



Ginger prevents Th2-mediated immune responses in a mouse model of airway inflammation

Marie Louise Berthe Ahui^{a,d}, Pierre Champy^{b,*}, Abdulraouf Ramadan^a, Linh Pham Van^a, Luiza Araujo^a, Konan Brou André^d, Séverine Diem^a, Diane Damotte^c, Séraphin Kati-Coulibaly^d, Michel Atté Offoumou^d, Michel Dy^a, Nathalie Thieblemont^a, André Herbelin^{a,*}

^a CNRS UMR 8147, Université Paris Descartes, Faculté de Médecine, Hôpital Necker, 161 rue de Sèvres; 75783 Paris Cedex 15, France

^b Laboratoire de pharmacognosie, CNRS UMR 8076, Faculté de Pharmacie, Université Paris-Sud 11, Châtenay-Malabry, France

^c Hôpital Européen Georges Pompidou, Service d'Anatomie et Cytologie Pathologiques, Paris, France

^d Laboratoire de Nutrition et de Pharmacologie, UFR Biosciences Université de Cocody-Abidjan, Côte d'Ivoire

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ABSTRACT

It is well documented that compounds from rhizomes of *Zingiber officinale*, commonly called ginger, have anti-inflammatory properties. Here, we show that ginger can exert such functions *in vivo*, namely in a mouse model of Th2-mediated pulmonary inflammation. The preparation of ginger aqueous extract (Zo.Aq) was characterized by mass spectrometry as an enriched fraction of *n*-gingerols. Intraperitoneal injections of this extract before airway challenge of ovalbumin (OVA)-sensitized mice resulted in a marked decrease in the recruitment of eosinophils to the lungs as attested by cell counts in bronchoalveolar lavage (BAL) fluids and histological examination. Resolution of airway inflammation induced by Zo.Aq was accompanied by a suppression of the Th2 cell-driven response to allergen *in vivo*. Thus, IL-4, IL-5 and eotaxin levels in the lungs as well as specific IgE titres in serum were clearly diminished in ginger-treated mice relative to their controls after allergen sensitization and challenge. Finally, we found that [6]-gingerol, a major constituent of ginger, was sufficient to suppress eosinophilia in our model of inflammation. This is the first evidence that ginger can suppress Th2-mediated immune responses and might thus provide a possible therapeutic application in allergic asthma.

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1. Introduction

Ginger (or Zo for *Zingiber officinale* Roscoe, Zingiberaceae) has a long history of medical use [1,2]. As a dietary supplement or herbal medicinal product, it is widely used to treat pregnancy-related nausea [3–5] as well as others forms of nausea such as motion sickness [6]. Ginger has also been valued for centuries for its anti-inflammatory properties. Thus, in traditional medicine, ginger has been used to treat a wide range of ailments including stomachaches, diarrhoea, toothache, gingivitis arthritis and asthmatic respiratory disorders [7]. During the past 35 years, many laboratories have renewed their interest in ginger as a treatment of chronic inflammatory conditions. Ginger was found to inhibit arachidonic acid metabolism *via* the cyclooxygenase and lipooxygenase pathways [8,9]. More recently, research in this field entered a new phase with the discovery that ginger inhibited the

induction of genes encoding cytokines and chemokines that are synthesized and secreted at sites of inflammation [10–12].

Asthma is characterized by a chronic inflammatory reaction in the airways. Numerous humoral and cellular immunological abnormalities have been reported in this disease [13–16]. It is well known that T-helper (Th)-lymphocytes play a key role in the regulation of immune and inflammatory reactions through the release of cytokines [17–19]. Th-lymphocytes can be divided into at least two subsets based on their cytokine secretion profiles. Th1-lymphocytes are characterized by the production of predominantly interleukin (IL)-2, interferon (IFN)- γ and tumour-necrosis factor (TNF)- α whereas Th2-lymphocytes are characterized by the production of predominantly IL-4, IL-5 and IL-6 that favour the synthesis of IgE. It is now well recognized that asthma is caused by Th2-driven inflammatory responses, which enhances airway eosinophilia and mucus production in the lungs [20].

Ginger is well known for its ability to inhibit the synthesis of several pro-inflammatory cytokines including IL-1, TNF- α and IL-8 by variable types of cells *in vitro* [21], and to affect Th1-derived responses *in vivo* [22]. However, whether ginger can also affect Th2-derived inflammatory responses *in vivo* has not been evaluated so far. Here, we provide the first evidence that ginger can suppress Th2-mediated immune responses in a mouse model of pulmonary inflammation.

* Corresponding authors. P. Champy is to be contacted at Laboratoire de pharmacognosie, CNRS UMR 8076, Faculté de Pharmacie, Université Paris-Sud 11, Châtenay-Malabry, France. Tel.: +33 1 46 83 56 39; fax: +33 1 46 83 56 39. Herbelin, CNRS UMR 8147, Hôpital Necker, 161 rue de Sèvres, 75783 Paris Cedex 15, France. Tel.: +33 1 44 49 53 88; fax: +33 1 44 49 06 76.

E-mail addresses: pierre.champy@u-psud.fr (P. Champy), Herbelin@necker.fr (A. Herbelin).

2. Materials and methods

2.1. Sample preparations of ginger

The rhizoma of Zo were collected from the market of Abidjan (Ivory Coast) and dried at 70 °C. The plant was authenticated at the National herbarium in Abidjan (CNF) where a voucher specimen was deposited. It was ground into a fine powder using a pestle and mortar and the powder was soaked in distilled water for 24 h at room temperature. The resulting solution was filtered and evaporated to remove excess water and the concentrated solution was freeze dried. From a 50 g sample of dried rhizoma, 5 g of solid material was obtained with a yield of 10%. Extract solutions were stored at 4 °C until use. The solid material was subsequently reconstituted in a known volume of distilled water and then serially diluted. In order to prepare the methanolic (MeOH) total extract, the powder of Zo (30 g) was refluxed in methanol (600 ml) in a Soxhlet apparatus for 2 days. MeOH was evaporated under reduced pressure, to give a brown extract (yield: 11%). The material was subsequently reconstituted in a known volume of sunflower oil. [6]-gingerol was purchased from VWR international company (Fontenay-sous-bois, France) and was reconstituted in distilled PBS 1X containing MeOH 1%. The preparation was sonicated just before use.

2.2. HPLC/ESI-MS/MS analysis of ginger extracts

High performance liquid chromatography–tandem mass spectrometry (HPLC/MS/MS) analysis of aqueous (Zo.Aq) and MeOH total (Zo.Me) extracts was conducted at 30 °C with a C18 Sunfire column (WATERS, 5 µm, 4.6×150 mm) equipped with a C18 Sunfire pre-column (5 µm), on a HPLC Agilent system 1100 series coupled to an electrospray (ESI)–quadrupole–time-of-flight (TOF) spectrometer (PE SCIEX QSTAR PULSAR, PE Sciex Instruments). A gradient of H₂O (A) and CH₃CN (B) was used (1→40 min: 20% B→100% B, 40→55 min: 100% B), at a flow rate of 1 ml/min. The acquisition parameters for positive and negative modes were respectively: GS1 (nebulizing gas): 50 psi, GS2 (drying gas): 55 psi, curtain gas: 35 psi, ionspray voltage: 5500 V or –4500 V, DP (declustering potential): 30 V or –30 V, FP (focalising potential): 180 V or –180 V, DP2: 20 V or –20 V, CAD (collision gas pressure): 2 mTorr (N₂). For MS/MS experiments, collision energy was set at 35 eV or –35 eV. Mass range measured was *m/z* 100–800. These were the optimized parameters for the maximum transmission of the gingerol-derived ions. The identity of [6]-gingerol was assessed by co-injection with a commercial standard.

2.3. Quantification of gingerols by HPLC-DAD

To determine the amounts of gingerols injected to animals, –diode array detection (HPLC-DAD) studies of Zo.Aq and MeOH total (Zo.Me) extracts were conducted. For quantifications, a commercial [6]-gingerol standard and the extracts were diluted extemporaneously in MeOH. UV quantification was conducted using a WATERS (St Quentin en Yvelines, France) chromatographic system (717 autosampler, 600 pump, photodiode array detector 996 equipped with an X-ACT in-line degazer, Jour Research) under the same chromatographic conditions as above. Full spectral scanning was performed from 210–400 nm, and DAD was set at 280 nm and 230 nm for analysis. Study was conducted at 280 nm, the limit of quantification being more satisfactory than at 230 nm (10^{-9} g versus $2.5 \cdot 10^{-8}$ g injected). The straight-line regression equation was obtained with 11 dilutions ranging from 0 to 0.075 mg/ml (injections 10 µl) by plotting peak area against concentration, with excellent correlation ($R^2 > 0.9997$). Precision was tested for three concentrations of the standard corresponding to the low, medium and high ranges of the calibration curve, and was comprised between 0.4 and 2.5% (relative standard deviation).

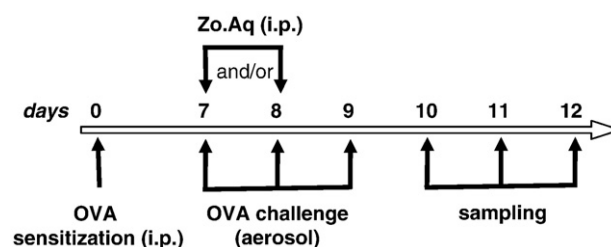


Fig. 1. Schematic diagram illustrating the protocol for OVA sensitization, challenges, and ginger treatment. NOD and C57BL/6 mice were sensitized with OVA and alum (day 0) and challenged by aerosol exposure on days 7, 8 and 9 with OVA or saline. Unless stated, mice were treated on days 7 and 8 1 h before the challenge via i.p. administration of ginger (Zo.Aq) at a dose of 360 mg/kg or PBS, and 1 day after the last challenge, the absolute number of eosinophils in the bronchoalveolar lavage fluid (BALF) was measured. In the same mice, cytokine protein levels were determined in lungs, sera and/or BALF. In a separate set of experiments, lungs were collected for tissue histopathology analysis.

2.4. Animals

NOD mice were bred and maintained in our animal facility under specific pathogen-free conditions whereas C57BL/6 mice were obtained from the Laboratory Animal Center Janvier (CERJ; le Genest-Isle, France). For experiments, 6–8 week old males weighing 20–25 g were used. Housing conditions and all *in vivo* experiments were performed according to the institutional ethical committee of France and the guidelines established by the European Union on Animal Care (CEE Council 86/609).

2.5. Sensitization, airway challenge, and Zo treatment

Mice were sensitized by intraperitoneal injection (i.p.) of 100 µg of ovalbumin (OVA, INC Biomedicals, Inc, OH) emulsified in 1.6 mg of alum hydroxide (Merck, Darmstadt, Germany) in a total volume of 400 µl on day 1. Sensitized and naïve (NaCl) mice received aerosolized allergen challenge (50 mg OVA for 20 min) on 3 consecutive days (days 7, 8, and 9) using an ultrasonic nebulizer (Ultra-Neb99, Devilbiss). An hour before the first and/or the second challenge (days 7 and 8), OVA-sensitized mice were injected i.p. with Zo at variable doses comprised between 45 to 720 mg/kg b.w. (Fig. 1).

2.6. Bronchoalveolar lavage (BAL) and lung homogenates

Animals were anesthetized by the intraperitoneal injection of urethane (ethylcarbamate, 2 g/kg b.m., Sigma Aldrich, Stenheim, Germany). After intubation, lungs were lavaged, and BAL fluid was recovered (0.5 ml of PBS; 5 times) through the trachea. Total leukocyte numbers were measured with a hemocytometer using Turk dye exclusion method. Differential cell counts were performed under light microscopy in a blinded manner, counting at least 200 cells according to the standard morphologic criteria on cytocentrifuged preparations (Shandon Cytospin 4, Thermo Electron Corporation) with a fast staining procedure (HAEME-Schnellfärbung, Labor+Technik Eberhard Lehmann, Berlin, Germany). Homogenates were prepared after cannulating the trachea and perfusing the airway. The lungs were then removed and vigorously vortexed. The resulting cell suspension was homogenized and centrifuged for 10 min at 1600 g at 4 °C. Supernatants were removed and stored at –20 °C until cytokine measurement.

2.7. Determination of cytokines and OVA-specific IgE titres by ELISA

The levels of eotaxin, IL-4, IL-5 and IFN-γ in BALF and lung homogenate were assessed by sandwich ELISA (R&D Systems Europe) according to the manufacturer's instructions. The levels of OVA-specific IgE were determined by sandwich ELISA, as previously described [8,9]. Levels of samples were related to an internal standard from pooled sera of hyperimmunized NOD mice. Data were expressed as IgE index

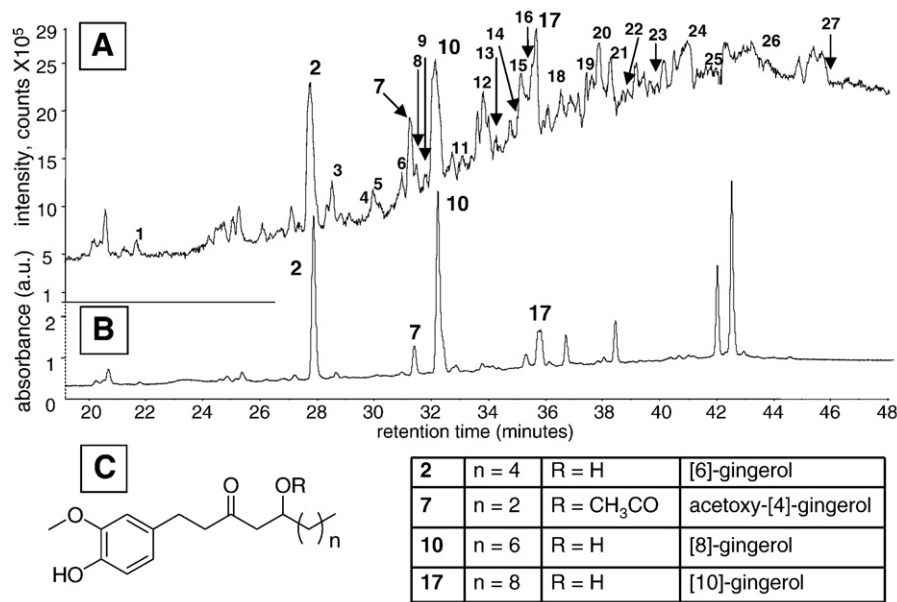


Fig. 2. LC/MS analysis of ginger rhizome extract Zo.Me. (A) Total ion current (TIC) chromatogram from positive ion ESI-HPLC-MS; (B) HPLC-DAD chromatogram set at 230 nm of the crude ginger rhizome extract. (C) Chemical structures of major gingerol-related compounds identified in extracts from ginger rhizome.

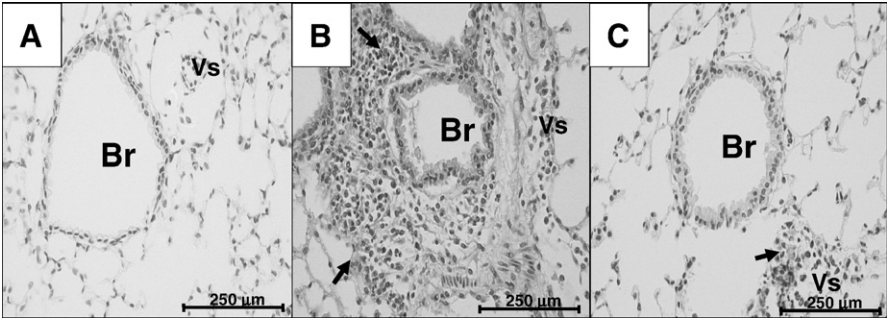


Fig. 3. An aqueous extract of ginger inhibits OVA-induced airway cell infiltration and mucus production in NOD mice. NOD mice were sensitized with OVA and challenged with OVA (B,C) or saline (A) as described in Fig. 1, and received (C) or not an aqueous extract of ginger (Zo.Aq; 360 mg/kg) 1 h before the first and second challenges. One day after the last challenge, lungs were collected, fixed and stained with hematoxylin and eosin (A–C; magnification ×400). Representative sections are shown for each treatment group. Dense perivascular and peribronchiolar infiltrates mainly composed of eosinophils are present in lung of OVA-challenged mice while they are dramatically decreased in lung of OVA-challenged mice treated with Zo.Aq and absent of their saline-challenged controls. Mucin is increased in the bronchiolar lining cells in OVA-challenged mice while it is absent from the epithelium of mice challenged with saline and present at low levels in mice treated with Zo.Aq. Arrows indicate locations of cell infiltration (B,C). Br: bronchioles; Vs: vessels.

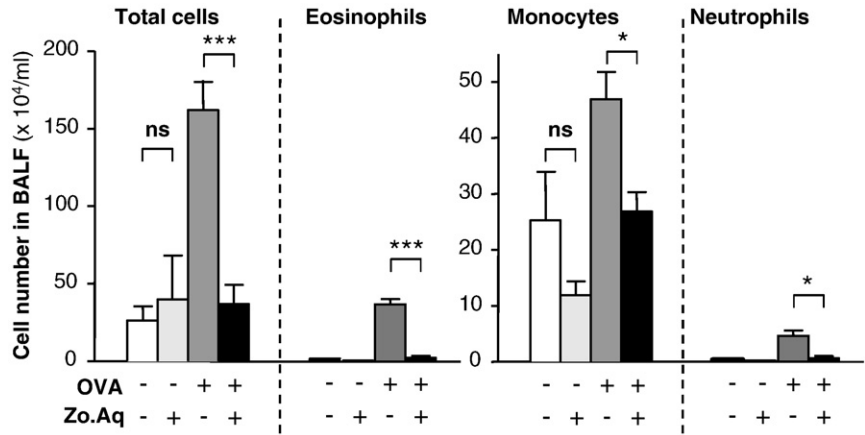


Fig. 4. Zo.Aq inhibits OVA-induced airway eosinophilia in NOD mice. NOD mice were sensitized with OVA and challenged with OVA or saline as described in Fig. 1, and received or not Zo.Aq 1 h before the first and second challenges. One day after the last challenge, the absolute number of total cells, eosinophils, monocytes and neutrophils in the BALF was measured. Data shown are mean ± S.E.M. of 3 to 12 mice per group. **p* < 0.05; ****p* < 0.001.

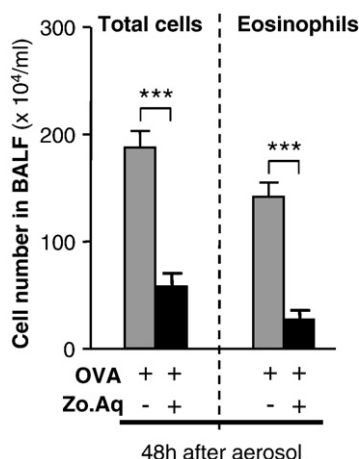


Fig. 5. The inhibitory effect of Zo.Aq on airway inflammation is maintained two days after the last challenge. NOD mice were sensitized with OVA and challenged with OVA or saline as described in Fig. 1, and received or not Zo.Aq (360 mg/kg) 1 h before the first and second challenges. One day or two days after the last challenge, the absolute number of total cells and eosinophils in BALF was measured. Data shown are mean $\pm$ S.E.M. of 3 to 12 mice per group. *** p < 0.001.

calculated as follows: IgE index = [OD sample - OD buffer only] / [OD positive control - OD buffer only].

2.8. Determination of lung histology

Lungs were perfused to eliminate residual blood, removed and fixed in paraformaldehyde. Paraffin-embedded tissues were sliced and 5 μ m thick paraffin sections were stained with hematoxylin and eosin (H&E) or with periodic acid Schiff (PAS) for determination of cell infiltration and mucus-producing cells. Histological examination was evaluated by light microscopy in a blinded fashion by a histopathologist (D.D.).

2.9. Statistical analysis

Statistical analysis was performed by using the Graph Pad Prism Software (version 3.03) to test whether treatment with ginger extracts leads to a significant reduction in several parameters associated with

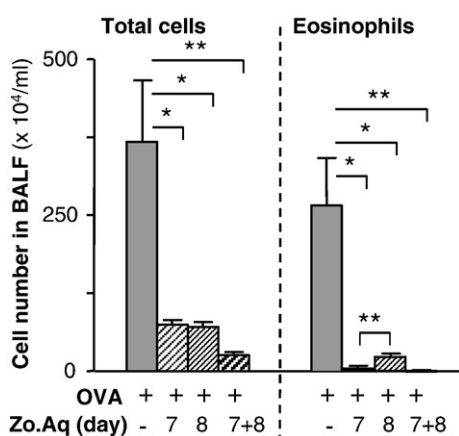


Fig. 6. Single dose of Zo.Aq is sufficient to inhibit airway inflammation in NOD mice. NOD mice were sensitized with OVA and challenged with OVA or saline as described in Fig. 1, and received Zo.Aq (360 mg/kg) on day 7, day 8, or on days 7+8 1 h before the challenge. One day after the last challenge, the absolute number of total cells and eosinophils in BALF was measured. Data shown are mean $\pm$ S.E.M. of 3 to 5 mice per group. * p < 0.05; ** p < 0.01.

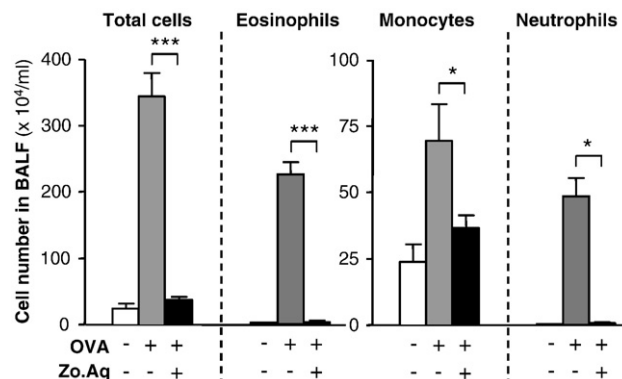


Fig. 7. Inhibition of OVA-induced airway inflammation by Zo.Aq applies to C57BL/6 mice. C57BL/6 mice were sensitized with OVA and challenged with OVA or saline as described in Fig. 1, and received or not Zo.Aq (360 mg/kg) 1 h before the first and the second challenges. One day after the last challenge, the absolute number of total cells, eosinophils, monocytes and neutrophils in the BALF was measured. Data shown are mean $\pm$ S.E.M. of 3 to 6 mice per group. * p < 0.05; *** p < 0.001.

allergic disease. Results are expressed as means $\pm$ standard error of the mean (SEM). Student's t -test was used for analysis of significance among the groups. Values of p < 0.05 were considered statistically significant.

3. Results

3.1. Characterization of the different constituents in the ginger extracts

Both Zo.Aq and Zo.Me total extracts were analysed by HPLC/DAD (230, 280 nm) and HPLC ESI-MS in positive and negative modes. The study of retention times, UV spectra, molecular ions and fragments [23,24] led to the identification of 27 gingerol-related compounds, such as gingerols, shogaols and paradols, as well as their acetylated and methylated derivatives. The major ones were [6]-gingerol (peak 2), acetoxy-[4]-gingerol (7), [8]-gingerol (10), and [10]-gingerol (17) (Fig. 2). [8]-gingerol and [6]-gingerol appeared to be the major constituents in the Zo.Aq extract (19.2% and 5.8%, w/w, respectively, as determined by HPLC-DAD quantitation). Similar values were found for the Zo.Me total extract. Their concentration in the rhizoma used for this study was thus calculated as being 30.3 and 9.2 mg/kg, respectively.

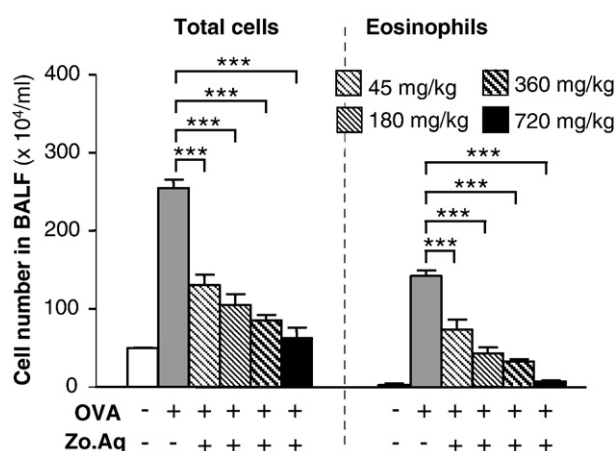


Fig. 8. Inhibition of OVA-induced airway inflammation by Zo.Aq is dose-dependent. NOD mice were sensitized with OVA and challenged with OVA or saline as described in Fig. 1, and received or not variable doses (45, 180, 360 and 720 mg/kg) of Zo.Aq on days 7 and 8 1 h before the challenge. One day after the last challenge, the absolute number of total cells and eosinophils in the BALF was measured. Data shown are mean $\pm$ S.E.M. of 3 to 10 mice per group. *** p < 0.001.

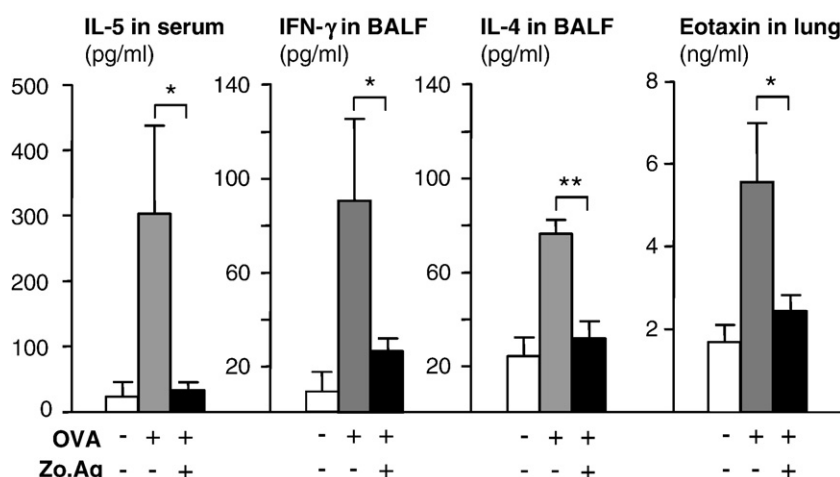


Fig. 9. Zo.Aq inhibits OVA-induced cytokine production in NOD mice. NOD mice were sensitized with OVA and challenged with OVA or saline as described in Fig. 1, and received or not Zo.Aq (360 mg/kg) 1 h before the first and second challenges. One day after the last challenge, BALF and lungs were collected. IL-4, IFN- γ , IL-5, and eotaxin protein levels were measured in BALF, serum and lung tissue homogenate supernatants by ELISA. Data shown are mean $\pm$ S.E.M. of 3 to 12 mice per group. * $p < 0.05$; ** $p < 0.01$.

3.2. Inhibition of airway inflammation in OVA-immunized and -challenged mice treated with aqueous ginger extract

Experimental OVA-dependent allergic asthma is characterized by airway eosinophilia and mucus production [25]. We have previously reported that the NOD mouse is a relevant model of experimental allergic asthma [26]. Thus, after sensitization and aerosol challenges with OVA, NOD mice develop eosinophilic airway inflammation (Fig. 3B versus Fig. 3A; Fig. 4) and intense mucus production (data not shown) in the lungs. These two typical features of Th2-mediated pulmonary inflammation were clearly reduced in NOD mice treated with Zo.Aq used at the dose of 360 mg/kg. Indeed, airway eosinophilia (Fig. 3C) and mucosal cells (data not shown) in the bronchiolar lining cells were virtually absent in OVA-immunized and -challenged animals receiving Zo.Aq 1 h before the first and the second challenges, relative to their OVA-immunized and -challenged controls (Fig. 3A). The effect of Zo.Aq on the recruitment of inflammatory cells into BALF in OVA-challenged mice is shown in Fig. 4. In OVA-immunized and -challenged mice used as positive controls, total cell numbers 1 day after the last challenge were significantly increased. The frequency of inflammatory cells in decreasing order was: eosinophils, monocytes and neutrophils. The administration of Zo.Aq dramatically reduced the number of cells recruited, including eosinophils, monocytes and neutrophils. In a single series of follow-up experiments we have extended observations up to 2 days after the last challenge, again demonstrating a comparable suppressive effect of Zo.Aq on eosinophilic recruitment in BALF (Fig. 5) and in the lungs (data not shown). We next addressed the question whether the inhibitory effect required repeated Zo.Aq exposures. To this end, we compared groups of OVA-sensitized animals that were exposed to single injection of Zo.Aq (1 h before the first or the second challenge) versus two successive injections of Zo.Aq (1 h before the first and the second challenge). Fig. 6 shows that a single Zo.Aq injection was sufficient to reduce markedly the number of eosinophils in BALF, although the two successive injections of the product resulted in a more complete suppressive effect. These data indicate that the inhibitory effect of Zo.Aq is robust and persistent.

Another important point was to examine whether the inhibitory effect of Zo.Aq was dependent on the NOD background or conversely if it applies to other strains susceptible to experimental allergic asthma. To address this issue, we used C57BL/6 mice, a strain that also develop mucus production and eosinophilic inflammation in the lungs after OVA challenge [27,28]. Fig. 7 shows that both total cell and eosinophil counts were dramatically decreased in the BALF of C57BL/6 after OVA challenge and treatment with Zo.Aq (360 mg/kg), relative to controls challenged with OVA.

We next examined whether the inhibition of airway inflammation obtained with Zo.Aq was dose-dependent. As shown in Fig. 8, treatment with increased doses of Zo.Aq comprised between 45 and 720 mg/kg showed that the efficiency of the product in terms of reduction of the number of eosinophils in BALF was closely dependent on the dose used. Statistical analysis indicates that the inhibition effect of Zo.Aq at the doses of 45 mg/kg, 180 mg/kg, 360 mg/kg, and 720 mg/kg are significantly different from control groups. Zo.Aq exhibited anti-inflammatory activity with a half-response value around 45 mg/kg. Altogether, these findings clearly show that treatment with Zo.Aq inhibits both eosinophilia and airway inflammation in OVA-immunized and -challenged mice.

3.3. Production of both Th1 and Th2 cytokines and eotaxin after OVA challenge is affected in Zo.Aq-treated NOD mice

Allergic airway inflammation is accompanied by the generation of IL-4, IL-5 and IL-13 as well as chemokines, such as eotaxin [29]. As shown in Fig. 9, levels of IL-4, eotaxin and IL-5 in BALF, lungs and sera, respectively, were significantly reduced in OVA-immunized and -challenged NOD mice treated with Zo.Aq (360 mg/kg). However, no significant difference was noted for IL-13 in lungs (data not shown). It should be noticed that the pro-Th1 cytokine IFN- γ that we were able to

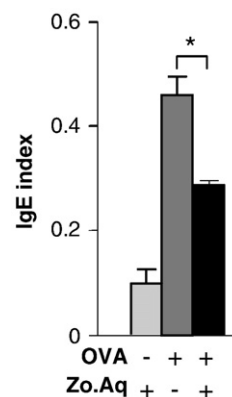


Fig. 10. Zo.Aq inhibits OVA-induced IgE production in NOD mice. NOD mice were sensitized with OVA and challenged with OVA or saline as described in Fig. 1, and received or not Zo.Aq (360 mg/kg) 1 h before the first and second challenges. Three day after the last challenge, blood was collected and anti-OVA-specific IgE were measured in serum by ELISA. Data shown are mean $\pm$ S.E.M. of 3 to 12 mice per group. * $p < 0.05$.

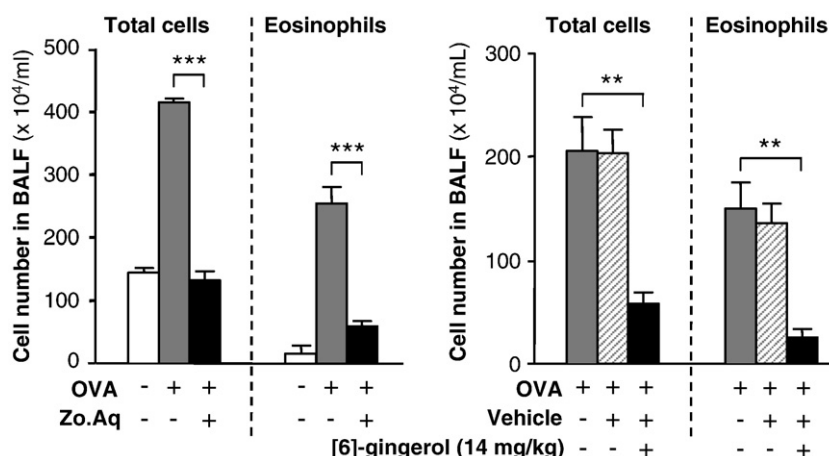


Fig. 11. [6]-gingerol inhibits OVA-induced airway inflammation. NOD mice were sensitized and challenged with OVA or saline as described in Fig. 1, and received or not the aqueous extract (Zo.Aq; 360 mg/kg) or the [6]-gingerol solution (14 mg/kg) only 1 h before the first challenge. One or two days after the last challenge, the absolute number of total cells and eosinophils in the BALF of Zo.Aq- or [6]-gingerol-treated mice was measured. Data shown are mean $\pm$ S.E.M. of 3 to 6 mice per group. ** $p < 0.01$; *** $p < 0.001$.

detect after OVA challenge was also significantly diminished in BALF of Zo.Aq-treated mice.

3.4. Production of OVA-specific IgE is affected in Zo.Aq-treated NOD mice

IL-4 is required for Th2 cell differentiation and isotype switching in B cells from IgM to IgE [30,31]. Sensitization and challenge with OVA caused significant increase in OVA-specific IgE in the serum 72 h after the last challenge, which were significantly reduced in mice treated with Zo.Aq (360 mg/kg) (Fig. 10).

3.5. [6]-Gingerol inhibits OVA-induced airway inflammation

[6]-Gingerol is a constituent of ginger which is well documented for its anti-inflammatory activities [32]. For this reason, we decided to examine whether [6]-gingerol shares with the Zo.Aq extract the capacity to suppress airway inflammation in our model. This was the case as attested by the capacity of [6]-gingerol at the dose of 14 mg/kg to suppress eosinophilia in BALF when administered 1–3 h before the first and the second challenges (Fig. 11). These findings suggest that the anti-inflammatory effect of ginger in our model might be mediated by [6]-gingerol.

4. Discussion

Ginger, the powdered rhizome of the herb *Zingiber officinale*, is widely used in traditional medicine as a treatment for several diseases, including chronic inflammatory diseases [1]. Although the anti-inflammatory properties of ginger are well documented, only a few studies have evaluated the effects of this natural product on the immune responses *in vivo* [22]. The objective of this study was to evaluate the effects of ginger on airway inflammation *in vivo* in a mouse model of allergen-induced allergic asthma.

Following sensitization, inhalation of OVA induces allergic inflammatory changes characterized by infiltration of inflammatory cells, mainly eosinophils, into regions around the airways and pulmonary blood vessels and by increasing in number of mucus-producing cells within the airways. The main finding in the present study was that these OVA-induced inflammatory changes in lung tissues were suppressed by ginger extracts.

Allergic reactions in the airways are well known to be mediated via two pathways. The first pathway involves induction of IgE by B cells via IL-4 released from Th2 cells and mast cells [33–37]. In the present study, ginger treatment led to a decreased production of IL-4 in lung tissues of OVA-sensitized and -challenged mice, a phenomenon which

was accompanied by a significant reduction of IgE antibody titres in the serum. Thus, the inhibitory effects of ginger on airway inflammation may be due in part to the decrease in IL-4 levels. The other pathway involves recruitment of eosinophils via release of IL-5 and eotaxin from Th2 cells or mast cells. Our results showed that the decrease in the number of eosinophils in BAL fluid and lung tissue, and the number of mucus-producing goblet cells in the airway mucosa were concomitant with the decrease in IL-5 and eotaxin. Therefore, from all these data, it can be assumed that the inhibitory effects of ginger extracts on airway inflammation are due to the decrease in Th2 cytokines and eotaxin.

An important point is to know whether the inhibitory effects of ginger apply to other cell types and molecules than those implicated in Th2-mediated inflammation. Although the predominant inflammatory cells recruited into asthmatic lung tissues are eosinophils, neutrophils and macrophages have been also found to be elevated in the BALF and lung tissues of OVA-sensitized and -challenged mice ([38], our data). Here, we showed that ginger inhibits the recruitment of neutrophils and macrophages too. Furthermore, we found reduced levels of the pro-Th1 cytokine IFN- γ in BALF of the sensitized- and challenged-mice treated with ginger. This latter finding is in agreement with previous studies showing that ginger extracts can inhibit the synthesis of several pro-Th1 cytokines [39,40].

In our study, 27 gingerol-related compounds were identified in the aqueous extract, [6] and [8]-gingerol being the principal compounds, as described in the literature [23,24]. It should be mentioned that most of the activities of ginger have been attributed to [6]-gingerol. Indeed, [6]-gingerol has been shown to have antioxidant activities, to promote angiogenesis, and to suppress the production of pro-inflammatory cytokines (TNF- α , IL-1 and IL-12) from macrophages [40]. From our study, two lines of evidence led us to assume that gingerol components are responsible for the inhibition of airway inflammation obtained with our ginger aqueous extracts. Firstly, the Zo.Me total extract which retains the gingerol constituents, shares with the Zo.Aq extract the capacity to suppress airway inflammation in our model (data not shown). Secondly, we found that a purified fraction of [6]-gingerol used alone has the same anti-inflammatory activity than the total extract. Although we cannot exclude that some of the effects of ginger extracts observed here are caused by synergistic action of several chemically homogeneous molecules, our findings suggest that the anti-inflammatory effect of ginger in our model is at least in part mediated by [6]-gingerol.

Ginger's rhizoma is used in several pharmacopoeias (African, Chinese, European and Indian) for his anti-inflammatory activities [41]. It is therefore expected that ginger extracts are particularly safe. Accordingly, neither side effects nor toxicological changes were observed in our hands (data not shown) as well as in several other studies [42].

In our study, ginger was in most cases administered before the induction of airway inflammation with allergen challenge and was effective in preventing its development. However, it should be mentioned that its administration only after the induction of the challenge phase remained efficient to inhibit eosinophilia (see Fig. 6). These results are important and useful for a future exploitation of ginger to develop natural substances not only to prevent allergic asthma but also to alleviate the established disease.

To summarize, our study provides the first evidence that ginger inhibits allergen-induced lung inflammation by reducing airway eosinophilia, Th2 cytokines, and allergen-specific antibodies, thus supporting its anti-inflammatory role during the allergic response in the lung. Ginger is therefore a promising new therapeutic approach for the preventive treatment of allergy.

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