Adenosine reduces reactive oxygen species and interleukin-8 production by *Trichomonas vaginalis*-stimulated neutrophils

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Abstract *Trichomonas vaginalis* is a flagellated protozoan that affects the human urogenital tract causing 276.4 million new infections a year. The parasite elicits a vaginal mucosal infiltration of immune cells, especially neutrophils which are considered to be primarily responsible for cytological change observed at the infection site as well as the major contributor in the inflammatory response against the parasite. Extracellular nucleotides and their nucleosides are signaling compounds involved in several biological processes, including inflammation and immune responses. Once in the extracellular space, the nucleotides and nucleosides can directly activate the purinergic receptors. Herein, we investigated the involvement of purinergic signaling on the production of reactive oxygen species (ROS) and cytokines by *T. vaginalis*-stimulated neutrophils. Parasites were able to induce an increase in ROS and IL-8 levels while they did not promote IL-6 secretion or neutrophil elastase activity. Adenine and guanine nucleotides or nucleosides were not able to modulate ROS and cytokine production; however, when *T. vaginalis*-stimulated neutrophils were incubated with adenosine and adenosine deaminase inhibitor, the levels of ROS

and IL-8 were significantly reduced. These immunosuppressive effects were probably a response to the higher bioavailability of adenosine found in the supernatant as result of inhibition of enzyme activity. The involvement of P1 receptors was investigated by immunofluorescence and A1 receptor was the most abundant. Our data show that the influence of purinergic signaling, specifically those effects associated with adenosine accumulation, on the modulation of production of proinflammatory mediators by *T. vaginalis*-stimulated neutrophils contribute to the understanding of immunological aspects of trichomoniasis.

Keywords *Trichomonas vaginalis* · Neutrophils · Reactive oxygen species · Interleukin-8 · Adenosine

Introduction

Trichomonas vaginalis is a flagellated parasite that exclusively affects the human urogenital tract causing 276.4 million new infections a year [1]. The infection was traditionally recognized as symptomatic in women and asymptomatic in men; however, recent data have mentioned that around 80% of *T. vaginalis* infections are asymptomatic in both men and women [2, 3]. The infection has been associated with serious health consequences, including adverse pregnancy outcomes [4], infertility [5], and predisposition to cervical [6] and prostate cancer [7]. In addition to the risk of transmission to sex partners, *T. vaginalis* infection has also been related with as much as a 2.7-fold increase in the risk of HIV acquisition [2]. The scenario of high prevalence and severe complications turns this sexually transmitted disease into an important public health concern.

Although *T. vaginalis* is known to be a non-invasive microorganism, it elicits the vaginal mucosal infiltration of immune cells, particularly polymorphonuclear leukocytes (PMNL).

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Neutrophils are considered to be primarily responsible for cytological change observed at the infection site as well as the major contributor in the inflammatory response against the parasite [8, 9]. *T. vaginalis* has already shown to be able to stimulate the production of a variety of chemoattractants by neutrophils, such as superoxide anions [10] nitric oxide (NO) [11] and interleukin-8 (IL-8) [12]. Furthermore, it has been demonstrated that *T. vaginalis* trophozoites and their secreted products are capable of producing leukotriene B₄ [13], which leads the production of IL-8 by neutrophils and mast cells [14, 15]. The tight control of the levels of these mediators is related to the recruitment of more immune cells or elimination of the parasites and consequently, maintenance or resolution of the inflammation, respectively. In this sense, more studies are important to elucidate the exact mechanisms of neutrophils mediating inflammatory responses against *T. vaginalis*.

Extracellular nucleotides such as ATP, ADP, and AMP, and their nucleoside derivative adenosine, are signaling compounds involved in several biological processes, including inflammation and immune responses [16]. While ATP acts as a “find-me signal” that conducts phagocytes to inflammatory sites, its breakdown product adenosine contributes to the engineering of inflammation and immune responses by providing a suppressive tissue-protecting signal [17]. Once in the extracellular space, the nucleotides and nucleosides can directly activate the purinergic receptors, namely P1 and P2 receptors. P1 receptors bind adenosine and they are subdivided into A₁, A_{2A}, A_{2B}, and A₃, while P2 receptors have affinity for nucleotides and they are subdivided into metabotropic P_{2Y} and ionotropic P_{2X} receptors [18]. The extracellular nucleotide and nucleoside levels may be dynamically controlled by a group of enzymes called ectonucleotidases. These enzymes include the ectonucleoside triphosphate diphosphohydrolase (E-NTPDase) family, which hydrolyze nucleoside tri- and diphosphates, and the ecto-5'-nucleotidase, which hydrolyzes nucleoside monophosphates [19]. Adenosine deaminase (ADA) is the enzyme responsible for the conversion of adenosine to inosine thus finishing the hydrolysis cascade [20].

In this sense, knowing that (i) *T. vaginalis* infection leads to inflammation within the genital tract and an increased number of PMNLs in vaginal fluid, and (ii) purinergic signaling plays important roles on immune responses, the aim of this study was to investigate the involvement of purinergic signaling on the production of cell mediators (reactive oxygen species, elastase activity, and cytokines) by neutrophils stimulated with *T. vaginalis*.

Materials and methods

T. vaginalis culture

T. vaginalis trophozoites (ATCC30236 isolate, from the American Type Culture Collection) were cultured axenically

in vitro on trypticase-yeast extract-maltose (TYM) medium, (pH 6.0), supplemented with 10% (v/v) heat-inactivated bovine serum, and incubated at 37 °C (± 0.5) [21]. Organisms in the logarithmic phase of growth exhibiting motility and normal morphology were used in all experiments.

Isolation of human neutrophils

Human neutrophils were isolated as previously described [22], with some modifications. Briefly, venous blood of healthy volunteers (ethical approved by UFRGS Ethical Committee, number 19346) was collected on heparin anticoagulant solution, centrifuged (250×g, 10 min, 24 °C), and the resulting platelet-rich plasma discarded. Leukocytes were obtained following erythrocyte sedimentation in 2.0% Dextran T-500 and centrifuged (525×g, 20 min, 24 °C) through a layering on Histopaque 1077. The neutrophil-enriched pellet was subjected to a 15-s hypotonic lysis to remove the remaining erythrocytes and centrifuged (1000×g, 5 min, 24 °C). The purified neutrophils were resuspended in RPMI 1640 supplemented with 10% fetal bovine serum and 10 mM HEPES for the next experiments.

T. vaginalis and neutrophils coculture

Neutrophils (1.0×10^5) were cocultured with live trophozoites (1.0×10^4) on 96-well microplates at 37 °C (5.0% CO₂) during 30 min or 6 h, for ROS or cytokines assays, respectively. The cells were incubated in the presence or not of 300 μM nucleotides (ATP, GTP, ADP, GDP, AMP, GMP), 300 μM nucleosides (adenosine, guanosine), 300 μM inosine, 300 μM AMPCP, an ecto-5'-nucleotidase inhibitor, and 50 μM EHNA, an ADA inhibitor. After the incubation, the microplates were centrifuged (1000×g, 5 min), the cell-free culture supernatants were collected for cytokines measurement, and the cells maintained on the bottom plate were used for ROS determination. For degranulation assay, 5.0×10^5 neutrophils were incubated with 5.0×10^4 trophozoites on 96-well microplates at 37 °C (5.0% CO₂) during different times (1, 2, 4, 6, or 12 h). Before the coculture, all neutrophils were primed with 5.0 μg/mL chytochalasin B, and 1.0 μM fMLP was used as positive control. After the incubation, the microplates were centrifuged (400×g, 5 min, 4 °C) and the cell-free supernatants were collected for neutrophil elastase measurement.

Reactive oxygen species production by neutrophils

Intracellular reactive oxygen species (ROS) accumulation in neutrophils was measured by flow cytometry using an oxidation-sensitive fluorescent probe, 2',7'-dichlorofluorescein diacetate (DCF-DA). After the coculture with *T. vaginalis*, the cells were washed once with PBS and stained using 10 μM

DCF-DA for 15 min at room temperature. Hydrogen peroxide (5.0 mM) was used as positive control of ROS production. The neutrophils fluorescence was measured by flow cytometry (FACSVerse, Becton Dickinson, CA) and 10,000 cells were gated and analyzed using CellQuest Pro software (Becton Dickinson) [23].

Cytokines production by neutrophils

The concentrations of IL-8 and IL-6 were determined in the cell-free coculture supernatants by ELISA kits (Invitrogen, Life Technologies, USA) according to the manufacturer's instructions.

Degranulation assay

Neutrophil degranulation was evaluated by elastase activity using MeO-SucAAPVpNA (AAPVpNA), the substrate for the enzyme. The cell-free coculture supernatants (50 μ L) were incubated at 37 °C for 2 h with 0.5 mM AAPVpNA. Finally, the absorbance was measured at 405 nm and the results were expressed as percentage of elastase released compared to the control (neutrophils alone) [24].

ADA enzymatic assay

For determination of ADA activity in the condition neutrophils incubated with *T. vaginalis* plus adenosine and EHNA, the neutrophils (1.0×10^5) and *T. vaginalis* trophozoites (1.0×10^4) were washed and resuspended with 50 mM sodium phosphate buffer (pH 7.5). The cells were then preincubated for 10 min at 37 °C and the reaction was initiated through addition of the substrate adenosine (300 μ M) and stopped, after 30 min, by adding the samples on 500 μ L of phenolnitroprusside reagent (50.4 mg phenol and 0.4 mg sodium nitroprusside/mL). The reaction mixtures were mixed with 500 μ L of alkaline-hypochlorite reagent (sodium hypochlorite to 0.125% available chlorine, in 0.6 M NaOH) and the samples were incubated at 37 °C for 15 min. The colorimetric assay was carried out at 635 nm [25] to measure the ammonia produced by the enzymatic reaction and the ADA activity was expressed as nanomole NH_3 hour/ 10^5 /neutrophils.

Adenosine determination by high performance liquid chromatography

The concentration of adenosine found in the cell-free supernatant from the coculture of neutrophils (1.0×10^5), *T. vaginalis* (1.0×10^4), adenosine (300 μ M), and EHNA (50 μ M) was determined by high performance liquid chromatography (HPLC). After 30 min of coculture, the supernatants were collected and submitted to a liquid-liquid extraction with a solution of 15% trichloroacetic acid (w/v), mixed by

vortexing (20 s, three times), and followed by cooling on ice (20 min) and centrifugation (3000 $\times$ g, 20 min, 4 °C). The supernatants (20 μ L) were injected in the chromatographic system (LC-20AT Prominence Liquid Chromatograph, Shimadzu) and data were acquired using the LC Solution Software (Shimadzu). The chromatographic separation was performed in a Shimadzu Shim-Pack column (C18, 4.6 mm $\times$ 150 mm, 5 μ m) using water and acetonitrile (7:93) (v/v) as mobile phase. The mobile phase was eluted at a flow rate of 0.8 mL/min and the analyte was detected at 259 nm. All of the peaks were identified by retention time (4.8 min for adenosine) and in comparison with the standards [26].

Immunofluorescence staining of P1 receptors

After the coculture of neutrophils and *T. vaginalis* in the presence or not of adenosine and EHNA, the microplates were centrifuged (1000 $\times$ g, 5 min) and the cells were washed with PBS (pH 7.4) and fixed with 4.0% paraformaldehyde for 20 min at room temperature. After two washes with PBS, potential sites for nonspecific antibody binding were blocked by 30 min incubation with 1.0% BSA in PBS. The cells were then incubated with specific A1, A2A, A2B, or A3 goat polyclonal IgG primary antibodies (1:100 dilution) overnight at 4 °C (Santa Cruz Biotechnology, Inc., USA). Subsequently, they were washed with PBS and incubated with donkey anti-goat IgG secondary antibody (1:1000) conjugated to Alexa Fluor™ 568 (Life Technologies, USA) for 90 min at room temperature. Finally, the cells were washed with PBS and their microtubules were labeled using 1 μ M fluorescent taxoid FLUTAX-2 [27] which was kindly provided by Dr. André A. Souto (Faculdade de Química, PUCRS, Brazil), and the DNA was stained with 10 μ g/mL of DAPI. Images were obtained using an Olympus IX81 confocal microscope and UPLSAPO 60X W NA: 1.20 objective and were overlaid using Olympus FV 1000 software. The fluorescence quantification was performed with Image J program.

Statistical analysis

All the experiments were performed in triplicate and with at least three independent assays. Data were expressed by mean $\pm$ standard deviation (S.D.) and analyzed by Student's *t* test, considering *p* < 0.05 as statistically significant. Confocal fluorescence quantification data were expressed by mean $\pm$ standard deviation (S.D.) and analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple range test, considering *p* < 0.05 as significant using the Statistical Package for the Social Sciences (SPSS) software version 18.

Results

The first inflammatory response investigated was the ROS production by neutrophils. The coculture of neutrophils and *T. vaginalis* was able to activate the ROS production when compared with neutrophils alone. In order to know if the purinergic signaling induced any influence on the ROS production by *T. vaginalis*-stimulated neutrophils adenine and guanine nucleotides and nucleosides as well as enzyme's inhibitors were included at the co-incubation medium. Adenine and guanine nucleotides such as ATP, ADP, and AMP or GTP, GDP, and GMP did not promote changes in ROS production, as well as the nucleosides adenosine and guanosine. However, when the ADA inhibitor, EHNA, was added with adenosine, a significant decrease in ROS production was observed. Inosine, the product of adenosine deamination, maintained ROS at the same level as the control (Fig. 1).

After ROS analysis, the involvement of purines in *T. vaginalis* inflammation was also explored focusing on cytokine secretion. IL-8 and IL-6 release were evaluated after coculture of neutrophils and parasites in the presence of ATP or GTP, adenosine or guanosine, adenosine plus EHNA, and inosine (Fig. 2a). Regarding IL-8, absolutely the same profile seen to ROS production was observed. The trophozoites by themselves were able to stimulate IL-8 secretion by neutrophils while neither adenine nor guanine nucleotides and nucleosides modulated the cytokine release. Once more, the condition that led to an important effect was that mediated by adenosine plus EHNA, which reduced IL-8 levels. In contrast to ROS and IL-8, the IL-6 production from *T. vaginalis*-stimulated neutrophils did not induce any changes in IL-6 levels. The incubation of nucleotides, nucleosides, and even the adenosine accumulation condition (with ADA inhibitor) did not modify the cytokine release (Fig. 2b).

In an attempt to investigate a distinct parameter of neutrophil function, we assessed its degranulation and the participation of *T. vaginalis* in this process. The results showed that even with long times of neutrophils and trophozoites coculture the parasites could not increase the neutrophil elastase activity. The level of the enzyme activity remained similar over the 12 h of incubation (Fig. 3).

Taking into account that in our experimental design the most pronounced effect was attributed to adenosine plus EHNA, we proposed that the anti-inflammatory profile observed through the reduction of ROS and IL-8 production could be attributed to adenosine accumulation in the supernatant. Aiming to access this hypothesis, the nucleoside concentration as well as the ADA activity was measured under this condition (Table 1). As expected, the ADA activity was consistently reduced (3.26 ± 0.21 to 1.46 ± 1.03 nmol $\text{NH}_3/\text{h}/10^5$ neutrophils) in the presence of EHNA, an ADA inhibitor. In the same way, the adenosine concentration measured in the supernatants was statistically higher when neutrophils and

Fig. 1 Participation of purinergic signaling on reactive oxygen species (ROS) production by neutrophils stimulated with *T. vaginalis* trophozoites. **a** Effect of adenine nucleotides (ATP, ADP, and AMP), nucleoside (adenosine), ecto-5'-nucleotidase and adenosine deaminase inhibitors (AMP + AMPCP and EHNA, respectively), and inosine. **b** Effect of guanine nucleotides (GTP, GDP, and GMP), nucleoside (guanosine), and ecto-5'-nucleotidase inhibitor (AMP + AMPCP). Hydrogen peroxide (5.0 mM) was used as positive control of ROS production (fluorescence = 1925). Data were expressed as percentage of ROS calculated in comparison with control group (neutrophils stimulated with *T. vaginalis*). Bars represent the mean $\pm$ S.D. of at least three independent experiments. Data were analyzed by Student's *t* test ($p < 0.05$). Asterisk symbol (*) represents difference when compared with the control group (neutrophils alone (white bar) or neutrophils stimulated with *T. vaginalis* (black bar))

parasites were cultivated in the adenosine plus EHNA condition (279.24 ± 6.42) compared to that obtained with adenosine incubation alone (226.26 ± 26.37 μM).

In order to know if the elevated adenosine concentration found in the presence of EHNA was really responsible for the anti-inflammatory effects observed, we analyzed the involvement of adenosine receptors by immunofluorescence (Fig. 4a). Neutrophils stimulated with *T. vaginalis* revealed that A1 receptor produced the highest fluorophore expression level, suggesting the participation of this adenosine receptor

Fig. 2 Participation of purinergic signaling on cytokine production by neutrophils stimulated with *T. vaginalis* trophozoites. Effect of adenine and guanine nucleotides (ATP and GTP) and nucleosides (adenosine and guanosine), adenosine deaminase inhibitor (EHNA), and inosine on **a** IL-8 and **b** IL-6 secretion. Bars represent the mean $\pm$ S.D. of at least three

independent experiments. Data were analyzed by Student's *t* test ($p < 0.05$). Asterisk symbol (*) represents difference when compared with the control group (neutrophils alone (white bar) or neutrophils stimulated with *T. vaginalis* (black bar))

subtype in the anti-inflammatory effects observed in the presence of high concentration of adenosine (Fig. 4b).

Discussion

In an inflammatory process, within a short period of activation of the innate immune system, the acute response is initiated by immune cells enabling secretion of various cytokines and chemokines in order to recruit more immune cells to the site of the infection [28]. Regarding trichomoniasis, neutrophils are the most common leukocytes found in the vaginal environment [8] and compounds produced by them may modulate the development of the infection.

ROS are key signaling molecules that play an important role in the progression of inflammatory disorders. An enhanced ROS generation by neutrophils at the site of inflammation causes endothelial dysfunction and tissue injury [28]. Oxidative attack is also a major weapon used by phagocytes against invading pathogens, and ROS production can kill microorganisms directly by causing oxidative damage to

biocompounds or indirectly by stimulating pathogen elimination through various nonoxidative mechanisms [29]. In *T. vaginalis* field, it has already been shown that human neutrophils produce superoxide anions when stimulated with either lysates, membrane components, or excretory-secretory products from the parasite [10]. Song et al. (2008) have also demonstrated that live *T. vaginalis* trophozoites increased the intracellular ROS accumulation in neutrophils playing an important role in caspase-3-mediated apoptosis of the human neutrophils [30]. In accordance with these previous studies, our results also showed that *T. vaginalis* is able to activate ROS production by neutrophils. Considering the involvement of purinergic signaling on this immune mechanism, the presence of nucleotides did not regulate the release of the mediator. Previous work from our group has already demonstrated the participation of nucleotides and nucleosides on the NO secretion by *T. vaginalis*-stimulated neutrophils [11]. Interestingly, the nucleoside adenosine by itself was not able to modulate ROS production by *T. vaginalis*-stimulated neutrophils. Adenosine is a known anti-inflammatory molecule and its role in the inhibition of the oxidative burst has already been confirmed [17]. Here, the decrease of ROS levels became evident only when EHNA, an ADA inhibitor, was co-cubated with adenosine. Our findings suggest that when adenosine deamination is blocked the nucleoside concentration increases and the anti-inflammatory response arises.

IL-8 (CXCL8) is an important chemokine that facilitates the development of inflammatory reactions by causing chemoattraction of leukocytes to the site of inflammation, activation of neutrophils to phagocytosis, and neutrophil degranulation [31]. Expression of IL-8 has been shown to be regulated by a number of different stimuli, and once triggered a wide variety of immune cell types, including monocytes/macrophages, neutrophils, T lymphocytes release the interleukin [31]. In the context of trichomoniasis, neutrophils [12], monocytes [13], vaginal, cervical [32], and prostate cells [23] have already been shown to release IL-8 in response to

Fig. 3 Effect of *T. vaginalis* stimulation (1, 2, 4, 6, or 12 h) on neutrophil degranulation. Percentage of neutrophil elastase activity when compared to control group (neutrophils alone). fMLP was used as positive control of degranulation. Bars represent the mean $\pm$ S.D. of at least three independent experiments

Table 1 Adenosine and EHNA treatment in *T. vaginalis*-stimulated neutrophils: adenosine deaminase (ADA) activity and adenosine concentration

Conditions	ADA activity (as nmol NH ₃ h/10 ⁵ neutrophils)	Adenosine concentration (μM)
Neutrophils	–	21.91 ± 3.03
Neutrophils + adenosine	1.23 ± 0.08	264.72 ± 14.50
Neutrophils + adenosine + EHNA	0.36 ± 0.17	316.48 ± 17.78
Neutrophils + <i>T. vaginalis</i>	–	24.35 ± 3.91
Neutrophils + <i>T. vaginalis</i> + adenosine	3.26 ± 0.21	226.26 ± 26.37
Neutrophils + <i>T. vaginalis</i> + EHNA + adenosine	1.46 ± 1.03	279.24 ± 6.42

T. vaginalis trophozoites. IL-8 is considered one of the main proinflammatory chemokine related to female and male genital tract infection or inflammation [33, 34] and our data demonstrated the reduction of the mediator by *T. vaginalis*-stimulated neutrophils in an adenosine-enriched medium obtained in the presence of an ADA inhibitor. ATP and extracellular nucleotides are well-established damage-associated molecular pattern (DAMPs) since they are released in response to injury, infection, or inflammatory stimuli and initiate immune responses [17]. In our study, the presence of ATP in the co-incubation medium promoted a mildly increase of IL-8 secretion by *T. vaginalis*-stimulated neutrophils, although the cytokine production was not significantly higher than those observed in the control. This finding is not surprising once Kukulski et al. (2012) showed that the effect of ATP on IL-8 secretion in human primary neutrophils was seen only upon NTPDase1 inhibition, suggesting that human blood neutrophils might be largely unresponsive to stimulation with nucleotides due to intrinsic NTPDase1 activity [35]. In our model, we also have to consider the action of NTPDases from *T. vaginalis* which efficiently hydrolyze extracellular nucleotides [36, 37].

To our knowledge, this is the first study evaluating the production of IL-6 by neutrophils with *T. vaginalis* trophozoites as stimulus. Parasites, in the presence or not of purines, failed in activating the IL-6 secretion from neutrophils. The capacity of neutrophils to express IL-6 has been extensively debated in the literature; however, an important study was recently published indicating that human neutrophils really produce IL-6 after activation of TLR8 or, less efficiently, TLR4 [38]. IL-6 is a multifunctional cytokine produced by a variety of cells that has both pro- and anti-inflammatory activities [39]. In *T. vaginalis* infection, the proinflammatory effects of IL-6 have already been identified in macrophages [40], ectocervical [32] and prostate cells [7] after contact with the parasite.

The neutrophil degranulation is a subsequent event to phagocytosis aiming the degradation of microorganisms [41]. The sequestration of pathogens induces the release of antimicrobial peptides and proteases from the neutrophil granules that are important regulators of the local inflammatory

responses [41]. In this sense, we investigated a possible participation of *T. vaginalis* stimulus on neutrophils degranulation induction assessed by measuring the elastase activity (present in azurophil granules). Our results demonstrated that, even with long exposure to the parasites, the enzymatic activity was not enhanced, suggesting that the activation of this phagocytosis pathway is not significant for the elimination of parasites by these immune cells.

The proinflammatory effect promoted by *T. vaginalis* in neutrophils through enhancing ROS and IL-8 production was reversed by adenosine and EHNA. The immunomodulatory activity was not observed in the presence of adenosine alone. This finding can be easily understood since adenosine, once generated, is rapidly degraded by ADA in inosine, or phosphorylated by adenosine kinase in AMP [42]. In our experimental condition, ADA activity inhibition condition obtained in the presence of EHNA from both neutrophils and *T. vaginalis* was confirmed by HPLC analysis as the concentration of adenosine was enhanced. A 20–25% increase in adenosine resulting from the addition of EHNA inhibited ROS and IL-8 production (Table 1). Even without an extensive increase in adenosine concentration, the difference observed on adenosine levels may be associated with the immunosuppressive effects, since it is known that adenosine can stimulate or inhibit neutrophil function depending on its concentration in the microenvironment and the receptor profile of the neutrophil [42].

P1 receptors are subdivided into A1, A2A, A2B, and A3 subtypes which bind extracellular adenosine with different affinities. In general, the anti-inflammatory effects of adenosine are attributed to A2A receptor based on its ability to increase cAMP accumulation [43]. The involvement of this receptor subtype was also observed in one previous study of our group where A2A was associated with reduction of NO production by *T. vaginalis*-stimulated neutrophils [11]. In the present study, the participation of A1 receptor was suggested by the overexpression of the receptor fluorescence in the presence of increased adenosine bioavailability. The highest activation was seen in A1 receptor which has been shown to display both pro- and anti-inflammatory effects in different experimental models [44]. Although the immunostimulatory

Fig. 4 Expression of P1 receptors after coculture of neutrophils and *T. vaginalis* in the presence or not of adenosine and EHNA. **a** Immunofluorescence of A1, A2A, A2B, and A3 subtypes. The specific fluorescence of P1 receptors is visualized on red (Alexa FluorTM 568). Green and blue represent FLUTAX-2 and DAPI staining, respectively, and merge means the association between the three colors. DIC, differential interference contrast. Microphotographs were done using a

1:20 objective and 3× zoom were applied on the images. **b** Quantification of A1, A2A, A2B, and A3 receptors expression. Red fluorescence intensity was measured using Image J program. Data were expressed by mean ± standard deviation (S.D.) and analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple range test, considering $p < 0.05$ as significant

responses after A1 binding are well established in the literature, protective activities have also been observed in neutrophils and lung tissue [42, 45].

In conclusion, the present report demonstrated the influence of purinergic signaling on the production of important immune cell mediators by *T. vaginalis*-stimulated neutrophils. Here we showed that the enhanced adenosine bioavailability caused by ADA inhibition played immunosuppressive responses, reducing ROS and IL-8 production by neutrophils.

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Compliance with ethical standards

Conflicts of interest Amanda Piccoli Frasson declares that she has no conflict of interest.

Camila Braz Menezes declares that she has no conflict of interest.

Gustavo Krumel Goelzer declares that he has no conflict of interest.

Simone Cristina Baggio Gnoatto declares that she has no conflict of interest.

Solange Cristina Garcia declares that she has no conflict of interest.

Tiana Tasca declares that she has no conflict of interest.

Ethical approval Human neutrophils were collected from venous blood of healthy volunteers with ethical approved by UFRGS Ethical Committee, number 19346.

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