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Nucleosides from *Phlebotomus papatasi* Salivary Gland Ameliorate Murine Collagen-Induced Arthritis by Impairing Dendritic Cell Functions

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Among several pharmacological compounds, Phlebotomine saliva contains substances with anti-inflammatory properties. In this article, we demonstrated the therapeutic activity of salivary gland extract (SGE) of *Phlebotomus papatasi* in an experimental model of arthritis (collagen-induced arthritis [CIA]) and identified the constituents responsible for such activity. Daily administration of SGE, initiated at disease onset, attenuated the severity of CIA, reducing the joint lesion and proinflammatory cytokine release. In vitro incubation of dendritic cells (DCs) with SGE limited specific CD4⁺ Th17 cell response. We identified adenosine (ADO) and 5’AMP as the major salivary molecules responsible for anti-inflammatory activities. Pharmacologic inhibition of ADO A₂ receptor or enzymatic catalysis of salivary nucleosides reversed the SGE-induced immunosuppressive effect. Importantly, CD73 (ecto-5’-nucleotidase enzyme) is expressed on DC surface during stage of activation, suggesting that ADO is also generated by 5’AMP metabolism. Moreover, both nucleosides mimicked SGE-induced anti-inflammatory activity upon DC function in vitro and attenuated establishment of CIA in vivo. We reveal that ADO and 5’AMP are present in pharmacological amounts in *P. papatasi* saliva and act preferentially on DC function, consequently reducing Th17 subset activation and suppress the autoimmune response. Thus, it is plausible that these constituents might be promising therapeutic molecules to target immune inflammatory diseases. *The Journal of Immunology*, 2011, 187: 4347–4359.

During their evolutionary process, several species of blood-feeding arthropods developed a number of sophisticated and redundant mechanisms to overcome the hemostatic and inflammatory/immune systems of their vertebrate hosts (1). Vasodilators, anticoagulants, inhibitors of platelet aggregation, and anti-inflammatory and immunomodulatory molecules are present in the salivary glands and are essential to a successful blood meal (2, 3). Furthermore, these active molecules may contribute in the transmission, as well as establishment, of arthropod-borne diseases (i.e., leishmaniasis by phlebotomines, malaria by anophelines, and Lyme disease by ixodid ticks), through modulation of the host immune response (4, 5). Indeed, arthropod saliva has been shown to inhibit several functions of the immune system, including activation of the alternative complement pathway, phagocytosis of pathogens, production of inflammatory cytokines by macrophages and dendritic cells (DCs), and activity of NK cells, as well as T and B cell proliferation (6–11).

In phlebotomines, it has been demonstrated that their saliva is able to selectively inhibit several DC and macrophage functions, including Ag presentation, NO and hydrogen peroxide production, and IFN-γ-induced inducible NO synthase gene expression, thus inhibiting intracellular killing by *Leishmania major* (9, 12). Furthermore, salivary proteins from certain sand fly species favor development of a Th2-type immune response, either in vitro or in the host, and may contribute to disease progression in leishmaniasis and bartonellosis (9).

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## Abbreviations and Acronyms

- ADA: Adenosine deaminase
- ADO: Adenosine
- A₂: Adenosine A₂ receptor
- A3: Adenosine A₃ receptor
- BMDC: Bone marrow-derived dendritic cell
- CIA: Collagen-induced arthritis
- CD73: Ecto-5’-nucleotidase enzyme
- COX: Cyclooxygenase
- DCs: Dendritic cells
- IFN: Interferon
- IL: Interleukin
- LPS: Lipopolysaccharide
- MDC: Macrophage-derived cell
- MHC: Major histocompatibility complex
- NK: Natural killer
- TNF: Tumor necrosis factor

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Abbreviations used in this article: ADA, adenosine deaminase; ADO, adenosine; BMDC, bone marrow-derived cell; CIA, collagen-induced arthritis; COX, cyclooxygenase; DC, dendritic cell; LN, lymph node; RA, rheumatoid arthritis; SGE, salivary gland extract; Treg, regulatory T cell.
in vivo, characterized by production of high levels of IL-4 (13, 14). Importantly, sand fly saliva induces release of immunomodulatory mediators such as IL-10 and PGE_2 and inhibits production of protective type 1 cytokines such as IL-12, IFN-γ, and TNF-α, all of which enhance survival of the *Leishmania* parasite (15–18).

We recently demonstrated that systemic pretreatment of mice with salivary gland extract (SGE) from the Old World species *Phlebotomus papatasi* and *Phlebotomus duboscqi* inhibited neutrophil migration during OVA-induced immune periitonitis (19). By exploring the specific mechanism of saliva action, we found that Phlebotomine saliva acts preferentially on APCs, inhibiting the ability of DC to present Ags to T cells. These anti-inflammatory effects seem to depend on a sequential production of PGE_2 and IL-10 by DCs, which act in an autocrine manner (19).

DCs are potent APCs specialized in the initiation of the immune response by direct activation and differentiation of naive T lymphocytes to specific subtypes (20). Inflamed synovia from arthritic patients contains high numbers of both DC subsets, myeloid and plasmacytoid, which strongly suggests a role for these APCs in disease perpetuation (21–23). During the Ag presentation process, depending on stimuli (i.e., pathogens or autoantigens), DCs that emigrate to inflamed joints produce proinflammatory mediators such as IL-1β, IL-6, IL-12p70, IL-15, IL-18, IL-23p19, and TNF-α that support expansion and differentiation of Th1 and/or Th17 cells, which play a pathologic role in arthritis (24–27). Given the ability of DCs to interact strongly with T cells, inducing and activating the lymphocyte CD4^+ Th17 subset, it is plausible to suggest that pharmacologic strategies aimed at blocking DC function may deserve attention as a potential therapeutic target of autoimmune diseases.

Taking into account this evidence, we examined in this article the potential therapeutic effect of *P. papatasi* SGE on collagen-induced arthritis (CIA). We also identify the constituents of *P. papatasi* saliva that are responsible for the immunomodulatory activity observed.

**Materials and Methods**

**Mice**

Male DBA/1J mice weighing 18–22 g were housed at the animal facility of the Department of Pharmacology or Immunology, School of Medicine of Ribeirão Preto, University of São Paulo (Brazil), in temperature-controlled rooms (22–25°C) and received water and food ad libitum. All experiments were performed in accordance with the Brazilian states of SP the Ethics Committee from the School of Medicine of Ribeirão Preto.

**Saliva**

Salivary glands were prepared from 7- to 10-d-old laboratory-bred females of *P. papatasi* from the Laboratory of Malaria and Vector Research at the National Institutes of Health as previously described (28). Briefly, 50 pairs of salivary glands were dissected under sterile conditions in endotoxin-free PBS, placed in 50 μl sterile PBS buffer, and kept at −70°C until needed. Immediately before use, the glands were disrupted by sonication using a Sonifier 450 homogenizer (Branson). Endotoxin levels were evaluated using the QCL-1000(c) Chromogenic LAL Endpoint Assay kit (Lonza, Switzerland), resulting in negligible levels of endotoxin in the salivary gland supernatant.

**Induction of CIA and assessment of arthritis**

CIA was elicited in mice, as previously described (29). Briefly, male DBA/1J mice (10 wk) received 200 μg diluted in acetic acid of bovine type II collagen (Sigma-Aldrich) emulsified in CFA (Sigma-Aldrich) on day 0. Mice were monitored daily for signs of arthritis, as described (29). Scores were assigned based on erythema, swelling, or loss of function present in each paw on scale of 0–3, giving a maximum score of 12 per mouse. When mice reached a score of 1 for clinical arthritis, they were treated with *P. papatasi* SGE (1 gland/animal) or 5'AMP plus adenosine (ADO) (∼20 μM each) by i.v. route daily for 2 wk. To evaluate the salivary nucleoside effectiveness, some animals were either treated when disease was scored 6 or the treatment was interrupted after 1 wk. Control mice received the same volume of PBS. Alternatively, in some groups, SGE were previously incubated with adenosine deaminase (ADA; 4 U), an enzyme that catalyzes ADO. Scoring was conducted in a blinded fashion. For histologic assessment, mice were euthanized at 50 d postchallenge, and the hind limbs were removed and demineralized thoroughly in 10% EDTA for 1–2 wk. The decalcified tissues were trimmed, dehydrated in graded ethanol, and embedded in paraffin. Serial sections (5 μm) were cut and mounted on glass slides precoated with 0.1% poly-L-lysine (Sigma-Aldrich). Histologic assessment was carried out following routine H&E staining. Ankle and joint sections were prepared and stained with H&E to study the inflammatory cell influx or using safranin-O to determine proteoglycan depletion and cartilage destruction. Quantification of cellular infiltrate was performed by ImageJ software (National Institutes of Health) in 40 fields (original magnification ×400) for each animal/group. To measure cytokine concentrations in the inflammatory site, articular tissues were harvested and titrated in 1 ml PBS containing protease inhibitor mixture Complete (Roche) by tissue blower. Articular homogenates were centrifuged, and their supernatants were collected and stored at −70°C for determination of IFN-γ, IL-10, IL-17, and TNF-α by ELISA (BD Biosciences) and PGE_2 by a RIA kit, according to the manufacturer’s instructions (DuPont NEN Research Products).

**DC generation**

BM cells were isolated from 6- to 8-wk-old DBA/1J naive mice and cultured with murine GM-CSF (20 μg/ml; PeproTech). On day 3, half of the supernatant was gently removed and replaced with the same volume of supplemented medium. Culture was continued with adherent cells were removed and depleted to positive selection using anti-CD11c magnetic beads according to the manufacturer’s instructions (Miltenyi Biotec), to eliminate residual macrophage and granulocyte contamination. Flow cytometric evaluation of purified DCs shows that 90% of cells express CD11c^high (marker of DC).

**SGE fractionation and HPLC procedures**

*P. papatasi* SGE (60 pairs) were diluted in 60 μl PBS and submitted to filtration in a Microcon YM-3 (Millipore) (cutoff <3 kDa), separating SGE into two fractions: filtrate (<3 kDa) and retentate (>3 kDa). Molecular sieving HPLC of the filtrate fraction was performed using a Superdex 75 column (3.2 × 300 mm; Amersham Biosciences) and eluted at 0.05 ml/min with 10 mM HEPES buffer (pH 7.2), containing 0.15 M NaCl. The solvent was delivered using a CM-4100 pump (Thermo Separation Products). The eluent was monitored for UV absorbance at 220 nm (SM-4100 UV spectrophotometer; ThermoSeparation Products), with frations being collected at 1-min intervals using a FC203-B fraction collector (Gilson). The column was calibrated using BSA, chicken OVA, carbonic anhydrase, myoglobin, cytochrome c, angiotensin 1, tryosine, and tryptophan. Reverse phase of filtrate was performed on a C18 column (0.5 × 150 mm; Thermo Separation Products) perfused at 25 μl/min using a PerkinElmer ABI 1400 pump. A gradient of 60-min duration from 5–90% acetonitrile in water, containing 0.1% trifluoroacetic acid, was imposed after sample injection. Aliquots of filtrate or these fractions were subjected to positive selection using anti-CD11c magnetic beads according to the manufacturer’s instructions (Miltenyi Biotec), to eliminate residual macrophage and granulocyte contamination. Flow cytometric evaluation of purified DCs shows that 90% of cells express CD11c^high (marker of DC).

**Effect of *P. papatasi* SGE, fractions, and nucleosides in LP$S$-induced BMDC maturation**

BMDCs (1 × 10^6/ml) in RPMI 1640 supplemented with 10% FBS were incubated with medium, total extract from *P. papatasi* salivary gland (16 μg/ml), or salivary fractions (retentate or filtrate; 16 μg/ml) each for 12 h. In some experiments, indicated concentrations of ADO, 5'AMP (100 μM), ADO synthetic analogues P101 (100–1000 μM), or medium were added to the BMDC culture. For the time-response studies, BMDCs were pretreated with ADO or 5'AMP (100 μM) for −3.0, −0.5, 0.0, +0.5, and +3.0 h of LP$S$ stimulation. In all experiments, LP$S$ (50 ng/ml) or medium was added to the culture and incubated at 37°C in 5% CO2 for 24 h in a total volume of 200 μl per condition. The supernatant was collected to measure TNF-α, IL-12p40, and IL-10 production by ELISA and PGE_2 by RIA. The cells were harvested, and their surface expression was characterized by flow cytometry using Abs against MHC class II, CD80, CD86, CD40, CD39, and CD73 conjugated to FITC, PE, or PerCP, as well as control isotypes.
In vivo depletion of IL-10 cytokine

The IgG1 2A5 anti-murine IL-10 was purified from ascite harvested of pristine (Sigma-Aldrich)-primed nude backcrossed BALB/c mice injected with the 2A5 hybridoma cells, as described elsewhere (30). The groups of vehicle- or nucleoside-treated arthritic mice received, at the onset of the disease, 250 μg 2A5Ab or normal rat IgG controls by i.p. route Ab over each 2 d, weekly during 2 wk thereafter. Scores were assessed, as described above. Draining lymph nodes (LNs) were harvested and DC maturation was evaluated through surface expression of CD11c and MHC class II characterized by flow cytometry using specific Abs.

T cell proliferation

To assess the influence of P. papatasi SGE treatment on Ag presentation, spleens and popliteal and inguinal LN cells harvested from arthritic mice were removed and washed twice with PBS. Tissues were minced, and the cells were filtered through a cell strainer, centrifuged at 500 × g for 10 min, and resuspended in RPMI 1640 medium at 2.5 × 106 cells/ml. For intracellular staining, nonadherent cells were cultured ± stimulation with collagen-pulsed BMDCs pretreated with PBS or SGE (8 glands/106 BMDC) for a final ratio of 10 total cells:1 BMDC for 96 h. Brefeldin A was added for the last 4 h of stimulation. For surface staining, cells were incubated with anti-CD4 for 30 min at 4°C, washed, and then fixed with BD Cytofix (BD Biosciences). Cells were permeabilized using PBS containing 1% FCS, 0.01% sodium azide, and 0.05% saponin and stained with anti-mouse IFN-γ and anti-mouse IL-17 (all Abs from BD Biosciences), acquired on FACS Canto II (BD Biosciences), and analyzed using FlowJo software (Tree Star).

To purify CD4+ T cells, total cells were incubated with microbeads coated with Abs against L3T4 (Miltenyi Biotec) and isolated using magnetic separation. The procedures were performed in accordance with manufacturer’s instructions. To perform in vitro coculture assay, BMDCs were preincubated with SGE, ADO, or medium for 3 h. Isolated CD4+ T cells were added to wells to a cell ratio of 10:1. In some wells, A2,3R antagonist was added 2 h before SGE or ADO preincubation. In all the experiments, CD4 (5 μg/ml), plate-bound anti-CD3 (5 μg/ml), or medium was added to the culture and incubated for 96 h in a total volume of 200 μl per condition. Supernatants were harvested for determination of IL-17 production, and cell proliferation was measured by overnight [3H]thymidine incorporation.

Quantitative RT-PCR

Total RNA of BMDCs preincubated with ADO (100 μM) was extracted 24 h after stimulation with LPs (50 ng/ml) using the Illumina RNA Prep Mini Kit (GE Healthcare). Gene expression was normalized to expression of the GAPDH gene. COX2, A2,3R, and A2αR primer sequences are as follows: GAPDH forward, 5′-TGCAGTGGCAAATGGAGATG-3′ and reverse, 5′-CTGGA-GTGAGATCATACTGGA-3′; COX-2 forward, 5′-GTGGAAAACCT-CGCCAGA-3′ and reverse, 5′-GCTGCGTCTCCATGTATTG-3′; A2,3R forward, 5′-TCTTCCGTCGTTTGCTC-3′ and reverse, 5′-ATACCC-GTCAACAGGCAT-3′; and A2B forward, 5′-CTGCTCATATGC- TGAGCTTGC-3′ and reverse, 5′-ATAGCTGCCATGTCGCT-3′. For CD39 and CD73 expression, total mRNA was extracted from DC culture harvested in different periods after LPs stimulation: CD39 forward, 5′-AACTC-TCTCTGAATTCCTGTCTC-3′ and reverse, 5′-ATGCCCTCGGCCAG-TTCTC-3′; CD73 forward, 5′-CAATCCACACACACTG-3′ and reverse, 5′-TATCTCTGGCGCTCCATA-3′.

Statistical analysis

Data are expressed as mean ± SEM and are representative of two to four independent experiments. Results of individual experiments were not combined, as they were analyzed individually. The means from different groups were compared by ANOVA, followed by Tukey’s test. Statistical significance was set at p < 0.05.

Results

Systemic treatment with SGE ameliorates CIA

We first investigated the potential therapeutic effect of P. papatasi SGE on the CIA model in DBA/1J mice. The disease was elicited as described in Materials and Methods. Mice were treated with SGE (one salivary gland/animal) by i.v. route daily for 14 d from the first day of clinical manifestation of disease. Collagen-immunized mice treated with PBS developed the expected disease progression, showing a prominent clinical severity score of 8.0 ± 2.23 (Fig. 1A) and a high number of affected paws (3.25 ± 0.51) (Fig. 1B) compared with naive mice. In contrast, SGE treatment ameliorated disease severity over the entire observation period, displaying a significant reduction of 37.5% in the clinical scores (5.0 ± 1.17) (Fig. 1A) and 38.5% in numbers of arthritic paws (2.0 ± 0.52). Histologic examination of the knees at the end of the monitoring period (14 d of treatment) revealed that untreated mice showed severe cellular infiltration (Fig. 1C, left columns, 1D, right columns) and marked reductions in matrix proteoglycan (Fig. 1C, right columns), suggesting joint cartilage damage. In contrast, these pathologic events were profoundly reduced in SGE-treated animals (Fig. 1C, left columns, 1D, right columns). Together, these data demonstrate that P. papatasi SGE attenuated development of CIA that could be clinically detected, and this activity can prevent progression of articular damage.

Taking into account that the tissue damage observed in several autoimmune diseases (including rheumatoid arthritis [RA]) is a consequence of proinflammatory mediators released into the inflammatory site (31), we investigated the concentration of TNF-α, IFN-γ, IL-17, and IL-15 in affected ankle joints. Paw samples from arthritic mice treated with PBS contained significantly higher concentrations of all above-mentioned inflammatory cytokines compared with those of naive mice (Fig. 2). In contrast, mice treated with SGE showed a significant reduction in the levels of TNF-α (Fig. 2A), IFN-γ (Fig. 2B), and IL-17 (Fig. 2C), but no effect on IL-15 levels (Fig. 2D) compared with control PBS-treated arthritic mice.

We previously demonstrated that PGE2 and IL-10 are involved in the anti-inflammatory activity of P. papatasi SGE in OVA-induced immune peritonitis (19). Thus, we attempted to address whether SGE treatment attenuated CIA by inducing production of these anti-inflammatory mediators. Levels of PGE2 and IL-10 were evaluated in the inflamed paws of CIA mice. Supporting our hypothesis, the treatment of CIA arthritic mice with salvia enhanced the levels of PGE2 (Fig. 2E) and IL-10 (Fig. 2F) production compared with PBS-treated arthritic mice (Fig. 2E, 2F, respectively). These data suggest that sand fly SGE triggers host anti-inflammatory mechanisms that may control progression of arthritis and protects the joint from extreme damage.

Blocking of DC function by SGE treatment inhibits CD4+ T cell activation

To elucidate the mechanism(s) of successful SGE therapeutic treatment on CIA, we investigated whether blocking the DC ability to present Ag by SGE affects CD4+ T cell activation and, consequently, inhibits proinflammatory cytokine release during CIA. To this purpose, nonadherent inflammatory cells from spleen and draining LNs of arthritic mice were cultured with syngeneic bone marrow-derived DC (BMDC) pretreated overnight with SGE (8 glands/ml) or PBS, followed by collagen (5 μg/ml) or medium stimulation for 96 h, after which the cells were phenotyped.

Collagen-pulsed BMDC markedly triggered an increase of CD4+ T cells and expression of IL-17 and IFN-γ by these cells when compared with BMDCs cultured with PBS only. Preincubation of collagen-pulsed BMDC with SGE reduced proliferating CD4+ T cells (56.0% reduction) as well as IL-17 (62.0% reduction) and IFN-γ-positive cells (76.0% reduction) (Fig. 3A).

To obtain more evidence for the role of SGE on CD4+ T cell activation via DC inhibition, we magnetically purified CD4+ T cells from draining LNs and cultured them with BMDCs preincubated with PBS or SGE (8 glands/ml) overnight, followed by stimulation with collagen (5 μg/ml), plate-bound anti-CD3 (5 μg/ ml), or medium for 96 h. As expected, BMDCs pretreated with PBS and pulsed with collagen induced an intense CD4+ lym-
phoproliferative response and IL-17 production compared with nonpulsed BMDCs (Fig. 3B, 3C). There was a significant reduction (49.0%; \( p < 0.05 \)) in Ag-specific CD4\(^+\) lymph proliferation when BMDCs were pretreated with SGE (Fig. 3B). Furthermore, IL-17 production was significantly inhibited (27.5% inhibition; \( p < 0.05 \)) in the supernatant of cocultures preincubated

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FIGURE 1. SGE from \( P. \) papatasi attenuated CIA. Naive (x) or collagen-immunized and challenged DBA/1 mice were injected i.v. daily with PBS (○) or \( P. \) papatasi SGE (1 gland/animal) (●) for 14 d. Mice were monitored for disease progression, as indicated by clinical scores (A) and number of affected paws (B). On day 15 of SGE treatment, mice were euthanized, the articular joints were harvested, and histopathologic analysis was performed. Knee joint sections were stained by H&E (left row) or with safranin-O (right row), a proteoglycan red marker showing profound cartilage damage in PBS-treated mice (absence of proteoglycan) and preservation of cartilage in SGE-treated mice (C). Quantification of cellular infiltrate was performed by ImageJ software (National Institutes of Health) in 40 fields with original magnification \( \times 400 \) for each animal per group (D). Morphometric histologic examination shows markedly less cellular infiltration in the SGE-treated than in the PBS-treated group (black bar versus striped bar). Results show the mean ± SEM, \( n = 4 \).

\( ^{#} p < 0.05 \) compared with naive group, \( ^{*} p < 0.05 \) compared with PBS-treated group.

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FIGURE 2. Decreased inflammatory cytokines in articular joints from SGE-treated arthritic mice. Ankle joints from naive (○) or PBS (●)- or SGE (♦)-treated arthritic mice were collected for determination of TNF-\( \alpha \) (A), IFN-\( \gamma \) (B), IL-17 (C), IL-15 (D), and IL-10 (F) levels by ELISA and PGE\(_2\) (E) by RIA in the homogenate supernatants. Results are expressed as mean ± SEM, \( n = 4 \) (naive) and 9–10 (arthritic groups).
with SGE (Fig. 3B, 3C); however, treatment of DCs with SGE did not alter either proliferative response or IL-17 production after polyclonal anti-CD3 stimulation (Fig. 3D, 3E). Together, these results suggest that SGE suppresses the specific immune response by acting on APCs, blocking the Ag presentation process, and, as a consequence, inhibiting proinflammatory mediator release by CD4+ T cells involved in RA.

Adenosine and adenosine monophosphate are the major anti-inflammatory molecules in P. papatasi SGE

To identify the molecule(s) responsible(s) for the successful therapeutic activity of P. papatasi SGE on CIA, the salivary extract was filtered using Microcon YM3 and separated into filtrate (representing molecules with mass <3 kDa) or retentate (compounds with mass >3 kDa). Filtrate, retentate, and SGE (control) samples containing ~16 μg protein/ml (equivalent to the protein concentration of 8 glands diluted in 1 ml) were tested in LPS-induced DC maturation. Following LPS stimuli, BMDCs produced high levels of both TNF-α and IL-12p40 compared with medium (Fig. 4A, 4B). When cultures were preincubated overnight with SGE, significant inhibitions of both proinflammatory mediators were observed (Fig. 4A, 4B). Interestingly, similar inhibitions of TNF-α and IL-12p40 synthesis were obtained with the filtrate, but not with the retentate, suggesting the presence of active salivary molecule(s) with <3 kDa (Fig. 4A, 4B). Anti-inflammatory cytokines such as IL-10 are also induced by LPS and may be upregulated by P. papatasi saliva (19). In fact, LPS-induced IL-10 production was increased upon preincubation of BMDCs with SGE or filtrate, but not with retentate salivary fraction (Fig. 4C).

To obtain further information on the biochemical nature of the low m.w. salivary molecule(s), the filtrate fraction was submitted to reverse-phase HPLC. The absorbance of eluted fractions was monitored at 258 and 280 nm. The chromatogram indicated two major peaks at 7 and 17 min of retention, having a large 258 nm absorption compared with absorption at 280 spectrum with expected retention times of the nucleosides adenosine (ADO) and adenosine monophosphate (5′AMP), respectively (Fig. 4D). These substances were previously described by our group in the salivary glands of P. papatasi (32, 33). Other compounds eluted at 40 min, although in minor concentrations, some of them also absorbing at 258 nm (Fig. 4D). In fact, the highest inhibition of TNF-α production was achieved using fractions 7 and 17 (Fig. 4E), suggesting that ADO and 5′AMP are the major salivary constituents with modulatory activity on DCs. Confirming this hypothesis, treatment of LPS-stimulated DCs with similar amounts of 5′AMP plus ADO mimics the SGE effect, that is, inhibits TNF-α and IL-12p40 production (Fig. 4F, 4G, respectively). Moreover, treatment of the SGE filtrate fraction or 5′AMP plus ADO with ADA, an enzyme that catabolizes ADO (34), prevented the inhibitory effect of SGE and 5′AMP plus ADO on TNF-α and IL-12p40 production by LPS-stimulated DCs (Fig. 4F, 4G, respectively). In addition, enhancement of IL-10 by SGE was also abolished by ADA-treated filtrate (Fig. 4H).

ADO and 5′AMP modulate DC maturation

To determine the relative contribution of ADO and 5′AMP in the SGE-dependent anti-inflammatory activity of SGE, a concentration-response curve and time-course inhibitory profile were conducted using standard commercial nucleosides.
ADO alone had no significant effect on basal cytokines released by BMDC in the absence of stimulus (medium); however, BMDC pretreated overnight with ADO before stimulation with LPS resulted in inhibition of TNF-α production, whereas IL-10 production was stimulated (Fig. 5A). Both effects were observed in a concentration-dependent manner (Fig. 5A). Furthermore, the immunomodulatory effect of ADO was partially observed even when it was added to the culture 3 h after LPS administration (Fig. 5C).

The impact of ADO on the maturation of DCs was evaluated by flow cytometry. Incubation of BMDCs with ADO alone for 24 h slightly affected MHC-II, CD86, and CD40 basal expression. As expected, exposure to LPS enhanced the expression levels of these surface markers. ADO did not alter the effect of LPS on the surface expression of CD86, although MHC-II and CD40 upregulation was decreased (Supplemental Fig. 1).

The anti-inflammatory effect of 5′AMP present in SGE gland could be a consequence of extracellular ADO generation. In fact, similarly to ADO, 5′AMP also inhibited TNF-α production and enhanced IL-10 release in a concentration-dependent manner (Fig. 5B). Whereas the kinetics of TNF-α inhibition in the presence of 5′AMP followed the same pattern as that displayed by ADO, IL-10 enhancement was seen only when 5′AMP was added to DC culture at −3.0, −0.5, and 0 h of LPS administration, but not at later time points (Fig. 5D), suggesting that some amount of time is required for 5′AMP to generate ADO.

Extracellular generation of ADO is mediated by two sequential enzymes, as follows: ecto-ATP disphohydrolase (CD39), which converts ATP and ADP to AMP and, subsequently, ecto-5′-nucleotidase (CD73), which converts 5′AMP to ADO (35). A recent study demonstrated that regulatory T cells (Treg) express both enzymes, and that ATP metabolic disruption by CD39 and CD73 is the mechanism by which these cells mediate immunosuppression (36); however, whether both nucleotidases are expressed in DCs is still unknown. Thus, we analyzed CD39 and CD73 expression in LPS-matured DCs as a putative mechanism by which 5′AMP could generate ADO and mediate a modulatory effect on DCs. Cytometric analysis revealed that both enzymes are expressed in BMDCs. Whereas CD39 expression seems to be constitutive and LPS activation did not alter its expression, CD73 can be upregulated by the maturation process. LPS stimulation enhanced CD73 expression by 34% compared with medium alone (Fig. 5E, 5F). Similarly, expression of CD39 transcripts has not changed upon LPS stimulus, whereas CD73 expression significantly increased under the same conditions (Supplemental Fig. 2A, 2B). Together, these results clearly demonstrate that ADO, already present in saliva and/or generated by 5′AMP metabolism, most likely accounts for, most if not all, anti-inflammatory activity presented by P. papatasi SGE.

ADO enhances LPS-induced PGE2 production

In a previous study, we demonstrated that the inhibitory effect of SGE on an Ag-immune inflammation model depends on sequential production of PGE2 and IL-10 by DCs, which in turn acts in an autocrine manner, reducing the Ag-presenting ability of these cells (19). Taking into account that ADO and 5′AMP are the molecules

**FIGURE 4.** ADO and 5′AMP are the major suppressive molecules in P. papatasi SGE. BMDCs were preincubated overnight with SGE (16 µg/ml) or SGE microcon YM-3 fractions: retentate (>3 kDa) and filtrate (<3 kDa), and then stimulated with LPS (50 ng/ml) for 24 h. TNF-α (A), IL-12 p40 (B), and IL-10 (C) production were measured in the culture supernatants. To characterize DC modulator(s), the filtrate fraction was chromatographed by reverse-phase HPLC, as described in Materials and Methods (D). The activity of each eluted fraction was evaluated in LPS-induced TNF-α production by BMDCs (E). Filtrate or ADO + 5′AMP commercial standards were previously incubated with vehicle (DMSO) or ADA (4.3 U) for 3 h before LPS stimulation, and cytokine production (F–H) was measured in the culture supernatant. *p < 0.05 compared with medium, †p < 0.05 compared with LPS-stimuli, ‡p < 0.05 when compared with vehicle treatment.
Effects of ADO and 5′AMP on TNF-α and IL-10 production by LPS-stimulated DCs. BMDCs were incubated without (open bars) or with (shaded bars) 50 ng/ml LPS for 24 h after overnight preincubation with increasing concentrations of ADO (A) or 5′AMP (B) (µM). Cytokine production was measured in the culture supernatants by ELISA. Time-course inhibition of cytokine production by 100 µM ADO (C) or 5′AMP (D) in LPS-stimulated BMDCs. The results are expressed as the mean ± SEM obtained from one of three independent experiments made in triplicate (n = 3 per group). *p < 0.05 compared with medium, #p < 0.05 compared with LPS stimuli. Surface molecules were labeled with FITC or PE conjugated with anti-CD11c, anti-CD39, or anti-CD73, representative mean fluorescence intensity (MFI) of BMDC staining from medium or LPS cultures is shown in each box (E). The filled histograms represent cells labeled with the specific mAb; the empty histograms represent the same cell suspension labeled with isotypic control mAb. F: Bars display the relative MFI obtained from one of four independent experiments made in quadruplicate (n = 4). *p < 0.05 compared with medium.

ADO present in SGE affected DC function during the Ag-presenting process and attenuated CIA

We investigated whether ADO inhibits DC function and determined which ADO receptors were expressed in BMDCs. Transcripts for A2a and A2b receptors (A2aR and A2bR) were detected in unstimulated cells, with A2aR being highly expressed in resting cells (Fig. 7B). After LPS stimulation, the transcript profile completely changed, with significant increase in A2bR expression, and decrease in A2aR expression (Fig. 7B). Given that the immunoregulatory effect of ADO is attributed to its engagement to A2aR (37) and was enhanced after inflammatory stimulation (Fig. 7A), the stable and selective A2aR antagonist (8,3, clorosterylcafeine) was tested on the SGE effect on BMDC-driven CD4+ T cell activation. The A2aR antagonist was added to DC culture 2 h before SGE addition, and DC function was assessed by Ag-induced lymphoproliferative response and IL-17 production. As expected, Ag-induced proliferation and IL-17 production were inhibited by BMDCs treated with SGE. This impaired ability of BMDCs treated with SGE to induce proliferation and IL-17 production by CD4+ T cells was completely prevented by A2aR blockage, suggesting that ADO mediates the immunosuppressive action of SGE on DCs through this receptor (Fig. 7C, 7D, respectively). Corroborating this hypothesis, standard commercial ADO also affected DC function, suppressing Ag proliferative response and IL-17 production (Fig. 7C, 7D). Interestingly, A2aR antagonist potentiated both proliferative response and IL-17 production, even in the absence of SGE, suggesting the participation of endogenous ADO in this process.

We examined the effect of nucleosides on CIA. In this set of experiments, arthritic mice were treated daily with comparable amounts of 5′AMP and ADO present in one salivary gland pair (20 µM each ones). Nucleoside treatment was as effective as SGE in ameliorating established CIA (Fig. 7E, 7F). To confirm that ADO
and 5′AMP are the compounds of *P. papatasi* SGE with therapeutic effect on CIA, we have depleted both nucleosides by treating SGE with ADA for 3 h. Strikingly, the therapeutic effect of SGE on CIA was completely abolished when SGE was deaminated by ADA (Fig. 7G, 7H). CIA mice that received ADA-treated SGE display no difference in clinical scores and numbers of affected paw compared with control group (ADA-treated vehicle). Taken together, these results show that ADO and AMP are, in fact, the active constituents present into *P. papatasi* saliva responsible for anti-inflammatory activity and amelioration of CIA symptoms.

Next, we investigated the effectiveness of nucleosides in two new experiments with different protocols of treatment of arthritic mice. To test whether salivary nucleosides have a therapeutic effect when the disease is already established, arthritic mice were treated when disease score reached 6 (representing a higher disease progression). In this condition, nucleoside treatment was not effective in reducing arthritis severity, because the clinical scores (Supplemental Fig. 3A) and numbers of affected paw (Supplemental Fig. 3B) were similar to those of vehicle-treated mice. In the second protocol, mice were treated only during the first week after disease onset. The beneficial effect of the therapy with the nucleosides lasted only for a few days after the treatment was stopped. Once the treatment ended, both clinical scores (Supplemental Fig. 3C) and numbers of affected paw (Supplemental Fig. 3D) reached the same level as those of vehicle-treated mice. Thus, salivary nucleosides present a beneficial effect on CIA only when administered on disease onset and maintained during all periods of disease.

**IL-10 released during treatment contributes to anti-inflammatory activity of nucleosides on CIA**

We demonstrated that both PGE2 (Fig. 2E) and IL-10 (Fig. 2F) were increased in the paws of SGE-treated arthritic mice. To assess whether these mediators are responsible for SGE anti-inflammatory activity in vivo, we first tested the effect of IL-10 depletion using specific Ab on treated CIA mice. Treatment of mice with anti–IL-10 Ab initiated at the onset of disease abrogated the animals from protective effect of nucleosides on CIA. The anti–IL-10 Ab concomitantly administered with ADO plus AMP (α–IL-10/ADO + AMP) induced in arthritic mice similar evolution of clinical scores as those observed in control groups (vehicle/ IgG control and vehicle/anti–IL-10) (Fig. 8A). In addition, the numbers of affected paw also were comparable to control groups (Fig. 8B). We have attempted to block endogenous PGE2 production using a COX-2 selective inhibitor, Rofecoxibe. However, mice succumbed within 1 wk of Rofecoxibe treatment, indicating a side effect of the COX2 inhibitor (38).

We further evaluated DC maturation stage in vivo after treatment. Nucleosides (ADO + AMP) or vehicle (PBS) were administered onset of CIA development daily for 14 d. Draining LNs were harvested, and DC maturation was analyzed by flow cytometry through CD11c and MHC-II markers on surface membrane. As expected, naive mice exhibited low frequency of CD11cMHC-IIhigh surface markers on DC from ADO plus AMP exhibited a significant downregulation of DC maturation with reduction of CD11cMHC-IIhigh that reached the basal levels, similar to naive group. These findings indicate that DCs exhibit selective defects induced by saliva that was associated with DC maturation. Such inhibitory effect could be, at least in part, dependent of IL-10 release induced by saliva or fractions. Accordingly, the i.p. administration of Ab against IL-10 (anti–IL-10) during nucleoside treatment reversed the immunosuppressive effect of salivary fractions, potentiating the expression of CD11cMHC-IIhigh surface markers on DC from ADO plus AMP-treated arthritic mice. Taken together, our data suggest that IL-10, and perhaps PGE2, released in the site of inflammation strongly contributes to anti-inflammatory activity of saliva or nucleosides on CIA.

**Discussion**

In the current study, we report the therapeutic effect of *P. papatasi* SGE in controlling the pathogenesis of CIA. We purified and identified ADO and 5′AMP as the active pharmacologic components in SGE responsible for such immunomodulatory activity.
Indeed, ADO and 5’AMP blocked Ag presentation by DCs, interfering with Th17 cell activation and consequently suppressing the inflammatory immune response. These data indicate that *P. papatasi* salivary compounds could be a selective therapy focusing on DC function during the effector phase of the immune response as well as targets to treat CIA.

SGE treatment at the onset of disease attenuated the severity of arthritis and protected joint tissue from degradation. In addition, SGE was associated with a marked decrease of CD4+ Th17 lymphocyte activation. Importantly, IL-17 production was measured by ELISA, and lymphocyte proliferation by [3H]thymidine incorporation was determined 96 h after C-II stimulation (5 μg/ml). *p < 0.05 compared with medium, *p < 0.05 compared with stimuli, &p < 0.05 compared with salivary nucleosides, %p < 0.05 compared with A2AR antagonist. Collagen-immunized DBA/1 mice were injected i.v. daily with salivary equimolar concentration of 5’AMP + ADO (20 μM each ones) (●) or PBS (○) for 14 d after start of disease. Mice were monitored for disease progression, as indicated by clinical scores (E) and numbers of affected paws (F). *p < 0.05 compared with PBS-treated group. Results show the mean ± SEM; n = 10. In some groups, salivary gland extract or vehicle (PBS) was previously incubated with ADA (4.3 U) for 3 h. Afterward, collagen-immunized and challenged DBA/1 mice were injected i.v. daily with ADA-treated vehicle (●) or ADA-treated P. papatasi SGE (1 gland/animal) (●) for 14 d. Mice were monitored for disease progression, as indicated by clinical scores (G) and number of affected paws (H). Results show the mean ± SEM, n = 5.

FIGURE 7. Blocking A2AR prevents SGE immunosuppressive effect on DC function and 5’AMP + ADO treatment-attenuated CIA. BMDCs (10⁶ cells/ml) were incubated with ADO (100 μM). Cells were harvested 24 h after LPS stimulation for A2AR (A) and A2BR (B) mRNA expression quantification. Purified CD4⁺ T cells (10⁷ cells/ml) from arthritic mice were added into BMDC culture overnight, pretreated with PBS, SGE (8 glands/ml), or ADO (100 μM). Specific A2AR antagonist was added into some wells 2 h before nucleoside or saliva treatment. IL-17 production (C) was measured by ELISA, and lymphocyte proliferation by [3H]thymidine incorporation (D) was determined 96 h after C-II stimulation (5 μg/ml). *p < 0.05 compared with medium, #p < 0.05 compared with stimuli, &p < 0.05 compared with salivary nucleosides, %p < 0.05 compared with A2AR antagonist. Collagen-immunized DBA/1 mice were injected i.v. daily with salivary equimolar concentration of 5’AMP + ADO (20 μM each ones) (●) or PBS (○) for 14 d after start of disease. Mice were monitored for disease progression, as indicated by clinical scores (E) and numbers of affected paws (F). *p < 0.05 compared with PBS-treated group. Results show the mean ± SEM; n = 10. In some groups, salivary gland extract or vehicle (PBS) was previously incubated with ADA (4.3 U) for 3 h. Afterward, collagen-immunized and challenged DBA/1 mice were injected i.v. daily with ADA-treated vehicle (●) or ADA-treated P. papatasi SGE (1 gland/animal) (●) for 14 d. Mice were monitored for disease progression, as indicated by clinical scores (G) and number of affected paws (H). Results show the mean ± SEM, n = 5.

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SGE treatment at the onset of disease attenuated the severity of arthritis and protected joint tissue from degradation. In addition, SGE was associated with a marked decrease of CD4⁺ Th17 lymphocyte activation. Importantly, IL-17 produced by CD4⁺ T lymphocytes is a key cytokine implicated in human autoimmune disease such as RA (31). In arthritis models, IL-17 promotes activation of synovial fibroblast and both leukocyte emigration and activation, resulting in the production of several mediators involved in the inflammatory response and tissue lesions, including cytokines (TNF-α, IL-1β), chemokines (MIP-2/CXCL2, KC/CXCL1, IL-8/CXCL8), adhesion molecules (ICAM-1), receptor activator of NF-κB ligand, and matrix metalloproteinases (39, 40).

The therapeutic efficacy of SGE treatment in collagen-induced rheumatoid arthritis seems to be due to blocking DC Ag presentation and consequently the CD4⁺ Th17 cell response suppression. Consistently, SGE selectively inhibits specific (i.e., collagen), but not polyclonal stimulation, which directly stimulates lymphocytes, confirming the interference of *P. papatasi* saliva in DC function. Interestingly, salivary nucleosides present a beneficial effect on CIA only when administered on disease onset and maintained during all periods of disease. This finding further supports the notion that in the absence of saliva treatment, DC is constantly activated and contributed to severity of CIA. In agreement with our results, saliva from other blood-feeding arthropods, such as *Ixodes scapularis* ticks (41), the Lyme disease vector, and *Ixodes ricinus* (42), also has been demonstrated to block the DC-dependent Ag-presenting process and attenuated experimental autoimmune encephalitis (43).
whole saliva and commercial ADO plus 5′-AMP. Moreover, the deamination of ADO with ADA, an enzyme that catalyzes ADO (34), maintains the maturation of BMDCs induced by LPS. Accordingly, ADO has a broad range of effects on inflammatory leukocytes, including DCs, downregulating the production of proinflammatory mediators and expression of costimulatory markers, which diminish the capacity of DCs to initiate and amplify the inflammatory immune response (44).

ADO effects are mediated by four surface receptors, named A1R, A2AR, A2BR, and A3R, which are members of the G protein-coupled receptor family (45). Whereas A1R and A3R are coupled to Gi (inhibitory) proteins and mediate the inhibition of adenyl cyclase, A2AR and A2BR interact with Gs (stimulatory proteins) and activate adenyl cyclase, generating the second messenger cyclic ADO monophosphate (cAMP) that downregulates host cell activation (46). Although all four ADO receptors are present on human and mouse peripheral blood monocytes and host cell activation (46). Moreover, ZM241385 prevented both anti-inflammatory effects and survival induced by low doses of ketamine, which promotes accumulation of ADO in mice when sepsis is induced by LPS or *Escherichia coli* (49). The immunomodulatory effect of 5′-AMP present on *P. papatasi* SGE is due to extracellular ADO generation. Extracellular ADO generation on inflammation foci is mediated via CD39, ecto-ATP diphosphohydrolase, which rapidly converts circulating ATP and ADP to 5′-AMP; subsequently, 5′-AMP is converted to extracellular ADO by ecto-5′-nucleotidase (CD73) (36). Recent studies show that Tregs express high levels of CD39 and CD73 on their surface, and ADO generation is a mechanism by which Tregs exert their suppressive effects (50). Monocytes also express CD39 and CD73, and these enzymes are active under inflammatory conditions (51, 52).

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Further exploring the biologic action of salivary nucleosides on DC function, similarly to SGE, ADO showed inhibitory effects on collagen-induced CD4+ T cell response. Interestingly, in the presence of 8,3′cloroesterylcafeine (a selective A2AR antagonist), the inhibitory effect of SGE on CD4+ Th17 activation was prevented; however, such a profile was exacerbated even in the absence of SGE, indicating the involvement of endogenous ADO downregulating the inflammatory process. The exacerbation of inflammation through blocking the A2AR has been demonstrated in a variety of inflammatory models. Treatment of mice with the selective A2AR antagonist ZM241385 potentiated liver injury and inflammation in response to Con A, *Pseudomonas aeruginosa*, and carbon tetrachloride stimuli (45). Moreover, ZM241385 prevented both anti-inflammatory effects and survival induced by low doses of ketamine, which promotes accumulation of ADO in mice when sepsis is induced by LPS or *Escherichia coli* (49).

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hyporesponsiveness of CD4+ T or CD8+ T lymphocytes is due to portion of pathogenic Th17 cells, reducing the severity and pro-
DCs. Although CD39 appears to be constitutive, the expression of CD73 is increased by proinflammatory stimuli. Thus, the con-
commitant expression of both enzymes on the surface of DCs helps to generate ADO in the extracellular compartment by cleaving 5’AMP present in the saliva of P. papatasi, contributing to pre-
vention of the tissue damage observed in CIA. Accordingly, 5’AMP represents a slow-release formulation of ADO, in addition to the preformed ADO present in P. papatasi saliva.

Although the increased levels of cAMP seem to be due, at least in part, to interaction and activation of A2AR, triggering a cascade of inhibitory factors of the inflammatory response, this interaction could also lead to an increase in expression of COX-2 (44, 53). Once activated, this enzyme converts arachidonic acid to PGG2, and subsequently to PGH2, which is rapidly metabolized by specific isomerases to the potentially active prostanoids PGD2, PGE2, PGF2α, PGI2, and thromboxane A2. Our data show that either ADO or A2R agonist was able to induce enhancement of PGE2 production by LPS-stimulated BMDCs. Prior evidence suggests that incubation of neutrophils with A2AR agonist increases expression of COX-2, leading to production of PGE2 via cAMP stimulation (53). Furthermore, incubation of polymorphonuclear cells with ADA drastically reduces prostaglandin production, with downregulation of cAMP and COX-2, establishing a direct relationship between ADO and PGE2 (53).

A great body of evidence shows that PGE2 and IL-10 are potent anti-inflammatory mediators, limiting tissue damage on different experimental autoimmune diseases, including RA (54, 55). Both mediators were increased in the paws of SGE-treated arthritic mice, and treatment of mice with anti–IL-10 mAb blocked the protective effect of nucleosides. Previously, we reported that SGE inhibits immune inflammation by sequential production of PGE2/ IL-10 acting autocrinally on DC function (19). Thus, it is reasonable that both PGE2 and IL-10 contribute to the therapeutic success of SGE on CIA.

In addition to PGE2 and IL-10 effect, we have shown that DCs from LN of treated arthritic mice also present a downregulation of MHC class II molecules on their surface in vivo. Importantly, we also demonstrated that such effect was also dependent on IL-10 production. Although we have not evaluated whether the inhibitory effect of salivary nucleosides on DCs affects T cell response in vivo, we have strong evidence that its function is impaired. We have previously published that the in vivo treatment of OVA-immunized mice with SGE reduced the Ag-induced proliferation of splenic T cells (19). Interestingly, saliva affected the proliferation of OVA-specific CD4+ T cells through DC inhibition, whereas the incubation of OVA-CD4+ T cells with SGE has not altered their proliferative response (19). The reduction in arthritis progression mediated by impairment of DC function has been demonstrated by others. For instance, treatment of arthritic mice with type II collagen-pulsed tolerogenic DCs, which presents a semimature phenotype (reduced the MHC-II, CD40, CD80, and CD86 costimulatory molecules on DC surface expression and reduced levels of inflammatory cytokines), decreases the proportion of pathogenic Th17 cells, reducing the severity and progression of arthritis in these animals (56). Moreover, the hyporesponsiveness of CD4+ T or CD8+ T lymphocytes is due to immunosuppressive mediators released by DC (i.e., IL-10, TGF-β, and PGE2) (57). Furthermore, DCs downmodulated by innate immune immunity improve CIA and induced Tregs in vivo (58). Thus, it is plausible to infer that the i.v. administration of whole extract or nucleosides (adenosine and adenosine monophosphate) present into P. papatasi saliva ameliorates CIA by inhibiting the effector’s phase of the immune-specific response through inhibition of the DC activation.

It was not the aim of the current study to determine the mechanism that SGE interferes in Ag-presenting process. Nonetheless, we cannot rule out the possibility that other salivary components inhibit Ag degradation and/or the displacement of CLIP peptide associated to MHC class II binding groove, preventing loading of antigenic peptides present on the membrane surface and subsequently affecting presentation to CD4+ T cells. In fact, it has been demonstrated that Sialostatin L, a cysteine protease inhibitor isolated from I. scapularis tick saliva, inhibits LPS-induced maturation of DCs, as well as it binds cathepsin S inside lysosomal compartment, thus blocking Ag-specific T cell proliferation (43).

An interesting finding of our study was that the amount of SGE that effectively reduced RA had low amounts of both salivary protein and nucleosides (∼2 μg protein and 20 μM 5’AMP and ADO). Stressing the relevance of our finding, we demonstrated that treatment of arthritic mice with similar amounts of each nu-
cleoside reduced CIA symptoms. Interestingly, the therapeutic effect of SGE on CIA was completely abolished when SGE was deaminated by ADA. Furthermore, several studies support the hypothesis that the efficacy of methotrexate on experimental and human RA is due to its ability to increase the levels of ADO (59). Indeed, methotrexate induces accumulation of aminomimidazole-
carboxamide, a competitive inhibitor of ADA, thus allowing in-
creased levels of ADO (60) and inhibition of chondrocytes (61) and synovial cells (62).

In conclusion, the results presented in this article indicate that ADO and 5’AMP are present in P. papatasi SGE in pharmaco-
logical concentrations and mediate the anti-inflammatory activity on RA. These constituents act through A2AR in the effector phase of the inflammatory process, inhibiting the ability of DCs to present Ag and thus leading to suppression of CD4+ Th17-induced inflammatory immune response. Our data support the idea that A2AR activation could be a novel therapeutic strategy for RA and other destructive chronic disorders.

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Disclosures
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