

Antigen-induced airway hyperresponsiveness in absence of broncho-obstruction in sensitized guinea pigs

Patricia Ramos-Ramírez,^{1,2} María G. Campos,³ Erasmo Martínez-Cordero,⁴
Blanca Bazán-Perkins,^{1,*} and Eduardo García-Zepeda^{2,*}

¹*Departamento de Hiperreactividad Bronquial, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Calzada de Tlalpan, México DF, México*

²*Departamento de Inmunología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Insurgentes Sur, México DF, México*

³*Unidad de Investigación Médica en Farmacología, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, México DF, México*

⁴*Departamento de Autoinmunidad, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Calzada de Tlalpan, México DF, México*

ABSTRACT

Background: Airway obstruction after antigen challenge is not always observed in patients with allergic asthma, even if they develop hyperresponsiveness. A similar event is observed in our guinea pig model of allergic asthma. Our aim was to study this phenomenon. **Methods:** Sensitized guinea pigs were challenged with ovalbumin (OVA) 3 times every 10 days. Animals were divided into 2 groups: (1) Guinea pigs exhibiting airway obstruction after antigen challenge (R = responders), and (2) guinea pigs lacking airway obstruction response (NR = nonresponders). After the third antigen challenge, antigen-induced airway hyperresponsiveness (AI-AHR), serum OVA-specific immunoglobulins, bronchoalveolar lavage fluid (BALF) inflammatory cells, histamine, cysteinyl leukotrienes and thromboxane A₂ (TxA₂) BALF levels, and in vitro tracheal contraction induced by contractile mediators and OVA were evaluated. **Results:** R group consistently displayed a transient antigen-induced airway obstruction (AI-AO) as well as AI-AHR, high TxA₂, histamine, OVA-IgG1, OVA-IgE and OVA-IgA levels, and intense granulocyte infiltration. NR group displayed no AI-AO and no changes in BALF measurements; nevertheless, AI-AHR and elevated OVA-IgG1 and OVA-IgA levels were observed. In all groups, histamine, TxA₂ and leukotriene D₄ induced a similar contraction. Tracheal OVA-induced contraction was observed only in R group. AI-AHR magnitude showed a direct association with OVA-IgG1 and OVA-IgA levels. The extent of AI-AO correlated directly with OVA-IgE and inversely with OVA-IgA levels. **Conclusions:** Our data suggest that TxA₂ and histamine participate in AI-AO likely through an IgE mechanism. AI-AHR might occur independently of AI-AO, contractile mediators release, and airway inflammatory cell infiltration, but IgA and IgG1 seem to be involved.

KEYWORDS airway hyperresponsiveness, asthma model, airway inflammation, airway obstruction, histamine, IgA, IgE, IgG1, leukotriene D₄, thromboxane A₂

INTRODUCTION

Asthma is a complex, heterogeneous, and variable airway disorder characterized by persistent inflamma-

tion, episodes of airflow obstruction and respiratory symptoms. Asthma exacerbations may include either immediate or late airway obstructive responses, and can be triggered by identifiable allergens in atopic or extrinsic asthma or by nonidentifiable allergens in nonatopic or intrinsic asthma [1, 2]. Nevertheless, allergen exposure in atopic individuals can also attenuate asthmatic responses and even induce tolerance [3]. Different factors have been associated to the great variability of asthmatic responses such as genetics, allergen dose, and degree of exposure or sensitization

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*These authors contributed equally to this work.

Address correspondence to Blanca Bazán-Perkins, Departamento de Hiperreactividad Bronquial, Instituto Nacional de Enfermedades Respiratorias, Calzada de Tlalpan 4502, Colonia Sección XVI, CP 14080, México DF, México. E-mail: perkins@unam.mx

[4]. For instance, in humans, a single high-dose of a potential allergen protects against allergen sensitization, whereas asthma symptom attenuation may be induced by frequent challenges of low antigen doses [5, 6]. In mice, high allergen doses can induce allergic responses, but prolonged allergen exposure eventually generates long-lasting immune tolerance [7]. Guinea pigs are able to show strong airway obstructive responses after antigen challenge; this response makes them an excellent model for studying acute and chronic allergic asthma [7–10]. In addition, many pharmacological studies designed to evaluate the effect of drugs in asthma have been conducted in guinea pigs as they display structural and functional airway features similar to humans [11, 12]. However, guinea pig asthma models show variability and heterogeneity [10, 11]. In our asthma model, most guinea pigs develop an intense immediate airway obstructive response, as well as airway hyperresponsiveness and inflammatory granulocyte infiltration and remodeling [13–15]. Nevertheless, approximately 20% of the total number of guinea pigs ($n = 200$; data not shown) do not develop airway obstructive response following one or various antigen challenges. Similar observations have been described by other authors in guinea pigs [16, 17], mice [18, 19], and humans [18, 20]. Unresponsiveness to antigen challenge might be an exclusion criterion for participating in asthma clinical trials, but unfortunately unresponsive individuals are poorly studied. The present study evaluates functional, pharmacological inflammatory and immunological features of guinea pigs with no airway obstructive response to antigen challenge.

MATERIALS AND METHODS

Animals

Male, outbred guinea pigs (350–400 g), purchased from Harlan Mexico (strain HsdPoc:DH) and housed at our institutional laboratory animal facilities, were used. The animals were maintained in filtered air-conditioned, 12/12-hour light/dark cycles, at $21 \pm 1^\circ\text{C}$ and 50%–70% humidity, fed with sterilized pellets (2040 Harlan Teklad Guinea Pig Diet), and water ad libitum. All animals were handled accordingly to protocols approved by the Scientific and Bioethics Committee of the Instituto Nacional de Enfermedades Respiratorias.

Sensitization Procedure

On day one, guinea pigs received a mixture of 60 $\mu\text{g/mL}$ ovalbumin (OVA) with aluminum hydroxide (1 mg/mL) dispersed in saline solution via

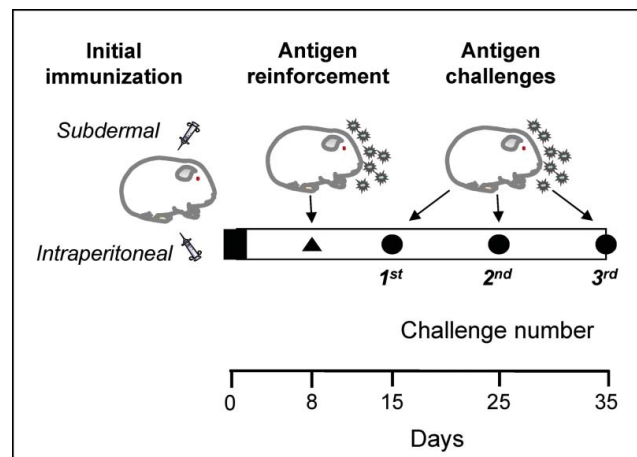


FIGURE 1. Sensitization and antigen-challenge procedures. After initial sensitization and reinforcement, guinea pigs received 3 consecutive antigen challenges every 10 days. Animals were sacrificed at day 35 (third challenge) after evaluation of antigen-induced airway responsiveness.

both intraperitoneal (0.5 mg/mL) and subdermal (0.5 mg/mL) injections (Figure 1). Antigen sensitization was reinforced 8 days later with OVA aerosol (3 mg/mL saline) delivered over 5 minutes (Figure 1). Aerosols were produced by a US-1 Bennett nebulizer (flow, 2 mL/minute; Multistage liquid impinger, Burkard Manufacturing Co., Rickmansworth, Hertfordshire, UK), releasing mixed particles whose size was $<4 \mu\text{m}$ (44%), $4\text{--}10 \mu\text{m}$ (38%), and $>10 \mu\text{m}$ (18%). From day 15 onward, 6 guinea pigs that developed immediate airway obstruction following antigen challenge, named the responder group (R), and other 6 animals with no broncho-obstructive response, named the nonresponder group (NR), were challenged over 1 minute with OVA aerosol every 10 days (1 mg/mL in the first challenge, and 0.5 mg/mL in the subsequent challenges) (Figure 1). This response pattern has been observed recurrently after adjusting the doses to reduce anaphylactic shock (data not shown). Two additional control groups of nonsensitized guinea pigs were included. Control group 1 received 3 challenges with vehicle (saline solution). Control group 2 received 2 challenges with vehicle. At third challenge, when hyperresponsiveness was evaluated, between histamine challenges, animals received OVA to demonstrate that the observed airway hyperresponsiveness is specific to OVA challenge. This procedure does not induce an evident late airway response in any group, either R or NR guinea pigs [13–15].

Barometric Plethysmography

Inhalation challenges were carried out by using a barometric plethysmograph to record acute airway

obstructive response to the antigen immediately after OVA delivery. We used whole-body single-chamber plethysmography for freely moving animals (Buxco Electronics Inc., Troy, NY, USA) to evaluate pulmonary function according to principles previously described [21].

Antigen-induced Airway Responsiveness

To evaluate airway responsiveness at day 35, corresponding to the third OVA challenge, animals were exposed to increased noncumulative doses of histamine (0.01 to 0.32 mg/mL) aerosols after an initial Penh baseline acquisition before and after OVA administration. Each histamine dose was delivered over 1 minute, and the average Penh value over the following 5 minutes was obtained. The interval between doses was 10 minutes. The dose-response curve finished when Penh reached 3 times its baseline level [13]. Once Penh returned to the initial baseline value (<50% increment) [22], the OVA challenge was administered. The second curve was performed 3 hours later.

Bronchoalveolar Lavage Fluid Cell Count

One hour after finishing the second histamine curve, animals received an intraperitoneal injection of sodium pentobarbital (65 mg/kg) and the trachea was cannulated to obtain the bronchoalveolar lavage fluid (BALF). Trypan blue stain was used to assess total cell number in a Neubauer hemocytometer, whereas Romanowsky stain was used for differential cell counting. Cell counts were expressed as number of cells per mL of BALF. All smears were coded and cells were counted blindly.

In Vitro Organ Bath Studies

After obtained BALF, the tracheae were dissected and connective tissue removed. The tracheal rings (~5 mm) were placed in an organ bath containing warmed (37°C) Krebs solution (pH 7.4) composed by (mM): NaCl 120, KCl 4.77, CaCl₂ 2.5, NaHCO₃ 25.0, KH₂PO₄ 1.2, MgSO₄ 1.2, and glucose 11, which was gassed with 95% O₂/5% CO₂. Indomethacin (1 µM) was added to inhibit endogen prostaglandin production. Tissues were attached to an isometric force transducer (Grass model FT03; Grass Instruments, CA, USA) and suspended under an initial tension of 1.0 g to equilibrate for at least 1 hour. To measure contractility of the tracheal rings, OVA was added at 3.74 µg/mL for 20 minutes. After washout, tissues were stimulated with 10 µM histamine, 10 nM U46619 (thromboxane A₂ (TxA₂))

agonist) and 1 µM leukotriene D₄ (LTD₄) for 20 minutes.

Immunoglobulin Levels in Serum

Serum OVA-specific IgG1, IgE, and IgA antibodies were measured by enzyme-linked immunolabeling sorbent assay as previously described [23]. Blood samples were obtained by intracardiac puncture and OVA-specific immunoglobulins were detected with biotinylated specific antibodies: anti-guinea pig IgG1 IgA and IgE (ICN ImmunoBiologicals, USA). Data were expressed as absorbance (A₄₉₂).

Histamine, TxA₂ and Cysteinyl Leukotrienes Levels in BALF

BALF samples were centrifuged at 1500 rpm for 10 minutes at 4°C and histamine, TxA₂ (measured as thromboxane B₂) and cysteinyl leukotrienes levels in BALF supernatant were measured by using enzyme immunoassay kits (Cayman Chemical Company, MI, USA).

Drugs and Reagents

Ovalbumin (chicken egg albumin) grade II, histamine dihydrochloride, Tween 20, orthophenylenediamine, sulphuric acid, LTD₄, reagents for Krebs and phosphate buffers and staining solutions for microscopy were purchased from Sigma Chemical Co. (St. Louis, MO, USA). U46619 was purchased from Tocris (Bristol, UK). Ovalbumin, U46619, LTD₄, and histamine were dissolved in saline solution. Aluminum hydroxide was purchased from J.T. Baker (Phillipsburg, NJ, USA). Pentobarbital sodium was acquired from Pfizer (Toluca, Mexico). Hydrogen peroxide was purchased from Merck (Merck, Darmstadt, Germany).

Statistical Analysis

Airway responsiveness to histamine was evaluated by means of the interpolated histamine dose that caused a 3-fold increase of basal Penh, i.e., the provocative dose 200% (PD₂₀₀). The PD₂₀₀ ratio corresponds to the PD₂₀₀ value observed after OVA challenge divided by the PD₂₀₀ value before challenge. One- or 2-way ANOVA followed by Tukey's test were applied for multiple comparisons. Associations between lung function parameters and either immunoglobulins were assessed through Pearson correlation coefficient. Statistical significance was set at 2-tailed *P* < .05. Data in the text and figures represent mean ± SE.

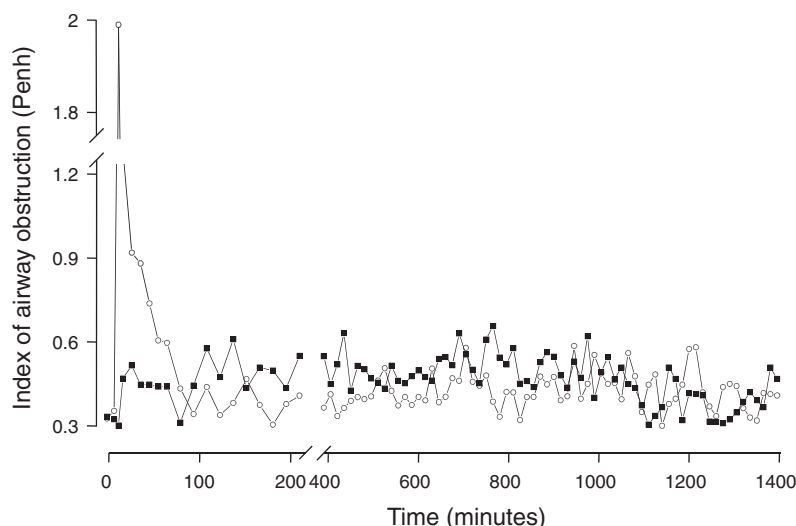


FIGURE 2. Temporal course of antigen responses during the first ovalbumin challenge in sensitized guinea pigs. Recordings show a typical transient airway obstructive response (open circles) and the absence of this response (closed squares) in 2 different guinea pigs, after the first antigen challenge (day 15).

RESULTS

Airway Obstructive Responses Induced by Antigen Challenge

Guinea pigs were selected in accordance with 1 of the 2 patterns of response to the first antigen challenge ($n = 6$ each; Figure 2), either the transient airway obstructive response typical of asthma model (R) [13–15] in which maximum value Penh ($R_{\max}$) was $>200\%$ baseline, or the nonnoticeable obstructive response, (NR) in which $R_{\max}$ did not exceed 200% basal Penh.

$R_{\max}$ values observed at the first antigen challenge (day 15) in R and NR guinea pigs were similar to those observed at the second and third challenges (days 25 and 35; Figure 3). $R_{\max}$ values in each challenge were significantly different ($P < .05$; $n = 6$ each group) between R and NR guinea pig groups, and between R and control group ($P < .05$, respectively; $n = 6$ each group; Figure 3). Maximum Penh values between control and NR groups were similar.

Antigen-Induced Airway Responsiveness

PD_{200} to aerosolized histamine was not different from PD_{200} obtained after saline challenge in the control groups (Figure 4). In both groups, R and NR, a significant decrease in histamine PD_{200} was observed after OVA challenge in comparison with both control groups ($P < .01$, respectively; Figure 4). Basal PD_{200} values among all groups were not significantly different.

Tissue Bath Studies

OVA induced tracheal contraction in R group in a manner significantly higher than the contraction

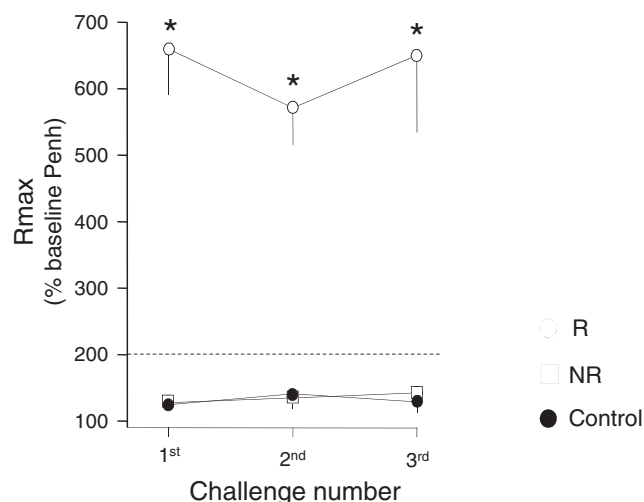


FIGURE 3. Maximum airway obstructive responses induced by ovalbumin challenges in sensitized guinea pigs. Values correspond to saline (closed circles, control 1 group) and OVA (open squares and circles) challenged guinea pigs, and were obtained as average of maximum Penh ($R_{\max}$) reached at the first hour. Open circles correspond to guinea pigs showing responses over 200% basal Penh (R group), whereas open squares illustrate guinea pigs with no airway obstruction response after antigen challenge (NR group). $*P < .05$, R group compared with both control and NR groups (2-way ANOVA with Tukey's multiple comparisons test). Symbols represent the mean $\pm$ SE of $n = 6$ animals for each group. Penh = index of airway obstruction.

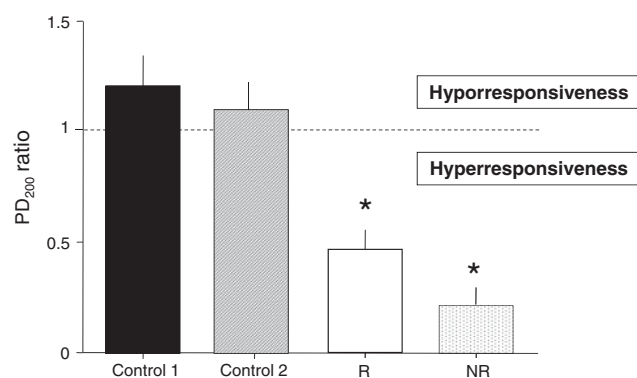


FIGURE 4. Airway responsiveness to histamine before and after ovalbumin challenge in sensitized guinea pigs. PD₂₀₀ ratio, i.e., the PD₂₀₀ value observed after OVA challenge divided by PD₂₀₀ value before challenge for: control 1 (nonsensitized and vehicle-challenged guinea pigs), control 2 (nonsensitized and OVA-challenged (day 35) guinea pigs), airway obstruction responder (R), and nonresponder (NR) groups. Bars represent the mean ± SE of $n = 6$ animals for each group. * $P < .01$ compared with both control groups (one-way ANOVA with Tukey's multiple comparisons test).

induced by OVA in control and NR guinea pigs ($P < .05$; Table 1). Histamine, LTD₄, and U46619 (a stable analog of TxA₂) administration induced a similar tracheal contraction in all groups (Table 1).

Specific anti-OVA IgG, anti-OVA IgA, and anti-OVA IgE in Serum

Serum anti-OVA IgG1 levels were similar in R and NR groups and significantly higher than control group ($P < .001$; $n = 6$ each group, respectively; Figure 5). Serum OVA-IgE levels only increased in R group in comparison with control group ($P < .001$; $n = 6$ each group; Figure 5). The serum levels of OVA-IgA increased in both R ($P < .05$; $n = 6$) and NR groups ($P < .001$; $n = 6$) in comparison with control group ($n = 5$; Figure 5).

In Table 2, it can be observed that the maximal Penh values were correlated directly with anti-OVA IgE levels and indirectly with anti-OVA IgA levels but were unrelated to OVA-IgG1 levels. Likewise,

TABLE 2. Pearson Correlation Coefficients of Lung Function and OVA-specific Immunoglobulins

	Lung function	
	Rmax	PD ²⁰⁰ ratio
Serum immunoglobulins		
IgE	0.39 ^a	0.11
IgG1	−0.02	−0.58 ^b
IgA	−0.40 ^a	−0.39 ^a

^a $P < .05$, ^b $P < .001$, $n = 17-18$

Rmax, maximum value Penh; PD²⁰⁰ ratio, change of PD²⁰⁰ after antigen challenge as compared with basal (pre-challenge) PD²⁰⁰ value; OVA, ovalbumin.

PD₂₀₀ ratio had an inverse correlation with OVA-IgG1 and OVA-IgA levels implying that the greater the OVA-IgG1 and OVA-IgA levels, the higher the airway hyperresponsiveness. However, this association of PD₂₀₀ ratio was not apparent for OVA-IgE. Since values of OVA-specific immunoglobulins among control groups (1 and 2) were not different statistically (data not shown), control group 1 was considered representative as control group.

Cell Counts in BALF

The number of total cells, macrophages, lymphocytes, eosinophils, and neutrophils in BALF from NR group was not significantly different compared to control group. Neutrophil and eosinophil counts in BALF from R group were significantly higher than control and NR groups ($P < .01$; $n = 6$ each group; Figure 6). Since cell counts among control groups were similar (data not show), control group 1 was considered the representative control group.

Histamine, TxA₂ and Cysteinyl Leukotrienes Levels in BALF

As shown in Figure 7, BALF levels of histamine and TxA₂ were increased significantly in R group in comparison with control and NR groups ($P < .01$ and $.05$, respectively). The levels of cysteinyl leukotrienes in all groups were similar.

TABLE 1. In vitro Maximal Response (g) to Ovalbumin, Histamine, U46619, and LTD₄ of Tracheae from Responder and Non-Responder Guinea Pigs

	OVA (3.74 μg/mL)	Histamine (10 μM)	U46619 (10 nM)	LTD ₄ (1 μM)
Controls	0.12 ± 0.04 ($n = 7$)	1.1 ± 0.4 ($n = 7$)	1.06 ± 0.19 ($n = 6$)	0.56 ± 0.08 ($n = 6$)
Responders	1.6 ± 0.4 ^a ($n = 10$)	1.9 ± 0.5 ($n = 10$)	0.98 ± 0.05 ($n = 3$)	0.53 ± 0.05 ($n = 6$)
Nonresponders	0.15 ± 0.08 ($n = 7$)	1.4 ± 0.6 ($n = 7$)	1.34 ± 0.28 ($n = 6$)	0.56 ± 0.07 ($n = 6$)

^a $P < .05$, Unpaired T-Student test

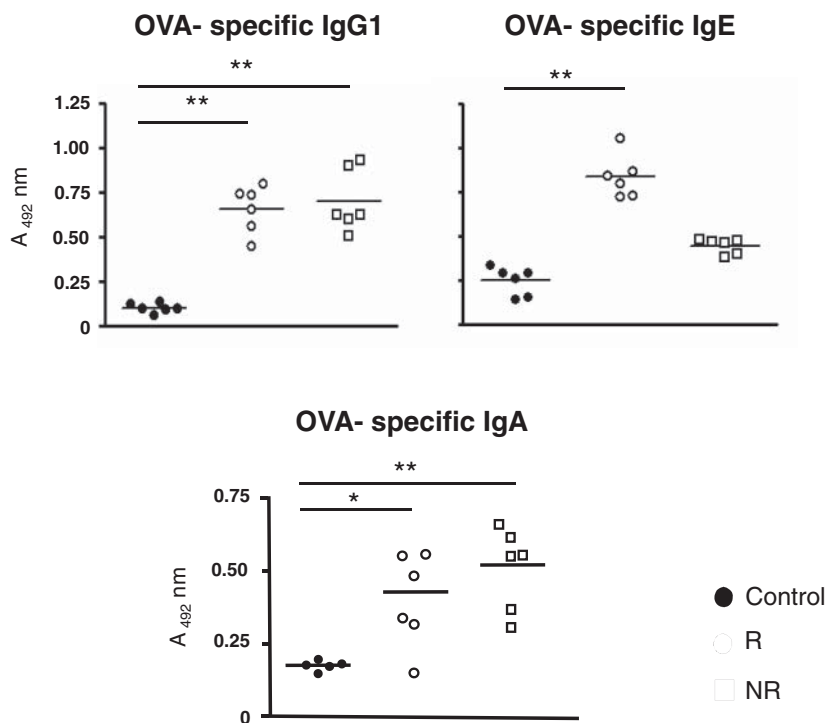


FIGURE 5. Scatter plots of specific immunoglobulins from challenged guinea pigs. Values for serum OVA-specific IgG1, IgE, and IgA antibodies for control, airway obstruction responder (R) and nonresponder (NR) guinea pig groups. * $P < .05$ and ** $P < .001$ compared with control group (one-way ANOVA with Tukey's multiple comparisons test). A₄₉₂ nm: absorbance, OVA: ovalbumin.

DISCUSSION

We have observed that guinea pigs that do not display airway obstruction after antigen challenge (NR group) are able to develop hyperresponsiveness in a similar way to guinea pigs which did develop airway obstruction (R). Airway obstruction is a central symptom of asthma exacerbation. Exposure to the antigen usually is followed by an immediate and transient early broncho-obstruction and, some hours later, by a late long lasting response [23]. Nevertheless, in some asthmatic patients only an isolated either early or late airway obstructive response has been observed [1–3]. Only early airway obstructive responses are observed in the guinea pig asthma model used in this study, such animals composed the R group. In a previously published guinea pig model, low antigen doses during challenges induced only early responses, while higher doses induced both, early and late responses [10]. Therefore, a provocative antigen dose is fundamental in the development of both responses in guinea pig. The doses used in this asthma model were adjusted to reduce anaphylactic shock during challenges (data not shown). Nevertheless, a nonasthma phenotype, i.e., guinea pigs Non-Responsive to in-

haled antigen challenge, was observed in this study. Unexpectedly, NR guinea pigs did display hyperresponsiveness to histamine. Although airway hyperresponsiveness is a hallmark of asthma, it is a variable feature among individuals [24], contributing to asthma heterogeneity. Furthermore, a significant proportion of healthy subjects also present airway hyperresponsiveness [25]. The proportion of asymptomatic subjects with airway hyperresponsiveness ranges from 20% to 60% [25–29]. Although the clinical significance of asymptomatic airway hyperresponsiveness remains a matter of debate, this phenomenon could precede the occurrence of asthma, representing a transition from the lack of symptoms to the asthmatic phenotype irrespectively of atopic status [25, 28].

The design of animal models of antigen-induced airway hyperresponsiveness without development of inflammatory cell infiltration and broncho-obstruction is essential to understand the pathophysiological mechanisms of this condition. An interesting observation in this study was that the treatment with OVA induced contraction only in tracheae from R guinea pigs, whereas histamine, the stable analog of TxA₂ (U46619), and LTD₄ produced similar contractile responses in bath tissues

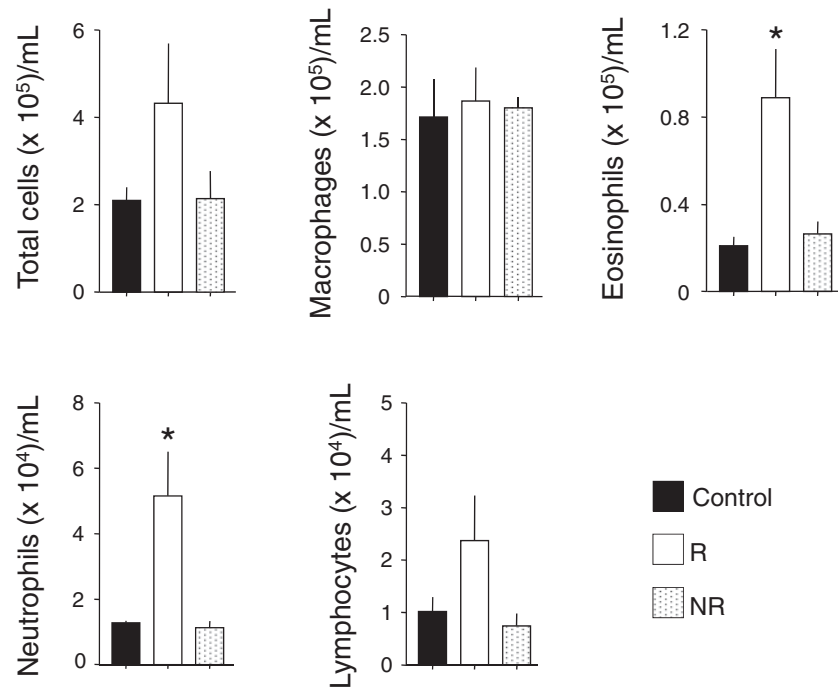


FIGURE 6. Total and differential cell counts in BALF from ovalbumin-challenged guinea pigs. Cell numbers recovered in bronchoalveolar lavage fluid are shown for each group. R group corresponds to guinea pigs with airway obstructive response; NR group corresponds to nonresponder guinea pigs after antigen challenge. Bars represent the mean $\pm$ SE of $n = 6$ guinea pigs in each group. * $P < .01$ compared with control and NR groups (one-way ANOVA with Tukey's multiple comparisons test).

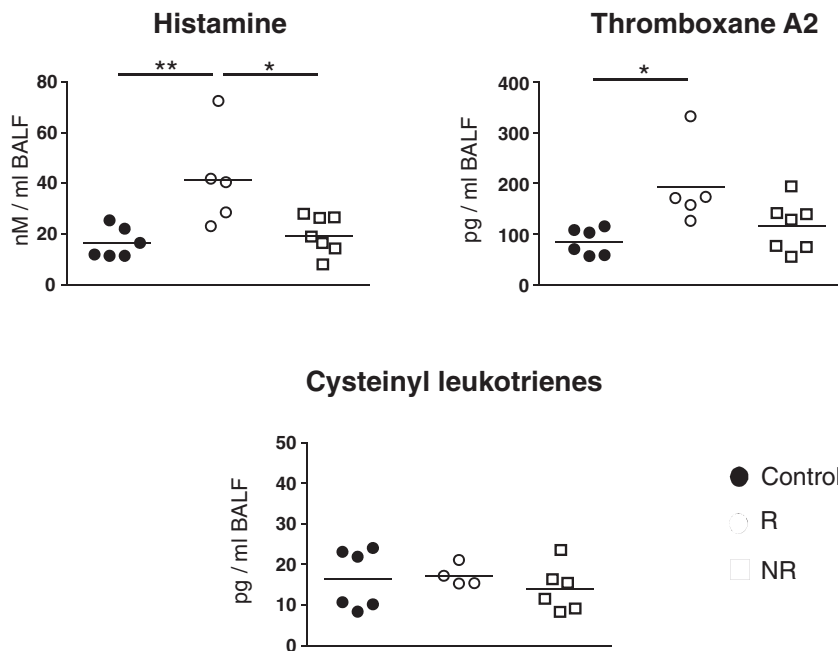


FIGURE 7. Scatter plots of histamine, thromboxane A2 and cysteinyl leukotrienes levels in BALF from challenged guinea pigs. Values for histamine, thromboxane A2, and cysteinyl leukotriene for control, airway obstruction responder (R) and nonresponder (NR) guinea pig groups. * $P < .05$ and ** $P < .001$ compared with control group (one-way ANOVA with Tukey's multiple comparisons test).

from all groups. This suggests that the smooth muscle receptors for these contractile mediators are functionally alike between guinea pig groups. Furthermore, since there is no response to OVA in both *in vivo* and *in vitro* models, it may be concluded that the response of an individual animal is not due to how effectively the antigen was delivered during the challenges.

In this study, we have observed that the BALF levels of contractile agonists of guinea pig airway smooth muscle such as histamine and TxA₂ suggest that these mediators, but not LTD₄, might be involved in the immediate or early asthmatic reaction in R group. None of these mediators were found in NR guinea pig BALF suggesting that the absence of contractile mediators after antigenic challenge might be the cause of the absence of airway obstruction in NR guinea pigs.

Human and guinea pig immunoglobulin classes during allergic responses are similarly regulated; nevertheless, the main immunoglobulin in guinea pig model of allergy is IgG1, while in human is IgE. Although it has been observed that both immunoglobulins contribute to broncho-constriction in guinea pigs, the obstructive response induced by IgG1 is very low in comparison with the strong response induced by IgE [30, 31]. Our results show that IgE production and broncho-obstruction extent are correlated, suggesting that IgE is essential in the development of obstructive responses induced by antigen in our model in guinea pig. High OVA-specific IgG1 production was observed in both groups, R and NR guinea pigs, confirming that NR guinea pigs are atopic. The role of IgG1 in the guinea pig includes participation in immediate hypersensitivity, pulmonary eosinophilia [32], and development of airway hyperresponsiveness evaluated *in vitro* [33] and *in vivo* [31]. In addition, the release of histamine in lung is greater in guinea pigs sensitized with IgG1 antibody than with IgE [34, 35], and it is known that histamine response is potentiated by TxA₂ in the IgG1- but not IgE-mediated anaphylactic airway obstruction [31]. In this context, anti-OVA IgG1 levels in our model were directly associated to the extent of airway hyperresponsiveness. This observation corroborates that IgG1 production may be crucial in the development of airway hyperresponsiveness induced by antigen in the guinea pig. Guinea pig IgE or IgG1 production depends on the antigen dose used during antigen sensitization. For example, low doses of antigen (OVA) during sensitization (10 µg *i.p.*) induced IgE and IgG1, while high doses of antigen (100 µg *i.p.*) favored IgG1 production [30, 36]. The dose of antigen used in our study, *i.e.*, 60 µg/mL, is in the range with the doses that produce IgG1 or both immunoglobulins, and it is

closer to the threshold production of the different immunoglobulin patterns. Then, it is likely that the antigen sensitizing dose used in this study is responsible for the development of R and NR phenotypes; however, more experiments are needed to demonstrate this hypothesis. IgA has been described as a noninflammatory antibody isotype that maintains homeostatic balance in mucosa. In humans, allergy has been observed associated with OVA-IgA and OVA-IgG [37]. In addition, mouse pulmonary OVA-IgA levels vary with OVA doses [38]. In guinea pig, the magnitude of broncho-obstruction is inversely associated with serum OVA-IgA levels; therefore, IgA seems not to be a fundamental factor in the development of allergic responses in guinea pig. In contrast, serum OVA-IgA and airway hyperresponsiveness magnitude are directly associated, pointing out the putative role of IgA in the mechanisms inducing hyperresponsiveness in the guinea pig. In this context, it has been reported that atopic patients and healthy individuals have an allergic response characterized by specific IgA [38]. Particularly, IgA can induce eosinophil degranulation, causing airway hyperresponsiveness via an indirect effect on airway smooth muscle [39–42]. Schneider and collaborators have shown that sensitization of mice with limiting doses of OVA can induce airway hyperresponsiveness accompanied by pulmonary IgA, but not allergic lung disease [38]. Then, our data suggest that antigen specific-IgA may play a dual role, this is, as the classical marker capable of offering protective effects on the allergen response, and it may also be involved in airway hyperresponsiveness.

In addition to broncho-obstruction, airway hyperresponsiveness, as well as IgA, IgE, and IgG1 production, responder guinea pigs showed an immediate increase of neutrophil and eosinophil in BALF. Asthma is considered an eosinophilic disease, and neutrophils are common during the acute phase of asthma [43]. The NR guinea pigs did not show infiltration of inflammatory cells in BALF, nor even neutrophils that are the earliest cells appearing in tissues after injury. Hyperresponsiveness observed in asymptomatic patients has been associated to inflammatory cell infiltration in a similar level than that found in mild asthma patients [44]. In addition, no association has been observed between asymptomatic hyperresponsiveness and inflammatory cell infiltration [45]. Antigen challenge in NR guinea pigs may not modify airways up to the point to induce injury and inflammation, but it does induce airway hyperresponsiveness and production of IgG1.

The precise mechanisms involved in the development of airway hyperresponsiveness are unknown,

but airway inflammation and remodeling have been suggested [24]; nevertheless, some studies suggest that airway hyperresponsiveness may occur even in the absence of inflammatory cells in the airway lumen or mucosa [46–49]. Our results confirm that this phenomenon occurs in guinea pig. Thus, other factors such as changes within the smooth muscle itself, airway remodeling or inflammatory mediators might be involved in airway hyperresponsiveness development.

Nowadays there are no syngeneic strains of guinea pigs; therefore genetic and epigenetic factors might be associated to the variability of allergic responses in our asthma model. To avoid variability in different guinea pig models, antigen doses are adjusted up to obtain an airway response [10, 50, 51]; to prevent a fatal anaphylactic reaction, airway obstructive responses were diminished by antihistaminergic [9, 52] and anticholinergic [52] pretreatments. It is noteworthy that in our asthma model the antigen-induced obstruction is not controlled by adjusting allergen doses or by drug pretreatments. Therefore, NR animals are likely revealing the biological variability of guinea pigs when are challenged with the same antigen dose.

In summary, our data suggest that the immediate asthmatic reaction in guinea pig might be associated to the release of TxA₂ and histamine through the action of IgE, whereas airway hyperresponsiveness can be observed independently of airway allergic responses after antigen challenge, and it depends on IgA and IgG1 expression. Furthermore, our results point it out that the sole determination of airway hyperresponsiveness or immunoglobulins is not enough to validate an asthma model. The putative guinea pig model of antigen-induced airway hyperresponsiveness (NR group) in absence of airway obstruction and inflammatory cell infiltration might represent an important tool to further understand the mechanisms underlying airway hyperresponsiveness. Finally, it is important to improve the method of sensitization to induce responder or asthma model guinea pigs.

Declaration of interests: Patricia Ramos-Ramírez was recipient of a Ph.D. scholarship from CONACyT (#256077) and is a doctoral student of Posgrado en Ciencias Biomédicas, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México (UNAM). The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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