



Preventive and curative glycoside kaempferol treatments attenuate the TH2-driven allergic airway disease

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ABSTRACT

Asthma is a chronic respiratory disease characterized by airway inflammation and airway hyperresponsiveness (AHR). One strategy to treat allergic diseases is the development of new drugs. Flavonoids are compounds derived from plants and are known to have antiallergic, anti-inflammatory, and antioxidant properties. To investigate whether the flavonoid kaempferol glycoside 3-O-[[β-D-glycopyranosil-(1→6)-α-L-ramnopiranosil]-7-O-α-L-ramnopiranosil-kaempferol (GRRK) would be capable of modulating allergic airway disease (AAD) either as a preventive (GRRK P) or curative (GRRK C) treatment in an experimental model of asthma. At weekly intervals, BALB/c mice were subcutaneously (sc) sensitized twice with ovalbumin (OVA)/alum and challenged twice with OVA administered intranasally. To evaluate any preventive effect, GRRK was administered 1 h (hour) before each OVA-sensitization and challenge, while to analyze the curative effect, mice were first sensitized with OVA, followed by GRRK given at day 18 through 21. The onset of AAD was evaluated 24 h after the last OVA challenge. Both treatments resulted in a dose-dependent reduction in total leukocyte and eosinophil counts in the bronchoalveolar lavage fluid (BAL). GRRK also decreased CD4⁺, B220⁺, MHC class II and CD40 molecule expressions in BAL cells. Histology and lung mechanic showed that GRRK suppressed mucus production and ameliorated the AHR induced by OVA challenge. Furthermore, GRRK impaired Th2 cytokine production (IL-5 and IL-13) and did not induce a Th1 pattern of inflammation. These findings demonstrate that GRRK treatment before or after established allergic lung disease down-regulates key asthmatic features. Therefore, GRRK has a potential clinical use for the treatment of allergic asthma.

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1. Introduction

Allergic asthma is a chronic inflammatory lung disease mediated by Th2 cells and characterized by airway eosinophilia, airway hyperresponsiveness (AHR), mucus hypersecretion and elevated IgE levels. It is a global health issue that results from a complex interplay between genetic and environmental factors [1].

Ovalbumin (OVA) induces allergic airway disease (AAD) in mice and has proven useful in investigating the mechanisms underlying airway inflammation [2]. The OVA–AAD experimental model produces measurable outcomes similar to those found in humans, including increased bronchoalveolar lavage fluid (BAL) total leukocyte, eosinophil and TCD4⁺ lymphocyte counts, inflammatory type 2 cytokines (IL-4, IL-5 and IL-13) and airway hyperresponsiveness to methacholine [3–5].

Medicinal plants have long been used in folk medicine as alternative treatments for a wide range of diseases including inflammatory and allergic diseases, resulting in symptom relief comparable to that achieved with the use of allopathic medicines [6].

Flavonoids are produced through plant metabolism and are found in abundance in seeds, citrus fruits, olive oil, tea and red wine. These compounds have profound effects on the function of immune and inflammatory cells as confirmed in a large variety of *in vitro* and *in vivo* studies [7]. Differences in the chemical structures, bioavailability, distribution and metabolism of flavonoids lead to variations in their pharmacological properties; therefore, many flavonoids are now known to present antiproliferative, antioxidant, anticarcinogenic, antiatherogenic, anti-inflammatory and antiallergic properties, while having very low rates of toxicity [8]. However, the molecular mechanisms underlying these biological effects have yet to be fully clarified, although evidence suggest the existence of undetermined molecular targets [8–10].

Recent studies have shown that flavonoids are able to inhibit mast cell degranulation, reduce histamine, tryptase, leukotrienes, prostaglandin D2, granulocyte-macrophage colony stimulating factor

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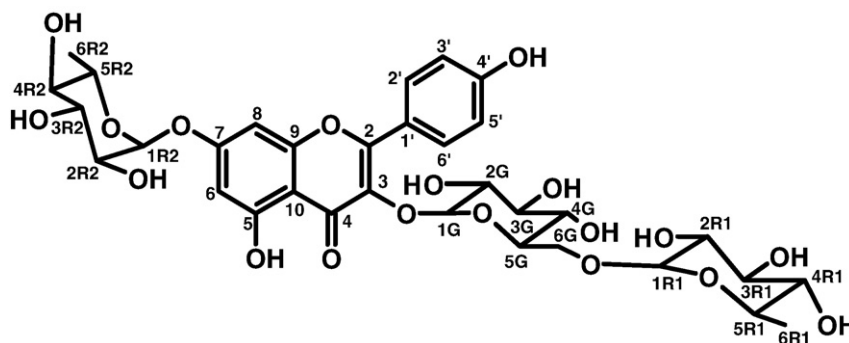


Fig. 1. Chemical structure of the flavonoid (kaempferol glycoside)-3-O-[[β-D-glycopiranosil-(1→6)-α-L-ramnopiranosil]-7-O-α-L-ramnopiranosil-kaempferol-GRRK.

(GM-CSF), IL-6 and IL-8 release from cultured mast cells as well as from macrophages [11–13]. In addition, flavonoids inhibit histamine, IL-4 and IL-13 production by activating human basophils [14]. All these data suggest that flavonoids may exert potent anti-inflammatory and antiallergic effects on allergic inflammatory diseases including asthma.

Previous reports have described several flavonoids that have been shown to exert antiallergic effects in murine models of asthma [15–18] and kaempferol is a flavonoid that has shown a promising anti-inflammatory effect [19].

The aim of this study was to demonstrate whether treatment with kaempferol, a defined chemical glycoside isolated from aerial parts of *Solanum asperum* Richer, 3-O-[[β-D-glycopiranosil-(1→6)-α-L-ramnopiranosil]-7-O-α-L-ramnopiranosil-kaempferol-GRRK) would modulate the onset of AAD in an OVA-induced murine allergic lung disease.

2. Materials and methods

2.1. Animals

Six- to eight week-old-age female mice on BALB/c background (25–30 g) were used throughout the study. They were obtained as breeding unit from the Institute of Biomedical Sciences (ICB), University of São Paulo (USP), São Paulo, Brazil and Laboratory of Pharmaceutical Technology (LTF), Federal University of Paraíba. Groups of 5–10 mice were used in each experiment. Mice were treated according to Animal Welfare guidelines of both Institutes.

2.2. GRRK identification and isolation

The aerial parts of *S. asperum* were collected in the State of Paraíba, Brazil, in September 2005 from a secondary vegetation of the Atlantic forest area at the campus of the Federal University of Paraíba, in the

municipality of João Pessoa, at 130 to 160 m elevation. The plant was identified by Dr. Maria de Fátima Agra (LTF-UFPB). Voucher specimen (Agra 1243) is deposited at the Prof. Lauro Pires Xavier (JPB) Herbarium, Federal University of Paraíba, João Pessoa, Brazil. The powdered (1.178 kg) was extracted with MeOH in a soxhlet apparatus. The extract was concentrated under vacuum. The crude residue was suspended in H₂O and extracted with hexane:ether (1:1) and AcOEt. The aqueous fraction, after standing in the refrigerator, furnished a yellow precipitate that was separated from the fraction and recrystallized from methanol to yield 720.0 mg of compound. The IR and NMR spectra, including 2D NMR were used to identify the structure of flavonoid as 3-O-[[β-D-glycopiranosil-(1→6)-α-L-ramnopiranosil]-7-O-α-L-ramnopiranosil-kaempferol-GRRK) (Fig. 1). The GRRK solution was sterilized by passing it through a 0.22 μm Millipore filter, and then analyzed for endotoxin level by a gel-clot *Limulus* amebocyte lysate assay (Zhejiang, China). The endotoxin level in the GRRK solution was less than 0.5 endotoxin units (EU)/ml.

2.3. Alum gel preparation

Alum (Al(OH)₃) gel was prepared by precipitating 0.184 M of ammonium aluminum sulfate dodecahydrate (AlH₄NO₈S₂ · 12H₂O) with an excess of 1 N NaOH, roughly 2.5 V/V. After precipitation, Al(OH)₃ was suspended in water (Milli Q) and washed 5 times at 3000 rpm for 15 min. The final precipitate was dissolved in water and the concentration Al(OH)₃ was calculated by determining the dry weight of 1 ml solution. For sensitization, alum gel stock solution (50 mg/ml) was dissolved in 10 ml PBS containing OVA (10 μg/ml).

2.4. Immunization and induction of allergic airway response

BALB/c mice were sensitized with 4 μg of OVA (grade V) adsorbed to 1.6 mg of Al(OH)₃ gel in 0.4 ml of PBS on days 0 and 7. On day 14 the

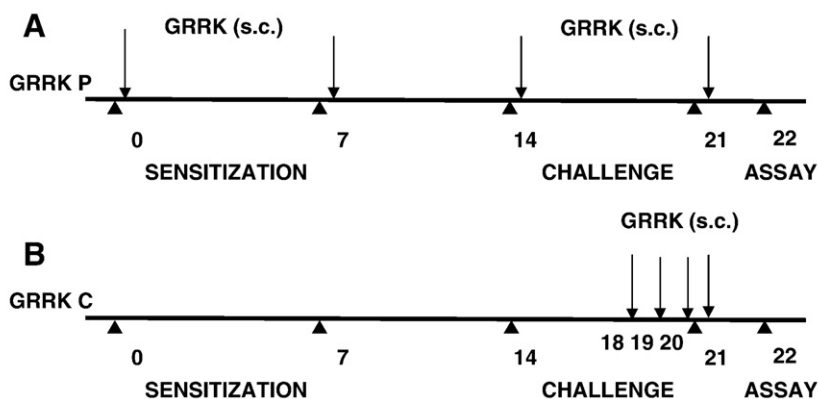


Fig. 2. Protocol for the preventive treatment of GRRK – GRRK P (A) and curative treatment of GRRK – GRRK C (B).

mice were challenged (i.n.) with 10 μ g of OVA/50 μ L in PBS to induce an allergic airway response. One week later (21th), mice were challenged with OVA (i.n.). This animal group was defined as AAD. The control group consisted of non-sensitized mice and received only the vehicle (PBS). All determinations were performed 24 h after the last OVA challenge.

2.5. GRRK treatment

To analyzed the preventive GRRK (AAD/GRRK P) effect, mice were treated with GRRK (3, 30 or 90 mg/kg, subcutaneous) or vehicle (PBS) 1 h before each ovalbumin sensitization and challenge (Fig. 2A). However to study the curative GRRK (AAD/GRRK C) effect, mice were

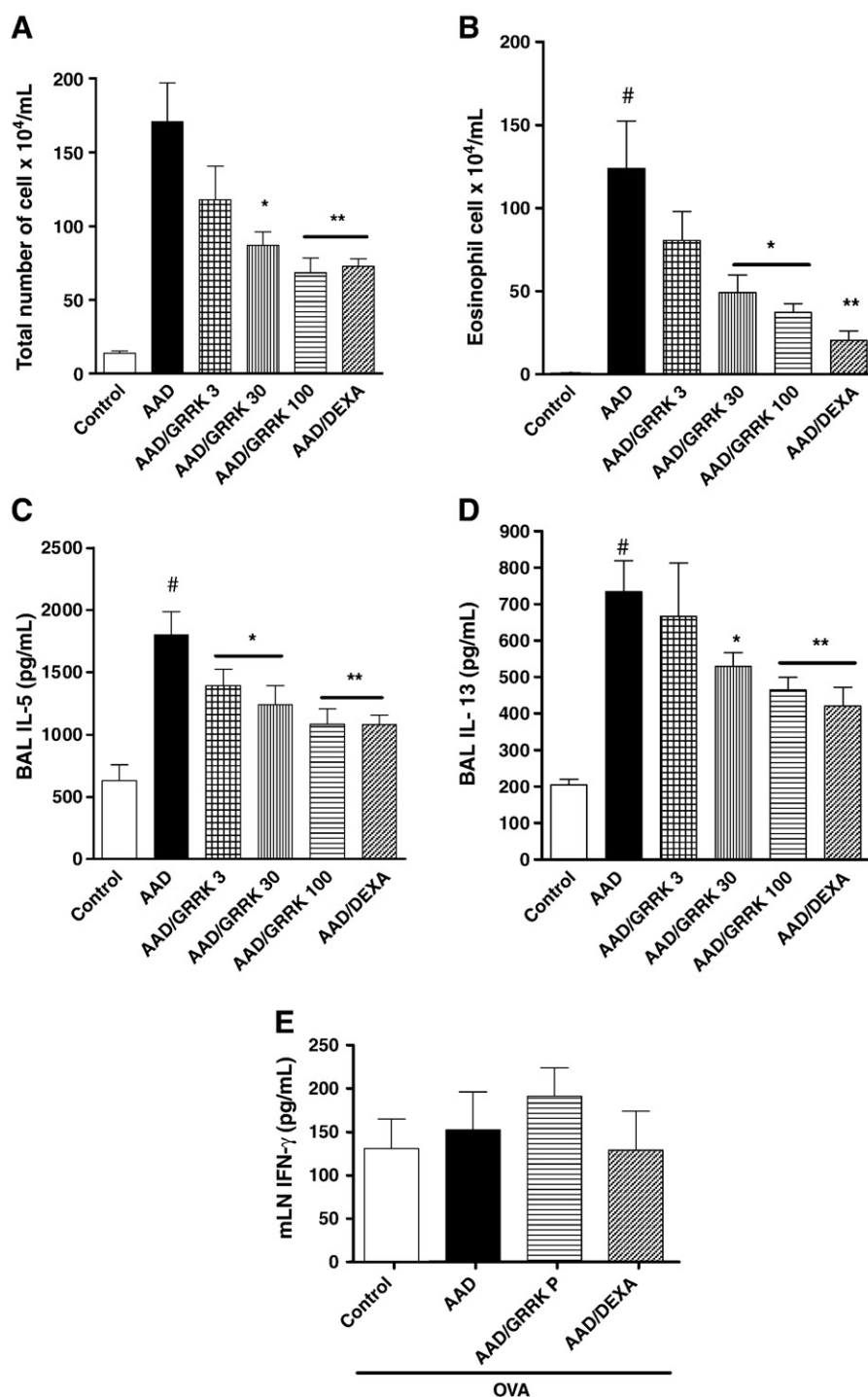


Fig. 3. Preventive GRRK treatment reduces the development of airway allergic inflammation. Groups of BALB/c mice were treated with GRRK (AAD/GRRK group), dexamethasone (AAD/DEXA group) or PBS (AAD group) 1 h before each OVA-sensitization co-adsorbed to alum and OVA-free challenge. Control group consisted of non-manipulated animals. The experiments were performed 24 h after the last OVA challenge. BAL total leukocyte counts (A); BAL eosinophil counts (B); IL-5 (C) and IL-13 (D) in BAL fluid. Cytokines in supernatants of OVA stimulated mediastinal lymph node cells (mLN) (E). Results are expressed as means $\pm$ SEM for groups of five mice and are representative of two experiments. Significant difference ($^{\#}P < 0.05$) when compared to control group. Significant difference ($^{*}P < 0.05$; $^{**}P < 0.01$) when compared to AAD group.

first sensitized and challenged as described before, but on the intervals of the days 18–21 the animals were treated with GRRK (3, 30 or 90 mg/kg, subcutaneous) (Fig. 2B). For comparison, a group of mice was treated with subcutaneous injection of dexamethasone (1 mg/kg) (AAD/DEXA).

2.6. Airways hyperresponsiveness

Airway responsiveness was assessed in unrestrained conscious mice placed in a plethysmographic chamber (Buxco Electronics, Sharon, CT), where respiratory parameters were measured before (1–3 min) and after (3–10 min) an aerosol of methacholine (Sigma-Aldrich) delivered for 20 s at 3, 6, 12 and 25 mg/ml in the aerosolator. The resistance was expressed as enhanced pause and was calculated as previously described [20].

2.7. Bronchoalveolar airways lavage (BAL)

Mice were deeply anesthetized by an i.p. injection of 4 mg/g body of chloral hydrate (Labsynth, São Paulo, Brazil) and blood samples from the retro-orbital plexus were collected for nitrate level and serum antibody determination. The trachea was cannulated and lungs were lavaged twice with 0.5 and 1.0 ml of PBS. Total and differential cell counts of BAL fluid were determined by hemocytometer and cytopspin preparation stained with Instant-Prov (Newprov, Pinhais, Brazil).

2.8. Lung-draining lymph node cultures

Cells from mediastinal lymph node (mLN) were adjusted to 5×10^6 cells/ml and cultured in a 24-well plate in medium consisting of RPMI supplemented with 10% FCS and 100 U/ml penicillin/streptomycin (1 ml cell suspension per well) for 48 h with OVA (100 µg/ml) at 37 °C in a humidified incubator with 5% CO₂. Supernatants were assayed for cytokine content by ELISA.

2.9. Antibodies and flow cytometric analysis

Single cell suspensions from BAL were pre-incubated with FcBlock (BD PharMingen, San Diego, CA). Cells were stained with the indicated antibodies in staining buffer (PBS containing 1% FBS and 0.01% NaN₃) for 30 min on ice, and analyzed by three color flow cytometry on a FACScan using FlowJo software (BDBiosciences, Mountain View, CA). All antibodies (CD4, B220, CD40, CD80, MHC II, CD11c, and CD11b) for flow cytometric analysis were purchased from Becton Dickinson (BD) PharMingen (San Diego, CA).

2.10. Cytokine measurements

The levels of cytokines (IL-5, IL-13 and IFN-γ) on the BAL fluid or on the supernatants were assayed by sandwich kit enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's suggestion (PharMingen, San Diego, CA). Values are expressed as pg/ml deduced from standards run in parallel with recombinant cytokines.

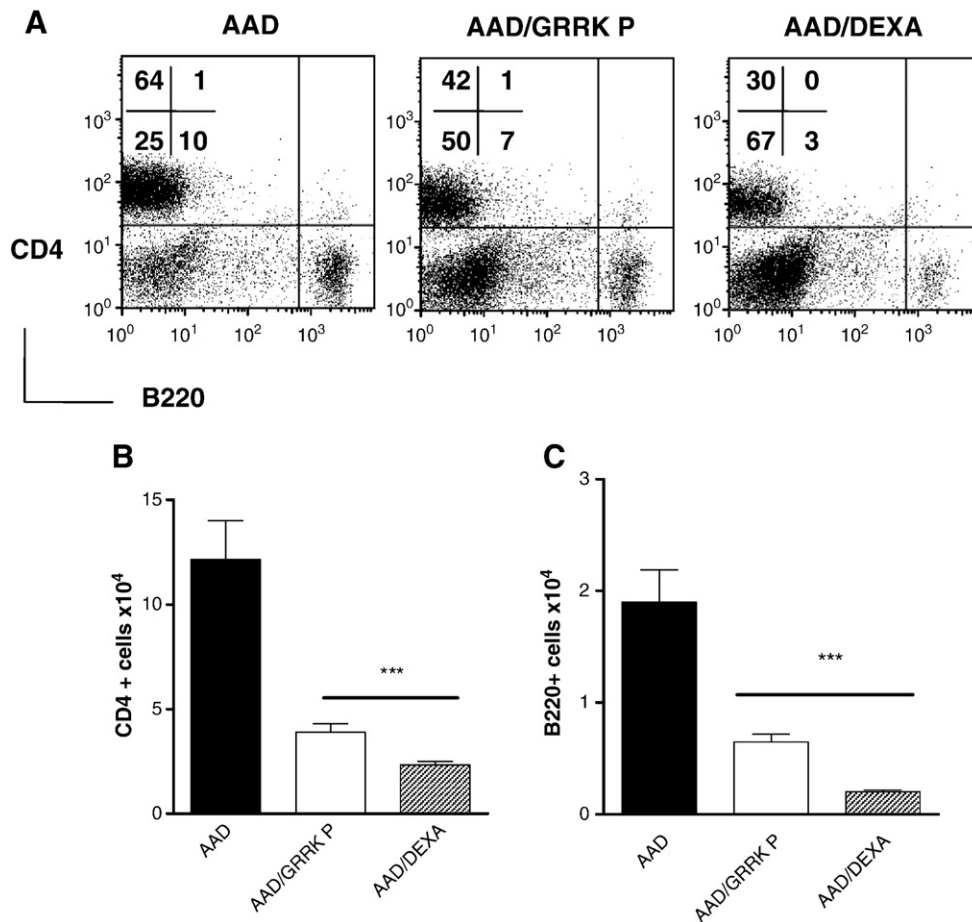


Fig. 4. Preventive GRRK treatment reduces the frequency and the total number of cells expressing CD4 or B220 present in the BAL fluid. The frequency of cells stained with anti-CD4 or anti-B220 by FACS plot for lymphocytes gated cells (FSC^{low} SSC^{low}) is shown in (A). The number of CD4⁺ (B) or B220⁺ (C) cells was deduced from the total number of BAL cell multiplied by the frequency of these cells shown in the plot. Results are expressed as means ± SEM for groups of four to five mice. Significant difference (****P* < 0.001) when compared to AAD group.

2.11. Lung histology and histocytometry

After BAL collection, lungs were perfused via the right ventricle with 10 ml of PBS to remove residual blood, immersed in 10% phosphate-buffered formalin for 24 h, and then in 70% ethanol until embedded in paraffin. Lung sections with 5 μ m were stained with hematoxylin/eosin for evaluation of peribronchial and perivascular lung inflammation or with periodic acid-Schiff (PAS)/hematoxylin for evaluation of mucus production as previously described [21]. Briefly, a quantitative digital morphometric analysis was performed using the application program Metamorph 6.0 (Universal Images Corporation, USA). The circumference area of bronchi and the PAS stained area were electronically measured and the mucus index was determined by the following formula: (PAS stained area/bronchial circumference area) $\times$ 100.

2.12. Statistical analysis

Data were expressed as a mean $\pm$ SEM. Multiple comparisons were performed by ANOVA with Bonferroni post hoc test using a computer program GraphPad Prism version 3.02. Results with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Anti-inflammatory effect of GRRK treatment for the prevention of AAD

Results of previous studies have suggested that flavonoids suppress Th2 immunity [15,16,22,23]. Therefore, in the present study, experiments were first performed to determine whether GRRK administered subcutaneously (sc) could prevent the development of allergic responses in the lung. In OVA-sensitized and challenged mice the total number of leukocytes and eosinophils found in the BAL were 8-fold and 5-fold higher, respectively, than that obtained in the control group (Fig. 3A and B). In the GRRK-treated animals (30 mg/kg or 100 mg/kg) and in the dexamethasone (DEXA) group, the total leukocyte count was 50% lower than that of the AAD group (Fig. 3A). In addition, eosinophil count in the 30 and 100 mg/ml GRRK groups was also 50% lower than that observed in the AAD group, while in the DEXA group eosinophil count was 75% lower than that found in the AAD group (Fig. 3B).

Next, Th2- or Th1-cytokine profiles were determined in the BAL fluid. A decrease occurred in the Th2-associated cytokines (IL-5 and IL-13) in the animals that received GRRK P (30 or 100 mg/ml) or DEXA compared to those of the AAD group (Fig. 3C and D). Since IFN- γ levels

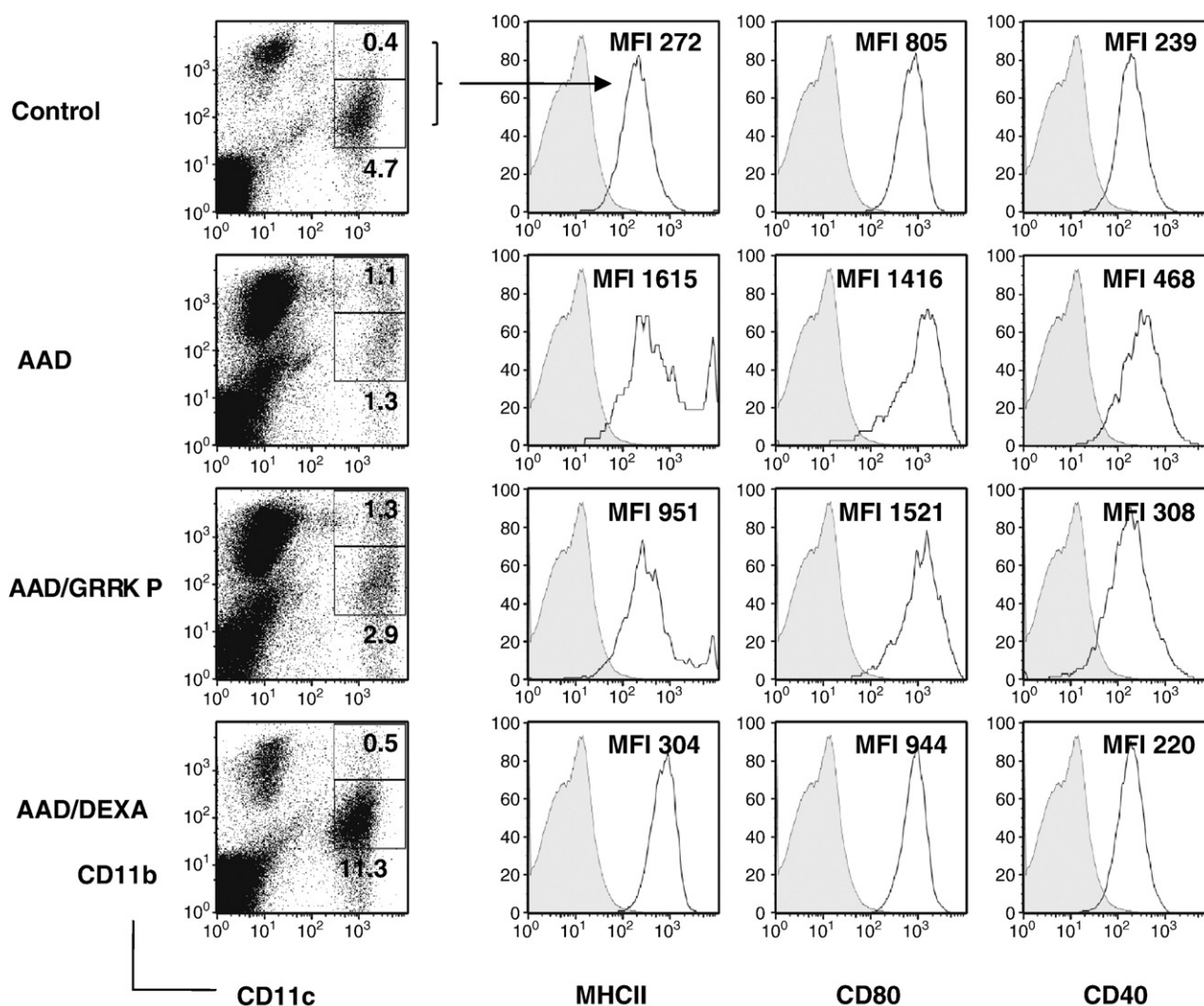


Fig. 5. Preventive GRRK treatment reduces MHC class II and CD40 expression in BAL cells expressing CD11c/CD11b molecules. BAL cells were stained with anti-CD11c, anti-CD11b, anti-CD80, anti-CD40, anti-I-A^d (MHC II) and anti-IgG2a κ (isotype control) antibodies and analyzed by FACS. The figure shows representative FACS plots of viable BAL cells expressing CD11c/CD11b (left) and histograms from gate of CD11c⁺ CD11b⁺ cells (right). Results are presented as the mean fluorescence intensity (MFI). Solid histograms correspond to isotype control antibody.

were not detected in BAL fluid, cells were cultured from the lung-draining mLN with OVA to boost IFN- γ production, however IFN- γ production following GRRK treatment was found to be similar to that found in the AAD group (Fig. 3E). These results indicate that when administered as preventive therapy, GRRK decreases airway Th2-allergic responses without increasing IFN- γ . It would therefore appear that GRRK did not shift airway inflammation towards a Th1 pattern.

3.2. GRRK preventive treatment reduced CD4⁺ T cells, B220 B cells, MHCII and CD40 expression on CD11b⁺/CD11c⁺ cells in AAD

To investigate the efficacy of GRRK P treatment on T and B cell populations, we determined the expression of CD4 and B220 molecules, respectively in BAL cells, using flow cytometry. Marked changes occurred in the CD4 and B220 cell counts in the BAL from the AAD group compared to the control group (Fig. 4). In the groups treated with GRRK or DEXA, a significant reduction occurred in CD4 (3-fold and 5-fold, respectively) (Fig. 4B) and in B220 cell counts (2-fold and 5-fold, respectively) (Fig. 4C). In addition, GRRK P or DEXA treatment also suppressed MHCII (1.8- and 5-fold, respectively) and CD40 (1.5- and 2-fold, respectively) expression on CD11b/CD11c cells (most probably alveolar dendritic cells) (Fig. 5). Taken together, these results show that GRRK treatment reduces the expression of the key markers involved in lung allergic inflammation.

3.3. The anti-inflammatory effect of GRRK treatment persist after asthma is already established – curative effect (GRRK C)

We next examined whether GRRK treatment could inhibit established allergic disease. For this purpose, animals were first OVA-sensitized and challenged and then GRRK treatment was initiated. As shown in Fig. 6A and B, the total leukocyte and eosinophil counts in the BAL were inhibited in a dose-dependent manner in the GRRK (30, 100 mg/ml) and DEXA groups compared with the AAD group. In addition, IL-5 (Fig. 6C) and IL-13 (Fig. 6D) productions were also found to be inhibited. As expected, the same parameters also decreased significantly in the DEXA group. These findings indicate that, even when asthma was already established, curative treatment with GRRK successfully impaired airway Th2-allergic responses.

3.4. GRRK treatment suppresses airway hyperresponsiveness (AHR) and airway mucus production in AAD

Asthma is associated with airway hyperresponsiveness (AHR) and mucus hyperproduction. To evaluate the effect of GRRK on methacholine-induced AHR, the animals were analyzed by plethysmography. As expected, AHR was intense in the AAD group compared to the control group (Fig. 7A). Dexamethasone treatment almost eliminated AHR compared to the AAD group ($P < 0.001$). Preventive

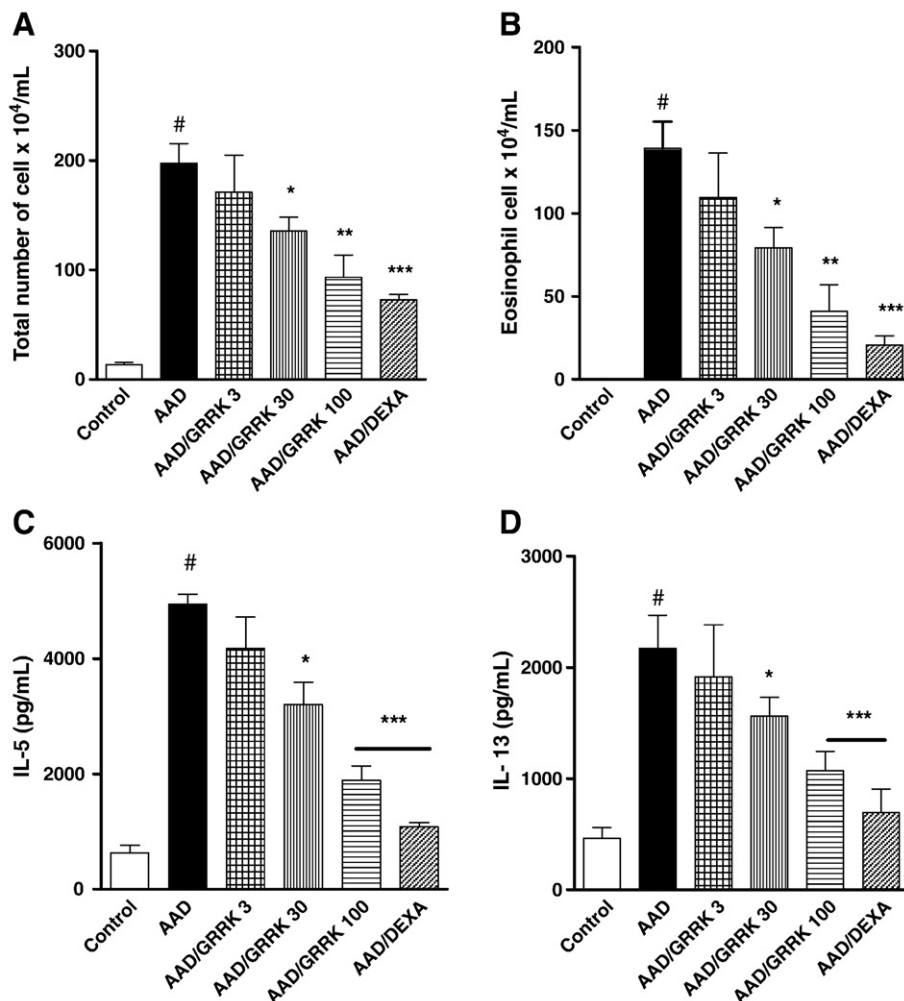


Fig. 6. Curative GRRK treatment suppresses established airway allergic inflammation. Groups of BALB/c mice were treatment from day 18 through 22 with GRRK (AAD/GRRK group), dexamethasone (AAD/DEXA group) or PBS (AAD group) 1 h before OVA challenge. Control group consisted of non-manipulated animals. The experiments were performed 24 h after the last OVA challenge. BAL total leukocyte counts (A); BAL eosinophil counts (B); IL-5 (C) and IL-13 (D) in BAL fluid. Results are expressed as means $\pm$ SEM for groups of five mice and are representative of two experiments. Significant difference ([#] $P < 0.05$) when compared to control group. Significant difference (^{*} $P < 0.05$; ^{**} $P < 0.01$; ^{***} $P < 0.001$) when compared to AAD group.

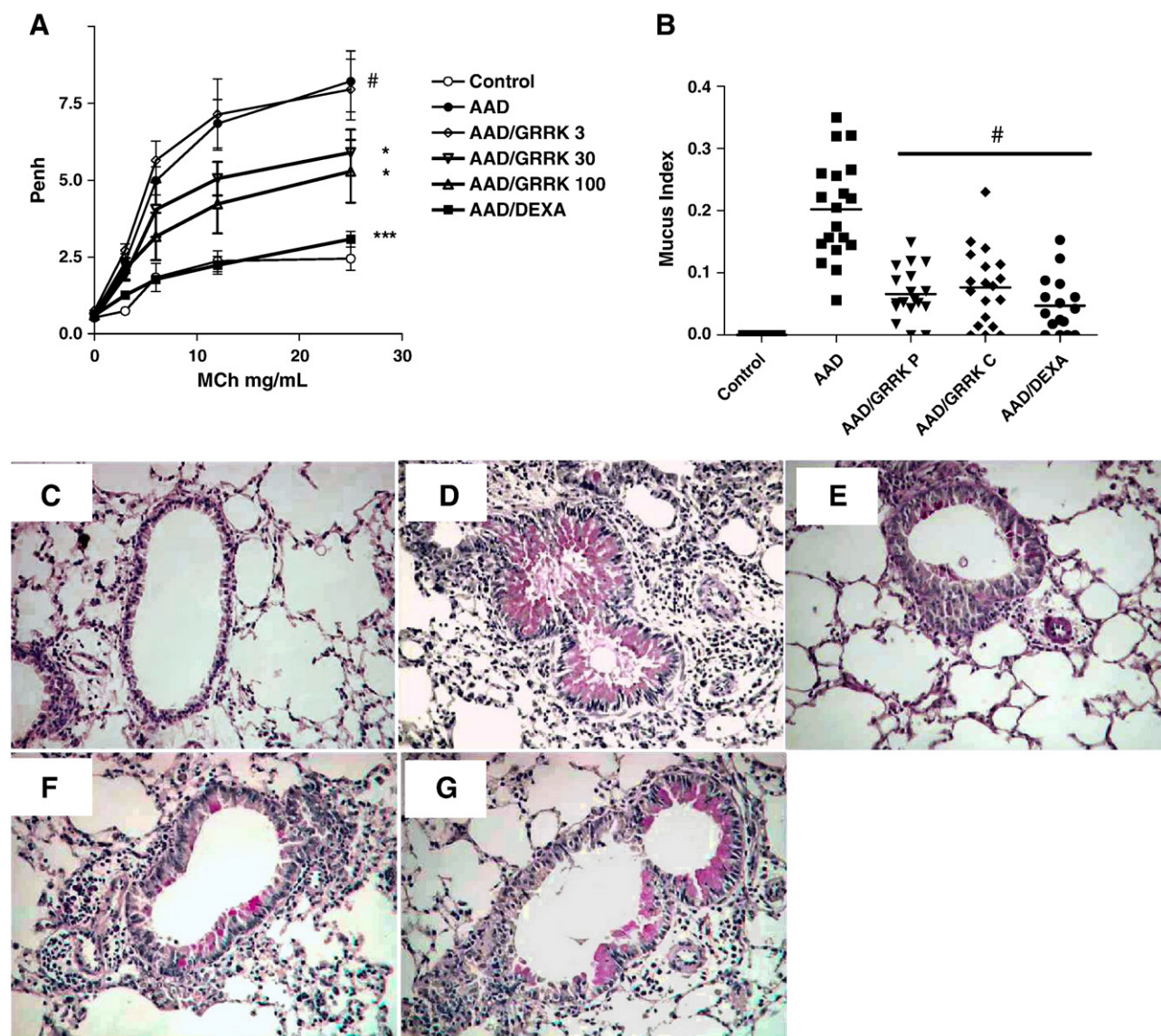


Fig. 7. GRRK treatment attenuates airway pathology. The experiments were performed 24 h after the last OVA challenge. Preventive GRRK treatment decreases airway hyperreactivity to increasing doses of methacholine (A). Mucus index in GRRK (30 mg/kg) preventive and curative treatments were determined by PAS staining as indicated in M&M (B). Significant difference (* $P < 0.05$) when compared to control group. Significant difference (** $P < 0.05$; *** $P < 0.001$) when compared to AAD group. Representative lung sections from control group (C), AAD group (D), AAD/DEXA (E), AAD/GRRK P (F), and AAD/GRRK (G). PAS staining as indicated in M&M (magnification $\times 400$).

GRRK (30 or 100 mg/kg) treatment significantly suppressed AHR ($P < 0.05$ and $P < 0.001$, respectively) (Fig. 7A). The GRRK curative treatment also suppressed the AHR (data not shown). Therefore, these findings show that GRRK treatment suppresses methacholine-induced AHR. In addition, the ability of GRRK treatment to change the lung inflammatory profile and mucus production in the AAD group was also evaluated. As shown in Fig. 7C bronchial and alveolar architecture were normal in the control group, while in the AAD group of mice, marked histopathology abnormalities were found in the lung, as characterized by bronchostenosis, epithelial hyperplasia, airway wall edema, inflammatory cells in the bronchial and vascular regions and the production of mucus plugging (Fig. 7D). However, in the groups of animals treated with GRRK, either preventively (Fig. 7F) or curatively (Fig. 7G), and in the DEXA group (Fig. 7E), the lung inflammation was attenuated (Fig. 7E) with fewer mucus-producing cells (Fig. 7B). Taken together, these results showed for the first time that GRRK treatment, either curative or preventive, inhibits the development of Th2-mediated lung pathology.

4. Discussion

Kaempferol is a flavonoid that has been shown to exert anti-inflammatory effect in experimental models such as graft-versus-host disease, atherosclerosis, paw edema and inflammatory disease [24–26]. Analysis of the structure–activity relationship showed that hydroxylation and the presence of a glycoside in the chemical structure of the flavonoid appear to be prerequisites for the anti-inflammatory effect. Based on these studies, we decided to investigate whether kaempferol glycoside (O-[β -D-glucopyranosyl-(1 $\rightarrow$ 6)- α -L-rhamnopyranosyl] 7-O- α -L-rhamnopyranosyl kaempferol) would attenuate ovalbumin-induced murine allergic lung disease.

The therapeutic objectives in asthma are to diminish the regional inflammatory response by reducing the migration of antigen-induced inflammatory cells, inflammatory cytokine production and to improve the impaired lung function. The findings of the present study suggest that preventive or curative GRRK treatment successfully attenuated the development of Th2-driven allergic airway disease by decreasing

airway eosinophilic inflammation, CD4 and B220 lymphocytes, CD40, MHCII (CD11b⁺/Cd11c⁺) expression and the production of Th2-type cytokine in BAL. In addition, this flavonoid decreased mucus hypersecretion and improved the AHR in a dose-dependent manner. These findings suggest that systemic GRRK treatment successfully reduced the two key features of asthma (inflammation and AHR), thereby confirming its potential as a therapeutic molecule.

The anti-inflammatory effect of glucocorticoids is thought to play a crucial role in their efficacy for the successful treatment of allergic diseases, although this effect is tempered by side effects. It would, therefore, be desirable to identify a compound that would act as a steroid but with fewer side effects [13]. Many flavonoids have been found to exert potent antiallergic and anti-inflammatory effects [11,16,18]. Previous studies have shown that flavonoids alleviate bronchoconstriction and airway hyperreactivity in ovalbumin-sensitized mice [15,27,28]. Furthermore, flavonoids suppress histamine release, Th2-type cytokine profile production and CD40 ligand expression in basophils and mast cells [23]. In fact, the higher the intake of flavonoids, the lower the incidence of asthma in humans [7]. In the present study, the results show that GRRK treatment (preventive or curative) in ovalbumin-sensitized and challenged mice successfully reduced the total leukocyte and eosinophil counts in the BAL and in the pulmonary parenchyma. These treatments were as effective as dexamethasone treatment when compared with AAD mice.

Since IL-5 has been shown to be one of the major cytokines responsible for the maturation, growth and infiltration of eosinophils into the lungs [29] in the present study IL-5 production was analyzed in order to study the effect of GRRK on eosinophil counts. Some studies showed that mice lacking IL-5 (IL-5 knockout mice) failed to develop airway eosinophilia in response to allergen exposure unless IL-5 was reconstituted in the animals [30,31]. Furthermore, treatment of wild-type mice with a neutralizing anti-IL-5 antibody strongly reduces the eosinophilic inflammatory response induced by allergens [32]. These results suggest that the inhibitory effects of GRRK on eosinophil recruitment may be mediated by IL-5 regulation rather than by a direct effect on eosinophils.

As IL-13 is the other key factor in the pathogenesis of asthma, it is reasoned that pursuing IL-13 or inhibiting its biological activity may prove to be effective in the treatment of asthma. In the present study, IL-13 production was diminished by GRRK treatment. IL-13 is involved in mucus hypersecretion and subepithelial fibrosis [33] and overexpression of IL-13 has been reported in asthma patients [34]. Similar studies reported that intratracheal IL-13 delivery successfully induces lung eosinophilia and airway hyperresponsiveness in mice [35–39]. Many flavonoids have been shown to alleviate bronchoconstriction and AHR in OVA-sensitized mice [15]. Although the mechanisms responsible for the development of airway hyperresponsiveness appear to be quite controversial, the usefulness of GRRK treatment has been confirmed in this study.

Antigen-activated CD4 T cells demonstrate to induce many of the features of asthma [40]. Here we demonstrated that GRRK treatment decreased CD4 T cell. These cells in the airways of asthmatic patients were increased following administration of allergen peptides that trigger a late asthmatic response [41]. In addition, mice lacking CD4⁺ T cells due to an inability to express major histocompatibility complex class II (MHCII) antigens fail to develop eosinophil-dominated inflammation or AHR following exposure to the antigen [42].

Beside the CD4⁺ T cells the costimulatory receptors play an important role in inflammation disease such as asthma. The CD40 costimulatory molecule is expressed on APCs such as dendritic cells, B cells, bronchial epithelial cells and airway macrophages. These CD40-expressing cells, together with CD40L expressed in leukocytes, such as activated T cells, modulate asthmatic airway inflammation via cytokine production by prolonging survival of inflammatory cells [43]. Previous studies have demonstrated that anti-CD40 and anti-CD80 antibodies decreased airway inflammation and hyperreactivity

in experimental asthma model [2,44]. In addition flavonoids such as luteolin, apigenin and fisetin demonstrated strong inhibition of CD40L expression mediated through the activation of nuclear factors of activated T cells and AP-1 [45]. The results presented in this study demonstrated for the first time that preventive or curative GRRK treatment reduced several key cell markers related with allergic asthma such as CD4⁺ and B220⁺, expressions of MHCII and CD40 costimulatory molecules on dendritic cells, suggesting the GRRK down-regulates APC activation to decrease the characteristic phenotype of asthma.

Many plant-derived secondary metabolites are capable of directly affecting inflammation by inducing the production of second messengers that dampen proinflammatory molecule production [46]. One study reported that kaempferol inhibited IL-4-induced transcription factor STAT6 activation by specifically targeting Janus kinase 3 (JAK3) in murine splenocytes, HL-60, U937, M12 and A1.1 cells [47]. Comparative analysis indicated that kaempferol is similar in size and planar structure to JAK 3 inhibitors [48,49]. Therefore, it is possible that the inhibitory effect of GRRK in AAD is indeed related to the inhibition of these transcription factors. However, the mechanisms through which GRRK treatment reduced asthmatic symptoms in the OVA-induced murine allergic lung disease remains elusive but do not appear to be related to the shift to the Th1 pathway.

In summary, glycoside kaempferol treatment (either preventive or curative) exerts a profound inhibitory effect on airway inflammation and hyperresponsiveness in a murine model of asthma by suppressing the Th2 cytokine profile suggesting a potential molecule candidate for novel antiasthmatic therapies.

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