected group (Fig. 5d).

Western blot analysis revealed the expression of the predicted full-length P2X7 receptor as a single band with an apparent molecular mass of 75 kDa in mesenteric endothelial cells from control Swiss (Fig. 5e) and infected mice (Fig. 5f). Corroborating the immunofluorescence data, the blot analysis showed that P2X7 receptor expression was reduced in the infected group (Fig. 5e, f).

Discussion

Endothelial cells exert an important physiological barrier regulating the passage of proteins and immune cells between blood and tissues [10, 45, 46]. In addition, the endothelium controls vascular tone through the synthesis of vasoconstrictor and vasodilator substances, such as NO [9]. However, under a pathological condition, endothelial cells change their phenotype [11, 47] favouring vascular permeability, leukocyte migration, platelet activation and reducing NO synthesis through eNOS [12, 13, 48].

S. mansoni modulates its host's immune system to survive. In the course of schistosomiasis, worms, eggs and their soluble antigens, *i.e.*, a complex mixture of proteins, glycoproteins and glycolipids, interact with vascular endothelium [49] and myocytes causing morphological alterations [4, 8, 34, 35, 50–52]. Previous data showed that schistosomiasis is associated with a reduction of mesenteric endothelial eNOS expression and ATP-induced NO production [15]. Despite these findings, the influence of schistosomiasis on host endothelial cells physiology and purinergic signalling has not been deeply explored.

Using ethidium bromide uptake as a first approach to evaluate P2X7 receptor function, our data showed that treatment of mesenteric endothelial cells from control mice with ATP (3 mM) and BzATP (10 μM) induced cell permeabilisation, suggesting

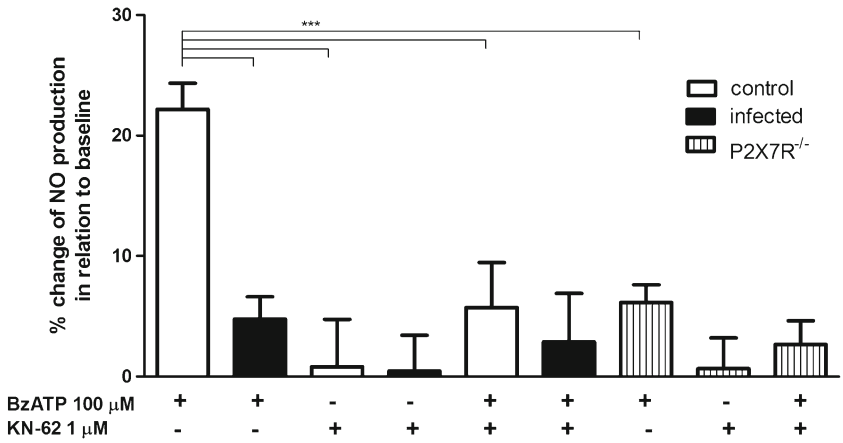

Fig. 3 Endothelial NO production induced by 100 μM BzATP. Experiments were performed in the absence or presence of the antagonist KN-62 (1 μM) in cultured endothelial cells from control (*white bars*), *S. mansoni*-infected (*black bars*; n=14–17 replicates, two different cultures for each group), and P2X7R$^{-/-}$ mice (*hatched bars*) (n=17 replicates using two different cultures). Data are expressed as the mean and S.E.M.; ***P<0.001 (one-way ANOVA followed by post hoc Newman–Keuls test)

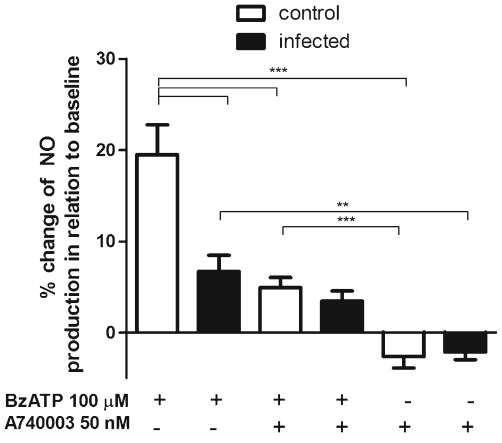

Fig. 4 NO production induced by 100 μM BzATP. Experiments were performed in the absence or presence of the antagonist A740003 (50 nM) in cultured endothelial cells from control (*white bars*) and *S. mansoni*-infected (*black bars*; n=6–14 replicates, four different cultures for each group). Data are expressed as the mean and S.E.M. $^{**}P<0.01$; $^{***}P<0.001$ (one-way ANOVA followed by post hoc Newman–Keuls test)

the presence of P2X7 receptors in this cell type. Both agonists had no effect when using endothelial cells from P2X7R$^{-/-}$ mice, suggesting that the agonists' effects were related to P2X7 receptor activation. Then, using another experimental protocol, namely NO measurement, we observed that endothelial P2X7 receptor activation with the agonist BzATP induced NO synthesis in an amount similar to that observed with ATP either in murine [15] or rat endothelial cells [40]. Two P2X7 receptor antagonists (KN-62 and A740003) blocked this effect. However, as KN-62 has other pharmacological actions beyond P2X7 receptor antagonism [44], we also performed the assays using P2X7R$^{-/-}$ mice to confirm the role of these receptors in the BzATP-mediated effect. KN-62-treated control cells and P2X7R$^{-/-}$ cells showed similar responses to BzATP. Similar results were also observed with A740003-treated cells. Altogether, these data confirm the presence of functional P2X7 receptors in mesenteric endothelial cells. The present data are in agreement with previous work where 10 μM BzATP induced endothelium-dependent rat mesenteric artery vasodilation, which was partially reduced by KN-62 [31]. Moreover, in the

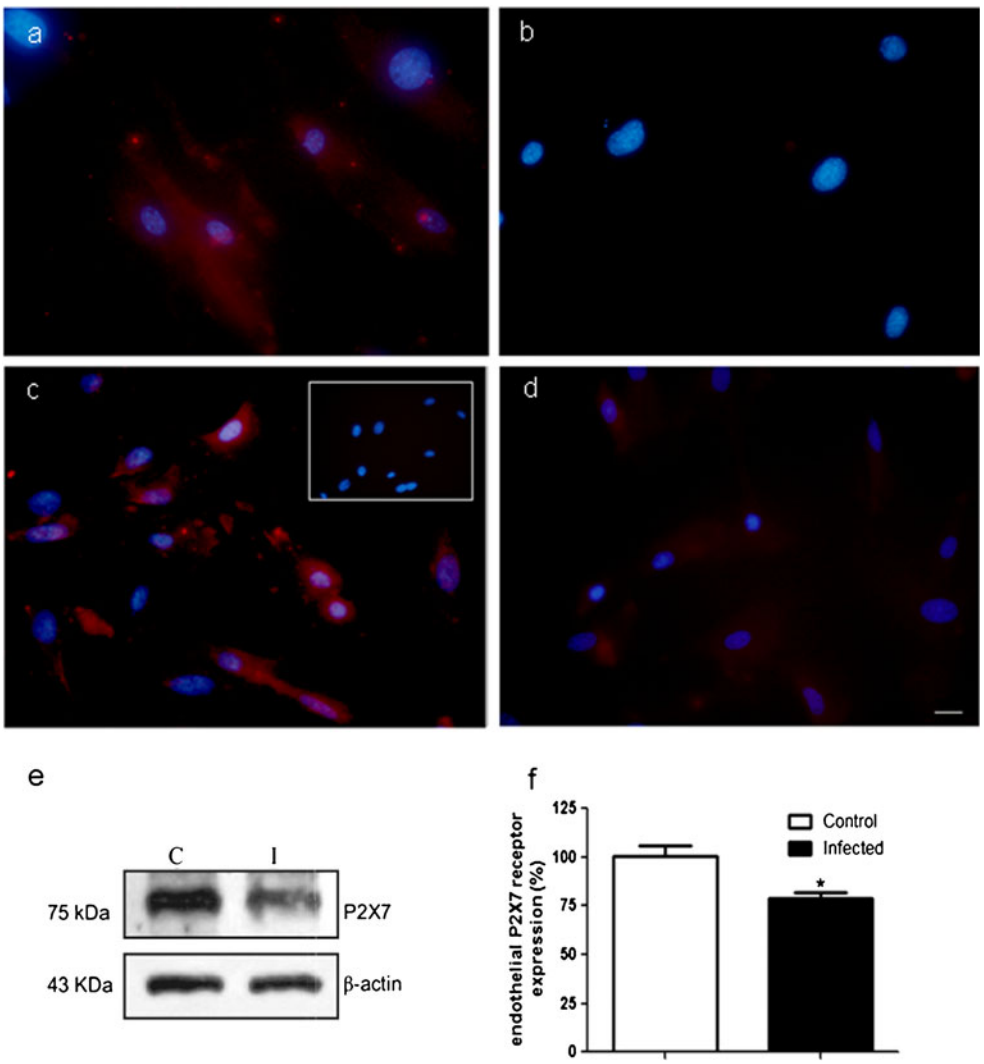

Fig. 5 P2X7 receptor expression in mesenteric endothelial cells. Immunocytochemistry of P2X7 receptor expression (*red*) on endothelial cells (10^4 cells/well) of C57BL/6 (**a**), P2X7R$^{-/-}$ (**b**), Swiss control (**c**) and *S. mansoni*-infected (**d**) mice. Nuclei were stained with DAPI (*blue*). *Inset:* secondary antibodies alone. Magnification, ×400; *scale*, 20 μm. **e** Representative Western blot assay (*C* control, *I* infected). **f** Densitometric analysis of the Western blot results. Data expressed as the mean and S.E.M. $^{*}P<0.05$; n= 4 experiments performed with four different cultures for each group (Student's t test)

infected group, NO production stimulated by BzATP was lower than in the control group, suggesting a reduction of P2X7 receptors' function or expression in our model.

Although BzATP is a potent agonist of P2X7 receptors, it may also activate other P2X receptors, such as P2X1 and P2X4 receptors [53], and murine mesenteric endothelial cells express the former receptors [32]. Therefore, the residual effect of BzATP observed in the control group in the presence of the P2X7 receptor antagonists, and in endothelial cells from P2X7R$^{-/-}$ mice, might reflect a P2X1 or P2X4 receptor activation and the related NO production. Notably, NO production in endothelial cells from *S. mansoni*-infected mice was proportional to the production observed in KN-62 or A740003-treated control endothelial cells and in P2X7R$^{-/-}$ mice. These effects, associated with cell permeabilisation data, indicate a downregulation of functional P2X7 receptors in mesenteric endothelium from *S. mansoni*-infected mice.

The expression of P2X7 receptors in cultured endothelial cells was confirmed through immunocytochemistry and Western blot assays. To our knowledge, this is the first report of P2X7 receptor expression and function in murine mesenteric endothelial cells. Furthermore, the infected group showed a reduced expression of P2X7 receptors when compared with control mice, corroborating the previously mentioned functional data.

Innate immunity is the most primordial system to protect hosts from infections and is regulated by a complex network of cytokines that orchestrate inflammation in response to invasion by infectious agents. The P2X7 receptor is a multifunctional protein related to multiple signalling pathways, including vasodilation, endothelial cell apoptosis, inflammasome activation and the production of pro-inflammatory cytokines, such as interleukin-1β (IL-1β). Thus, the P2X7 receptor causes a wide variety of biological effects associated with infection and inflammation [24, 54–56], eventually leading to killing of intracellular parasite [57, 58]. IL-1β is a multifunctional cytokine upregulated in the initial phase of schistosomiasis [59]. Endothelial cells are targets of IL-1β. For instance, it has been shown that IL-1β not only reduces eNOS expression [60] and NO production [61] but also stimulates endothelial cells apoptosis [62].

Nevertheless, after egg deposition, the immune system polarises to a Th2 profile balancing ongoing immune responses to establish a chronic infection [3, 45]. In this scenario, several cytokines are produced. For instance, high TGF-β1 levels in schistosomiasis protect animals against severe liver injury [63]. Previous data have shown that this Th2 cytokine prevents P2X7 receptor upregulation in monocytes during inflammation [64]. Whether this effect also occurs in endothelial cells remains to be established. However, as a constant high extracellular ATP concentration is detrimental for endothelial cells [54, 65], we propose that this stage of the illness could be related to a downregulation of endothelial P2X7 receptors. Moreover, *S. mansoni* express an ATP diphosphohydrolase and Sch-P2X receptors, suggesting that some evolutionary adaptation of host–parasite interaction may have occurred [36, 66, 67]. The overall balance of this interaction could limit the P2X7 receptor-mediated damage of the mesenteric endothelium associated with persistent inflammation in schistosomiasis [34, 35]. In conclusion, we observed that schistosomiasis reduces murine mesenteric endothelial cell expression of P2X7 receptors and their downstream activation of NO production.

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